3 Management of Soilborne Microbial Plan Pathogens

Biological Management of Crop Diseases

Biological control or biocontrol of crop diseases has been considered both in a narrow sense to indicate the control of one organism by another organisms and also in a broad sense to indicate the use of natural or modified organisms, genes or gene product to reduce the effects of pathogens and to favor development of crop plant species (Beirner 1967; Cook 1987). Biological disease management involves utilization of biotic and abiotic agents that act through one or more mechanisms to reduce the potential of the pathogen directly or indirectly by activating host defense systems to reduce the disease incidence and intensity. Several investigations have established that the combination of biotic and abiotic agents results in synergism, improving the effectiveness of pathogen suppression. Biological control agents (BCAs) that effectively reduce incidence and spread of crop diseases caused by microbial pathogens may be divided into two major groups: (i) biotic agents and (ii) abiotic agents. They display varying degrees of biocontrol potential, depending on the host-pathogen combination and the environmental conditions in different geographical locations. Biotic BCAs are nonpathogenic, living organisms that exhibit antagonistic potential against microbial pathogens – oomycetes, fungi, bacteria and viruses that are present in a free-state or in vectors living in the soil environments. They are able to suppress pathogen development through one or more mechanisms. Abiotic BCAs are derived from inorganic or organic sources and they may also act directly on the pathogens or indirectly by activating host plant defense systems. They may be classified based on their chemical constitution or on the mode of action on the target pathogens. The biotic BCAs vary in their biocontrol efficiency due to their sensitivity to environmental conditions, capacity to adapt to new locations, rhizosphere competence, quantum of production of metabolites toxic to target microbial pathogen(s). Hence, it is necessary to establish the precise identity of the strains/isolates, based on the cultural, biochemical and genetic characteristics (Narayanasamy 2013).

3.1 ASSESSMENT OF BIOLOGICAL CONTROL POTENTIAL OF BIOTIC AGENTS

The biotic biological control agents (BCAs) have been isolated from soils, plants and water bodies, using appropriate growth media. Polymerase chain reaction (PCR)-based molecular techniques have been more frequently used to identify, differentiate and quantify the BCAs in different substrates. These techniques may be modified, if necessary, and the biological control agents can be identified up to strains. Precise identification of the BCAs is required, since the strains/isolates of one species of the fungus or bacteria may differ widely in their biological control potential. In addition, biological control activity may also vary, depending on the crop plant species/cultivar, type of soil or environmental conditions existing in different ecosystems.

3.1.1 FUNGAL BIOLOGICAL CONTROL AGENTS

The importance of precise identification of the strain of the fungus to be used as a biocontrol agent is underscored by the example of Trichoderma harzianum (Th), which has been registered as commercial biocontrol agent for the management of several crop diseases. But T. harzianum is also known to be the incitant of green mold disease of mushroom (Ospina-Giraldo et al. 1999). Furthermore, some isolates of T. harzianum were able to produce the mycotoxin belonging to the tricothecene class, which can induce serious ailments in humans and animals, when the contaminated grains are consumed (Sivasithamparam and Ghisalberti 1996). By using the specific tri5 primers in a PCR assay, it was possible to select suitable T. harzianum isolate that did not produce the mycotoxin (Gallo et al. 2004). The relationship between functional group within Trichoderma spp. and their biocontrol activity based on a combination of physiological, biochemical (enzyme production) and molecular (ITS sequences) criteria, was inferred. The efficacy of particular strains of T. harzianum depended on the intended target and the required functions of biocontrol. The results highlighted the importance of selection of the most efficient strains for the target pathogen to be controlled (Grondona et al. 1997). Maintenance of fungal strains to be used as BCAs is essential to have a reliable source of authenticated fungal strains and to ensure consistency of performance of the BCA both in vitro and in vivo. Predominantly, DNA markers that allow authentication of strains and permit monitoring of contamination have been employed (Markovic and Markovic 1998). The quality control test for Trichoderma is based on production of PCR fingerprints, by using semi-random primers designed to primarily target intergenic, more variable areas in the genome (Bulat et al. 1998). Distinct and reproducible fingerprints of strains of Trichoderma spp. were generated by applying appropriate universally-primed (UP) primers in the PCR assay. The fingerprints permitted also
differentiation of a collection of other strains of *Trichoderma* spp. The UP-PCR analysis was combined with dilution plating method and a semi-selective medium was used to recover *Trichoderma* spp. strains, after application in commercial greenhouses. The isolates from the commercial biocontrol products applied in different greenhouse were also identified by UP-PCR analysis. In addition, the presence of a *Trichoderma* strain in the untreated bench was also detected, indicating the possible spread of the BCA to untreated plants (Lübeck and Jensen 2002). Investigation was taken up to determine diversity of *Fusarium* strains from soils suppressive to banana wilt caused by *Fusarium oxysporum* f.sp. *cubense* (Foc). *Fusarium* strains (> 100) isolated from rhizosphere of banana plants were identified up to species level. The PCR-based RFLP analysis of intergenic spacer region of the ribosomal RNA operon was used to characterize non-pathogenic strains. The species-specific primers FOFl and FORI, in addition to morphological characteristics were used to establish the identity of the isolates. Twelve different genotypes could be distinguished and identified using a six-letter code allotted to each isolate following digestion with restriction enzymes *Hae*I III, *Hha*I, *Hinf*I, *Msp*I and *Scrl*. Eleven of these included nonpathogenic *F. oxysporum* isolates from South Africa (Nel et al. 2006a).

### 3.1.1 Assessment of Biocontrol Potential of Fungal Isolates

The interactions between microbial plant pathogens and the biological control agents (BCAs) may vary and they may be differentiated into mutualism, antagonism, parasitism, competition and predation. Mutualism refers to the association between two or more organisms deriving benefits from such association. Many of the BCAs can be considered as facultative mutualists, since survival rarely depends on any specific host. Degree of disease suppression may vary depending on the environmental conditions. An association in which one organism is benefited and the other is neither benefited nor harmed, is known as commensalism. If the interaction results in adverse effects exerted by one organism on the other, the interaction is designated antagonism. Organisms exhibit competition to obtain nutrients or occupy available niche for their survival. Such competition may lead to decreased growth, activity or sporulation of the poor competitor. Many BCAs are able to outgrow the slow-growing plant pathogens, which are ‘starved out’ or prevented from gaining access to the specific host tissues that favor their development. Parasitism indicates the ability of one organism to obtain required nutrition from another organism. Predation is generally seen in the interaction between animals resulting in killing of one organism by another for consumption. Protection to plants may be provided by exploiting suitable forms of interactions between biocontrol agents and the microbial plant pathogens. The biological control potential of the fungus-like and fungal species is determined by screening their isolates/stains under in vitro and in vivo conditions by performing various tests for determining the adverse effects of the BCAs directly or indirectly on the pathogen development.

#### 3.1.1.1 Laboratory Tests

The dual culture method, a preliminary test, is followed to select fungi with antagonistic activity against the target microbial pathogen(s). Various isolates of the pathogen and the test fungal species/isolates are grown and maintained in a suitable medium. The mycelial plugs or disks of the test isolates and fungal pathogens are placed at 4 cm apart from each other in the same petriplate with mycelia in direct contact with agar, so that they grow toward each other. Observations on the adverse effects of the test isolates on pathogen development are recorded and the isolates most effectively inhibiting the growth and sporulation and abnormalities induced are tentatively selected for further testing, as in the case of *F. oxysporum* against *Pythium ultimum* infecting cucumber (Benhamou et al. 2002) and *F. oxysporum* f.sp. *cubense* infecting banana (Nel et al. 2006a). Different species of *Trichoderma* possess the ability to parasitize fungal pathogens (mycoparasitism) and to produce antibiotics or other metabolites inhibitory to the pathogens (antagonism). *Trichoderma virens* (known earlier as *Gliocladium virens*) produced glitoxin, inhibitory to *Rhizoctonia solani* (see Figure 3.1). Another antibiotic isolated from *T. virens* was inhibitory to *Pythium ultimum*, but not to *R. solani* (see Figure 3.2). *T. virens* was able to parasitize *R. solani* by forming coils around the pathogen hyphae. The mutant strains of *T. virens* produced by ultraviolet light irradiation (G6-2, G6-15 and G6-57), were unable to parasitize *R. solani* like the wild-type strain, although they were able to produce the antibiotics (see Figure 3.3) (Howell 2003). The basis of antifungal activity of *T. harzianum* SQR-T037, effective against *F. oxysporum* f.sp. *cucumeris* (Foc), causal agent of cucumber Fusarium wilt disease, was investigated. Presence of 6-pentyl-α-pyron (6PAP) in the culture filtrate of *T. harzianum* was identified by mass spectrometry and nuclear magnetic resonance spectroscopy. The antifungal activity increased with increasing concentrations of 6PAP, as reflected in inhibition of mycelial growth, spore germination, sporulation and fusaric acid production by the pathogen. Furthermore, addition of 6PAP at 350 mg/kg to

![FIGURE 3.1 Inhibition of mycelial growth of *Rhizoctonia solani* by glitoxin produced by *Trichoderma virens* Restriction of pathogen growth in glitoxin-amended medium (A) and unrestricted pathogen development in nonamended medium (B).](image-url)

[Courtesy of Howell 2003 and with kind permission of the American Phytopathological Society, MN]
cucumber-continuously cropped soil decreased population of indigenous *F. oxysporum* by 41.2% and reduced disease incidence by 78.1 to 89.6% in pot experiments. The dry weight of cucumber seedlings showed an increase by 60.0 to 92.6%. The results indicated the potential of 6PAP for the management of Fusarium wilt of cucumber (Chen et al. 2012).

Biocontrol efficiency may be assessed by examining the interactions between the BCA and the pathogen under light microscope and/or electron microscope. The interaction between *Rhizoctonia solani* and the mycoparasitic endophyte *Chaetomium spirale* ND35 was investigated under light and electron microscopes. Coiling of *C. spirale* around *R. solani* hyphae and its intracellular growth in *R. solani* was observed. Immmunochemistry procedure and transmission electron microscopic observations revealed that contact between the antagonist and the pathogen was mediated by an amorphous β-1,3-glucan-enriched matrix originated from cell wall of *C. spirale* and stick to *R. solani* cell surface. At the same time, *R. solani* cells reacted by forming hemispherical wall appositions which were intensely labeled by antibodies specific to β-1,3-glucan. The appositions were formed at sites of potential antagonist entry. However, the antagonist could overcome this barrier effectively, indicating that production of β-1,3-glucanase might facilitate breaching of the host barrier (Gao et al. 2005). The fungal endophytes, isolated from leaves of Norway maples, were screened for their efficacy in inhibiting the development of *Rhizoctonia solani*, infecting potatoes and several other crops. The mycelial growth inhibition by different fungi was determined. *Trichoderma viride* inhibited the growth of *R. solani* at a faster rate than that of *Phomopsis* sp., *Alternaria longipes* and *Epicoccum nigrum*. The culture filtrates also inhibited the pathogen growth to different extent. Culture filtrate of *T. viride* was the most effective in inhibiting the growth of *R. solani*. Confocal microscopic examination of the interaction between *R. solani*, causing black scurf of potato tubers and *Trichoderma viride* revealed that the BCA could establish close contact with the pathogen hyphae by coiling. The coils were very dense and appeared to tightly encircle the hyphae of *R. solani*. Penetration of the pathogen hyphae by *T. viride* could be seen at 7 days after contact, followed by loss of turgor of hyphal cells. *Phomopsis* sp. and *Epicoccum nigrum* did not penetrate pathogen cells, but induced abnormal cell morphology and lysis of cells, possibly due to production of extracellular chitinolytic enzymes produced by these fungal biocontrol agents (Lahlali and Hijri 2010).

Agar drop test was performed as a preliminary screen to evaluate the potential of biological control agents from large number of samples. Five drops of potato dextrose agar (PDA) (0.7 ml/petri dish) were dispensed in the petridishes. Mycelial macerate (10 µl) of the 1-week-old culture of the pathogen, *Sclerotium cepivorum* was used to inoculate the agar drops in petridishes. An agar plug (2 mm diameter) from the edges of the culture of each test fungus was added to each of the precolonized agar drops. The plates were then incubated for 5 weeks at 20°C. Sclerotia (100) from each agar drop were examined under the microscope for degradation (soft or collapsed) by squeezing them using a pair of forceps (Clarkson et al. 2001). In another method, petridish-grown test plants were used to assess the biocontrol potential of biological control agents. Fungal endophytes (349) were isolated from root segments of eggplant, melon, barley and Chinese cabbage grown as bait plants in a mixed soil containing forest soils. These isolates were inoculated onto axenically raised Chinese cabbage seedlings grown in petridishes to assess their efficacy in suppressing the development of Verticillium
yellows disease of Chinese cabbage caused by *Verticillium longisporum*. Three isolates almost completely suppressed the development of virulent strain of *V. longisporum*. Two of these isolates were identified as *Phialophora fortinii* obtained from the roots of eggplant and Chinese cabbage plants. Third isolate was a dark septate endophyte (DSE) fungus obtained from barley roots. Seedlings grown for one week in the presence of the endophytes were challenged with *V. longipes*. In DSE-treated roots, some cell walls in the epidermal and cortical layers showed cell wall appositions and thickenings, which appeared to limit the ingress of the pathogen in the adjacent cells. Such marked host responses were seen in the root cells of cabbage seedlings preinoculated with the endophytes. Development of *Verticillium* yellows was more effectively hampered by DSE. External and internal disease symptoms were reduced by 84% and 88%, respectively, by DSE. Chinese cabbage plants treated with DSE yielded desirable level of marketable quality produce (Narisawa et al. 2004).

The antagonistic effects of *Trichoderma asperellum* and *Bacillus* spp. on *Macrophomina phaseolina* and *Fusarium solani*, causing charcoal rot and root rot diseases respectively in strawberry, were assessed by using dual culture (confrontation) assay. Radial growth of *M. phaseolina* and *F. solani* was inhibited by more than 36%. Preventive application of *T. asperellum* by root-dipping reduced the incidence of charcoal rot up to 44% under growth chamber conditions and up to 65% under field conditions. Likewise, crown rot and root caused by *F. solani* was reduced up to 100% under greenhouse conditions and up to 81% under field conditions. Application of *Bacillus* sp. (in formulation) was also effective in reducing charcoal rot incidence, but its effectiveness was variable against *F. solani*, depending on the growth conditions (Pastrana et al. 2016). *Trichoderma viride* strain 110 was transformed with the *gfp* gene from the jelly fish *Aequorea victoria*, coding for green fluorescent protein (GFP), as a reporter gene. The effect of transformation with *gfp* gene on the mycoparasitic activity of *T. viride* was determined by inoculating sclerotia of *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and transformed *S. minor* and wild-type strains of *T. viride*. Transformation with *gfp* gene did not alter the mycoparasitic activity on the sclerotia of the pathogens tested. Colonization of sclerotia was followed by fluorescent microscopy which revealed intracellular growth of the antagonist in the cortex (in *S. Rolfsii*) and intracellular growth in the medulla (in *S. rolfsii* and *S. sclerotiorum*). Uniform distribution of mycelium of *T. viride* just beneath the rind of sclerotia of both *S. rolfsii* and *S. sclerotiorum* suggested that sclerotia became infected at several randomly distributed sites without any preferred point of penetration into the sclerotia (Sarrocco et al. 2006).

The biocontrol potential of *Microsphaeropsis* sp. strain P130A for suppressing the development of tuberborne inoculum of *Rhizoctonia solani*, causing potato black scurf disease was investigated, based on the viability of sclerotia produced in vitro and on both viability and production of sclerotia on potato tubers. Transmission electron microscopy (TEM) technique was employed to study the interactions between *R. solani* and *Microsphaeropsis* sp. The BCA significantly reduced germination, the percent reduction increasing with increasing period of incubation. Similar reduction in sclerotial germination was seen in tuberborne sclerotia, following treatment with the BCA, in addition to reduction in the number of sclerotia present on the tubers during 8 months of storage at 4°C, whereas the number of sclerotia increased in untreated control tubers. TEM observations showed that the BCA-induced rupture of the pathogen plasma membrane and that a chitin-enriched matrix was deposited at the sites of potential antagonist penetration. Host penetration was not associated with pathogen cell wall alterations, which occurred at the time of antagonist development in the pathogen cytoplasm. The cells of *R. solani* treated with crude extract of *Microsphaeropsis* sp. showed disorganization of cytoplasm and breakdown of plasma membranes. The results indicated that *Microsphaeropsis* sp. suppressed the pathogen development through antibiosis and mycoparasitism (Carisse et al. 2001). *Coniothyrium minitans* and *Microsphaeropsis ochracea* did not show any mutual antagonism in dual culture assays, indicating the feasibility of using them together for their suppression of *Sclerotinia sclerotiorum*. Combined application of both mycoparasites increased the sclerotia mortality in a temperature range of 16 to 26°C, compared to application of single BCA. With increasing temperature, the efficacy of *M. ochracea* decreased, but not that of *C. minitans*. Sclerotia degeneration by *C. minitans* was slightly faster than by *M. ochracea*. The BCAs were transformed via *Agrobacterium tumefaciens*-mediated transformation (ATMT), employing reporter genes, encoding GFP and DsRed and the degradation process was visualized using fluorescent microscopy. Both antagonists were able to penetrate effectively the sclereria rind, and growth forming pycnidia in the cortex and medulla, ultimately degrading the sclereria entirely in a period of 25 days after single inoculation. The ability to colonize the pathogen sclerotia by both BCAs was revealed by the independent production of pycnidia in the sclerotial medulla and on the sclereria rind. Combined inoculation of *C. minitans* and *M. ochracea*, although had slightly delayed growth, the degradative effects on sclerotia were additive. The results indicated the advantage of enhancing the biocontrol efficiency by combining both BCAs which show no mutual antagonism to each other (Bitsadze et al. 2015). In confrontation assays, *Trichoderma* isolates with antagonistic activity against *Rhizoctonia solani* and *Sclerotium rolfsii* were identified with degree of antagonism being greater against *R. solani* than against *S. rolfsii*. Production of metabolites in all isolates of *Trichoderma* did not correlate with biocontrol efficacy. However, one *T. viride* isolate T14 produced highest amounts of inorganic phosphate, indole acetic acid (IAA) and siderophores and showed high antagonistic activity and growth-promoting activity (Kotasthane et al. 2015).

### 3.1.1.1.2 Greenhouse/Growth Chamber Assessments

Laboratory tests may not be useful to select biological control agents (BCAs) that have mechanisms other than mycoparasitism. Hence, various tests are performed to determine the biocontrol potential of BCAs using live plants under greenhouse/growth chamber conditions. The BCAs are applied for treatment of seeds and/or soil to suppress the soilborne pathogens...
present in the seeds/propagules or soils. Seed treatment with BCAs is preferred, because it is easy and economical. The biocontrol potential of T. harzianum KUEN 1585 (a commercial product) was assessed under pot culture conditions. The soil was infested with a pathogenic isolate of F. oxysporum f.sp. cepae (Foc) and onion seeds were coated with T. harzianum at 10 g/kg seed. The basal rot disease incidence was reduced by T. harzianum to a level comparable with the fungicides imidazole and prochloraz. Similar results were obtained in the field experiment also. Seed treatment with the BCA enhanced the bulb diameter of sets. Extracts from onion sets grown from treated seeds contained compounds with high antifungal activity against F. oxysporum f.sp. cepae (Coşkun'tuna and Özer 2008). Cotton seeds were treated with Trichoderma virens strain G-6, T. koningii strain TK-7 and T. harzianum strain TH-23 and planted in sterile vermiculite seedling trays. Biocontrol efficacy was determined, based on the percentage of seedlings affected by damping-off diseases caused by Rhizoctonia solani (Howell et al. 2000). The efficiency of T. harzianum T22 was assessed along with the fungicide by treating spinach seeds against infection by damping-off pathogens, F. oxysporum f.sp. spinaciae, Pythium ultimum, and Rhizoctonia solani. The BCA was applied as a commercial product, was as effective as the fungicide mefenoxam against P. ultimum. None of the treatments was able to reduce the incidence of diseases caused by all three pathogens (Cummings et al. 2009).

Soilborne fungal pathogens have been managed by fumigating soils with chemicals like methyl bromide, the use of which has been either banned or restricted in many countries. As an alternative to the chemical application, the effectiveness of natural volatiles from fungi like Muscodor spp. was assessed. Populations of soilborne pathogens, Rhizoctonia solani, Pythium ultimum, Aphanomyces cochlioides were reduced by mycofumigation with Muscodor albus and M. roseus. Consequently, sugar beet stand establishment was increased, while disease severity was reduced. The mycofumigants were applied as colonized agar strips, ground pasta, and alginate formulations. The Stabileze formulation containing a mixture of water-absorbent starch, corn oil, sucrose, and fumed silica was applied, and the Verticillium wilt disease incidence and severity showed marked reduction in eggplant (brinjal). Both M. roseus and M. albus reduced disease severity. M. albus mycofungicide reduced the populations of the pathogens significantly (Stinson et al. 2003). A biorational synthetic mixture of organic components mimicking key antimicrobial gases produced by Muscodor albus was employed in place of live BCA for the suppression of Pythium ultimum, Rhizoctonia solani AG2-2 and Aphanomyces cochlioides, causing sugar beet seedling diseases. The biorational mixture was as effective as the live M. albus starch-based formulation in suppressing the development of all three pathogens, causing damping-off and also reduced the number of galls induced in the roots of tomato by the nematode, Meloidogyne incognita (Grimme et al. 2007).

Phytophthora capsici, incitant of Phytophthora blight disease, is one of the important diseases, accounting for heavy losses in pepper. The effectiveness of Muscodor albus as a potential biofumigant was assessed under greenhouse conditions. P. capsici infested potting mix was treated with M. albus at three concentrations, mefenoxam (Ridomil) and with no treatment (control). Seedlings of five sweet pepper cultivars and one butternut squash cultivar were transplanted into the potting mix. A significant interaction between pepper cultivars and soil treatment was observed. M. albus at the highest concentration reduced disease severity slightly, but significantly on cvs. Alliance, Aristole, Paladin and Revolution, compared with control plants. The extent of reduction in disease severity by M. albus on cv. Paladin, the most tolerant cultivar, was up to commercially acceptable levels (Camp et al. 2000). The role of antimicrobial volatiles from M. albus in suppressing Rhizoctonia solani in soil and potting mix was studied. The volatiles reduced damping-off of broccoli seedlings, when pots containing soil or soilless potting mix infested with R. solani were placed in the presence of active M. albus culture without physical contact in closed containers. In contrast, agar plugs of R. solani on PDA were inhibited, when they were placed in the presence of M. albus incorporated in garden soil or soilless potting mix. Gas chromatographic analysis with solid-phase micro-extraction showed that isobutyric acid and 2-methyl-1-butanol were released from treated substrates. A significant relationship between production of isobutyric acid and extent of disease reduction was observed. Isobutyric acid was produced only for short time, peaking at 24 h in potting mix and 48 h in soil. Amounts of isobutyric acid released from soil were several times more in soil than in potting mix. The results suggested that soil environment was better for the biological activity or viability of M. albus than that of soilless potting mix (Mercier and Jiménez 2009).

Soil sterilization using chemical fumigants such as dichloropropene, organic sulfur and dazomet (3,5-dimethyl-1,3,5-thiadiazine-2-thione) and biofumigants, has been shown to reduce soilborne pathogens, particularly nematodes. The fungus Clonostachys rosea 67-1, a promising mycoparasite was evaluated either alone or in combination with dazomet for their effectivity in suppressing the development of cucumber Fusarium wilt disease caused by F. oxysporum f.sp. cucumerinum (Foc) KW2-1. When C. rosea 67-1 was applied, after dazomet fumigation, incidence of disease was fully controlled (100%), compared with 88.1% and 69.8% for dazomet and the BCA applied alone treatments, indicating a synergistic effect between the chemical and BCA. The effects of chemical fumigation on colonization and activity of Foc and the interaction between the BCA and Foc were investigated. The growth of Foc decreased with increasing concentration of dazomet. When exposed to dazomet (100 ppm), the pathogen sporation decreased by 94.4%. Observations under scanning electron microscope revealed severe damage due to fumigation. In the greenhouse, disease incidence was significantly decreased, following fumigation. By contrast, germination of spores of C. rosea increased by > sixfold in fumigated soil and its ability to parasitize fumigated F. oxysporum f.sp. cucumerinum was also significantly enhanced (Tian et al. 2014).

Various mushroom species produce many antimicrobial compounds that can inhibit fungi and bacteria. The spent
mushroom substrate (SMS) of *Lentinula edodes* derived from sawdust bag cultivation was evaluated for its control potential against *Phytophthora capsici*, incitant of pepper Phytophthora blight disease. Water extract from SMS (WESMS) of *L. edodes* inhibited mycelial growth of *P. capsici* and also suppressed disease development in pepper seedlings by 65% and promoted plant growth by over 30%. In high performance liquid chromatography (HPLC) analysis, oxalic acid was detected as the principal organic compound in WESMS and it inhibited mycelia growth at a minimum concentration of 200 mg/l. In quantitative real-time PCR assay, the transcriptional expression of CaBPR-1 (PR protein-1), CaBGLU (β-1,2-glucanase), CaPR-4 (PR protein-4) and CaPR-10 (PR protein-10) were significantly enhanced on WESMS- and DL-β-amino butyric acid (BABA)-treated pepper leaves, compared to water-treated leaf sample. The results suggested that WESMS of *L. edodes* might suppress Phytophthora blight disease through multiple effects including antifungal activity, plant growth promotion and induction of defense gene(s) (Kang et al. 2017). Avocado white root rot disease is primarily caused by *Rosellinia necatrix*. Both the pathogen isolates (19) and the biocontrol agent *Entoleuca sp.* isolates (21) were obtained by burying bait twigs around avocado escape trees. *Entoleuca sp.* was able to colonize roots of avocado and remained persistent up to 2 years, when it could be recovered from the inoculated avocado roots. Most isolates (86%) of *Entoleuca sp.* reduced disease incidence and hence, they were considered as the effective biocontrol agents with potential for the suppression of avocado white root disease (Arjona-Girona and Lopez-Herrera 2018).

Fungal pathogens belonging to the genera *Rhizoctonia* and *Sclerotium* produce sclerotia, as survival structures resistant to adverse environmental conditions, and they are produced on crop residues and organic matter present in soil. Sclerotia of pathogens in soil samples can be retrieved by flotation and sieving and the biocontrol activity of BCAs may be assessed by sclerotial degradation assay. The degree of degradation of sclerotia was determined by squeezing them with forceps (as reflected by soft or collapsed sclerotia) under the light microscope. The BCAs causing greater level of degradation were further tested in pot trial, as in the onion white root disease caused by *Sclerotium cepivorum*. Soil amended with medium-grade vermiculite and sclerotia of the pathogen were mixed and the BCA as wheat bran cultures (1 g/100 g) or spore suspension (1 × 10^7 spores/100 g) was incorporated into the soil in pots. Onion seeds were planted at one seed/pot, maintaining appropriate control. Seedling emergence percentage and appearance of disease symptoms were recorded at weekly intervals up to 14 weeks after planting (Clarkson et al. 2001). *Sclerotinia sclerotiorum* infects oilseed rape (*Brassica napus*) causing stem rot disease. *Penicillium oxalicum* PY-1 was evaluated for its biocontrol potential, using the hyphaemediated infection procedure. The mycelial homogenate, culture filtrate and spores of PY-1 were sprayed on oilseed rape plants. The inoculated plants were incubated at 20 ± 2°C and 100% RH for 1 week. The number and size of lesions induced by *S. sclerotiorum* were recorded for each treatment. Spore suspension and culture filtrate (10-fold dilution) suppressed infection by *S. sclerotiorum* (Yang et al. 2008).

The biocontrol efficacy of *F. oxysporum* F047, nonpathogenic isolates obtained from banana rhizosphere and two field strains of *Trichoderma* T22 and T5 was assessed by treating the banana plantlets with spore suspension of the BCA isolates applied as drench. Pathogenic isolates of *F. oxysporum* f.sp. *cubense* (Foc) was multiplied in PDA (half strength). Mycelial plugs taken from cultures were multiplied in sterilized millet seeds. Pathogen-colonized millet seeds were transferred to steam-sterilized soil at the rate of 15 ml seeds/500 ml of soil. The infested soil was transferred to pots in which banana plants treated with BCAs were planted. Prior to planting, the banana roots were slightly bruised by manually squeezing the root system to facilitate initiation of infection by *Foc*. Disease development was verified by cutting the rhizome open using a scalpel, based on the disease severity rating according to Inihab’s Technical Guidelines (Nel et al. 2006a). The combined effects of *T. harzianum* and *T. asperellum* on the development of bean root rot disease caused by *Fusarium solani* were assessed under greenhouse conditions. The roots of bean plants were dipped in fungal BCA biomass suspension (1.8 × 10^7 CFU) and planted in pots containing wheat bran-corn meal. Development of disease symptoms was recorded up to 6–8 weeks after planting. Stem sections of bean plants were examined for the presence of *F. solani*. Isolation of the pathogen from stem sections was made after sterilization. The BCAs either alone or in combination provided significant protection against the root rot disease (Akrami et al. 2009). The nonpathogenic isolate *F. oxysporum* F221-B inhibited effectively in vitro the mycelial growth of *Rhizoctonia solani*, which causes the serious root rot and wilt disease on lettuce grown under hydroponics system. The strain F221-B reduced the disease incidence and severity by about 60 to 89%, compared to control. The BCA strain also promoted the growth of three lettuce cultivars, increasing the fresh weight by 2 folds over healthy control. The results indicated the effectiveness of nonpathogenic *F. oxysporum* F221-B in reducing the disease and also promoting plant growth (Thongkamngam and Jaenaksorn 2017).

The influence of inoculum density of *Sclerotium cepivorum*, causal agent of onion white rot disease on the efficiency of *Trichoderma koningii* strain Tr5, was investigated. Soil amendment with the BCA grown on autoclaved white millet grain provided 63 to 79% control of white rot disease in soil containing 10, 25, 50 or 100 sclerotia of *S. cepivorum* g of soil added at the time of sowing. Pathogen sclerotial density did not have any significant change in the antagonistic activity of *T. koningii*. Rhizosphere colonization by *T. koningii* was determined by incubating onion roots sampled from plants growing in soil with appropriate sclerotial density on a *Trichoderma* selective medium. Isolates of *T. koningii* were identified based on morphologic characteristics and polygalacturonase (PG, EC 3.2.1.15) and pectinesterase (PE, EC 3.1.1.11) isozyme profiles. The extent of disease suppression increased with increase in concentrations of sclerotia of *S. cepivorum* and *T. koningii*. Effect of colonized millet grain
was proportional and no further increase in the disease suppression could be seen, when *T. koningii* Tr5-colonized millet was added at > 1,590 kg/ha, at any concentrations of sclerotia of *S. cepivorum* (Metcalfe et al. 2004). The effect of *T. harzianum* on the dynamics of *Rhizoctonia solani*, incitant of potato stem canker and black scurf was investigated. *T. harzianum* reduced the severity of symptoms, expressed as ‘*Rhizoctonia* stem lesion index’ (RSI), during the first 7 days postinoculation (dpi), when the inoculum was placed at certain distances (varying from 30 to 60 mm) from the host. With inoculum at 40 mm from the host, RSI were 6 and 40 with and without *T. harzianum*, respectively. The antagonistic effect was overcame at longer period after inoculation. *T. harzianum* reduced black scurf severity on progeny tubers. The mean number of progeny tubers infected per potato plant was reduced by BCA application, as well as the proportion of small tubers. Furthermore, the number of malformed and green-colored tuber was reduced in pots treated with *T. harzianum* than in the pots without the BCA (Wilson et al. 2008). *Trichoderma* strains have been shown to suppress the development of diseases caused by soilborne pathogens through multiple mechanisms of action, and also possess the ability to promote plant growth significantly. Strains of *Trichoderma atroviride* and *T. harzianum* grown on organic materials were applied as biopreparations in the soil in open-field lettuce cultivation. Populations of the BCAs were monitored over time. The multiplex PCR-*Trichoderma*-identification technique showed that populations of *Trichoderma* spp. increased in the soil and they were not detected in the untreated soil plots. The results of multiplex PCR assays were confirmed by the standard plating method. Indigenous species were identified in the field soil. However, the abundance of the BCAs was estimated to be relatively low (10³ CFU/g dry soil), after application of biopreparations. *Trichoderma* spp. persisted at this population level even after two years (Oskiera et al. 2017).

Environmental conditions have marked influence on the development of both the pathogen and the biological control agents (BCAs) in addition to host plants. The influence of different environmental and cropping conditions on nonpathogenic strain of *F. oxysporum* (CS-20 and CS-24) and *F. solani* (CS-1) was assessed under greenhouse and growth chamber conditions. Liquid spore suspensions (10⁷/ml) of BCA isolates were applied to soilless potting mix at the time of tomato seedling establishment and seedlings were transplanted in the pathogen-infested field soil 2 weeks later. Temperature regimes ranging from 22 to 32°C significantly affected disease development and plant physiology. Strain CS-20 significantly reduced Fusarium wilt disease incidence at all temperature regimes tested with disease reduction of 59 to 100%, compared to control treatment. Strains CS-24 and CS-1 reduced disease incidence in the greenhouse at high temperatures but were less effective at optimum temperature (27°C) for disease development. Both CS-1 and CS-20 strains were equally effective in suppressing the development of all three races of the wilt pathogen *F. oxysporum* f.sp. *lycopersici*, as well as multiple isolates of each race, reduction in disease incidence being 66 to 80%. Among the three strains, CS-20 could effectively reduce Fusarium wilt disease of tomato under different environmental conditions (Larkin and Fravel 2002). In a later investigation, the effectiveness of *F. oxysporum* Fo-B2 in suppressing the development of *F. oxysporum* f.sp. *lycopersici* was assessed in three different environments viz., growth chamber with sterile soilless medium, greenhouse with fumigated and non-fumigated soil and nonfumigated field plots. The Fo-B2 strain reduced disease severity significantly, but the efficiency of disease suppression decreased, as the experimental environment became less controlled. The BCA was most effective, when it colonized vascular tissues intensively. The degree of Fo-B2 colonization was markedly reduced in plants grown in nonfumigated soil. The results suggested that indigenous soil microorganisms were a primary factor negatively impacting the efficiency of Fo-B2 (Shishido et al. 2005). Isolates of binucleate *Rhizoctonia* (BNR) obtained from soybean were screened for their biocontrol potential against *Rhizoctonia solani* AG-4 and AG2-2. Eight BNR isolates, when combined with AG-4 or AG2-2, significantly increased seedling emergence and survival of cv. Ozzie and reduced severity of disease, compared with pathogen alone (AG-4 or AG2-2). No interaction between BNR isolates (BNR-4, BNR 8-2 and BNR3) and seven soybean cultivars was evident. With AG-4, BNRs significantly increased emergence and survival of cultivars and reduced disease severity. On the other hand, with AG2-2, BNRs reduced disease severity. BNRs effectively suppressed *R. solani* in both potting soil mix and natural soil. BNRs alone significantly increased plant growth, compared with noninoculated controls. It was possible to isolate BNRs consistently from hypocotyls and roots, indicating colonization of tissues was associated with control. The results indicated the potential of BNR isolates for use against the soilborne *R. solani* which has a wide host range, including many crop plant species (Khan et al. 2005).

Fusarium crown and root rot (FCRR) disease of tomato caused by *F. oxysporum* f.sp. *radicis-lycopersici* (FORL) seriously impacts production in hydroponic rock wool systems. Six isolates of plant growth promoting fungi (PGPF), nonpathogenic *F. oxysporum* and five isolates of bacteria were evaluated for their efficacy in suppressing FCRR development. *F. equisetti* was the most effective and disease reduction rate by this BCA was consistently high and significant. Stem extracts from *F. equisetti*-treated and pathogen-challenged tomato plants inhibited the germination and germ tube length of FORL microconidia. Similar inhibitory activity of stem extracts of BCA-treated and uninoculated control plants was also observed (Horinouchi et al. 2007). Nonpathogenic *F. oxysporum* endophytes isolated from healthy banana roots were evaluated for their biocontrol potential against Fusarium (Panama) wilt of banana caused by *F. oxysporum* f.sp. *cubense*. The identity of the isolates was established using PCR-RFLP analysis. Under greenhouse conditions, 10 nonpathogenic *F. oxysporum* isolates significantly reduced disease incidence in banana plantlets generated via tissue culture technique. *Pseudomonas fluorescens* WCS417 and the most effective nonpathogenic *F. oxysporum* isolate were tested under field conditions. None of the fungal and bacterial BCAs
could reduce the disease development, indicating the need for investigating factors contributing to protection of banana plants under field conditions (Belgrove et al. 2011). Two soil-borne fungal endophyte strains, almost entirely suppressed development of virulent strain of *F. oxysporum f.sp. melonis*, causing melon Fusarium wilt disease, when inoculated into axenically raised melon seedlings in petridishes. The endophytes were identified as *Cadophora* sp., based on morphological characteristics and ITS1-5.8S rDNA-ITS2 sequences. The hyphae of *Cadophora* sp. grew along the surface of the root and colonized root cells of the cortex and reduced the ingress of the pathogen into adjacent cells. Endophyte-treated seedlings grown in soil amended with valine showed decrease in Fusarium wilt incidence in field plots (Kasthini et al. 2014).

*Coniothyrium mimitans* isolate Conio, grown on maize meal perlite and ground maize meal-perlite medium produced high sporulation (1.6 × 10^7 conidia/g inoculum). Preplanting × meal perlite and ground maize meal-perlite medium produced seedlings grown in soil amended with valine showed decreaseing ingress of the pathogen into adjacent cells. Endophyte-treated seedlings grown in soil amended with valine showed decrease in Fusarium wilt incidence in field plots (Kasthini et al. 2014).

*Coniothyrium mimitans* isolate Conio, grown on maize meal perlite and ground maize meal-perlite medium produced high sporulation (1.6 × 10^7 conidia/g inoculum). Preplanting soil incorporation of Conio at 10^11 CFU/m^2 significantly reduced Sclerotinia rot in a sequence of three lettuce crops grown in the greenhouse. Reduced dosages (10^8/m^2) were not effective in suppressing disease development. After harvest of the second and third crops, application at full rate (10^11 CFU) of the BCA reduced the number and viability of sclerotia recovered on soil surface and increased infection of the pathogen by *C. mimitans*, compared with spore suspension or reduced rate of maize meal-perlite inocula. In the pot culture, *C. mimitans* decreased carpogenic germination, recovery and viability of sclerotia and increased infection of sclerotia by the BCA, compared to the spore suspension, as in the glasshouse experiments. In addition, reduced maize meal-perlite treatment also decreased apothecial production, recovery and viability of sclerotia. The inoculum density of *C. mimitans* appeared to be the key factor influencing the effectiveness of the biological management of Sclerotinia rot of lettuce (Jones and Whips 2002). *Sclerotinia sclerotiorum*, causing white mold disease of common bean, produces sclerotia as survival structures in the soil, which contribute significantly to development of epidemics. The mycoparasitic activity of *Coniothyrium mimitans* on pathogen sclerotia was investigated. Even a single conidium could infect pathogen sclerotia. Under optimal conditions, use of 2 conidia/sclerotium produced maximum infection of sclerotia (c. 90%) and produced up to 1,000 conidia. Similar results were obtained with stem pieces infected by *S. sclerotiorum*. Under field conditions, application of conidial suspensions of *C. mimitans* to the bean crop, soon after white mold outbreak, resulted in higher percentage of sclerotal infection than later applications. Infection of sclerotia was high (90%), when conidial suspensions were applied within 3 weeks. Inoculum concentration or the BCA isolate did not have significant influence on sclerotal infection by *C. mimitans*. The results showed that a suspension of 10^6 conidia/ml in 1,000 l/ha sprayed immediately after first symptom appearance led to > 90% infection of sclerotia of *S. sclerotiorum*. Infection of sclerotia, capable of preventing pathogen carryover, occurred within a broad range of inoculum quality (Gerlagh et al. 2003). Effects of different inocula of *Coniothyrium mimitans* on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* applied at different times of the year were investigated. Five spore-suspension inocula of *C. mimitans*, including three different isolates (Conio, IVT1 and Contans) with a standard maize meal-perlite inoculum were evaluated. Maize meal-perlite inoculum at 10^7 CFU/cm^3 soil reduced sclerotial germination and apothecial production in a series of three glasshouse bioassays, with decrease in sclerotal recovery and viability in the second bioassay and increasing *C. mimitans* infection of sclerotia in the first bioassay. Spore suspensions were less effective and inconsistent. Sclerotal germination was delayed or inhibited, when bioassays were made in the summer. High temperatures inhibited infection of sclerotia by *C. mimitans* also, although the BCA could survive the high temperatures. Apothecial production is critically influenced by inoculum level of *C. mimitans* (Jones et al. 2004). Lettuce drop disease caused by *Sclerotinia minor* accounts for appreciable losses. *Coniothyrium mimitans* effectively suppressed the development of lettuce drop, but a commercial product of *Trichoderma* or an isolate of *T. virens* was ineffective. *C. mimitans* significantly increased the percentage of sclerotia infected and reduced the percentage of viable sclerotia, when applied to lettuce plants exhibiting initial symptoms of the disease. *C. mimitans* could be detected always in untreated control plants in the experiments conducted in greenhouse, indicating the likelihood of *S. minor*, being a natural resident in the soils and its natural suppressing effect on the pathogen (Ismaini and Keane 2007).

*Polymyxa betae* is the vector of one of the destructive diseases of sugar beet rhizomania caused by *Beet necrotic yellow vein virus* (BNYVV). In addition, *P. betae* infects the sugar beet roots causing enlarged roots (sori) containing the resting spores internalized with BNYVV. As the resting spores can remain viable for several years, BNYVV is persistent in infested soil. In order to eliminate resting spores of *P. betae*, the antagonistic fungi, including *T. harzianum* and *Talaromyces flavus* were isolated from soil samples from infested fields. The antagonistic potential of the BCAs was assessed in split plot trial under greenhouse conditions. The cystosorous population of *P. betae* was determined by staining seedling roots with lactic acid and fuchsin (lactofuchsin) at 60 days after sowing, under light microscope. Different methods of application of antagonists viz., soil treatment, seed treatment and combination of both were tested. The number of cystosori in one gram of the roots varied with treatments. Soil application of the BCAs was more effective in reducing cystosorous population than seed treatment and root weight consequently showed increase over the control (Naraghi et al. 2014).

### 3.1.1.1.3 Field Trials

Many factors in different combinations influence the competence and capacity of biocontrol agents (BCAs) to inhibit the development of microbial plant pathogens and disease incidence/severity. The availability of suitable ecological stages in the life cycle of the pathogens, favorable environmental conditions for rapid buildup of BCA populations and persistence for long periods in the absence of crop hosts by
producing resistant spores (resting) are important factors affecting the performance of BCAs under field conditions and they are considered for development of commercial products for large scale application. Field trials are conducted to determine the efficacy of the selected species/strains/isolates of the BCA that are found to be effective in the in vitro and/or greenhouse tests. Field experiments are carried out for two or more seasons/years at as many locations as possible. Treatments are arranged in suitable statistical designs, such as randomized block design (RBD) or split-plot design, depending on the number of treatments to be tested and compared. In the case of soilborne pathogens, sites that have past history of incidence of target disease at high levels are selected for testing. Alternatively, high populations of target pathogen are incorporated into the field soil, using pathogen biomass raised on artificial media or large quantities of infected plant materials are used for amending the soils. The efficacy of the biocontrol agents may be affected by the form of BCA preparations – spore suspensions, mycelial cultures with media, pellet or powder/ granule – formulations (Narayanasamy 2013).

A high percentage of microorganisms isolated from different substrates, exhibited antagonistic activity under laboratory conditions, proved to be ineffective in the greenhouse and/or field assessments. Such a situation may probably be due to poor competitiveness and unavailability of environmental conditions required for the growth and proliferation rapidly. The putative BCAs should be fast-growing and aggressive against target pathogen(s) and possess a wide spectrum of activity. The putative BCA with two or more mechanisms may be preferred, because of the possibility of obtaining more effective suppression of pathogen development, compared with the BCA functioning via single mode of action on the pathogen. The fungal BCA Talaromyces flavus (anamorph – Penicillium dangeardi) was able to suppress Verticillium dahliae, incitant of Verticillium wilt disease of tomato, potato and eggplant (brinjal) and could also parasitize Sclerotinia sclerotiorum, Rhizoctonia solani and Sclerotium rolfsii. Chitinase produced by T. flavus effectively arrested the development of S. rolfsii and V. dahliae. Spore germination, hyphal growth and melanization of newly formed sclerotia of V. dahliae were significantly retarded by the antifungal compounds produced by T. flavus. Microsclerotia were killed, when treated with culture filtrate (CF) of T. flavus and toxicity of the CF was considered to be due to the glucose oxidase activity of T. flavus (Madi et al. 1997). Rapid colonization of plant tissues by the biocontrol agent (BCA), making them unavailable for infection by the fungal pathogen is one of the effective mechanisms of action by the BCAs. The biocontrol potential of Talaromyces flavus against Verticillium albo-atrum, causing Verticillium wilt disease of potato was assessed. T. flavus was applied as tuber treatment and soil application and the treatments were randomized in complete block design with four replications. The isolate Tt-Po-V52 was the most effective in suppressing disease development, when applied as tuber treatment with minimum infection index. Under field conditions, the BCA-treated plots had an infection index of 0.15 as against 3.5 in the untreated control plots. The results indicated the effectiveness of T. flavus for reducing the incidence and severity of Verticillium wilt disease in potato (Naraghi et al. 2010).

