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Current Advances in the Fusarium Wilt Disease Management in Banana with Emphasis on Biological Control

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1. Introduction

Banana (Musa spp.) is the fourth most important global food commodity after rice, wheat and maize in terms of gross value production. At present, it is grown in more than 120 countries throughout tropical and subtropical regions and it is the staple food for more than 400 million people (Molina and Valmayor, 1999). Among the production constraints, Fusarium wilt caused by the fungus *Fusarium oxysporum f.sp. cubense* (Foc) is the most devastating disease affecting commercial and subsistence of banana production throughout the banana producing areas of the world (Ploetz, 2005). The disease is ranked as one of the top 6 important plant diseases in the world (Ploetz & Pegg, 1997). In terms of crop destruction, it ranks with the few most devastating diseases such as wheat rust and potato blight (Carefoot and Sprott, 1969). The disease almost destroyed the banana export industry, built on the Gros Michel variety, in Central America during the 1950's (Stover, 1962). In addition, the widely grown clones in the ABB 'Bluggoe' and AAA 'Gros Michel and Cavendish' sub groups are also highly susceptible to this disease worldwide. Presently, Fusarium wilt has been reported in all banana growing regions of the world (Asia, Africa, Australia and the tropical Americas) except some islands in the South Pacific, the Mediterranean, Melanesia, and Somalia (Stover, 1962; Anonymous, 1977; Ploetz and Pegg, 2000).

The fungus *Foc* is the soilborne hyphomycete and is one of more than 100 formae speciales of *F. oxysporum* that causes vascular wilts of flowering plants (Domsch et al. 1980; Nelson et al. 1983). Although Fusarium wilt probably originated in Southeast Asia, (Ploetz and Pegg, 1997), the disease was first discovered at Eagle Farm, Brisbane, Queensland, Australia in 1876 in banana plants var. Sugar (Silk AAB) (Bancroft, 1876). The fungus infects the roots of banana plants, colonizing the vascular system of the rhizome and pseudostem, and inducing characteristic wilting symptoms mostly after 5-6 months of planting and the symptoms are expressed both externally and internally (Wardlaw, 1961; Stover, 1962). Generally, infected plants produce no bunches and if produced, the fruits are very small and only few fingers develop. Fruits ripen irregularly and the flesh is pithy and acidic. The fungus survives in soil for up to 30 years as chlamydospores in infested plant material or in the roots of alternative hosts (Ploetz, 2000).

Since the discovery of Fusarium wilt of banana, though various control strategies like soil fumigation (Herbert and Marx, 1990); fungicides (Lakshmanan et al., 1987); crop rotation
(Hwang, 1985; Su et al., 1986), flood-fallowing (Wardlaw, 1961; Stover, 1962) and organic amendments (Stover, 1962) have been evolved and attempted, yet, the disease could not be controlled effectively except by planting of resistant cultivars (Moore et al., 1999). Planting of resistant varieties also cannot be implemented because of consumer preference (Viljoen, 2002). Under these circumstances, use of antagonistic microbes which protect and promote plant growth by colonizing and multiplying in both rhizosphere and plant system could be a potential alternative approach for the management of Fusarium wilt of banana.

Besides, biological control of Fusarium wilt disease has become an increasingly popular disease management consideration because of its environmental friendly nature which offers a potential alternative to the use of resistant banana varieties and the discovery of novel mechanisms of plant protection associated with certain microorganisms (Weller et al., 2002; Fravel et al., 2003). Biological control of soil borne diseases caused especially by *Fusarium oxysporum* is well documented (Marois et al., 1981; Sivan and Chet, 1986; Larkin and Fravel, 1998; Thangavelu et al., 2004). Several reports have previously demonstrated the successful use of different species of *Trichoderma*, *Pseudomonas*, *Streptomyces*, non pathogenic *Fusarium* (npFo) of both rhizospheric and endophytic in nature against Fusarium wilt disease under both glass house and field conditions (Lemanceau & Alabouvette, 1991; Alabouvette et al.1993; Larkin & Fravel, 1998; Weller et al. 2002; Sivamani and Gnanamanickam, 1988; Thangavelu et al. 2001; Rajappan et al. 2002; Getha et al. 2005). The details on the effect of these biocontrol agents in controlling Fusarium wilt disease of banana are discussed in detail hereunder.

### 2. *Trichoderma* spp.

*Trichoderma* spp., are free-living fungi that are common in soil and root ecosystems. They are highly interactive in root, soil and foliar environments. They produce or release a variety of compounds that induce localized or systemic resistance responses in plants. This fungal biocontrol agent has long been recognized as biological agents, for the control of plant disease and for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients. It can be efficiently used as spores (especially, conidia), which are more tolerant to adverse environmental conditions during product formulation and field use, in contrast to their mycelial and chlamydospore forms as microbial propagules (Amsellem et al. 1999). However, the presence of a mycelial mass is also a key component for the production of antagonistic metabolites (Benhamou and Chet 1993; Yedidia et al. 2000). Several reports indicate that *Trichoderma* species can effectively suppress Fusarium wilt pathogens (Sivan and Chet, 1986; Thangavelu et al. 2004). Thangavelu (2002) reported that application of *T. harzianum* Th-10, as dried banana leaf formulation @ 10 g/plant containing 4X10³¹ cfu/g in basal + top dressing on 2, 4 and 6 months after planting in cv. Rasthali recorded the highest reduction of disease incidence (51.16%) followed by *Bacillus subtilis* or *Pseudomonas fluorescens* (41.17%) applications as talc based formulation under both glass house and field conditions. The talc based formulation of *T. harzianum* Th-10 and fungicide treatment recorded only 40.1% and 18.1% reduction of the disease respectively compared to control. In the Fusarium wilt-nematode interaction system also, soil application of biocontrol agents reduced significantly the wilt incidence and also the root lesion and root knot index. In addition to this, 50 to 82% of reduction in nematode population viz., *Pratylenchus coffeae* and *Meloidogyne incognita* was also noted due to application of bioagents and the maximum reduction was due to *T. harzianum* treatment (Thangavelu, 2002). Raghuchander et al. (1997)
reported that *T. viride* and *P. fluorescens* were equally effective in reducing the wilt incidence. Inoculation of potted abaca plants with *Trichoderma viride* and yeast showed 81.76% and 82.52% reduction of wilt disease severity respectively in the antagonist treated plants. (Bastasa and Baliad, 2005).

