Delivery Systems for Introduction of Microbial Inoculants in the Field

13

Prashant P. Jambhulkar, Pratibha Sharma, and Rakesh Yadav

Abstract

Undoubtedly choosing correct microbial inoculants is the foremost factor governing the success of a biocontrol program. But making it reach to the field with a suitable delivery method maintaining consistent performance is the next most important challenge. Microbial inoculants are delivered through several means based on the survival nature and mode of infection of the pathogens. These bioagents cannot be applied as spore suspension in field but are applied as powdered or liquid formulation primarily through seed treatment, soil application, root dip, or foliar application. Application of microbial inoculants can influence, at least temporarily, the resident microbial communities and offer protection against a wide range of pathogens. The biocontrol agent applied through different delivery methods multiplies in the soil and remains near the root zone of plants and offers protection even at later stages of crop growth. In this chapter, we have discussed about various microbial bioformulations commercially available and their mode of application in the field. Along with conventional methods of delivery system, other methods such as microbigation, seed biopriming, seed encapsulation, fluid drilling, and consortia method of application are discussed with recent research updates.

Keywords

Microbes • Inoculants • Seed treatment • Biopriming • Microbigation

P.P. Jambhulkar (✉)
Agricultural Research Station (MPUAT, Udaipur), Banswara 327001, Rajasthan, India
e-mail: ppjambhulkar@gmail.com

P. Sharma
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012, India

R. Yadav
Krishi Vigyan Kendra, (RVSKVV, Gwalior), Jhabua, MP, India

13.1 Introduction

Plant diseases are caused by various biotic and abiotic factors viz. fungi, bacteria, viruses, viroids, phanerogamic parasites, protozoans, and nematodes are taking heavy toll of crops. These pathogens are causing substantial losses in differ-

D.P. Singh et al. (eds.), *Microbial Inoculants in Sustainable Agricultural Productivity*,
DOI 10.1007/978-81-322-2644-4_13

ent crops and therefore need to be managed. Several chemicals have been used in the past to manage the diseases caused by various pathogens. No doubt, some degree of control was achieved, but it posed new problems of residual toxicity and development of resistant strains of the pathogens. Delivery of microbial inoculants is being a very attractive option since it would substantially reduce the use of agrochemicals (Berg 2009). Microorganisms play a vital role in cropping systems, particularly plant-growth-promoting microorganisms (PGPMs). Soil or seed inoculation with microbial inoculants may lead to changes in the structure of the indigenous microbial population, which is important with regard to the safety of introduction of microbes in plant microenvironment (Trabelsi and Mhamdi 2013). Many reports indicate that the application of microbial inoculants can influence, at least temporarily, the resident microbial communities; therefore, screening of biocontrol agents (BCAs) with broad-spectrum activity and capacity to elicit systemic resistance in plants and offer protection against a wide range of pathogens needs to be done. Success in the identification of new microbial inoculants that exhibit significant control of various root and foliar diseases in the past few decades has contributed to the rising interest in the biological control of various phytopathogens. Further, an in-depth study of the action of BCAs is needed before using them on a large scale. End number of formulations approved by regulatory authorities around the globe are available for use in disease management. Therefore, biocontrol agents or antagonists as a means of plant disease control has gained importance in recent years. The biocontrol agent multiplies in the soil and remains near the root zone of plants and offer protection even at later stages of crop growth. The antagonistic activity of biocontrol agents against plant pathogens is highly specific against a particular pathogen and/or different races of the pathogen. Delivery system of BCAs mainly depends on the type of pathogen to be managed, the stage of the crop to be protected, the nature and severity of the disease, and the climatic conditions of the region (Desai et al. 2000). For application of a good formulation, a proper delivery method of microbial inoculants is essential. Since bioformulation incorporated in the soil have high densities of viable and efficient microbes for a rapid colonization of host rhizosphere, it may induce at least a transient perturbation of the equilibrium of soil microbial community. However, a modification in the microbial community structure caused by inoculation could be buffered by ecosystem resilience, which is driven by the level of diversity and interactions of the plant–soil biota (Kennedy 1999).

Seed treatment is a practical method of delivery system for both fungal and bacterial biocontrol agents. Biological control agents applied to seed have been shown to protect the seed against many seed-borne pathogens of crops, as well as increase plant growth and vigor (Jambhulkar and Sharma 2013). The use of biological agents as seed treatment is a valuable and an equally effective protection as chemical seed treatment. Physiological seed treatment such as seed priming has been used to quicken seed germination and improve the survival of seedlings (Burelle 2000). Bioformulation may directly be applied to plant roots in the form of root dip, spray, drip, or flood application for the management of soil-borne pathogens (Gasic and Tanovic 2013). Commercialization of good biocontrol agents becomes difficult due to impractical dosage recommendations, limited or inconsistent control efficacy, and improper delivery system. A better understanding of the ecological and epidemiological relationship between microorganisms and suitable delivery systems that will carry fungal strains with enhanced fungicide resistance will help to reduce the gap between experimental results and commercial use of biopesticides. Developing accurate delivery system is foremost important to assure effectiveness of bioformulation under field conditions. Unlike agrochemicals, in which chemical is dissolved in a solvent, most microbial inoculants are particulate suspensions. Problems with suspensions include settling of the microbial pesticides, nozzle blockages, stress affecting the viability of spores, inappropriate droplet size, large number of infective spores packed to a droplet, etc. (Bateman et al. 2007). Researches are in progress to optimize the delivery system for each group of biopesticides. The application of

fluorescent pseudomonads by seed treatment (Niranjana et al. 2009), seedling root dip (Verma 2009), and soil drenching (Jeyalakshmi et al. 2010) has been attempted my many workers to control phytopathogens in various crops. Jambhulkar and Sharma 2013 reported the efficacy of various carrier formulations of *Pseudomonas fluorescens* through seed treatment, seedling root dip and soil drenching in unity and in combination. Work on droplet size revealed that smaller droplets would enhance effectiveness of microbial inoculants (Alves and Bateman 2013), whereas larger droplets are suitable for entomopathogenic nematodes (Bateman et al. 2007). Stable, effective formulations and appropriate delivery system are needed to convince farmers to adopt bioformulations.

13.2 Microbial Inoculants as Biocontrol Agents

Indiscriminate use of chemical compounds as pesticides damages the entire agroecosystem, which encourages the use of biopesticides. In fact, there is great potential of biopesticides in organic and conventional agriculture. Biological control by antagonistic microbial inoculants is a potential nonchemical means for crop protection, which is seen as a very attractive plant protection measure as it would substantially reduce the use of chemical pesticides and fungicides, and there are now an increasing number of inoculants being commercialized for various crops (Berg 2009). There is immense role of microorganisms in agricultural ecosystem, particularly plant-growth-promoting microorganisms (PGPMs). Plant growth benefits are mainly attributed to three major mechanisms: (1) PGPMs acting as biofertilizers (such as nitrogen-fixing bacteria and phosphate-solubilizing bacteria) assist uptake of plant nutrients by providing fixed nitrogen and other nutrients; (2) phytostimulators (microbes expressing phytohormones such as *Azospirillum*) can directly promote the growth of plants usually by producing phytohormones, and (3) biological control agents (such as *Trichoderma, Pseudomonas*, *Bacillus*, etc.) protect plants against phytopathogenic organisms (Mohiddin et al. 2010; Dawar et al. 2010). Microbial inoculants have various benefits over chemical pesticides: they (a) are more safe, (b) show reduced environmental damage, (c) show more targeted activity, (d) are effective in smaller quantities, (e) are able to multiply but are also controlled by the plant and indigenous microbes, (f) have quicker decomposition procedures, (g) are less likely to induce resistance by the pathogens and pests, and finally (g) can be used either in organic and conventional agriculture (Berg 2009).

Bacillus is a genus of bacteria known to elicit induced systemic resistance (ISR) in plants. In addition, *Bacillus* spp. have reduced incidence of viral diseases, for example, cucumber mosaic virus on tomato. On plants that are not challenged with pathogens, it has been reported that *Bacillus* can increase fresh weight and number of fruits and flowers (Kloepper et al. 2004). *Pseudomonas*, a genus of bacteria that can colonize plant roots and suppress pathogens through the production of antibiotics, is a genus that can elicit ISR as well (Kloepper et al. 2004). Bacteria in this genus have a strong potential as biocontrol and growth-promoting agents due to the following characteristics: a) rapid growth in vitro; b) rapid utilization of seed and root exudates; c) ability to colonize and multiply in the rhizosphere and the spermosphere, as well as inside the plants; d) production of metabolites like antibiotics, siderophores, and growth promoters; e) competition with other microorganisms; and, finally, f) ability to adapt to environmental stress (Weller 2007). In early attempts, products made of this *Bacillus* spp. failed due to the instability of the culture and lack of long-term viability (Kloepper et al. 2004). It is known that the majority of bacteria that promote plant growth are rhizosphere inhabitants; they have been designated as plant-growth-promoting rhizobacteria (PGPR). The most promising group of PGPR for biocontrol of plant diseases is fluorescent pseudomonads. Fluorescent pseudomonads associated with plants include *Pseudomonas fluorescens*, *P. putida*, *P. aeruginosa*, and *P. aureofaciens*. Plant-growth-promoting rhizobacteria may stimulate the production of biochemical compounds associated with host defense; mas-

sive accumulation of phytoalexins and phenolic compounds; increase in the activities of PR proteins, defense enzymes, and transcripts; and enhanced lignification. The induction of SAR using various ISR inducers has been of recent interest with quite reasonable success (Meena 2014).