Sclerotinia sclerotiorum with wide host range, produces sclerotia externally on affected plant parts and also internally in the stem pith cavities. Sclerotia from infected plant tissues reach the soil, where they overwinter and produce apothecia or mycelium during the next season. Pathogens of this nature have to be managed by biological destruction at sites, where the inoculum for new infections is likely to be produced by establishing the biological control agents at the source of inoculum production. Sclerotia of S. sclerotiorum could be killed on root surface, inside roots and stems of sunflower plants by applying Coniothyrium minitans could be attributed to prevention of infection by S. minor. It was suggested that for effective management of lettuce drop disease, application of Contans to reduce
sclerotial inoculum in soil and fungicide Endura to prevent new infection by S. minor might be a practical strategy to be adopted (Chitrampalam et al. 2011).

The effects of a multicomponent treatment, consisting of oilseed rape seedcake fertilizer (amendment) and rice straw component augmented with Trichoderma sp. Tri-1 (Tri-1) on the development of Sclerotinia stem rot in oilseed rape and yield were assessed, in comparison with fungicide, carbendazim. Oilseed rape seed yield was significantly increased by the multicomponent treatment, compared with the fungicide in the fields infested with Sclerotinia sclerotiorum. Disease incidence in the field trials with residual rice straw + formulated Tri-1 (in oilseed rape seedcake) was significantly lower than that of untreated plots, suggesting that the increase in oilseed rape seed yield was at least in part was associated with biological control of the pathogen (see Table 3.1). Disease suppression was more effective than that provided by control + fungicide spray treatment. Field experiments with sclerotia applied to soil in mesh bags, allowing both recovery of sclerotia and the colonization of sclerotia by Tri-1 and the indigenous microflora showed that inhibition of sclerotial germination by straw + formulated Tri-1 treatment was greater than the untreated control at 90, 120 and 150 days, but the difference between treatments was not statistically significant. The persistence of Tri-1 in bare soil, on oilseed rape and in the presence of rice straw was determined in the greenhouse pot experiment. Populations of hygromycin-resistant Tri-1-like fungi were high in the control straw treatment, but significantly lower than that was found in the straw + Tri-1 treatment. Oilseed rape appeared to support populations of Tri-1-like fungi, although not to the same extent as the rice straw. Populations of hygromycin-resistant Tri-1-like fungi declined very rapidly in the soil. Only straw + Tri-1 treatment, had population below 10^2 CFU/g of soil, after 30 days. The results indicated that the multicomponent treatment had the potential to reduce the incidence of Sclerotinia stem rot and also increase seed yield of oilseed rape crops (Hu et al. 2015).

Among the Trichoderma spp. isolated from rhizosphere of various crops, T. asperellum (NVTA2) applied as talc formulation for root dipping and soil treatment reduced carnation stem rot disease caused by Sclerotinia sclerotiorum incidence by 11.8%, compared to control. In addition, treatment with NVTA2 strain, stimulated growth of plants and increased the flower yield (Vinodkumar et al. 2017).

The efficacy of three strains of Trichoderma viride (L4, S17A, 99-27) in suppressing the development of onion white rot disease caused by Sclerotium cepivorum was assessed in a three-stage screening system to degrade sclerotia of the pathogen on agar medium and in soil and to reduce white rot disease incidence on onion seedlings. The strains L4 and S17A were tested under field conditions, because of the greater efficacy shown in the laboratory and greenhouse assessments. These strains of T. viride were consistent in their antagonistic activity by reducing white rot symptoms, when they were used for seed treatment in 2000 and 2001. The BCA application was equally effective as the fungicide tebuconazole which induced phytotoxic symptoms. Application of T. viride strains L4 and S17A could be preferred because of the precise placement of BCA using a special drilling equipment and this requirement might limit the BCA application (Clarkson et al. 2001). In a later investigation, the effects of combination of onion waste compost (OWC) and spent mushroom compost (SMC) and Trichoderma viride S17A on viability of sclerotia of Sclerotium cepivorum were assessed under glasshouse and field conditions. Incorporation of OWC into the soil reduced the viability of sclerotia and also the incidence of the disease. In two field trials, OWC reduced sclerotial viability and also reduced white rot disease incidence, as effectively as the fungicide tebuconazole (Folicur). Addition of T. viride S17A to SMC facilitated proliferation of T. viride S17A in the soil and increased the healthy onion bulb yield. The results indicated that infection of onion plants from sclerotia could be prevented by amendment of soil with OWC, SMC or T. viride S17A (Coventry et al. 2006).

Phytophthora ramorum and P. pini cause the destructive sudden oak death (SOD) disease, which killed over a million oak trees in the California and Oregon States in the United States. Trichoderma asperellum was evaluated for its potential of suppressing the pathogen development in the container nursery beds. The effectiveness of soil solarization for eradicating P. ramorum in the surface soil of nursery beds and its effect on the establishment of T. asperellum in the solarized soil were assessed. In the field trials, the leaf inoculum of P. ramorum was buried at 5, 15 and 30 cm below the soil surface. Solarization for 2- or 4-weeks during summer of 2012, eliminated the inoculum buried at all depths in one trial, at 5 and 15 cm in another trial conducted in California, but only at 5 cm in Oregon trial. T. asperellum was applied in the solarized soil. BCA application did not result in reduction in pathogen recovery. Population of densities of the introduced T. asperellum at 5cm depth, often increased by two- to fourfold in solarized soil, compared to nonsolarized plots and the effect

**TABLE 3.1**

Extent of Suppression of Take-All and Rhizoctonia Root Rot Diseases of Wheat by Different Mutants and Wild-Type Strain of *Pseudomonas fluorescens* HC1-07 (Yang et al. 2014)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Take-all Pasteurized</th>
<th>Raw</th>
<th>Rhizoctonia root rot Pasteurized</th>
<th>Raw</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC1-07 nf</td>
<td>2.8d 3.2c</td>
<td>2.5c 2.4c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC1-07 vissB</td>
<td>3.5c 3.6b 2.3b 2.9b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC1-07 vissB-</td>
<td>3.1d 3.4c 2.6c 2.6c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC1-07 prnR2</td>
<td>3.9b 3.7b 2.8c 2.8bc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC1-07 prnR2-1</td>
<td>3.0d 3.3c 2.6c 2.5bc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control + pathogen</td>
<td>4.4a 3.9a 4.3a 4.3a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control + MC +pathogen</td>
<td>4.1ab 4.1a 4.1a 4.2a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

x- nontreated; control + MC (seed treated with methyl cellulose).
y- disease rating scale: 0 to 8; means followed by the same letter are not significantly different at P = 0.05 according to the Kruskal-Wallis all-pairwise comparison test.
of enhanced BCA population was not significantly reflected on pathogen recovery from the soil. The results suggested that soil solarization might be employed to disinfest the upper layers of soil in container nurseries in some locations, where suitable environmental conditions prevail (Funhashi and Parke 2016).

Verticillium dahliae, causal agent of strawberry Verticillium wilt disease, has a wide host range that includes several crops. As the use of fumigants was restricted and reliable sources of resistance to Verticillium wilt pathogen were not available, the need for finding out alternate strategies for restricting wilt disease was realized. The biocontrol potential of nonpathogenic strains of Verticillium dahliae was assessed by infesting the soil with test strains for two years. Application of nonpathogenic strains had positive effect on 20% of plants, whereas 30% of plants remained unaffected. But 50 to 60% of plants were impacted negatively, as they showed severe wilting symptoms, resulting in total loss. The results indicated that other factors such as use of certified plant materials, and presence of other pathogens infecting strawberry have to be considered for enhancing effectiveness of nonpathogenic strains of V. dahliae as biological control agent to protect the strawberry crops (Diehl et al. 2013).

An atoxigenic strain of Aspergillus flavus, that did not produce aflatoxin, was evaluated for its biocontrol potential against toxigenic strain of A. flavus, contaminating corn grains and cotton seeds. Atoxigenic strains could reduce aflatoxin contamination by displacing or excluding aflatoxin-producing strains of A. flavus. The strain AF36 has been applied as a BCA in commercial corn and cotton production in Texas and Arizona to reduce aflatoxin contamination. The efficiency of the strain AF36 in reducing adverse effects of A. flavus in pistachio orchards were assessed during 2008–2011. AF36 strain was applied as hyphae-colonized steam-sterilized wheat seed. In all pistachio orchards, application of the wheat-AF36 product substantially increased the proportion of vegetative compatibility group (VCG) YV36, the VCG group associated with biofumigation activity was investigated, for suppressing the development of common bunt (CB) disease of wheat caused by Tilletia caries by applying as a seed treatment or an in-furrow soil treatment. Dry rye grain culture of M. albus was ground into powder and applied as seed treatment at 125 mg/g seed to wheat seed infected with T. caries teliospores. For soil treatment, the culture was broken into small particles and applied in the furrow at 48 mg/m of row, along with teliospores-infested seed during planting. Treatments were evaluated during two growing seasons and two planting dates beginning in early spring, when soil temperatures were optimal for disease development (5–10°C) and then about 3 weeks later. In the first year, treatments in the first seeding date reduced common bunt (CB) from 44% diseased spikes in untreated controls to 12% and 9% in seed and in-furrow treatments, respectively, and from 6% in controls to 0% in both treatments in the second seeding date. In the second year, CB was reduced from 8% in controls to 0.5% and 0.25% for seed and in-furrow treatments in the second seeding date. The usefulness of M. albus application might be greater for organic wheat production, where it is obligatory to apply nonchemical methods (Goates and Mercier 2011). The higher fungi, Ascomycetes and Basidiomycetes have been underexplored for the presence of antimicrobial compounds effective against plant pathogens, particularly bacterial pathogens. Several proteins have been isolated from mushrooms and used in medicine. Protein extracts (150) from 94 different basidiomycete- and ascomycete-wild mushroom species were evaluated for antibacterial activity against Ralstonia solanacearum, which infects a wide range of plants, including several crops using microtiter plate assays. The mushroom extracts varied in their effectiveness in suppressing the development of R. solanacearum from partial inhibition to complete inhibition. Three extracts reduced disease progress and severity in artificially inoculated tomato and potato plants. The in vitro inhibitory activities of the extracts did not always correlate with in vivo disease suppression. The extracts from Amanita phalloides and Clitocybe geotropa showed that the active substances were proteins (180 kDa size). The results indicated the possibility of identifying effective biocontrol agents in higher fungi, which have not received attention of researchers (Erjavec et al. 2016).

3.1.2 Mycorrhizal Biological Control Agents

Mycorrhizal fungi have symbiotic associations with roots of several plant species and such associations are common benefitting both partners. Several plant species have coevolved
Management of Crop Diseases Caused by Soilborne Microbial Plant Pathogens

with these symbionts, indicating their dependence on the association with mycorrhizae. Plants provide the fungi with photosynthates and they, in turn, obtain mineral nutrients from mycorrhizal fungi. In contrast, pathogenic fungi proliferate at the expense of host plants which ultimately succumb to the damages inflicted by the fungal pathogens. However, in some cases, the mycorrhizal association might be less mutualistic or even parasitic to the plants (Klironomos 2003). Two major types of mycorrhizal associations have been differentiated: (i) arbuscular mycorrhizal (AM) symbiosis and (ii) ectomycorrhizal (ECM) symbiosis. The AM symbiosis is formed between the roots of the higher plants and zygomycete fungi belonging to the order Glomales. The AM fungi are obligate biotrophs that depend on their ability to colonize suitable host plant species for completion of their life cycle. The mycelia of AM fungi have an extraradical phase that grows out into the soil and an intraradical phase that proliferates inside the root. These two phases grow in very different environments and distinctly differ in their morphology and physiology (Dodd et al. 2000). AM fungi transfer organic nutrients and water to plants and obtain carbohydrates in exchange. The role of AM fungi in providing protection to the plants against microbial plant pathogens is an important contribution of these fungi for the survival of the host plants. Ectomycorrhizal (ECM) symbiosis is between fungi belonging to Basidiomycetes and Ascomycetes and plants belonging to the taxons of Gymnosperms and Angiosperms. Large trees, shrubs and sometimes herbs develop ECM symbiosis. ECM fungi often form an extensive, fanlike network of hyphal structures that grow from ECM root. The ECM fungi can be grown in pure cultures. These fungi, while colonizing plant roots, form a fungal sheath known as mantle, which covers the rootlets. The hyphae can penetrate between root cells and form a network of intercellular hyphae, which do not penetrate into plant cells. Extraradical mycelium produced from the mantle spreads on the soils and also absorbs nutrients from the soil. The extraradical hyphae get aggregated, forming a rootlike structure called rhizomorphs. Hyphae of the mantle form the Hartig net behind the root cap cells. The Hartig net forms the interface between the plant and ECM fungus and is involved in the bidirectional nutrient transfer. The ECM mycelium forms a physical barrier, preventing the entry of root pathogens (Campbell 1989).

3.1.2.1 Assessment of Biological Control Potential

The biocontrol potential of various species and strains of the mycorrhizal biocontrol agents (BCAs) has been assessed under in vitro, greenhouse/growth chamber and/or field conditions. In vitro experiments have been used to select the most effective BCAs from a large number of isolates of species/strains in suppressing the development of the target pathogen(s) and to study the mechanisms of biocontrol actions of the selected BCAs. Greenhouse/growth chamber investigations have been useful to investigate the biocontrol potential under different environmental and growth conditions and to forward the ones for testing for their efficacy under natural field conditions.

3.1.2.1.1 Laboratory Tests

Biochemical changes, following colonization of roots by arbuscular mycorrhizal fungi (AMFs) and fungal pathogens were determined. Polyacrylamide gel electrophoresis (PAGE) technique was employed to assess the protective effects of Glomus mosseae (Gm) and G. intraradices (Gi) against Phytophthora parasitica, causing tomato root rot disease. P. parasitica was inoculated on non-mycorrhizal and mycorrhizal tomato plants precolonized for four weeks with either AMF species. In non-mycorrhizal plants two acidic β-1,3-glucanase isoforms were constitutively expressed and their activity was higher in mycorrhizal roots. Two additional acidic forms were detected in extracts from Gm-colonized tomato roots, but not in Gi-colonized roots. Roots infected by P. parasitica exhibited greater enzyme activities. However, P. parasitica did not induce the isoforms related to Gm colonization. In tomato plants colonized by Gm, infection by P. parasitica induced two additional basic isoforms that could be easily recognized. The results indicated that differences in the expression of isoforms of β-1,3-glucanases in AMF-colonized and non-mycorrhizal plants following infection by P. parasitica might have a role in development of root rot disease in tomato (Pozo et al. 1999).

The molecular basis of the bioprotective effect of Glomus mosseae (Gm) on tomato root infection by Phytophthora nicotianae var. parasitica (Pnp) was investigated, using immunoenzyme labeling technique on whole root segments. Infection intensity by the pathogen was lower in mycorrhizal roots. Immunogold labeling of Pnp in cross-section of infected tomato roots showed that inter- or intracellular hyphae developed mainly in the cortex and their presence induced necrosis of host cells. The cell walls and the contents showed strong autofluorescence in reaction to the pathogen. The hyphae of Gm and Pnp were present in most cases in different root regions and sometimes in the same root tissues. The number of Pnp hyphae, growing in the root cortex, was greatly reduced in mycorrhizal root systems. In mycorrhizal tissues infected by the pathogen, arbuscule-containing cells surrounded by intercellular Pnp hyphae did not show any necrosis. These host cells showed only weak autofluorescence (Cordier et al. 1998). The role of hydrogen peroxide (H₂O₂) in disease development was studied. It is difficult to study the mechanism of H₂O₂ generation and relieving its stress in intact plant. The role of mycorrhizal inoculation in chilli plants challenged with Phytophthora capsici was investigated to study the effect on hypersensitive response. In the control (without mycorrhizal BCA) T3 and with mycorrhizal BCA (T4), visible disorders were detected at two days after inoculation with P. capsici, but in the following days, T3 plants developed 25% more necrotic lesions on the leaves than plants in T4 treatment. Leaf necrosis correlated with H₂O₂ accumulation and the greater damage observed in T3 plants coincided with larger accumulation of H₂O₂, at 12 h after inoculation, accompanied by an increase in peroxidase (POX) and superoxide dismutase (SOD) activity. The T4-infected and mycorrhizal plants exhibited an earlier accumulation of H₂O₂ starting...
Arbuscular mycorrhizal fungi (AMF) have the ability to suppress the development of soilborne pathogens like *F. oxysporum* f.sp. *lycopersici* (*Fol*), incitant of tomato *Fusarium* wilt disease. The dynamics of root exudation of tomato in any intercropping system due to the AM fungus, *Glomus mossae* (*Gm*) and/or *Fol*, its effect on *Fol* development in vitro and effects of compounds identified in the root exudates were investigated. Gas chromatography-mass spectrometry (GC-MS) analyses showed an AMF-dependent increase in sugars and decrease in organic acids, mainly glucose and malate. In high-performance liquid chromatography (HPLC) analyses, an increase in chlorogenic acid was observed in the combined treatment of AMF and *Fol*, indicating the ability of AMF and *Fol* to induce changes in the composition of root exudates of tomato. Root exudates of AMF-colonized tomato roots stimulated spore germination rate of *Fol*, whereas coinoculation with AMF and *Fol* resulted in reduction in spore germination rate. In vitro assays revealed that citrate and chlorogenic acid in the root exudates were likely to be responsible for reduced spore germination rate in the combined AMF + *Fol* treatment, since these compounds occurring at the concentration naturally in the root exudates were inhibitory to *F. oxysporum* f.sp. *lycopersici*, infecting tomato plants (Hage-Ahmed et al. 2013).

Following infection by *F. oxysporum*, plants respond innately via a complex and integrated set of defenses, encompassing both constitutive and induced responses. They include production of signaling compounds such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and abscisic acid (ABA) or reactive oxygen species (ROS). Changes in the concentration of the plant hormones in melon shoots, as a consequence of interaction between the plant, *F. oxysporum*, the antagonistic *T. harzianum* and the AMF *Glomus intraradices* were studied. Infection by *F. oxysporum* activated a defense response in melon plants, mediated by SA, JA, ET and ABA, similar to the one induced by *T. harzianum*.

When inoculated with the pathogen, both *T. harzianum* and *G. intraradices* attenuated the plant response mediated by the hormones ABA and ET, elicited by pathogen infection. *T. harzianum* was also able to attenuate the SA mediated response. In the three-way interaction (pathogen-antagonist-AMF), a synergistic effect on the modulation of the hormone disruption induced by the pathogen, was observed. The results suggested that induction of plant basal defense response and attenuation of hormonal disruption caused by *F. oxysporum* could be both mechanisms by which *T. harzianum* might control *Fusarium* wilt in melon plants, whereas the mode of action of *G. intraradices* appeared to be independent of SA and JA signaling (Martínez-Medina et al. 2010).

Systems for investigating the interactions among plants, arbuscular mycorrhizal fungus (AMF) and fungal pathogens under in vitro conditions offer advantages over pot culture experiments. Avoidance of unwanted contaminants and the possibility of highly controlled and nondestructive dynamic observations on the interactions are the main advantages. An in vitro system was developed for relating infection of premycorrhized soybean plantlets by *Fusarium virguliforme*, causing sudden death syndrome (SDS) in soybean and for monitoring the early step of *F. virguliforme* infection process in the presence of AMF *Rhizopagus irregularis*. Plantlets premycorrhized with *R. irregularis* were inoculated with *F. virguliforme* either locally using a plug of gel supporting medium (method 1) or using macroconidial suspension of the pathogen applied on the medium surface (method 2). In method 1, the hyphae of *F. virguliforme* reached the surface of the roots at 2 days after inoculation (dai), independently of the presence/absence of AMF associated with soybean plantlets. After contact with roots, the pathogen developed profusely and formed a dense network of hyphae during the following 48 h. *F. virguliforme* grew both inter- and intracellularly within the roots. Many hyphae were observed in the root tip zone also. Fungal structures with a swollen and pigmented cell could be visualized under the light microscope (see Figure 3.4). Faster and denser hyphal development occurred, when conidial suspension was used as inoculum. The root system was infected by the pathogen, regardless of the method of inoculation. Small necrotic

![Figure 3.4 Biocontrol activity of *Rhizopagus irregularis* against *Fusarium virguliforme* determined using premycorrhized soybean plantlets](image-url)

[A courtesy of Giachero et al. 2017 and with kind permission of Frontiers in Plant Science Open Access Journal]
areas of 1–3 mm were produced near the point of penetration by the pathogen. The results showed that in the presence of AMF, the intensity of symptoms was reduced and root infection level was also less, compared with the control. The in vitro cultivation system showed the potential for investigating interactions involving AMF, pathogen and plant root system (Giachero et al. 2017).

3.1.2.1.2 Greenhouse Tests

The effects of AM fungi Glomus fasciculatum and G. eutunicatum on the growth of three strawberry cultivars and root infection by Phytophthora fragariae were investigated. Root necrosis induced by the pathogen was reduced in cv. Cambridge Favourite and Elsanta in mycorrhizal plants by about 60% and 30%, respectively, compared with non-mycorrhizal plants. No significant reduction in infection by P. fragariae in mycorrhizal plants of the least susceptible cv. Rhapsody was observed (Norman et al. 1996). In another investigation, non-mycorrhizal tomato plants showed widespread root necrosis induced by Phytophthora nicotianae var. parasitica, as well as significant reduction in growth parameters. In contrast, Glomus mosseae-colonized tomato plants showed less infection and reduction in growth parameters. Reduction in necrosis of adventitious roots, ranging from 63 to 89% was recorded in AMF-colonized tomato plants, compared with non-mycorrhizal plants (Trotta et al. 1996). In a later investigation, tomato plants colonized by Glomus mosseae were inoculated with zoospores of P. nicotianae var. parasitica with nonmycorrhizal inoculated control plants. At harvest, the control plants showed extensive root necrosis, reaching 61% infection, whereas infection was limited to 31% in mycorrhizal plants with increase in root system by 50%, indicating the additional advantage through AMF association (Vigo et al. 2000). The effects of association of asparagus with three AM fungal species were assessed under glasshouse conditions. Incidence of Fusarium wilt disease caused by F. oxysporum f.sp. asparagi was reduced significantly in AMF colonized asparagus plants, indicating enhancement of tolerance to Fusarium wilt disease. No significant differences could be detected in phosphorus contents of plants due to association with any of the AM fungi, Gigaspora margarita, Glomus fasciculatum and Glomus sp. R10 tested (Matsubara et al. 2001).

Biological control potential of Glomus intraradices in suppressing the development of Fusarium wilt disease caused by Fusarium solani f.sp. phaseoli (Fsp) in bean plants and pathogen population in rhizosphere soil was assessed, using a compartmentalized experimental system. Specific primers in real-time PCR assay, culture-dependent methods and microscopic determination procedures were applied to quantify the pathogen and the AMF in the plant tissues, soil regions of mycorrhizosphere (rhizosphere + mycosphere) and the bulk soil. Nonmycorrhizal bean plants infected by Fsp exhibited characteristic disease symptoms, whereas infected plants and colonized earlier by G. intraradices appeared normal. The content of genomic DNA of Fsp was significantly reduced in mycorrhizal bean plants and in mycorrhizosphere of soil compartment. Presence of G. intraradices in the mycorrhizosphere was not significantly modified, although the mycorrhizal colonization of roots was slightly increased in the presence of the pathogen. Reduction in pathogen population, as reflected by DNA contents and the root symptom might be due to biotic and/or abiotic modification of the mycorrhizosphere, as a result of colonization with G. intraradices (Fillon et al. 2003). The effects of AMF Glomus intraradices and G. claroideum on the pea root rot disease, caused by Aphanomyces euteiches were investigated under greenhouse pot culture conditions, over the duration of three harvests, using oospores as inoculum. Incidence of root rot disease was reduced in AMF-colonized plants, the beneficial effect being provided more effectively by G. intraradices than by G. claroideum. At the final harvest, percentage of root length with oospores did not show variation between treatments. The mycorrhizal plants suffered less in terms of shoot growth and disease severity, compared to nonmycorrhizal plants. The degree of induction of tolerance varied, depending on the AMF species (Thygesen et al. 2004).

The AM fungi Glomus mosseeae, G. eutunicatum, G. fasciculatum and Gigaspora margarita were evaluated for their ability to suppress the development of Phytophthora blight in pepper seedlings, caused by Phytophthora capsici and to promote the growth of pepper plants. Among the AM fungi tested, G. mosseeae was the most effective in reducing the disease severity due to P. capsici by 91.7, 43.0 and 57.2% under pot, greenhouse and field conditions, respectively. The phytoalexin, capsidol concentration was increased in pepper plants preinoculated with P. capsici. The results showed that the AM fungus could improve the plant growth, in addition to suppression of Phytophthora blight disease in pepper plants (Ozgonen and Erkiliç 2007). Plant growth depression in tomato + AM fungi is known in certain plant-AMF combinations. The AM fungi Glomus intraradices, G. mosseeae and G. claroideum were evaluated for their efficiency in enhancing tolerance of tomato plants to Pythium root rot caused by Pythium aphanidermatum by employing fully factorial experiment. Two weeks after inoculation of tomato seedlings with AM fungi, roots were challenged with P. aphanidermatum. All AMF species caused significant growth suppression, but did not affect PR-1 gene expression or the phosphorus concentration in tomato plants. G. intraradices only reduced root infection by P. capsici, measured by ELISA and pathogen could be isolated by selective media at harvest time. Likewise, root infection by P. aphanidermatum reduced levels of colonization by G. intraradices, but not by the other two species of AM fungi. Although adverse effect on plant growth was known, the beneficial effect of AMF may be considered as an advantage of employing AM fungi for protecting tomato plants (Larsen et al. 2012).

The AM fungi Glomus mosseeae, G. monosporum, G. deserticola, G. intraradices and two other unidentified species were evaluated for their efficacy in reducing the adverse effects of tomato wilt pathogen F. oxysporum f.sp. lycopersici (Fol) under greenhouse conditions. G. mosseeae and G. monosporum improved the plant growth parameters such as
plant height and fruit yield. In mycorrhizal plants challenged with Fol, root infection by the pathogen was significantly reduced, compared with nonmycorrhizal plants inoculated with Fol (Utkhade 2006). The combined effects of Glomus mosseae BEG12 and the bacterial biological control agent Pseudomonas fluorescens A6R1 on the development of root rot disease of tomato caused by Rhizoctonia solani 1556 were assessed under greenhouse conditions. The root and shoot growth of tomato plants infected by R. solani were compared, when protected or not by the BCAs. Microscopical examination of epiphytic and parasitic growth of the pathogen was studied in the presence and absence of the BCAs, allowing the quantification of roots with hyphae appressed to epidermal cells (epiphytic growth) and of roots with intraradical infection (parasitic growth). G. mosseae BEG12 and P. fluorescens A6R1 could fully overcome the growth depression of tomato caused by R. solani 1556. The suppression of disease development was associated with a significant decrease of the epiphytic and parasitic growth of the pathogen, along with increase in root length and number of root tips of inoculated tomato plants (Berta et al. 2005).

The comparative biocontrol potential of Glomus mosseae, T. harzianum and Pseudomonas fluorescens in suppressing the development of banana Panama (Fusarium) wilt disease caused by F. oxysporum fsp. cubense (Foc) was assessed by inoculating in single, dual and tripartite combinations. The BCAs were allowed to colonize banana plants at 0, 45 and 90 days prior to challenge inoculation with the pathogen (1.5 × 10^6 CFU/g). Appropriate control treatments were maintained. ELISA tests were performed to determine the populations of Foc every month. Banana plants preinoculated with G. mosseae + T. harzianum and challenged with Foc could sustain 61% and 70% improvement in plant height and girth respectively and 75% in bunch weight over plants with pathogen inoculation alone. These controls finally succumbed to the disease. Pathogen populations as determined by ELISA tests, were reduced to 0.58 OD in seven months in G. mosseae + T. harzianum treatments, compared to the level of 1.90 OD in control plants. The results suggested that the protection to banana by the BCAs might be due to physical modification in the cell wall, growth promotion and through induction of resistance to the Panama wilt disease (Mohandas et al. 2010). Isolates of T. harzianum and fluorescent Pseudomonas spp. were evaluated for their biocontrol potential for suppressing the development of tomato wilt disease caused by F. oxysporum fsp. lycopersici. Application of T. harzianum and Pseudomonas sp. increased seed germination by 22 to 48%. All BCAs reduced the incidence of wilt disease in pot and field trials and combinations of BCAs were more effective than application of single BCA. Combination of Pseudomonas, T. harzianum and Glomus intraradices protected the plants more effectively, compared to unprotected control plants. Disease incidence and severity were reduced by 74% and 67% respectively in pots and field experiments. The combination treatments increased the yield also by 20%. Further, combinations of all three BCAs with cowdung compost (CDC) reduced the disease incidence by 81% and 74% in pot and field experiments respectively and increased the yield by 33% (Srivastava et al. 2010).

3.1.2.1.3 Field Tests

Commercial formulations containing vesicular arbuscular mycorrhiza (VAM) Glomus intraradices were assessed for its effectiveness in suppressing Allium (onion) white rot (WR) in organic soils in comparison with the fungicide Folicur 3.6 F (430 g a.i./l, tebuconazole) under field conditions during 2000-2001. The AM product MIKRO-VAM used in transplanted onions reduced the incidence of white rot by about 50%, compared with untreated control and the extent of disease reduction was comparable to the fungicide Folicur 3.6F. Onion cultivar Hoopla was more susceptible than cv. Fortress to white rot disease in 10 of 13 field trials. A significant negative correlation between disease incidence and AM root colonization was recorded, indicating the effectiveness of AM root colonization in suppressing development of onion white rot disease (Jaime et al. 2005). The effects of treatment of soybean seeds with mefenoxam, fludioxonil, mefenoxam + fludioxonil, were assessed, maintaining control without seed treatment. Soil fumigation with a mixture of 1,3-dichloropropene and chloropicrin was used as a base to determine the direct effect of the fungicide on plant growth and yield parameters. A significant fumigation x seed treatment interaction was indicated in 2005. Seed treatment with fludioxonil supported AM colonization on nonfumigated soil, where fludioxonil-treated plants had double the root colonization, as in the control and five times root colonization than in plants treated with mefenoxam. In the fumigated soil, plants treated with mefenoxam alone, or in combination with fludioxonil had lower AM colonization than the control and fludioxonil-treated plants. Fumigation did not significantly reduce mycorrhizal colonization across locations. No differences in grain yield, final stand or grain composition were recognized among seed-treated fungicides or between nonfumigated and fumigated soil. With the exception of mefenoxam in fumigated soil in 2005, no evidence of reduction in mycorrhizal colonization of soybean roots due to seed treatment with fungicide could be obtained under field conditions (Murillo-Williams and Pedersen 2008).

3.1.3 BACTERIAL BIOLOGICAL CONTROL AGENTS

Bacterial species existing in various substrates such as seeds, propagules, plants, soil and water have been shown to possess antagonistic properties against soilborne microbial plant pathogens, resulting in suppression of disease development. The bacterial species are screened in vitro to eliminate less effective ones and the more efficient isolates are advanced to further testing in the greenhouse/growth chamber, followed by field testing under natural/ inoculated conditions, where the putative biocontrol agents have to adapt to variations in environmental conditions and to compete with other microorganisms present on the plant surface and soil. The in vitro assessments are useful to demonstrate the ability of the bacterial species/strain to secrete enzymes, toxic metabolites or
siderophores, hormones involved in pathogen suppression and plant growth promotion. Caution has to be exercised in not eliminating the species/isolates that are not inhibitory directly to the target pathogen(s). Such species/isolates may act indirectly by stimulating innate defense systems of the host plant.

3.1.3.1 Assessment of Biological Control Potential of Bacterial Isolates

Various kinds of assessment methods have been applied to determine the biocontrol activities of the bacterial species/isolates against microbial pathogens.

3.1.3.1.1 Laboratory Methods

The inhibitory (antagonistic) activities of the bacterial biocontrol agents (BCAs) against fungal/bacterial pathogens are demonstrated by measuring the extent of inhibition of spore germination and mycelial growth of fungal pathogens, whereas the colony development/morphology of bacterial pathogens as affected by the bacterial BCAs are determined. The conventional dual culture or confrontation assay is performed in petriplates containing potato dextrose agar (PDA) or other media, favoring the differential development of the pathogen and the test bacterial isolates. The bacterial isolates are streaked on the medium at 2 cm from the periphery of the plate. Agar plates containing the mycelium of the fungal pathogens are placed at the rate of one plug/plate at the center of the plate and incubated at room temperature for 5–7 days, depending on the rate of growth of the fungal pathogen. An inhibition zone is formed between the bacteria and the fungus, if the bacterial isolate has antagonistic properties. The percentage of inhibition is calculated. The effectiveness of the fungus, if the bacterial isolate has antagonistic properties.

The conventional dual culture or confrontation assay is performed in petriplates containing potato dextrose agar (PDA) or other media, favoring the differential development of the pathogen and the test bacterial isolates. The percentage of inhibition is calculated. The effectiveness of the fungus, if the bacterial isolate has antagonistic properties.

The percentage of inhibition is calculated. The effectiveness of the fungus, if the bacterial isolate has antagonistic properties. The antagonistic activity of Myxococcus coralloides, M. flavescens, M. flavus and four other species entirely inhibited the mycelial growth of Sclerotinia sclerotiorum, Pythium ultimum, Rhizoctonia spp. and Phytophthora capsici, whereas Verticillium dahliae and V. albo-atrum, Cylindrocarpon spp. and F. oxysporum f.sp. api were less sensitive to inhibition by the myxobacteria (Bull et al. 2002).

Different variants of dual culture method have been developed to assess the antagonistic properties of bacterial biocontrol agents. The endorhizosphere bacteria obtained from root tips of tomato were screened for their antagonistic activity against Verticillium dahliae, causal agent of Verticillium wilt disease. Selected bacterial isolates (53 of 435) were antagonistic to V. dahliae and several other soilborne pathogens in dual cultures. Three efficient antagonistic isolates were identified as Bacillus sp. Two of the most effective bacterial isolates designated K-165 and 5-127 were rhizosphere colonizers and very efficient in inhibiting mycelial growth of V. dahliae and successfully suppressed the development of Verticillium wilt of solanaceous hosts (Tjamos et al. 2004). A variant of dual culture method was applied to select Rhizobium isolates effective against F. oxysporum f.sp. ciceris race O, infecting chickpea. The bacterial isolates were streaked across the petriplates at the center and the second streak was made at right angles to the first streak. Discs (5 mm diameter) of the mycelium of a 7-day old culture were placed at each side of the bacterial streak maintaining a distance of 2.5 cm between the reactants. Percent inhibition was calculated after an incubation period of 7 days (Arfaoui et al. 2006).

The biocontrol potential of Bacillus strains (400) isolated from roots of cucumber plants grown in the greenhouses and fields was assessed against F. oxysporum f.sp. ciceris, causing cucumber wilt disease. The strain BO68150 was the most effective in suppressing the pathogen development under laboratory and greenhouse conditions. At seedling stage, the biocontrol efficiency was 50.68%. The results indicated that the mechanism of action of the strain BO68150 was likely to be other than antagonism against F. oxysporum f.sp. cucumerinum (Li et al. 2012).

Paenibacillus polymyxa strains (25) isolated from rotten ginseng roots were screened for their antifungal activity against Phytophthora capsici in vitro. The strain GBR-462 was the most efficient in suppressing the development of the pathogen. Antimicrobial activity was influenced by the initial inoculum density. No inhibitory activity was observed on mycelial growth and zoospore germination of the pathogen, when tested at lower inoculum density of 10^6 CFU/ml of P. polymyxa GBR-462. However, sporangium formation and zoospore release were significantly inhibited at the lower inoculum density. Furthermore, light and electron microscopic observations showed aberrant sporangia with no or few nuclei, indicating that the sporangium and zoospore formation were inhibited even at lower inoculum density. Application of P. polymyxa GBR-462 into potted soil suppressed the disease development and severity by 30%, compared with untreated control (Kim and Knudsen 2009). Isolates of Bacillus amyloliquefaciens, B. licheniformis, Paenibacillus pabuli, B. atrophaeus, B. subtilis, B. pumilus and B. endophytycus were evaluated for their antagonistic activities against Phytophthora parasitica var. nicotianae, using microbioassay. The assay involved the use of tobacco seedlings grown in petriplates for quantification of initial zoospore inocula and high throughput screening of antagonistic bacterial isolates. Zoospore inocula (10^2–10^3 spores/petri dish) were applied on 14 days old tobacco seedlings for susceptibility test. The optimum inoculum for infection of tobacco seedlings was 10,000 spores. Fifteen isolates of the above species were tested and four of them exhibited 100% protective activity in planta, as in petridishes. The microbioassay was rapid, reproducible and efficient for screening the bacterial isolates for their potential to protect tobacco seedlings under hydroponic conditions in petridishes (Wang et al. 2012).

The antagonistic activity of Paenibacillus polymyxa strains against Phytophthora palmivora and Pythium aphanidermatum was observed, using agar plates, liquid media and soil. P. polymyxa reduced colonization of pathogens in liquid assays. Most plants treated with P. polymyxa survived and infection by P. aphanidermatum was well correlated with mycocidal substance production by the BCA (Timmusk et al. 2009). Isolates of lactic acid bacteria (LAB) were evaluated for their efficacy against Pythium ultimum infecting tomato. The LAB isolates (294) obtained from soils and rhizospheres of maize, rye, carrot, garden soil and compost were tested by...
confrontation assay. About 75% of the isolates showed inhibitory effect on mycelial growth of *P. ultimum*. The most promising isolates protected tomato plants in the pot culture also, indicating their potential for application against *P. ultimum* (Lutz et al. 2012). *Paenibacillus elgii* HOA73 was isolated from soil samples and its identity was established, based on the 16S rRNA gene sequence. *P. elgii* was antagonistic to *F. oxysporum* f.sp. *lycopersici* and other plant pathogens. The bacterial culture filtrate (CF) was highly effective in inhibiting the mycelial growth (by 86.1%) at 50% concentration. The bacterial crude extract was able to inhibit pathogen growth by 72.5%. An antifungal compound was purified from bacterial crude extract, using different chromatographic techniques and it was identified as butyl-2,3-dihydroxybenzoate (B2,3DB). This compound displayed potent antifungal properties and inhibited pathogen growth by 83.2%, when applied at 0.6 mg. The minimum concentration of B2,3DB that inhibited any visible growth of *F. oxysporum* f.sp. *lycopersici* was 32 µg/ml, and the conidial germination of the pathogen was also inhibited at the same concentration of B2,3DB produced by *P. elgii*. The results showed the potential of *P. elgii*, as a biological control agent that could be applied against tomato wilt disease (Nguyen et al. 2015).

The comparative efficacy of the bacterial BCAs *Bacillus subtilis* QST713 and Streptomyces hyicus WYECl08 and fungal BCAs *Coniothyrium minitans* CON/M/91-08 and *T. harzianum* T-22 in reducing the survival of sclerotia and production of apothecia of *Sclerotinia sclerotiorum* was assessed under the growth chamber conditions. In general, the efficacy was positively correlated with the rate of application of BCAs. *B. subtilis* reduced production of apothecia and sclerotia by 81.2% and 50%, respectively. *S. hyicus* could inhibit production of apothecia completely (100%), but sclerotial production by 29.6% only. *T. harzianum* was less effective than other BCAs, in reducing apothecia production, but equally effective in reducing sclerotial production as the other BCAs. The results showed the variations in the biocontrol potential of the BCAs, indicating the need for selecting suitable BCA for application to effectively suppress the development of the pathogen and consequently incidence of the disease(s) (Zeng et al. 2012). *Bacillus amylovoricfaciens* Y1 was evaluated for its efficacy in suppressing the Fusarium wilt disease of tomato caused by *F. oxysporum* f.sp. *lycopersici* (Fol), under in vitro and greenhouse conditions. The strain Y1 was effective in inhibiting the growth of Fol and it also produced indole acetic acid (IAA), both in the presence and absence of tryptophan. The culture developed in Black White (BW) medium (Y1), BW medium amended with a commercial fungicide (BW + F) and BW medium alone were tested in the greenhouse conditions. Application of Y1 culture and BW + F resulted in significant reduction in disease incidence than in the BW. The shoot length and fresh and dry weight of both roots and shoots were greater in Y1 than in either BW or BW + F treatments. A similar trend was observed for chitinase and 1,3-glucanase activities in roots and leaves of tomato plants in Y1 culture treatment during most of the experimental duration. Presence of Y1 strain in the rhizospheric soils of Y1-treated plants resulted in significant reduction in the populations of other bacteria. The results indicated the usefulness of *B. amylovoricfaciens* Y1 strains for disease suppression and growth promotion in tomato (Maung et al. 2017).

The biocontrol potential of eight isolates of *Bacillus* obtained from rhizosphere soil samples against *Sclerotinia sclerotiorum*, incitant of white mold disease of mustard was assessed. All isolates were identified as *Bacillus amylovoricfaciens* subsp. *plantarum* (Bap), based on cultural, biochemical and molecular analyses of 16S rDNA and gyrase subunit A (gyrA). These isolates inhibited mycelial growth and suppressed formation of sclerotia in vitro. Deformities and cell wall lysis of mycelia, abnormalities of apothecia and inhibition of germination of ascospores, following interaction with *Bacillus* isolates were observed under light and scanning electron microscopes, indicating the antagonistic potential of Bap. Seed bacterization with Bap isolates provided protection to the mustard seedlings up to 98% against *S. sclerotiorum* in vitro conditions (Rahman et al. 2016). In another investigation, strains of *Bacillus* spp. (105) isolated from soils were screened for their antagonistic activities against soil-borne pathogens. The strain B14 most effectively inhibited the pathogen, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Fusarium solani* and *Mucrosephoma phaseolina*, with percentages of inhibition varying from 60 to 80. In addition, the strain B14 was able to produce siderophores and also auxins, which promoted plant growth. Based on 16S rRNA gene sequence, the strain B14 was identified as *B. amylovoricfaciens* (Sabate et al. 2017). Pink root disease caused by *Setothophora terrestres* is a major disease of onion, accounting for considerable yield losses in Argentina. *Bacillus subtilis* subsp. *subtilis* (BSS) isolated from the rhizosphere soil from onion plants was evaluated for its antagonistic activity against *S. terrestres*. The BSS strain showed strong inhibitory capacity specifically affecting pathogen growth, but it was not inhibitory to other onion pathogens *F. oxysporum* f.sp. *cepea* and *F. proliferatum*. The cell-free culture filtrate (CF) of BSS showed high growth inhibition of *Foc* in petriplates. Electron microscopy of cocultures of *S. terrestres* and BSS revealed thickened, tortuous, coiled fungal hyphae with granules and globule-like terminations. The results indicated the potential of *B. subtilis* ssp. *subtilis* as a biocontrol agent against *S. terrestres* (Orio et al. 2016).

### 3.1.3.1.2 Greenhouse Tests

The isolates of putative biological control agents that are highly antagonistic to soilborne microbial pathogen(s) under in vitro conditions are advanced to the next stage of determining their efficacy in suppressing disease development in whole plants under greenhouse/growth chamber conditions. These bioassays are useful in identifying the BCAs which may function directly by inhibiting pathogen proliferation or indirectly on the pathogen by activating host plant defense systems, resulting in enhancement of resistance to target disease(s). Take-all decline (TAD) of wheat is the resultant of natural suppressiveness of soils occurring in different geographical regions. The key role of fluorescent *Pseudomonas* spp., producing
the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG), contributing to two Dutch TAD soils was demonstrated. The 2,4-DAPG-producing fluorescent Pseudomonas spp. were present on roots of wheat plants in both the TAD soils at densities at or above the threshold density required to suppress take-all of wheat caused by Gaemannomyces graminis var. tritici (Ggt) and in a complementary take-all conducive soil, population density of 2,4-DAPG-producing Pseudomonas spp. were below the threshold level. Furthermore, introduction of 2,4-DAPG-producing strain SSB-17 into the take-all conducive soil suppressed development of take-all to the level observed in the TAD soil. Furthermore, a mutant of strain SSB-17, deficient in 2,4-DAPG production was not able to suppress the take-all development, indicating the key role of 2,4-DAPG-producing Pseudomonas spp. in TAD soils. The results indicated that the genotypic composition of 2,4-DAPG-producing Pseudomonas spp. varied between the Dutch TAD soils and the TAD soils from Washington State, although quantitatively both were similar (de Souza et al. 2003a).

Two isolates of endorhizospheric bacteria K-165 and 5-127, which most effectively suppressed the development of Verticillium dahliae, were tested in the greenhouse experiments. Root dipping or soil drenching of eggplants with bacterial suspensions (10⁷ CFU/ml) resulted in reduced severity of wilt symptoms induced by V. dahliae, compared with untreated controls, under high pathogen inoculum level (40 microscerotia/g of soil). In potato fields heavily infested with V. dahliae, seed tuber treatment with a bacterial talc formulation (10⁸ CFU/g formulation) showed significant reduction in symptom development, expressed as percentage of diseased potato plants and the yield was increased by 25% in comparison with untreated control plots. The antagonistic bacterial isolates preferentially colonized the endorhizosphere of tomatoes and eggplants. The fatty acid analysis indicated that the isolate K-165 could be Paenibacillus alvei, whereas the isolate 5-127 might be Bacillus amyloliquefaciens (Tjamos et al. 2004). Antagonistic efficiency of Brevibacillus brevis in suppressing development of tomato wilt disease caused by F. oxysporum f.sp. lycopersici (Fol) was assessed, using plants raised in petridish microcosms and in pots in the greenhouse. Coinoculation of Fol and B. brevis resulted in marked decrease in symptom development, compared with inoculation with Fol alone. In addition, beneficial effect on tomato growth was also observed in plants coinoculated with the pathogen and BCA, indicating the usefulness of B. brevis as a potential biocontrol agent that could be considered for further investigation (Chandel et al. 2010). Antagonistic Paenibacillus xylanlyticus YUPP-1, P. polymyxa YUPP-8 and Bacillus subtilis YUPP-2 were isolated from cotton respectively at seedling, squaring and boll-setting stages. The effects of combined application of these bacterial species on Verticillium wilt disease development were assessed in pot experiments. Cotton plants in the combined BCA application were not infected before squaring stage, whereas infection rates of cotton treated with a single strain at seedling stage were 6.7, 6.7 and 13.3%, respectively for strains YUPP-8, YUPP-1 and YUPP-2. The control plants showed infection rate of 80%.