Similarly, soil application of *T. viride* NRCB1 as chaffy grain formulation significantly reduced the external (up to 78%) and internal symptoms (up to 80%) of Fusarium wilt disease in tissue cultured as well as sucker derived plants of banana cv. Rasthali (Silk-AAB) and increased the plant growth parameters significantly as compared to the talc powder formulation under pot culture and field conditions (Thangavelu and Mustaffa, 2010).

The possible mechanisms involved in the reduction of Fusarium wilt severity due to *Trichoderma* spp. treatment might be the mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites, and induction of plant defence system. The mycoparasitism involves in coiling, disorganization of host cell contents and penetration of the host (Papavisas, 1985; University of Sydney, 2003). During the mycoparasitism, *Trichoderma* spp. parasitizes the hyphae of the pathogen and produce extracellular enzymes such as proteolytic enzymes, β-1, 3-glucanolytic enzymes and chitinase etc., which cause lysis of the pathogen. The toxic metabolites such as extracellular enzymes, volatiles and antibiotics like gliotoxin and viridin which are highly fungistatic substances (Weindling, 1941) are considered as elements involved in antibiosis. In addition, *Trichoderma* spp. could compete and sequester ions of iron (the ions are essential for the plant pathogen,) by releasing compounds known as siderophores (Srinivasan et al. 1992). There are several reports demonstrating control of a wide range of plant pathogens including *Fusarium* spp. by *Trichoderma* spp. by elicitation of induced systemic or localized resistance which occur due to the interaction of bioactive molecules such as proteins avr-like proteins and cell wall fragments released by the action of extracellular enzymes during mycoparasitic reaction. Thangavelu and Mustaffa, (2010) reported that the application of *T. viride* NRCB1 as rice chaffy grain formulation and challenge inoculation with *Foc* in cv. Rasthali resulted in the induction of defense related enzymes such as Peroxidase and Penylalanine Ammonia lyase (PAL) and also the total phenolic content significantly higher (>50%) as compared to control and *Foc* alone inoculated banana plants and the induction was maximum at 4-6th day after treatment. They suggested that this increased activities of these lytic enzymes and thus increased content of phenols in the *T. viride* applied plants might have induced resistance against *Foc* by either making physical barrier stronger or chemically impervious to the hydrolytic enzymes produced by the pathogen (Thangavelu and Mustaffa, 2010). Morpurgo et al. (1994) reported that the activity of peroxidase was at least five times higher in the roots and corm tissues of *Foc* resistant banana variety than in the susceptible variety. Inoculation of resistant plants with *Foc* resulted in 10-fold increase in PO activity after seven days of inoculation, whereas the susceptible variety exhibited only a slight increase in PO activity.


*Pseudomonas* spp. are particularly suitable for application as agricultural biocontrol agents since they can use many exudates compounds as a nutrient source (Lugtenberg et al.1999a); abundantly present in natural soils, particularly on plant root systems, (Sands & Rovira, 1971); high growth rate, possess diverse mechanisms of actions towards phytopathogens
including the production of a wide range of antagonistic metabolites (Lugtenberg et al. 1991; Dowling & O’Gara, 1994; Dunlap et al. 1996; Lugtenberg et al., 1999b), easy to grow in vitro and subsequently can be reintroduced into the rhizosphere (Lugtenberg et al. 1994; Rhodes & Powell, 1994) and capable of inducing a systemic resistance to pathogens (van Loon et al. 1998; Pieterse et al. 2001).

Several studies have investigated the ability of *P. fluorescens* to suppress Fusarium wilt disease of banana. Fluorescent pseudomonad species such as *Pseudomonas fluorescens* (Sakthivel and Gnanamanickam 1987), *Pseudomonas putida* (de Freitas and Germida 1991), *Pseudomonas chlororaphis* (Chin-A-Woeng et al. 1998) and *Pseudomonas aeruginosa* (Anjaiah et al. 2003) have been used to suppress pathogens as well as to promote growth and yield in many crop plants. Sivamani and Gnanamanickam (1988) reported that the seedlings of *Musa balbisiana* treated with *P. fluorescens* showed less severe wilting and internal discoloration due to *Foc* infection in greenhouse experiments. The bacterized seedlings also showed better root growth and enhanced plant height.