Another group of microbes corresponds to some types of algae that have biotechnological potential as soil fertilizers for plant production and some macroscopic marine algae (*Eklonia maxima*) that improve the growth and yield of plants (Reisser 2010; Crouch and van Staden 1992). Finally, several genera and species of PGPR and different microbes are used as inoculants; the diversity represents an opportunity to start research in this area and provide new solutions for the current necessities of agriculture.

Soil microorganisms form a very complex and dynamic community between different compartments and levels. Microorganisms can survive in the spermosphere, a zone influenced by the seeds, which is full of nutrients that support their growth. In addition, they can inhabit the phyllosphere, a zone that comprises the above-ground parts of the plants, whose most relevant characteristic is the fact that it is in constant fluctuation related to external facts as temperature, radiation, and water availability. Other zones correspond to the vascular tissue, the rhizosphere, and the endophytic sites. Several strains of *Trichoderma* have been described as antagonistic fungi that are able to attack a wide range of phytopathogenic fungi. The production and secretion of fungal-cell-wall-degrading enzymes and compounds affecting the integrity of fungal membrane and cell walls are considered as the key steps in the antagonistic process by *Trichoderma* (Chet et al. 1998). Antagonism may be accomplished by competition, parasitism, and antibiotics or by a combination of these. Parasitism involves the production of several hydrolytic enzymes that degrades cell wall of pathogenic fungi. β-1,3 glucanase and chitinase are the key enzymes responsible for fungal cell and sclerotial cell wall lysis. These enzymes are produced by several fungi and bacteria and may be an important factor in biological control. Moreover, in the rhizosphere, region that includes plant roots and surrounding soil, intensive interactions between plants, soil, and microfauna take place due to its high energy and carbon content. This accumulation in the rhizosphere corresponds to all the compounds produced by plant roots, most of which are organic derived from photosynthesis and other plant processes (Pinton et al. 2001). Different and varied biochemical signal exchanges take place between these communities and their host plants; indeed, a wide diversity of bacteria and plant-associated microbes can interact with plants in a beneficial way, either by enhancing their growth and/or controlling phytopathogens (Beattie 2006; Nihorimbere et al. 2011).

13.3 Formulations Used for Inoculation of Microbial Inoculants

Development of a bioformulation is necessary to commercialize biocontrol technologies by industries. The commercial use of plant-growth-promoting rhizobacteria requires inoculum that retains high cell viability and can easily be transported and applied to seed. It needs extensive studies for large-scale multiplication of a biocontrol agent (BCA), which include suitable and inexpensive medium, method of fermentation (solid or liquid), type of formulation (wettable powder, liquid, granular), nature of filler material, delivery systems, optimum shelf life, and storage conditions. Application guidelines are set by considering all these aspects of a bioformulation. Delivery of free cell form is usually impractical to achieve satisfactory bioremediative effect because microbes are encumbered by biotic and abiotic stresses from the environment (Ting et al. 2010). The aims of formulating viable cells are to ensure that adequate cell viability is sustained to increase the efficacy of the cells and to facilitate the delivery and handling processes (Filho et al. 2001). A bioformulation can improve product stability and shelf life and also protect microbial inoculants against different environmental conditions and provide initial food source (Jambhulkar and Sharma 2014). Application of microbial

inoculants either to increase crop health or to manage plant diseases depends on the development of commercial formulations with suitable carriers that support the survival of microorganism for considerable length of time (Aeron et al. 2011). A formulated microbial product is a product composed of one or more biological control agents mixed with ingredients that will improve its survival and effectiveness (Schisler et al. 2004). Those microbial inoculants formulated for delivery to soil are of especial importance due to their specific court of action, which is the rhizosphere. Microbial inoculants can be applied to the soil as fluid suspensions, as powder formulations, and as granules for soil and spray application. Fluid suspensions are prepared based on culture concentrates diluted in water or a buffer solution prior to application. They can also be prepared as dormant aqueous suspensions, obtained after harvesting the bacteria from a liquid culture, washed free of the spent medium and stored at a specific concentration in sterile water at room temperature (Miranda 2012). Microbial inoculants are formulated as dry formulation for direct application dusts (DP), seed dressing formulations-powders for seed dressing (DS), granules (GR), microgranules (MG), dry formulations for dilution in water-water dispersal granules (WG), and wettable powders (WP); liquid formulations for dilution in water emulsions, suspension concentrates (SC), oil dispersion (OD), suspoemulsion (SE), capsule suspension (CS), ultra low volume formulations (Knowles 2005, 2006). Powdered formulations are more commonly used. They consist of organisms concentrated into dry or wet powders. Depending on the composition of the powders, they can be applied directly to the soil, suspended in water, or dusted onto seeds. The commonest method to formulate granular products is to mix the organism and the ingredients with the granules (Burges 1998). In general, product formed from solid or semi solid-state fermentation does not require sophisticated formulation procedures prior to use. For example, grain or other types of organic matter upon which antagonists are grown are simply dried ground and added to the area to be treated. There are several problems with solid-state fermentation, which may make the system inappropriate for commercial product development. The preparations are bulky, they may be subject to a greater risk of contamination, and they may require extensive space for processing, incubation, and storage. The liquid-state fermentation is devoid of such problems, and large quantities of biomass can be produced within a few days. Either the biomass can be separated from medium and concentrated or the entire biomass with medium can be incorporated into dusts, granules, pellets, wettable powders, or emulsifiable liquids. The carrier material may be inert or a food base or a combination of both. Inoculant formulations depend directly on the carrier used for the delivery of the products because target microorganisms are mixed with it before being applied. Carriers are the inert ingredients that hold or dilute the microorganism to the desired concentration and improve coverage and distribution (Burges 1998). Commercial application of PGPR either to increase crop health or to manage plant diseases depends on the development of commercial formulations with suitable carriers that support the survival of bacteria for a considerable length of time. Carriers constitute the key for the effective release of the different products; they need to be effectively chosen due to their diversity (e.g., water, vermiculite, calcium sulphate, mineral soil and sand, vegetable oil, corn cob) that starts from classic ones to new and unconventional ones (Bashan 1998; Burges 1998). Certain specific conditions might increase the efficacy of a formulation. Addition of organic acids to *T. koningii* formulations and polysaccharides and polyhydroxyl alcohols to *T. harzianum* increases the activity of BCAs (Connick et al. 1991). The carrier represents the principal portion of inoculants. The materials from which they are made define their effectiveness. Moreover, they have to fill certain requirements in order to be efficient. First, they need to have the capacity to deliver the correct concentration of viable cells at the time they are needed. The reason is because there are certain ranges of concentrations that can be inoculated in certain crops. In addition, as inoculants should be sterile; carriers should be chemically consistent and able to provide enough

water-holding capacity for microbial growth (Bashan 1998). Moreover, carriers need to be easily available and able to be mixed with other compounds like nutrients in order to provide a good environment for the live cells. In fact, they need to be easy to mix and easy to fabricate as they are intended to be used massively. Furthermore, they need to be easy to handle and have longer shelf life because they will be used by farmers who will use them periodically and will need to have reservoirs of the products for rapid use (Bashan 1998). Foliar application of fluorescent pseudomonads was attempted by few workers (Gnanamanickam and Mew 1992; Bahadur et al. 2007; Prathuangwong et al. 2013), all of whom used bacterial cell suspension for seed treatment, soil application, or foliar sprays. Use of bacterial suspensions is impractical for large-scale application to control foliar diseases in the field. A powder formulation with longer shelf life would be beneficial (Tables 13.1 and 13.2).

13.4 Delivery Systems

Plant-growth-promoting rhizobacteria are delivered through several means based on the survival nature and mode of infection of the pathogen. It is generally delivered through seed treatment, root dip, soil application, and irrigation water. An ideal formulation is expected to facilitate the delivery of the living biocontrol agents in its active state, at the right place, at the right time. While the formulated microbial products must be effective at the site of action and compatible with agronomic practices, they should be easy to apply to and adhere to plant parts such as seeds, tubers, cuttings, seedlings, transplants, and mature plants or be available in the soil medium.

