Chapter 7
Pathogen and Management of Fungal Wilt of Banana Through Biocontrol Agents

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Abstract Banana is an important and affordable source of food for millions of people of the developing tropical countries. It comes in the category of export fruits in the world. Banana wilt is a limiting factor for growth of the banana industry. It is one of the most destructive diseases of banana. The main causal organism of wilt of banana is *Fusarium oxysporum* f. sp. *cubense* (Foc). Right from the discovery of *Fusarium* wilt in banana, many control measures like fumigation of soil and crop rotation along with organic amendments have been attempted. But the problem could not be resolved fully except by planting resistant cultivars. Use of resistant varieties also is not effective and can't be implemented easily because of lack of consumer preference. Due to these problems, use of antagonistic agents is being adopted largely. They have the potential to protect and promote plant growth by colonizing and multiplying both in the rhizosphere and plant system. They are useful as an effective eco-friendly alternative for field management of the banana wilt.

To control *Fusarium* wilt, biocontrol method is now gaining popularity. It is eco-friendly in nature. Biocontrol has potential and various mechanisms for plant protection. *Trichoderma* sp. acts as a major interactive agent in root, soil, and foliar environments through releasing an array of compounds producing localized or systemic resistance in plants. *Pseudomonas* sp. also has varying mechanisms towards the control of phytopathogens through release of a wide range of antagonistic metabolites. This chapter records updated information about the pathogen and how *Fusarium* can be managed through the biocontrol agents.

7.1 Introduction

Banana (*Musa* spp.) flourishes well under tropical and moisture-rich farmlands and propagates from the underground rhizome. The whole plant is a false stem and consists of broad leaves with their long petioles arranged in disc-like fashion. During

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2011 banana worth $44 billion, having production of 145 million metric tons, was produced in over 130 countries. *Fusarium* wilt (also known as Panama disease) is the most severe and destructive disease of the crop (Ploetz 2015). *Fusarium* wilt of banana is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc) (Stover 1962). It is a major constraint for banana production throughout the world. A strain of the fungus that affects Cavendish and other dessert bananas in the tropics, called Foc tropical race 4 (TR4; VCG 01213/16), has been confined to five Asian countries (Indonesia, Malaysia, Philippines, mainland China and Taiwan) for more than three decades. Cavendish banana production is expanding in Asia to Laos, Myanmar and Vietnam where local varieties still dominate the market. This is due to an increase in Cavendish banana consumption and a decline in areas of production caused by Foc TR4 in China (Hung and Hung 2018). The fungus enters the plant through the roots and spreads its colony in the xylem vessels which blocks the flow of water and nutrients. The pathogen had evolved along with its host in the region of Indo-Malaya. Then it spread to other banana-growing places through infected planting material (Mostert et al. 2017).

The pathogen that originated in Asia and evolved with the host, the wild banana plant *Musa acuminate* Foc, consists of 8 lineages and 3 races along with 24 VCGs (vegetative compatibility groups). Maximum damage occurs due to Foc tropical race 4 (TR4) found only in Asia (Li et al. 2013).

It causes serious problems in parts of Africa and in Central and South America (Viljoen 2002), Indonesia, Burma, Sri Lanka, Thailand and the Philippines causing in enormous losses every year (Stover and Simmonds 1987; Ploetz 1994). In the last 10 years, banana plantations in China have decreased dramatically due to *Fusarium* wilt in Hainan, Guangdong and Fujian provinces. The fungus produces external symptoms like gradual wilting, progressive yellowing of banana leaves and resultantly collapse at the petiole (Yin et al. 2011). Distinguishing symptoms for the disease are discoloration of vascular tissues (fruit stalk, pseudostem, roots, corm) varying from dark brown to light yellow appearing first on the outer or oldest leaf sheath and then extending to pseudostem (Ploetz 2006), and finally the disease causes death of banana plants. The Foc isolates have been grouped into four physiological races based on pathogenicity to host cultivars in fields (Fourie et al. 2009). *F. oxysporum* f. sp. *cubense* races 1, 2, 3 and 4 attack many cultivars (Table 7.1), and all cultivars show susceptibility to Foc1 and Foc2 (Persley 1987; Ploetz 1990).