Thangavelu et al. (2001) demonstrated that *P. fluorescens* strain pf10, which was isolated from the rhizosphere of banana roots, was able to detoxify the fusaric acid produced by *Foc* race-1 and reduced wilt incidence by 50%. Dipping of suckers in the suspension of *P. fluorescens* along with the application of 500 g of wheat bran and saw dust inoculation (1:3) of the respective bio-control agent effectively reduced Fusarium wilt incidence in banana (Raghuchander et al. 1997). Rajappan et al. (2002) reported that the talc based powder formulation of *P. fluorescens* strain pf1 was effective against *Foc* in the field. *Pseudomonas fluorescens* strain WCS 417, known for its ability to suppress other Fusarium wilt diseases, reduced the disease incidence by 87·4% in Cavendish bananas in glasshouse trials (Nel et al. 2006). Saravanan et al. (2003) demonstrated that either basal application of neem cake at 0.5 kg/plant + sucker dipping in spore suspension of *P. fluorescens* for 15 min + soil application of *P. fluorescens* at 10 g/plant at 3, 5 and 7 months after planting or the basal application of neem cake at 0.5 kg/plant + soil application of *P. fluorescens* at 10 g/plant at 3, 5 and 7 months after planting showed the greatest suppression of wilt disease in two field trials conducted in Tamil Nadu, India.

Fishal et al. (2010) assessed the ability of two endophytic bacteria originally isolated from healthy oil palm roots, *Pseudomonas* sp. (UPMP3) and *Burkholderia* sp. (UPMB3) to induce resistance in susceptible Berangan banana against *Fusarium oxysporum f. sp. cubense* race 4 (FocR4) under glasshouse conditions. The study showed that pre-inoculation of banana plants with *Pseudomonas* sp UPMP3 recorded 51% reduction of Fusarium wilt disease severity, whereas, the combined application of UPMP3+UPMB3 and single application of UPMB3 alone recorded only 39 and 38% reduction of Fusarium wilt disease severity respectively. Ting et al. (2011) reported that among six endobacteria isolates, only two isolates (*Herbaspirillum* spp and *Pseudomonas* spp.) produced volatile compounds which were capable of inhibiting the growth of *Foc* race 4. The compounds were identified as 2-pentane 3-methyl, methanethiol and 3-undecene. They found that the isolate *Herbaspirillum* spp. recorded 20.3% inhibition of growth of *Foc* race 4 as its volatile compounds contained all the three compounds whereas *Pseudomonas* isolate AVA02 recorded only 1.4% of growth inhibition of race 4 *Foc* as its volatile compounds contained only methanethiol and 3-undecene. They concluded that the presence of all these three compounds especially 2-pentane 3-methyl and also in high quantity is very important for the antifungal activity.
against *Foc*. Of the 56 fluorescent pseudomonad isolates obtained from banana rhizosphere, *Pseudomonas aeruginosa* strain FP10 displayed the most potent antibiosis towards the *Foc*. This strain was found to produce IAA, siderophores and phosphate-solubilizing enzyme which indicated that this strain is having potential of plant-growth-promoting ability. The presence of DAPG gene (ph1D) in the strain FP10 was confirmed by PCR and the production of DAPG was confirmed by TLC, HPLC and FT-IR analyses. The *in-vivo* bioassay carried out showed that the banana plants received with pathogen and the strain FP10 exhibited increased height (30.69cm) and reduced vascular discolouration (24.49%), whereas, the pathogen *Foc* alone-inoculated plants had an average height of 21.81 cm and 98.76% vascular discolouration (Ayyadurai et al. 2006).

Saravanan and Muthusamy (2006) reported that soil application of talc-based formulation of *P. fluorescens* at 15 g/plant in banana, suppressed Fusarium wilt disease significantly (30.20 VDI) as compared to pathogen *Foc* alone-inoculated plants (88.89 VDI). It was found that the ability of *P. fluorescens* to suppress Fusarium wilt pathogens depends on their ability to produce antibiotic metabolites particularly 2, 4- Diacetylphloroglucinol (DAPG). The metabolite DAPG extracted from the rhizosphere of *P. fluorescens* applied to soil showed significant inhibition of growth and spore germination of *Foc*. They also showed that the quantity of DAPG production was less in the extracts of soil, inoculated with *P. fluorescens* and challenge inoculated with *F. oxysporum* f. sp. *cubense* as compared to *P. fluorescens* alone inoculated soil. In plants pretreated with *P. fluorescens* and challenged with pathogen *Foc*, there was reduction in the number of *Foc* colonies (14 numbers) as compared to the plants treated with *Foc* alone (41 number). A 72% reduction in the pathogen infection was noticed as a result of *P. fluorescens* treatment. Colonies of *P. fluorescens* in plants challenged with *F. oxysporum* were reduced to 33 in number, perhaps due to competition for infection loci (Sukhada et al. 2004). Electron microscopic studies revealed that in the root samples of bacteria treated and pathogen challenge inoculated plants, there was extensive fungal proliferation in the cortex and had wall appositions made of electron-dense materials lining the host cortical cell wall. The wall appositions formed were highly significant in restricting the further growth of the fungus. They opined that electron-dense materials might have been produced either by the bacteria or the host tissue in response to the attacking pathogen. Massive depositions of unusual structures at sites of fungal entry was also noticed, which clearly indicated that bacterized root cells were signalled to mobilize a number of defence structures for preventing the spread of pathogen in the tissue (Sukhada et al. 2004). Pre-inoculated *P. fluorescens* helped the banana plant to resist pathogen attack to some extent due to the structural modification of the root system and due to the accumulation of newly formed electron-dense molecules, which may be providing the defense mechanism to the host plant. Treatment of ‘Maçã’ banana (*Musa* spp.; group ABB) with endophytic diazotrophic bacteria *Herbaspirillum* (BA234) and *Burkholderia* (AB202) also resulted in significant reduction of *Foc* unit propagules as well as increase in biomass of the plant in four and two months after plant inoculation with AB202 and BA234 respectively suggesting that these endophytic diazotrophic bacteria may be used as potential bio-fertilizer and bio-control agents for banana (Weber et al. 2007).