Field evaluations also showed that combined application of three BCAs was more effective than the individual strains. Verticillium wilt mortality rate and disease index of cotton at the boll-setting period were 9.4% and 6.5%, respectively, for combined application, whereas the control group had 47% and 32.8%. The results showed the effectiveness of combined application of three BCAs to keep the disease incidence and severity under check (Yang et al. 2013).

The biocontrol potential of isolates of Mitsuaria and Burkholderia obtained from rhizospheric soils of soybean and tomato against soilborne fungal pathogens was assessed, using seedling bioassays. Formation of lesions on developing seedlings was suppressed by isolates of Mitsuaria. Severity of lesion production in soybean challenged with Pythium aphanidermatum and tomatoes challenged with P. aphanidermatum and Rhizoctonia solani was significantly reduced by all isolates of Mitsuaria. Infection by Phytophthora sojae was also reduced by Mitsuaria. By contrast, Burkholderia isolates suppressed lesion production by R. solani in soybean (15%) and tomato (20%) (Benitez and Mc Spadden Gardener 2009). The knowledge of the genetic mechanisms of biocontrol activity provides the basic information for targeting bacterial population of interest, using gene-specific primers. Sequence analyses of those genes are employed to identify the strains of bacterial strains of interest. PCR-based approach to select novel biocontrol bacterial strains was applied by characterizing 2,4-diaceylphloroglucinol (2,4-DAPG)-producing Pseudomonas populations, based on amplification of phID gene sequences. This procedure was employed to quantify the abundance and directly characterize the genotype of most abundant phID+ populations inhabiting the rhizosphere of various crops. The use of functional markers revealed information on the ecology of bacterial biocontrol agents. On corn and soybean, native populations of bacterial strains exceeded levels required for in situ pathogen suppression (McSpadden Gardener et al. 2005).

The biological control potential may be assessed by applying the test isolates as soil application, seed treatment or root treatment to determine the extent of disease suppression. Strains of Pseudomonas spp. isolated from agricultural soils, river silt and rhizosphere soils, were evaluated for their antagonistic activities against Rhizoctonia spp. and Pythium spp., causal agents of root rot of wheat. Strains 14B2r, 15G2R, 29G9R, 39G2R, 48G9R and Wood 3R reduced the severity of symptoms induced by R. solani AG-8 and P. ultimum, under greenhouse conditions. The latter two strains suppressed the development of R. oryzae and P. irregulare also. In addition, four strains promoted growth of wheat plants, which correlated with disease suppression. Based on the 16S rDNA typing, the strains were identified as Pseudomonas borealis, P. chlororaphis, P. fluorescens, P. marginalis, P. poae, P. putida, P. syringae and P. vanovensis (Mavrodi et al. 2012). In a later investigation, of the 420 bacterial strains tested, Bacillus subtilis strain JN2, Myroides odorantimimus 3YW8, Bacillus amyloliquefaciens 5YN8 and Stenotrophomonas maltophilia 2JW6 were more efficient (>50%) in suppressing the development of ginger wilt disease caused by Ralstonia.
solanacearum under greenhouse conditions (Yang et al. 2012). The biocontrol potential of 12 bacterial isolates against Pythium sp. and Rhizoctonia spp. infecting plants in soilless system was assessed. One strain of Pseudomonas sp. P4 closely related to P. protegens (earlier known as Pseudomonas fluorescens) was highly antagonistic against two strains of AG1-IB. Strains P4 was tested for its effectiveness in protecting lamb’s lettuce against Rhizoctonia solani, causing root rot in small-scale hydroponics with significant reduction in root rot infection. The assessment of survival and population density of P4 on root showed that a density of BCA above the threshold value of 10^5 CFU/g of root tissues was required for effective disease suppression. PCR assay detected the presence of known loci in the P4 strain of Pseudomonas sp. The gene clusters plt, phl, ofa and fit-rx required for biosynthesis of secondary metabolites very similar to those of P. protegens P15 were identified in P4 strain of Pseudomonas sp., revealing the potential of the strain for the management of root pathogens P. aphanidermatum and R. solani (Moruzzi et al. 2017).

Soilborne actinomycetes are known to produce antibiotics that have been used in plant disease management. The biocontrol potential of four isolates of soilborne Streptomyces spp. viz., J-2, B-11, B-5 and B-40 was assessed against Sclerotium rolfsii. Culture inoculum of isolates was more effective in inhibiting scerotal germination than the culture filtrates. The isolate J-2 was the most effective in inhibiting sclerotial germination to the extent of 88 to 93%. The disease severity was significantly reduced in seedlings grown from BCA-treated seeds, the isolate J-2 being the most effective than the other isolates of Streptomyces spp. (Errakhi et al. 2007). Chickpea is seriously attacked by black root rot disease caused by Fusarium solani f.sp. pisi. Isolates of actinomycetes (100) obtained from soil samples were evaluated for their antagonistic activity against Fusarium solani f.sp. pisi. Based on dual culture test assessments in vitro, three most effective isolates S3, S12 and S40 were selected for greenhouse tests. The identity of these isolates was established based on 16S tDNA sequences. The isolates S3 and S12 were most similar to Streptomyces antibioticus, whereas the isolate S40 was very similar to S. peruviensis. Under greenhouse conditions, these isolates effectively suppressed the development of black root rot symptoms. The isolate S12 provided highest reduction on severity of the disease caused by F. solani f.sp. pisi (Soltanzadeh et al. 2016).

The biocontrol potential of Burkholderia cepacia strain 5-5B and Pesta formulations of binucleate Rhizoctonia B (BNR) isolates (BNR621 and P9023) was assessed against Rhizoctonia solani, causing Rhizoctonia stem and root rot of poinsettia was assessed. Application of B. cepacia suppressed stem infection during propagation, whereas application of either isolate of BNR did not affect the disease development. In contrast, after transplanting rooted poinsettia, one application of either BNR isolate was more effective in suppressing stem and root rot than the application of B. cepacia. Sequential application of B. cepacia at propagation followed by a BNR isolate at transplanting was more effective over the crop production cycle than the multiple applications of both BCAs individually or combined application of both BCAs. Root colonization by both BCAs after transplanting rooted poinsettias was affected by application method. The least root colonization by both biocontrol agents occurred in the combined application. The highest root colonization by the BNR isolates was observed in the sequential application that provided the most effective disease control. The results showed that application of different biocontrol agents during the different production phases of poinsettia was effective for disease control (Hwang and Benson 2002). Pseudomonas CMR12a strain was evaluated for its potential to suppress the development of Rhizoctonia root rot of bean. The involvement of phenazines and cyclic lipopeptides (CLPs) in the antagonistic activity of the BCA was investigated under growth chamber conditions. The wild-type strain CMR12a significantly reduced disease severity caused by the intermediate aggressive AG2-2 and the highly aggressive AG4HG1 strains of R. solani. A CLP-deficient and phenazine-deficient mutant of CMR12a could also protect bean plants, but less efficiently, compared to the wild-type strain. Two mutants deficient in both phenazine and CLP production lost the entire biocontrol activity. Disease-suppressive capacity of CMR12a decreased, after washing the bacteria, before application to soil and thereby removing the metabolites produced during growth on the plate. In addition, microscopic observations, revealed pronounced branching of hyphal tips of both strains of R. solani in the presence of CMR12a. More branched and denser mycelium was also observed for phenazine-deficient mutant treatment. However, neither the CLP-deficient mutant nor the mutants deficient in both CLPs and phenazins influenced hyphal growth. The results indicated the involvement of phenazines and CLPs during Pseudomonas CMR12a-mediated biocontrol of Rhizoctonia root rot of bean (D’aes et al. 2011).
were able to significantly reduce mycelial growth and sclerotic stem rot disease. These strains significantly reduced the viability of sclerotia in pot experiments in the greenhouse. Spray application of the BCA strains reduced disease incidence. The strains SC-1, when applied in the field at 10% flowering stage, reduced the disease incidence more efficiently than the strain W-67. However, the BCA strains were less effective than the fungicide Prosaro® 420SC (Kamal et al. 2015).

**Streptomyces** isolates (717) obtained from the rhizosphere of cucumber were evaluated for their antagonistic activity against *Phytophthora drechsleri*, incitant of damping-off disease of cucumber. Two isolates, C201 and C801 of *Streptomyces*, showing high inhibitory activity against the pathogen (> 70%) and cellulase activity in the presence and absence of NaCl, were tested under greenhouse conditions. The strains C201 and C801 reduced seedling damping-off disease incidence by 77% and 80%, respectively, in artificially infested soils. Strain C201 increased the dry weight of seedlings up to 21%. Analyses of 16S rRNA sequences revealed the close relationship of C201 and C801 to *S. rimosus* and *S. mononcynici* respectively. Induction of systemic resistance (ISR) in *Streptomyces*-treated cucumber plants was inferred by increased activity of polyphenoloxidase (PPO) and peroxidase (POX) enzymes (Sadeghi et al. 2017). Two isolates J-2 and B-11 of *Streptomyces* sp. were tested under greenhouse conditions for their ability to protect sugar beet seedlings. Soil application of biomass and culture filtrate (CF) mixture of the isolates significantly reduced the root rot incidence. The isolate J-2 was more effective and increased fresh weight of sugar beet, indicating its potential for large scale use for the management of sugar beet root rot caused by *S. rolfsii* (Errakhi et al. 2009). In a later investigation, the antagonistic activities of species of *Streptomyces*, viz., *S. mycavariensis* SS-2-243, *S. philanthi* RL-1-178 and *S. philanthi* RM-1-138 against *Sclerotium rolfsii* (stem rot) and *Ralstonia solanacearum* (bacterial wilt), infecting pepper (chilli) were investigated under greenhouse conditions. *S. philanthi* RL-178 suppressed Sclerotium root rot and stem rot as efficiently as the fungal BCA *T. harzianum* NR-1-52 and the fungicide carboxin. *S. mycavariensis* SS-2-243 and *S. philanthi* RL-1-178 showed antagonistic activity against the pepper bacterial wilt pathogen *R. solanacearum* and the degree of disease suppression was similar to that of the antibiotic streptomycin sulfate. Under field conditions, soil inoculation with both pathogens was carried out. *S. philanthi* RL-1-178 protected the chilli plants against *S. rolfsii* and *R. solanacearum* more effectively than *S. mycavariensis* SS-2-243 or *T. harzianum*, resulting in the survival of 58.75% of the chilli plants growing in the infested soil. The efficacy of the BCA was in equivalence to carboxin or streptomycin treatment (Boukaew et al. 2011). Actinomycetes isolated from groundnut (peanut) rhizospheric soils were screened by dual culture technique for their antagonistic activity against *Sclerotium rolfsii*, incitant of groundnut (peanut) stem rot disease. The effective isolates were evaluated under greenhouse conditions, using their culture filtrates and crude extracts to determine the extracellular antifungal activity and characterize their biocontrol and plant growth-promoting traits. The most effective isolate RPIA-12 exhibited high level of antagonism against *S. rolfsii*. The isolate RPIA-12 produced hydrogen cyanide (HCN), lipase, siderophores and indole-acetic acid (IAA). Production of oxalic acid, a pathogenicity factor of *S. rolfsii*, was inhibited by crude extracts of RPIA-12, resulting in reduction of stem rot severity. RP-IA-12 was identified as closely related to *Streptomyces flocculus*, based on 16S rRNA gene sequencing (Jacob et al. 2016).

The biocontrol potential of *Rhizobium leguminosarum* bv. *viciae* (*Rlv*), applied as seed treatment, against damping-off disease caused by *Pythium* spp. affecting pea and lentil was evaluated. Treatment of pea seeds with *Rlv* strains R12, R20 and R21 increased root nodule mass. Seed treatment with the strain R21 was the most effective among the strains of *Rlv* in providing disease suppression to a level in equivalence to that of the fungicide Thiram™. By contrast, the strain 12 was the most effective in protecting lentil plants against damping-off and the treatment was as effective as the fungicide thiram. The strain 12 also enhanced plant growth, root nodule mass and shoot biomass. The disease suppression was strain-specific, as the strain R21 was more effective against damping-off affecting pea, whereas strain 12 was more effective in protecting lentil against damping-off disease (Huang and Erickson 2007). The efficacy of *Lysobacter capsici* strain PG4 in suppressing tomato Fusarium wilt disease caused by *F. oxyysporum* f.sp. radicis-lycopersici (FORL) was assessed under greenhouse conditions. The strain PG4 effectively suppressed the development of the disease and it was found to be a good root colonizer. High population of PG4 (ca. 10⁵ CFU/g of roots) could be recovered from the roots of plants growing from PG4-coated seeds. Disease incidence in treated plants was 24%, as against 86% in untreated plants inoculated with FORL. Further, PG4-treatment enhanced plant fresh weight, indicating the growth-promoting effect of *L. capsici* PG4 (Puopolo et al. 2010). Four isolates of *Streptomyces* sp. exhibited strong antagonistic activities against potato soft rot pathogens, *Pectobacterium carotovorum* and *P. atroseptica* in vitro and they were evaluated for their efficacy in suppressing soft rot symptoms in potato slices of cvs. Bintje, Yokon Gold, Russet and Norland. The biomass inoculum and culture filtrates of the BCA isolates were applied on tuber slices. The isolate identified as *Streptomyces* sp. OET reduced the disease symptom severity by 65 to 94% caused by both bacterial pathogens inducing soft rot in potato tubers (Baz et al. 2012).

The nonpathogenic strain of *Agrobacterium vitis* VAR03-1 was evaluated for its efficacy in suppressing the crown gall, by soaking the roots of grapevine, rose and tomato to protect against *A. vitis*, *A. rhizogenes* and *A. tumefaciens* respectively infecting these crops. The soil was infested with these pathogens, before planting the root-treated plants. Treatment with the strain VAR03-1 significantly reduced the number of plants with tumors and decreased disease severity in grapevine, rose and tomato. The degree of protection provided by VAR03-1 was greater than that by strain K84 used earlier. Furthermore, the strain VAR03-1 provided protection to a wide range of...
host plants against three tumorigenic Agrobacterium spp. (Kawaguchi et al. 2008a, 2008b). The nonpathogenic strains ARK-1, ARK-2 and ARK-3 isolated from graft unions were identified as Agrobacterium vitis. Stems of grapevine seedlings were inoculated with cell suspensions of strains of A. vitis (Ti), strain VAR03-1 and one of the three nonpathogenic strains, as competitors to assay the suppression of tumor formation by A. vitis. The ratio 1:1 of cells of pathogen/strains ARK-1, ARK-2 and ARK-3 reduced tumor incidence, the strain ARK-1 being the most effective in inhibiting tumor formation by A. vitis. The strain ARK-1 established its population well on roots of grapevine tree rootstock and persisted on roots for one year. The strains ARK-1, -2 and -3 did not inhibit pathogen in YMA medium and the culture filtrate of ARK-1 also did not suppress tumor development. The results suggested that the nonpathogenic strains might suppress tumor development in grapevines through a mechanism different from that of VAR03-1 strain. The strain ARK-1 appeared to have potential for suppressing crown gall disease in grapevine effectively (Kawaguchi and Inoue 2012). In a later investigation, the nonpathogenic Agrobacterium vitis strain ARK-1 an endophyte in grapevine-controlled grapevine crown gall more effectively than the nonpathogenic strain VAR03-1 used earlier. The biocontrol potential of ARK-1 strain to reduce the crown gall disease affecting apple, Japanese pear, peach, rose and tomato was assessed. The roots of these plants were soaked in a cell suspension of ARK-1 before planting into the soil infested with tumorigenic Agrobacterium spp. The number of plants developing crown gall tumor was reduced due to treatment with the BCA. Analysis of results of greenhouse experiments showed that the integrated risk ratio (IRR) after treatment with ARK-1 was 0.29 for rose crown gall and 0.16 for tomato crown gall, indicating the effectiveness of ARK-1 treatment against Agrobacterium spp. The results of six field trials with peach conducted during 2010-2013, showed that IRR, was 0.38 for apple crown gall, 0.16 for Japanese pear crown gall and 0.20 for peach crown gall after treatment with ARK-1, indicating that the crown gall disease incidence could be significantly reduced by treatment with nonpathogenic ARK-1 strain of Agrobacterium vitis (Kawaguchi et al. 2013).

The biocontrol potential of Bacillus subtilis HS93, B. licheniformis LS674 and T. harzianum was assessed for suppressing Phytophthora blight (Phytophthora capsici) and root rot (Rhizoctonia solani) affecting pepper. Seed treatment and root drenching with suspension of HS93 with 0.5% chitin was more effective against P. capsici and R. solani infection than the bacteria alone. Likewise, enhancement of biocontrol activity of LS674 and T. harzianum by combining with 0.5% chitin was more significant against R. solani, but this combination was ineffective against P. capsici. In the presence of chitin, the antagonistic activity appeared to increase against some fungal pathogens (Ahmed et al. 2007). The biocontrol potential of bacterial strains (231) isolated from soils and roots of cucumber, pepper and tomato plants from different locations were screened by seedling assay. Two-week old pepper seedlings were inoculated with zoospore suspension of Phytophthora capsici, causing Phytophthora blight disease. Four strains, KJ1R5, KJ2C12, KJ9C8 and 11S16, were consistently effective in protecting pepper plants against P. capsici. These BCA strains could protect pepper plants under field conditions also (Kim et al. 2008). Strains of Pseudomonas putida FC-6B, Pseudomonas sp. FC-7B, Pseudomonas sp. FC-24B, P. putida FC-8B, isolated from used rockwool soilless substrates, were evaluated for their efficacy in suppressing Fusarium wilt disease of tomato caused by F. oxysporum f.sp. lycopersici (Fol). The pathogen was mixed with soil (at 5 × 10⁶ chlamydospores/ml) and distributed in pots. Roots of tomato seedlings were dipped in 100 ml of bacterial suspension (10⁶ and 10⁷ CFU/ml) for 10 min and planted in pots. Pseudomonas chlororaphis MA342 strain applied at 7.5 × 10⁶ CFU/ml, as root dipping treatment reduced wilt disease incidence by 20 to 55%. Pseudomonas sp. FC-9B strain (at 10⁷ CFU/ml) was the most effective in reducing disease incidence and this strain promoted plant growth also (Srinivasan et al. 2009). Another bacterial BCA, Brevibacillus brevis suppressed tomato wilt disease incidence significantly compared with untreated and inoculated control treatment (Chandel et al. 2010).

The biocontrol potential of soilborne Streptomyces spp. against Sclerotium rolfsii, incitant of damping-off disease of sugar beet, was assessed. Four isolates of Streptomyces spp. J-2, B-11, B-5 and B-40 inhibited sclerotial germination in infested soil, compared to control. The isolate J-2 was the most effective, inhibiting sclerotial germination by 93% and 88%, respectively, when mycelial inoculum and culture filtrate (CF) were applied. Disease severity was reduced in seedlings growing from the BCA-treated seeds (Errakhi et al. 2007). The disease suppressive effect of putative BCA isolates in reducing the severity of potato scab disease caused by Streptomyces turgidiscabies was assessed. Of the 26 isolates, five actinomycetes isolated from either rhizosphere of soil of wild oats grown earlier in potato fields or soil adhering to potato stolons and tubers were effective antagonists. These isolates were identified as Streptomyces spp. The isolate WsRs-501 exhibited stronger inhibition of growth of S. turgidiscabies, compared to other isolates. Furthermore, WoRs-501 was the most effective in suppressing potato scab disease in pot experiments. A 10% (v/v) mix of WoRs-501 (6.2 × 10⁹ CFU/g dry mass) reduced the disease severity by 78 to 94%, in comparison to untreated control at a pathogen concentration of 5 × 10⁴ to 5 × 10⁶ CFU/g dry soil. The isolate WsoRs-501 could tolerate a wide range of pH levels and temperatures of the soil, indicating that this isolate with ability to adapt to different soil environmental conditions, might be an effective candidate suitable for a large-scale field application (Kobayashi et al. 2012). Bacillus velezensis BAC03 effectively suppressed the development of scab disease of radish caused by Streptomyces scabies. The strain BAC03 applied at 5 days before planting significantly reduced S. scabies population and completely suppressed scab disease development. But delaying the BCA application adversely affected its efficiency in proportion to the time interval between BCA application and planting. The strain BAC03 at 10⁹ CFU/cm³ potting mix or higher concentration was effective in reducing radish scab. Increasing the frequency of BCA application
had no advantage in terms of disease suppression. The strain
BAC03 increased the plant growth, whether the pathogen was
present or not in the soil (Meng and Hao 2017).

Plant growth-promoting rhizobacteria (PGPRs) Bacillus
pumilus SE34 and Pseudomonas putida SE89B61 were
evaluated for their efficacy in suppressing the bacterial wilt
disease caused by Ralstonia solanacearum, by treating the
tomato seeds cv. Solar Set. After sowing the BCA-treated
seeds on flats, the soil was drenched with suspensions of R.
solanacearum (5 ml at 6 x 10^7 CFU/ml). Tomato plants were
transplanted at 3 days after challenge inoculation into pots
containing moist soil. Treatment of tomato plants with B. pum-
ilus and P. putida reduced wilt disease incidence significantly,
compared to controls. Two applications of the BCAs were
more effective than single application (Amith et al. 2004).

Pseudomonas fluorescens strain 1100-6 effective against
Agrobacterium vitis, causing crown gall disease in grapevine
was transformed with gfp gene encoding green fluorescent
protein (GFP). The transformed mutant P. fluorescens 1100-
6-gfp was injected into 3-year-old potted grapevine cuttings,
rooted in perlite and then transferred to pasteurized potting
mix. The disposition of genetically tagged strain 1100-6-gfp
was evaluated, after 6 months by PCR amplification of the
gfp gene in extracts from grapevine inoculated with tagged
strain at the time of planting. Roots of plants inoculated with
tagged strain either did not have or showed detectable popula-
tions near the soil line. But the gfp-gene sequence was detect-
able in stem sections cut from vines up to 6 cm above the
soil line. Extracts of soil revealed detectable concentrations of
the gfp-gene sequence in (3/5) containers, as well as the stem
extracts from plants that cohabited with an inoculated vine.
Observations on inoculated tissues, using epifluorescence
microscope, revealed the presence of the BCA predominantly
in the xylem tissues. Both the pith and xylem vessels had the
BCA at the point of inoculation. Occasionally, persistence
of the BCA in the external surface of grapevines was seen
(Eastwell et al. 2006).

3.1.3.1.3 Field Tests

The putative bacterial biological control agents selected
based on their effectiveness under laboratory and greenhouse/
growth chamber conditions, are advanced to field evalua-
tion for assessing their biocontrol potential under prevailing
natural environmental conditions. The selected BCAs have
to adapt to the various environmental conditions existing in
the soils and also compete with numerous microorganisms
for nutrients and appropriate niche, where they can proliferate
to the required population levels to suppress microbial
pathogens.

The effects of treatment of seed with strains of rhizosphere
bacterial strains on development of damping-off disease of
sugar beet, canola, safflower and pea caused by Pythium
spp. were assessed. Based on in vitro assessments, 12 strains
belonging to Pseudomonas fluorescens (708, 1-2, 1105, 1809,
2106 and 2201), Bacillus cereus PS1, Bacillus megaterium
SB6, Arthrobacter sp. 2101, Pantoea agglomerans (909, 2-2)
and Erwinia rhapontici A123 were found to be effective in
reducing the incidence of sugar beet damping-off. Strains of
P. fluorescens 708, 1-2, 2202, B. cereus PS1, E. rhapontici
A123 and Pantoea agglomerans 2-2 were efficient in sup-
pressing the development of damping-off of canola, dry pea
and sugar beet in fields naturally infested with Pythium spp.
when applied as seed treatment (Bardin et al. 2003). Isolates
(14) of Rhizobium were evaluated for their biocontrol efficacy
against chickpea Fusarium wilt disease caused by F. oxyspor-
rum f.sp. ciceris. The isolates Pch43 and Rh4 of Rhizobium
were the most effective in reducing wilt incidence to less than
8% in chickpea, as against 48.7% infection in control plots. In
addition, growth-promoting activity of the isolates was also
clearly revealed under field conditions (Arfaoui et al. 2006).

The biological control agents (BCAs) have been applied as
liquid cultures or formulated products on the soil at required
concentrations. In general, soil application of BCAs is both
expensive and difficult to achieve uniform coverage of patho-
gen-infested patches present in a field. Pseudomonas putida
06909-rif/nal was applied in irrigation water for suppressing
the development of Phytophthora parasitica, infecting citrus.
Application with irrigation water increased the BCA popula-
tion, as well as its biocontrol efficacy against P. parasitica
over that of single yearly applications at the commencement
of irrigation season (Steddom and Merge 1999). The efficacy
of Pseudomonas fluorescens SS101 and its surfactant (cyclic
lipopeptides surfactant massetolide A)-deficient mutant
massA 10.24 to suppress the population and root infection
of apple and wheat seedlings by Pythium sp. was assessed.
Both parent (wild-type) strain and the mutant effectively sup-
pressed resident Pythium populations to an equivalent level
in the presence or absence of plant roots and ultimately sup-
pressed Pythium infection to the same degree on all host
plants investigated. The split-root plant assays were con-
ducted, using strain SS101 or mutant 10.24 in the orchard soil
to study the role of induced resistance in suppressing disease
development. Strain SS101 or the mutant 10.24 significantly
reduced the infection by Pythium spp. on the component
of wheat or root system of plants grown on the soil treated
with respective bacterial strain. Infection of wheat roots and
Gala apple roots and Gala apple seedlings roots was reduced to
11% and 15%, respectively, as against 34 and 60–70% in
untreated soil, following application of wild-type and mutant
strains of Pseudomonas fluorescens. The results of split-root
assays indicated that strain SS 101 did not limit root infection
by Pythium spp. via induced systemic resistance (Mazzola et
al. 2007).

The actinomycete Streptomyces misionensis (Sm) strain
PMS101, Bacillus thermoglucosidasis (Bt) strain PMB207
and S. siyaeensis (Ss) strain PMS502 were evaluated for their
biocontrol potential against F. oxysporum f.sp. lilli, causing
Fusarium seedling blight and basal rot of lily, in comparison
with the fungicide Sporgon (50% procloraz-Mn complex).
A large-scale trial in an automated and environment-controlled
commercial greenhouse showed that treatment of scale bulb-
lets of lily with B. thermoglucosidasis (Bt) or Sporgon (100
µg/ml) and Sm resulted in a significant reduction in the inci-
dence of seedling blight. The difference between these two
treatments was not significant (P > 0.05). The results of greenhouse and field trials showed that the treatment of scale bulblets or 1-year-old bulbs of lily with Bt strain PMS101 at 1–1.2 × 10^8 CFU/ml or Sm strain PMS101 at 1–1.4 × 10^8 CFU/ml without Sporgen was also effective in suppressing the development of basal rot disease of lily. The results showed the effectiveness of B. thermoglucosidasis and S. misionensis as BCAs for the management of Fusarium seedling blight and basal rot of lily (Chung et al. 2011). The protective effects of compatible endophytic bacterial strains Bacillus subtilis EPCO16 and EPC5, and rhizobacterial strains Pseudomonas fluorescens Pf1 for the protection of pepper (chilli) against wilt pathogen Fusarium solani were assessed. Application of the bacterial strains singly or in combination under greenhouse and field conditions reduced the disease incidence by enhancing host plant resistance. Higher levels of activities of peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL), β-1,3-glucanase, chitinase and phenolics indicated the induction of systemic resistance (ISR) in treated pepper plants. Combinations of the three strains were more effective in reducing disease incidence than the individual strains (Sundaramoorthy et al. 2012).

The biocontrol potential of four bacterial strains Pseudomonas chlororaphis PA-23, Bacillus amyloliquefaciens BS5, B. amyloliquefaciens E16 and Pseudomonas sp. DF41 was assessed for suppressing the development of stem rot disease of canola caused by Sclerotinia sclerotiorum under field conditions. The strains PA-23 and BS6 were as effective as suppressing disease development as the fungicide Rovral Flo® (iprodione). Double-spray application of PA-23 and BS6 did not provide enhancement of protection level to canola plants, compared with plants receiving single sprays of BCA strains. The results suggested that P. chlororaphis and B. liquefaciens could be used for the management of canola stem rot disease under field conditions (Fernando et al. 2007). Pseudomonas fluorescens biotype F isolate DF37 and the Canada milk vetch extract (MVE) were selected, based on their effectiveness in suppressing the development of potato Verticillium wilt disease under in vitro and growth room conditions. Bacillus pumulis M1 was able to reduce the disease incidence on cv. Kennebec (highly susceptible), whereas isolate DF37 effectively reduced Verticillium wilt parameters (disease incidence, severity and vascular discoloration), in both cvs. Russet Burbank (moderately susceptible) and Kennebec. The bacterial isolate and plant extract were evaluated under field conditions, using potato cultivars Russet Burbank and Kennebec. In the first year of field testing, isolate DF37 and plant extract MVE effectively reduced the disease on Russet Burbank and Kennebec, respectively. The extent of reduction in per cent infection and vascular discoloration, due to DF37 application were respectively 26% and 67%, and 45% and 55% for MVE application, compared to control. In the second-year trial, DF37 and M1 and MVE treatments reduced all wilt parameters (ranging from 19 to 31%) and increased the yield (18%) in cv. Kennebec. The isolate DF37 could reduce wilt incidence by 29 to 43% and increase the yield by 24% on cv. Russet Burbank (Uppal et al. 2008).

Bacillus subtilis QST713 strain produced lipopeptides and surfactant antibiotics, which did not have inhibitory effect on resting spores of Plasmodiophora brassicae, incitant of clubroot disease of canola. The biofungicide Serenade (formulation containing B. subtilis QST713) suppressed clubroot on canola under controlled conditions, but its performance in the field was inconsistent. The effect of timing of application of Serenade or its components (product filtrate and bacterial suspension) on infection by P. brassicae was assessed under controlled conditions, by applying as a soil drench at 5% concentration (v/v). The BCA and its components were applied to the planting mix infested with P. brassicae at seeding or at transplanting 7 or 14 days after seeding (DAS) to target primary and secondary zoospores of P. brassicae. QPCR assay was used to assess root colonization by B. subtilis, as well as P. brassicae. Serenade was more effective in reducing infection by P. brassicae than the individual components. Two applications of Serenade were more effective than one application, providing complete suppression of disease development. By contrast, the individual components could reduce the disease severity only to the extent of 62 to 86%. The pathogen DNA content was reduced in canola roots by 26 to 99% by Serenade at 7 and 14 DAS. The results of QPCR assay were strongly correlated with root hair infection (%) assessed at the same time (r = 0.84 to 0.95). Genes encoding jasmonic acid (BnOPR2), ethylene (BnACO) and phenylpropanoid (BnOPCL and BnCCR) pathways were upregulated by 2.2- to 2.3-fold in plants treated with the biofungicide, relative to the control plants. The results suggested the possibility of involvement of mechanisms, antibiosis and induced systemic resistance, in suppression of clubroot disease development by Serenade (Lahlah et al. 2013).

The ethanol extract of the bacterial biocontrol agent (BCA) Serratia marcescens N4-5 strain, applied as seed treatment effectively suppressed the development of damping-off of cucumber caused by Pythium ultimum in potting mix and in sandy clay loam soil. The ethanol extract of N4-5 strain was found to be compatible with isolates of T. harzianum. The Th23::hph-egfp isolates of T. harzianum and GL-21 showed no inhibitory effect of ethanol extract of N4-5 in vitro. Isolate Th23::hph-egfp consistently colonized the cucumber seed coat (100%) and emerging root system (> 94%), when combined with ethanol extract of N4-5 strain. Microscopic observations showed that N4-5 ethanol extract-treated seed coat was more extensively colonized by Th23::hph-egfp strain than on thiram-treated seed (see Figure 3.5). The efficiency of colonization of cucumber rhizosphere by Th23::hph-egfp strain was determined by applying this strain as a drench to the soil area in combination with N4-5 ethanol extract and Thiram. There was no evidence of inhibition of colonization of Th23::hph-egfp by seed treatment with N4-5 ethanol extract. Similar populations of Th23::hph-egfp were detected in the cucumber rhizosphere over the period of experimentation (3 weeks) (see Figure 3.6). The inoculum sandwich technique was applied to determine biocontrol potential of suppressing damping-off in natural soil (sandy loam). The N4-5 ethanol extract applied as seed treatment was very effective, as indicated by greater
plant stand, compared with untreated control. The ethanol extract of N4-5 strain, as seed treatment was generally more effective than the in-furrow application of Th23::hph-egfp and GL-21 strains and the commercial product Mycostop, containing Streptomyces griseoviridis strain K61. Combining N4-5 extract with isolates of T. harzianum isolates did not result in reduction in effectiveness of disease suppression, but improved in another field soil. The results indicated the potential of ethanol extract of N4-5 strain of S. marcescens for suppression of damping-off disease of cucumber either alone or in combination with isolates of T. harzianum (Roberts et al. 2016).

Four strains of Pseudomonas sp. FP22, FP23, FP30 and FP35 and strain of Serratia plymuthica HRO-C48 were evaluated for the impact on cotton Verticillium wilt development, parameters of cotton growth and yield in a naturally infested field. The cotton seed bacterization with BCA isolates reduced the AUDPC values, ranging from 22.1 to 50.9% in field trials in 2005 and 2006. The growth parameters were also beneficially impacted by the BCA treatments. The increase in seed cotton yield by treatment with BCA isolates ranged from 13.1 to 23.0% in cv. Sayar314 and from 4.2 to 12.8% in cv. Acala Maxxa in 2005, whereas there was no significance in seed cotton yield among the treatments in 2006 (Erdogan and Benlioglu 2010). Pseudomonas fluorescens strains HC1-07 and HC9-07 produce, respectively, cyclic lipopeptide (CLP) and phenazine-1-carboxylic acid (PCA), which determine the biocontrol potential of the strains against wheat take-all pathogen Gaeumannomyces graminis var. tritici (Ggt) The seven-gene operon for the synthesis of PCA from P. syringae 2-79 was introduced into the P. fluorescens HC1-07 rif strain to enhance the biocontrol activity of the recombinant strain HC1-07PH2 against Ggt. The strain HC1-07PHZ consistently inhibited the hyphal growth of three isolates of Ggt. The strain HC1-07PH2 applied at a dose of 10^2 CFU/kg seed suppressed development of take-all disease more effectively than the strain HC1-07rif and H09-07rif applied either individually or in combination. At 10^4 CFU/seed concentration, the strain combination HC1-07rif + HC9-07rif provided significantly greater level of protection to wheat plants than the individual strains including HC1-07PHZ (Yang et al. 2017).

Bacillus amyloliquefaciens IUMC7 and its culture filtrate (CF) showed potential for suppressing the development of Ralstonia solanacearum, causal agent of bacterial wilt disease affecting several crops. The mushroom compost infested with the strain IUMC7 was incorporated into the soil and it reduced the bacterial wilt disease severity in tomato plants under greenhouse conditions, compared to the control. Population of R. solanacearum decreased in soil inoculated with IUMC7 strain. Thin layer chromatography-bioautography assay showed that one of the antimicrobial compounds produced by the strain IUMC7 might be an iturin-like lipopeptide. The mushroom compost amended with B. amyloliquefaciens IUMC7 might be useful for reducing the bacterial wilt disease severity in tomato and possibly in other crops also (Sotoyama et al. 2017).

![FIGURE 3.5 Effects of different treatments on colonization of cucumber seeds on by T. harzianum Th23::hph-egfp strain A: seed treated with thiram; B: seed treated with ethanol and Serratia marcescens N4-5 ethanol extract; C: seed treatment with gelatin and live N4-5 strain; D: magnified view of seed treated with thiram and colonization by Th23::hph-egfp strain; E: magnified view of untreated seed; F: magnified view of seed treated with N4-5 ethanol and G: live N4-5 strain.](Courtesy of Roberts et al. 2016 and with kind permission of the American Phytopathological Society, MN)
3.1.4 Viral biological control agents

3.1.4.1 Viruses infecting fungal pathogens

Reduction in virulence of fungal pathogens, following infection by viruses, is termed as hypovirulence. The phenomenon of hypovirulence is considered to have a role in counterbalancing plant diseases in nature. Mycoviruses are able to infect all major taxa belonging to the Kingdom, Fungi. The mycoviruses may have double-stranded (ds)-RNA or single-stranded (ss)-RNA as their genome. The ds-RNA mycoviruses are classified into three families, Totiviridae and Partitiviridae, which include viruses with nonenveloped isometric particles of 25–50 nm in diameter, inducing latent infections frequently in their host fungi. Members of the genus Totivirus have nonsegmented genome, while members of the genus Partitivirus genus have segmented genomes (Ghabrial 1998). Viruses included in the family Hypoviridae lack conventional virions and their ds-RNAs are enclosed in the host-encoded vesicles (Dawe and Nuss 2001).

Many fungal species have been shown to intraspecifically transmit hypovirulence-associated ds-RNA by anastomosis, following transfection. The ascospore progeny derived from the debilitated strain Ep-1PN of Sclerotinia sclerotiorum exhibited normal growth rate and typical colony morphology, indicating the failure of S. sclerotiorum debilitation-associated RNA virus (SsDRV) passing through sexual cycle. The homothallic nature of sexual reproduction of S. sclerotiorum would also impede the transmission of SsDRV through hyphal anastomosis. The ascospore progeny had similar virulence levels as the wild-type strain Sunf-M, when allowed to colonize detached leaves of oilseed rape. The debilitated strain Ep-1PN could survive on leaves of oilseed rape for more than 1 week and it could protect leaves from attack by Ep-1PN1, a virulent ascospore progeny of EP-1PN with normal colony morphology. The debilitation phenotype of EP-1PN also could be transmitted to EP-1PN1 in the soil and subsequently protected seedlings against invasion by normal virulent strains (Xie et al. 2006). A geminivirus-related DNA mycovirus conferring hypovirulence to Sclerotinia sclerotiorum was isolated and characterized. Intraspecific transmission of a hypovirulence-associated ds-RNA and hypovirulent phenotype from hypovirulent isolate Ss275 of S. sclerotiorum (with high mycelial incompatibility) to five virulent isolates of Sclerotinia minor (with fewer mycelial compatibility groups) was observed. The hypovirulent phenotype was successfully transmitted to one isolate of S. minor Sm10. Three putatively converted isolates of Sm10 had all cultural characteristics of the hypovirulent phenotypes and pathogenicity as well. In northern hybridizations, ds-RNA isolated from one of the converted isolates Sm10T hybridized with a DIG-labeled cDNA probe prepared from ds-RNA isolated from Ss275. The RAPD analysis confirmed that the isolate Sm10T was derived from Sm10 and not from Ss275. The results revealed that intraspecific transmission of ds-RNA was possible (Melzer et al. 2002). The later investigation showed that S. sclerotiorum hypovirulence-associated DNA virus (SsHADV-1) could be transmitted from strain DT-8 to vegetatively incompatible S. sclerotiorum strain with relatively high frequency (Yu et al. 2010).

Phytophthora root rot disease of citrus is induced by Phytophthora nicotianae and P. palmivora in Florida. P. nicotianae isolate Pn117 was characterized as hypovirulent on citrus roots. The biocontrol efficacy of this hypovirulent isolate was indicated by nonrequirement for additional applications to sustain rhizosphere activity for 7 months, after citrus trees were planted. The isolate Pn117 induced less severe symptoms, compared to virulent isolate of P. nicotianae Pn 198 and P. palmivora Pp99. Inoculation of all rootstocks with hypovirulent Pn117 strain at 3 days prior to inoculation with virulent isolates, resulted in significantly less disease and
production of greater amounts of roots, compared to seedlings inoculated with virulent isolates alone. Recovery of virulent strain Pp99 from cooinoculated citrus was reduced, if Pn117 was preinoculated, compared with single inoculation with *P. palmivora* alone, indicating the possible competition between the hypovirulent and virulent isolates for space and nutrients (resources) available in host roots. Preestablishment of the hypovirulent isolate in root cortex might lead to loss of non-structural carbohydrates, reducing subsequent colonization by *P. palmivora*. The results indicated that the isolate Pn117 had the potential for use as an effective BCA against virulent isolates of *P. nicotianae* and *P. palmivora*, infecting citrus, since the hypovirulent isolate effectively colonized the host roots and persisted with soil amendments or repeated application. By contrast, the BCAs *Pseudomonas putida* and *Trichoderma viride* required weekly augmentations or addition of organic amendments that favor differential proliferation of the biological control agents (Colburn and Graham 2007).

*Rosellinia necatrix*, incitant of white root rot disease, infects fruit trees and other woody plants. *R. necatrix* isolate W370 contains ds-RNA with 12 segments, considered to represent a possible member of the family Reoviridae. The strain W370 was weakly virulent and its hyphal-tips became ds-RNA-free and strongly virulent. The 12 segments of W370 ds-RNA could be transmitted to hygromycin B-resistant strain RT37-1, derived from a ds-RNA-free strain of W370 in all or none fashion through hyphal contact with W370. The W370 ds-RNA-transmitted strains were less virulent than their parent strain RT37-1 on apple seedlings, with mortality ranging between 0% and 16.7% in apple seedlings that were inoculated with the W370 ds-RNA-containing strains and 50 to 100% for seedlings inoculated with the ds-RNA-free strains. Some W370 ds-RNA-containing strains killed more than 16.7% seedlings, but these became free of the ds-RNA in planta. The results indicated that W370 ds-RNA was the hypovirulence factor in *R. necatrix*. Furthermore, one strain lost one segment (S8) of W370 ds-RNA during subculture and the S8-deficient mutant strain also exhibited hypovirulence in *R. necatrix* (Kanematsu et al. 2004). In the further study, a member of the genus *Mycoreovirus* within the family Reoviridae isolated from *Rosellinia necatrix* was named as *Rosellinia necatrix mycoreovirus* 3 (W370) (RnMYRV-3) and established as the hypovirulence factor of the white rot pathogen. Two virus-free fungal isolates (W37 and W97) that were somatically incompatible with virus-harboring field isolate (W370) were transferred with purified RnMTRV-3 particles. Electrophoresis and northern hybridization analysis confirmed infection of *R. necatrix* by RnMYRV-3, which could be back-inoculated to respective virus-free isolates via hyphal anastomosis. Virus-infected strains produced smaller lesions on apple fruits than the virus-free isolates. Virus-cured strains were indistinguishable from wild-type strains in culture morphology and displayed similar level of virulence on apples. The level of virus accumulation varied among virus-transfected subcultures and within its single colonies (Sasaki et al. 2007). The host range of two viruses infecting *S. sclerotiorum* was investigated. Two mycoviruses, the partivirus RnPVI-W8 (RnPVI) and the mycovirus RnMyRV3/W370 (MYRV3) from *Rosellinia necatrix* were inoculated onto the protoplasts of fungal plant pathogens with purified virus particles. The presence of ds-RNA of viral genomes in regenerated mycelia of *Diaporthe*, *Cryphonectria parasitica* and *Valsa ceratosperma* was confirmed. Horizontal transmission of both viruses from newly infected strains to virus-free, wild-type strains through hyphal anastomosis was readily demonstrated by dual culture method. However, vertical transmission through conidia was rarely observed. The results showed that protoplast inoculation method was useful in extending the host range of mycoviruses (Kanematsu et al. 2010).

3.1.4.2 Viruses Infecting Bacterial Pathogens

Bacteriophages or phages are one of the most abundant biological entities present in the biosphere. Phages are viruses specifically infecting bacteria that subvert the metabolism of their bacterial hosts for their replication. The phages are classified into three different groups, Podoviridae, Myoviridae and Siphoviridae with different head sizes and tail lengths. The biocontrol potential of the selected phages has to be confirmed by further tests under field conditions. Bacteriophage therapy was applied for the control of dysentery in human beings in the preantibiotic era. Use of phages, as a management approach, was indicated by the investigation on cabbage black rot caused by *Xanthomonas campestris pv. campestris* (Mallmann and Hemstreet 1924). Application of bacteriophages for the management of bacterial plant diseases commenced in the early 19th century. Kotila and Coons (1925) demonstrated that bacteriophages isolated from the soil suppressed the growth of *Pectobacterium carotovorum subsp. carotovorum*, causing potato blackleg disease. Coinoculation of the phage and pathogen prevented rotting of potato tubers. In addition, they isolated phages effective against *P. carotovorum* subsp. *carotovorum* and *Agrobacterium tumefaciens* were isolated from a number of environmental sources such as river water and soil (Coons and Kotila 1925). The potential of phages for suppressing the development of bacterial diseases of crops has been exploited, because of the relative ease of preparing the phage isolates and low cost of production of phages. The effectiveness of phages in suppressing soft rot caused by *Pectobacterium atrosepticum* and *P. carotovorum* subsp. *carotovorum* was demonstrated, using slices of carrot and potato tuber, respectively (Coons and Kotila 1925; Kotila and Coons 1925). Use of bacteriophage biocontrol for suppressing some soilborne bacterial pathogens has been reported to be effective in some investigations. The principal factor for determining the applicability of bacteriophage biocontrol is the exclusive lytic cycle (virulent) nature of the phage. Virulent phages are those that cause infections resulting in lysis (death) of the host bacterium ultimately, leading to the release of progeny phage particles. Ideally, a phage for biocontrol applications should be exclusively lytic and possess a host range, which allows productive infection on all strains of the pathogen species being targeted. Unlike chemical bioicides, phages occur naturally in the environment without any
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harm. After application, the number of phages increases, if the susceptible bacterial species are available to them. They tend to persist in high numbers in any environment as long as the host bacterial species is present, since they are obligate pathogens. Phages generally have a narrow host range, typically being limited to strains within a species of bacteria. Many soil factors such as pH, moisture, organic matter content and soil type may cause phage inactivation either individually or in combination. However, under favorable conditions, phages have been shown to persist in the soil at relatively stable concentrations for several weeks (Buttimer et al. 2017).