13.4.1 Seed Treatment

Biological formulations applied to seeds greatly help to deliver the agents to the spermosphere of plants, where, in general, extremely conducive environments prevail. The BCAs are therefore provided an excellent opportunity to survive, multiply, persist, and exercise control of soil-borne phytopathogens (Cook and Baker 1983). Seed treatment has the potential to deliver microbial agents "in the right amount, at the right place and at the right time." With increasing public awareness of the potential environmental and health hazards of both agrochemicals and the advances in biotechnology to improve the performance in microbial products, the application of microbial inoculants to seeds (Chandra and Greep 2010; Chandra et al. 2006) is likely to increase in the future. With an aim to deliver the active ingredients as close to the target as possible, this approach continues to receive considerable attention from end users. Significant advances in seed treatment technology has been achieved due to consistent work done around the globe, and this approach is an attractive means for introducing biological control agents into the soil–plant environment, as these introduced organisms are offered the selective advantage to be the first colonizers of plant roots. At the time of planting seedlings, the formulated products can be used directly (powders, liquids) without stickers. Powders for seed treatment are formulated by mixing an active ingredient, inert carrier to facilitate product adherence to seeds by mixing seeds with formulated product (Woods 2003). Additives such as gum arabic and xanthan gum are used to prolong the survival of microbial agents applied to seeds. Alginate hydrogel, used as a seed encapsulation material, maintains the entity in a viable state and protects it from other stresses. Seed priming, in which seeds are mixed with an organic carrier and then moisture content, is brought to a level just below that required for seed treatment which has been used to deliver *T. harzianum* to control *Pythium*-induced damping-off on cucumber (Callan et al. 1990). In another process of seed treatment, an industrial film-coating process which was developed for the application of chemicals and biological crop protection agents is being utilized for the application of *Trichoderma* spp. on radish and cucumber seeds through a film coating and was shown to be effective against damping-off (Cliquet and Scheffer 1997). Prathuangwong et al. (2013)

Table 13.1 Commercial formulations of biocontrol agents available in India

Product	Bioagent(s)	Target organism	Delivery system	Developing agency
Antagon-TV	*T. viride*	*R. solani, Macrophomina phaseolina*	Seed treatment, soil application	Green Tech Agro Products, Coimbatore
Biocon	*P. fluorescens*	Bacterial wilt and rot diseases	Spray	Tockalai Experimental Station, Tea Research Association, Jorhat, ASSAM
Bioguard	*T. viride*	*Fusarium*	Spray	Krishi Rasayan Export Pvt. Ltd. Solan (HP)
Bioshield	*Pseudomonas fluorescens*	*Fusarium, Rhizoctonia, Pythium, Colletotrichum, Phytophthora*	Seed treatment, spray	POABS Biotech, Kuttoor, Kerala
Biotik	*Metarhizium anisopliae*	Termites, red ants, root grubs, grasshoppers	Seed treatment, spray, soil application	SS Biotech Guwahati Assam
Ecoderma	*T. viride*	*Fusarium, Rhizoctonia, Pythium, Phytophthora*	Seed treatment, drenching, soil application, seedling dip	Margo Biocontrol Pvt. Ltd., Bangalore
Bioderma	*T. viride*+ *T. harzianum*	*Pythium, Rhizoctonia, Phytophthora, Fusarium*	Seed treatment and spray	Biotech International Ltd., New Delhi, India
Ecofit	*Trichoderma viride*	*R. solani, Macrophomina phaseolina*	Seed treatment	Hoechast and Schering AgrEvo Ltd., Mumbai
Funginil	*T. harzianum*	*Botrytis, Pythium, Fusarium, Macrophomina, Rhizoctonia, Sclerotinia*	Seed treatment, soil application	Crop Health Bioproduct Research Centre, Gaziabad
Kalisena SD Kalisena SL	*Aspergillus niger* AN-27	*Pythium, Fusarium, Macrophomina, Rhizoctonia, Sclerotinia*	Seed treatment, foliar spray, soil application	Cadila Pharmaceuticals Limited, Ahmedabad
Pant Biocontrol Agent-1 (Biowilt-X)	*T. harzianum*	*Pythium, Fusarium, Macrophomina, Rhizoctonia, Sclerotinia*	Seed treatment, soil application	G. B. Pant University of Agriculture Technology, Pantnagar
Pant Biocontrol Agent-2	*Pseudomonas fluorescens*	*Fusarium*	Seed treatment, soil application	G. B. Pant University of Agriculture Technology, Pantnagar
Pusa Th3	*Trichoderma harzianum*	*Fusarium, Rhizoctonia, Sclerotium*	Seed treatment, soil application	Div. of Plant Pathology, IARI, Pusa, New Delhi
Sun Agro Derma Sun Agro Derma H	*T. viride* *T. harzianum*	*Fusarium, R. solani, Macrophomina phaseolina, Colletotrichum*	Seed treatment, seedling root dip, soil application	Sun Agro Chemicals, Chennai
Tricho-X	*T. viride*	*Fusarium, Rhizoctonia, Sclerotium, Pythium*	Seed treatment, foliar spray, soil application	Excel Industries Limited, Mumbai
Trichostar	*T. harzianum*	*Fusarium, Rhizoctonia, Sclerotium, Pythium*	Seed treatment	GBPUAT, Pantnagar
Gliostar	*Gliocladium.* spp.	*Fusarium, Rhizoctonia, Sclerotium, Pythium*	Seed treatment, drenching	GBPUAT, Pantnagar

(continued)

Table 13.1 (continued)

Product	Bioagent(s)	Target organism	Delivery system	Developing agency
Monitor	*Trichoderma* sp.	*Fusarium, Rhizoctonia, Sclerotium, Pythium*	Seed treatment and spray	Agricultural and Biotech Pvt. Ltd. Gujrat
Trichoderma	*Trichoderma* sp.	*Fusarium, Rhizoctonia, Sclerotium, Pythium*	Seed treatment	Innovative Pest control Lab, Bangalore
Phule Trichokill	*Trichoderma* sp.	*Fusarium, Rhizoctonia, Sclerotium*	Seed treatment	Department of Plant Pathology, MPKV, Rahuri
Biowilt-X Bionem-X Biocomp-X	*T. harzianum* *Pochonia chlamydosporia* *P. fluorescens*	*Fusarium oxysporum* f.sp. *ciceris* and *F. udum*, *Meloidogyne incognita*, and wilt disease complex (*Fusarium* + *Melodiogyne*)	Seed treatment	Dept. of Plant Pathology, AMU, Aligarh
Soil Guard	*T. viride*	*Fusarium, Rhizoctonia, Pythium, Colletotrichum, Phytophthora*	Seed treatment, soil application	POABS Biotech, Kuttoor, Kerala
Myco-Jaal	*Beauveria bassiana*	Diamond black moth	Spray	Pest Control of India, Bangalore

applied kaolin-based formulation of *Pseudomonas fluorescens* SP007s as seed treatment and spray to reduce fungal population in rice plants.

13.4.2 Seed Biopriming

Biopriming, a seed treatment system that integrates the biological and physiological aspects of disease control, involves coating the seed with fungal or bacterial biocontrol agents (El-Mougy and Abdel Kader 2008). It is a method of treating seeds with microbial inoculants and incubating under warm and moist conditions until just prior to radical emergence. Priming is one of the simple techniques which improve the vigor, seedling establishment, and plant efficiency in the field. There are three main large-scale priming approaches using different methods to regulate water potential, which are quite popular in European countries: (1) Osmoconditioning: seeds are incubated in an aerated solution of an osmoticum such as polyethylene glycol, or an inorganic salt such as potassium nitrate or phosphate, using high liquid–seed ratio (e.g., 10:1) in stirred bioreactors of various designs. At the end of the process, seeds are rinsed before further processing. (2) Solid-matrix priming technique: seeds are mixed with equivalent quantity of friable, nonclumping, inert material, e.g., a carbonaceous, preferably ligneous shale or coal, with osmotic component at least 90 % of the equilibrium water potential, moistened sufficiently to equilibrate seeds to the correct water content. Extraneous solid material is sieved off after incubation. (3) Basic priming method: incubate damp seeds and bring the seed directly to predetermined moisture content by various means, without using external matrix or osmotic agent to regulate seed water potential (McQuilken et al. 1998). Priming allows the early DNA transcription and RNA and protein synthase which repair the damaged parts of the seeds and reduce metabolic exudation (Entesari et al. 2013). These agents thus improve the seed germination charac-

Table 13.2 Commercial formulations of biocontrol agents available worldwide

Biocontrol agent	Product	Target disease/organism	Manufacturer	Delivery system
Agrobacterium radiobacter strain 84	Galtrol	*Agrobacterium tumefaciens*	AgroBioChem, USA	Spray
A. radiobacter strain 1026	Nagol	*Agrobacterium tumefaciens*	Bio-Care	Spray
Bacillus subtillus strain GB34	GB34	*Rhizoctonia, Pythium, Phytophthora, Fusarium*	Gustafon, USA	Drenching during sowing and transplanting
B. subtillus strain GB 03	Kodiac, companion	*Rhizoctonia, Aspergillus*	Growth Products, USA	Drenching during sowing and transplanting
Pseudomonas aureofaciens strain TX-1	Bio-jet, spot less	*Pythium, Rhizoctonia solani*	Eco Soil Systems	Overhead irrigation, can only be used with BioJet automatic fermentation system
Pseudomonas fluorescens A506	Frostban, Blightban A506	Fire blight, frost damage, bunch rot	Plant Health Technologies	Spray at blooming flower and fruiting
Streptomycine griseoviridis K61	Mycostop	Soil-borne pathogens	Kemira Agro Oy, Finland	Drenching, spraying, or through irrigation
Trichoderma harzianum T-22	Root shield or BioTrek T-22G	Soil-borne pathogens	BioWorks, Inc., USA	Granules mixed with soil or potting medium, powder mixed with water and added as soil drench
T. harzianum T-39	Trichodex	*Botrytis cinerea*\	BioWorks, Inc., USA	Spray
T. asperellum T34	T34 Biocontrol	*Fusarium oxysporum* f.sp. *dianthi*	Fargro Ltd., Littlehamptom, West Sussex, UK	Drenching during sowing and transplanting, root dip of cuttings
Ampelomyces quisqualis M-10	AQ10	Powdery mildew	Ecogen, USA	Spray
Aspergillus flavus AF 36	Alfa guard	*Aspergillus flavus*	Circle One Global, USA	Seed treatment, foliar spray, soil application
Gliocladium catenulatum strain JI446	Prima stop soil guard	Soil-borne pathogens	Kemira Agro Oy, Finland	Seed treatment, foliar spray, soil application
Trichoderma sp.	Bio-Fungus	*Sclerotinia, Phytophthora*, *R. solani, Pythium* spp., *Fusarium, Verticillium*	De Cuester, Belgium	Seed treatment, foliar spray, soil application
Candida oleophila	Aspire	*Botrytis* spp., *Penicillium* spp.	Ecogen, Inc., Langhorne, PA	Postharvest to fruit as drench, drip, or spray