4. *Bacillus* spp.

*Bacillus subtilis* has been identified as a potential biological control agent. These strains could produce a wide range of antifungal compounds, such as subtilin, TasA, subtilosin, bacilysin,
mycobacillin and some enzymes, which can degrade fungal cell wall (Berg et al. 2001). It was suggested that these antibiotic production plays a major role in plant disease suppression (Knox et al. 2000; Leelasuphakul et al. 2006). In addition, some antagonistic mechanisms of these *Bacillus* species involves in the competition for nutrients and space, the induction of plant resistance, etc. (Guerra-Cantera et al., 2005; Van loon et al., 1998).

Sun et al. (2011) isolated an antagonistic *Bacillus* strain, KY-21 from the soil of banana’s rhizosphere and tested against *Foc* both under *in-vitro* and *in-vivo* conditions. Under lab condition, mycelium growth of the pathogen was seriously inhibited after treatment with the fermentation filtrate of KY-21. The microscopic examination of mycelium revealed that the tips of the hypha were deformed into spherical structures that were remarkably constricted by dual culture. Besides, the inoculation of banana plants with *Bacillus* strain, KY-21 also increased the activities of polyphenol oxidase (PPO) and peroxidase (POD) significantly compared to control. The *in-vivo* biocontrol assays showed that at 60 days after *Foc* inoculation, the plantlets treated with KY-21 exhibited 35% severe wilt symptom and 18.3% severe vascular discoloration as against 68.4% and 48.3% of severe wilt symptom and severe vascular discoloration respectively in control plantlets. Besides, plantlets inoculated with KY-21 showed significantly reduced development of disease as compared to the control.

5. **Actinomycetes**

Actinomycetes particularly *Streptomyces* spp. are important soil dwelling microorganisms, generally saprophytic, spend majority of their life cycle as spores and are best known for their ability to produce antibiotics. They may influence plant growth and protect plant roots against invasion by root pathogenic fungi (Crawford et al. 1993). Streptomyces species have been used extensively in the biological control of several formae speciales of *F. oxysporum*, which caused wilt disease in many plant species (Reddi and Rao 1971; Lahdenpera and Oy, 1987; Smith et al. 1990). *Streptomyces violaceusniger* strain G10 isolated from a coastal mangrove (*Rhizophora apiculata* (Blume)] stand, was shown to exhibit strong *in-vitro* antagonism toward several plant pathogenic fungi including *Foc* race 4. Under *in-vivo* bioassay, treating the planting hole and roots of tissue-culture-derived ‘Novaria’ banana plantlets with *Streptomyces* sp. strain g10 suspension (10⁸ cfu/ml), resulted in 47% reduction of leaf symptom index (LSI) and 53% of rhizome discoloration index (RDI) with reduced wilt severity when the plantlets were inoculated with 10⁴ spores/ml *Foc* race 4 compared to untreated plantlets. However, the reduction in disease severity was not significant when plantlets were inoculated with a higher concentration (10⁶ spores/ml) of *Foc* race 4 (Getha et al. 2005). Getha and Vikineswary (2002) studied the interaction between *Streptomyces violaceusniger* strain g10 and *F. oxysporum* f.sp. *cubense* and demonstrated the production of antifungal metabolites especially antibiotics by the antagonists which caused swelling, distortion, excessive branching and lysis of hyphae and inhibition of spore germination of *Foc* pathogen by the antagonist.

Among 242 actinomycete strains, isolated from the interior of leaves and roots of healthy and wilting banana plants, *Streptomyces griseorubiginosus*-like strains were the most frequently encountered strains. The screening of these strains for antagonistic activity against *Fusarium oxysporum* f. sp. *cubense* revealed that 50% of the *Streptomyces* strains isolated from healthy trees especially from the roots had antagonistic activities against *Foc* and only 27% of strains isolated from wilting trees showed the same activity (Cao et al.
Similarly in 2005, out of 131 endophytic actinomycete strains isolated from banana roots, the most frequently isolated and siderophore producing endophytic *Streptomyces* sp. strain S96 was found to be highly antagonistic to *Foc*. The subsequent *in vivo* biocontrol assays carried out showed that the disease severity index of Fusarium wilt was significantly reduced and mean fresh weight of plantlets increased compared to those grown in the absence of the biocontrol strain S96 (Cao et al. 2005).

6. General mode of action of antagonistic bacteria

Generally biocontrol agents can antagonize soil-borne pathogens through the following strategies: (1) Competition for niches and nutrients (niche exclusion), (2) Production of secondary metabolites which are used in direct antagonism (3) Growth promotion by changing the physiology of the plant and (4) Induction of resistance to disease.

Antagonistic bacteria are more effective against root pathogens only if they have a strong ability to colonize the root system (Weller, 1988) and also the fungal hyphae. This is widely believed to be essential for biocontrol (Weller et al. 1983; deWeger et al. 1987; Parke, 1990). The scanning and transmission electron microscopy study revealed that colonization on banana roots, on the hyphal surface and macrospores of *Foc* fungus race 4, by the endophyte *Burkholderia cepacia*. The study also showed that *B. cepacia* exists mainly in the intercellular space of the banana root tissues. Benhamou et al. (1996) provided evidence that root colonization by the endophytic bacterium *Pseudomonas fluorescens*, involved in a sequence of events that included bacterial attachment to the plant roots, proliferation along the elongation root, and local penetration of the epidermis. M’Piga et al. (1997) also confirmed the entry of *P. fluorescens* into the root system and their colonization inside. Once inside the host tissue, these bacteria produce an array of antifungal metabolites like siderophores and different antibiotics like phenazine-1 carboxylic acid, and 2, 4-diacetylphloroglucinol preventing the further advancement of the fungus (Beckman et al. 1982; Mueller & Beckmann,1988) by inducing severe cell disturbances in pathogenic fungi (Dowling & O’Gara, 1994). Sukhada et al. (2004) also located the colonies of *P. fluorescens* and *Foc* in banana using respective FITC-conjugated antibodies. They found that the bacterial population was relatively greater towards the cortex region of the root as compared to the stele region. In plants pretreated with *P. fluorescens* and challenged with *Foc*, there was reduction in the number of *Foc* colonies (14 numbers) as compared to the plants treated with *Foc* alone (41 number).