Once Foc R1 threatened banana production most severely. Which ruined the 'Gros Michel' industry of banana in the Central America and Caribbean in the mid-1900s (Ploetz 1994). This persisted till the development of Cavendish banana

Table 7.1 Infection of *F. oxysporum* f. sp. *cubense* on different cultivars

SN	Race	Infected cultivar
1.	Race 1 (Foc R1)	'Gros Michel', Lady Finger (AAB) and Silk (AAB) varieties
2.	Foc R2	Bluggoe (ABB)
3.	Foc R3	*Heliconia* spp.
4.	Foc R4	Cultivar Cavendish

cultivars which had no susceptibility for Foc R1 and Foc R2 in tropical regions (Fernández-Falcón et al. 2003). However, it was not long-lasting. Cavendish cultivars soon became highly susceptible to Foc R4, a new race (Ploetz and Pegg 2000) causing worldwide losses. It brings the spiteful change that the banana industry is once again threatened by *Fusarium* wilt. *F. oxysporum* is able to infect more than 100 plant species which can be divided into more than 120 host-specific forms called *formae speciales* (Minerdi et al. 2008). Foc is one of the most destructive pathogen f. sp. of *F. oxysporum* (Ploetz 1990) easily soil-borne and strongly saprophytic. This survives in soil up to several decades through spores (specifically as chlamydospores) that reinfect the susceptible varieties of banana (Stover 1962). This creates problem in disease management. The results of the wilt management studies have been disappointing, i.e. effective and efficient control strategies are still not available to meet the world demand.

7.2 Symptoms

The *Fusarium* wilt symptoms usually become prominent at the time of flowering. The fungus infects the plant roots through forming colony in the vascular tissues in the rhizome and pseudostem. Wilt symptoms are induced after 5–6 months of planting when symptoms get expressed both internally and externally (Figs. 7.1 and 7.2) (Wardlaw 1961; Stover 1962). Usually wilt-infected plants bear no bunches or have very few small fruits that ripen irregularly, have pithy flesh and are acidic. Purplish brown discoloration of the vascular bundles, which can be seen in cross sections of the corm and pseudostem (Fig. 7.2), is the typical internal symptom. In the corm, the discoloration appears as a collection of tiny dots. When root portions

Fig. 7.1 Collapsed leaves of banana plant due to fungal infection

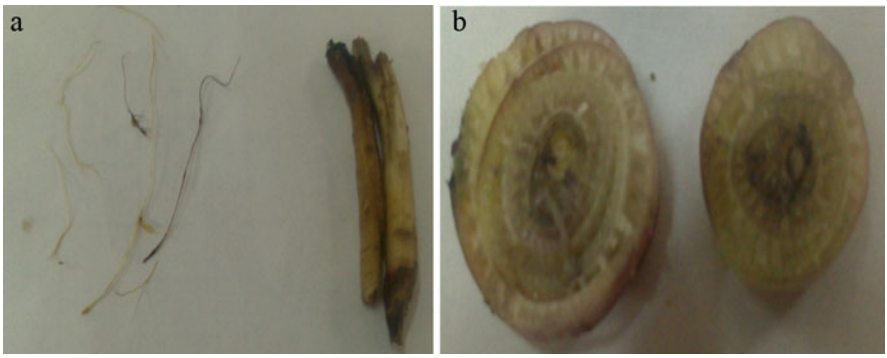

Fig. 7.2 Discolouration in wilted banana roots and pseudostems

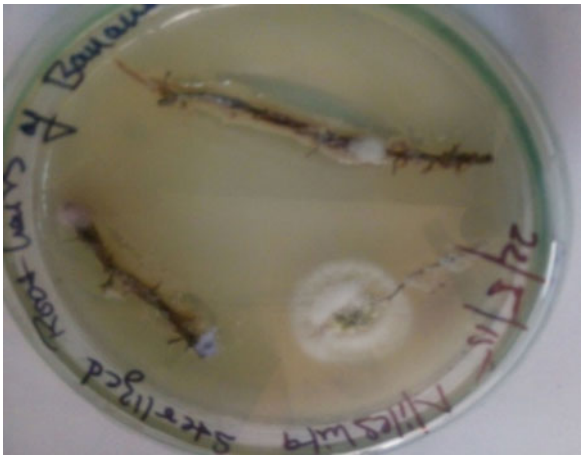

Fig. 7.3 Colonization of *Fusarium oxysporum* on banana roots and root hairs

are cultured on PDA medium, fungal colonies develop (Fig. 7.3). The mycelia of *Fusarium oxysporum* are delicate white and may be sparse to abundant. The fungus develops three types of spores: microconidia, macroconidia and chlamydospores. Microconidia arise laterally on simple phialides and are abundant. They are ellipsoid to oval and straight to curved 4–11 × 2.1–3.4 μm and nonseptate. Macroconidia are also sparse to abundant, borne on branched conidiophores or on the surface of sporodochia. They are thin-walled, three- to five-septate, subulate-fusoid and pointed at both ends with pedicellate base. Three septate are the most common conidia measuring 26–45 × 3–4 μm while five-septate conidia measure 32–57 × 3–5 μm. Chlamydospores may be both smooth and rough walled, produced abundantly and formed either terminally or on intercalary basis (Fig. 7.4) (Kumar and Saxena 2015).