(continued)

Table 13.2 (continued)

Biocontrol agent	Product	Target disease/organism	Manufacturer	Delivery system
T. harzianum (ATCC20476) and *T. polysporum* (ATCC20475)	Binab T	Wilt, tale-all, root rot	Bio-Innovation AB, Sweden Henry Doubleday Research Association, UK	Spray, mixing with potting substrate, as paste painting on tree wounds
Fusarium oxysporum (nonpathogenic)	Biofox C	*F. oxysporum, F. moniliforme*	SIAPA, Bologna, Italy	Seed treatment or soil incorporation
Pseudomonas syringae ESC-10	Bio-save 100 Bio-save 1000	*Botrytis cinerea, Penicillium* spp., *Mucor pyriformis, Geotrichum candidum*	EcoScience Corp, Orlando, Florida	Pellets, postharvest to fruit as drench dip or spray
P. syringae ESC-11	Bio-save 110	*Botrytis cinerea, Penicillium* spp., *Mucor pyriformis, Geotrichum candidum*	EcoScience Corp, Orlando, Florida	Pellets, postharvest to fruit as drench dip or spray
P. chlororaphis	Cedomon	Net blotch, stripe disease, *Fusarium* spp., spot blotch, leaf spots	BioAgri AB, Sweden	Seed dressing
P. fluorescens	Conquer	*Pseudomonas tolaasii*	Mauri Foods, Kittanning, PA	Spray
Coniothyrium minitans	Contans	*Sclerotinia sclerotiorum* and *S. minor*	Prophyta Biologischer Pflanzenschutz, Germany	Spray
Burkholderia cepacia	Deny	*Rhizoctonia, Pythium, Fusarium*	Stine Microbial Products	Seed treatment, aqueous suspension for drip irrigation
Bacillus subtilis	Epic	*R. solani, Fusarium* spp., *Alternaria, Aspergillus* spp.	Gustafson Inc., Dallas, TX	Added to slurry, mix with chemical fungicides for seed treatment
F. oxysporum (nonpathogenic)	Fusaclean	*F. oxysporum*	Natural Plant Protection, France	In drip to rock wool, incorporate in potting mix; in rows
Pseudomonas cepacia	Intercept	*R. solani, Fusarium spp., Pythium spp.*	Soil Technologies, Fairfield, IA	Seed treatment, foliar spray, soil application
Trichoderma spp.	Monitor SD	Soil-borne plant pathogens	M/s Agriland Biotech Pvt Ltd., Baroda, India	Seed dressing
Trichoderma spp.	Monitor WP	Soil-borne plant pathogens	M/s Agriland Biotech Pvt Ltd., Baroda, India	Soil application
Agrobacterium radiobacter	Nogall, Diegall	*Agrobacterium tumifaciens*	Bio-Care Technology Pvt. Ltd, Australia	Root dips
A. radiobacter K84	Norbac 84C	*Agrobacterium tumifaciens*	New BioProducts, Corvalis, OR	Root, stem, cutting dip, or slurry

(continued)

Table 13.2 (continued)

Biocontrol agent	Product	Target disease/organism	Manufacturer	Delivery system
Paecilomyces lilacinus	Bioact or Paecil	Various nematodes	Technological Innovation Corporation Pvt Ltd	Drenching
Pythium oligandrum	Polygandron	*Pythium ultimum*	Plant Production Institute, Slovak Republic	Seed treatment and soil incorporation
Gliocladium catenulatum	Primastop	*Pythium* spp., *R. solani, Botrytis* spp.	Kemira Agro Oy, Finland	Drenching and soil incorporation
Bacillus subtilis FZB24	Rhizo-Plus	*R. solani, Fusarium* spp., *Alternaria* spp., *Sclerotinia, Streptomyces scabies*	KFZB Biotechnik GmBH, Germany	Seed treatment, soil drenching, root dip application
T. harzianum	Root Pro	*R. solani, Fusarium* spp., *Alternaria* spp., *Sclerotium rolfsii*	Mycontrol Ltd., Israel	Mix with growing media at time of seeding or transplanting
B. subtilis	Serenade	*Pythium, Cercospora, Alternaria, Helmithosporium,* fire blight	AgraQuest Inc., Davis, CA	Spray
Gliocladium virens GL-21	SoilGard	Damping-off, root rot pathogens, *R. solani, Pythium* spp.	Thermo Trilogy, Columbia, MD	Granules incorporated in soil
B. subtilis GB03	System 3	Seedling pathogens	Helena Chemicals Co., Memphis, TN	Seed treatment
T. viride	Trieco	Soil-borne pathogens	Ecosense Labs Pvt. Ltd., Mumbai, India	Seed treatment, tuber or seed dressing, soil drenching
Trichoderma sp.	Trichoderma 2000	Soil-borne pathogens	Mycontrol Ltd., Israel	Seed treatment, tuber or seed dressing, soil drenching

teristics and the seedling emergence under unfavorable conditions and priming results in a stronger plant. Seed priming can improve the physiological responses and increase seed tolerance to environmental stress (Khan et al. 2008).

This technique is more useful over simple coating of seeds as it results in rapid and uniform seedling emergence. *Trichoderma* conidia germinate on seed surface and form a layer around bioprimed seeds. These bioprimed seeds tolerate adverse soil conditions better. Biopriming may also reduce the amount of biocontrol agents that is applied to seeds (Ramanujam et al. 2010). Enhancements of seed inoculation with biological agents in combination with priming which will stabilize the efficiency of biological agents have been reported by previous workers (Callan et al. 1990; Warren and Bennett 1999). Nayaka et al. (2008) bioprimed maize seeds with conidial suspension of *T. harzianum* for the control of *F. verticillioides* and fumonisins in maize. It was found that the pure culture of *T. harzianum* was more effective in reducing the *F. verticillioides* and fumonisin incidence, followed by talc formulation. Biopriming with microbial inoculants is potentially able to promote rapid and uniform seed germination and plant growth. Moeinzadeh et al. (2010) reported the application of UTPf76 and UTPf86 strains of *Pseudomonas fluorescens* on improving sunflower seed germination and promotion of seedling growth. These bioprimed strains enhanced seed factors such as germina-

tion index, germination percentage, germination rate and vigor index, and also seedling growth indices, including root length, shoot height, dry and wet weight of seedlings, and numbers of lateral roots. In biopriming, the selected strains were applied to the seed during osmopriming with NaCl.

13.4.3 Seed Encapsulation

The reproducible results following introduction of microbial inoculants into soil relies on the survival rate of the inoculated microbes in heterogenous soil environment, and it can be achieved by improved encapsulation technique (Young et al. 2006). It is a specialized seed-coating process which involves enveloping the seed, microbes, and possibly few other components such as pesticides or micronutrients, in a gelatinous or polymer gel matrix, thereby prolonging the survival of microbial inoculants on seed. A gel-encapsulation system developed with hard alginate prill to coat or pellet seeds for making formulation of biocontrol fungi (Lumsden and Lewis 1989). The gel-like matrix allows the cell to remain viable with its catalytic ability for longer duration. Alginate forms microbeads immediately in the presence of polyvalent cations by binding the cation to guluronic acid units (Witter 1996) in a single step with a sufficient mechanical strength. Digat (1991) has patented a process to produce granules of up to about 8-mm diameter, with a core containing liquid microbial inoculants and an outer protective coating layer. GEL COAT is an example of seed encapsulation which is an alginate hydrogel product patented as a delivery system for entomopathogenic nematodes (Boyetchko et al. 1999). This method of delivery system has a distinct advantage of being user friendly and environmentally safe, since the active ingredients are effectively sealed until they are released during germination. Major factors that need to be taken care of while adopting this technique are seed inoculum density, coating stability, both for microbes viability and coat integrity, in association with user feasibility and cost of production. *Pseudomonas putida* CC-FR2-4 and *Bacillus subtilis* CC-pg104 encapsulated in alginate supplemented with humic acid and inoculated to *Lactuca sativa* L. seedlings and observed significant plant growth by Rekha et al. (2007). Encapsulation of *Bacillus megaterium* was attempted by Sivakumar et al. (2014), with bacterial alginate by enriching the bead microenvironment with humic acid, and high viability of encapsulated bacteria with minimum cell loss after 5 months of storage was observed, thereby achieving successful plant-growth promotion of rice seedlings. This novel technique clearly demonstrates that inoculation of encapsulated microbial inoculants promotes plant growth and is feasible for application in agricultural industry.