Competition for nutrients such as carbon, nitrogen or iron is one of the mechanisms through which biocontrol strains can reduce the ability of fungal pathogens to propagate in the soil (Alabouvette, 1986; Buyer & Leong, 1986; Leong, 1986; Loper & Buyer, 1991; Fernando et al., 1996; Handelsman & Stabb, 1996). Already established (pre-emptive competitive exclusion) or aggressively colonizing biocontrol bacteria can therefore prevent the establishment and subsequent deleterious effects of a pathogen. Most organisms, including fluorescent *Pseudomonas* species, take up ferric ions through high-affinity iron chelators, designated as siderophores that are released from bacterial cells under Fe3+ limiting conditions. The role of siderophores produced by pseudomonads has been well correlated with the biocontrol of disease suppressive soils and on the plant growth by supplying the plant with sequestered iron. Kloeper et al. (1980) reported that inhibition of the wilt pathogen was attributable to iron deprivation caused by pseudomonad siderophores compounds produced in low-iron conditions.
environments that function in iron transport. It is suggested that the management of Fe availability in the infection court, through Fe competition, can induce suppressiveness to a Fusarium wilt pathogen.

Dowling and O’Gara (1994) reported that bacterial endophytes like *P. fluorescens* produced an array of antifungal metabolites like siderophores and different antibiotics like phenazine-1-carboxylic acid, and 2, 4-diacylphloroglucinol that could induce severe cell disturbances in a number of pathogenic fungi. These compounds have direct effect on the growth of the pathogens. Biocontrol bacteria producing chitinase (Shapira et al., 1989; Dunne et al., 1996; Ross et al., 2000), protease (Dunlap et al., 1997; Dunne et al., 1998), cellulase (Chatterjee et al., 1995) or β-glucanases (Ruiz-Duenas & Martinez, 1996; Jijakli & Lepoivre, 1998) were shown to suppress plant diseases as these enzymes are involved in the breakdown of fungal cell walls by degrading cell wall constituents such as glucans and chitins, resulting in the destruction of pathogen structures or propargules. The bacteria also play a major role in growth promotion by producing phytohormones such as auxins, gibberellins, cytokinins and ethylene (García de Salamone et al., 2001; Remans et al., 2008). Besides promoting growth, they induce resistance in plants against pest and disease. There are two types of induced resistance exist called Systemic acquired resistance (SAR) and Induced systemic resistance (ISR). SAR is dependent on the salicylic acid pathway and is mainly associated with pathogen attack or in response to the exogenous application of chemicals such as salicylic acid and produces pathogenesis-related (PR) proteins such as β-1,3-glucanases, endo-chitinases and thaumatin-like proteins (Ward et al. 1991; Uknes et al. 1992; Rahimi et al. 1996; Van Pelt-Heerschap et al. 1999). Bacteria induced defenses in plants are expressed through structural and biochemical mechanisms. Structural mechanisms include the reinforcement of plant cell walls by deposition of newly formed molecules of callose, lignin and phenolic, occlusion of colonized vessels by gels, gums and tyloses (He et al. 2002; Jeun et al. 2004 Gordon and Martyn 1997; Olivain and Alabouvette, 1999). Whereas, the biochemical mechanism of resistance includes accumulation of secondary metabolites such as phytoalexins and production of PR proteins such as β-1,3-glucanases and chitinases. In the case of induced systemic resistance (ISR), the resistance induced only after the colonization of plant roots by bacteria. After colonization, they produce secondary metabolites and volatiles and defense related enzymes (Stougard, 2000; Han et al., 2006), which give resistance to plants. The level of defense related enzymes are known to play a crucial role in the degree of host resistance. Peroxidase (PO) and Polyphenol oxidase (PPO) are believed to be one of the most important factors of the plant’s biochemical defense against pathogens, and are actively involved in the self-regulation of plant metabolism after infection (Kavitha and Umesha, 2008; Dutta et al., 2008). Peroxidase is involved in substrate oxidation and cell wall lignifications; the PPO can oxidize phenolic compounds to quinines. Both of these defense mechanisms are associated with disease resistance. ISR elicited by PGPR has shown promise in managing a wide spectrum of plant pathogens in several plant species under greenhouse and field environments (Radjacommare et al., 2004; Thangavelu et al., 2004). Fishal et al. (2010) observed increased accumulation of resistance-related enzymes such as peroxidase (PO), phenylalanine ammonia lyase (PAL), lignithioglycolic acid (LTGA), and pathogenesis-related (PR) proteins (chitinase and β-1,3-glucanase) in banana plantlets treated with endophytic bacteria UPMP3 and UPMB3 singly or as mixture under glasshouse conditions.
7. Non-pathogenic Fusarium (npFo)