Soil samples for phage isolation were collected from tomato, pepper and tobacco fields. After sieving, the soil samples were placed in flasks and the moisture level was adjusted to 40% waterholding capacity with distilled water. Each flask was seeded with overnight cultures of strains of *Ralstonia solanacearum*, causal agent of bacterial wilt disease and incubated for 48 h. The soil samples were suspended in phosphate buffered saline, centrifuged and the supernatant containing the phages was assayed by soft agar overlay procedure. The phages produced plaques (the clear areas formed due to lysis of the susceptible bacterial culture) on the lawns formed by host bacterial pathogen colonies developed on CPG soft agar. The plaques are proportional to the concentration of the phage tested. Plaques were formed on the lawn of *R. solanacearum* culture at 30°C, after incubation for 24 h (Murugaiyan et al. 2010). Nine strains of *R. solanacearum*, including race 1 (biovars 3 and 4) and race 3 isolated from pepper, tomato and tobacco were used to test the host specificity of the phages obtained from the rhizospheres of the three crop plants infected by *R. solanacearum*. A filamentous phage PE26 had a relatively wide host range of bacterial hosts. PE226 and TM227 generated clear plaques on all nine strains of *R. solanacearum* tested. The morphological properties and the genomic characteristics of the two phages showed that they were probably related, although they were isolated from pepper and tomato, respectively. Both PE226 and TM227 had long, filamentous shape, which was different from other phages, infecting *Ralstonia* spp. A filamentous phage ØRSS1, infecting *R. solanacearum* strains was reported earlier by Kawasaki et al. (2007). But the genome organization of PE226 was different from that of phage ØRSS1. As the phage PE226 consistently formed clear plaques on nine strains of *R. solanacearum*, it might be a typical temperate phage carrying the properties of lysis and lysogenicity of life cycles (Murugaiyan et al. 2010).

*Ralstonia solanacearum* can survive in the soil for long time. Furthermore, *R. solanacearum* is easily disseminated via soil, contaminated irrigation water, surface water, farm equipment and infected propagules, requiring an effective management system for containing the bacterial wilt disease induced by this pathogen. Phage therapy in agricultural settings, faced two major problems: (i) extracellular polysaccharides produced by bacterial pathogens, prevents phage adsorption and (ii) levels of susceptibility of bacterial strains to phages differed significantly. Different kinds of phages that specifically infected strains of *R. solanacearum*, belonging to different races and/or biovars, were isolated and characterized. Phage ØRSA1, P2-like head-tail virus (Myoviridae) with a very wide host range infected all 15 species, strains of race 1, 3 or 4 and biovar 1, N2 or 4. Phage ØRSB1, another mycovirus, lysed 10 of 15 tested strains. Another phage ØRSB1 with T7-like morphology (Podoviridae) was able to lyse 14 of 15 strains from race 1, 3 or 4 and formed very large plaques (10–15 mm diameter) on assay plates (Yamada et al. 2007; Fujiwara et al. 2008; Kawasaki et al. 2009).

Application of bacteriophages for the management of bacterial diseases of crops appeared to be a practical proposition. The efficacy of three lytic phages ØRSA1, ØRSB1 and ØRSL1 in suppressing the development of tomato bacterial wilt disease was assessed. Infection with ØRSA1 and ØRSB1 either alone or in combination with other phages resulted in a rapid increase in the host cell density. The cells resistant to infection by these phages could be recognized at 30 h after addition of phage to the bacterial cell culture. In contrast, infection by ØRSL1, resulting in lysis of bacterial cells and lower host cell density (1/3 of control) were maintained over a long period, indicating the susceptibility of *Ralstonia solanacearum* to ØRSL1. Pretreatment of tomato seedlings with ØRSL1 phage drastically limited penetration, growth and movement of root-inoculated bacterial cells. Tomato plants treated with ØRSL1 phage did not exhibit wilting symptoms during experimental duration of 18 days, whereas the control plants without phage treatment wilted. Phage ØRSL1 was relatively stable in soil, especially at high temperatures (37–50°C). Phage could be recovered from roots of tomato plants and soil at 4 months postinoculation, indicating its persistence in plants and soil. As all *R. solanacearum* cells were not killed by ØRSL1, the coexistence of bacterial cells and phage might provide effective prevention of wilt disease incidence (Fujiwara et al. 2011). The filamentous phage ØRSM infected *R. solanacearum* and its virulence was also reduced. Inoculation of ØRSM3-infected bacterial pathogen cells into tomato plants did not induce bacterial wilt disease. But these cells enhanced expression of pathogenesis-related (PR) genes, including PR-1, PR-2b and PR-7 in tomato plants. Furthermore, pretreatment with ØRSM3-infected pathogen cells protected tomato plants against infection by virulent *R. solanacearum* strains. The effective dose of ØRSM3-infected cells for disease prevention was approximately 10⁶ CFU/ml. As the bacterial cells infected by ØRSM3 could grow and produce infections, under appropriate conditions phage particles were produced continuously. It might be possible to develop the approach of employing phages as a management strategy for tomato bacterial wilt disease (Addy et al. 2012).

Persistence of phages at high populations in the phyllosphere and rhizosphere in close proximity to the target bacterial pathogens is of critical importance, as the efficacy is significantly influenced by inoculum densities of both phages and target pathogens. In order to determine the survival of phages in the rhizosphere and translocation into stems, the phages were applied to soil surrounding tomato plants. Phages were detected in foliar plant tissues at levels as high as 10⁶–10⁷ plaque forming units (PFU)/g plant tissue in the upper
leaves and stems at two days after application. Phage populations decreased drastically and plummeted below the limit of detection by the seventh day in plants with damaged roots and by the 15th day in plants with undamaged roots. Suspension of phage specific to *Ralstonia solanacearum*, causing tomato bacterial wilt disease was applied to soil surrounding tomato plants as preinoculation and postinoculation pathogen treatments. Minimum disease reduction was observed, when the phage suspension was applied at 3 days before inoculation and at time of inoculation. Phage application was ineffective, if applied at 3 days after pathogen inoculation, indicating the lack of therapeutic activity of phage against tomato bacterial disease pathogen (Iriarte et al. 2012). *Pectobacterium* spp. cause soft rot diseases of potato and carrot. The effectiveness of bacteriophages in suppressing the development of soft rot disease was investigated. Bacteriophages capable of lysing diverse *Pectobacterium* species and isolates from plant and soil were identified. Repeated isolations from plaques resulted in the isolation of 189 single phages showing wide spectrum of host specificity. Core phages (24) were selected and a dendrogram was generated. Phylogenetic analysis based on DNA fingerprints of phage isolates indicated the extent of genetic diversity of the selected phages for investigation. The phages were stable at 16°C–40°C and pH 6.7. The viability (stability) of the phages differed significantly, depending on the phage isolate (Lee et al. 2017).

### 3.1.4.3 Cross-Protection with Mild Strains of Plant Viruses

Nonpathogenic isolates or strains of fungal and bacterial pathogens are known to protect plants against infection of virulent strains of the pathogens. The phenomenon of cross-protection involves application of mild strains of a virus to protect the plants against infection by severe strain or related virus. The mild strain-inoculated plants do not develop symptoms of infection by the severe strain of the same virus or related virus. The extent of protection depends on the relatedness of the mild strain with the severe strain. Cross-protection as a disease management strategy has been effectively applied for protection of perennial crops like citrus and annuals like tomato. Mild strains may be selected from naturally occurring strains or they may be artificially produced by exposing viruses or virus-infected plants to chemicals or unusual temperature (high or low) regimes (Narayanasamy 2002, 2013, 2017).

*Grapevine fan leaf virus* (GFLV) causes grapevine degeneration responsible for substantial losses in production. GFLV is transmitted by the soilborne ectoparasitic nematode *Xiphinema index* from vine to vine. Mild strains of GFLV and the closely related virus *Arabis mosaic virus* (ArMV) were identified by comparative performance analysis of grapevines infected with different strains and comparative severity of symptoms on *Chenopodium quinoa*, a systemic herbaceous host of GFLV (Huss et al. 1989; Legin et al. 1993). Protection with mild GFLV strains against GFLV infection on *Gomphrena globosa* was earlier reported (Bianco et al. 1988). In a later investigation, healthy scions were grafted onto rootstocks that were healthy or infected with mild protective strains of GFLV-G Hu or ArMV-Ta. Challenge inoculation with GFLV was performed using *X. index*. Development of disease symptoms on test plants was monitored over 9 consecutive years in control plants. Presence of GFLV in vines cross-protected by ArMV-Ta was verified, by employing GFLV-specific antibodies in DAS-ELISA tests. In the case of GFLV-G Hu-protected vines, GFLV infection was monitored by characterizing coat protein gene of superinfecting isolates by immunocapture (IC)-RT-PCR-RFLP analysis. Cross-protected vines had significantly reduced challenge infection rate consistently. However, fruit yield was reduced by 9% and 17%, respectively, by ArMV-Ta and GFLV-G Hu strains, but the quality of the fruit was not affected. The results indicated that cross-protection of grapevines with mild strains might not be an economically viable approach for managing the GFLV disease (Komar et al. 2008).

#### 3.1.5 MECHANISMS OF ACTION OF BIOTIC BIOCONTROL AGENTS

Soilborne microbial plant pathogens may have additional modes of dispersal, in addition to the dissemination via soil. Some of them are transmitted through seeds and propagules, irrigation water and farm equipments. The biological control agents applied for suppression of development of the pathogens and diseases induced by them have to be stable, persistent in soil environment and versatile in action against the target microbial pathogen(s). It is necessary to understand various mechanisms of action of the biocontrol agents on the pathogens and their requirements and limitations for exploiting their potential in the most effective manner for reducing disease incidence and spread within and outside the field.

##### 3.1.5.1 Fungal Biological Control Agents

Suppression of development of fungal pathogens by the biocontrol agents may be the resultant of different mechanisms of action such as parasitism, antibiosis, competition for nutrients and/or space, prevention of colonization of specific host (root) tissues by the pathogen and induction of local and/or systemic resistance to the target pathogens. Further, host plant growth may be promoted, resulting in enhancement of levels of resistance to microbial pathogens (Narayanasamy 2002, 2013). Microbial plant pathogen development may be suppressed by fungal biocontrol agents (BCAs) via three types of antagonism: (i) direct antagonism, (ii) indirect antagonism and (iii) mixed-path antagonism (Pal and Gardener 2006). The ability of the BCA to parasitize and kill the pathogen represents direct antagonism, whereas in the case of indirect antagonism, no physical contact is made by the BCA with the fungal pathogen, but the level of host resistance is enhanced by activating host defense system. Several fungal BCAs induce resistance against microbial plant pathogens. Competition between the BCA and the pathogen for space and/or nutrients may also inhibit the pathogen development indirectly by starving-out the pathogen or making required plants inaccessible. Mixed-path antagonism includes antagonistic activities based on
the ability of the BCA to produce various different kinds of enzymes, antibiotics or metabolites toxic to pathogens.

The genus *Trichoderma* includes many species found in different ecosystems. Some strains can reduce the severity of crop diseases by inhibiting the development of fungal pathogens, primarily in the soil or on plant roots through their antagonistic and mycoparasitic potential. The competitive genome sequence analysis of two important biocontrol agents (BCAs) was performed. The mechanism of mycoparasitism was studied by using *T. atroviride* and *T. virens* to understand how the BCAs suppressed the development of fungal pathogens (Kubíček et al. 2011). The presence of fungal pathogens as the prey and availability of root-derived nutrients are the factors that facilitate the establishment of *Trichoderma* spp. in the rhizosphere. The ability of *Trichoderma* spp. to suppress a broad range of plant pathogens, including oomycetes, fungi, bacteria and viruses, through elicitation of induced systemic resistance (ISR) or localized resistance has been revealed by various investigations. Furthermore, some *Trichoderma* rhizosphere-competent strains are able to promote the growth and yield of crop plants through enhancement, nutrient use efficiency, seed germination and stimulation of plant defenses against biotic and abiotic damage (Shoresh et al. 2010). *Trichoderma* strains are present in the rhizospheres of many plants and plant-derived sucrose is an important resource available to *Trichoderma* spp., facilitating root colonization, coordination of defense mechanisms and increased rate of leaf photosynthesis. Adherence of *Trichoderma* to the root surface may be mediated by hydrophobins, which are small hydrophobic proteins of the outer-most cell wall layer that coat the fungal cell surface and expansin-like proteins related to cell wall development. *Trichodermaasperellum* produces class I hydrophobin Tas Hyd1, which supports colonization of plant roots, possibly by enhancing its attachment to the root surface and protecting the hyphal tips from plant defense compounds (Viterbo and Chet 2006). Plant cell wall-degrading enzymes are also involved in active root colonization, as it occurs with endopolygalacturonase, the PG1 from *T. harzianum* (Morán-Diez et al. 2009). The proteome analysis showed that a small secreted cysteine-rich protein (SSCP) was present in *T. harzianum* and *T. atroviride* and it was a homologue of the avirulence protein Avr4 from *Cladosporium fulvum*. It is possible that binding of Avr4 to chitin could protect *Trichoderma* against plant chitinases (Stergiopoulos and de Wit 2009).

3.1.5.1.1 Mycoparasitism

Parasitization of fungal pathogen by fungal biocontrol agents (BCAs) is required for obtaining nutrition from the host pathogen. Among fungi that have potential for use as biocontrol agents, *Trichoderma* spp. appears to be the leader, as reflected by the availability of more than 50 different *Trichoderma*-based agricultural products registered in different countries for use to protect and improve yield of vegetables, ornamentals and fruit trees (Woo et al. 2006). Strains of *Trichoderma* secrete various enzymes and antimicrobial compounds. *T. harzianum* (Th) produces trichodermin and a small peptide that inhibit *Rhizoctonia solani*, which in turn, secretes a coumarin-derivative, capable of inhibiting the mycelial growth of *T. harzianum*. However, the antifungal compound produced by *T. harzianum* is a more powerful inhibitory compound, effective even at low concentration than those produced by *R. solani* (Bertagnolli et al. 1998). Several fungal BCAs like *Trichoderma virens* function as aggressive mycoparasites penetrating the hyphae, as well as resting bodies (sclerotia), reducing the survivability/viability of the pathogen in the soil. Destruction of sclerotia by *T. virens* is likely to result in reduction in the inoculum potential of *R. solani* (Howell 1987). Penetration of hyphae of *Rhizoctonia solani* by haustoria of *T. virens* could be visualized under light microscope (Howell 2003). *Coniothyrium minitans* (Cm) is a mycoparasite of *Sclerotinia sclerotiorum* (S). The effect of oxalic acid (OA) degradation on the β-1,3-glucanase activity of *C. minitans* involved in the mycoparasitism was assessed. OA degradation by *C. minitans* to an extent of 86 to 92% at 20°C on potato dextrose broth (PDB) medium and the pH of cultures was increased from 3.4–4.8 to 8.3–8.6. The spread of *C. minitans* toward the colonies of *S. sclerotiorum* was correlated with increase in the ambient pH from 2.9 to 6.6. OA degradation was correlated with enhanced production of β-1,3-glucanase by *C. minitans* and the stimulated activity of this enzyme. Degradation of OA by *C. minitans* might also be a mechanism by which the BCA might protect host plants, in addition to parasitism of *S. sclerotiorum* (Ren et al. 2007). In a later investigation, amendment of synthetic oxalate in PDA (0.25–2.0/g) suppressed the aggressiveness of parasitism by *C. minitans* on colonies of *S. sclerotiorum* strain PB. The results suggested that infection of hyphae of *S. sclerotiorum* was negatively affected by the presence of oxalate. The role of oxalate degradation by *C. minitans* in its mycoparasitism on *S. sclerotiorum* provided a key clue for improvement of the biocontrol potential of *C. minitans* (Huang et al. 2011).

*T. harzianum* antagonistic to *Phytophthora capsici*, either alone or in combination with a compatible bacterial BCA, *Streptomyces rochei* was evaluated for the efficacy in suppressing the development of the Phytophthora blight in pepper. *T. harzianum* not only arrested the spread of mycelial growth of *P. capsici* in the petridish, but also invaded the whole surface of the pathogen colony and sporulate over it. The hyphae of *P. capsici* were surrounded by those of *T. harzianum*, resulting in their subsequent disintegration and eventual suppression of the growth of *P. capsici*, as revealed by observation under scanning electron microscope (SEM). By contrast, *S. rochei*, secreted an antifungal compound (1-propanone-4-chlorophenyl), primarily responsible for its biocontrol activity against *P. capsici* (Ezziyyani et al. 2007). In a later study, mycoparasitism of *Sclerotinia sclerotiorum* by *T. harzianum* was investigated by nucleic acid-based techniques to detect and quantify the genomic DNAs of both the BCA and pathogen. Sclerotia of *S. sclerotiorum* were incubated on *T. harzianum* culture. Germination of sclerotia by producing mycelium was reduced by 50% within one day, and the decrease in germination continued with increase in interval after incubation. Quantification of *Sclerotinia* DNA in older sclerotia by qPCR assay revealed a decrease in the genomic
DNA, indicating decrease in pathogen population. In contrast, the Trichoderma DNA increased, and the increase persisted in the older sclerotia, reflecting the higher BCA population. Fresh sclerotia did not appear to be affected by T. harzianum (Kim and Knudsen 2009). The mechanism of antagonism of T. harzianum (Ths97) against Fusarium solani (Fso14), causing Fusarium root rot of olive trees was investigated. Optical microscopic examination of the confrontation zone in petriplates used for dual culture procedure showed the strain Ths97 grew alongside Fso14 with numerous contact points, suggesting parasitic activity on the pathogen. The Ths97 strain exhibited a strong protective role against root infection by F. solani Fso14 whether inoculated before or after inoculation with the pathogen (Amira et al. 2017).

The mechanism of antagonism of Trichoderma atroviride against Rhizoctonia solani AG3, causal agent of potato black scurf disease was studied, employing confocal microscopy. The mycelium of T. atroviride established close contact with those of R. solani by coiling. The coils were very dense encircling the pathogen hyphae very tightly. At 7 days after establishing contact, the hyphae of the BCA penetrated R. solani hyphae, resulting in loss of turgor (Lahlali and Hijri 2010). Another fungal BCA, Trichoderma asperellum showed antagonistic activity against Rhizoctonia solani in confrontation assay. The effects of mitogen-activated protein kinase-encoding gene task1 on morphological development, mycoparasitic interaction, production of cell wall-degrading enzymes and secondary metabolites were investigated in T. asperellum. The Atask1 mutant of T. asperellum showed altered growth morphology and lost its ability to parasitize R. solani. The mutant also showed increased expression of several cell wall-degrading enzymes during confrontation with R. solani. T. asperellum task1 expression was negatively correlated with cell wall-degrading enzyme activities during inducing assays. In antibiosis assays, task1 gene deletion enhanced output of 6-pentyl-α-pyrone and inhibition of pathogen growth (Yang 2017).

Acremonium strictum is a mycoparasite of Helminthosporium solani, causing silver scurf disease of potato tubers. Both A. strictum and H. solani invariably occurred together in the tubers. Axenic culture of H. solani was obtained by repeated hyphal tip isolation. A. strictum was tightly linked to and partially dependent on H. solani in culture. It appeared that A. strictum was dependent on H. solani for its survival and for its growth in culture. However, growth and sporulation and germination of H. solani was reduced in the presence of A. strictum. Observations under scanning electron microscope (SEM) showed shrunken and shrunken conidia of H. solani, when present along with A. strictum. The adverse effects of A. strictum on H. solani might be due to either direct parasitism or inhibition due to antifungal compounds secreted by A. strictum (Rivera-Varas et al. 2007). The mechanism of biocontrol activity of Pythium oligandrum against Rhizoctonia solani AG-3, causing potato black scurf was investigated. Seed tubers infected by R. solani were dipped for a few seconds in a suspension of P. oligandrum oospores, followed by air-drying. Confocal laser scanning microscopic examination with an immuno-enzymatic staining technique revealed that the hyphae of P. oligandrum colonized the sclerotia of R. solani and established close contact by coiling around hyphae of the pathogen present on the surface of seed tubers, in a manner similar to that was observed in dual culture assay. Quantification of R. solani DNA on seed tubers by PCR showed that R. solani population was reduced on seed tubers treated with P. oligandrum, compared with untreated control tubers (Ikeda et al. 2012).

Molecular biology of mycoparasitic interaction between the fungal pathogen and biocontrol agents has been studied in some pathosystems. The role of cell wall-degrading enzymes (CWDEs) and antibiotics in mycoparasitism of BCAs was investigated. The strain P1 of T. harzianum was genetically modified by target disruption of the single copy of ech-42 gene encoding for the secreted 42-kDa endochitinase (CHIT-42). The stable mutants lacked the ech-42 transcript, the protein and endochitinase activity in culture filtrates. Other chitinolytic and glucanolytic enzymes expressed during mycoparasitism were not affected by disruption of ech-42. The mutant was as effective as P1 strain against Pythium ultimum, whereas its effectiveness against the foliar pathogen Botrytis cinerea on bean leaves, was reduced by 33%. However, the endochitinase deficient mutant was more effective against another soilborne pathogen Rhizoctonia solani than the wild-type P1 strain. The results indicated that the biocontrol activity of T. harzianum might be significantly influenced by the nature of fungal pathogen involved in the interaction (Woo et al. 1999). The activities of several cell wall-degrading enzymes including proteases, chitinases and glucanases of the BCAs may be required for mycoparasitism of the fungal pathogens. Purified host cell walls, compounds secreted by hosts and also live host may stimulate the expression of the genes encoding these enzymes. Such enhanced gene expression may improve the biocontrol potential of the BCAs. Expression of novel genes in Trichoderma hamatum effective against Sclerotinia sclerotiorum, S. minor, Rhizoctonia solani and Pythium spp., causing diseases in various crops, was investigated, by applying southern subtractive hybridization (SSH) technique. The homologues of chit42 and prb1, two genes considered to be essential for mycoparasitism in other Trichoderma spp. were expressed at higher levels by T. hamatum in medium containing glycerol differed significantly from T. atroviride, suggesting that substantial differences might exist in mycoparasitism in these two Trichoderma spp. The sequence, Northern and Southern analyses of the subtraction products revealed 19 novel T. hamatum genes upregulated during mycoparasitism, representing a substantial increase in the number of T. hamatum genes. Four sequences had no significant similarity to any sequences in the GenBank and they may be perhaps restricted to mycoparasites to facilitate mycoparasitism. The SSH technique was found to be useful for identifying genes upregulated during mycoparasitism (Carpenter et al. 2005). Trichoderma spp. with significant biocontrol potential against Sclerotinia sclerotiorum, causal agent of carnation stem rot disease, were identified as T. asperellum (NVTA1, NVTA2), T. harzianum (NVTH1, NVTH2), T. citrinoviride (NVTC1,
NVTC2) and *T. erinaceum* (NVTE1). Presence of both cellobiohydrolase (*cbh1*) and endochitinase (*ech42*) genes was detected in NVTA2 strain of *T. asperellum*. The crude metabolite from NVTH2 inhibited the mycelial growth of *S. sclerotiorum* most effectively (Vinodkumar et al. 2017).

Mycoparasitism of *Sclerotinia sclerotiorum* by *T. harzianum* was investigated, using green fluorescent protein (GFP)-expressing *T. harzianum* ThzID1-M3 mutant. A specific PCR primer 1 probe set for detecting the GFP-expressing isolate was employed for monitoring its presence. Quantitative real-time PCR along with epifluorescence microscopy and image analysis was applied to study the dynamics of colonization of sclerotia in nonsterile soil. It was possible to quantify the amounts of ThzID1-M3 DNA and wild-type *S. sclerotiorum* DNA from individual sclerotia using real-time PCR assay. Epifluorescence from transformants was quantified using computer image analysis for estimating colonization on a per-sclerotium basis. Colonization of sclerotia by *T. harzianum* on agar plates was observed, using confocal laser scanning microscopy (CLSM) to detect the GFP-fluorescing hyphae of ThzID1-M3 mutant. This method, although highly labor intensive, provided high spatial resolution of colonization dynamics. Both techniques quantified colonization of sclerotia by *T. harzianum* over a period of time. The real-time PCR provided a more precise assessment of the extent of sclerotial colonization by the BCA and it could be more easily applied to sample entire sclerotia (Kim and Knudsen 2011). *T. harzianum* effectively suppressed the development of bean foot rot disease caused by *Fusarium solani* through mycoparasitism. A transcriptome analysis was performed, using expressed sequence tags (ESTs) and quantitative real-time PCR (RT-qPCR) in order to study the mechanism of disease suppression by *T. harzianum*. A cDNA library from *T. harzianum* mycelium (ALL-42 isolate) grown on cell walls of *F. solani* (CEF) was constructed and analyzed. High quality sequences 2,927 of 3,845 were selected and 37.7% were identified as unique genes. The ontology analysis indicated that majority of the annotated genes were involved in metabolic processes (80.9%), followed by cellular processes. Twenty genes that encoded proteins with potential role in biological control were investigated. RT-qPCR analysis showed that none of these genes were expressed, when *T. harzianum* was challenged with the pathogen. These genes showed different patterns of expression during in vitro interaction between *T. harzianum* and *F. solani* (Steindorff et al. 2012).

### 3.1.5.1.2 Antibiosis

The fungal biocontrol agents (BCAs) may produce different kinds of enzymes and antimicrobial compounds that may facilitate their successful establishment in the soil environment to interact with the microbial plant pathogens. Some BCAs may suppress the pathogen development via more than one mechanism. Mycoparasitism and antibiotics may overlap and one mechanism may operate predominantly, depending on the pathogen, substrate or environment prevailing in the soil. Different steps in the process of parasitism, such as recognition of the host, attachment and subsequent penetration and killing of the host cells have been recognized. During parasitism, *Trichoderma* spp. secrete hydrolytic enzymes that hydrolyze cell wall of the host (pathogen) (Woo et al. 2006). The proteolytic activity of *T. harzianum* preceded lysis of the protein matrix of the pathogen cell and for inactivation of the hydrolytic enzymes secreted by the pathogen, resulting in decrease in its pathogenicity. It is possible to select the isolate(s) of the BCA with greater potential for secretion of hydrolytic enzymes from different natural sources such as compost or disease suppressive agricultural soil or through transformation of the BCA isolate with multiple copies of the genes involved in the biosynthesis of the hydrolytic enzymes (Narayanasamy 2013).

*T. harzianum* inhibited the mycelia growth of *F. oxysporum* f.sp. *lycopersici* (Fol), causing Fusarium wilt disease in tomato. The culture filtrate (CF) with volatile and nonvolatile metabolites of *T. harzianum* inhibited all isolates of *Fol* tested (Mishra et al. 2010). Mechanisms of biocontrol activity of strains of *Trichoderma* sp. against *Sclerotiorum rolfsii* and *F. oxysporum* f.sp. *ciceris* were investigated. The BCA strains were plated on media amended with colloidal chinin and cell wall extracts of *S. rolfsii*. Chitinolytic activity was detected in all isolates of *Trichoderma* sp. tested. Two strains, with high activities of endochitinase and exochitinase, produced cellulase which might also contribute to the biocontrol potential of *Trichoderma* sp. (Anand and Reddy 2009). *Trichothecium roseum* MML003 with strong suppressive activity against rice sheath blight pathogen *Rhizoctonia solani* did not have either mycoparasitic activity or ability to produce siderophores and *H₂O₂*. The culture filtrate (CF) of *T. roseum* inhibited the mycelia growth and formation of sclerotia by *R. solani*. Sclerotial germination and viability were also substantially reduced by treatment with CF of *T. roseum*. Application of *T. roseum* resulted in significant suppression of sheath blight development under greenhouse conditions. Antifungal compounds produced by *T. roseum* might be involved in arresting the development of rice sheath blight disease (Jayaprakashvel et al. 2010).

The fungal BCAs may vary in the range of fungal pathogens against which they are effective. Isolates of *Trichoderma harzianum* have been shown to be effective against a wide spectrum of fungal pathogens, including *F. oxysporum* f.sp. *melonis*, causing melon Fusarium wilt disease. Isolates (31) of *Trichoderma* sp. were analyzed by random amplified polymorphic DNA (RAPD)-PCR technique. Five most effective isolates of *T. harzianum* (T-30, T-31, T-32, T-57 and T-78) were characterized by their ability to secrete hydrolytic enzymes viz., chitinases, glucanases and proteases. In the plate cultures, the greatest mycoparasitic activity was exhibited by the isolates T-30 and T-78, as reflected by the total and extracellular hydrolytic activities of N-acetyl glucosaminidase (NAGases), chitinase and β-1,3-glucanase, which were greater than other isolates tested. The expression of genes encoding for NAGases (exc1 and exc2) or glucanases (bgn13.1) activities and their respective enzyme activities in vitro were measured. Different profiles of gene expression between various *T. harzianum* isolates were related to the activity values and dual
plate confrontation test. The high NAGase activity detected in T-30 and T-38 corresponded to the levels of expression of the gene *excl* for T-30, but not for T-78. The high NAGase activity of the isolate T-28 might be due to a higher expression of *excl* over previous hours before sampling. The high chitinase activity of T-78, both total and extracellular, could be linked to the levels of expression of genes *chit42* and *chit33*, as both were highest for this isolate. These two isolates exhibited the maximum activity of β-1,3-glucanase. These corresponded with the expression level of gene *bgn13.1* for T-30. The isolates T-30 and T-78 exhibited the greatest mycoparasitic potential against *F. oxysporum* f.sp. *melonis* (López-Mondejar et al. 2011).

Molecular mechanism of biocontrol activity of *Trichoderma virens* against *Rhizoctonia solani* was investigated. The gene encoding for chitinase (*chit42*) in *T. virens* was disrupted or overexpressed. Decrease or increase in biocontrol activity of the transformants matched with the disruption or overexpression of *chit42* gene in the cotton-*Rhizoctonia solani* pathosystem. As the differences in the biocontrol activity of transformants and wild-type strain were less, it might be possible for the involvement of other factors in the biocontrol efficiency of *T. virens* against *R. solani* (Baek et al. 1999). Variable results on the role of chitinase in the biocontrol potential of *T. harzianum* were obtained depending on the BCA-pathogen combination. Disruption of *ech42* of *T. harzianum* did not alter the biocontrol activity against *Pythium ultimum*, but the biocontrol activity was greater in the transformants than in the wild strain of *T. harzianum* against *R. solani* (Woo et al. 1999). Investigations on molecular genetics of the fungal BCAs, were focused on the role of genes encoding the enzymes involved in the biocontrol activity. Identification of enzymes required for biocontrol activity, was difficult due to redundancy of the cell wall-degrading enzymes (CWDEs) encoding genes in the genome of *Trichoderma*. *T. longibrachiatum* was effective in suppressing development of damping-off disease caused by *Pythium ultimum*. *T. longibrachiatum* was transformed with the gene *egl1*, encoding for β-1,3-glucanase. The transformants overexpressing *egl1* were slightly more effective in reducing the disease incidence in cucumber than the wild-type strain. As the antagonistic efficiency was not enhanced in the transformants, it is likely that biosynthesis of several CWDEs might be necessary to increase the biocontrol efficiency to significant levels (Migheli et al. 1998). The effectiveness of *T. harzianum* in suppressing development of many fungal pathogens such as *Rhizoctonia solani* and *Sclerotinia sclerotiorum* might be due to the release of lytic enzymes primarily chitinases, glucanases and proteases in susceptible hosts (Chet and Chernin 2002). The involvement of proteases in the biocontrol activity of *Trichoderma* sp. was indicated. *T. harzianum* T334 produced low levels of protease constitutively. Mutants of T334 were generated by UV-irradiation. Some mutants of T334 were more effective against *P. debaryanum* and *R. solani* than the wild-type strain. The mutants were able to produce greater amounts of extracellular trypsin- and chymotrypsin-like proteases with manifold levels of activities of the wild-type strain T334 (Sezekeres et al. 2004). The aspartic proteases were reported to have a major role in the biocontrol potential of *Trichoderma*. The gene SA76, encoding an aspartic protease, was cloned for 3′ rapid amplification of cDNA ends from T88. The Northern blot analysis indicated that SA76 was induced in response to different fungal cell walls. Analysis of SA76 expression confirmed that aspartic protease activity was induced in a simulated parasitism by the presence of cell walls of *R. solani*, *Phytophthora sojae*, *F. oxysporum* and *Sclerotinia sclerotiorum*. The enhanced activity was due to induction at the transcription level, because the transcripts accumulated abundantly shortly after induction (Liu and Yang 2007).

Involvement of endochitinase of fungal BCAs, as a mechanism of biocontrol activity, has been demonstrated. The effectiveness of a 42-kDa endochitinase encoded by *Tho*42 gene from *T. harzianum* against *Rhizoctonia solani* AG-8 and/or *R. oryzae*, causing barley root rot was assessed. Purified endochitinase strongly inhibited both *R. solani* and *R. oryzae*. By contrast, the endochitinase showed only moderate level of inhibition against *Gaemumonomycetes graminis* var. *tritici* (Ggt), causal agent of wheat take-all disease and it was ineffective against *Fusarium graminearum*, *F. pseudogrimearum* and *F. culmorum*, causal agents of wheat head blight disease (Wu et al. 2006). Proteomic, genomic and transcriptomic methods were applied for the isolation and characterization of a novel plant cell wall (PCW)-*Trichoderma* gene coding for a plant cell wall-degrading enzyme (CWDE). A proteomic analysis, using a three-component (*Trichoderma* spp.-tomato-pathogen) system facilitated the identification of a differentially expressed *T. harzianum* endopolygalacturonase (endo-PG). A specific spot (0303) remarkably increased only in the presence of *R. solani* and *Pythium ultimum* and corresponded to an expressed sequence tag (EST) from a *T. harzianum* T34 cDNA library that was constructed in the presence of PCW polymers and used to isolate the *Thpgl* gene. The *Thpgl*-silenced transformants had lower PG activity, less growth on pectin medium and reduced capacity to colonize tomato roots. The results were confirmed by real-time PCR assay, which revealed the presence of a pathogen in the system triggering the expression of *Thpgl* (Morán-Díez et al. 2009).

A nonpathogenic strain of *F. oxysporum* S6 isolated from soil suppressive to *Sclerotinia sclerotiorum* exhibited antagonistic activity in dual culture assay. The toxic nonvolatile metabolic compounds from S6 strain were isolated by chromatographic technique. After purification, they were identified as cyclosporine A by spectroscopic methods. The antibiotic inhibited mycelial growth and suppressed sclerotal formation. The antifungal activity against *S. sclerotiorum* was correlated with the presence of cyclosporine A by dilution plate method. When the sclerotia were placed at the center of BCA colony, the percentage of germination of sclerotia was significantly reduced due to infection of sclerotia by the BCA. In the greenhouse assessment, the number of surviving soybean plants significantly increased, when the BCA and the pathogen were coinnoculated. The results indicated that the antifungal activity of *F. oxysporum* S6 against *Sclerotinia*
sclerotiorum was primarily due to secretion of cyclosporine A by the BCA (Rodriguez et al. 2006). Similar association of antagonistic activity of Trichoderma spp. against S. sclerotiorum infecting potato with nonvolatile toxic compounds was reported by Ojaghian (2011). T. harzianum T23 produced viridofungin A (VFA) in culture. Bioautography assay showed that three fractions F223, F323 and F423 were produced by T23 strain, whereas the T16 strain formed two fractions F416 and F516 in culture. VFA appeared to have wider antifungal activity against Verticillium dahliae, Phytophthora infestans and Sclerotinia sclerotiorum. VFA was fungistatic, rather than fungicidal (El-Hasan et al. 2009).

Mycofumigation with antimicrobial volatiles produced by fungal BCAs has been applied for suppression of fungal pathogens. The fungi Muscodor albus and M. roseus were employed for mycofumigation to enhance sugar beet stand and to decrease severity of diseases caused by Rhizoctonia solani, Pythium ultimum and Aphanomyces cochlioides. Five classes of compounds viz., alcohols, esters, ketones, acids and lipids were the key components of the mycofumigant gas volatiles. These compounds were tested either singly or as mixtures in vitro against Pythium ultimum, F. oxysporum f.sp. betae, Rhizoctonia solani, Phytophthora cinnamomi, Verticillium dahliae and Sclerotinia sclerotiorum. No single class of the natural volatiles from M. albus was toxic individually to the test pathogens. The most effective single compound belonged to the esters group (Strobel et al. 2001). In a later investigation, the efficiency of five different formulations containing M. roseus was assessed for the control of sugar beet Pythium damping-off and eggplant Verticillium wilt diseases. The Stabileze formulation was effective in reducing consistently disease severity and population of Verticillium dahliae in vivo. The results indicated that mycofumigation efficacy could be maximized by selecting an appropriate formulation (Stinson et al. 2003). The efficacy of Muscodor albus in suppressing the development of Rhizoctonia solani in greenhouse soilless-growing mix was assessed. The treatment showed only local effect essentially, indicating the inability of volatiles to move through the growing mix. The temperature range of 4–22°C was suitable for fumigation activity of M. albus. The ability of M. albus to suppress damping-off disease developing in broccoli seedlings declined rapidly after its incorporation in the growing mix. In treated mix, disease incidence remained at low levels, regardless of planting time after treatment, suggesting that biofumigation could eliminate R. solani effectively. In the case of pepper, M. albus provided high level of protection against Phytophthora capsici. Improved growth of pepper was attributed to the suppression of other deleterious microorganisms that often contaminate commercial growing mix (Mercier and Mankan 2005). In a later investigation, 12 isolates of M. albus were found to produce volatile compounds that were biologically active (Strobel et al. 2007).

Muscodor albus strain MFC2 was evaluated for its efficacy in protecting kale (Brassica oleracea) against Pythium ultimum, causing damping-off disease under greenhouse conditions. Kale seeds were sown in soils infested with P. ultimum, followed by inoculation with M. albus culture. Seedling emergence in pots inoculated with the BCA and pathogen was equal to a level close to that in the control without the pathogen. The volatiles from M. albus appeared to have no harmful effect on plant development and the biocontrol activity of M. albus might be due to volatiles from the BCA (Worapong and Strobel 2009). Results of another investigation showed that volatiles from M. albus suppressed the development of damping-off of broccoli seedlings, when pots containing soil or soilless potting mix infested with Rhizoctonia solani were placed in the presence of active M. albus culture without physical contact in closed containers. Gas chromatographic analysis revealed that isobutyric acid and 2-methyl-1-butanol were released from the treated soil/substrates. Production of isobutyric acid showed positive correlation with the extent of disease control. Amounts of isobutyric acid released from the soil were several folds greater than that released from potting mix. In addition, higher populations of the BCA were required to achieve effective control of damping-off disease in soilless potting mix than in soil, suggesting that soil environment was better for the biological activity or viability of M. albus than the soilless potting mix (Mercier and Jiménez 2009). The endophyte Oidium sp. isolated from Terminalia catappa (tropical chestnut) effectively suppressed the mycelial growth of Pythium ultimum. The BCA produced primarily esters of propionic acid, 2-methyl-y-butyric acid and 3-methyl-y-butyric acid. Addition of exogenous volatile naphthalene-1-oxybis caused substantial synergistic increase in the antibiotic activity of the volatile organic compounds (VOCs) of Oidium sp. against P. ultimum. The development of the pathogen was entirely arrested, resulting in ultimate death of the pathogen. The results suggested that the VOCs of different endophytic fungi might act both additively and synergistically to suppress the development of different pathogens colonizing the same plant species (Strobel et al. 2008).

Different mechanisms may operate in the interaction between T. harzianum strain SQR-T037 and F. oxysporum f.sp. cucumerinum, causing Fusarium wilt in cucumber continuous cropping (CCC) system. The allelochemicals exuded from cucumber cause stress and these chemicals have to be biodegraded for better growth of cucumber plants. The allelochemicals isolated from cucumber rhizosphere included 4-hydroxy-benzoic acid, vanillic acid, ferulic acid, benzoic acid, 3-phenylpropionic acid and cinnamic acid. The allelochemicals were entirely degraded by SQR-T037 after 170 h of incubation. Inoculation of SQR-T037 in the CCC soil also led to degradation of allelochemicals exuded from cucumber roots. The degradation of allelochemicals was accompanied by significant decrease in disease index and increase in dry weights of cucumber plants in pot experiments, following application of T. harzianum. The results indicated that allelochemicals and allelopathic stress could be attributed to the activity of the BCA strain (Chen et al. 2011). In the further investigation, the allelopathic stress was characterized. One allelopathic compound was purified and identified as 6-pentyl-α-pyrone (6PAP), using mass spectrometry and nuclear magnetic resonance
spectrum. The antifungal activity of 6PAP at different concentrations (50, 150, 350 and 450 mg/l) was assayed using growth inhibition tests in petriplates. Antifungal activity increased with increase in concentrations of 6PAP. At 350 mg/l, 6PAP inhibited mycelial growth and spore germination by 73.7% and 79.6%, respectively, compared with control. In addition, 6PAP at 150 mg/l decreased sporulation and fusaric acid production by Foc by 88% and 52.68% respectively. Application of 6PAP to cucumber continuously cropped soil reduced pathogen population by 41.2% and the incidence of cucumber Fusarium wilt disease by 78 to 89.6%, in addition to stimulation of cucumber plant growth due to elimination of allelochemicals from soil (Chen et al. 2012).

3.1.5.1.3 Competition for Nutrients and Space

A biocontrol agent, to be effective in suppressing soilborne pathogens, should have rhizosphere competence (the ability to grow in the rhizosphere) and compete with other microorganisms, including microbial pathogens for nutrients and space. Three-way interactions among plants, pathogens and BCAs are complex and variable, depending on the environment existing in the soil and microclimate around the plants. Competition and rhizosphere competence may result in replacement of endogenous fungi on root surface. Trichoderma sp could suppress the growth of endogenous fungi on agar medium and mask the presence of these fungi. T. virens-treated root segments taken from soil heavily infested with propagules of Macrophomina phaseolina, when plated on agar medium yielded T. virens from the root tissues at room temperature. But at 40°C, the BCA did not grow, the pathogen readily developed, because of its tolerance to higher temperature. It was not possible to detect the BCA without suppressing the development of the pathogen (see Figure 3.7) (Howell 2003).

Several nonpathogenic strains of F. oxysporum, that have an important role in microbial ecology of soil, have been isolated from soil, particularly from soils naturally suppressive to diseases caused by soilborne pathogens. Soils suppressive to Fusarium wilts supported large populations of nonpathogenic Fusarium spp. of which F. oxysporum strain Fo47 has been shown to be efficient biocontrol agents. In order to generate mutants, insertional mutagenesis was employed to tag genes involved in the biocontrol activity of F. oxysporum Fo47. The mutants of Fo47 were evaluated for their antagonistic activity against F. oxysporum f.sp. lini, causing linseed wilt disease. The biocontrol activity of Fo47 was primarily due to competition with the pathogen involving their saprophytic ability. The mutants were characterized by their saprophytic traits. Mutants 83 and 94, the most significantly effective in their biocontrol activity, had the same ability to grow and elongate on MMA-nitrate medium, as the wild-type strain Fo47. The mutants 83 and 94 exhibited significant differences with respect to their antagonistic ability. Mutant 83 inoculated in the ratio of 10:1 was as effective as the parent Fo47 strain inoculated in the ratio of 1,000:1, whereas mutant 94 inoculated in the ratio of 1,000:1 was no more effective than strain Fo47 inoculated in the ratio of 10:1. The results indicated that the mutants were not impaired in saprophytic phase. As the mutants were either less or more antagonistic than the wild-type strain, the biocontrol activity of the strains Fo47 was not dependent entirely on its saprophytic ability of the nonpathogenic Fo47 strain (Trouvelot et al. 2002).

_Fusarium oxysporum_ F2, a nonpathogenic strain, effectively suppressed the development of symptoms of Verticillium wilt disease induced by *Verticillium dahliae* in eggplant under greenhouse and field conditions. Parasitism or antibiosis was not involved in the interaction between the pathogen and the biocontrol agent (BCA). In order to investigate the mechanism of biocontrol activity, the pathogen strain and BCA F2 strain were transformed respectively with the EGFP and DsRed2 reporter genes to facilitate visualization of their presence on the root surface of eggplant. In addition, the real-time PCR analysis was applied to monitor the ramification of both fungi into the vascular system. The strain F2 colonized the root surface along the intercellular junctions excluding _V. dahliae_ from the same ecological niche. The QPCR analysis also showed that application of F2 reduced the levels of _V. dahliae_ vascular colonization, as well as disease severity. The results of the split-root experiment showed that the strain F2 did not trigger the defense mechanisms of eggplant against _V. dahliae_. The results seemed to support the view that the mechanism of biocontrol activity of the strain F2 against _V. dahliae_ was through competition for space or nutrients on the root surface of eggplants (Pantelides et al. 2009). In a further study, the F2 strain of _F. oxysporum_ was applied by stem injection of a conidial suspension, as root drenching might adversely affect the native beneficial microbial community. Stem injection of the strain F2 at 7 days prior to transplanting the seedlings on soil infested with _V. dahliae_ microsclerotia, resulted in reduced disease severity, compared to untreated control plants. Ramification of F2 into the plant vascular system of eggplant stems was visualized by inoculating an EGFP-transformed F2 strain. The transformed F2 strain colonized the plant vascular tissues effectively over a long period of time as determined by the levels of DNA.
QPCR analysis showed that application of F2 strain reduced significantly the DNA contents of V. dahliae in stem tissues, compared to the untreated control plants (Gizi et al. 2011).

3.1.5.1.4 Prevention of Colonization of Host Tissues by Pathogens

In some interactions between soilborne microbial pathogens and biocontrol agents (BCAs), the biocontrol activity may be due to prevention of the pathogen gaining access to the susceptible host (root) tissues. Treatment of cotton seeds with Trichoderma (Gliocladium) virens reduced colonization of cotton roots by Rhizoctonia solani, causing root rot disease, resulting in reduction in disease incidence and severity (Howell and Stipanovic 1995). Maintenance of adequate population levels of BCAs at target sites and timing of their application contribute significantly to the effectiveness of biocontrol of soilborne pathogens causing root diseases. The nonpathogenic strain Fo47 of F. oxysporum protected tomato roots against infection by F. oxysporum f.sp. radicis-lycopersici (FORL) causing tomato foot and root rot (TFRR) disease. When tomato seedlings were planted in sand amended with spores of Fo47, hyphae attached to the roots earlier than FORL, whereas root colonization by the pathogen was arrested at the stage of initial attachment to tomato roots. The percentage of spores of Fo47 germinating in the tomato root exudates in vitro was higher than that of FORL. By using different autofluorescent proteins as markers and observing under confocal laser scanning microscope, the pathogen and the BCA could be visualized simultaneously on tomato roots and colonization of root surface by them was quantified. The preferential germination of Fo47 spores in root exudates components was considered to reduce pathogen growth toward roots and consequently to reduce the number of FORL for attachment sites on roots (Bolwerk et al. 2005).