13.5 Soil Application

Soil treatment is preferred when biocontrol agents are too sensitive to desiccation (Warrior et al. 2002). The biocontrol agent (BCA) establishes a high population in the soil, making them suppressive to the disease. Niche exclusion also becomes operative in such cases, as the increase in number of the introduced microbes renders essential nutrients unavailable to soil pathogens and other less beneficial microflora (Lumsden et al. 1995). Soil acts as repertoire of both beneficial and pathogenic microbes; delivering of microbial inoculants to soil will increase the population dynamics of augmented bacterial antagonists and thereby suppress the establishment of pathogenic microbes on to the infection court. Many species of *Trichoderma* have also been formulated extensively, using cellulosic carriers and binders and modern thin-film coating techniques, in an attempt to introduce them into the rhizosphere regions of seedlings to protect them from diseases such as *Rhizoctonia solani* and *Pythium ultimum*. However, the major limitation of fungi as seed coatings remains; so they do not colonize the rhizosphere as readily as the bacterial agents (Warrior et al. 2002). Numerous attempts have been made to control several soil-borne pathogens by incor-

porating natural substrates colonized by antagonists of pathogen into soil (Sesan and Csep 1992). Though drenching of soil with aqueous suspensions of bioagent propagules was carried out, there will not be any even distribution of bioagents in the soil. Bankole and Adebanjo (1998) reported that soil drenching with suspension of *T. viride* was very effective in reducing infection from cow pea seeds infected with *Colletotrichum truncatum* (brown blotch). Soil drenching with *T. harzianum* has given good control of stem rot of groundnut caused by *S. rolfsii* (Kulkarni and Anahosur 1994). An aqueous drench containing conidia of *T. harzianum* controlled wilt of chrysanthemum by preventing reinvasion by *F. oxysporum*.

Weststeijn (1990) found that root rot in tulip caused by *P. ultimum* was reduced by mixing *Pseudomonas* suspensions thoroughly through the soil to a concentration of 10^8 cells per gram dry soil before planting the bulbs. Wilt disease of sunflower was found to be suppressed when *P. cepacia* strain N24 was applied to the seedbeds at the rate of 500 ml per m^2 under greenhouse conditions (Hebber et al. 1991).

A technique of enrichment of farmyard manure (FYM) with *Trichoderma* culture for soil and nursery bed application is widely accepted and appreciated by farmers for soil treatment against soil-borne pathogens. This technique involves less labor and time to multiply *Trichoderma* culture to manifold for soil application. Vidhyasekaran and Muthamilan (1995) stated that soil application of peat-based formulation of *P. fluorescens* (Pf1) at the rate of 2.5 Kg of formulation mixed with 25 Kg of well-decomposed farm yard manure, in combination with seed treatment, increased rhizosphere colonization of Pf1 and suppressed chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceris*. Application of *Trichoderma harzianum* Th3-enriched farm yard manure in soil, along with seed treatment, before sowing of chickpea to ward off against root rot caused by *Rhizoctonia solani* exceptionally reduce the disease and increased yield (Jambhulkar et al. 2015).

13.6 Foliar Application

Liquid formulations are being commonly applied on foliar parts of the plants for control of foliar pathogens. The efficacy of the foliar application mainly governs by the microclimate of the crop canopy. The crop canopy has varied concentration of nutrients like amino acids, organic acids, and sugars exuded through stomata, lenticels, hydathodes, and wounds. It affects the efficacy and survival of antagonists in phylloplane.

Kelly Cartwright (1995) reported that three spray applications of *Pseudomonas cepacia* to cuttings during a 2-week period were more effective than either one or two bacterial sprays in the control of *Rhizoctonia* stem rot of poinsettia. Rice blast (*P. oryzae*) can be effectively controlled by foliar spray of talc-based powder formulation of *P. fluorescens* strain Pf1 (1 kg ha^{-1}). The effectiveness of spraying persisted up to 2 weeks. When the bacterial product was sprayed on plants grown from treated seed, the effectiveness was higher than when spraying was carried out without any prior seed treatment (Vidhyasekaran et al. 1997). Foliar application of *Pseudomonas chlororaphis* (PA-23), *Bacillus amyloliquifaciens* (BS6), *Pseudomonas* sp (DF41), and *B. amyloliquifaciens* (E16) was found very effective against causal agent of stem rot of canola, *Sclerotinia sclerotiorum* in field (Fernando et al. 2007). *Trichoderma* species can be applied as foliar spray to control diseases affecting above-ground parts. Biological control of foliar diseases is not so developed as biocontrol of soil-borne diseases. The reasons for the paucity of examples of biocontrol of foliar diseases may be the availability of cheap and effective chemical fungicides and the ease of application to the foliage, and results obtained with biocontrol agents were not so good as those obtained with common fungicides (Elad and Kirshner 1992).

The dosage and frequency of application have to be standardized based on the crop value, which could be a reliable and practical approach. Selected strains from many genera

of bacteria isolated from these suppressive soils have the potential to reduce plant diseases when applied to the plant root environment (Weller et al. 2002). Today liquid bioformulations with high potency, cost-effective with good suspension properties, and good stability are available and being successfully adopted globally. Additives are important for application in monocots which facilitates adhesion of microorganisms on plant tissues. Additives such as stickers, spreaders, adjuvants, and emulsifiers in foliar sprays facilitate adhesion of microorganisms on plant tissues (Harvey 1991).

13.7 Root Dip

The nature of pathogen may be seed borne or soil borne; it may establish host parasite relationships by entering through the root. Hence, protection of the rhizosphere region by prior colonization with PGPR will prevent the establishment of host–parasite relationship. Seedling roots can be treated with spore or cell suspension of antagonists either by drenching the bioagents in nursery bed or by dipping roots in microbial inoculant suspension before transplanting. This method is suitable for the vegetable crops and rice where transplanting is practiced (Singh and Zaidi 2002). In an experiment, Jambhulkar and Sharma (2013) dipped paddy seedlings in suspension of talc-based bioformulation of *Pseudomonas fluorescens* for 2 h before transplanting, and it showed reduction in bacterial leaf blight of rice. Similarly, dipping of rice seedlings in talc-based formulation of *P. fluorescens* (PfALR1) prior to transplanting reduced sheath blight severity and increased yield in Tamil Nadu, India (Rabindran and Vidhyasekaran 1996). Nandakumar et al. (2001) reported that *P. fluorescens* strain mixtures by dipping the rice seedlings in bundles in water containing talc-based formulation of strain mixtures (20 g/l) for 2 h and later transplanting it to the main field suppressed sheath blight incidence.

13.8 Fluid Drilling Technology

Fluid drilling, also referred to as fluid sowing or gel seeding, is the technology of sowing seeds that have been germinated, using a gel to suspend and transfer them to the seedbed. This delivery system involves the incorporation of biocontrol agents into fluid drill gels. The major advantage of sowing germinated seed compared to dry seed is earlier and more uniform emergence. The gel protects the exposed radicle from mechanical damage and also provides the growing seedling with an initial water source. Unfortunately, the gel tends to attract microorganisms, including soilborne pathogens, which may result in an increased incidence of disease. Conway 1986 has used fungicides as adjuvant to the gel matrix to decrease damping-off disease caused by *R. solani* in chili peppers. Fluid drilling offers an ideal system for the delivery of a biocontrol agent such as *Trichoderma* for the control of soil-borne disease problems (Fisher et al. 1983). In one study, vegetable or fruit tree seedlings were dipped into gels incorporated with antagonists so that the root area was surrounded by a thin layer of gel before the seedlings were planted. Fluid-drilling gels have been used to deliver *T. harzianum* for the control of *R. solani* and *S. rolfsii* on apple (Conway 1986). This innovative approach, utilizing the benefits derived from fluid drill technology, offers considerable promise for the formulation and application of biocontrol microorganisms. But in future, the technique of fluid drilling will be successful only if sowing of primed seeds rather than germinated seeds are used in carrier gel (Pill 1991). The positional advantage due to additive incorporation in the fluid-drilling gel shows an efficient, cost-effective, and environmentally sound application method for bioagents.

13.9 Microbigation

Applying microbial biocontrol agents to control weeds, soil pathogens, and soil insect through drip irrigation system is called “microbigation” (Boari et al. 2008). The uniform and precise

application of microbial particles close to the target organism and to the plant to be protected can increase the success of a biological control treatment. To make acceptability of biocontrol application among farmers, use of systems or technologies that are usually available in agriculture can be modified and enlarge the market. An exploratory drip irrigation system was carried out by Boari et al. 2013, using dripper lines, drippers, filters, and other tools commonly used in irrigation and precision agriculture in the greenhouse to evaluate their suitability for applying microbial biocontrol agents. Conidial suspensions of marketed or marketable agents were used, i.e., *Fusarium oxysporum, F. solani*, *T. harzianum*, and *Paecilomyces lilacinus*. They demonstrated that conidial suspensions (10^6 conidia ml^{-1}) can pass through the drippers without causing clogging, regardless of their size, and remained viable. A further advantage could be the limitation of the applied doses to the crop root zone and not the whole field, and therefore a reduction of the costs for treatment. Several biocontrol agents could be applied at the soil level through this system, such as mycoherbicides (Charudattan 2001), antagonists (Whipps and Lumsden 2001), and biopesticides (Copping 1999). They can be applied at plant transplanting or through soil drenching or root dip (Alabouvette et al. 1993).