The severity of the disease depends on host susceptibility, fungal virulence and environmental conditions such as rainfall and temperature. In highly susceptible

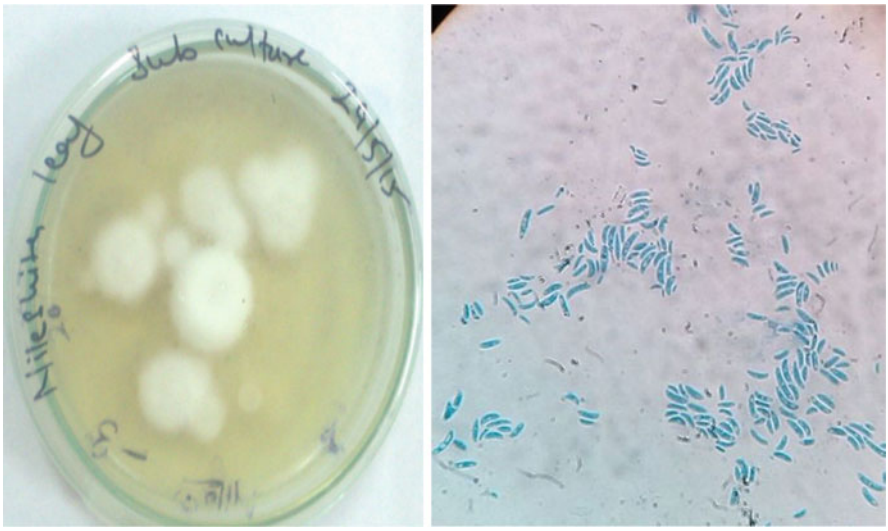

Fig. 7.4 Pure colonies of *Fusarium oxysporum* on PDA medium and the conidia

cultivars, under the conditions of water stress or waterlogging, the entire foliage may become yellow, the growth may cease, emergence of bunch may stop and finally the whole plant may collapse. The pathogen spreads to suckers, producing internal symptoms within 1 or 2 months of emergence of suckers (Moore et al. 1995).

Fusaric acid (FA) is a phytotoxin produced by *F. oxysporum* f. sp. *cubense*, and different members of the *F. oxysporum* species complex cause leaf chlorosis (Dong et al. 2012, 2014). Chloroplast damage reduces photochemical efficiency of plant photosynthesis and net CO_2 assimilation. It has been confirmed through artificially inoculated plants of Gros Michel FA. The pathogen however occurs in symptomatic leaves, but chlorosis is induced upon injecting FA into the leaf lamina. There was a reduction in transpiration in diseased leaves due to stomatal closure with reduction in hydraulic conductivity. This was detected in diseased stems associated with development of the above symptoms (Dong et al. 2012, 2014). Biochemical and structural alterations occur in affected plants showing senescence (Dong et al. 2014).

7.3 Diversity of the Disease

For the first time, Smith (1910) isolated *Fusarium oxysporum* f. sp. *cubense*. It has abundant ellipsoid oval microconidia on short lateral phialidic conidiophores. In due course of time, clusters of typical fusoid 3–5 macroconidia are produced. In culture, the fungus produces a reddish pigment and upon ageing develops globose chlamydospores. These characteristics remarkably separate this from other similar-looking species *F. solani* and *F. moniliforme* also having abundant microspores

(Booth 1971). So far, four races of *F. oxysporum* f. sp. *cubense* (Foc) have been reported (Moore et al. 1995) based on pathogenicity to different banana cultivars: race 1, which occurs throughout the world, attacks cultivars like Pome (AAB) and Silk (AAB) groups; race 2 is widely distributed throughout banana-growing areas which is pathogenic to Monthan, Bluggoe and also cooking bananas; race 3 occurs in Honduras, Costa Rica and Australia and pathogenic to *Heliconia* spp.; and race 4 occurs in maximum banana-growing countries like Malaysia, Australia, South Africa, Canary Islands, Taiwan, Brazil, etc. but not in India, and it attacks Cavendish group of banana (AAA) and also the race 1- and race 2-susceptible banana varieties (Pushpavathi et al. 2015). A sum of 594 *F. oxysporum* isolates from ten (10) Asian countries were identified and grouped on vegetative compatibility group (VCG) analysis. The isolates were divided in DNA lineages through PCR-RFLP analysis. For representing 3 Foc races, they identified 6 lineages and 14 VCGs in this study. VCG complex 0124/5 was the most common in the Indian subcontinent, Cambodia and Vietnam, but the VCG complex 01213/16 showed dominance in the rest of Asia (Mostert et al. 2017).