The rhizosphere competence and capacity to adapt and maintain adequate population of the biocontrol agent in the rhizosphere, in addition to timing of application of the BCA, are important requirement of the BCA for providing effective protection to host plants against soilborne pathogens like Rhizoctonia solani, causing stem and root disease of poinsettia. One application of the binucleate Rhizoctonia (BNR) isolate was not effective in suppressing the development of stem rot of poinsettia. In contrast, one application of BNR isolate, after transplanting rooted poinsettia was, was more effective than the bacterial BCA Burkholderia cepacia. Root colonization by BNR isolate reached the maximum, when the B. cepacia was applied at propagation, followed by BNR application after transplantation. Both BNR isolates and B. cepacia were good colonizers of poinsettia roots and maintained initial high population levels up to 5 weeks, after application (Hwang and Benson 2002). In a later investigation, BNR isolates were found to effectively protect cotton against soilborne pathogens. The BNR isolates could be consistently recovered from hypocotyls and roots of soybean, indicating that colonization of root tissues was associated with the suppression of development of R. solani, infecting soybean plants (Khan et al. 2005). The yeast species Candida valida, Rhodotorula glutinis and Trichosporon asahii showed significant biocontrol potential against R. solani, causing postemergence damping-off of sugar beet seedlings. Root colonization in plant assay indicated that C. valida and T. asahii colonized 95% of roots of sugar beet at 5 days after application, whereas R. glutinis colonized 90% of the roots after 8 days. All yeast species were present at all depths of rhizosphere soils adhering to the tap roots up to 10 cm. The yeast species had high levels of rhizosphere competence, as reflected by the extent of their colonization of roots. In addition, the yeast BCAs promoted plant growth, when applied individually or in combination (El-Tarbily 2004).

Naturally occurring root endophytic fungi, such as Heteroconium chaetospora and Phialocephala fortinii suppressed the development of Verticillium wilt disease of eggplant. Colonization patterns of P. fortinii and a dark septate endophytic (DSE) fungus were investigated for their antagonistic potential against Verticillium longisporum, causing Verticillium yellow disease in Chinese cabbage. Hyphae of P. fortinii and DSE taxon extensively colonized roots of Chinese cabbage seedlings without inducing visible symptoms. Hyphae of P. fortinii grew along the surface of roots and formed microsclerotia on or in the epidermal layer, whereas the hyphae of the DSE taxon heavily colonized some root cortical cells. P. fortinii suppressed the effects of postinoculated virulent strains of Verticillium in vitro. The DSE taxon was able to colonize Chinese cabbage roots and suppressed the development of Verticillium yellows. The protective values of DSE taxon against the disease were significantly higher, compared to other fungal endophytes as reflected by higher marketable value of the produce obtained from DSE taxon-treated plots (Narisawa et al. 2004). Fusarium equisetti, naturally occurring endophyte of barley roots, significantly reduced the severity of take-all disease caused by Gaumannomyces graminis var. tritici (Ggt). F. equisetti colonized barley roots endophytically and competed with other fungal root colonizers present in the rhizosphere. The BCA isolates reduced the mean root lesions length induced by Ggt. However, the suppressive effect by F. equisetti was not distinct (Maciá-Vicente et al. 2009).

Accumulation of phenolic acids, occurring in continuously cropped soils, is increased due to infection by soilborne fungal pathogens. The biocontrol potential of Phomopsis liquidambari against Fusarium solani in soil enriched with phenolic acids was assessed by inoculating the BCA in the soil. Inoculation of P. liquidambari significantly inhibited the reproduction of F. solani. The extent of degradation of soil phenolic acids by P. liquidambari was determined. No direct antagonistic relationship between F. solani and P. liquidambari was observed, implying the alleviated stimulation of phenolic acid was the major factor in suppressing the development of F. solani. Further, presence of glucose did not significantly impact the biocontrol activity of the BCA. Inoculation with P. liquidambari reduced the severity of disease caused by F. solani in peanut. The results showed that P. liquidambari could be employed to suppress F. solani in phenolic acids-rich continuous cropping soils (Xie et al. 2017).
### 3.1.5.1.5 Induction of Resistance in Plants to Diseases

Biotic biocontrol agents (BCAs) may have single or multiple modes of action, involving direct or indirect effect on the microbial plant pathogens, resulting in suppression of disease development. The BCAs have been employed as inducers of disease resistance in several agricultural and horticultural crops. Induction of resistance by BCAs has been considered as an important disease management strategy, since enhancement of genetic resistance of crops through conventional breeding or biotechnological methods, has been found to be difficult or time-consuming or not feasible. Fungal BCAs have been shown to induce resistance to crop diseases, in addition to other mechanisms of biocontrol activity against microbial pathogens. Inoculation of tomato with the nonpathogenic *Penicillium oxalicum* resulted in reduction in severity of Fusarium wilt disease caused by *F. oxysporum* f.sp. *lycopersici* (Fol) as reflected by area under disease progress curve (AUDPC) values and extent of dwarfing of plants. *P. oxalicum* was shown to induce systemic resistance in tomato to *Fol*. Histological examination showed that BCA-inoculated plants did not lose cambium, had lower number of bundles and less vascular colonization by *Fol*. Renewal or prolonged cambial activity in treated plants, leading to the formation of additional secondary xylem might result in reduced disease severity. As no adverse effect of treatment with the BCA in tomato cultivars susceptible or resistant to Fusarium wilt disease was observed, *P. oxalicum* could be employed for protecting tomatoes against infection by *F. oxysporum* f.sp. *lycopersici* (de Cal et al. 1997, 2000). Application of *Crinipellis perniciosa*-chitosan filtrate (MCp) significantly delayed the development of Verticillium wilt disease in tomato induced by *Verticillium dahliae*. Activation of synthesis of pathogenesis-related (PR) protein with tissue lignification of tomato leaves was observed (Cavalcanti et al. 2007).

*Trichoderma viride* with multiple mechanisms of biocontrol activity was evaluated for its effectiveness against *F. oxysporum* f.sp. *adzuki* and *Pythium arrhenomanes*, infecting soybean. In vitro experiments revealed its mycoparasitic nature, whereas the pot experiments showed the disease suppressive ability of the BCA against both diseases. Furthermore, enhancement of growth of shoot and root systems, as well as pod yield of soybean plants treated with *T. viride* was also observed. *T. viride* seemed to be an avirulent opportunistic symbiont in the soybean rhizosphere. Enhancement of resistance against secondary infection of soybean by the fungal pathogen was also discernible (John et al. 2010). The dynamics of expression of defense response genes in the root tissues of potato plantlets were studied, following treatment with *T. harzianum* and challenge with *Rhizoctonia solani*. Gene expression analysis showed that genes for *PR1* at 168 h postinoculation (hpi) and phenylalanine ammonia lyase (PAL) at 96 hpi. In plants treated with *T. harzianum* strain Rifai MUCL 2907, induction of *PR1*, *PR2* and *PAL* at 48 hpi in plants inoculated with *R. solani* and induction of LOX at 24 hpi and *PR1*, *PR2*, *PAL* and *GST1* at 72 hpi in plants inoculated with both BCA and pathogen were observed. The results suggested that in the presence of BCA isolate, expression of LOX and GST1 genes might be primed in potato plantlets with *R. solani* at an early stage of infection (Gallou et al. 2009).

*T. harzianum* produces various metabolites with different functions that influence plants and pathogens to different extent. The role of oxidant-antioxidant metabolites of *T. harzianum* isolates in induction of resistance in sunflower against *Rhizoctonia solani* was investigated. Changes in the apoplast of sunflower challenged by *R. solani* in the presence or absence of *T. harzianum* NBRI-1055 were determined. Analysis of oxidative stress response revealed a reduction in hydroxyl radical concentration. The protection by the BCA strain against the pathogen was associated with accumulation of the reactive oxygen species (ROS) gene network, involving catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and ascorbate peroxidase (APx). In NBRI-1055-treated plants challenged with *R. solani*, these enzymes registered maximum activity after different periods (7–8 days). The enhanced enzymatic activities were accompanied by inhibition of lipid and protein oxidation in *Trichoderma*-treated plants. In addition, synthesis of secondary metabolites of phenolic nature was stimulated by the BCA strain, reaching a fivefold increase in concentration. Strong antioxidant activity at 8 days postinoculation resulted in the systemic accumulation of phytoalexins. The results suggested that the mechanism of biocontrol activity of *T. harzianum* against *R. solani* might be related to neutralizing *R. solani*-induced oxidative stress (Singh et al. 2011). The multianalysis technique was applied to quantify endogenous levels of salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA) and 1-aminocyclopropane-1-carboxylic acid (ACC) and the ethylene (ET) precursor, in melon plants inoculated with *Glomus intraradices* and *T. harzianum* in the presence of Fusarium wilt pathogen *F. oxysporum* f.sp. *melonis* (*Fom*). Infection by *Fom* activated defense response in plants, mediated by plant hormones SA, JA, ET and ABA, similar to the activation of *T. harzianum*. Both *T. harzianum* and *G. intraradices* attenuated plant response mediated by ABA and ET, elicited by the pathogen infection. Furthermore, *T. harzianum* attenuated the SA-mediated plant response. A synergistic effect of *T. harzianum* and *G. intraradices* in reducing the disease incidence was observed. But no such effect was noted in the hormonal disruption induced by the pathogen. The results suggested that the mechanisms of biocontrol activity of *T. harzianum* might be induction of the hormonal disruption induced by *F. oxysporum* f.sp. *melonis*, causing Fusarium wilt disease of melons, whereas the mechanisms involving *G. intraradices* appeared to be independently of SA and JA signaling (Martinez-Medina et al. 2010).

The interaction between *Trichoderma virens* and *Rhizoctonia solani*, incitant of cotton root rot disease, was investigated to evaluate the mechanism of disease suppression by the biocontrol agent (BCA). Defense-related compounds were induced in root tissues, following colonization by *T. virens*. The effect of seed treatment with BCA on elicitation of defense responses of cotton plants was studied. The role of terpenoid compounds in the control of cotton root rot disease
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was investigated by analyzing the extracts of cotton roots and hypocotyls grown from *T. virens*-treated seeds. Terpenoid synthesis and peroxidase activity were enhanced in the roots of treated plants, but not in the untreated controls. The terpenoid pathway intermediates deoxyhemigossypol (dHG) and hemigossypol (HG) strongly inhibited the development of *R. solani*, indicating that terpenoid production was the major contributor for the control of root rot disease. In addition, a strong correlation between the biocontrol activity and induction of terpenoid was revealed, when the strains of *T. virens* and *T. koningii* were compared. The results indicated that induction of resistance by *T. virens* occurred through activities of terpenoids acting as elicitors of defense responses in cotton (Howell et al. 2000). Treatment of roots with effective strains of *T. virens* resulted in elicitation of heat stable proteinaceous compounds. One of the compounds with MW of 3–5 kDa, was sensitive to proteinase K. SDS-PAGE analysis revealed the presence of several bands in the gel used for separating the proteins in the active material. One band showed cross-reaction with an antibody to ethylene-inducing xylanase from *T. viride*. Another band (18 K) induced production of terpenoids, in addition to increase in peroxidase activity, in cotton radicles and this protein showed maximum similarity to a serine proteinase from *Fusarium sporotrichoides* (Hanson and Howell 2004).

*Trichoderma virens* TRS106 protected tomato plants, against *Rhizoctonia solani* by inducing host resistance to the pathogen. Tomato plants treated with the strain TRS 106, showed limited lesion development on inoculation with *R. solani*. No direct inhibition of *R. solani* by TRS106 was evident in in vitro test. The strain TRS stimulated systemic defense responses in tomato plants by activating defense enzymes, including guaiacol peroxidase (GPX), syringaldazine peroxidase (SPX) and phenylalanine ammonia lyase (PAL). Simultaneously it enhanced accumulation of phenolics and *H₂O₂*, accompanied by decrease in lipid peroxidation in the leaves. Remarkable increases in the contents of 22 phenolics occurred in leaves of *Trichoderma*-treated tomato plants, both uninoculated and inoculated with *R. solani*. Some phenolics were present in a free form, while others were accumulated in bound forms glycosylated conjugates belonging to phenylpropanoids, hydroxybenzoic and cinnamic acid derivatives and flavonoids. Several of the detected phenolics, furfural and salicylic acids, pyrocatechol and hesperidin were strongly toxic to *R. solani* in plate tests. The systemic mobilization of phenolic metabolism might be a possible factor of tomato defense response positively involved in biocontrol of *R. solani* by TRS106 strain. The results indicated the suitability of using *T. virens* TRS106 strain as a potential biofungicides in the integrated management of diseases caused by *R. solani* in various crops (Malolepsza et al. 2017).

The effectiveness of the biotic inducer of resistance, *T. harzianum* strain 382 and was assessed for the management of Phytophthora blight of pepper caused by *Phytophthora capsici*. The biotic inducer *T. harzianum* remained spatially separated from *P. capsici* in split root and leaf blight bioassays. The results suggested that resistance induced by *T. harzianum* was systemic in nature (Khan et al. 2004). Treatment of pepper seeds with spores of *T. harzianum* significantly reduced stem necrosis caused by *P. capsici*. Drenching the roots of pepper plants with spore suspension of *T. harzianum* also provided similar level of protection against Phytophthora blight. The necrotic lesions yielded only the pathogen, but not the BCA, suggesting the absence of direct contact between the pathogen and the BCA. The percentage of *P. capsici* isolated at 9 days after inoculation was higher in untreated inoculated plants than in treated inoculated plants. *T. harzianum* applied in the subterranean part of plants, could induce defense response against *P. capsici* in the aerial parts of the plants. Concentrations of capsidol in stems of treated and inoculated plants were > sevenfold greater than in nontreated inoculated plants at 6 days after inoculation. The capsidol concentration was reduced at later stages. Accumulation of capsidol in the earlier stages of BCA-pathogen interaction with pepper plants might contribute to enhancement of resistance to the pepper blight disease (Ahmed et al. 2000).

*Trichoderma asperllum* activated metabolic pathways in cucumber involved in plant signaling and biosynthesis, eventually leading to systemic accumulation of phytoalexins. Penetration of epidermis and subsequent ingress into the outer cortex of cucumber seedlings by *Trichoderma* required secretion of cell wall lytic enzymes. Two differentially secreted arabinofuranosidases were detected by SDS-PAGE procedure, when *T. asperllum* was grown in the presence of cucumber roots. Furthermore, an aspartyl protease was also detected. Differential mRNA display performed on *Trichoderma* mycelia, interacting and noninteracting with plant roots showed that another aspartyl protease was present along with differentially regulated clones. RT-PCR assays revealed that the proteases were induced in response to plant root attachment and were expressed in planta. The gene *papC* (similar to *papA* from *T. harzianum*) was induced in plate confrontation assay with *Rhizoctonia solani*. The expression studies indicated that *T. asperllum papA* was upregulated during the first 48 h of interaction by cell wall proximity. The gene *papB* did not appear to be regulated by the presence of the pathogen. The results suggested that the protease identified, might play a role in *Trichoderma* to function both as a mycoparasite and as a plant opportunistic symbiont (Viterbo et al. 2004). *T. harzianum* isolate T39 is versatile in its biocontrol activity against many plant pathogens. The mode of action of *T. harzianum* differed, based on pathogen involved. Activation of defense responses locally, as well as systemically in cucumber plants treated with T39 strain, was observed against different fungal pathogens (Elad 2000). Treatment of onion seeds with *T. harzianum* strains TR1C7 or TR1C8 induced acceleration of production of antifungal compounds, suppressing the development of black mold disease caused by *Aspergillus niger* (Ozer 2011).

Plants have an immune system that is able to detect motifs or domains with conserved structural traits, typical of entire class of microorganisms, but not present in their hosts. These are named as pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs). MAMP-triggered plant responses
are elicited rapidly and involve iron fluxes across the plasma membrane, the generation of reactive oxygen species (ROS), nitric oxide, ethylene (ET) and also, but later, the deposition of callose and the synthesis of antimicrobial compounds by the host plant. Effective *Trichoderma* strains produce a wide range of MAMPs which may be either proteins or xylanase (Xyn2/Eix) by *T. viride*, cellulases by *T. longibrachiatum*, cerato-platanins (Sml/Epl1) by *T. virens/T. atroviride*, swollenin TasSw by *T. asperelloides*, endopolygalacturonase (ThPGI) by *T. harzianum* or secondary metabolites alamethicin, 18-mer peptaiolbs by *T. virens* and 6 pentyl-α-pyrene, harzianolide and harzianopyridone by different *Trichoderma* species (Hermosa et al. 2012). The potential of *Trichoderma* sp. to induce systemic resistance was not realized until the demonstration of induction of defense responses by *T. harzianum*, following colonization of bean roots (De Meyer et al. 1998) and penetration of roots of cucumber seedlings by *T. asperellum* resulting in triggering of induced systemic resistance (ISR) (Yedida et al. 1989). Development of ISR in plants colonized by *Trichoderma* has been demonstrated by inoculating leaves of plants with the pathogen, the roots of which were treated with the BCA. However, the effectiveness of ISR against soilborne pathogens is yet to be demonstrated. The growth-promoting activity of *T. atroviride* on tomato seedlings was suggested to be associated with reduced ethylene (ET) production, resulting from a decrease in its precursor 1-aminoacyclopropane-1-carboxylic acid (ACC) through the microbial degradation of indole-3-acetic acid (IAA) in the rhizosphere and/ or through the ACC deaminase (ACCD) activity in the microorganisms. Putative sequences of ACCD were detected in *Trichoderma* genomes (Kubicek et al. 2011).

Nonpathogenic binucleate *Rhizoctonia* spp. (np-BNR) was reported to protect plants against damping-off and crown and root rot diseases caused by *Pythium* spp. and *Rhizoctonia solani*. The greenhouse and field assessments indicated that strain np-BNR strain 232-CG could induce systemic resistance (ISR) in the stem and cotyledons of bean to challenges with *R. solani* AG-4 or *Colletotrichum lindemuthianum*, causing root rot and anthracnose disease respectively (Xue et al. 1998). The mechanism of biocontrol activity of np-BNR was investigated in comparison with the chemical inducer benzothiadiazole (BTH) against *Rhizoctonia solani* and *Alternaria macrospora*, causing pre- and postemergence damping-off of cotton. Pretreatment of cotton seedlings with np-BNR isolates protected the plants effectively against a virulent strain of *R. solani* AG-4. Several isolates significantly reduced disease severity. The combination of BTH and np-BNR provided significant protection against seedling rot and leaf spot of cotton. However, the degree of disease reduction obtained with np-BNR treatment alone was comparable to effectiveness of combined treatment. The results indicated that np-BNR isolates could protect cotton from infections by both root and foliar pathogens and they were more efficient than the chemical inducer of resistance, BTH (Jabaji-Hare and Neate 2005). Nonpathogenic Fo47 strain of *F. oxysporum* reduced wilt disease incidence in tomato from 100 to 75%, when applied as seed treatment. As the presence of the BCA hyphae could be observed only just below the crown region, the disease suppression might be due to induction of resistance in tomato to *F. oxysporum* f.sp. *radicis-lycopersici* (FORL). Induction of resistance in tomato by Fo47 functioned through a systemic acquired resistance (SAR)-like mechanism (Duijff et al. 1998; Bolwerk et al. 2005).

The biocontrol activity of nonpathogenic isolates of *F. oxysporum* (npFo) was investigated, through induction of systemic resistance (ISR) in asparagus against *F. oxysporum* f.sp. *asparagi* (Foa). In the split-root system experiments, roots inoculated with npFo strain showed hypersensitive response and those subsequently inoculated with Foa exhibited resistance. Development of ISR in npFo-treated plants resulted in significant reduction in the number of necrotic lesions and reduced wilt disease severity, compared with untreated control plants. In hyphal-sandwich root inoculation experiments, activation of POX and PAL and lignin content were higher in npFo-treated plants and increased more rapidly than in npFo-nontreated plants, after Foa inoculation. Presence of antifungal compounds in the exudates of roots inoculated with Foa was observed for npFo-treated plants, but not for npFo-nontreated plants. The results indicated that the isolates of npFo might function as inducers of systemic acquired resistance (SAR) and defense responses against Foa invasion in asparagus (He et al. 2002). Mutagenesis via ultraviolet (UV) irradiation has been applied successfully to generate mutants for use as biocontrol agents against crop pathogens. Two nonpathogenic mutants (4/4 and 15/15) were obtained from the cucurbit wilt pathogen *F. oxysporum* f.sp. *melonis* (Fom, race 1,2) by a continuous dip-inoculation technique, following UV mutagenesis. The strain 15/15 induced mortality of susceptible cultivars to a lesser level, compared to the wild-type strain. The strain 4/4 could colonize 100% of the roots and 33 to 70% of lower stem tissues at 7 days after inoculation of seedlings. The nonpathogenic strain lacking only pathogenicity might more efficiently compete with the pathogenic strain, than other BCAs that might require different set of conditions for their survival and development in the soil environment (Freeman et al. 2002).

*Fusarium solani* Fs-K, an endophytic isolate was obtained from root tissues of tomato plants grown on the compost which suppressed soilborne pathogens. Strain Fs-K colonized root tissues and subsequently protected plants against *F. oxysporum* f.sp. *radicis-lycopersici* (FORL) and also elicited ISR against the foliar pathogen *Septoria lycopersici*. The attenuated expression of genes of PR-proteins like, PR-5 and PR-7 was detected in tomato roots inoculated with strain Fs-K, compared with noninoculated plants. The expression pattern of PR genes was either not affected or aberrant in leaves. A genetic approach, using mutant tomato plant lines, was used to determine the role of ethylene (ET) and jasmonic acid in the response of plant to infection by FORL, in the presence or absence of isolate Fs-K. Mutant lines Never ripe (*Nr*) and epinastic (*epi* 1), both impaired on ethylene-mediated plant responses, inoculated with FORL, were not protected by Fs-K isolate, indicating that ethylene signaling pathway is required for the mode of action used by the endophytic BCA to induce...
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resistance. In contrary, defl mutants defective in jasmonate biosynthesis, showed reduced susceptibility to FORL, in the presence of Fs-K, which suggested that jasmonic acid was not required for mediation of biocontrol activity of isolate Fs-K (Kavroulakis et al. 2007). In a later investigation, the non-pathogenic mutant generated from \textit{F. oxysporum f.sp. melonis (Fom) (rev157)} could not protect muskmelon plants against infection by pathogenic strain \textit{Fom 24}. The mutant rev157 was unable to protect nonhost flax plants against wilt pathogen \textit{F. oxysporum f.sp. lini}. In contrast, the parental strain \textit{Fom 24} of the mutant rev157, was able to protect the flax plants against \textit{F. oxysporum f.sp. lini}. The comparative molecular genetics of the pair of strains \textit{Fom24/rev157} might shed light on the identity of genes involved in the biocontrol activity of \textit{F. oxysporum} (L’Haridon et al. 2007). Effective suppression of Verticillium wilt and Phytophthora blight caused by \textit{Verticillium dahliae} and \textit{Phytophthora capsici} respectively in pepper could be achieved by employing nonpathogenic isolate \textit{F. oxysporum Fo47}. The isolate Fo47 inhibited the growth of \textit{V. dahliae}, but not that of \textit{P. capsici} in plate confrontation assay, indicating that at least part of the protective effect of the BCA strain against \textit{V. dahliae} was due to antagonism or competition for nutrition. In order to determine the role of induction of resistance as a mechanism of biocontrol activity of Fo47, three defense genes previously related to pepper resistance were monitored over time. The genes encoded a basic pathogenesis-related (PR)-1 protein (CABPR1) a class II chitinase (CACHII2) and a sesquiterpene cyclase (CASC1) involved in the synthesis of capsidol, a phytoalexin. These three genes were transiently upregulated in the roots of Fo47-treated plants in the absence of inoculation with the pathogen, but in the stem only CABPR1 was upregulated. In the plants inoculated with \textit{V. dahliae} prior to the treatment with Fo47, three genes had a higher relative expression level than the control in both roots and stem of pepper plants, indicating the involvement of induction of resistance as another mechanism of the biocontrol activity of Fo47 strain in treated pepper plants (Veloso and Díaz 2012).

\textit{Pythium oligandrum}, a mycoparasite, produces oligandrin, an elicitin-like protein, which was evaluated along with crude glucan obtained from the cell wall of \textit{P. oligandrum} and crab-shell chitosan for their ability to induce resistance in tomato root tissues to Fusarium root rot and wilt caused by \textit{F. oxysporum f.sp. radicis-lycopersici} (FORL). These compounds were applied to decapitated tomato plants and induction of defense mechanisms on root tissues were monitored. Disease incidence was significantly reduced in plants treated with oligandrin and chitosan, whereas glucans did not have any effect on disease incidence. In oligandrin-treated tomato plants, restriction of fungal growth to outer root tissues, decrease in pathogen viability and formation of aggregated deposits accumulating at the surface of invading pathogen hyphae were the striking features of the defense responses. The results showed that oligandrin could induce systemic resistance in tomato and exogenous foliar application of fungal protein could sensitize susceptible tomato plants to react rapidly and efficiently to infection by \textit{F. oxysporum f.sp. radicis-lycopersici}. Reduction in disease incidence might be primarily through accumulation of fungitoxic compounds at sites of attempted pathogen penetration (Benhamou et al. 2001). In the later investigation, four elicitin-like proteins (POD-1, POD-2, POS-1 and oligandrin) were identified as elicitor proteins in \textit{P. oligandrum}. Two groups of \textit{P. oligandrum} isolates were differentiated, based on the nature of cell wall proteins (CWP) as D-type containing POD-1 and POD-2 and the S-type isolate containing POS-1. The distribution of genes encoding elicitin-like proteins among ten \textit{P. oligandrum} isolates was analyzed, using genomic fosmid library of the D-type isolate MMR2. Based on Southern blot analyses, the isolates were divided into the same two groups, as those based on the CWP. The D-type isolates contained pod-1 and pod-2 and two oligandrin genes designated \textit{oli-d1} and \textit{oli-d2}, whereas S-type isolates had \textit{pos-1} and one oligandrin gene \textit{oli-s1}. These genes were single copies present only in \textit{P. oligandrum}, but not in nine other \textit{Pythium} spp. tested. All genes were expressed during colonization of tomato roots by \textit{P. oligandrum}, as indicated by RT-PCR tested. It is possible that these genes encode potential elicitor proteins, resulting in the enhancement of resistance in plants against pathogens. Analyses of genetic relationships suggested that the D-type isolates might be derived from S-type isolates by genetic duplication and deletion events (Matsunaka et al. 2010). The ability of \textit{Pythium oligandrum} to induce resistance in potato against black scurf caused by \textit{Rhizoctonia solani} was assessed, using potato tuber disk assay. Treatment of tuber disks with cell wall protein fraction of \textit{P. oligandrum} enhanced the expression of defense-related genes such as 3-deoxy-d-arabino-heptulosonate-7-p phosphatase synthase, lipoxigenase and basic PR-6 genes and reduced severity upon challenge with \textit{R. solani}, compared with untreated controls. The results suggested that the biocontrol mechanisms employed by \textit{P. oligandrum} against \textit{R. solani} might involve induction of disease resistance, as well as mycoparasitism (Ikeda et al. 2012).

### 3.1.5.2 Bacterial Biological Control Agents

The bacterial biocontrol agents (BCAs) may act against soil-borne microbial plant pathogens directly or indirectly by different mechanisms such as antagonism, antibiotic, competition for nutrients and space, colonization of specific sites required for establishing infection by the pathogens and inducing resistance to the pathogens by activating host plant defense systems. In addition, the plant growth-promoting rhizobacteria (PGPRs) may enhance plant growth to different extent. The PGPRs are included under the genera \textit{Pseudomonas}, \textit{Bacillus}, \textit{Azospirillum}, \textit{Rhizobium} and \textit{Serratia}.

#### 3.1.5.2.1 Antibiosis

Antagonistic activity of \textit{Pseudomonas jassentii} against \textit{Aphanomyces cochlioides} AC5 and \textit{Pythium aphidermatum} PA-5 was investigated. The antagonist produced two related secondary metabolites, 3-[(1R)-hydroxyoctyl]-5-methylene-2(5H) furanone (4,5-dihydroactein) and 3-[(1R)-hydroxyhexyl]-7-5-methylene-2 (H)-furanone. These compounds inhibited radial growth and also induced...
morphological abnormalities like hyperbranching and swellings in treated pathogen isolates, AC-5 and PA-5 respectively. Staining with rhodamine-phalloidin, which bound to plasma membrane-associated filamentous-actin (F-actin), revealed that tip-specific actin filaments were remodelled into a plaque-like form at an early stage of interaction up to 24 h with any one of the secondary metabolites. At later stages (48 h), the plaques were eliminated, reflecting the disorganization of actin arrays in the morphologically abnormal hyphae of AC-5 and PA-5 isolates. A similar response of actin disorganization was seen in AC-5 and PA-5 hyphae following treatment with latrunculin B, an actin-assembly inhibitor produced by a sea sponge. The results showed that actin disorganization and inhibitory activities of the secondary metabolites of _P. jessenii_ were similar to those induced by the actin-assembly inhibitor latrunculin (Deora et al. 2010). The native _Streptomyces_ isolates C and S2 were evaluated for their biocontrol potential against the fungal pathogens, _Rhizoctonia solani_ AG-2, _Fusarium solani_ and _Phytophthora drechsleri_ involved in sugar beet root rot disease. In vitro antagonism assays showed the antagonistic nature of the isolate C with percentages of inhibition of mycelial growth being 45, 53 and 26% for _R. solani_, _F. solani_ and _P. drechsleri_. Treatment with NaCl increased the biocontrol activity of soluble and volatile compounds of isolates C and S2. Both isolates showed protease, chitinase and α-amylase activity. In addition, both isolates were able to produce siderophores. Addition of salt enhanced the production of siderophores and activities of protease and α-amylase. In contrast, salt treatments reduced chitinase activity significantly. Production of salicylic acid (SA), β-glucanase and lipase by isolate S2 and biosynthesis of cellulase by isolate C was significant both in the presence and absence of NaCl. Soil application of isolate C reduced root rot of sugar beet by the soilborne fungal pathogens. The results indicated that the isolates C and S2 had the potential for use as biocontrol agents for suppressing sugar beet root rot disease, particularly in the saline soils (Karimi et al. 2012).

The mechanism of biocontrol activity of _Streptomyces griseocarneus_ strain Di944, isolated from the rhizosphere of field tomato plant, against damping-off and root rot pathogens affecting tomato plug transplants, was investigated. The biocontrol agent (BCA) inhibited _Rhizoctonia solani_, _Pythium_ spp., _Phytophthora_ spp., _F. oxysporum_ f.sp. lycopersici, _F. oxysporum_ f.sp. radicis-lycopersici, _F. solani_, _Thielaviopsis basicola_ and _Verticillium dahliae_, but not bacterial pathogens of tomato, as indicated by agar diffusion bioassay. The culture filtrates of _S. griseocarneus_ contained an antifungal compound, a pentaene macrolide complex, designated rhizostreptin, which was detected also in the extracts from rhizospheres of tomato transplants grown from seeds treated with the BCA. The antifungal compound suppressed the development of damping-off caused by _R. solani_. Spore germination and mycelial growth of fungi pathogenic to tomato were inhibited by rhizostreptin at concentrations between 0.5 and 2.0 μg/ml. Rhizostrepmin was more fungitoxic than other polyene macrolides, amphotericin B, nystatin, filipin and candidin-type heptanes. Growth of _S. griseocarneus_ was substantial in mineral medium supplemented with cell wall components of _R. solani_ and this indicated the ability of the BCA to utilize pathogen cell wall components as carbon and nitrogen sources. In addition, production of hydrolytic enzymes, chitinase, glucanase, phospholipase and proteinase by _S. griseocarneus_ Di944 was observed in mineral medium supplemented with glucose and ammonium sulfate or cell wall components of _R. solani_ as carbon sources. Secretion of extracellular antifungal pentaene macrolide and fungal cell wall-degrading enzymes (CWDEs) was considered to be the major mechanism by which the BCA was able to inhibit pathogenic fungi and oomycetes involved in the damping-off and root rot of tomato transplants (Sabaratnam and Traquair 2015).

_Pseudomonas chlororaphis_ strain JP1015 and _P. fluorescens_ strain JP2175, capable of inhibiting the growth of _Aspergillus flavus_ in vitro, were evaluated for their antifungal activity in soil coculture. Growth of _A. flavus_ was inhibited up to 100-fold by _P. chlororaphis_ and _P. fluorescens_ within three days following soil coinoculation. _A. flavus_ propagule densities after 16 days remained 7- to 20-fold lower in soil treated with either bacterial strain. Under bench-scale wind chamber conditions, treatments of soil with _P. chlororaphis_ and _P. fluorescens_ reduced airborne spores dispersed across a 1 m distance by 75- to 1,000-fold and 10- to 50-fold, respectively, depending on the soil type and inoculum level. The results suggested the possibility of employing bacterial BCAs to reduce soil population of mycotoxigenic fungal pathogens like _A. flavus_ that could infect crop plants via airborne propagules (Palumbo et al. 2010). The antagonistic activity of two _Rhizobium_ strains PchAzm and PchS. Nir2 against _Rhizoctonia solani_ was assessed. These strains reduced fungal growth observed in vitro. Further, these isolates reduced chickpea infection by _R. solani_, due to the direct effect of _Rhizobium_ strains on the pathogen. Concomitantly reduction in infection was accompanied by enhanced level of defense-related enzymes, phenylalanine ammonia lyase (PAL) and peroxidase (POX). Phenol contents of the roots were increased following bacterization of plants. The results showed the direct effect of _Rhizobium_ strains on pathogen and indicated the possible induction of resistance by the BCA strains (Hemissi et al. 2013).

Indigenous plant growth-promoting bacteria from solarized soil effective against _Monosporascus cannonballus_, incitant of root rot and vine decline of melon were evaluated for their antagonistic activity. Two bacterial species were identified as _Bacillus subtilis_ and _Pseudomonas putida_ (PpF4), based on phenotypic, physiological characteristics and analysis of 16S rDNA sequences. Antagonism by BsCR was characterized by a consistent inhibition of the pathogen growth in vitro. PpF4 strain strongly inhibited the development of perithecia of _M. cannonballus_. Under greenhouse conditions, BsCR alone and in combination with PpF4 consistently decreased the disease symptoms. BsCR and the combination of bacterial strains significantly increased root biomass in both inoculated and noninoculated control plants. Following seed treatment with BsCR, the
accumulation and the isoenzyme induction of peroxidase in roots as biochemical marker for induction of resistance were detected, indicating that BsCR might reduce disease severity by the activation of the plant defense responses also. The results showed that the synergistic biocontrol activities of *B. subtilis* BsCR and *P. putida* PfF4 might be included as a component in the integrated management of root rot and vine decline of melon caused by *M. cannonballus* (Antonelli et al. 2013). Biological control potential of *B. subtilis* strain Z-14 against wheat take-all disease pathogen *Gaeumannomyces graminis var. tritici* (Ggt) was assessed by amending the medium with crude extract of the bacterial culture filtrate in petridish assay. The severity of take-all in wheat seedlings grown on petridishes was reduced by 91.3% and in potted plants by 69.8%, compared to Ggt-inoculated control plants. Treatment with crude extract significantly increased growth and fresh weight of roots. The culture filtrate of strain Z-14 was relatively thermostable with 88.2% of antifungal activity being retained at 100°C for 30 min. The pH range (3 to 8) did not significantly affect the antifungal activity of the culture filtrate, under basic conditions. The activity was not transferable to the organic solvent phase after treatment with organic solvent extractants. The culture filtrate showed a broad spectrum of antifungal activities against fungal pathogens (Zhang et al. 2017a).

Although the plant growth-promoting rhizobacteria (PGPRs) have multiple mechanisms of biocontrol activities, production of different kinds of antibiotics appears to be the principal mechanism of action against microbial pathogens causing soilborne crop diseases. These BCAs secrete phloroglucinols, phenazines, pyoluteorin, pyrrolnitrin and rhamnolipids. Production of antibiotics by PGPRs has been demonstrated in different BCA-pathogen-crop plant species has been demonstrated. Among phloroglucinols, 2,4-diacetylphloroglucinol (2,4-DAPG) is commonly detected in the presence of *Pseudomonas* spp. Abundance of 2,4-DAPG-producing *Pseudomonas* spp. was correlated with natural suppression of wheat take-all disease caused by *Gaeumannomyces graminis var. tritici*. The fluorescent pseudomonads have been reported to suppress the development of root and seedling diseases caused by soilborne fungal pathogens. *P. fluorescens* CHA0 suppressed tobacco black root disease and tomato wilt and crown and root rot disease (Duffy and Defago 1997). Suppression of development of sugar beet damping-off disease by *Pseudomonas* spp. (Shanahan et al. 1992) and wheat take-all disease by *P. fluorescens* strains Q2-87 and Q8r-96 (Raaijmakers and Weller 1998) has been reported. The effects of 2,4-DAPG on fungal pathogens, which can produce different spore forms and survival structures have been investigated. The responses of *Pythium* spp. infecting several crops to 2,4-DAPG were assessed. Variations in the sensitivity of 14 *Pythium* isolates obtained from different hosts to 2,4-DAPG were recorded. Different propagules of *P. ultimum* var. *sporangiferum* exhibited differences in their sensitivity to 2,4-DAPG. Zoospores were the most sensitive, followed by sporangia, the mycelium being the most resistant structure. Ultrastructural changes in the hyphal tips of *P. ultimum* var. *sporangiferum* exposed to 2,4-DAPG were assessed, using the transmission electron microscope (TEM). Different extents of disorganization in hyphal tips of the pathogen were visualized. Localized alteration (proliferation or disruption) in plasma membrane organization, development of an extensive network of smooth membranous vesicles, degenerated cytoplasm bordered by a retracted plasma membrane and hyphal senescence accompanied by vacuolization and degeneration of its contents were the frequently observed alterations in the fungal pathogen structure. It seemed that 2,4-DAPG did not affect the cell wall structure and composition of hyphal tips of the pathogen, since B(1,3)-1, B(1,4)- and B(1,6)-glucans were present at the same concentrations in hyphal tips both in the presence or absence of 2,4-DAPG, as revealed by immunolocalization experiments, using the primary antibody (de Souza et al. 2003a, 2003b). The sensitivity of 76 plant pathogenic and/or saprophytic strains of *F. oxysporum* to 2,4-DAPG produced by *Pseudomonas fluorescens* was assessed. *F. oxysporum* strains (17%), including *F. oxysporum* f.sp. *melonis* (strain Fom38 and Fom1127) and *F. oxysporum* f.sp. *cubense* (strains Focub 1, 2 and 13) were relatively tolerant to high concentrations of 2,4-DAPG. Some tolerant strains (18) could metabolize 2,4-DAPG. In two tolerant strains, deacetylation of 2,4-DAPG to less fungitoxic derivatives, monoacetylphloroglucinol and phloroglucinols occurred. Fusaric acid produced by *F. oxysporum* strain might directly affect 2,4-DAPG biosynthesis by repressing the expression of the biosynthetic gene *phiA*. Fusaric acid-mediated repression of 2,4-DAPG synthesis in *Pseudomonas* spp. was strain-dependent, as fusaric acid blocked the 2,4-DAPG biosynthesis in strain CHA0, but not in the strain Q21-87 (Schouten et al. 2004). *Pseudomonas fluorescens* UP61 was effective in suppressing the development of *Sclerotium rolfsii*, infecting beans and *Rhizoctonia solani* infecting tomato. The strain UP61 produced three antibiotics, viz., 2,4-diacetylphloroglucinol (2,4-DAPG), pyrrolnitrin and pyoluteorin, contributing to its antagonistic activity. Molecular techniques like 16S rDNA sequencing, random fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and rep-PCR assays and partial sequencing of the *phiD* gene, governing the biosynthesis of 2,4-DAPG showed similarity between the strain UP61 with other biocontrol agents isolated from other geographical locations that have been shown to produce these antibiotics (De La Fuente et al. 2004). A simple and rapid method was developed to detect the presence and assess the genetic diversity of *phiD* *Pseudomonas* strains directly in the rhizosphere samples, without the need for enrichment on nutrient media and prior isolation of the BCA strains. The denaturing gradient gel electrophoresis (DGGE) analysis of the 350-bp fragments of *phiD* allowed discrimination between genotypically different *phiD* reference strains and indigenous isolates. The DGGE analysis of the *phiD* gene allowed identification of new genotypic groups of specific antibiotic-producing *Pseudomonas* with different abilities to colonize the rhizosphere of sugar beet seedlings (Bergsma-Vlami et al. 2005).
**Pseudomonas fluorescens** strain KD did not produce 2,4-DAPG which is the main antibiotic involved in the biocontrol activity of several strains of *Pseudomonas* spp. However, strain KD effectively protected cucumber plants against damping-off disease caused by *Pythium ultimum*. The type III secretion system (TTSS) is employed by bacteria for pathogenic or symbiotic interactions with plant and animal hosts. The presence of TTSS genes in *P. fluorescens* KD strain was detected. In spite of the presence of pathogenic attribute, the strain KD was not pathogenic to cucumber. Inactivation of hrcV strongly reduced the biocontrol potential of the strain KD against *P. ultimum*. The reduced biocontrol efficacy was not due to a lower ecological fitness of hrcV mutant, since the mutant persisted in the potting mix and colonized the plant roots to the same level as that of the wild-type strain, regardless of whether the pathogen was present or not. The expression of the operon containing hrcV in the strain KD was strongly stimulated in vitro and in situ by *P. ultimum*, but not by cucumber (Rezzonico et al. 2005). The polar growth of *Aphanomyces cochlioides*, causing damping-off disease of sugar beet and spinach was inhibited by *P. fluorescens* strain ECO-001 isolated from *Plantago asiatica*. The antibiotic 2,4-DAPG secreted by ECO-001 induced excessive branching of hyphae of the pathogen. Confocal laser scanning microscopic (CLSM) observations revealed that both ECO-001 and syn-hyphae of the pathogen. Confocal laser scanning microscopic of *Longisporum* Verticillium in suppressing production of microsclerotia by *Plantago asiatica* ECO-001 isolated from sugar beet and spinach was inhibited by *PCA to PCN*. A asparagine synthetase-like enzyme, capable of converting *phzH* phzABCDEFG boxamide (PCN) formed by the BCA. A seven-gene operon for HCN was under the regulation of quorum sensing as part of its lifestyle. The gacS and *lp* mutants could not protect canola against stem rot disease caused by *Sclerotinia sclerotiorum*. Further, both mutants were unable to sustain themselves in canola phyllosphere. The results indicated that suppression of stem rot disease development in canola by DF41 strain was dependent on LP production and presence of a functional Gac system (Berry et al. 2010). A gacS mutant of *Pseudomonas* sp. lost biocontrol activity against *Leptosphaeria maculans*, causal agent of blackleg of canola. The biocontrol activity could be restored in the mutant PA23-314 by incorporating the gacS gene. The phenazine mutant PA23-63 showed an antifungal and biocontrol activity to the same level as the wild-type strain (Selin et al. 2010).

*Pseudomonas chlororaphis* isolated from the rhizosphere of green pepper plants produced phenazines mainly phenazine-1-carboxylic acid (PCA) and 2-hydroxyphenazine (2.OH-PHZ) which had broad spectrum of antifungal activity against plant pathogens (Liu et al. 2007). The biocontrol activity of *P. aeruginosa* PNA1 against *Pythium splendens*, infecting bean and *P. myriotylum* infecting cocoyam was found to be due to production of phenazine-1-carboxylic acid (PCA) and phenazine-1-carboxamide (PCN). The tryptophan autotrophic mutants, FM13 deficient in phenazine production, were unable to protect the plants against oomycete pathogens. Exogenous supply of tryptophan restored the biocontrol activity of the mutant FM13, as reflected by reduction in disease severity in treated cocoyam plants (Tambong and Höfte 2001). *Pseudomonas* CMR56 and CMR12a strains isolated from the rhizosphere of cocoyam plants were highly efficient in protecting cocoyam plants against *Pythium myriotylum*. These two strains produced phenazines and surfactants. The strain CMR5c produced pyrrolnitrin and pyoluteorin also (Perneel et al. 2007).