13.10 Coaggregation Assay

Microbial inoculant formulation has a very important effect in the inoculation process as it determines the potential of the bioagents (Bashan et al. 1984). Poor performance of bioformulations in agriculture was reviewed by Van Veen et al. (1997), and suggested to use multiple microbial consortia for multipronged attack against phytopathogens and to thrive together in unique ecological niches in ideal proportions, instead of using a single strain, for a single trait. Coaggregation is a bacteria–bacteria, fungus–fungus, or fungus–bacteria interaction, and the interactions are highly specific that only few or certain species are consortial partners.

Coaggregation was first reported by Gibbons and Nygard (1970), who referred to it as interbacterial aggregation, and it was readily observed with naked eyes (Cisar et al. 1979). Coaggregation is effective only when equal numbers of partners are present and genetic stability of coaggregation is mediated by surface components that recognize a carbohydrate on the cell of the partner (Kolenbrander and Phucus 1984). Coaggregation has been reported earlier among certain bacterial species. Bougeu and Mc Bride (1976) and Kolenbrander and Phucus (1984) reported that *Actinomyces viscosus* T14V and *Streptococcus sanguis* 34 co-aggregated by a mechanism which is not inhibited by 1 M NaCl and is independent of dextran, requires calcium and pH in the range of 8.0 to 8.5. Recently, Sivakumar and Joe (2008) attempted coaggregation of *Azorhizobium caulinodans* with *A. brasilense*, *A. chroococcum*, *Bacillus megatherium*, and *Pseudomonas fluorescens* to develop coaggregates with multiple benefits using seed powders of many plants, viz., *Moringa oleifera, Strychnos potatorum*, and *Sappindus emaignatus*. There is wide scope of using coaggregates to deliver microbial inoculants for obtaining multiple benefits in different crops against various soil-borne pathogens. Studies on coaggregates open up the possibilities for further investigation of the genetic basis of effective coaggregation and also the nature of cellular mechanism.

13.11 Consortia Application

Judicious use of microbial inoculants as biocontrol agent (BCA) is a potentially important component of sustainable agriculture. The principal biocontrol mechanism involved includes mycoparasitism, antibiosis, competition, and induced resistance; additional mechanisms are hypovirulence mediated through fungal viruses and inhibition of enzymes involved in plant pathogenicity (Kapat et al. 1998). Individual biocontrol mechanism could be predominant for some BCAs, but there are also many instances where more than one mechanism may operate in a given BCA iso-

late. The ecological processes determining the fate of such biological control are complex. Thus, it is not surprising that, in addition to variable control efficacies, biocontrol success in field crops has been limited despite much research effort. Most biocontrol success has been achieved in greenhouse cultivation (Paulitz and Belanger 2001), where ecological parameters are less variable. Because of the inconsistent or limited biocontrol achieved in the field, BCAs have also been used in combination with fungicides or cultural practices (Shtienberg and Elad 1997).

Use of mixtures of cultivars (Mundt 2002) or fungicides (Brent and Hollomon 2007) has been successfully adopted in many crops to increase and maintain disease control efficacy when individual cultivars or fungicides may not be able to control disease effectively. To improve biocontrol efficacies achieved through the use of a single BCA, there has been increasing interest recently among researchers in using mixtures of BCAs to exploit potential synergistic effect among them (Xu et al. 2011). Number of biocontrol mechanism may operate in mixed BCA populations, and we need to consider both direct and indirect interactions between different BCA populations. Compared with the more efficacious BCA, combined use of two or more BCAs may lead to increased, reduced, or similar biocontrol efficacy (Xu et al. 2011).

Biocontrol agents applied individually are not likely to perform consistently against all pathogens of the crop or under diverse crop conditions. A combination of biocontrol agents is more likely to have a greater variety of traits responsible for the suppression of one or more pathogens, and also it is likely to have these traits expressed over a wide range of environmental conditions (Crump 1998). Numerous studies (Meyer and Roberts 2002; Roberts et al. 2005) have reported increased performance in the suppression of pathogens or disease by combinations of biocontrol agents. Incompatibility among microbes combined in biocontrol preparations is possible since biocontrol agents are typically selected based on their antagonistic behavior towards other microbes (Leeman et al. 1996; Meyer and Roberts 2002). Several researchers have indicated that strains combined in biocontrol preparations must be compatible for increased disease suppression to occur (Raupach and Kloepper 1998; Roberts et al. 2005). Accumulating evidence from literature has shown that compatible multiple strains appear to be an important prerequisite for the desired effectiveness of strains and more consistent disease suppression (Ganeshmoorthi et al. 2008). Compatible strains of *P. fluorescens* (Pf1, Py15 and Fp7) and *Bacillus subtilis* strains (EPCO 16 and EPC 5) were found to effectively inhibit the growth of *Alternaria solani* in tomato crop (Sundaramoorthy and Balabaskar 2012). Similarly, experiments for the biological control of the bacterial blight pathogen revealed that different species of *Bacillus* applied to rice plants as a seed treatment before sowing, a root dip prior to transplantation, and two foliar sprays prior to inoculation could afford up to 59 % suppression of the disease. These treatments could also bring about a twofold increase in plant height and grain yield (Vasudevan and Gnanamanickam 2000). Efforts are in progress, including formulation of synergy hypothesis in relation to biocontrol mechanism to exploit microbial mixture for uses in biocontrol of plant diseases.

13.12 Conclusion

Today in the light of the growing concern towards environmental safety, suppression of plant diseases through biocontrol agents is gaining ground as an alternative to traditional disease management strategies. Now it is necessary to focus on the challenges involved in testing, formulating, and delivering newer potential biocontrol agents within the context of integrated disease management. Undoubtedly choosing correct microbial inoculants is the foremost factor governing the success of biocontrol program. But making it reach to the field with a suitable delivery method maintaining consistent performance is the next most important challenge. Thus, the major challenge for plant pathologists is to develop a specific delivery system for a particular bioagent against a specific pathogen. There is a trend prevalent among farmers to use or apply a

single biocontrol agent, but research in the area of consortia application with multiple mechanism of disease control against more than one pathogen is the need of the hour. Also, future research strategies should emphasize on achieving viable and stable biological product. In addition, future research should be attempted to develop a systematic approach to select a suitable method of delivery system based on the characteristics of the microbial inoculants. Another research area in the wake of less labor and increasing mechanization in agriculture, a model of pilot irrigation system such as microbigation, needs to be invented for applying microbial inoculants in field level to the diseased plant in the form of viable spores or fungal conidia through drippers. Overall, a multifaceted management program will require helping the end user to grow a disease-free crop.

References

Aeron A, Dubey RC, Maheshwari DK, Pandey P, Bajpai VK, Kang SC (2011) Multifarious activity of bioformulated *Pseudomonas fluorescens* PS1 and biocontrol of *Sclerotinia sclerotiorum* in Indian rapeseed (*Brassica campestris* L.). Eur J Plant Pathol 131:81–93

Alabouvette C, Lemanceau P, Steinberg C (1993) Recent advances in the biological control of *Fusarium* wilts. Pestic Sci 37:363–373

Alves RT, Bateman RP (2013) Evaluation of formulation and volume application rate on the secondary pick-up of *Metarhizium acridium* (Driver and Milner) Bischoff, Rehner and Humber conidia on *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). Bioassay 8(4):1–6

Bahadur A, Singh UP, Sarma BK, Singh DP, Singh KP, Singh A (2007) Foliar application of plant growth-promoting rhizobacteria increases antifungal compounds in pea (*Pisum sativum*) against *Erysiphe pisi*. Microbiology 35(3):129–134

Bankole SA, Adebanjo A (1998) Efficacy of some fungal and bacterial isolates in controlling wet rot disease of cowpea caused by *Pythium aphanidermatum*. J Plant Prot Trop 11:37–43

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16:729

Bashan Y, Reem Y, Levanony H, Sade A (1984) Non specific response in plant growth, yield and root colonization on noncereal crop plants to inoculation with *Azosprillium brasilense*. Can J Bot 67:1317–1324

Bateman RP, Matthews GA, Hall FR (2007) Ground based application equipment. In: Lacey LA, Kaya HK (eds) Field Manual of Techniques in Invertebrate Pathology. Springer, Dordrecht, pp 73–98

Beattie GA (2006) Plant-associated bacteria: survey, molecular phylogeny, genomics and recent advances. In: Gnanamanickam S (ed) Plant-associated bacteria. Springer, Dordrecht, pp 1–56

Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Boari A, Zuccari D, Vurro M (2008) 'Microbigation': delivery of biological control agents through drip irrigation systems. Irrig Sci 26:101–107

Boari S, Josens R, Parisi DR (2013) Efficient egress of escaping ants stressed with temperature. PloS One 8(11):e81082