7.4 Survival and Disease Cycle of the Wilt Pathogen

The pathogen *F. oxysporum* f. sp. *cubense* (Foc) is a facultative parasite capable of saprophytic growth and classified as a root-inhabiting fungus with populations unevenly distributed which decline speedily in absentia of the host (Gowen 1995). However, Moore et al. (1995) reported that the fungus can survive in the field for up to 30 years as chlamydospores in the infested plant debris or in the roots of alternative hosts. It also survives in the roots of several species of common grasses and weeds such as *Paspalum*, *Panicum*, *Ixophorus* and *Commelina* which are the nonsymptomatic hosts (Gowen 1995). Ramakrishnan and Damodaran (1956) reported that liming of soil reduced the survival period of the pathogen to 2 months. The Indian strain of the pathogen could survive under water stagnation for a month (Rawal 2000). The texture and organic matter content of the soil greatly influence the survival of the pathogen. The pathogen colony tends to be higher and survive for long in light texture soils but not in heavy alkaline soils. Certain crop residues may enhance antagonistic microflora which reduce pathogen survival (Sequiera 1992). However, Thangavelu et al. (2001) observed the incidence of the wilt disease from loose soil to heavy clay soil with the pH ranging from 4.80 to 8.45 and EC of 0.12 to 1.10 dsm^{-1}. In the suppressive soils having high microbial populations, the pathogen development gets inhibited. Such soil is common in Australia, Central America, South Africa and Canary Islands (Moore et al. 1995). Figure 7.5 depicts naturally occurring cycle of banana wilt pathogen.

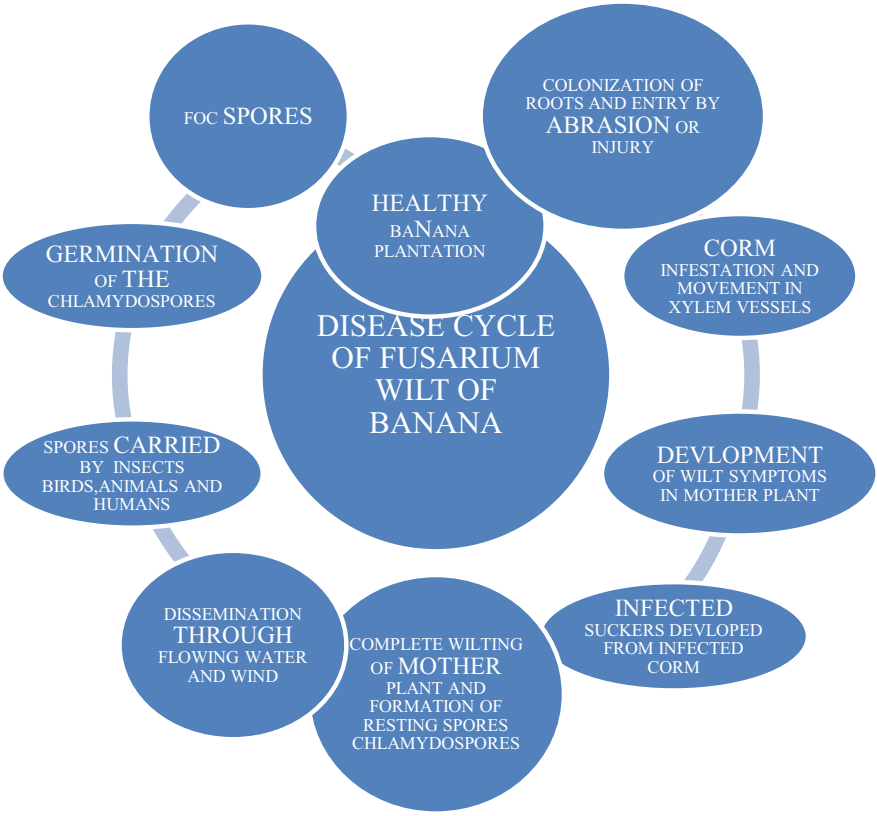

Fig. 7.5 Disease cycle of *Fusarium* wilt of banana

7.5 Management of the *Fusarium* Wilt

The exclusion of pathogen is essential for having pathogen-free areas. *F. oxysporum* f. sp. *cubense* can never be eradicated completely from a region when it gets infested. So quarantine regulations are essentially needed. The regional awareness and contingency programmes should be kept in mind in all threatened regions (Ploetz 2015).

To prevent Foc infection, many control methods were developed, viz. crop rotation, soil fumigation, fungicidal application, flooding fallow lands (Wardlaw 1961) and organic amendments. But none proved effective. The only effective method is cultivation of wilt-resistant banana plantations. Strict quarantine procedures regulate spread into areas where this pathogen does not occur yet possible only with great vigilance (Dita et al. 2010). Through involvement of quarantine practices, checking of infection in plant materials and along with farm implements cleaning can reduce disease spread (Nel 2004). Destruction of infected rhizomes and suckers can retard the flow of chlamydospore distribution in soil. The burning and

sterilization of the diseased plant parts such as pseudostem and rhizome along with leaves in soils help in reducing the pathogen populations (Thakker et al. 2013). One of the alternates is to use pathogen-free tissue-cultured banana plants because this may be a substitute of suckers. This will reduce the flow of infection. Another investigation revealed an enhancement of susceptibility in micropropagated Cavendish cultivars for Foc race 4 with comparison to plants grown through conventional planting materials (Smith et al. 1998). This study highlights the value of agronomic practices in solving the disease.