Mechanisms of action of two bacterial biocontrol agents *Pseudomonas chlororaphis* PCL 1391 and *P. fluorescens* WCS365 against *F. oxysporum* f.sp. *radicis-lycopersici* (FORL), causal agents of tomato foot and root rot disease were investigated, using confocal laser scanning microscope (CLSM) and different autofluorescent proteins as markers. Tomato seedlings were bacterized with PCL1391 and WCS 365 strains and planted in sand system infested with (QS) (Chin-A-Woeng et al. 2001). Development of canola stem rot disease caused by *Sclerotinia sclerotiorum* was suppressed effectively by *Pseudomonas* sp. DF41. Two mutants *gacS* (DF41-469) and *lp* (DF469-1278) mutants were generated via transposon mutagenesis. The GacS/GacA system is known to control expression of genes required for the synthesis of secondary metabolites such as antibiotics in several *Pseudomonas* spp. The gacS mutant had an insertion in gacS, forming part of the GacS/GacA regulatory system. By contrast, mutation occurred in a gene involved in lipopeptides (LP) synthesis in the *lp* mutant. The wild-type strain DF41 produced a number of compounds including hydrogen cyanide (HCN), proteases, alginate and LP molecules that might contribute to biocontrol. All of these compounds were generated under Gac control. Furthermore, DF41 strain produced autoinducers, suggesting that this strain might employ quorum sensing as part of its lifestyle. The gacS and *lp* mutants could not protect canola against stem rot disease caused by *Sclerotinia sclerotiorum*. Further, both mutants were unable to sustain themselves in canola phyllosphere. The results indicated that suppression of stem rot disease development in canola by DF41 strain was dependent on LP production and presence of a functional Gac system (Berry et al. 2010). A gacS mutant of *Pseudomonas* sp. lost biocontrol activity against *Leptosphaeria maculans*, causal agent of blackleg of canola. The biocontrol activity could be restored in the mutant PA23-314 by incorporating the gacS gene. The phenazine mutant PA23-63 showed an antifungal and biocontrol activity to the same level as the wild-type strain (Selin et al. 2010).
The BCA strains reached the root surface earlier and multiplied faster than the pathogen. The bacterial strains and pathogen hyphae colonized the same niches on tomato root, the intercellular junctions, probably due to chemotaxis toward and utilization of compounds in the exudates. By colonizing these sites and utilizing the nutrients in the exudates, the bacteria prevented colonization and penetration of root tissue by FORL. The strain PCL1391 produced phenazine-1-carboxamate (PCN), which altered the growth and morphology of pathogen hyphae both in vitro and in vivo (greenhouse conditions). The lack of PCN production in the strain PCL1391 resulted in a delay in the appearance of morphological abnormalities in pathogen hyphae. By contrast, the strain WCS 365 might suppress pathogen development via induced systemic resistance (ISR). However, there was no difference in biocontrol activity of the bacterial BCAs that could be associated with ISR. It is possible that the effects of ISR by WCS365 strain were nearly compensated by the antibiosis effect of the strain PCL1391. The extensive root colonization by both bacterial BCAs might represent a new mechanism in biocontrol by these Pseudomonas strains (Bolwerk et al. 2003). Pseudomonas chlororaphis strain PA23 effectively suppressed the development of disease symptoms induced by Sclerotinia sclerotiorum on canola and sunflower crops. The strain PA23 produced nonvolatile antibiotics phenazine and pyrrolnitrin, as well as volatile antibiotics nonanal, benzothiazole and 2-ethyl-1-hexanol. The role of nonvolatile antibiotics on root colonization and biocontrol potential of PA23 against S. sclerotiorum on sunflower was investigated. Application of strain PA23 alone or in combination with phenazine- and pyrrolnitrin-deficient Tn mutants resulted in significantly higher root bacterial number and suppression of Sclerotinia wilt disease (P = 0.05). The bacterial population decreased considerably and it seemed that the bacterial population was negatively correlated with the number of antibiotics produced by the strain PA23. The strains producing at least one antibiotic were able to maintain relatively higher population than nonproducers and an increase in bacterial population at 6 weeks after sowing was recorded for strains producing at least one antibiotic. The results did not indicate a clear role for phenazine or pyrrolnitrin in the antagonistic activity of P. chlororaphis PA23 (Athukorala et al. 2010).

Wheat-take-all disease caused by Gaemannomyces graminis var. tritici (Ggt). The strain HC1-07 produced a cyclic lipopeptides (CLP) with a MW of 1,126 and the extracted CLP strongly inhibited the growth of Ggt and Rhizoctonia solani AG-8, causing Rhizoctonia root rot of wheat at concentration of 100 µg/ml. In order to determine the role of the CLP in biological control, plasposon mutagenesis was applied to generate two nonproducing mutants, HC1-07visCB and HC107prtR2. These two mutants were deficient in swimming and swarming mobility. Furthermore, HC1-07prtR2 mutant lost the ability to secrete exoprotease, whereas HC1-07visCB mutant retained wild-type levels of exoprotease production. Production of siderophores was not affected in both mutants. But the biofilm formation was reduced in both mutants, compared to the wild-type strain. The genomic complementation of the mutants restored the surface mobility and production of exoprotease to the wild-type strain levels and partially restored biofilm formation. The mutants were introduced individually...
into Quincy Virgin soil and the population sizes of each strain in the rhizosphere of wheat plants were determined at 2-week interval. Of the two mutants, HCl-07prtR2 was mildly, but significantly impaired in the ability to persist in the wheat rhizosphere. Complementation of the prtR mutation restored plant colonization to the wild-type strain levels (see Figure 3.8). The CLP-deficient mutants HCl-07viscB and HCl-07prtR2 and complemented mutants were tested for the biocontrol potential against Ggt and R. solani under controlled conditions. Strain HCl-07 applied as seed treatment significantly suppressed development of both wheat take-all and Rhizoctonia root rot diseases, compared with control (see Table 3.1). Both mutants were less efficient in suppressing the development of both diseases. Genetically complemented mutants regained the ability to suppress disease development. The results suggested that the viscosia-like CLP appeared to be a more important determinant of suppression of wheat take-all and Rhizoctonia root rot diseases (Yang et al. 2014).

The mechanism of biocontrol activity of Pseudomonas fluorescens Pf29Arp against the wheat take-all pathogen, Gaeumannomyces graminis var. tritici (Ggt) was investigated, using the confrontation assay in petriplates. In addition, disease development in roots, rates of bacterial and fungal root colonization, the transcript levels of candidate fungal pathogenicity genes and plant-induced genes were monitored during the 10-day infection process. The BCA inoculation of wheat roots reduced the development of Ggt-induced disease expressed as attack frequency and necrosis length. The growth rates of Ggt and Pf19Arp, monitored through qPCR assay of DNA contents with a part of the Ggt 18S rDNA gene and a specific Pf29Arp strain deletion probe, respectively, increased throughout the interactions. Bacterial antagonism and colonization did not have any significant effect on root colonization by Ggt. Expression of fungal and plant genes was quantified in planta by qRT-PCR assay, during interaction. During the early stages of the tripartite interaction, several pathogen genes were downregulated by Pf29Arp, including two laccases, a β-1,3-glucanase and a mitogen-associated protein kinase. The host plant glutathione-S-transferase gene was induced by Ggt alone and upregulated by pf29Arp in interaction with the pathogen. The results showed that the antagonism of BCA strain Pf29Arp might act through alteration of fungal pathogenesis and probably through the activation of host defenses (Daval et al. 2011). The interactions among bacterial BCA Pseudomonas spp., wheat-infecting root pathogens and the 10 representative Swiss agricultural soils with cereal cropping history were investigated. The Swiss agricultural soils that were highly suppressive to Ggt were often highly conducive to P. ultimum and vice-versa. No significant relationship could be established between soil suppressiveness and the abundance of Pseudomonas spp. carrying DAPG. PHZ and pyrrolnitrin biosynthetic genes and ability of the soil to support expression of the antimicrobial genes. Correlation analyses indicated that certain soil factors such as silt, clay and some nutrients might influence both abundance and expression of antimicrobial genes. The results suggested that Pseudomonas spp. producing DAPG, PHZ or pyrrolnitrin were abundantly present in Swiss soils and the expression of genes encoding antimicrobial compounds in the bacteria might have a role in the suppression of development of the soilborne diseases infecting wheat. However, the precise role of the BCAs in soil suppression could not be clearly understood (Imperiali et al. 2017).

The genes phzM and phzS genes in Pseudomonas aeruginosa PA01 coded for eight enzymes that modify phenazine into related derivatives. The gene phzM is located in upstream of phzAIBCIDEIFIG1 operon which is involved in the production of pyocyanin antibiotic (Mavrodi et al. 2001). Purified pyocyanin from P. aeruginosa PA01 inhibited the mycelial growth of M. phaseolina infecting peanut. Using the well-diffusion method, the effect of pyocyanin on disease suppression and biofilm formation by the rhizobial strain Ca 12 on radicles of peanut was assessed. Pyocyanin suppressed disease more effectively at high concentration. However, at lower concentration, pyocyanin increased CFUs of Ca 12 strain on radicles of peanut seedlings. Application of pyocyanin-producing pseudomonads together with rhizobia contributed to the enhancement of nodulation ability and sustained the growth and productivity of peanut even in the presence of M. phaseolina (Khare and Arora 2011). Pseudomonas chlororaphis strains DF190 and PA23, Bacillus cereus strain DFE4 and B. amyloliquefaciens strain DFE16 were evaluated for the antagonistic activity against canola blackleg pathogen, Leptosphaeria maculans (anamorph, Phoma lingam). Application of the bacteria at 24 or 48 h prior to pathogen inoculation of the canola cotyledon was an important factor for suppression or prevention of formation of blackleg lesions. The strains PA23 and DF190 produced phenazines and pyrrolnitrin, whereas the strains

FIGURE 3.8 Colonization of wheat roots by Pseudomonas fluorescens strain HCl-07rif, cyclic lipopeptide-deficient mutants HCl-07prtR2-1 under growth chamber conditions Cycle 0: populations of each strain in soil at planting and populations determined at 2-week interval for cycle 1, 2, and 3; same letter above bars for the same cycle indicates that means are not significantly different (P = 0.05) as per Fischer’s protected least significant difference test.

[Courtesy of Yang et al. 2014 and with kind permission of the American Phytopathological Society, MN]
DFE4 and DFE16 produced lipopeptides, antibiotics iturin A, bacillomycin D and surfactin. The strains PA23 and DFE4 were tested as representative producers of each set of antibiotics for the split inoculation (SPI) of the extracts for induction of induced systemic resistance (ISR). The assays showed a small, but significant reduction in severity via a systemic response. The local SPI inoculation of the extract (for direct antagonism) showed significantly greater reduction of disease severity, which was also consistent with the SPI of the bacterial cells, establishing a more important role for the antifungal metabolites present in the culture extracts for the direct suppression of development of blackleg symptoms. The localized inhibition of pycnidiospores by the bacteria could be due to the successful colonization of the infection site which in turn most possibly function as a suitable delivery system for antifungal metabolites. The biocontrol activity of the mutant PA23-63, though deficient in phenazine production, was as effective as the wild strain in suppressing *L. maculans* development, indicating the nonrequirement of phenazines for its antagonistic activity. Strains of *P. chlororaphis* did not induce defense-related enzymes at the point of inoculation. Direct antifungal activity of *P. chlororaphis* strains appeared to be the dominant mechanism mediating control of blackleg disease of canola caused by *L. maculans* (Ramarathnam et al. 2011).

Hydrogen cyanide (HCN) is a volatile antibiotic produced as a secondary metabolite by *Pseudomonas* spp. HCN is highly toxic to most aerobic microorganisms, due to its ability to block the cytochrome oxidase pathway even at very low concentrations (pmol). Suppression of plant disease development is attributed to the action of HCN on some oomycetes. *P. fluorescens* CHA0 produces HCN, other antibiotics and siderophores. The mutants of CHA0 deficient in synthesis of HCN, antibiotics and exoenzymes were unable to protect tobacco plants against tobacco black rot pathogen *Thielaviopsis basicola*. HCN was considered to be primarily responsible for the antagonistic activity against the pathogen (Voisard et al. 1989). In *Pseudomonas fluorescens* strains Q2-87 and CHA0, HCN synthase required for HCN production was encoded by the *hcnABC* gene (Haas and Défago 2005). Primers targeting *hcnABC* genes with MultiAlin were employed for identification of isolates containing these sequences. A single amplicon of about 570-bp in length was amplified from the DNA of HCN-producing strains. No amplicon was generated by two negative HCN pseudomonads tested (Svercel et al. 2007). Bacterial species with antagonistic activity have been shown to produce cyclic lipopeptides (CLP) effective against fungal pathogens. A derivative of *Bacillus subtilis* BBG110, overproducing the CLP mycosubtilin exhibited enhanced suppressive activity, against *Pythium* spp. infecting tomato seedlings (Leclerc et al. 2005). Application of viscosinamide-producing *P. fluorescens* strain DR54 to sugar beet considerably increased seedling emergence and root length in soil infested with *Pythium ultimum* (Thrane et al. 2000). Sugar beet seeds were coated with CLP-producing *P. fluorescens* strains and subsequently germinated in nonsterile soil. The strain DR54 maintained a high and constant level of viscosinamide in the rhizosphere of plants growing from treated seeds for about 2 days, whereas strains 96.578 and DSS73 produced higher concentrations of CLPs tensin or amphisin. All three CLPs were detectable for several days in the rhizosphere. The results suggested that production of CLPs might occur only in specific habitats like rhizosphere of germinating sugar beet seeds, rather than in the bulk soil (Nielsen and Soenensen 2003). An antifungal polyketide 2,3-deepoxy-2,3-didehydrohizoxin (DDR) was produced by *Pseudomonas* spp. The DDR was effective in suppressing the development of wheat seedlings blight disease caused by *Fusarium culmorum* (Johansson and Wright 2003). *Bacillus* strains DE07, QST713 and F2B24 were evaluated for the antagonistic activity against *F. oxysporum*. The cellulases, cell-free supernatants and volatiles from BCA strains exhibited varying degrees of suppressive effects. Proteomic analysis of secreted proteins from EU07 and F2B24 revealed the presence of lytic enzymes, cellulases, protease, 1,4-ß-glucanase and hydrolases. All these enzymes contributed to degradation of seedling cell wall. Further, the proteins involved in the metabolism of protein folding, protein degradation, translocation, recognition and signal transduction cascade may play an important role in the suppression of *F. oxysporum* development (Baysal et al. 2013). The microbial population of a Korean soil suppressive to *Fusarium* wilt of strawberry was characterized. In this suppressive soil, infection of strawberry roots by *F. oxysporum* resulted in a response by microbial defenders, of which members of the Actinobacteria appeared to have a vital role. *Streptomyces* genes responsible for ribosomal synthesis of a novel heat-stable antifungal thiopetide antibiotic inhibitory to *F. oxysporum* and the mode of action of the antibiotic against fungal cell wall biosynthesis were studied. The results revealed the role of natural antibiotics as weapons in the fight against the pathogen and antagonists in the rhizosphere that is required for plant health, vigor and development (Cha et al. 2016).

The biosurfactants produced by *Pseudomonas* spp. interact with cell surface of fungal plant pathogens and also can act on lipids creating pores on the membrane layer (Raaijmakers et al. 2006). *Pseudomonas fluorescens* SS101 strain effectively prevented infection of tomato leaves by *Phytophthora infestans*, causing late blight disease and also restricted the expansion of existing lesions and sporangial production. As the sporangia are required for primary and secondary infections by *P. infestans*, destructive effect of the strain SS101 on both lesions area and sporangia formation might result in reduction of rate of disease development and spread. The biosurfactants produced by SS101, massetolide A was involved in the antagonistic activity of the BCA. The massetolide A-deficient mutant 10.24 was evaluated for the antagonistic activity against *F. oxysporum* spp. The DDR was effective in suppressing the development of wheat seedlings blight disease caused by *Fusarium culmorum* (Johansson and Wright 2003). *Bacillus* strains DE07, QST713 and F2B24 were evaluated for the antagonistic activity against *F. oxysporum*. The cellulases, cell-free supernatants and volatiles from BCA strains exhibited varying degrees of suppressive effects. Proteomic analysis of secreted proteins from EU07 and F2B24 revealed the presence of lytic enzymes, cellulases, protease, 1,4-ß-glucanase and hydrolases. All these enzymes contributed to degradation of seedling cell wall. Further, the proteins involved in the metabolism of protein folding, protein degradation, translocation, recognition and signal transduction cascade may play an important role in the suppression of *F. oxysporum* development (Baysal et al. 2013). The microbial population of a Korean soil suppressive to *Fusarium* wilt of strawberry was characterized. In this suppressive soil, infection of strawberry roots by *F. oxysporum* resulted in a response by microbial defenders, of which members of the Actinobacteria appeared to have a vital role. *Streptomyces* genes responsible for ribosomal synthesis of a novel heat-stable antifungal thiopetide antibiotic inhibitory to *F. oxysporum* and the mode of action of the antibiotic against fungal cell wall biosynthesis were studied. The results revealed the role of natural antibiotics as weapons in the fight against the pathogen and antagonists in the rhizosphere that is required for plant health, vigor and development (Cha et al. 2016).

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The role of biosurfactants in *Pseudomonas*-mediated suppression of viability of microsclerotia of *Verticillium longisporum*, infecting cauliflower was studied. The biosurfactant-deficient mutants of *Pseudomonas* CMR12a and *P. aeruginosa* PN1 strains were less efficient in reducing viability of *Verticillium* microsclerotia, compared with the wild-type strain. The biosurfactants were effective at a BCA cell density of 2 x 10^6 CFU/ml. However, the biosurfactant production by the BCA strains did not fully account for *Verticillium* microsclerotial germination. The biosurfactants had residual adverse effect on microsclerotial germination and/or formation of secondary microsclerotia (Debode et al. 2007). The potential of a biosurfactants produced by *Pseudomonas koreensis* was assessed by applying crude extract of the BCA cells. Assessment of the effect of the crude extract on *Pythium ultimum*, infecting tomato plants in hydroponic system showed that the incidence of the disease was significantly reduced. Application of the biosurfactants did not affect the indigenous microflora (Hultberg et al. 2010a). The effectiveness of the biosurfactants from *P. koreensis* 2,742 strain against *Phytophthora infestans* was assessed, using a detached leaf assay. The biosurfactants inhibited the motility of zoospores, but mycelial growth was not significantly affected. The sporangia production was also not reduced by the surfactant from *P. koreensis* (Hultberg et al. 2010b). Likewise, the biosurfactants of *P. koreensis* effectively suppressed the development of damping-off caused by *Pythium ultimum* in tomato (Hultberg et al. 2011).

### 3.1.5.2.2 Competition for Nutrients and Space

Competition for nutrients and space available in various substrates is a natural phenomenon for the survival of all microorganisms, including soilborne bacterial pathogens. Availability of essential micronutrients such as iron is a crucial factor for all microorganisms. Iron becomes a limiting factor in the rhizosphere, depending on the soil pH. The iron-binding ligands known as siderophores with high affinity for iron are produced by microorganisms for sequestration from the micro-environment. Ability to produce siderophores confers competitive advantages to bacteria for colonizing plant tissues and to exclude other microorganisms from the same ecological niche. The siderophores may be of two kinds: catechol type or tyroxamate type (Neilands 1981). The fluorescence of pseudomonads is attributed to the presence of an extracellular diffusible pigment known as pyoverdin (Pvd) or pseudobactin, which has affinity for Fe^{3+} ions. It is a siderophore (iron-carrier) of the producer strain. In iron-depleted media, Pvd-producing *Pseudomonas* spp. may inhibit the growth of fungi and bacteria with efficient siderophores. Under certain conditions, Pvd may function as a diffusible, bacteriostatic or fungistatic antibiotic (Kloeper et al. 1980).

Under greenhouse conditions, *Pseudomonas putida* strain B10 suppressed Fusarium wilt and take-all diseases of wheat. The suppressive effect was lost, when the soil was amended with iron which repressed siderophores production by the strain B10 (Kloeper et al. 1980). The strain B324 of *Pseudomonas* was inhibitory to all seven isolates of *Pythium ultimum* var. *sporangiferum*, infecting wheat, in addition to induction of growth-promoting effect on wheat plants under iron-limiting conditions (Becker and Cook 1988). *Pseudomonas fluorescens* strain CV6 effectively suppressed the development of cucumber root rot disease caused by *Phytophthora drechsleri*. The BCA also produced substantial amount of siderophores, antifungal compounds and indole acetic acid (IAA). In addition, strain PN1 produced enzymes involved in induction of systemic resistance, indicating that its biocontrol activity against *P. drechsleri* was successfully achieved through multiple mechanisms operating during the interaction between the pathogen and biocontrol agent (Maleki et al. 2010). *Pseudomonas aeruginosa* (PN1–PN10) strains (10) isolated from the rhizosphere of chir-pine were evaluated in vitro and in vivo for their antagonistic activities against *Macrophomina phaseolina*, causing root rot. The strain PN1 produced siderophores, IAA, cyanogens and solubilized phosphorus, in addition to chitinase and β-1,3-glucanase. In dual culture assay, the BCA inhibited the mycelial growth of *M. phaseolina* by 69%. PN1 showed strong chemotaxis toward root exudates, resulting in effective root colonization. The BCA PN1 strain showed strong antagonistic effect on *M. phaseolina* and also significantly promoted the growth of chir-pine seedlings (Singh et al. 2010).

Microorganisms may exert positive or negative effects on others for their survival in the rhizosphere and the nature of the interaction may, in turn, have influence on the development of diseases caused by root pathogens. *Pseudomonas putida* WCS 358 suppressed radish Fusarium wilt disease caused by *F. oxysporum* f.sp. *raphani* by competing for iron through the production of the siderophore pseudobactin. Addition of iron to nutrient solution fed to the radish plants, reduced the suppressive effect of the strain WCS358. The pseudobactin negative mutant WCS358 was as effective in suppressing development of Fusarium wilt disease as the wild-type strain, suggesting that the mutant might act on the pathogen through an alternative mechanism. Another strain RE8 of *P. putida* did not depend on the production of pseudobactin for its suppressive activity on wilt pathogen. The strain RE8 could induce systemic resistance in treated radish plants. When the mixture of WCS358 and RE8 strains was applied to the soil, the level of disease suppression was enhanced, compared to application of single strains. This additive effect on disease suppression could be due to pseudobactin-mediated competition for iron and induction of systemic resistance by these BCA strains. The results indicated the possibility of exploiting the complementary mechanisms of disease suppression by two compatible strains of *P. putida* on *F. oxysporum* f.sp. *raphani*, causing Fusarium wilt disease of radish (de Boer et al. 2003).

The competition between the pathogens and the biocontrol agents (BCAs) for available nutrients, as a mechanism of biocontrol activity has been investigated in other pathosystems also. The efficiency of strains of *Pseudomonas fluorescens* in suppressing the development of sugar beet root rot caused by *Pythium ultimum* was assessed. All strains of the BCA reduced the number of lesions and the root and soil
populations of *Pythium*, whereas the strains of SBW25 and CHA0 increased lateral roots formed in treated plants. The strain SBW25 did not produce any antifungal compounds and its biocontrol activity was related to its greater colonization ability and rhizosphere competition (Naseby et al. 2001). *P. fluorescens* strain F113 protected the sugar beet roots effectively against *Pythium ultimum*. During colonization of alfalfa rhizosphere, the strain F113 produced variants that were characterized by the translucent and diffused colony morphology. The phenotypic variation in this strain appeared to be mediated by the activity of two site-specific recombinases Sss and XerD. The mutants with disruption in either of the genes involved in the biosynthesis of Sss or XerD, showed drastic reduction in rhizosphere colonization and the mutants had reduced chemotactic motility. Large number of variants (mutants), overexpressing the genes sss or xerD were generated. All isolated variants were more motile than the wild-type strain and appeared to contain mutations in the gacA and gacS genes. The highly motile mutants were more competitive than the wild-type strain, displacing it from the root tip within 2 weeks (Martínez-Granero et al. 2006).

### 3.1.5.2.3 Prevention of Pathogen Colonization of Host Roots

Colonization of host plant roots may be prevented by the bacterial biocontrol agents. Application of *Pseudomonas fluorescens* strain CH31 to cucumber significantly reduced root colonization by *Pythium aphanidermatum*, resulting in reduction in incidence of root rot disease in cucumber (Moulin et al. 1996). Likewise, treatment of sugar beet seeds with *P. putida* 40RNF as pellets significantly reduced the incidence of sugar beet damping-off disease caused by *Pythium ultimum* by 43% at 48 h after planting. Further, the number of sporangia was reduced by 68% in the soil around sugar beet plants growing from treated seeds. Seed treatment with *P. putida* 40RNF was effective as the fungicide hymexazole in reducing disease incidence in infested soil (Shah-Smith and Burns 1996). Based on the competitive tomato root tips colonization assay used to select efficient root colonizers, 24 isolates of *Pseudomonas rhodesia* isolates with equal or better colonizing ability as *P. fluorescens* WCS365 were selected. These isolates of *P. rhodesia* effectively reduced the incidence of tomato foot and root rot disease caused by *F. oxysporum* f.sp. radicis-lycopersici (Validov et al. 2007).

### 3.1.5.2.4 Induction of Resistance in Plants to Disease

Resistance to crop diseases caused by soilborne microbial plant pathogens may be induced by biotic and abiotic agents. Induced resistance has been broadly differentiated into two forms as systemic acquired resistance (SAR) and induced systemic resistance (ISR). In response to pathogen infection or chemical application ‘SAR’ process is initiated, whereas colonization of host plant roots by plant growth promoting rhizobacteria (PGPR) leads to ISR. The direct or indirect effects of treatment of wheat or apple with *Pseudomonas fluorescens* SS101 on the development of *Pythium* spp. were assessed by the split-root plant assay. The assays were performed in orchard soils to explore the possible role of ISR. The strain SS101 or the massA mutant 10.24 significantly reduced infection by *Pythium* spp. on the apple or wheat root system raised in soil treated with the respective bacteria. Wheat root infection was reduced from 34% for plants grown in non-treated soil to 11% for the component of root systems grown in SS101- or mutant 10.24-treated soils. The frequencies of *Pythium* root infection in SS101 and 10.24 treatments did not show significant differences for the component of the root system physically separated from bacterially treated soil, compared to the nontreated control (Mazzola et al. 2007). The efficacy of *Pseudomonas fluorescens* strain FP7 in suppressing the development of turmeric rhizome rot disease caused by *Pythium aphanidermatum* was assessed. A combination of rhizome dip and soil drench of FP7 liquid formulation resulted in the reduction of disease incidence to the minimum (19.0%) under greenhouse conditions and to 10.18 and 13.29% under field conditions respectively in trial I and II. Twelve differentially expressed tripartite interaction between host-pathogen-bioagent through protein profiling could be identified. Further, mass spectrometry (MS) analysis revealed that proteins such as tryptophan synthase beta subunit-like phosphoglycerate kinase subunit beta, cysteine-rich peptide, ribosomal protein S3, clathrin assembly protein and disease resistance protein RPP13-like were differentially regulated. The differentially expressed proteins during tripartite interaction might be directly or indirectly involved in disease resistance in turmeric plants (Karthikeyan et al. 2017).

Induction of systemic resistance in lupine by *Pseudomonas fluorescens* and *P. putida* against Fusarium wilt disease caused by *F. oxysporum* Esp. *lupine* (FOL) was investigated. Both BCAs significantly reduced the disease incidence under greenhouse and field conditions. A time-course of defense-related enzymes showed substantial increases in their activities in induced infected seedlings, compared to nontreated infected or healthy control plants. The extent of enhancement of enzyme activities varied among treatments. Increases in chitinase and β-glucanase activities attained maximum levels at 12 and 18 days after inoculation with FOL, respectively. Furthermore, phenylalanine ammonia lyase (PAL) activity increased significantly at 8 days after inoculation. Accumulation of phenolic compounds and specific flavonoids occurred significantly, following infection by the pathogen in induced and/or infected seedlings, compared with noninoculated control plants. The BCAs increased the growth parameters and seed yield, compared with untreated control plants (Abd El-Rhaman et al. 2012). *Didymella bryoniae*, causing gummy stem blight of watermelon is a serious threat to production in Mekong Delta of Vietnam. *Pseudomonas aeruginosa* strain 23 effectively suppressed directly the pathogen development by producing antibiotics locally and/or indirectly by stimulating the defense systems systemically. Foliar infection by *D. bryoniae* was significantly reduced by seed treatment with the BCA, indicating the induction of resistance by the strain 23 under field conditions. *P. aeruginosa* colonized watermelon plants endophytically, more actively in *D. bryoniae* infected plants than in healthy plants. Treatment
with the BCA, inhibited penetration, which was associated with accumulation of \( \text{H}_2\text{O}_2 \), followed by enhanced peroxidase activity and production of new peroxidase isofoms. Suppression of watermelon gummy blight development was achieved by production of antibiotics and ISR under greenhouse and field conditions, following treatment with \( \text{P. aeruginosa} \) (Nga et al. 2010).

Strains of bacterial biological control agents with multiple mechanisms of action against the fungal pathogens are likely to be more effective in suppressing pathogen development than BCAs with single mode of action. \( \text{Pseudomonas chlororaphis} \) strain PA-23 could produce phenazine-1-carboxylic acid (PCA), which inhibited the mycelial growth of \( \text{Sclerotinia sclerotiorum} \), incitant of canola stem rot disease. Ascospore germination in canola flower petals was inhibited by the strain PA-23. In addition, two applications of strain PA-23 induced resistance against infection by \( \text{S. sclerotiorum} \). Enhanced accumulation of PR-proteins and oxidative enzymes, including chitinase and \( B-1,3 \)-glucanase by PA-23 in canola leaf tissues might account for reduction of pathogen infection. The combination of antibiotic production along with induction of systemic resistance might act in tandem, resulting in restriction of pathogen growth, colonization of canola tissues and consequent suppression of disease intensity significantly (Fernando et al. 2007). Two strains of \( \text{P. putida WCS358r} \) and \( \text{P. fluorescens WCS374r} \) could trigger ISR in \( \text{Eucalyptus urophylla} \) against bacterial wilt disease caused by \( \text{Ralstonia solanacearum} \), when infiltrated into two lower leaves at 3–7 days before challenge inoculation with the pathogen, but not when the BCA strains were incorporated into the soil. The mutant strain WCS358r, deficient in the biosynthesis of pseudobactin (siderophores), did not induce ISR. The purified pseudobactin from WCS358r induced ISR, suggesting that pseudobactin 358 was the determinant of ISR in WCS358r strain. In contrast, the siderophores-deficient mutant of WCS374r was able to induce ISR to the same level as the wild-type strain. The purified siderophores from this strain induced ISR, indicating that both the siderophores and other uncharacterized ISR determinants of WCS374r could trigger ISR in \( \text{Eucalyptus} \) (Ran et al. 2005). Development of tobacco soft rot disease caused by \( \text{Pectobacterium carotovorum} \) subsp. \( \text{carotovorum} \) \( \text{(Pcc)} \) was reduced by \( \text{Pseudomonas chlororaphis} \) 06 and the mechanism of the biocontrol activity of the BCA was via induction of resistance in tobacco plants. The bacterial determinant involved in ISR was identified using high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) mass spectrometry. The active compound was \( \text{2R, 3R-butane-diol, which induced systemic resistance against Pcc, but not against P. syringae pv. tabaci (Pst). The global sensor kinase GacS of P. chlororaphis} \) 06 was a key regulator for ISR against \( \text{P. carotovorum} \) subsp. \( \text{carotovorum} \) through regulation of \( \text{2R, 3R-butane-diol, a fermentation product, as well as to the sensor kinase GacS for its production} \) (Han et al. 2006).

\( \text{Pseudomonas fluorescens PICF7} \) strain, a native root endophyte of olive trees effectively suppressed the development of olive vascular disease caused by \( \text{Verticillium dahliae} \). The bacterial BCA could trigger a broad range of defense responses in root tissues of olive. The mechanism of induction of systemic resistance to olive wilt pathogen by PICF7 strain was studied. Root bacterization of olive plants with PICF7 strain was performed and aerial tissues were sampled at different time interval after bacterization. The suppression subtractive hybridization (SSH) cDNA library, enriched in upregulated genes was generated. Of the 376 expression sequence tags (ESTs), five involved in the defense responses, were selected to perform time course quantitative real-time PCR (qRT-PCR) experiments. Induction of olive genes potentially coding for lipoygenase 2, catalase, 1-aminoxylopropene-1-carboxylic oxidase and PAL was confirmed at some stages of interaction. The results revealed that root colonization by the endophytic strain of \( \text{P. fluorescens} \) could trigger defense responses in this organ. In addition, root colonization by the BCA could induce a range of systemic defense responses in tissues away from the roots (Cabanás et al. 2014).

\( \text{Bacillus amyloliquefaciens} \) BS6 and \( \text{Pseudomonas chlororaphis} \) PA-23 were evaluated for their biocontrol potential against canola stem rot disease caused by \( \text{Sclerotinia sclerotiorum} \) under greenhouse and field conditions. The BCAs were equally effective in suppressing the development of stem rot disease, as the fungicide Rovral Flo® (iprodione). Suppression of disease development by the BCAs was attributed to reduction of canola flower infections by \( \text{S. sclerotiorum} \) through direct antimicrobial activity and/or induction of plant defense enzymes (Fernando et al. 2007). The mechanism of biocontrol activity of \( \text{Bacillus pumilus} \) 7 Km isolated from wheat rhizosphere against wheat take-all disease pathogen \( \text{Gaemanynomyses graminis var. tritici} \) was investigated. The soil was drenched with bacterial suspension and changes in the defense-related enzymes and total phenolics contents were monitored. Disease severity was significantly reduced in the bacterized roots of wheat plants and plant growth was also promoted by treatment with the BCA. The activities of soluble peroxidase (SPOX), ionically cell wash bound peroxidase (CWPOX), \( B-1,3 \)-glucanase, 8-1,4-glucanase were increased in plants treated with \( \text{Bacillus pumilus} \). Further, the phenolics contents were at higher levels in BCA-treated plants, compared to untreated control plants. The activities of enzymes reached the peak at 4–8 days after application of BCA to wheat roots. The disease suppressive activity of \( \text{B. pumilus} \) might be due to its ability to induce local/systemic defense system operating in the host root system (Sari et al. 2007). Seed bacterization and soil application of a mixture of \( \text{Bacillus subtilis} \) strains S2BC-1 and GIBC-Jamog reduced the incidence of tomato wilt disease caused by \( \text{F. oxysporum} \) f.sp. lycopersici significantly as indicated by localized and split-root experiments. Activities of chitinase and \( B-1,3 \)-glucanase were enhanced in root samples from treated tomato plants. High concentrations of peroxidase isofoms were detected in samples from localized and ISR experiments. The results indicated the possibility of operation of both antibiosis and induction of ISR mechanisms functioning in concert in tomato plants protected with the BCA strains (Shanmugam and Kanoujia 2011).
Elicitation of induced systemic resistance (ISR) by *Bacillus* sp. strain BS107 in tobacco against soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) was observed. A determinant of ISR secreted by the strain BS107 was isolated from the cell-free culture supernatant and identified, using mass spectrometry and NMR analyses, as 2-amino-benzoic acid (2-AB) as the principal ISR determinant. Purified 2-AB displayed effective ISR activity against soft rot disease development in the tobacco leaves. Treatment of tobacco roots with 2-AB resulted in protection against Pcc. The pathogen was not inhibited at the concentration that induced resistance. Reverse transcription (RT)-PCR assays of tobacco leaves of plants treated with 2-AB on the roots, showed upregulation of the induced marker genes such as *PR1a*, *PR1c*, *PR2* and *PR-4*. The inducer determinant 2-AB was biosynthesized from chorismic acid which is a precursor of salicylic acid (SA) and SA is known to have a vital role in mediating plant defenses (Yang et al. 2010). *Bacillus valvismoris* strain EXTN-1 isolated from red pepper suppresser tomato bacterial wilt disease caused by *Ralstonia solanacearum*. *Bacillus subtilis* strain 816-6, *B. pumilus* strain 228-7, *Bacillus* sp. strain 113-3 and *Paenibacillus polymyxa* strain H321-5 were also evaluated for their ability to induce resistance to tomato bacterial wilt pathogen, along with the strain EXTN-1. The tomato seedlings roots were bacterized with the test strains and the plants were grown in perlite-hydroponic system, followed by challenge inoculation with *R. solanacearum*. All bacterial strains suppressed bacterial wilt development to different extent. The strain EXTN-1 was the most effective in reducing infection to 65%, as against 95% in untreated control plants. The movement of the pathogen from the site of inoculation was hampered in plants treated with the strain EXTN-1 was probably due to a mechanism other than antibiotics, since no direct antagonistic activity of this strain against *R. solanacearum* could be detected. The strain EXTN-1 produced an elicitor which efficiently induced systemic resistance in many crops (Park et al. 2007). The bacterial mixture of *Bacillus atrophaeus* S2BC-2 and *Burkholderia cepacia* TEPP-Sungal protected gladiolus plants against *F. oxysporum* f.sp. *gladioli*, causal agent of vascular wilt and corm rot disease to the maximum extent. Protection by these strains was attributed to induction of systemic resistance as deduced from stimulation of PR-proteins and activities of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL), in addition to phytoalexins and/or chalcone synthase (Shanmugam et al. 2011).

The mechanisms underlying the interactions of *Streptomyces*- host plant-microbial plant pathogens have been investigated. The biocontrol potential of *Streptomyces coelicolor*, *S. griseus*, *S. albus*, *S. antibioticus* and *S. champavatti* against *Rhizoctonia solani* infecting tomato was assessed. *Streptomyces* spp. have been shown to induce systemic resistance in different pathosystems. Association of changes in H$_2$O$_2$ production, lipase peroxidation (LPO) and antioxidant enzymes with ISR induction was studied. Production of H$_2$O$_2$ at 2 days after inoculation (dai) of *R. solani* was 1.1-fold higher in BCA-treated tomato plants, compared with untreated inoculated control plants. Increases in catalase and ascorbate peroxidase activities were recorded at 4 dai, whereas enhancement of activities of guaiacol reductases and glutathione peroxidase were observed at 5 dai. Similarly, LPO reached the maximum at 6 dai in untreated inoculated plants, whereas there was a decrease of 1.3–1.5 fold in BCA-treated plants, compared with untreated inoculated plants. Priming by *Streptomyces* in tomato as a mechanism of biocontrol activity against *R. solani* was revealed, highlighting the capacity of *Streptomyces* spp. to activate plant defense responses in tomato plants through induction of antioxidant enzymes along with improved reactive oxygen species management (Singh et al. 2016). The endophytic *Streptomyces* spp., viz., *S. diastaticus*, *S. fradiae*, *S. olivochromogenes*, *S. collinus*, *S. ossamyceticus* and *S. griseus* were evaluated for their biocontrol potential against *Sclerotium rolfsii*, infecting chickpea. Stimulation of systemic resistance was monitored by treating the chickpea seeds, followed by inoculation with the pathogen. Substantial increase in defense-related enzymes such as PAL and PPO, along with the accumulation of total phenolics and flavonoids occurred in *S. griseus*-treated and pathogen-inoculated chickpea plants. Likewise, significant increase in superoxide dismutase (SOD), peroxidase (PO), ascorbate oxidase (APX) and guaiacol peroxidase (GPX) was recorded in the same treatment, which favored least lipid peroxidation and chickpea pathogenic stress. Further, in *S. griseus*-primed plants, collar tissues remained undamaged, as revealed by scanning electron microscopic observations. Real-time PCR analysis of genes encoding SOD, PO, PAL, APX, catalase, chitinase and β-glucanase showed significant increases. The results indicated that the chickpea defense pathway might be triggered after perception of endophytes to synthesize various enzymes, resulting in enhancement of resistance in chickpea against *S. rolfsii* (Singh and Gaur 2017). The extracellular polysaccharide (EPS) produced by *Ralstonia solanacearum*, incitant of eggplant bacterial wilt, functions as an elicitor of resistance in eggplant against bacterial wilt disease. Eggplants treated with intact crude EPS showed a significant decrease in bacterial wilt incidence under both in vitro and greenhouse conditions. In the eggplant cultivars, EPS induced significant upregulation of guaiacol peroxidase and ascorbate peroxidase defense genes, providing significant enhancement of resistance to bacterial wilt disease (Prakash et al. 2017).

### 3.1.6 Formulations of Biological Products

Several species/strains of fungi or bacteria have been shown to possess biocontrol potential against soilborne microbial plant pathogens. However, only some of them have been found to be suitable for development of formulation and commercialization as biological products or microbial biocides for use as alternatives to chemicals for the management of diseases affecting field or glasshouse crops.

#### 3.1.6.1 Development of Formulations

Development of formulations of biotic biocontrol agents (BCAs) depends on several factors, such as the efficiency and
adaptable to the BCAs that have been found to effectively suppress the development of the soilborne fungal/bacterial pathogens through single or multiple mechanisms of action on target pathogen(s). The knowledge of mechanisms of action of the selected BCA in combination with analysis of the sequence of corresponding genes can provide gene targets to develop high throughput screening procedures. The effectiveness of the BCA at low doses, production of antimetabolites or toxins against target pathogen, tolerance to fungicides or other plant protection chemicals and plant activators and adaptability to general crop management practices are considered as desirable attributes of the BCA to be advanced to formulation stage (Narayanasamy 2013).

Nonpathogenic *F. oxysporum* strain CS-20 with significant antagonistic activity against *F. oxysporum* f.sp. *lycopersici* was inhibited by the fungicide azoxystrobin and chlorothalonil, whereas mfenoxam (Ridomil Gold) and mfenoxam + copper did not affect the growth of the BCA. However, *F. oxysporum* CS-20 was found to be incompatible with all fungicides tested under greenhouse conditions, since the disease incidence was not reduced by the combination of the BCA and fungicides (Fravel et al. 2005). In another investigation, the possibility of combining soil application of *Coniothyrium minitans*, an effective biocontrol agent capable of suppressing the development of Sclerotinia stem rot of oilseed rape, caused by *Sclerotinia sclerotiorum*, with compound fertilizer (N, P and K), was examined. Simultaneous application of the BCA and fertilizer at various concentrations significantly reduced the number of apothecia formed by sclerotia of *S. sclerotiorum* in both pot and field-plot experiments. The compound fertilizer did not affect the ability of *C. minitans* to infect sclerotia of the pathogen in vitro or suppress carpogenic germination of sclerotia of *S. sclerotiorum*. The results indicated that *C. minitans* was compatible with fertilizer, when applied at planting of oilseed rape. Thus, application of the BCA along with fertilizer could save labor cost, resulting in greater monetary benefit to the grower and enhancement of production efficiency (Yang et al. 2011).

After assessment of the biocontrol potential and investigation of mechanisms of the biocontrol activity, the most effective strains/isolates are tested in pilot trials under conditions as close as possible to the natural field conditions under which the BCA is to be placed in practice. At this stage, it would be desirable to expose the BCA in an environment where the host plants develop. In addition, some endophytic fungal strain may colonize shoots, roots or stems and are able to increase resistance to water stress and salt or temperature tolerance. *T. harzianum* T22 enhanced expression of proteins involved in photosynthesis and starch accumulation (Shoresh and Harman 2008). *Trichoderma* strains may alleviate intrinsic stresses like loss of seed vigor and improve seed germination (Shoresh et al. 2010).

### 3.1.6.1.1 Preparation of Formulations

Strains of biocontrol agents with high level of antagonistic ability to suppress the development of the soilborne microbial plant pathogens by acting through one or more mechanisms are selected for preparing formulations. These strains should be genetically stable, effective at a wide range of temperatures, easy to mass-produce in culture on inexpensive media and be effective against several microbial pathogens. Furthermore, the putative BCA should be available in an easily distributable form and it should be nontoxic and nonpathogenic to human beings and other plant species grown in the same ecosystem. Different methods have been applied for preparing formulations containing fungal and bacterial biocontrol agents. Formulated microbial products may have biomass of the selected BCA and ingredients to improve its survival and effectiveness of the products as the major components. The microbial mass may be of two types: culture grown on semisolid or liquid media. Liquid formulations may be flowable or aqueous suspensions consisting of biomass suspensions in water, oils or emulsions. Dry (powder) formulations may be in the form of wettable powder, dusts or granules. Wettable powders and dusts contain dry inactive or active ingredients and granules are free-flowing, aggregated product consisting of active or inactive ingredients (Schisler et al. 2004). The formulated product should be shelf-stable, retaining the biocontrol activity at a level similar to that of the freshly isolated microorganism. The shelf-life of a biocontrol product is the duration for which the microorganisms remain viable and antagonistic to the required level. The biocontrol product should be easy to prepare and apply and produce abundant viable propagules with long-shelf life, in addition to being efficacious and economical. The shelf-life of the product should be at least 12–18 months under unrefrigerated conditions to provide protection to crops at commercially acceptable level. Various aspects of product formulations are described in detail in earlier publications (Narayanasamy 2006, 2013).

*Strains of Pseudomonas syringae* pv. *syringae* produce syringomycin E (SRE), a cyclic lipodepsinonapeptide with potent antifungal activity. The potential of the compound as an organic-compatible agrofungicide and vegetable seed treatment against soilborne pathogen *Pythium ultimum* var. *ulitnimum* was assessed. A variant of *P. syringae* pv. *syringae* B301D with enhanced SRE-producing ability was isolated and grown in bioreactor with SRE yields averaging 50 mg/l in 40 h. SRE was extracted and purified through a large-scale
chromatography system using organic-compatible processes and reagents. The minimum concentrations of the purified product required to inhibit 50% and 90% of *P. ultimum* oospore germination were determined as 31.3 and 250 μg/ml, respectively. Drench treatment of cucumber seeds in *P. ultimum*-infested potting medium (500 oospores/g) with 50 μg/ml SRE or water with no SRE resulted in 90.2 ± 4.5% and 65.7 ± 4.6% germination rates, respectively. Seed coating with 0.05% (w/w) SRE allowed 65.7 ± 4.6% seed germination on naturally infested soil, whereas 100.0 ± 0.0% of noncoated seeds were unable to germinate due to infection by *P. ultimum*. Organic-compatible SRE suitable for large-scale production, has the potential for use as organic fungicide seed protectant in organic systems of crop production (Kawasaki et al. 2016). Large-scale production of Trichoderma sp. formulations is confronted with many difficulties, although these BCAs have been employed for the management of several crop diseases. Development of Trichoderma sp. formulations in encapsulated granules (CG) for extended period of viability of conidia during storage was attempted. An ionic gelling method was followed for producing encapsulated granules containing sodium alginate matrix modified with different polymers. Granules characterization showed that there was interaction between the alginate matrix and polymers used in the formulation of the encapsulated granules, which ensured stability of formulations, maintaining the viability of Trichoderma during different stages of production and storage. After 14 months, the product stored at 28°C had viable concentration maintained above 10⁶ CFU/g, at which the BCA might be effective against target pathogens (Locatelli et al. 2018).