Bougeu G, McBride BC (1976) Dextran-mediated interbacterial aggregation between dextran-synthesizing *Strepcocci* and *Actinomyces viscosus*. Infect Immun 13:1228–1234

Boyetchko S, Pedersen E, Punja Z, Reddy M (1999) Formulations of biopesticides. In: Hall FR, Menn JJ (eds) Biopesticides: Use and delivery, Methods in Biotechnology: 5. Humana Press, Totowa, pp 487–508

Brent KJ, Hollomon DW (2007) Fungicide resistance in crop pathogens: How can it be managed, 2nd edn, FRAC Monograph No. 1. Croplife International, Brussels

Burelle NK (2000) Biological control of tomato diseases. In: Gnanamanickam SS (ed) Biological control of crop diseases. Markel Dekker Inc, New York. ISBN 0-8247-0693-5

Burges HD (1998) Formulation of microbial biopesticides: beneficial microorganisms, nematodes, and seed treatments. Kluwer Academic, Dordrecht/Boston

Callan NW, Mathre DE, Miller JB (1990) Bio-priming seed treatment for biological control of Pythium ultimum preemergence damping-off in sh2 sweet corn. Plant Dis 74:368–372

Chandra K, Greep S (2010) Liquid based bio-fertilizers. J Eco-Friend Agric 5(1):1–7

Chandra K, Greep S, Ramarathinam S (2006) Bio efficacy of liquid formulation of *Beauveria bassiana* on Tea shoot hole borer *Euwallacea fornicutus.* In: Veeraragavatham D, Balakrishna Murthy G (eds) National seminar on convergence of technologies for organic horticulture, pp 58–61

Charudattan R (2001) Biological control of weeds by means of plant pathogens: significance for integrated weed management in modern agro-ecology. BioControl 46:229–260

Chet I, Benhamou N, Haran S (1998) *Trichoderma* and *Gliocladium*. In: Harman GE, Kubicek CP (eds) Mycoparasitism and Lytic Enzymes. Taylor and Francis, London, pp 153–172

Cisar JO, Kolen Brander PE, Melnlme FC (1979) Specialty of coaggregation reactions between human oral *Strepcocci* and *Actinomyces viscosus*. Infect Immun 46:453

Cliquet S, Scheffer RJ (1997) Influence of culture conditions on growth and survival of conidia of *Trichoderma* spp coated on seeds. Biocontrol Sci Technol 7:171–181

Conway KE (1986) Use of fluid drilling gels to deliver biological control agents to soil. Plant Dis 70:835–839

Connick WJ Jr, Daigle DJ, Quimby PC Jr (1991) An improved inert emulsion with high water retention for mycoherbicide delivery. Weed Technol 5:442–444

Cook RJ, Baker KR (1983) The nature and practice of biological control of plant pathogens. American Phytopathological Society, St Paul

Copping LG (1999) The biopesticide manual. BCPC, Farnham

Crouch I, van Staden J (1992) Effect of seaweed concentrate on the establishment and yield of greenhouse tomato plants. J Appl Phycol 4:291–296

Crump DH (1998) Biological control of potato and beet cyst nematodes. Asp Appl Biol 53:883–386

Dawar S, Wahab S, Tariq M, Zaki MJ (2010) Application of *Bacillus* species in the control of root rot diseases of crop plants. Arch Phytopathol Plant Protect 43(4):412–418

Desai S, Reddy MS, Kloepper JW (2000) Comprehensive testing of biological control. In: Gnanamanickam SS (ed) Biological control of crop diseases. Markel Dekker Inc, New York, 5pp. ISBN 0-8247-0693

Digat B (1991) A new encapsulation technology for bacterial inoculants and seed bacterization, in Plant Growth-Promoting Rhizobacteria – Progress and Prespects, 2nd International Workshop on Plant Growth-Promoting Rhizobacteria, Interlaken, Switzerland, October 1990 (eds C Keel, B Koller and G Defago), International Union of Biological Sciences, WPRS Bulletin, 1991/XIV/8, pp 383–391

Elad Y, Kirshner B (1992) Calcium reduces *Botrytis cinerea* damages to plants of *Ruscus hypoglossum*. Phytoparasitica 20:285–291

El-Mougy NS, Abdel Kader MM (2008) Long term activity of bio-priming seed treatment for biological control of faba bean root rot pathogens. Australas Plant Pathol 37:464–471

Entesari M, Sharifzadeh F, Ahmadzadeh M, Farhangfar M (2013) Seed biopriming with *Trichoderma* species and *Pseudomonas fluorescence* on growth parameters, enzymes activity and nutritional status of soybean. Int J Agron Plant Prod 4(4):610–619

Fernando WGD, Nakkeeran S, Zhang Y, Savchuk S (2007) Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Pseudomonas* and *Bacillus* species on canola petals. Crop Prot 26:100–107

Filho AB, Alves AB, August NT, Pereira RM, Alves LFA (2001) Stability and persistence of two formulations containing *Anticarsia gemmatalis* Nuclear Polyhedrovirus (AgMNPV). J Neotrop Entomol 30:411–416

Fisher CG, Conway KE, Motes JE (1983) Fluid drilling: A potential delivery system for fungal biological control agents with small seeded vegetables. Proc Okla Acad Sci 63:100–101

Ganeshmoorthi P, Anand T, Prakasam V, Bharani M, Ragupathi N et al (2008) Plant growth promoting rhizobacteria (PGPR) bioconsortia mediates induction of defense related proteins against infection of root rot pathogen in mulberry plants. J Plant Interact 3:233–244

Gasic S, Tanovic B (2013) Biopesticide formulations, possibility of application and future trends. Pestic Phytomed (Belgrade) 28(2):97–102

Gibbons RJ, Nygard M (1970) Interbacterial aggregation of plaque bacteria. Arch Oral Biol 15:1317–1400

Gnanamanickam SS, Mew TW (1992) Biological control of blast disease of rice (*Oryza sativa* L.) with antagonistic bacteria and its mediation by a *Pseudomonas* antibiotic. Ann Phytopathol Soc Jpn 58:380–385

Harvey LT (1991) A guide to agricultural spray adjuvants used in the United States. Thompson Publications, Fresno

Hebber P, Berge O, Heulin T, Singh SP (1991) Bacterial antagonists of sunflower (*Helianthus annuus* L.) fungal pathogens. Plant Soil 133:131–140

Jambhulkar PP, Sharma P (2013) Promotion of rice seedling growth characteristics by development and use of bioformulation of *Pseudomonas fluorescens*. Indian J Agric Sci 83(2):136–142

Jambhulkar PP, Sharma P (2014) Development of bioformulation and delivery system of *Pseudomonas fluorescens* against bacterial leaf blight of rice (*Xanthomonas oryzae* pv. *oryzae*). J Environ Biol 35(5):843–849

Jambhulkar PP, Sharma P, Meghwal ML (2015) Additive effect of soil application with *Trichoderma* enriched FYM along with seed treatment and drenching with *Trichoderma* formulation for management of wet root rot caused by *Rhizoctonia solani* in chickpea. J Pure Appl Microbiol 9(1):405–412

Jeyalakshmi C, Madhiazhagan K, Rettinassababady C (2010) Effect of different methods of application of *Pseudomonas fluorescens* against bacterial leaf blight under direct sown rice. J Biopestic 3(2):487–488

Kapat A, Zimand G, Elad Y (1998) Effect of two isolates of *Trichoderma harzianum* on the activity of hydrolytic enzymes produced by *Botrytis cinerea*. Physiol Mol Plant Pathol 52:127–137

Kelly Cartwright D (1995) Comparison of Pseudomonas species and application techniques for biocontrol of *Rhizoctonia* stem rot of Poinsettia. Plant Dis 79:309–313

Kennedy AC (1999) Bacterial diversity in agroecosystems. Agric Ecosyst Environ 74(1–3):65–76

Khan A, Shad Khan K, Khan AZ, Marwat KB, Afzal A (2008) The role of seed priming in semi-arid area for mung bean phenology and yield. Pak J Bot 40(6):2471–2480

Kloepper JW, Ryu CM, Zhang S (2004) Induce systemic resistance and promotion plant growth by *Bacillus* spp. Phytopathology 94:1259–1266

Knowles A (2005) New developments in crop protection product formulation. (pp. 153–156). Agrow Reports UK: T and F Informa UK Ltd.

Knowles A (2006) Adjuvants and additives. (pp. 126–129). Agrow Reports: T&F Informa UK Ltd.