The accumulation of host-specific pathogens in the soil can be reduced through intercropping and rotations which in turn together can alter the microbiological niche of the soil. The banana plantations can be changed to crops like sugarcane, paddy, cereals and cassava (Buddenhagen 2009). Besides, Huang et al. (2012) investigated that crop rotation of Chinese leek-banana efficiently controls *Fusarium* wilt in banana, reducing wilt incidence by 88–97%. The crop rotation practices however are less effective for potent soil microbes like Foc. The destruction of asymptomatic alternate hosts and effective weed management schedule in infested fields prevent and easily check the spread of *Fusarium* wilt (Hennessy et al. 2005).

Chemical methods have proved effective in field control of the *Fusarium* wilt of banana. The fungicides of benzimidazole group such as carbendazim, benomyl and thiabendazole showed effectiveness in suppressing Foc under in vitro and even in greenhouse conditions (Nel et al. 2007). Chemical compounds, viz. propiconazole, prochloraz and cyproconazole, also reduced *Fusarium* banana wilt incidence by about 80% (Nel 2004).

Fusarium population is maximum in the rhizosphere (83.4%) but least in the rhizoplane (46.3%). Its occurrence was 71.6% in the infected stems while only 51.6% in collars. The pathogenicity tests confirmed it to be dominantly responsible for wilt in banana. Extracts of *Ranunculus sceleratus* showed the highest inhibition of mycelial growth (97.3%) (Kumar 2016).

7.5.1 Biological Control

In the developing countries, banana comes in the category of staple food and consumed daily for breakfast or lunch and even exported widely to the developed countries. Since *Fusarium* wilt is a big problem for banana, so control strategies like soil fumigation, fungicides (Lakshmanan et al. 1987), crop rotation (Su et al. 1986), flooding fallow lands (Stover 1962), organic amendments (Stover and Simmonds 1987) and plant extracts (Kumar 2016) were tried, but the problem could be resolved only by planting the resistant cultivars (Moore et al. 1999). Resistant varieties however are not acceptable everywhere due to lack of consumers' preference (Viljoen 2002).

Use of antagonistic microbes is a potential alternative and has become increasingly popular (Weller et al. 2002). Therefore the search is on for novel mechanisms of plant protection and for other bioagents (Pushpavathi et al. 2016). Biological

control of many soil-borne pathogens like *Fusarium oxysporum* is well studied (Thangavelu et al. 2004). There are reports demonstrating the successful use of different species of *Trichoderma*, *Pseudomonas*, *Streptomyces* and nonpathogenic *Fusarium* (npFo) of both rhizospheric and endophytic nature against *Fusarium* wilt under both glasshouse and field conditions (Rajappan et al. 2002; Getha et al. 2005). Results of the glasshouse evaluations of Nel et al. (2006) revealed that two of the nonpathogenic *F. oxysporum* isolates, CAV 255 and CAV 241, reduced *Fusarium* wilt incidence by 87.4 and 75.0%, respectively.

Pushpavathi et al. (2015) reported that sucker treatment before planting with biocontrol agents either *Trichoderma viride* or *Pseudomonas fluorescens* and soil drenching with the same biocontrol agent (twice at 30 and 180 DAP as booster application) effectively reduced the disease incidence and intensity thereby significantly increasing the yields.

7.5.1.1 *Trichoderma* spp.

It is a free living and common fungus in soil and root ecosystems. This interacts well in root, soil and foliar environments and releases a variety of compounds causing localized or systemic resistance in plants. It has long been known as biological agent used in the disease management having the ability for increasing root development, growth, productivity, resistance to abiotic stresses, nutrient uptake and use. This may be efficiently applied as spores which are tolerant to odd conditions for formulation and field application in comparison to their mycelia and chlamydospores (Amsellem et al. 1999). The mycelial mass produces antagonistic metabolites (Yedidia et al. 2000). The investigations have revealed that *Trichoderma* species effectively suppress *Fusarium* wilt (Thangavelu et al. 2004). Thangavelu (2002) reported application of *T. harzianum* Th-10 preparation @ 10 g/plant having 4×10^{31} cfu/g in basal and top dressing in 2, 4 and 6 months after planting showed maximum reduction of wilt incidence (51.16%).This was succeeded by *Bacillus subtilis* and *Pseudomonas fluorescens* (41.17%) applications as talc-based preparations both in glasshouse and fields. *T. harzianum* Th-10 talc-based preparation and 'carbendazim' (0.1%) showed only 40.1% and 18.1% reduction of the wilt problem. In the *Fusarium* wilt-nematode complex, soil application of biocontrol agents significantly checked the wilt severity and the root lesions and root-knot index also and besides caused 50–82% of reduction in population of nematode, viz. *Pratylenchus coffeae* and *Meloidogyne incognita*. The maximum reduction was because of *T. harzianum* use (Thangavelu 2002). Raguchander et al. (1997) found *T. viride* and *P. fluorescens* to be equally effective in reducing the wilt problem. The potted abaca plants when inoculated with *T. viride* and yeast together also showed 81.76% and 82.52% decrease of wilt disease incidence (Bastasa and Baliad 2005).