### 3.1.6.1.2 Application Methods

The formulated bioproducts containing fungal or bacterial biocontrol agents (BCAs) may be applied on seeds, propagules, soils and/or foliage of crops to suppress development of soilborne microbial plant pathogens either as protective or curative treatments. The efficacy of formulated products in reducing the disease incidence or severity has to be compared with that of the unformulated fresh fungal or bacterial species to determine the extent of loss of biocontrol activity, if any, due to the process of formulation and storage for different duration. The commercial bioproducts are recommended to the growers, after their evaluation by state-owned research centers/universities.

#### 3.1.6.1.2.1 Seed/Propagule Treatment

Seeds or vegetatively propagated plant materials (propagules) are treated with liquid, powder or granular formulations containing single or mixture of strains of fungal or bacterial BCAs. Seed treatment with biocontrol products is generally preferred, because of ease of application and lesser cost of treatment. Seed treatment not only suppresses the pathogen present on the spermosphere, but also protects the emerging young seedlings against infection by the soilborne pathogens. Various kinds of stickers, such as carboxymethyl cellulose (CMC, 1%), methocel (2%), polysulf (0.8%) and polyvinyl alcohol (20%) have been used for efficient adherence of the BCA to seeds or propagules.

The comparative efficacy of biocontrol agents and chemicals used for treating the seeds and soil was assessed for suppressing the development of damping-off and wilt diseases of spinach caused by *Pythium ultimum*, *Rhizoctonia solani* and *F. oxysporum* f.sp. *spinaciae* in organic production system. Two experimental seed treatments GTGI and GTGII (each comprised of a proprietary organic disinfectant and the latter also containing *T. harzianum* T22) provided protection as efficiently as the fungicide mefenoxam against *P. ultimum* in one trial and significant reduction of damping-off in the second trial. Natural II and Natural X (*Streptomyces* products) and Subtilex (*Bacillus subtilis*) applied as seed treatments suppressed damping-off significantly in one of two trials. For suppression of development of *R. solani*, GTGI and Natural II seed treatments reduced damping-off as effectively as a drench with the fungicide Terralor (pentachloronitrobenzene). Soil drench with Prestop (*Gliocladium catenulatum*) suppressed postemergence wilt caused by *F. oxysporum* f.sp. *spinaciae* in both trials. Compost tea drench and seed treatment with Yield Shield (*Bacillus humilis*) each suppressed postemergence wilt in only one of two trials. None of the treatments was effective against all three pathogens and some treatments intensified symptoms of damping-off disease (Cummings et al. 2009).

Treatment of cotton seeds with Trichoderma (*Gliocladium* virices or *Bacillus subtilis*) reduced colonization of roots by *F. oxysporum* f.sp. *vasinfectum*, causing Fusarium wilt disease, resulted in lower levels of disease incidence and severity (Zhang et al. 1996). Corn damping-off disease caused by *Pythium ultimum*, *P. arrhenomanes* and * Fusarium graminearum* was more effectively suppressed by coating the seeds with *T. virices* isolate GI3, leading to greater seedling stand and better plant growth due to significant reduction in root rot severity compared to the fungicide captan or other BCAs tested (Mao et al. 1997). Seed treatment with *Bacillus* sp. strain L32-49-94 suppressed the development of *Pythium* root rot caused by *P. ultimum*, *Rhizoctonia* root caused by *Rhizoctonia solani* and wheat-take-all caused by *Gaeumannomyces graminis* var. *tritici*, when the treated seeds were directly drilled into soil in both winter and spring seasons (Kim et al. 1997). Antibiotic-producing isolate J-2 of *Streptomyces* sp. efficiently reduced damping-off of sugar beet caused by *Sclerotium rolfsii* by treating the seed a few weeks before planting. The BCA isolate was able to multiply and survive in the rhizosphere soil from naturally infested soil for more than 3 weeks (Errakhi et al. 2007). *Streptomyces rochei* strain PTL2 was highly antagonistic to *Rhizoctonia solani*, causing damping-off of tomato seedlings and also exhibited remarkable capacity for production of hydrogen cyanide, siderophores, L-aminocyclopropane-L-carboxyoxlate deaminase and phytohormones, chitinolytic activity and inorganic phosphate solubilization. Talc-based formulation of the strain PTL2 showed highest protective activity by reducing the disease incidence to 14.1 from 89.3% (in control), whereas the fungicide Thiram®-treated plots had 16.7% infection by *R. solani*. Furthermore, the talc-based formulation increased the root length, shoot...
length and dry weight of tomato seedlings to the maximum extent. The ability to reduce disease incidence and enhance plant growth parameters, indicates the advantages of employing the strain PT2 of S. rochei for large-scale application to protect tomato nurseries (Zamoum et al. 2017).

3.1.6.1.2.2 Treatment of Propagules and Transplants Potato silver scurf disease caused by Helminthosporium solani primarily spread from infected to healthy tubers during storage. The minitubers of Red Norland potato were immersed in a suspension of formulated preparation of Acremonium strictum for 3 min and air-dried. The treated and untreated tubers were inoculated with H. solani by spraying conidial suspension. The BCA reduced sporulation and spore germination of H. solani. The BCA was effective, when applied as a protective treatment only, as A. strictum did not have any curative effect on already infected tubers (Rivera-Varas et al. 2007). Dipping of wounded roots of tomato seedlings in a suspension of cells of nonpathogenic Agrobacterium radiobacter strains K84 or K1026 or the commercial product ‘Nogall’ entirely inhibited crown gall formation by A. tumefaciens (Fakhouri and Khalaf 1996). Pseudomonas aureofaciens and P. fluorescens with wide spectrum of antagonistic activity, when applied as root dip to grapevine cuttings, showed differential effect, depending on grapevine cultivar. Disease incidence was reduced by 50 to 80% and disease severity index (DSI) by 75 to 86%. Both P. aureofaciens and P. fluorescens persisted on the root surfaces of treated grapevine cuttings and in nonsterile soil. Treatment of rooted raspberry seedlings with P. aureofaciens strain B4117 protected the seedlings effectively against infection by A. tumefaciens, infecting raspberry (Khmel et al. 1998). Soaking roots of grapevine seedlings in a cell suspension of nonpathogenic Agrobacterium vitis strain VAR03-1 for 24 h before a 1-h soak in a cell suspension of tumorigenic A. vitis and subsequent planting in infested soil resulted in reduction of crown gall incidence significantly (Kawaguchi et al. 2007). The effectiveness of treatment of roots of grapevine, rose and tomato with A. vitis strain VAR-03, for reducing infection and disease severity induced by respective pathogens A. vitis, A. rhizogenes and A. tumefaciens was demonstrated under field conditions. VAR-03 strain was efficient in suppressing development of grapevine crown gall and the BCA could multiply and establish a population averaging 10^6 CFU/g of roots in the rhizosphere of grapevine and persist on grapevine roots for 2 years (Kawaguchi et al. 2008b). Tomato bacterial wilt disease caused by Ralstonia solanacearum was reduced to 65% by root bacterization with Bacillus vallismortis strain EXTN-1, as against 95% infection in nonbacterized control plants (Park et al. 2007) The roots of banana plantlets were immersed in a suspension of cells of the endophytic actinomycete Streptomyces griseorubigenosus at 1 hour prior to planting, followed by inoculation of Fusarium wilt disease. The pathogen inoculum was reduced by 50%. In addition, the plant growth was also improved by the BCA treatment (Cao et al. 2005). Tomato Fusarium wilt disease incidence could be reduced by dipping the roots in suspension of cells of Achromobacter xylosoxydans at a concentration of 10^6 CFU/ml by about 50%. The symptoms induced by the phytotoxin produced by the pathogen F. oxysporum f.sp. lycopersici were not observed in BCA-treated tomato plants. In addition, BCA treatment enhanced plant growth significantly, compared to control plants (Moretti et al. 2008).

3.1.6.1.2.3 Soil Treatment Biocontrol agents with required levels of biocontrol activity against target pathogen(s) have to remain viable as actively proliferating propagules under natural field conditions. The biocontrol agents either forming a natural component of the microflora or as introduced organisms have to compete with pathogens or other soil microflora for available nutrients and niches for establishment. Both fungal and bacterial BCAs applied to the soil have been found to be aggressive colonizers of the rhizospheres of plants to be protected. Some of the BCAs have multiple mechanisms of action on the fungal and bacterial pathogens, resulting in suppression of disease development and also enhanced plant growth and consequently higher yields. Some of the plant growth-promoting rhizobacteria (PGPRs) have the ability to induce systemic resistance in treated plants to soilborne diseases as well as to foliar diseases. The fungal biocontrol agents like Trichoderma viride, T. harzianum and T. virens have been more frequently employed for the management of a wide range of soilborne pathogens such as Pythium spp., Phytophthora spp., Rhizoctonia solani, F. oxysporum, Sclerotinia spp. and Verticillium spp. The nonpathogenic F. oxysporum Fo47, Coniothyrium minitans and Pythium oligandrum have also been found to be effective in suppressing the development of soilborne pathogens infecting several crops. Among the bacterial BCAs, strains of Pseudomonas fluorescens, Bacillus subtilis and Agrobacterium radiobacter have been effective in suppressing different soilborne pathogens and also in enhancing plant growth significantly. Soil application of BCAs is less preferred, because of the requirement of large quantities of the products. Further, uniform spread of the products to cover the infested areas of the soil may be difficult to achieve. The BCAs may be applied as a broadcast incorporation or as placement in the seed furrows at the time of sowing (Narayanasamy 2013).

Nonpathogenic isolates of F. oxysporum isolated from wilt-suppressive soils were more effective in suppressing the development of Fusarium wilt diseases of tomatoes, watermelon and muskmelon, compared to Burkholderia cepacia, Trichoderma virens, T. hamatum and Pseudomonas fluorescens (Larkin and Fravel 1998). Trichoderma koningii, with multiple mechanisms of action, suppressed the saprophytic growth of Gaeumannomyces graminis var. tritici in natural soils and consequently reduced incidence of wheat take-all disease (Simon and Sivasithamparam 1989). T. koningii applied to the seed furrows in the field reduced crown rot infection in root by 40% and also increased yield of spring wheat by 65%. The combination of T. koningii and Pseudomonas fluorescens Q292-80 provided more effective protection, resulting in greater reduction in disease incidence and greater yield levels (Duffy et al. 1996). The ability of the hypovirulent binucleate Rhizoctonia (HBNR) to protect tomato plants...
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against Fusarium crown and root rot (FCRR) throughout the growing period and their effects in reducing the population of *F. oxysporum* f.sp. *radicis-lycopersici* (FORL) in roots and stem under soilless systems were assessed under greenhouse conditions. In the greenhouse and soilless systems, HBNR isolates significantly reduced vascular discoloration and discoloration of total root system by 90 to 100% and by 73 to 89% respectively. Under field conditions, HBNR isolate WI significantly reduced vascular discoloration by 7%. Application of HBNR resulted in increases in marketable and total yields of tomatoes by 70% and 73% respectively, compared to untreated control plants (Muslim et al. 2003). A plant growth-promoting rhizobacterium (PGPR) *Bacillus* sp. strain HN09 isolated from neem tree rhizosphere inhibited the development of *F. oxysporum* f.sp. *radicis-lycopersici* (FORL) in vitro. Substantial level of disease suppression was achieved by soil supplementation with a preparation containing neem cake seeded with the strain HN09. Dry sterilization of neem cake before fermentation provided comparable protection against FCR disease, as that provided by unsterilized treatment, whereas moist sterilization treatment decreased the effectiveness significantly. Application of the bioformulation activated the tomato genes encoding PR-proteins, resulting in enhancement of resistance of tomato plants to Fusarium crown and root rot (FCRR) disease (Lin et al. 2017).

The bioplastic formulation Master-Bi® granules were inoculated with conidial suspension of a non aflatoxigenic strain of *Aspergillus flavus* NRL30797 (ca 10^7 conidia/granule). Incubation of 20-g samples receiving a single Master-Bi® granule for 60 days, resulted in 10^{+2} and 10^{+3} propagules of *A. flavus* /g of soil in microbiologically active (nonsterile) and sterilized soil respectively. The bioplastic granules were highly stable, in addition to supporting the proliferation of the BCA. The results indicated that Master-Bi® had the potential for substituting the wheat grains, used earlier as carrier for *A. flavus* NRRL30797 (Accinelli et al. 2009). In a later investigation, the effect of field application of the BCA-inoculated bioplastic granules on soil population of aflatoxigenic strains of *A. flavus* was assessed. A rapid shift in composition of soil population of *A. flavus* with a significant decrease in relative abundance of indigenous aflatoxigenic isolates was observed. Application of bioplastic granules at 30-kg/ha was more efficient in replacing aflatoxigenic isolates was observed than a 15-kg/ha dosage. Soil application of 15- and 30-kg/ha bioplastic granules treated with the BCA, reduced aflatoxin contamination by 59% and 86% in 2009 and 80% and 92% in 2010, respectively (Accinelli et al. 2012).

Members of endophytic fungal genus *Trichoderma* are well established as plant-beneficial microorganisms functioning as the highly successful biological component in the form of biofertilizers, biocontrol agents and plant growth activators. Variable interactions among different lentil genotypes and *Trichoderma* strains were studied both in the presence and absence of the root pathogen *Aphanomyces euteiches*. Two commercial *Trichoderma* formulations, RootShield® (RS) and RootShield® Plus (RSP) based on *T. harzianum* T22 and *T. virens* G41 respectively were evaluated for their efficacy in suppressing development of Aphanomyces root rot and plant growth promotion in 23 wild and cultivated lentil genotypes. No significant disease control was observed with either formulation in any lentil genotype. Significant genotype-specific plant growth promotion was recorded. The overall effect of *Trichoderma* treatment was markedly higher in the presence of the pathogen compared to pathogen-free conditions. In many cases, negative responses were evident, particularly in the absence of root infection by *A. euteiches*. The genotype PI572390 of *L. tomentosus* alone exhibited positive responses for most tested parameters. The results showed that lentil genotype played a major role in interactions among tested *Trichoderma* strains and the plant. The beneficial effect of *Trichoderma* could be realized under biotic stress (infection) and hence, blanket recommendation of the BCA is likely to be a negative strategy affecting the yield in fields free from the root pathogen(s) (Prashar and Vandenberg 2017).

Three endophytic bacterial strains, *Stenotrophomonas maltophilia* H8, *Pseudomonas aeruginosa* H40 and *Bacillus subtilis* H18, were evaluated for their efficacy in suppressing the development of root rot disease caused by *Rhizoctonia solani* in cotton. The BCAs were applied as soil drench or talc-based formulation on the pathogen-infested soil under greenhouse conditions. Soil drench was more effective than talc-based formulation. Increase in emergence and survival of seedlings with significant reduction in disease severity was observed. In addition, the BCAs enhanced growth parameters markedly. Gas chromatography–mass spectrometry analysis revealed the presence of bacterial bioactive compounds with a broad-spectrum antifungal activity and capacity to induce systemic resistance in cotton seedlings (Selim et al. 2017).

### 3.2 ASSESSMENT OF BIOCONTROL POTENTIAL OF ABIOTIC AGENTS

Abiotic agents of plant and animal origin and organic and inorganic compounds have been evaluated for their capacity to suppress development of soilborne microbial pathogens and the diseases induced by them in various crops.

#### 3.2.1 NATURAL PRODUCTS OF PLANT AND ANIMAL ORIGIN

Natural plant products such as plant residues, green manures and composts are added to the soil after harvest or before planting next crop. The effects of plant residues and green manures on soil microflora and soilborne pathogens affecting various crops have been investigated.

#### 3.2.1.1 Effects of Composts

Composts are the most frequently applied amendments to improve the soil fertility and to encourage the proliferation of microorganisms antagonistic to soilborne microbial plant pathogens. Composts have been reported to be effective, especially in controlled environment or container-based production systems. The inability to produce predictable and reproducible compost composition, has hampered a meaningful interpretation of the results obtained from the investigations directed
toward standardization of compost treatments (Mazzola 2007). Application of composts, because of the significant beneficial effect by disease suppression, has been employed as a component of integrated management system for soilborne diseases. The composts carry antagonists that can suppress development of soilborne pathogens. In addition, they encourage the development of antagonists already present in the field soils. The microflora of three composts were investigated to isolate and test the biocontrol potential of microorganisms in the composts, against *Pythium ultimum*, incitant of damping-off of greenhouse-grown cucumber. A more diverse bacterial population was present in the compost from paper mill sludge (170 groups) than in composts from plant waste and manure (75 and 88 groups respectively). Among the bacteria and fungi tested, *Zygorhynchus moelleri* and *Bacillus marinus* were the most effective in reducing incidence of damping-off disease under greenhouse conditions, followed by *Penicillium thomii*, *Pseudomonas fluorescens*, *P. aeruginosa* and *Graphium putredinis*. *Z. moelleri* proved to be consistently effective in the second trial also, indicating its suitability for large-scale application (Carisse et al. 2003). The effectiveness of seed-colonizing microbial communities presents in municipal biosolids compost in suppressing the development of Pythium damping-off in greenhouse-grown cucumbers was demonstrated by Chen and Nelson (2012).

Composts may serve as a food base for endogenous microorganisms, including plant pathogens or introduced biocontrol agents to sustain suppression based on the activities of microbial communities. Two composted swine wastes CSW1 and CSW2 were incorporated into peat moss-based potting mix at 4 to 20% (v/v) to determine the degree of suppression of pre-emergence damping-off of impatients caused by *Rhizoctonia solani*. Disease incidence was reduced to a greater extent in potting mix amended with 20% CSW1, compared with CSW2. Mixes amended with CSW1 (20%), after 35 weeks or more of curing, were consistently suppressive to *Rhizoctonia* and *Pythium* damping-off (Diab et al. 2003). Fish emulsion (2% and 4%) in naturally infested muck soil effectively and consistently suppressed damping-off in cucumber caused by *R. solani*, as well as in peat substrate (Abbasi et al. 2004). The composts consisting of dairy and greenhouse wastes significantly reduced the severity of cucumber Fusarium root rot and stem rot caused by *F. oxysporum* lsp. *radicis-cucumerinum* (FORC). Strains of *Pseudomonas aeruginosa* isolated from the composts showed greatest degree of antagonism against FORC. Furthermore, internal stem colonization by FORC was also reduced by *P. aeruginosa*. The results indicated that suppressiveness of composts against FORC depended primarily on the production of antibiotics by *P. aeruginosa* (Bradley and Punja 2010). The biocontrol potential of two types of date palm composts and indigenous microorganisms present in them for their efficacy in suppressing the development of potato stem canker and black scurf caused by *R. solani* was assessed. The microorganisms inhibitory to *R. solani* in confrontation assay were further examined under light microscope. Mycelial lysis, mycoparasitism and/or formation of mycelial cords via anastomosis between hyphae were observed. The compost extracts lost its antagonistic activity, after heating or filtration, indicating chemical components of the compost had no inhibitory effect on *R. solani*. Suppressive effect of the date palm compost was due to the antagonists. Under greenhouse conditions, incidence of stem canker and black scurf of potato was significantly reduced in peat-sand amended with compost, compared to the untreated control. Plant growth was not affected by the application of fungal antagonists from the compost, suggesting that antibiosis might be the principal mechanism of action of date palm compost in reducing the incidence of stem canker and black scurf caused by *R. solani* (El-Khaldi et al. 2016).

Household waste compost, composted cow manure and fresh *Brassica* tissues were evaluated for their biocontrol potential against tomato Verticillium wilt caused by *Verticillium albo-atrum*. The composts reduced the disease severity significantly. The biological activity increased with increase in organic matter input levels. The organic matter suppressive to soilborne pathogens either alone or in combination with chitin/chitosan soil amendments might be effective to achieve disease suppression to the desired levels (Giotis et al. 2009). The efficacy of onion waste compost (OWC), spent mushroom waste (SMC) amended with *Trichoderma viride* S17A for the control of Allium white rot (AWR) caused by *Sclerotium cepivorum* was assessed. Incorporation of OWC into the soil reduced viability of sclerotia and the incidence of AWR on onion plants in the glasshouse pot assays. On the other hand, SMC or *T. viride* reduced only the incidence of AWR. In the field trials, OWC reduced sclerotial viability and disease incidence as effectively as the fungicide. Addition of *T. viride* to SMC facilitated proliferation of the BCA in the soil and increased healthy onion bulb yield. The suppressive activity of OWC could be attributed to the presence of sulfur compounds in the compost (Coventry et al. 2006). Composted cotton-gin trash (CGT) showed highly suppressive effect on tomato southern blight disease caused by *Sclerotium rolfsii* and it could be used as an alternative to or in combination with conventional soil fumigation. Propagules density of *Trichoderma spp.* was higher in soils amended with CGT than in soils receiving synthetic fertilizers. The disease incidence was at low level in soil amended with CGT (23%), compared with synthetic fertilizer-applied (61%). Organic amendments enhanced soil fertility, in addition to suppression of soilborne diseases (Bulluck III and Ristaino 2002). Cow manure amendment significantly reduced the incidence of potato brown rot caused by *Ralstonia solanacearum* in Dutch sandy soils but did not affect pathogen population. However, cow manure reduced the bacterial pathogen densities in Egyptian sandy soils, most probably due to microbial competition, as a clear shift in population was detected by denaturing gradient gel electrophoresis (DGGE) technique (Messiha et al. 2007).

Compost teas are fermented watery extracts of composted materials that have beneficial effects on plants including antimicrobial activities. Compost teas may be employed after active aeration and with additives to enhance microbial population density required for effective suppression of plant pathogens. Aerated compost tea (AET) and nonaerated...
compost tea (NCT) with or without additives were applied as soil drenches for suppressing development of *Pythium ultimum*, causing damping-off in container system. ACT fermented with kelp and humic acid additives was suppressive to the maximum extent. Suppression of the formulation was significantly reduced by heat treatment (Scheuerell and Mahaffee 2004). Non-aerated compost teas (NCTs) prepared from seaweed compost, shrimp powder compost and chicken, bovine and sheep manure composts decreased the percentage of necrotic tomato seedlings inoculated with *P. ultimum* from 100 to 42%, but did not reduce necrosis in *Rhizoctonia solani*-inoculated seedlings. Sterilization of NCT resulted in complete or partial inhibition of inhibitory effect on pathogen growth. The NCTs, when applied on tomato seedlings, promoted plant growth to different levels (Dionne et al. 2012). Microbe-fortified composts and compost tea preparations were evaluated for their efficacy. The composts and compost teas amended with *Anabaena oscillarioides* C12 and *Bacillus subtilis* B5, respectively enhanced tomato seed germination and growth parameters, in addition to significant reduction in severity of diseases caused by *Pythium debaryanum*, *P. aphanidermatum*, *R. solani* and *F. oxysporum* and also reduced the fungal load (Dukare et al. 2011).

Biochar is a heterologous material generated through a process (pyrolysis) carried out at temperatures ranging from 200°C to 900°C, under limited oxygen availability. Biochar has been generated from a wide range of organic materials such as crop residues, wood, municipal biowastes, sewage sludge, manure and animal bones. Biochar differs basically from charcoal by its final end use–agriculture and environmental management for biochar and fuel and energy for charcoal (Lehmann and Joseph 2009). Use of biochar as soil amendment has been shown to enhance crop yields significantly (Jeffery et al. 2011). The beneficial effects on plant growth and health may be related to its ability to stimulate development of beneficial microbes, in the bulk soil as well as in the rhizosphere (Thies et al. 2015). In addition, biochar application to soil may result in suppression of diseases caused by airborne and soilborne pathogens. The disease suppressive effects of biochar, the response of different crop plants and the mechanisms of action of biochar on development of soilborne microbial pathogens and diseases induced by them have been studied in some pathosystems (see Table 3.2) (Bonanomi et al. 2015).

Biochar may be an alternative to crop residues or composts, applied as soil amendments, since it selectively enhances the activity of beneficial microorganisms without stimulating pathogen populations and virulence. Biochar made from animal bones was used as a carrier for an effective delivery of biocontrol agents. Scanning electron microscopic observations revealed that *Pseudomonas chlororaphis, Bacillus pumilus* and *Streptomyces pseudoovensuelae* could extensively colonize the porous structure of biochar. They were highly effective in suppressing damping-off and Fusarium crown and root rot of tomato caused, respectively, by *Pythium aphanidermatum* and *F. oxysporum* f.sp. *radicis-lycopersici*, following soil application of biochar (Postma et al. 2013). Phytotoxic compounds may be released into agricultural soils from decomposed organic materials, including crop residues. By contrast, biochar can protect plant roots by absorbing phytotoxic compounds and thus indirectly protect the roots from pathogen infection. Asparagus decline was due to accumulation of allelopathic toxins and Fusarium crown and root rot (FCRR) caused by *F. oxysporum* f.sp. *radicis-lycopersici* (FORL) and *F. proliferatum* (Fp). A commercial formulation of biochar from hardwood dust was evaluated for its efficacy in reducing the adverse effects of allelopathy on arbucular mycorrhizal (AM) root colonization and on FCRR disease. In the greenhouse experiments, addition of biochar at 1.5% and 3.0% (w/w) to asparagus field soil caused by proportional increases in root weight and linear reductions in the percentage of root lesions by FORL and Fp, compared with control. Concomitantly, there was a 100% enhancement in root colonization by AM fungi at incorporation rate of 3% of biochar. Under microplot conditions, biochar was added at 3.5% (w/w). Plots with biochar amendment, had plants with greater AM colonization in the first year of growth. This effect was due to absorption of phytotoxic and fungitoxic phenolic compounds (cinnamic-, coumaric- and ferulic-acids) released from decaying *Asparagus* crop residues. However, in the

<table>
<thead>
<tr>
<th>Plant pathogens</th>
<th>Plant host</th>
<th>Source material for biochar</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phytophthora cinnamomi</em></td>
<td><em>Quercus rubra</em></td>
<td>wood</td>
<td>Zwart and Kim (2012)</td>
</tr>
<tr>
<td><em>Pythium aphanidermatum</em></td>
<td><em>Acer rubrum</em></td>
<td>wood</td>
<td>Zwart and Kim (2012)</td>
</tr>
<tr>
<td><em>Plasmodiophora brassicae</em></td>
<td><em>Brassica rapa</em></td>
<td>Miscanthus</td>
<td>Knox et al. (2015)</td>
</tr>
<tr>
<td><em>F. oxysporum</em> f.sp.</td>
<td><em>Asparagus officinalis</em></td>
<td>coconut, charcoal</td>
<td>Matsubara et al. (2002)</td>
</tr>
<tr>
<td><em>F. oxysporum</em> f.sp.</td>
<td><em>tomato</em></td>
<td>carbonized chaff</td>
<td>Matsubara et al. (2002)</td>
</tr>
<tr>
<td><em>F. proliferatum</em></td>
<td><em>Asparagus spp.</em></td>
<td>pig bone</td>
<td>Matsubara et al. (2002)</td>
</tr>
<tr>
<td><em>Rhizoctonia solani</em></td>
<td><em>cucumber</em></td>
<td><em>Asparagus spp.</em></td>
<td>Quest Biochar</td>
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<td></td>
<td><em>French bean</em></td>
<td><em>Eucalyptus wood</em></td>
<td>Jaiswal et al. (2014a)</td>
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<td></td>
<td></td>
<td><em>crop wastes</em></td>
<td>Jaiswal et al. (2014b)</td>
</tr>
</tbody>
</table>
subsequent years, biochar-treated plants were smaller in size, probably due to greater than average rainfall and the ability of biochar to retain moisture, creating conditions favoring root rot disease development. The results indicated variations in effectiveness of biochar in mitigating the deleterious effects of allelopathic residues in replant soils on *Asparagus* (Elmer and Pignatello 2011).

Induced systemic resistance (ISR) was suggested as mechanism of action of biochar against soilborne pathogens. Strawberry plants grown in substrates amended with biochar had higher expression of genes encoding three pathogenesis-related (PR)-proteins *FaPR1, Faolp2, Fraa3*), one gene encoding lipoxygenase (*Falox*) and one gene (*FaWRKY1*), encoding transacting factor that belongs to the WRKY family (Harel et al. 2012). The effects of two biochars obtained from peanut shell (BC1) and wheat straw (BC2) on severity of bacterial wilt disease of tomato caused by *Ralstonia solanacearum* and also on soil microbial properties were assessed. The biochar formulations were applied at 2% (w/w) to field soil infested with *R. solanacearum*. BC1 and BC2 treatments reduced the disease index of bacterial wilt by 28.6% and 65.7%, respectively, and increased level of resistance to bacterial wilt in tomato (see Figure 3.9 (a), (b)). BC2 was more effective in suppressing disease severity. Biochar treatments significantly reduced the pathogen density by 51.63% in BC1-treatment and 68.22% in BC2 treatment, whereas *R. solanacearum* density increased by 80.43%, after pathogen inoculation (see Figure 3.10). Following inoculation of soil with the pathogen, contents of soil bacteria and actinomycetes were reduced in control (without biochar) plots. In contrast, the contents of biochar treated plots showed significant increases in soil bacteria (57.3% and 96.43% in BC1 and BC2 respectively). Soil actinomycetes populations also registered increases, whereas soil fungi populations were reduced. Biochar treatments increased soil neutral phosphatase and urease activity. Higher metabolic capabilities were observed in biochar amendment treatments, indicating high substrate utilization by potential of microorganisms. Resistance of tomato grown in soils amended with biochars was closely related to the changes in soil microbial activity and community structure (Lu et al. 2016).

The mechanisms of suppression of soilborne diseases caused by microbial plant pathogens and enhancement of plant growth by biochar application are not clearly understood. The relationships between biochar-induced changes in the rhizosphere microbial community structure and composition and
activity associated with suppression of development of soil-borne diseases were investigated. Biochar application suppressed Fusarium crown and root rot caused by *F. oxysporum f.sp. radicis-lycopersici* (FORL) and simultaneously improved the growth of treated plants. Furthermore, biochar reduced Fusarium colonization and survival in soil, and increased the cultivable counts of several biocontrol and plant growth promoting microorganisms. Illumina sequencing analyses of 16S rRNA gene revealed substantial differences in the rhizosphere bacterial taxonomical composition between biochar-amended and non-amended treatments. Furthermore, biochar amendment significantly increased the microbial and taxonomic and functional diversity, microbial activities and an overall shift in carbon-source utilization. High microbial taxonomic and functional diversity and activity in the rhizosphere was found to be associated with suppression of diseases caused by soil-borne pathogens by biochar application (Jaiswal et al. 2017).

### 3.2.1.2 Effects of Plant Residues

The residues of cruciferous plants have been found to have the high disease suppressive effect, among various kinds of crop residues that have been evaluated. Cruciferous plants contain glucosinolates, sulfur-containing secondary metabolites. The glucosinolates are hydrolyzed by the enzyme myrosinase to produce isothiocyanates (ITCs). Many ITCs are volatile with inhibitory effects on a wide range of soil-borne pathogens. Although incorporating *Brassica napus* residue was effective in suppressing soilborne fungal pathogens, the adoption of this approach was impracticable. The impact of *B. napus* seed meal on apple replant disease caused by *Rhizoctonia solani* and *Pythium* spp. was assessed under greenhouse conditions. Seed meal amendment of soil, irrespective of glucosinolate content, significantly suppressed apple root infection by *Rhizoctonia* spp. and nematode *Pratylenchus penetrans*. On the other hand, seed meal amendment of the cv. Dwarf Essex with high glucosinolate content did not consistently suppress soil populations of *Pythium* sp. Seed meal amendment enhanced *Pseudomonas* spp. and total bacterial populations. Dwarf Essex seed meal was toxic, when applied at the rate of 2% (v/v) (Mazzola et al. 2001). Among the individual isothiocyanates tested, propenyl- and ethyl-isothiocyanates were the most fungistatic and inhibited mycelia growth and completely suppressed conidial and chlamydospore germination of *F. oxysporum* pathogens infecting conifer seedlings (Smolinski et al. 2003).

Incorporation of lettuce residues into the pathogen-inoculated soil significantly reduced the incidence of root and stem rot disease of cucumber caused by *F. oxysporum f.sp. radicis-cucumerinum* (FORC) and also increased the total cucumber yield. Lettuce residue incorporation could form an effective component of integrated disease management (IDM) system (Pavlou and Vakalounakis 2005). In a later investigation, residues of various other plant species, *Diplotaxis tenuifolia* (wildrocket, WR), *Artemisia dracunculus* (tarragon), *Salvia officinalis* (sage) and *Brassica oleracea* var. *italica* (broccoli) were evaluated for their efficacy in suppressing the development of cucumber crown and root rot disease caused by *F. oxysporum f.sp. radicis-lycopersici* (FORL). Disease incidence and severity of the disease in cucumber plants inoculated with FORL were reduced by 20 to 80%, when seedlings were planted in soils incorporated with residues of different plant species. Effective soil suppressiveness persisted, after repeated inoculation and planting in the same soil without additional treatment between inoculations. In addition, residues of WR induced soil suppressiveness in two additional tested soils, differing in their physical and chemical properties. Soil suppressiveness to Fusarium crown and root rot disease was induced, when cucumber seeds were sown in soils that were initially amended with WR residues and later infested with FORL, chlamydospores. The results indicated the possibility of reducing incidence of soilborne diseases caused by *F. oxysporum* by incorporating suitable plant residues that contribute to development of soil suppressiveness (Klein et al. 2011). Incorporation of organic amendments into the soil may induce soil suppressiveness against specific soil-borne pathogens. Soil suppressiveness could be induced by incubating sandy soil with debris of wildrocket (*Diplotaxis tenuifolia*, WR) under field conditions. The microbial dynamics in the roots of cucumber seedlings were investigated, following transplantation into WR-amended or nonamended soil, as influenced by inoculation with *F. oxysporum f.sp. radicis-cucumerinum* (FORC). Appearance of disease symptoms on plants grown on nonamended soil was discernible at 6 days after inoculation, whereas the symptoms appeared only at 14 days after inoculation of WR-amended soil. The pathogen propagules were quantified using real-time PCR assay. The pathogen population was significantly lower (66%) at 6 days after inoculation in WR-amended soil compared to unamended soil. The decrease in root colonization by FORL was correlated with a reduction in disease incidence at 21 days after inoculation and transplanting into suppressive soil. Quantitative analyses and mass-sequencing methods indicated a qualitative shift in the root’s bacterial community composition in suppressive soil, rather than a change in bacterial community composition. The shift in bacterial community was related primarily to the increase in *Streptomyces humilus*, which was antagonistic to fungal pathogens (Klein et al. 2013).

The effects of green manure crops (buckwheat and canola) and crop sequences on potato scab caused by *Streptomyces scabies* and Verticillium wilt caused by *Verticillium dahliae* were assessed in the 2-year field trials. Tubers grown in buckwheat-treated soil had significantly lower Verticillium wilt ratings, whereas tuber yield was increased significantly. Potatoes grown in soil planted to corn or alfalfa in the previous year had significantly lower Verticillium wilt ratings, whereas tuber yield was increased significantly. Potatoes grown in soil planted to corn or alfalfa in the previous year had significantly lower Verticillium wilt and potato scab ratings, as well as higher yields than potatoes grown in soil previously planted to potato. Green manure crops may selectively enrich the abundance or activity of antibiotic producers like *Streptomyces* spp. within the soil microbial community (Wiggins and Kinkel 2005). The comparative suppressive effects of Austrian winter pea (*Pisum sativum*) cv. Melrose, broccoli cv. Excelsior and Sudangrass amendment on Verticillium wilt severity and yield of Russet Burbank.
potato were assessed. The disease was consistently reduced by all three green manure types applied at the highest rate tested. A positive correlation between amendment rate and degree of disease reduction was also observed. Austrian winter pea was more effective than the other two green manure types even at a reduced rate of amendment applied (Ochiai et al. 2007). The efficacy of green manure *Brassica* crops in functioning as biofumigant was assessed for suppression of *F. oxysporum f.sp. conglutinans* (Foc) and *F. oxysporum f.sp. raphani* (For), causal agents of Fusarium yellows of cabbage and Fusarium wilt disease respectively in cabbage and radish. Green manure treatment carried out by growing nine cycles of biocidal plants, with a short crop cycle (30–35 days) did not reduce Fusarium wilt incidence on susceptible *Brassica* crops. The population of pathogen was partially increased as a result of incorporation of tissues of susceptible plants. In crops. The population of pathogen was partially increased as a result of incorporation of tissues of susceptible plants. In contrast, *Brassica* crops resistant to both *Foc* and *For* proved to be biocidal to both pathogens. The results indicated that biofumigation with *Brassica* spp. might not be effective for soil disinfection on crops susceptible to the pathogen concerned. *Brassica* crops resistant to Fusarium yellows disease of cabbage might be grown for biofumigation to suppress the disease development (Lu et al. 2010). Sweet corn (cv. Jubilee Sweet corn and Jubilee Super Sweet corn) as green manure was evaluated for its efficacy in reducing incidence of potato Verticillium wilt disease. The sweet corn cultivars reduced disease incidence by 60 to 70% by reducing colonization of potato feeder roots and potato stem apices by the pathogens. Feeder root colonization was positively correlated with Verticillium wilt incidence (P < 0.05) and negatively correlated with yield. Further, corn green manures increased populations of several fungi like *Ulocladium* and *Fusarium equiseti*. When potato crop was grown consecutively for 2 years, the beneficial effect of sweet corn green manures was almost entirely lost. But, following two consecutive years of potato, a single sweet corn crop was enough to restore the level of disease suppression to the original level and increase potato yield by fourfold. Austrian pea, Sudangrass, oilseed rape, oats and rye grown as green manure crops, provided similar beneficial effects of disease suppression and enhancement of potato yields (Davis et al. 2010).

Disease suppressive effects of condensed distiller’s solubles (CDS), a coproduct of ethanol production from corn (*Zea mays*) were investigated, using them as amendment in the pathogen-infested soils and peat-based substrate prior to planting. The CDS amendment (1% and 3% w/w) to sandy-loam soil, displayed a low level of toxicity to microsclerotia of *Verticillium dahliae* and reduced their germination by 46 to 63% after 1 week in the laboratory soil microcosm tests. The CDS contained moderate levels (~ 144 mmol/l) of volatile acetic acid and formic acid, as well as nonvolatile glycolic organic acids, some of which are known to be toxicants. In solution assays, the viability of *V. dahliae* microsclerotia treated for 24 h in 1, 2, 5 and 10% (v/v) CDS (pH 3.6–4.5) or mixture of organic acids at the same parent composition as in CDS, was reduced by 2, 7, 22 and 18% or 6, 32, 53 and 69% respectively. The mixture of organic acids with the same volumetric ratios as 2% and 4% CDS entirely inhibited the growth of *Pythium ultimum*. Treatment of *P. ultimum*-infested muck soil with 2% CDS (v/w) reduced damping-off severity by 45 to 52% and increased the percentage of healthy seedlings from 164 to 180% over untreated control. Preplanting amendment of glycolic acid (0.075% and 0.15% v/w) to infested muck soil significantly increased the percentage of healthy cucumber seedlings by 107% and 122%, respectively, and decreased the damping-off severity by 33% and 40%, respectively, over control. The results suggested that organic acids in the CDS might have suppressive effects on soilborne fungal pathogen in sandy-loam and muck soils (Abbasi et al. 2009). Rice bran (RB), as amendment to correct soil pH, has been employed for reducing the incidence of potato common scab (PCS) disease caused by *Streptomyces scabiei* in Japan. The mechanism underlying the beneficial effect of rice bran was investigated. RB amendment-reduced PCS incidence by repressing the pathogenic *Streptomyces* population in young tubers. Amplicon sequencing analyses of 16S rRNA genes from rhizosphere microbiome revealed that RB amendment dramatically altered bacterial composition, resulting in enhancement of relative abundance of Gram-positive bacteria such as *Streptomyces* spp. and this was negatively correlated with PCS disease severity. Most actinomycete isolates were antagonistic to *S. scabiei* and *S. turgidiscabies* on R2 medium. Under field conditions, inoculation of potato plants with *Streptomyces* isolates reduced common scab incidence. The results suggested that the rice bran amendment differentially enhanced antagonistic bacterial populations in the potato rhizosphere (Tomihama et al. 2016).

Broccoli residue or crabmeal (chitin) amendments were added to the naturally infested Verticillium wilt-conducive soils from Salinas Valley of coastal California. Illumina sequencing of a 16S rRNA gene library generated from 160 bulk soil samples was employed to monitor changes in the soil prokaryote community. Under greenhouse conditions, extent of suppression of Verticillium wilt, plant height, soil microsclerotia density and soil chitinase activity were assessed using eggplant as assay host. In soil with high soil microsclerotia density, all amendments significantly reduced only in the brocoli-amended treatments. Error-corrected sequence variants (8,790) representing 1,917,893 different sequences were included in the analyses. The treatments had significant impact on soil microbiome community structure, but measures of α diversity did not vary between treatments. Community structure correlated with disease score, plant height, microsclerotia density and soil chitinase activity, suggesting that the prokaryote community might affect the disease-related response variables or vice-versa. Likewise, the abundance of 107 sequence variants correlated with disease-related response variables which included variants from genera with known antagonists of filamentous fungal pathogens. Generally, fungal genera with antagonistic activity were more abundant in amended soils than in unamended soils, and constituted up to 8.9% of all sequences in broccoli + crabmeal-amended soil. The results showed that substrate-mediated shifts in soil prokaryote communities might be associated with the transition
of Verticillium wilt-conducive soils to Verticillium wilt-suppressive soils (Inderbitzin et al. 2018).

Reductive soil disinfestation (RSD), performed under anaerobic conditions, is an ecofriendly alternative to chemical soil disinfestation (CSD) for the management of soilborne plant pathogens. Damping-off disease caused by *Rhizoctonia solani* is responsible for serious losses in cucumber. Accumulation of soilborne pathogens including *Rhizoctonia solani*, in addition to soil degradation occurred, due to intensive vegetable cultivation in the greenhouses in China. Effects of flooding soil along with incorporation of alfalfa (Al-RSD-F), alfalfa-RSD (Al-RSD) irrigated to the maximum field capacity and covered with plastic film, Al-RSD + T37 (*Tricoderma harzianum* T37) inoculated at the end of Al-RSD, ethanol (Et)-RSD incorporated into the soil covered with plastic film and ammonia (AW)-incorporated into the soil and covered with plastic film on cucumber damping-off development were assessed. Al-RSD treatment reduced the populations of *R. solani* and incidence of damping-off disease in cucumber seedlings. AW treatment was toxic to cucumber seedlings which could not survive under greenhouse conditions. The results showed that alfalfa-amended soil disinfestation with or without *Trichoderma* and ethanol soil disinfection could be applied for effective suppression of damping-off disease and improving the quality of degraded greenhouse soils (Huang et al. 2016b). Reductive soil disinfestation (RSD) was performed using ethanol (Et)-RSD and alfalfa (Al-RSD) as organic carbons in soil infested by *R. solani*. At the conclusion of RSDs, *Chaetomium* (a fungal biocontrol agent) population increased, while *Rhizoctonia* and *Aspergillus* decreased significantly. Furthermore, some nitrification, denitrification and nitrogen-fixing genes were apparently increased in the RSD-treated soils. But the effect of Al-RSD was greater than that of Et-RSD. Overall, Et-RSD could induce more antagonists belonging to Firmicutes under anaerobic condition. By contrast, Al-RSD could continuously stimulate some functional microorganisms (Lysobacter and Rhodanobacter), facilitating efficient nitrogen transformation activities in the soil in the following cropping season (Huang et al. 2016a).

Effectiveness of application of organic matter and reductive soil disinfestation (RSD) were evaluated for reducing the disease incidence/severity. Real-time PCR and MiSeq pyrosequencing were employed to monitor changes in microbial community during the progress of biocontrol activity of organic matter. Antagonists (Ant) significantly reduced pathogen population and also disease incidence. Soil microbial population and activity were also increased. By contrast, combination of RSD and antagonists (RSD + Ant) was more effective and facilitated to maintain the antagonist population and activity. The results showed that combination of organic matter and antagonists altered the soil microbial community to a greater extent and decreased incidence of damping-off disease in cucumber caused by *Rhizoctonia solani*, whereas combination of RSD and antagonists was useful in improving soil microbial community structure and stability, resulting in reduction in disease incidence (Huang et al. 2017). In a later investigation, the effects of RSD with ethanol (10 t/ha), sugarcane bagasse (SB, 15 t/ha) and bean dregs (BD, 15 t/ha) were compared with chemical soil disinfection (CSD) with dazomet (DZ, 0.5 t/ha) on suppression of soilborne pathogen development and soil fungal community structure. Quantitative PCR and high-throughput sequencing techniques were employed to determine the populations of microorganisms. BD treatment effectively alleviated soil acidification and salinization. Both RSD-related treatments and CSD significantly reduced the populations of *F. oxysporum* causing wilt diseases. Furthermore, RSD and CSD treatments harbored a distinct unique microbiome in the treated soils. The results indicated that BD treatment could considerably alleviate soil deterioration, improve soil microbial activity and support development of disease-suppressive microorganisms (Zhao et al. 2018).

### 3.2.1.3 Effects of Plant Products

Various secondary metabolites of plants such as essential oils and tissue extracts of plants have antifungal, antibacterial, antiviral and cytotoxic properties. A wide range of plant species has been screened for the presence of antimicrobial compounds, some of which have been shown to be effective under field conditions.