Several endophytic isolates of non-pathogenic *F. oxysporum* (npFo) derived from symptomless banana roots provided some degree of protection against *Foc* race-4 for the Cavendish cultivar Williams in the green house (Gerlach et al.1999). Similarly, pre-treatment of banana plants with endophytic bacterial strain UPM39B3 (*Serratia*) and fungal strain UPM31P1 (*Fusarium oxysporum*), isolated from the roots of wild bananas either singly or in combination resulted in significant increase in plant growth parameters in the *FocR4* inoculated plants than the diseased plantlets that were not infected with endophytes (Ting et al. 2009). It was also observed that the diseased plantlets benefited from the improved plant growth were able to survive longer than diseased plantlets without endophytes. Nel et al. (2006) evaluated several npFo and *Trichoderma* isolates obtained from suppressive soils in South Africa for the suppression of Fusarium wilt disease under glass house conditions. The results of the study indicated that two of the nonpathogenic *F. oxysporum* isolates, CAV 255 and CAV 241 recorded 87.4 and 75.0% reduction of Fusarium wilt incidence respectively. Forsyth et al. (2006) isolated three non-pathogenic *F. oxysporum* isolates from the roots of banana grown in Fusarium wilt suppressive soils and evaluated for their capability for suppressing Fusarium wilt of banana in glasshouse trials. The results showed that among the three npFo isolates examined, one isolate BRIP 29089, was associated with a significant reduction in internal disease symptom development, with 25% of plants showing mild vascular discoloration caused by *Foc* race 1 and race 4 in Lady Finger and Cavendish (cv. Williams) group of banana respectively. Interestingly, Cavendish plants treated with isolate BRIP 45952, and inoculated with *Foc*, displayed a significant increase in internal symptom development, with 50% of the plants showing severe vascular discoloration. Hence, it is important to understand that npFo can either reduce or increase the disease severity based on the nature of the strains used and hence one should be cautious while selecting strain for disease control (Forsyth et al. 2006). Ting et al. (2008) demonstrated the potential of endophytic microorganisms in promoting the growth parameters (plant height, pseudostem diameter, root mass and total number of leaves) of their host plant by artificially introducing five isolates of bacterial and fungal strains isolated from the roots of wild bananas into both healthy and diseased banana plantlets (Berangan cv. Intan). The results indicated that among the five isolates tested the bacterial isolate UPM39B3 (*Serratia*) and fungal isolate UPM31P1 (*Fusarium oxysporum*) showed tolerance towards Fusarium wilt via improving vegetative growth of the plant. This "tolerance" to disease may also be attributed to direct inhibition of the pathogen through the production of antifungal compounds (White and Cole 1985; Koshino et al. 1989). Thangavelu and Jayanthi (2009) selected two npFo isolates (Ro-3 and Ra-1) out of 33 obtained from banana rhizosphere soil based on mycelial growth and spore germination under *in-vitro* condition. These two npFo isolates were evaluated under both pot culture and field conditions by application: (i) at planting; (ii) at planting + 2 months after planting; and (iii) at planting + 2 months after planting + 4 months after planting; in tissue-cultured as well as in sucker derived plants of cv. Ra, Rashali (Silk-AAB). The result showed that soil application of Ro3 npFo isolate three times in both tissue-cultured and sucker derived plants of banana registered 89% reduction of Fusarium wilt severity and significant increase in plant growth parameters when compared with *Foc* alone inoculated banana plants.
The modes of actions of non-pathogenic Fusarium isolates suggested commonly are: competition for nutrients (Couteaudier and Alabouvette, 1990), competition for infection sites at the root surface or inside the roots (Fravel et al. 2003) production of secondary metabolites, which cause antibiosis and antixenosis and induced resistance (Clay, 1991; Dubois et al. 2006). Some endophytes with growth promoting properties are also useful in enhancing tolerance to diseases by growth promotion (Ting et al. 2009).

Although the non-pathogenic Fusarium isolates are useful in controlling the Fusarium wilt disease, the main concern are: i) whether the biocontrol agent is truly nonpathogenic, ii) whether it may be pathogenic on a species of plant on which it has not yet been tested and iii) whether the biocontrol agent could become pathogenic in the future.

8. Biocontrol agents for tissue cultured plants

In the case of micro-propagated banana plants, its usage as planting material leads to a reduction in the spread of *Foc*, but at the same time, resulted in enhanced susceptibility to *Foc* under field conditions (Smith et al. 1998) due to the loss of native endophytes during tissue culture, including beneficial plant growth promoting rhizobacteria and fungi (Nowak, 1998; Smith et al. 1998). Therefore, biotization of tissue culture plantlets with native effective non-pathogenic endophytic microbes including mycorrhizal fungi during first or second stage hardening but before planting, enhance plant resistance to tissue cultured plants against Fusarium wilt (Nowak, 1998). Lian et al. (2009) reported that re-introduction of naturally occurring endophytes to tissue culture banana plantlets resulted in a substantial reduction in the infection and severity of Fusarium wilt disease (67%) as well as increased plant growth parameters (height, girth, leaf area). Arbuscular mycorrhiza (AM) fungi are the most beneficial symbiotic fungi, increases nutrient uptake ability of the plant roots, by enhancing the water transport in the plant thus increasing the growth and yield. Besides, these fungi have also been shown to provide physical barrier against invading pathogens and thus reduce disease severity in short-term greenhouse studies. The application of *Glomus* spp to micropropagated banana plantlets (Grand Naine) reduced the internal and external symptoms of *Foc* race 4 and enhanced plant development and nutrient uptake of the plants (Jaizme-vega et al. 1998). Jie et al. (2009) re-introduced mixture of naturally-occurring uncultivated endophytes (dominated by γ-Proteobacteria) isolated from native healthy banana plant into tissue culture banana plantlets led to 67% suppression rate of wilt disease at the fifth month after pathogen infection on plantlets in the greenhouse. In addition to disease suppression, growth of host plantlets was also promoted with the inoculation of these endophytes both in pathogen-infected and healthy control plants. They proposed that the suppression of wilt disease was due to increased activities of PPO, POD and SOD enzymes in the plantlets inoculated with endophytic communities.