Soil application of chaffy grain formulation of *T. viride* NRCB1 greatly checked the external (up to 78%) and internal (up to 80%) symptoms of *Fusarium* wilt in tissue-cultured as well as sucker-derived banana plants also increasing the plant

Table 7.2 Bioagents and mechanisms of action the control of banana *Fusarium* wilt

S. no.	Bioagent	Mode of action	References
1.	*Streptomyces violaceusniger*	On incubation in agar plates showed in vitro antibiosis, microscopic studies evidenced lysis of hyphal ends in the inhibited fungal colonies It also evidenced in vitro antagonistic actions against *F. oxysporum* f. sp. *cubense* in the form of swelling and distortion, also showing excessive branching in hyphae finally inhibition of spore germination	Getha and Vikineswary (2002)
2.	*P. fluorescens*	Banana roots precolonization with *Pseudomonas fluorescens* could reduce *Fusarium oxysporum* f. sp. *cubense* colonization by 72%. Unusual deposits at sites to prevent fungal entry with a number of defence activity against spread of pathogen	Sukhada et al. (2004)
3.	*Streptomyces violaceusniger*	Through antibiotics produced	Getha et al. (2005)
4.	*Pseudomonas fluorescens*	Antibiotics (2,4-diacetylphloroglucinol) production inhibiting the growth and spore germination of *F. oxysporum* f. sp. *cubense*	Saravanan and Muthusamy (2006)
5.	*Serratia* sp.	Shows promising growth-promoting properties	Ting et al. (2008)
6.	*Proteobacteria*	Increase in PO and SOD wilt for defence	Jie et al. (2009)
7.	*Trichoderma viride*	Induction of PO and PAL activities and increase of defence-related enzymes and antibiosis	Thangavelu and Mustaffa (2010)
8.	*P. fluorescens*	Induction of defence enzymes such as PO and PAL	Akila et al. (2011)
9.	*Bacillus subtilis*	Induction of defence enzymes PO and PAL	Akila et al. (2011)
10.	*Pseudomonas* spp.	Production of volatile compounds such as methanethiol, 2-pentane 3-methyl and 3-undecene	Ting et al. (2011)
11.	*Herbaspirillum* spp.	Production of volatile compounds 2-pentane 3-methyl, methanethiol and 3-undecene	Ting et al. (2011)
12.	*Trichoderma viride*	Through production of antibiotics	Pushpavathi et al. (2015)
13.	*P. fluorescens*	Through production of metabolites and antibiotics	Pushpavathi et al. (2015)

growth parameters significantly both under pot culture and field conditions (Thangavelu and Mustaffa 2010).

The mechanisms reducing the *Fusarium* wilt severity due to *Trichoderma* spp. may be mycoparasitism, spatial and nutrient competition, antibiosis because of enzymes and secondary metabolites and improvement of plant defence system (Table 7.2). The mycoparasitism shows coiling, disorganization of cell contents and penetration of the host (Papavizas 1985). *Trichoderma* sp. parasitizes the pathogen hyphae and produces proteolytic enzymes like 1,3-glucanolytic, chitinase, etc. resulting in lysis. They produce metabolites, extracellular enzymes, volatiles and

antibiotics (e.g. gliotoxin, viridian) which are fungistatic (Weindling 1941) causing antibiosis. Further *Trichoderma* spp. can also compete by forming siderophores (Srinivasan et al. 1992). Thangavelu and Mustaffa (2010) highlighted the application of *T. viride* in the form of rice chaffy grain formulation upon infection produces defence-related enzymes [peroxidase and phenylalanine ammonia lyase (PAL)]. The phenolic content was also significantly higher (>50%) in comparison. Increased activities of lytic enzymes and also phenols in the *T. viride*-treated plants impart resistance to Foc by making physical barrier stronger or chemically impervious to the pathogen (Thangavelu and Mustaffa 2010).

7.5.1.2 *Pseudomonas* spp.