An immobile phytohormone, 24-epibrassinolide (EBL) was evaluated for its biocontrol potential in suppressing the development of *Fusarium* wilt disease of cucumber caused by *F. oxysporum* f.sp. *cucumerinum* (Foc). Pretreatment with EBL of either the roots or shoots significantly reduced disease severity and as well as improved the plant growth, regardless of the treatment methods applied. EBL applications decreased the *Fusarium* populations on root surfaces and in nutrient solution, but increased the population of fungi and actinobacteria on root surfaces. The PCR-DGGE analysis showed that *Foc* inoculation had significant effect on the bacterial community on root surfaces, as expressed by diversity index and evenness index. But EBL applications alleviated these changes. In addition, several kinds of decomposing bacteria and growth-promoting bacteria were identified from root surfaces of *Foc*-inoculated plants and EBL-pretreated plants, respectively. The results, in general, indicated that the microbial community on root surface was affected by a complex interaction between phytohormone-induced resistance and plant pathogens (Ding et al. 2009). Formulations containing 1, 5 and 10% aqueous emulsions of clove oil, neem oil, mustard oil, synthetic cinnamom oil, pepper extract and cassia extract were evaluated for their efficacy in pathogen suppression. Treatment of soil with 5% and 10% aqueous emulsions reduced significantly populations of *Phytophthora nicotianae*. The population densities were reduced at 1 day after treatment to the level below the limit of detection (< 0.04 CFU/cm³). Soil was treated with 10% aqueous emulsions of two pepper extract-mustard oil emulsions and two cassia extract formulations. Populations of *P. nicotianae* in soil treated with one of the pepper-mustard oil formulations, were still not detectable at 21 days after application. The neem oil formulations were still not detectable at 21 days after application. The neem oil formulation and metalaxyl did not reduce pathogen populations at all rates.
tested. In the greenhouse assays, 10% aqueous emulsions of a pepper extract–mustard oil formulation, cassia extract and cinnamon oil formulation at 35 days after treatment suppressed the disease development in periwinkle by 87 to 93% of the disease incidence, compared with untreated, infested soil (control) (Bowers and Locke 2004). The efficacy of 14 essential oil products commercially available was assessed by in vitro and in vivo tests for suppressing the development of *Phytophthora capsici*, infecting zucchini (*Cucurbita pepo*) fruit. Oregano, palmarosa and red thyme essential oils (EOs) had the lowest EC$_{50}$ values (< 0.15 µg/ml) for inhibiting the production and germination of sporangia and zoospores and mycelial growth of *P. capsici*. Populations of *P. capsici* in soil were significantly reduced by all three EOs. Zucchini fruits sprayed with red thyme (0.1 µg/ml) or oregano and palmarosa (0.2 µg/ml) were effectively protected against infection by *P. capsici*. Emergence of zucchini seedlings was affected by oregano but not by red thyme. Zucchini seedlings remained unaffected by *P. capsici* in soil treated with red thyme at 0.1 µg/ml, whereas all seedlings were killed in the untreated control soil. The results indicated the effectiveness of red thyme EO in protecting zucchini seedlings against infection by *P. capsici* (Bi et al. 2012).

The biocontrol potential of thymol and palmarosa (antibacterial agents produced respectively by *Thymus vulgaris* and *Cymbopogon martini*), against soilborne bacterial pathogen *Ralstonia solanacearum* was assessed. Thymol, palmarosa and lemongrass oil were applied at 400 mg and 700 mg/l of soil as a soil fumigant. At 7 days after application, pathogen population declined to undetectable levels in thymol, palmarosa oil and lemongrass oil treatments at both concentrations. Tomato seedlings planted in soil treated at 700 mg of essential oils were not infected by *R. solanacearum*. Further, all plants in thymol treatment were free of the pathogen. Thyme oil-producing plants such as thyme, creeping thyme and Greek Oregano were found to be symptomless carriers of the pathogen. Hence, these plant species should not be grown as rotation crops (Pradhanang et al. 2003). The ability of essential oil from *Pimenta racemosa* to suppress the development of tomato bacterial wilt caused by *Ralstonia solanacearum* (phytotype IIB/4NPB) was assessed. Lemongrass (chemotype 1)-, aniseed (chemotype 2)- and clove (chemotype 3)-scented chemotypes of *P. racemosa* var. *racemosa* essential oils were tested under in vitro conditions. The chemotype 3 displayed most effective suppressive activity on disease development, as no incidence of bacterial wilt was observed on tomato plants grown in soil treated with chemotype 3 at a concentration of 0.14%. In untreated control treatment, infection by bacterial wilt up to the extent of 62% was recorded. In addition, treatment of soil with chemotype 3 increased growth of tomato plants, compared with control plants. The results indicated the effectiveness of essential oil from *P. racemosa* var. *racemosa* for advancing to large scale application for managing an important bacterial disease affecting tomato and other crops (Deberdt et al. 2018). Field evaluation of the biocontrol activity of essential oils against bacterial wilt of tomato caused by *Ralstonia solanacearum* was carried out. Thymol and palmarosa at 0.7% concentration were applied to field soil at 2 h after infesting with the pathogen, followed by sealing off the soil with plastic mulch for 3 or 6 days. Tomato seedlings were planted 7 days later. Both thymol and palmarosa oil treatments reduced bacterial wilt incidence significantly in susceptible cv. SolarSet (12%), compared with untreated control plots (65.5%). Thymol can be artificially synthesized, and it is available commercially, resulting in reduction in chemical cost, compared to cost of production of essential oils from plants. Further, thymol could be applied through drip irrigation system and this may reduce the cost of application further. As thymol has a wide spectrum of activity against bacteria, fungi and nematodes, it has the potential for large scale application for the management of bacterial wilt disease affecting tomato (Ji et al. 2005).

The biocontrol potential of leaf extracts of *Moringa oleifera* either alone or in combination with *Trichoderma* Kd63, *Trichoderma* IITA508 or *Bacillus subtilis* was assessed in vitro, greenhouse and field tests for the suppression of development of *Sclerotium rolfsii* causing damping-off and stem rot in cowpea. Under field conditions, seed treatment with *Moringa* combined with *Trichoderma* soil sprinkle reduced disease incidence and severity by more than 70% with significant yield increase in cowpea (Adandonon et al. 2006). Similar approach of combining leaf extract and fungal and bacterial biocontrol agents was applied for suppressing development of pre- and postemergence damping-off of pepper caused by *Pythium aphanidermatum*. Among the 66 medicinal plant species tested, zimmu leaf extract (*Allium sativum* x *A. cepa*) was found to be the most effective in suppressing pathogen growth in vitro and the presence of 22 antimicrobial compounds was detected in zimmu leaf extract. Assessment under pot culture conditions, showed that seed treatment with a combination of *T. viride*, *P. fluorescens* and zimmu leaf extract protected the pepper seedlings effectively and also promoted plant growth and increased yield as well (Muthukumar et al. 2010).

### 3.2.1.4 Effects of Animal Products

Various animal products such as cattle, chicken, duck and pig manures are applied to the soil as organic amendments and their usefulness as nutrients, enhancer of soil fertility and activator of antagonistic microorganisms have been discussed earlier. Chitosan, among the animal products, has been evaluated for its biocontrol potential more frequently and extensively, for suppressing the development of crop diseases caused by soilborne microbial plant pathogens. The shells of marine crustaceans such as crabs and shrimps are very affordable sources for commercial production of chitin. They are available as waste from seafood processing industry. Chitosan, a deacetylated form of chitin, is a natural biodegradable fiber (polymer), whose principal characteristics correspond to its polycationic nature. Low molecular weight chitosan possesses high antimicrobial activity, which increases with decreasing MW. Use of chitosan as a film coating as a delivery system for fertilizers, plant protection products and micronutrients for crop growth promotion forms an important application.
Chitosan is dissolved in an acid solution to activate its antimicrobial properties. Direct suppressive effect of chitosan on fungal pathogen is due its fungicidal properties. In addition, chitosan can also function as an inducer of resistance in plants against pathogens by activating host defense responses.

Chitosan was applied as a soil amendment either alone or in combination with other treatments. In soilless tomato, root rot caused by *F. oxysporum* f.sp. *radicis-lycopersici* was effectively suppressed by chitosan amendment (Lafontaine and Benhamou 1996). Infection in forest tree seedlings in the nurseries by *Fusarium acuminatum* and *Cylindrocladium* was drastically reduced by applying chitosan as soil amendment (Laflamme et al. 1999). Chitosan was applied as seed treatment for suppression of diseases caused by *F. oxysporum* in many crops (Rabea et al. 2003). The biocontrol potential of different organic amendments, along with crab shell (chitin) against cotton *Verticillium* wilt pathogen *Verticillium dahliae* was assessed. Crab shell was the most effective in reducing disease severity by 72% in pots, whereas soybean stalk and alfalfa reduced the disease severity by 60% and 56%, respectively. Crab shell stimulated the proliferation of antagonists effective against *V. dahliae* in the rhizosphere. The extracts of crab shell chitin were inhibitory to *V. dahliae* in vitro. Changes induced by crab shells in the composition and structure of microorganisms in the rhizosphere might contribute to the suppression of development of *Verticillium* wilt disease of cotton (Huang et al. 2006). Chitosan was inhibitory to *F. oxysporum* f.sp. *radicis-lycopersici* in vitro. Ultrastructural investigations, using transmission electron microscope (TEM), revealed marked changes in the sensitive fungal cells. Confocal laser microscopy showed that Rhodamine-labeled chitosan entered rapidly into the conidia via an energy-dependent process. The results suggested that chitin application might be combined with other compatible biocontrol agents to enhance the level of effectiveness of disease suppression (Palma-Guerrero et al. 2008). Pearl millet downy mildew caused by *Sclerospora graminicola* is both soilborne (ooospores remaining in soil) and airborne (ooospore and sporangia spreading via wind). The efficacy of seed priming with chitosan in reducing the incidence of downy mildew disease was determined. Chitosan treatment of seeds at 2.5 g/kg increased seed germination and seedling vigor, compared with untreated seeds. Chitosan did not affect the sporulation and release of ooospores from sporangia. In seedlings growing from chitosan-treated seeds, activities of defense-related enzymes, chitinase and peroxidase were stimulated, following challenge inoculation with the pathogen. Assessment of effects of chitosan treatments under greenhouse and field conditions showed 79% and 76% protection, respectively, against downy mildew disease in pearl millet (Manjunatha et al. 2008).

Oligochitosan, obtained through hydrolysis or degradation of chitosan, is water soluble and more effective than chitosan, in suppressing disease development due to its ability to induce resistance in treated plants and to inhibit pathogen growth directly. Among the nine fungal pathogens tested for their sensitivity to oligochitosan, *Phytophthora capsici*, lacking chitin in the cell wall, was the most sensitive, with EC$_{50}$ and mean inhibitory concentration (MIC) values of 100 and 580 µg/ml, respectively. Oligochitosan at low concentrations inhibited different stages in the life cycle of *P. capsici*, including production of zoospores, zoospore release, cystospore germination and it induced leakage of electrolytes from the pathogen mycelium. Oligochitosan might act on the cell membrane by altering osmotic pressure. Most drastic structural alteration in the hyphae treated with oligochitosan (10 µg/ml) was the disruption of endomembrane system, especially vacuole and secretory vesicles, such as plasmamembranes in the hyphal tips as revealed by observations under electron microscope. The polycationic nature of oligochitosan might contribute to its antifungal and multiple modes of action, including induction of resistance to diseases in plants treated with oligochitosan (Xu et al. 2007). The efficacy of a heterogenous chitosan suspension (MCp) and commercial plant activator acibenzolar-S-methyl (ASM) for inducing resistance in cocoa against *V. dahliae* was assessed. The MCp and ASM enhanced the level of protection to susceptible cocoa cv. SIAL70 against *Verticillium* wilt. MCp treatment reduced the disease severity to a level equivalent of 80% of ASM protection level. Local induced resistance was basically associated with activities of peroxidase (POX) and polyphenol oxidase (PPO) in leaves and with lignin deposition at 13 days after application. Induction of resistance locally was indicated by enhanced activities of chitinase and 1,3-glucanase in the leaves at 4–18 days after treatment with MCp and ASM. Increase in lignin deposition level following treatment with MCp and ASM and challenge with *V. dahliae*, was considered as the defense strategy operating in cocoa against *V. dahliae* (Cavalcanti et al. 2008).

Comparative biocontrol potential of chitosan and salicylic acid (SA) against soilborne fungal pathogens infecting tomato was assessed. Chitosan (0.5–4 mg/ml) and SA (1–25 mM) inhibited mycelial growth of *F. oxysporum* f.sp. *lycopersici* (Fol), *F. oxysporum* f.sp. *radicis-lycopersici* (FORL), *V. dahliae*, *Rhizoctonia solani*, *Colletotrichum coccodes*, *Pythium aphanidermatum* and *Sclerotinia sclerotiorum* in a concentration-dependent manner. *Aphanidermatum* and *S. sclerotiorum* were the most sensitive to both resistance inducers (RIs). When applied as soil drench, single treatments with chitosan (4 mg/ml) or SA (10 mM) provided varied degree of protection to tomato plants against *V. dahliae*, *Fol* and FORL. Disease severity to *V. dahliae*, *Fol* and FORL, respectively, was reduced to the extent of 42.1 to 73.68%, 60.86 to 78.26% and 45 to 50% following chitosan- and SA-based treatments. In addition, treatments with chitosan or SA enhanced growth of tomato plants, compared to untreated control plants (Jabnoun-Khiareddine et al. 2015). Chitosan can be used as a vehicle and for protecting other antimicrobial compounds such as essential oils, which are volatile and can ensure better persistence of the active ingredient of thyme (*Thymus vulgaris*) and tea tree (*Melaleuca alternifolia*) which are applied against plant pathogens. The effectiveness of chitosan in reducing foot and root rot of wheat caused by *Fusarium graminearum* was assessed by seed treatment in combination with essential oils. The pathogen growth on seeds was...
reduced without affecting seed germination. Disease severity was reduced in wheat seedlings. Chitosan seed treatment increased the contents of resistance markers such as phenols and activities of defense-related enzymes like phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), peroxidase (PO) and chitinase in the seedlings. Greenhouse and field experiments also indicated that chitosan could enhance the level of resistance of plants growing from chitosan-treated seeds (Orzali et al. 2017).

Chitosan has been used for seed treatment and soil application. Treatment of seeds with chitosan (low MW 5–20 kDa) induced resistance in tomato plants against Phytophthora infestans (Kipruskhina et al. 2017). Seed soaking followed by foliar application of chitosan (0.25–2.0 g/l) were effective in suppressing development of wilt and root rot diseases of bean caused by Fusarium solani and Rhizoctonia solani (El-Mohamedy et al. 2017). In vitro and in vivo experiments revealed the efficacy of chitosan in suppressing bacterial wilt of potato. Tubers were soaked for 30 min (0.5–2.0 g/l) and spraying on leaves of potato effectively protected plants against infection by Ralstonia solanacearum. In addition, chitosan treatments promoted growth of potato plants, compared with untreated control (Farag et al. 2017). In another investigation, chitosan was mixed with a biofertilizer containing phosphate and potassium rocks, supplemented with addition of Cunninghamella elegans containing chitin and they were incorporated in the soil prior to planting green peppers or tomato. The treatment protected green peppers, but not tomato against R. solanacearum infection (Stamford et al. 2017).

### 3.2.2 Synthetic Organic Compounds

Different kinds of synthetic organic compounds have been evaluated for their biocontrol potential against soilborne microbial plant pathogens infecting various crops. Most of the organic compounds seem to act on the pathogens indirectly by activating host defense responses. The compounds activate the same spectrum of systemic acquired resistance (SAR) genes to levels comparable to those induced by the biotic inducers of disease resistance.

#### 3.2.2.1 Salicylic Acid

The molecular mechanism underlying systemic acquired resistance (SAR) has been elucidated using the model plant Arabidopsis thaliana. The SAR regulatory protein nonexpressor of PR (NPR1) gene is activated by salicylic acid (SA) through redox changes. These changes, in turn, drive systemic expression of antimicrobial PR proteins and facilitate their secretion by upregulating protein secretory pathway genes (Mou et al. 2003; Wang et al. 2005). Further, the long distance signaling in Arabidopsis appeared to depend on a peptide signal system mediated by the Asp protease constitutive disease resistance (CDR1) (Xia et al. 2004). The reactive oxygen species (ROS)-mediated systemic signaling network also contributed to SAR. The SA-induced defense expression via nonexpressor of PR gene-1 (NPR1), a key mediator of SAR functions in both dicotyledons and monocotyledons (Dong 2004). Interaction between wilt pathogen, F. oxysporum (Fo) and Arabidopsis thaliana (At) was investigated to understand the mechanism of activation of host defense responses directed against wilt pathogens. The expression of salicylate- and jasmonate-responsive defense genes in Fo-challenged roots of At plants, as well as in the roots of plants whose leaves treated with salicylate or jasmonate were analyzed. The genes (PR1.PDF1.1 and CHIB) encoding proteins with defense functions or transcription factors (AtERF1, AtERF2, AtER4 and ATMYC2) known to positively or negatively regulate defenses against Fo were not activated in Fo-inoculated roots. In contrast, the jasmonate responsive gene PDF1.2 was induced in the leaves of plants, whose roots were challenged with Fo, but salicylate-responsive PRI gene was not induced in the leaves of inoculated plants. Exogenous SA application prior to inoculation, however, activated PRI and BGL2 defense gene expression in leaves and provided enhanced level of resistance to Fo, as indicated by foliar necrosis and subsequent plant death. Exogenous SA treatment of the foliar tissues did not activate defense gene expression in the roots of treated plants. The results suggested that salicylate-dependent defenses may function in foliar tissues to reduce the development of pathogen-induced wilting and necrosis. Although jasmonate application induced defense gene expression in leaves, it did not increase the resistance level to Fo (Edgar et al. 2006).

The effects of salicylic acid application were assessed to elucidate the mechanism of biocontrol activity of nonpathogenic F. oxysporum (npFo) against F. oxysporum f.sp. asparagi (Foa), causing Fusarium wilt disease of asparagus (Asparagus officinalis). Split-root system was employed, where one-half of the root system of asparagus seedling was drenched with salicylic acid and the other half of the root system was examined for the activation of defense responses. Treatment of asparagus root with SA by soil drench (20 mg/l) at 2 days before challenge inoculation with Foa, was sufficient to protect the plants systemically. Diphenyleniodonium chloride (DPI), an SA synthesis inhibitor, prevented induction of resistance to Foa by npFo. In vitro assays showed lack of inhibitor effect of SA on conidial germination and mycelial growth of Foa. SA-treated plants showed enhancement of systemic resistance with significant reduction in disease severity of the roots inoculated with Foa, compared with untreated control plants. SA-activated peroxidase and phenylalanine ammonia lyase (PAL), as well as lignification upon Foa infection, in a manner similar to that observed with npFo pretreatment. In addition, pretreatment of asparagus roots with SA or npFo primed the plants for a potential defense response to Foa. The results suggested the involvement of an SA-dependent SAR pathway in the npFo-induced potential defense responses and resistance in asparagus to F. oxysporum f.sp. asparagi (He and Woly 2005). The mechanism of activity of SA in suppressing tomato wilt disease caused by F. oxysporum f.sp. lycopersici (Fol) was studied. SA at a concentration of 200 mM was supplied through root feeding and foliar spray on tomato plants. Endogenous accumulation of free SA in tomato
roots was detected by high performance liquid chromatography (HPLC) technique and its identity was confirmed by LC-MS/MS analysis. The endogenous level of SA in the roots increased at 168 h after application to about ten times higher than in untreated control plants. Similar increase in SA content in leaves also was observed, following foliar application of SA. The activities of PAL and PO were significantly stimulated, following SA application through either root or foliage. SA-treated tomato plants challenged with Fol, showed significant reduction in the intensity of vascular browning, leaf yellowing and wilting. However, the mycelial growth of Fol was significantly inhibited as shown by in vitro assays. The results indicated that SA-induced SAR might be responsible for suppression of wilt disease development in tomato, as the principal mechanism of biocontrol activity of SA (Mondal et al. 2005).

Perception of both general and specific pathogenesis-associated molecules by plants triggers defense responses via signal transduction cascades and transcriptional activation of numerous genes. In chickpea (Cicer arietinum), putative genes potentially involved in defense responses, including the rapid synthesis of PR-proteins, presence of an oxidative burst and synthesis of putative cell wall-strengthening proteins and antimicrobial proteins have been identified (Coram and Pang 2006). The responses of three chickpea genotypes treated with defense signaling compounds SA, methyl jasmonic acid (MeJ) and acinomyceloncarboxylic acid (ACC) to Ascoscyta rabiei were investigated, using microarray technique, followed by validation with QRT-PCR assay. Of the 715 experimental microarray features, 425 (59.4%) were differentially expressed (DE) at least in one condition. According to treatment applied, 69, 15.8 and 57.6% were differentially expressed respectively by ACC, MeJ and SA. The coregulation of transcripts between treatments for each genotype with varying levels of disease resistance showed large proportions of transcripts were independently regulated by ACC, MeJ or SA. Of the coregulated transcripts, the ACC-SA category contained the most for all genotypes, lending support to the view of cross talk and overlap occurring between signaling pathways (Salzman et al. 2005; Jalali et al. 2006).

### 3.2.2.2 Benzothiadiazole

Benz (1, 2, 3)-thiadiazole-7-carbothioic acid-S-methyl ester (BTH) is a nontoxic functional analogue of salicylic acid and its mechanism of suppression of development of diseases caused by microbial plant pathogens has been investigated. The biocontrol potential of BTH and an avirulent strain of *Pseudomonas syringae* pv. *maulicola* (Psm) in inducing systemic acquired resistance (SAR) in canola (Brassica napus) against *Leptosphaeria maculans* was assessed. Application of BTH enhanced resistance against virulent strains of *Psm* and *L. maculans* to a great extent, then localized preinoculation of plants with avirulent strain. Pretreatments of plants with BTH and avirulent strain resulted in enhancement of defense-related responses to a greater level than in untreated plants. Development of SAR in *B. napus* plants expressing a bacterial salicylate hydroxylase transgene (NahG) that is known to metabolize SA to catechol was significantly compromised. The plants accumulated only reduced levels of PR gene transcripts, compared with nontransformed plants (control). The results indicated that BTH induced SAR including long-lasting and broad host range resistance, associated with PR gene activation and requirement of SA (Potlakayala et al. 2007). The effectiveness of benzothiadiazole (BTH) in inducing resistance in cocoyam (*Xanthosoma sagittiflorum*) against root rot pathogen *Pythium myriotylum* was assessed. Under controlled conditions, BTH (0.2 mg/ml) applied on leaves induced resistance to the pathogen effectively, resulting in significant reduction in disease incidence and severity. The activities of peroxidase (PO) and polyphenol oxidase (PPO) and total phenol contents registered increases. The enhancement of peroxidase activity was correlated with two new isoforms in a white (sensitive) cultivar inoculated, after stimulation. In a yellow (resistant) cultivar, stimulation was characterized by the appearance of one isoform. Quantitative analysis of phenolic compounds by HPLC showed an increase of hydroxycinnamomeric and flavonoid derivatives after inoculation. Presence of a new caffeoylshikimic acid derivative was also detected after stimulation, following inoculation of both cultivars. The pattern of induction for resistance to *P. myriotylum* appeared to be cultivar-dependent (Mboubda et al. 2010).

Acibenzolar-S-methyl (ASM), a derivative of BTH was evaluated for its biocontrol potential against *Phytophthora cactorum*, incitant of crown rot and red stele disease of strawberry. The comparative efficacy of ASM and chitosan in suppressing the development of crown rot and red stele disease in strawberry was assessed. Both ASM and chitosan reduced crown rot symptoms and the suppressive effect was enhanced, when the interval between treatment and challenge inoculation with *P. cactorum* was increased from 2 to 20 days. Increase in concentration of ASM from 10 to 1,000 μg a.i./plant did not provide any additional advantage. Inoculation of alpine strawberry plants (*Fragaria vesca* var. *alpina*) cv. Alexandria with *P. fragariae* var. *fragariae*, after treatments with ASM, chitosan or fosetyl-Al (fungicide) showed that ASM provided effective protection to alpine strawberry, whereas chitosan was ineffective in protecting the plants. No significant difference was observed between the effectiveness of ASM and fosetyl-Al in protecting alpine strawberry plants against the pathogen (Eikeno et al. 2003). The effect of resistance inducers may differ, based on the method of application. In the greenhouse experiments, soaking seeds of cotton in ASM solutions (25 or 50 μg/ml) for 3–5 h, before planting the seeds in soils naturally infested with *Thileaviospsis basicola*, incitant of black root disease of cotton, resulted in consistent reduction in disease severity on tap roots by 20 to 30%; under field conditions. ASM was applied as sprays over seeds during sowing (in-furrow spray) as seed soaking or as foliar spray over seedlings. Seed-soaking reduced symptom severity by 33%, whereas in-furrow spray reduced disease severity by 24%, increased the number of relatively healthy roots by 35% and increased boll number by 29%. Foliar sprays were ineffective in reducing severity of disease. The results indicated
the need for selecting suitable method of application of ASM for providing effective protection against cotton black root disease (Mondal et al. 2005). The comparative protective effects of ASM (BTH), methyl jasmonate (MeJ) and K$_2$HPO$_4$ against Monosporascus cannonballus, causing melon decline disease was assessed under pot and field conditions for 2 years. Seed treatment with MeJ significantly reduced root rot symptom severity and wine decline, whereas plants from seeds treated with BTH and K$_2$HPO$_4$ were slightly more resistant to the pathogen. Greenhouse assessments in 2006 using soil naturally infested with M. cannonballus showed that MeJ seed treatment followed by foliar application, decreased disease severity. Both MeJ and BTH treatments reduced root rot and vine decline in 2007, but K$_2$HPO$_4$ was ineffective. The resistance inducers differentially activated the synthesis of a number of PR protein isozymes, markers of induced systemic resistance (ISR) in the root system. Application of MeJ, as inducer of resistance in melon to root rot and vine decline in melon appeared to be a feasible strategy to manage the disease (Aleandrí et al. 2010).

Acibenzolar-S-methyl (ASM) showed direct inhibitory activity (up to 40% inhibition) against Rhizoctonia solani AG-4, causing soybean root rot disease. At a concentration of 0.08 and 0.5 g/l, ASM induced systemic resistance responses in soybean hypocotyls, resulting in reduction of rotting severity. Reduction in disease severity by ASM application was correlated with stimulation of chitinase activity. The protective effect of ASM against R. solani AG-4 was, possibly due to a combination of induced resistance and its direct antifungal activity against the pathogen. Further, ASM adversely affected growth of soybean plants. A dose-dependent inhibition of root growth was observed, following seed treatment with ASM. However, the growth retardation of soybean attributed to ASM treatment was overcome and the treated plants recovered and attained normal growth condition, in the case of plants treated with lower dose of ASM (0.08 g/l). The need for determining optimum dose of ASM was revealed by the results of investigation (Faessel et al. 2008). The comparative effectiveness of ASM and chitosan in suppressing the development of Verticillium wilt disease of potato was assessed under in vitro and greenhouse conditions. ASM did not significantly reduce the mycelial growth of V. dahliae, whereas chitosan inhibited the mycelial growth (5.4 to 16.9%), depending on the concentration. Potato tubers were dipped in different concentrations of the elicitors of resistance and planted in soil artificially infested with V. dahliae. ASM and chitosan were sprayed on the foliage of potato seedlings at 100 µg a.i./plant at 15, 25 and 40 days after planting. All treatments reduced Verticillium wilt severity and increased fresh tuber weight. ASM was more effective in reducing disease severity and enhancing yield level than chitosan. The results showed that ASM and chitosan had the potential to effectively reduce Verticillium wilt disease by inducing systemic resistance and increase tuber yield (Amini 2015).

Ceratocystis fimbriata causes mango wilt disease. Induction of systemic resistance to the pathogen in mango by applying acibenzolar-S-methyl (ASM) and potassium phosphate (Phi), a salt of phosphorous acid with systemic mobility in treated plants was investigated. The effects of treatment with the inducers were assessed by microscopic and biochemical analyses. Disease development in test plants was monitored using fluorescence and light microscopy. High performance liquid chromatography (HPLC) procedure was employed to quantify secondary metabolites in the stem sections of treated and control plants. Spraying mango plants with ASM and Phi resulted in reduction of internal necrosis and disease development. Both chemicals induced defense responses in the stem tissues against C. fimbriata infection. HPLC analysis showed that concentration of two alkaloids (theobromine and 7-methylxanthine) and 10 phenolic compounds (catechin, epicatechin, epigallocatechin, gallic acid, myricetin, p-coumaric acid, p-hydroxybenzoic acid, phloridzin, sinapinic acid, salicylhydroxamic acid) were in present in higher concentrations in stem tissues of plants treated with ASM or Phi, compared with untreated control plants. By contrast, higher concentrations of secondary metabolites were detected in the stem tissues at early stages of infection by C. fimbriata, particularly in plants treated with resistance inducers. The results suggested that phenylpropanoid pathway in the stem tissues of treated mango plants might be induced, following infection by the pathogen (Araujo et al. 2015).

Acibenzolar-S-methyl (ASM) was evaluated for its ability to induce resistance in tomato against the bacterial wilt disease caused by Ralstonia solanacearum (Rs). Tomato plants were treated with ASM (25 µg/ml) as foliar spray and soil drench (12.5 µg/ml) and inoculated with different populations of Rs and suitable controls were maintained. Growth reduction in untreated and inoculated plants was observed. Application of ASM significantly reduced bacterial wilt disease incidence, when inoculated with low concentration of Rs, suggesting that ASM might be effective only when the pathogen inoculum level was low (low disease pressure conditions) (Haciasalihoglu et al. 2007). The mechanism of action of ASM and Pseudomonas fluorescens Pf2 in suppressing development of bacterial wilt disease in tomato was studied. Treatment of tomato seedlings with either Pf2 strain or ASM significantly reduced bacterial wilt disease severity by 58% and 56%, respectively. An increase in the effectiveness of disease suppression was observed, when both Pf2 and ASM were combined providing 72% reduction in disease severity. The seedling biomass showed increase due to ASM treatment, relative to the control. Significant changes in the activities of polyphenol oxidase (PPO), β-glucosidase (B-GL) and peroxidase (PO) were recorded in tomato plants treated with ASM or Pf2. Under field conditions, application of ASM as foliar spray or soil drench was effective in suppressing bacterial wilt disease development in tomato (Abo-Elyour et al. 2012).

3.2.2.3 β-Aminobutyric Acid

The biocontrol potential of the nonprotein amino acid β-aminobutyric acid (BABA) against fungal pathogens, infecting different crops has been assessed. The protective effect of BABA against potato late blight disease caused by Phytophthora infestans was demonstrated, using two potato
cultivars, Bintje and Pampena with different levels of horizontal resistance to late blight disease. Foliar treatment at 30 days after emergence provided protection (60%) in cv. Pampena against *P. infestans*. BABA treatment stimulated the expression of defense molecules, such as glucanases, chitinases and phenolic compounds (Altamiranda et al. 2008). Potato cultivars with different levels of resistance to *P. infestans* received four applications of BABA throughout the crop season and they produced tubers with greater resistance to *P. infestans* and also to *Fusarium solani* infection of tubers, compared with those from untreated potato plants. Tuber slices from treated plants, inoculated with *P. infestans* showed an increase in contents of phenolics and phytoalexins. Aspartyl protease StAPI accumulation was also higher in tubers from BABA-treated plants and inoculated with *F. solani*. Infected tubers of BABA-treated plants showed minor fungal proteolytic activity than those from nontreated plants. Application of BABA improved the plant growth, in addition to protection against tuber infection by fungal pathogens (Olivieri et al. 2009).

The protective efficacy of β-aminobutyric acid (BABA) against the white mold pathogen *Sclerotinia sclerotiorum* infecting artichoke was investigated, using cvs. C3 and Explorer. Soil treatment by drenching with BABA induced high level of resistance in artichoke plantlets of both cvs. C3 and Explorer with similar levels of protection. A consistent increase in peroxidase activity paralleled with the differential induction of an alkaline isoenzyme with a pI of 8.6 also was detected. The results indicated that BABA-induced resistance and an augmented ability to express basal defense responses were positively correlated in a more pronounced manner in the cv.C3 (Marcucci et al. 2010). In another investigation, the ability of BABA to protect *Brassica napus* plants against infection by *Leptosphaeria maculans* was assessed. BABA showed direct inhibitory effect against *L. maculans* in in vitro tests. The EC<sub>50</sub> value of BABA was similar to that of the fungicide tebuconazole. Both spore germination and hyphal growth were inhibited. Suppression of disease progression in plants and antifungal activity in vitro were weaker for α-aminobutyric acid and negligible for γ-aminobutyric acid. In contrast to benzothiadiazole (BTH), another resistance inducer, the effect of BABA on disease development was nearly independent of the timing of treatment, indicating possible antifungal activity in plants. By contrast, quantification of multiple hormones and an expression analysis indicated that BABA treatment induced synthesis of salicylic acid (SA) and expression of SA marker gene PR-1. However, no evidence for priming SA responses to *L. maculans* could be obtained. The antifungal activity of BABA against *L. maculans* could be another possible mechanism of action of BABA to provide protection to canola against microbial pathogens (Šásek et al. 2012).

### 3.2.2.4 Glycerol

Glycerol is an environment-friendly, nontoxic, edible and biodegradable sugar alcohol. Glycerol and its derivative glycerol-3-phosphate (G3P) were considered to have the ability to participate in plant defense-against stresses. Exogenous application of glycerol as foliar spray was evaluated for its potential in suppressing infection by *Phytophthora capsici* in cocoa. Glycerol applied over a period of 4 days on cocoa leaves increased endogenous level of G3P and decreased the level of oleic acid (18:1). Reactive oxygen species (ROS) known as a defense activation marker, were produced and the expression of many pathogenesis-related genes was induced. The effect of glycerol application on G3P and 18:1 fatty acid content, and gene expression levels, in cocoa leaves were dosage dependent. Spray application of glycerol at 100 mM concentration was sufficient to stimulate the defense response without causing any observable damage and resulted in a significantly decreased lesion formation by *P. capsici*. However, at higher concentration (500 mM), chlorosis and cell death were observed on treated leaves (Zhang et al. 2015).

### 3.2.2.5 Ethanol

Biological soil disinfestation (BSD), an environmentally safe approach, is increasingly becoming acceptable for management of soilborne diseases, because of rising concerns related to environmental risks. The efficacy of soil disinfestation using ethanol was evaluated for the control of *Fusarium* wilt disease of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*). Survival of bud cells and chlamydospores of *Fol* declined significantly in soil saturated with diluted ethanol solution in vitro. In the field trials, artificially added nonpathogenic *F. oxysporum* and indigenous *F. oxysporum* were both strongly suppressed in soil saturated with 1% ethanol solution. Wheat bran treatment was not as effective as ethanol treatment. Artificially added *F. oxysporum* could not be detected in three of four sites amended with wheat bran. Ethanol application did not show any suppressive activity in preautoclaved soil, indicating the requirement of presence of native microorganisms for disease suppression. The ethanol-mediated biological soil disinfestation (Et-SBD) transiently increased the number of all aerobic bacteria, but the number of fungi and aerobic bacteria was stable. Bacterial community structure in the soil treated with Et-SBD showed slight, but apparent differences, compared with soils that received irrigation or other treatments, as indicated by polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) analysis. The results indicated the potential of ethanol-based soil disinfestation for suppressing soilborne fungal diseases affecting various crops (Momma et al. 2010).

### 3.2.3 Inorganic Chemicals

The beneficial effects of inorganic chemicals on plant growth and disease suppression by enhancing host resistance have been reported in some pathosystems. Phosphates are considered to generate an endogenous systemic acquired resistance (SAR) signal, because of calcium sequestration at points of phosphate application (Reuveni et al. 1994). Phosphites (Phi) are alkali metal salts of phosphorous acid with the ability to protect plants against microbial plant pathogens. The effects of treatment of potato seed tubers and foliage with Phi on development of *Phytophthora infestans*, (late blight and tuber...
Rot, *Fusarium solani* (dry rot) and *Rhizoctonia solani* (black scurf) infecting potatoes were assessed, using cvs. Shepody and Kennebec. Protection provided by Phi was variable, being high against *P. infestans*, intermediate against *F. solani* and low against *R. solani*. In addition, seed tubers treated with calcium or potassium phosphites (CaPhi and KPhi) at 1% commercial product, emerged earlier than untreated controls. When Phi applied as foliar sprays four times at different doses, high levels of protection against *P. infestans* were observed on both cultivars. Higher protection was recorded in Kennebec, when CaPhi was applied, whereas the protective effect of KPhi was greater in cv. Shepody. Expression of β-1,3-glucanase was induced at different times after treatment, but no correlation between β-1,3-glucanases expression and level of foliar protection could be established. By contrast, Phi did not induce any negative effect on plant growth. Leaves of treated plants were darker green than those in untreated plants. Increase in Rubisco protein and a delay on setting of leaf senescence in the Phi-treated leaves were also seen (Lobato et al. 2008).

The comparative effectiveness of phosphate (Phostrol) and metalaxyl-m (Ridomil Gold 480EC) applied as planting-infurrow treatment was assessed for reducing the incidence of potato pink rot caused by *Phytophthora erythroseptica* under field conditions in 2005 and 2006. Inoculum of a metalaxyl insensitive isolate of *P. erythroseptica* was applied either in-furrow as vermiculite slurry at planting or as a zoospore drench in soils adjacent to plants in late August. After harvest, disease incidence and severity and effect on tubers were determined. The mean percentages of diseased tubers were 1.7% (2005) and 1.3% (2006) for the fungicide-treated plots and 10.1% (2005) and 3.1% (2006) for phosphate-treated plots. The potato cv. Shepody was significantly more susceptible to pink rot (9.9% and 3.3% diseased tubers in 2005 and 2006 respectively) than Russet Burbank (3.4% and 1.2% in 2005 and 2006 respectively). The results indicated that metalaxyl was more effective than phosphate in reducing the incidence of pink rot affecting potato tubers (Al-Mughrabi et al. 2007).

The protective ability of potassium nitrate (KNO₃) was determined for suppressing the development of soybean stem rot caused by *Phytophthora sojae*. Application of KNO₃ (4–30mM) prior to challenge inoculation with *P. sojae* reduced the incidence of disease in two soybean cultivars. The extent of disease suppression depended on concentration of potassium in the plants of both cultivars. Observations under scanning electron microscope revealed marked accumulation of potassium at penetration stopping sites of *P. sojae* in the cortex layer of soybean plants treated with 30 mM of KNO₃, compared to untreated control plants. Reduction in release of zoospores of *P. sojae* was observed in the presence of 0.4–30 mM KNO₃, indicating some direct effect of the chemical on the pathogen development. The results indicated the potential of KNO₃ as a strategy to reduce fungicide use for managing soybean stem rot disease caused by *P. sojae* (Sugimoto et al. 2009). The biocontrol activity of potassium sorbate (PS), potassium bicarbonate (PB) and dipotassium hydrogen phosphate (DPHP) against soilborne pathogens *F. oxysporum* f.sp. *lycopersici* (Fol), *F. oxysporum* f.sp. *radicis-lycopersici* (FORL), *F. solani* (Fs), *Verticillium dahliae* (Vd), *Rhizoctonia solani*, *Pythium aphanidermatum* and *Sclerotinia sclerotiorum* was assessed. The effects on growth were variable, depending on the sensitivity of different fungal pathogens tested. Single treatment of soils with PS (0.25%), PB (50 mM) and DPHP (50 mM) reduced wilt diseases to different extent. PS treatment reduced wilt incidence by 50, 78, 26 and 65% respectively, compared to Vd-, Fol- and FORL-inoculated control plants. In addition, PS treatment improved plant growth significantly. PB-based treatment resulted in reduction to an extent of 60, 86 and 30% in Fol, FORL and Fs infections and severity, but it had no effect on Vd wilt disease. DPHP suppressed Fusarium wilt by 65.2%. PB treatment reduced Rhizoctonia root rot also to some extent. The results indicated the need for selecting suitable potassium salt for suppressing different diseases affecting tomato (Jabnoun-Khiareddine et al. 2016).

The potential of silicon (Si) applied as soil amendment in inducing resistance in banana (*Musa acuminata*) against *Cylindrocladium spathiphyllici*, incitant of banana toppling disease was assessed. Banana plantlets, inoculated by dipping the root system in the suspension of pathogen conidia, were planted on desilicated ferrosol and amended with 2 mM of soluble Si under greenhouse conditions and control without Si amendment. Image analysis program WinRHIZO was applied at 7, 14 and 21 days after inoculation. Root necrosis (lesions) was reduced by about 50% at 14 days after inoculation in the plants on Si-amended soil, compared with control plants. Furthermore, Si amendment also improved plant growth because of the suppression of pathogen development by enhancing the level of resistance of banana plants supplied with Si. The results indicated the possibility of using Si amendment of soil as an ecofriendly disease management strategy and an alternative to chemical control (Vermeire et al. 2011). The extent of protection provided by silicon to tomato against crown and root rot disease caused by *F. oxysporum* f.sp. *radicis-lycopersici* was assessed using sand culture system. Hoagland’s nutrient solution with (100 mg Si/l, Si⁺) was used as a nutrient source for tomato plants without Si (Si⁻) and control plants were also maintained. At 8 weeks after transplantation, the plants were inoculated with three inoculum levels (0, 10⁶ and 10⁷ conidia/plant). Disease severity was significantly reduced by Si treatment at 4 weeks after inoculation. Si contents of roots and stems of treated tomato plants were significantly greater, compared to control plants. The increase in the Si contents of the roots was positively correlated with reduction in disease severity in roots, crown and stem of treated tomato plants. The results suggested that the decrease in disease severity due to Si treatment, might be because of the delay in the onset of initial infection of roots and movement of the pathogen from roots to stem (Huang et al. 2011).

The effects of silicon on development of symptoms of Fusarium wilt disease caused by *F. oxysporum* f.sp. *cubense* (*Foc*) on banana plants were assessed, using seedlings of Grand Nain (resistant) and Maca (susceptible) cultivars grown in plastic trays amended with 0.39g Si (Si⁺) and without.
amendment (Si\(^+\)). The soil was inoculated with *Foc* at 60 days after transplanting banana seedlings. The Si concentrations in the roots and rhizome-pseudostem significantly increased by 30.26% and 58.82%, respectively, compared with Si\(^-\) treatment. The Si-treated plants showed a reduction in disease severity determined, based on area under reflex leaf symptoms progress curve, the area under root symptom progress curve and the area under asymptomatic fungal colonization of tissues progress curve, compared to Si\(^-\) plants. The area under darkening rhizome-pseudostem progress curve (AUDRPPC) of Maca significantly increased by 15.98% for the Si\(^-\) treatment, compared with Si\(^+\) treatment. The area under relative lesion length progress curve (AURLLPC) of Maca plants significantly reduced by 45.54% for the Si\(^+\) treatment. Grand Nain plants showed no difference in AUDRPPC and AURLLPC values due to treatment with Si. The plants in the Si\(^-\) treatment of cvs. Maca and Grand Nain plants did not show difference in AUDRPPC and AURLLPC values. The results showed that amendment with Si in soil for growing susceptible banana cultivars showed potential for reducing wilt severity in Si-deficient soils (Fortunato et al. 2012a). In further investigation, plants of banana cv. Grand Nain and Maca were grown in plastic pots with soil amended with Si and inoculated with *F. oxysporum* f.sp. *cubense* (*Foc*) race 1. Relative lesion lengths (RLLs) and asymptomatic fungal colonization were reduced to a great extent at 40 days after inoculation in the resistant Grand Nain, then in the susceptible Maca. Reduction in severity of symptoms was the greatest in resistant variety. The activities of PAL, PPO, POX, chitinases and β-1,3-glucanase were enhanced in the roots of banana plants grown on soil with Si amendment (Fortunato et al. 2012b). *Phytophthora pistacia* causes pistachio gummosis, one of the major diseases impacting production adversely. The biocontrol potential of sodium and potassium silicate in suppressing the disease development was assessed. The silicon salts inhibited mycelial growth, sporangial production, cyst germination and fungal biomass. Further, zoospore release was significantly reduced by potassium silicate, but not by sodium silicate. Under pot experiments, broad bean (annual host crop) and seedlings of pistachio grown on sterilized soil, following application of silicon salts were inoculated with *P. pistacia*. Significant reduction was observed in disease incidence, percentage of roots colonized by the pathogen and mortality of plants, compared with nontreated control plants was observed. The silicon salts did not have significant effect on pH and electrical conductivity of soil. Coupled plasma mass spectrometry (ICP-MS) analyses showed that silicon concentration was increased in Si-treated broad bean and pistachio plants. The results showed the potential of silicon salts could be exploited for suppressing the development of an economically important disease of pistachio (*Mostowizadeh-Ghalamfarsa et al. 2017*).

The biocontrol potential of silver nanoparticles (AgNPs) synthesized with aqueous extract of *Artemisia absinthium* against *Phytophthora* spp. was assessed. The AgNPs (10 μg /ml) inhibited mycelia growth of *Phytophthora parasitica*, *P. infestans*, *P. palmivora*, *P. cinnamomi*, *P. tropicalis*, *P. capsici* and *P. katsurae*. The AgNPs were highly inhibitory to mycelial growth, zoospore germination, germ tube elongation and zoospore production of *P. parasitica* and *P. capsici*, as revealed by detailed dose-response analyses. Under greenhouse conditions, AgNP treatment prevented infection by the pathogen and improved plant survival. Further, no adverse effects of AgNPs in plant growth were evident. The results indicated that AgNPs could be applied for effective suppression of diseases caused by *Phytophthora* spp. as an alternative to fungicides (Ali et al. 2015). *Stenotrophomonas* spp. (earlier known as *Pseudomonas maltophilia*) have wide distribution and they have potential for use as plant growth promoter and biocontrol agents. Biosynthesis of gold and silver nanoparticles (AgNPs) was achieved using *Stenotrophomonas* sp. BHU-S7 (MTCC5978) strain. Biosynthesis of AgNPs by this strain was monitored by UV-visible spectrum, showing surface plasmon resonance (SPA) peak at 440 nm. The antifungal activity of AgNPs (~12 nm size) was assessed. *Sclerotium rolfsii* exposed to AgNPs failed to germinate on potato dextrose agar (PDA) medium, as well as in soil system. Furthermore, treatment with AgNPs reduced collar rot disease incidence in chickpea caused by *S. rolfsii* under greenhouse conditions. Induction of phenolics, altered lignification and H\(_2\)O\(_2\) production in plants treated with AgNPs indicated possible involvement of induced systemic resistance (ISR) as a possible mechanism of action of AgNPs against *S. rolfsii* (Mishra et al. 2017).

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Management of Crop Diseases Caused by Soilborne Microbial Plant Pathogens