Kolenbrander PE, Phucus CS (1984) Effect of saliva and co-aggregation of oral Actinomycetes and *Streptococcus* species. Infect Immun 44:228–233

Kulkarni SA, Anahosur KH (1994) Effect of Age of Groundnut Plant to Infection of *Sclerotium rolfsii* Sacc A Causal Agent of Stem Rot Disease. Karnataka J Agric Sci 7:367–368

Leeman M, Den Ouden FM, Van Pelt JA, Matamala-Garros A, Bakker PAHM, Schippers B (1996) Suppression of *Fusarium* wilt of radish by co-inoculation of fluorescent pseudomonas spp. and root colonizing fungi. Eur J Plant Pathol 102:21–31

Lumsden RD, Lewis JA (1989) Selection, production, formulation and commercial use of plant disease biocontrol fungi : problems and progress. In: Whipps JM, Lumsden RD (eds) Biotechnology of Fungi for Improving Plant Growth. Cambridge University Press, Cambridge, pp 171–190

Lumsden RD, Lewis JA, Fravel DR (1995) Formulation and delivery of biocontrol agents for use against soil borne plant pathogens. In: Hall FR, Barry JW (eds) Biorational Pest Control Agents. Formulation and Delivery, ACS Symposium Series 595. ACS, Washington, DC, pp 166–182

McQuilken MP, Halmer P, Rhodes DJ (1998) Application of microorganisms to seeds. In: Burges HD (ed) Formulation of microbial biopesticides: Beneficial microorganisms, nematodes and seed treatments. Kluwer Academic, Dordrecht

Meena B (2014) Biological control of pest and diseases using fluorescent pseudomonads. In: Sahayaraj K (ed) Basic and Applied Aspects of Biopesticides. Springer, New Delhi, pp 17–29. doi:10.1007/978-81-322-1877-7-2

Meyer SLF, Roberts DP (2002) Combinations of biocontrol agents for management of plant parasitic nematodes and soilborne plant pathogenic fungi. J Nematol 34:1–8

Miranda MVC (2012) Effect of microbial inoculants on biocontrol and plant growth promotions. Thesis submitted to The Ohio State University

Moeinzadeh A, Zadeh FS, Ahmadzadeh M, Tajabadi FH (2010) Biopriming of sunflower (*Helianthus annuus* L.) seed with *Pseudomonas fluorescens* for improvement of seed invigoration and seedling growth. Aust J Crop Sci 4(7):564–570

Mohiddin FA, Khan MR, Khan SM (2010) Why *Trichoderma* is considered super hero (super fungus) against the evil parasites?". Plant Pathol J 9:1–11

Mundt CC (2002) Use of multiline cultivars and cultivar mixtures for disease management. Annu Rev Phytopathol 40:381–410

Nandakumar R, Babu S, Viswanathan R, Sheela J, Samiyappan R (2001) Induction of systemic resistance in rice against sheath blight diseases by plant growth promoting rhizobacteria. Soil Biol Biochem 33:603–612

Nayaka SC, Niranjana SR, Uday Shankar AC, Niranjan Raj S, Reddy MS, Prakash HS, Mortensen CN (2008) Seed biopriming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisins in maize. Arch Phytopathology Plant Protect 41:1–19

Nihorimbere V, Ongena M, Smargiassi M, Thonart P (2011) Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnol Agron Soc Environ 15:327–337

Niranjana SR, Lalitha S, Hariprasad P (2009) Mass multiplication and formulations of biocontrol agents for use against fusarium wilt of pigeonpea through seed treatment. Int J Pest Manag 55(4):317–324

Paulitz TC, Belanger RR (2001) Biological control in greenhouse systems. Annu Rev Phytopathol 39:103–133

Pill WG (1991) Advances in fluid drilling. Hort Technology Oct/Dec. 59–65.

Pinton R, Varanini Z, Nannipieri P (2001) The rhizosphere as a site of biochemical interactions among soil components, plants and microorganisms. In: Pinton R, Varanini Z, Nannipieri P (eds) The Rhizosphere. Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp 1–17

Prathuangwong S, Athinuwat D, Chuaboon W, Chatnaparat T, Buensanteai N (2013) Bioformulation Pseudomonas fluorescens SP007s against dirty panicle disease of rice. Afr J Microbiol Res 7(47):5274–5283

Rabindran R, Vidhyasekaran P (1996) Development of a formulation of Pseudomonas fluorescens PfALR2 for management of rice sheath blight. Crop Prot 15:715–721

Ramanujam B, Prasad RD, Sriram S, Rangeswaran R (2010) Mass production, formulation, quality control and delivery of *Trichoderma* for plant disease management. J Plant Protect Sci 2(2):1–8

Raupach GS, Kloepper JW (1998) Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88:1158–1164

Reisser W (2010) The future is green: on the biotechnological potential of green algae. Springer, Dordrecht

Rekha PD, Lai WA, Arun AB, Young CC (2007) Effect of free and encapsulated *Pseudomonas putida*. CC-FR 2-4 and *Bacillus subtilis* CC-pg 104 on plant growth under gnotobiotic conditions. Bioresour Technol 98:447–451

Roberts DP, Scott M, Lohrke S, Meyer LF, Jeffrey S, Buyer JH, Bowers CJ, Baker WL, de Jorge T, Souza JAL, Chung S (2005) Biocontrol agents applied individually and in combination for suppression of soilborne diseases of cucumber. Crop Prot 24:141–155

Schisler DA et al (2004) Formulation of *Bacillus* spp. for biological control of plant diseases. Phytopathology 94:1267–1271

Sesan T, N Csep (1992) Prevention of white rot (*Sclerotinia sclerotiorum*) of sunflower and soybean by the biological control agent *Coniothyrium minitans*. International Organization for Biological and Integrated Control for Noxious Animals and Plants, West Palearctic Region Section 15: 60 – 63

Shtienberg D, Elad Y (1997) Incorporation of weather forecasting in integrated, biological-chemical man-

agement of *Botrytis cinerea*. Phytopathology 87:332–340

Singh U S, Zaidi N W (2002) Current Status of formulation and delivery of fungal and bacterial antagonists for disease management in India, pp 168–179. In *Microbial Biopesticide Formulations and Application* (Eds Rabindra RJ Hussaini SS Ramanujam B) Project Directorate of Biological Control, Bangalore 269 pp.

Sivakumar PK, Joe MM (2008) Development of co-aggregated cells as bioinoculants using plant seed powders-A novel delivery system for rice grown under lowland condition. Agric Conspec Sci 73(4):253–257

Sivakumar PK, Parthasarthi R, Lakshmipriya VP (2014) Encapsulation of plant growth promoting inoculant in bacterial alginate beads enriched with humic acid. Int J Curr Microbiol App Sci 3(6):415–422

Sundaramoorthy S, Balabaskar P (2012) Consortial effect of endophytic and plant growth promoting rhizobacteria for the Management of Early Blight of Tomato Incited by *Alternaria Solani*. J Plant Pathol Microbiol 3:145

Ting ASY, Fang MT, Tee CS (2010) An in vitro assessment on the efficacy of clay-based formulated cells of *Pseudomonas* isolate UTAR EPA2 for Petrol Degradation. Am J Appl Sci 7(2):178–184

Trabelsi D, Mhamdi R (2013) Microbial inoculants and their impact on soil microbial communities: A review. BioMed Research International, Article ID 863240, http://dx.doi.org/10.1155/2013/863240

Van Veen AJ, Van LS, Van Eles JD (1997) Fate and activity of microorganisms introduced into soil. Microbiol Mol Biol Rev 61:121–133

Vasudevan P, Gnanamanickam S S (2000) Progress and prospects for biological suppression of rice diseases with bacterial antagonists. Biological control and plant growth promoting rhizobacteria (PGPR) for sustainable agriculture, Hyderabad, India, April 3–4

Verma KK (2009) Management of *Meloidogyne javanica* by bacterial antagonist Pseudomonas fluorescens as seedling root dip in tomato. Indian J Nematol 39(2):207–210

Vidhyasekaran P, Muthamilan M (1995) Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Dis 79:782–786

Vidhyasekaran P, Rabindran R, Muthamilan M, Nayar K, Rajappan K, Subramanian N, Vasumathi K (1997) Development of powder formulation of *Pseudomonas fluorescens* for control of rice blast. Plant Pathol 46:291–297

Warren JE, Bennett MA (1999) Bio-osmopriming tomato *Lycopersicon esculentum* Mill. seeds for improved stand establishment. Seed Sci Technol 27:489–499

Warrior P, Konduru K, Vasudevan P (2002) Formulation of biological control agents for pest and disease management. In: Gnanamanickam SS (ed) Biological control of crop diseases. Markel Dekker Inc, New York, 5pp. ISBN 0-8247-0693

Weller D (2007) *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 Years. Phytopathology 97:250

Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Weststeijn WA (1990) Fluorescent pseudomonads isolate E11-2 as biological agent for *Pythium* root rot in tulips. Neth J Plant Pathol 96:262–272

Whipps JM, Lumsden RD (2001) Commercial use of fungi as plant disease biological control agents: status and prospects. In: Butt TM, Jackson C, Magan N (eds) Fungi as biocontrol agents: progress problems and potential. CABI Publishing, Wallingford

Witter L (1996) Immobilized microbial cells. In: Baianu IC, Pessen H, Kumosinski TF (eds) Physical chemistry of food processes. Van Nostrand Reinhold, New York, pp 475–486

Woods TS (2003) Pesticide formulations. In: AGR 185 in encyclopedia of agrochemicals. Wiley, New York, pp 1–11

Xu XM, Jeffries P, Pautasso M, Jeger MJ (2011) Combined use of biocontrol agents to manage plant diseases in theory and practice. Phytopathology 101:1024–1031

Young CC, Rekha PD, Lai WA, Arun AB (2006) Encapsulation of plant growth promoting bacteria in alginate enriched with humic acid. Biotechnol Bioeng 95(1):76–83