Pseudomonas spp. belong to the category of useful biocontrol agents in agriculture because they can use many exudates/compounds serving as nutrient (Lugtenberg et al. 1999). They show high rate of growth and have diverse mechanisms against plant pathogens by producing a broad range of antagonistic metabolites (Lugtenberg et al. 1999). They are easy to culture in vitro. They can be easily inoculated in the rhizosphere (Rhodes and Powell 1994) inducing systemic resistance (Pieterse et al. 2001). *P. fluorescens* also suppresses *Fusarium* wilt disease in banana. Fluorescent pseudomonads such as *P. fluorescens* (Sakthivel and Gnanamanickam 1987), *P. putida* (de Freitas and Germida 1991), *P. chlororaphis* (Chin-A-Woeng et al. 1998) and *P. aeruginosa* (Anjaiah et al. 2003) have been found to inhibit pathogens and promote better growth and yield of many crops. Sivamani and Gnanamanickam (1988) observed that *P. fluorescens*-treated seedlings of *Musa balbisiana* expressed less severe wilting and internal discoloration because of Foc infection. The bacterized seedlings have enhanced root system enhancing plant height. *P. fluorescens* strain pf10 from banana rhizosphere is potent in detoxifying fusaric acid of Foc race 1 reducing wilt incidence up to 50% (Thangavelu et al. 2001). Dipping suckers in the *P. fluorescens* suspension and biocontrol agent effectively checked *Fusarium* wilt of banana (Raguchander et al. 1997). Rajappan et al. (2002) also found that the talc-based preparation was effective in the field against Foc. *P. fluorescens* strain WCS 417 suppressed other *Fusarium* wilts by 87.4% in Cavendish bananas under glasshouse (Nel et al. 2006). Basal application of neem cake @ 0.5 kg/plant + sucker through dipping in spore suspension of *P. fluorescens* for 15 min + soil application of *P. fluorescens* @10 g/plant at 3, 5 and 7 months after planting also checked the wilt (Saravanan et al. 2003).

Fishal et al. (2010) evaluated capability of *Pseudomonas* sp. (UPMP3) and *Burkholderia* sp. (UPMB3) obtained from healthy oil palm roots in induction of resistance fighting against *F. oxysporum* f. sp. *cubense* race 4 on susceptible Berangan banana under glasshouse. Preinoculation of banana through *Pseudomonas* sp. UPMP3 had 51% less in wilt incidence while in combined use of either UPMP3 + UPMB3 or only UPMB3 led to 39% and 38% decrease, respectively.

Two isolates of *Herbaspirillum* spp. and *Pseudomonas* spp. produced volatile compounds having potential to inhibit growth of Foc race 4 (Ting et al. 2011). The

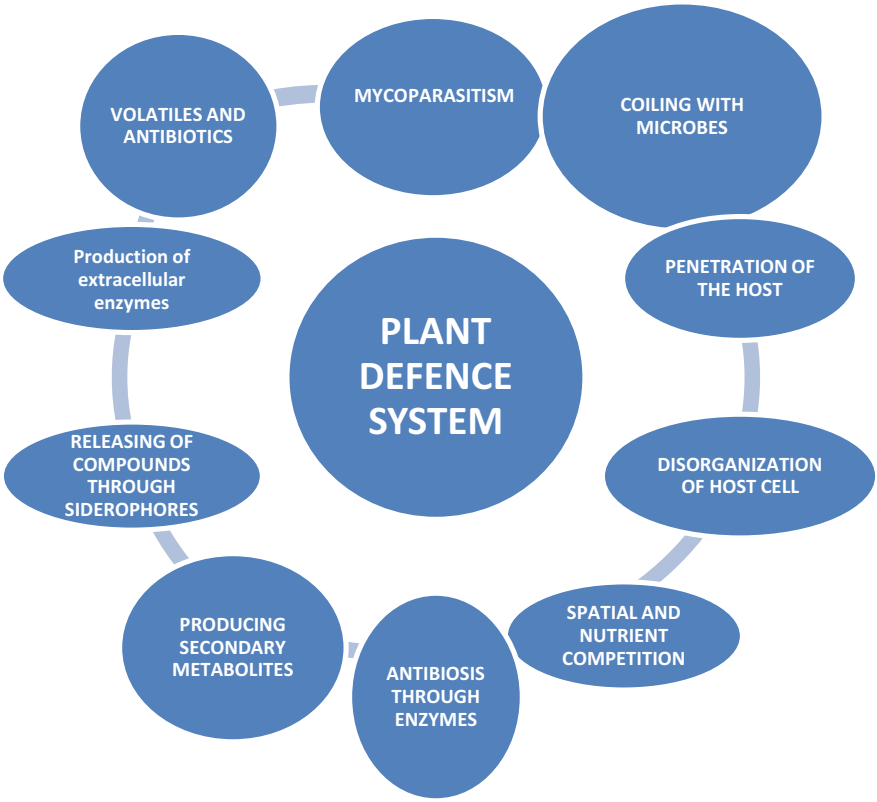

Fig. 7.6 Mechanism of action of bioagents for invoking plant defence

identified compounds were methanethiol, 3-undecene and 2-pentene 3-methyl. *Herbaspirillum* spp. isolate had 20.3% check of growth of Foc race 4 as its volatiles have all the three compounds, while *Pseudomonas* isolate AVA02 showed only 1.4% inhibition because it contained only 3-undecene and methanethiol. All three compounds, such as 2-pentane 3-methyl, in high dose have value for the antifungal activity against Foc. *Pseudomonas aeruginosa* strain FP10 proved to be the most potent in antibiosis and the plant growth-promoting activity (Ayyadurai et al. 2006).