9. Suppressive soil for the biological control of Fusarium wilt

Suppressive soils are sites where, despite the presence of a virulent pathogen and susceptible host, disease either does not develop, or the severity and spread of disease through the site is restricted (Alabouvette et al. 1993). This type of suppressive soils for
Fusarium wilt has been reported in many regions of the world. Although the suppression has generally been shown to be due to soil physical structure (type of soil, drainage condition, presence of montmorillonoid soils and pH) nutritional status and microbial composition (Fungi, bacteria and Actinomycetes) and biological factors also said to play a major role (Scher and Baker, 1982; Alabouvette et al. 1993). Biological control of Fusarium wilts of numerous crops by application of antagonistic fungi and bacteria isolated from suppressive soils has been accomplished during the last two decades all over the world (Leeman et al., 1996; Lemanceau et al., 1992; Park et al., 1988; Raaijmakers et al., 1995). Most of the studies have found that non-pathogenic strains of \textit{F. oxysporum} are associated with the natural suppressiveness of soil to Fusarium wilt diseases (Smith and Snyder, 1971; Alabouvette, 1990; Postma & Rattink, 1992). These npFo colonize the plant rhizosphere and roots without inducing any symptoms in the plants (Olivain and Alabouvette, 1997). Nel et al. (2006) evaluated the ability of non-pathogenic \textit{F. oxysporum} and \textit{Trichoderma} isolates from suppressive soils in South Africa to suppress Fusarium wilt of banana in the glasshouse. The results revealed that only npFo isolates CAV 255 and CAV 241, reduced Fusarium wilt incidence by 87.4 and 75.0\%, respectively. Smith et al. (1999) proposed that application of biocontrol agents isolated from banana roots grown in Fusarium wilt suppressive soil of tissue culture plantlets in the nursery. By application of these biocontrol agents, the banana roots had a better chance of protection against \textit{Foc}. Generally, the microbial activity in suppressive soil is influenced by type of clay minerals present in the soil. In tropical America, a close relationship was found between suppression of Fusarium wilt and presence of clay (montmorillonoid type) soils, where as in the Canary Islands, suppression was associated with host mineral nutrition (Ploetz, 2000).

10. Integrated approach of Fusarium wilt management

In general, most of the available approaches for biocontrol of plant diseases are involved in the use of a single biocontrol agent to a single pathogen (Raupach and Kloepper, 1998). This has led to inconsistent performance of biocontrol agents and poor activity in all soil environments in which they are applied or against all pathogens that attack the host plant. To overcome these problems, applications of mixtures of biocontrol agents having multiple mode of actions are advocated particularly under field conditions, where they are highly influenced by abiotic and biotic conditions (Duffy et al., 1996; Raupach and Kloepper, 1998; Guetsky et al., 2001). Integration of biocontrol with agronomic practices may also improve the efficacy of the biocontrol organisms and the health of the host plants, which may be sensitive to environmental changes. Under this situation, compatible interactions are an important pre-requisite for the successful development of an integrated approach for the control of plant diseases. In the case of banana, integration of multiple control methods was more effective than single method for controlling Fusarium wilt disease in banana. Saravanan et al. (2003) carried out both \textit{in-vitro} and \textit{in-vivo} studies with biocontrol agents along with organic manures to develop integrated disease management practices to control Fusarium wilt disease. They found that basal application of neem cake at 0.5 kg/plant + sucker dipping in spore suspension of \textit{Pseudomonas fluorescens} for 15 min+soil application of \textit{P. fluorescens} at 10 g/plant at 3,5 and 7 months after planting showed the greatest suppression of wilt disease and this was on par with basal application of neem cake at 0.5
kg/plant + soil application of *P. fluorescens* at 10 g/plant at 3, 5 and 7 months after planting. They also reported that *Trichoderma viride* applied as soil or sucker dipping or their combinations or along with the neem cake also had a significant reduction in disease index, but less than that of *P. fluorescens*. Raghuchander et al. (1997) reported that dipping of suckers in the suspension of *T. viride* along with application of 500 g of wheat bran and saw dust inoculation (1: 3) of the respective bio control agent effectively reduced Fusarium wilt incidence in banana. Kidane and Laing (2010) developed integrated method of controlling Fusarium wilt by integrating biological and agronomic control methods. Single and combined applications of non-pathogenic, endophytic *Fusarium oxysporum* N16 strain by dipping their roots in a spore suspension containing 10⁷ cfu ml⁻¹, *Trichoderma harzianum* Eco-T® (Plant Health Products (Pty) Ltd. KwaZulu-Natal, South Africa) @ 4L pt at a concentration of 10⁵ conidia ml⁻¹ at the time of planting, monthly application of plants with 4 L of silicon solution per plant containing 900mg silicon L⁻¹ and placing coarse macadamia husks at the bottom of banana plants as mulching were tested against *F. oxysporum* f. sp. *cubense* on bananas under greenhouse and field conditions. The results showed that treatments involving combinations of nonpathogenic *F. oxysporum*, *T. harzianum* Eco-T®, silicon and mulch had significantly higher number of leaves, stem height and girth size than single applications of the treatments. They found that the mulching increased the growth of feeder roots and created a conducive microenvironment, thereby increased the microbial activity in the soil. The combined application of non-pathogenic Fusarium strain along with silicon also resulted in reduction of corm disease index by more than 50% and shoot yellowing and wilting by 80%. Therefore, integration of biocontrol with agronomic practices improved the efficacy of the biocontrol organisms and the health of the host plants. Recently Zhang et al. (2011) evaluated the effects of novel bio-fertilizers, which combined an amino acid fertilizer and mature pig manure compost with the antagonists *Paenibacillus polymyxa* SQR21, *Trichoderma harzianum* T37 and *Bacillus subtilis* N11 (isolated from the healthy banana roots) in a severely Fusarium wilt diseased field for the suppression of Fusarium wilt of banana as pot experiments. The results showed that the bio-organic fertilizers which contained the bio-agents significantly suppressed the incidence of wilt disease (by 64–82%), compared to the control. The best biocontrol effect was obtained in the treatment with the BIO2 that contains *Bacillus subtilis* N11. The reason for more effect might be due to the application of the antagonists in combination with suitable organic amendments.