Talc-based preparation of *P. fluorescens* when applied in soil @ 15 g/plant in banana significantly checked wilt disease (Saravanan and Muthusamy 2006). The power in *P. fluorescens* for suppressing *Fusarium* depends on its potential to produce antibiotic 2,4-diacetylphloroglucinol (DAPG). DAPG obtained from *P. fluorescens* when applied to soil significantly inhibited spore germination and growth of Foc.

The biocontrol agents cause, viz. coiling, penetration in the mycelia, mycoparasitism, disorganization in contents of host cell, spatial, competition for nutrients and antibiosis. This all is through production of secondary metabolites,

released compounds by siderophores, volatiles, extracellular enzymes and antibiotics and thus induces plant defence system (Fig. 7.6).

7.6 Strategies to Enhance the Biocontrol Potential

There has to be a systematic approach for use of bioagents for the control of banana *Fusarium* wilt. For the best results, the following points are considered: (1) types of bioagents and their various attributes and (2) the problems in initial colonization of antagonists and related variations after initial colonization.

The first step to find effective biocontrol agents is to check their potential. The foremost thing is the known type of antagonists and properties that result in their production, maintain efficacy and transportation. Cost of BCAs needs to be low with yield viable and very effective propagules in high concentration and long-term storage under dry condition (Jackson 1997). *Bacillus* species strains are ideal candidates for viable BCAs (Farhana et al. 2011; Govindasamy et al. 2011; Tan et al. 2013) and advantageous as they survive in adverse environments by producing endospores (Schallmey et al. 2004). A large number of strains of *Bacillus* spp. are widely used as BCAs for soil-borne pathogens (Gurr et al. 2005), including *Rhizoctonia* (Yu et al. 2002) and *Fusarium* (Sun et al. 2011) which have proved to be highly effective after long storage and transportation (Schallmey et al. 2004). Nonpathogenic *F. oxysporum* (Nel et al. 2006) and *Trichoderma* spp. (Thangavelu et al. 2004) have also been demonstrated but preferred less due to their difficulty in storage and transportation.

7.7 Hurdles in Colonization of the Antagonists

The second factor which needs to be taken into consideration is the efficacy or potential of the antagonistic microbes for initially colonizing the rhizosphere and production of substances inhibiting the pathogens. There are natural barriers which hamper colonization of antagonistic microbes indicating problems encountered upon soil application of BCAs. This includes predation and phagocytosis from soil protozoa (Ronn et al. 2002) and suppression of microbes of the niche (Bolwerk et al. 2003) or in plant roots (Chao et al. 1986), fighting with local microbes in different ecosystems available for nutrients. They drastically reduce the population of most antagonistic microbes in the first 2 to 3 days after application of BCAs (Christoffersen et al. 1995). The BCAs must maintain a certain level to result into acceptable levels of pathogen control (Wang et al. 2011). To promote promising efficacy of bioagent measures need to be taken to help BCA tide over such initial adverse phase, the recommendation therefore has to be by repeatedly applying BCAs.

7.8 Conclusions

The causal organism of banana wilt is soil-borne *Fusarium oxysporum* f. sp. *cubense* (Foc) and a strain of the fungus that affects Cavendish and other dessert bananas in the tropics. It is called Foc tropical race 4. This affects the roots of banana plants by colonizing the vascular system of the rhizome and pseudostem producing wilt. For its management a lot of biocontrol agents have been tried but it cannot be controlled completely. The bioagents are under evaluation both under lab and greenhouse conditions. The field experiments have been conducted only in a few cases and however need large-scale confirmation. Most effective biological control methods are (1) the Foc pathogen present in a particular area or country may be tried under both in vitro and in vivo conditions, (2) select biocontrol agents in having multiple actions and functions, (3) test the compatibility of bioagents and their tolerance to the chemicals, (4) suitable easy method for mass production and delivery system need to be standardized for producing maximum number of durable propagules, (5) bioagents need to be incorporated in the right quantity in the right place (in the soil near the rhizosphere) at the appropriate time and physiological state, (6) use of bioagents through other organic amendments and (7) incorporation of biocontrol agents with easily adaptable agronomic practices to effectively manage the disease.

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