Botanical fungicides are also gaining momentum as these are considered as an alternative source for chemicals in the management of soil borne pathogens. The active principles present in both bio-agents and botanicals may either act on the pathogen directly or induce systemic resistance in the host plants resulting in reduction of disease development (Paul and Sharma, 2002). Akila et al. (2011) tested two botanical fungicides from *Datura metel-Wanis 20 EC* and *Damet 50 EC* along with *Pseudomonas fluorescens*, Pf1 and *Bacillus subtilis*, TRC 54 individually and in combination for the management of Fusarium wilt under greenhouse and field conditions. Combined application of botanical formulation and biocontrol agents (Wanis 20 EC + Pf1 + TRC 54) reduced the wilt incidence significantly under greenhouse (64%) and field conditions (75%). The reduction in disease incidence was positively correlated with the induction of defense-related enzymes peroxidase and polyphenol oxidase.
<table>
<thead>
<tr>
<th>Sl. no</th>
<th>Name of biocontrol agents</th>
<th>Mode of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td><em>Pseudomonas</em> spp.</td>
<td>Production of volatiles (2-Pentane 3-methyl, methanethiol and 3-undecene, antibiotics DAPG and Siderophore production.)</td>
<td>Ting et al. 2011</td>
</tr>
<tr>
<td>3.</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Production of antibiotics (2,4-Diacetyl Phloroglucinol</td>
<td>Saravanan and Muthusamy, 2006</td>
</tr>
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<td>4.</td>
<td><em>P. fluorescens</em></td>
<td>Competition for space, cell wall appositions lining the cortical cell wall</td>
<td>Sukhada et al. 2004</td>
</tr>
<tr>
<td>5.</td>
<td><em>Bacillus</em> spp.</td>
<td>Antibiotics, induction of defense related enzymes such as Peroxidase and Polyphenol oxidase.</td>
<td>Sukhada et al. 2004</td>
</tr>
<tr>
<td>6.</td>
<td><em>Streptomyces violaceusniger</em></td>
<td>Production of Antibiotics</td>
<td>Getha et al. 2005</td>
</tr>
<tr>
<td>7.</td>
<td><em>Streptomyces violaceusniger</em></td>
<td>Production of Antibiotics</td>
<td>Getha and Vikineswary, 2002</td>
</tr>
<tr>
<td>10.</td>
<td><em>F. oxysporum</em></td>
<td>Plant growth promotion</td>
<td>Ting et al. 2008</td>
</tr>
<tr>
<td>11.</td>
<td>γ-Proteobacteria</td>
<td>Increase in Polyphenol oxidase, Peroxidase, Superoxide dismutase,</td>
<td>Jie et al. 2009</td>
</tr>
<tr>
<td>12.</td>
<td><em>P. fluorescens</em></td>
<td>Induction of defense related enzymes such as Peroxidase &amp;Polyphenol oxidase</td>
<td>Akila et al. 2011</td>
</tr>
<tr>
<td>13.</td>
<td><em>Bacillus subtilis</em></td>
<td>Induction of defense related enzymes such as Peroxidase &amp;Polyphenol oxidase</td>
<td>Akila et al. 2011</td>
</tr>
</tbody>
</table>

Table 1. Summary of Bio-control agents used in the management of Fusarium wilt disease of banana with their mode of action.

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Fig. 1. A) External symptoms (yellowing and buckling of leaves) of Fusarium wilt infected banana plant. B) Brown vascular discoloration in the Pseudostem C) Brown vascular discoloration in the corm of Fusarium wilt infected plant D) Microscopic view of both macro and micro conidia of Foc.

11. Conclusion

Although several biocontrol agents including botanicals have been tried against Fusarium wilt disease, still this lethal disease could not be controlled completely. Besides most of the biocontrol experiments were conducted either under lab condition or green house conditions and only in few cases, field experiments were conducted. Therefore, most of the bioagents tested against Fusarium wilt of banana have not yet registered and reached the end users i.e. banana growers. This is mainly because of lack of confidence on the efficacy and consistency of the bioagents in controlling the disease. Therefore, for evolving consistent and effective biological control methods for the management of Fusarium wilt disease are i) the Foc pathogen present in a particular area or country must be characterized thoroughly up to VCG level and the bio-agents isolated must be screened under both in vitro
and in vivo conditions ii) the bio-agents having multiple mode of actions and functions should be selected rather than selecting bioagents with one or two mode of actions. In addition, mixture of bioagents of different genera or mixture of fungal and bacterial bioagents along with or without fungicides or botanicals have to be tried to improve the level and extent of disease control under different environmental and soil conditions iii) the compatibility between bioagents or tolerance of bioagents to chemicals or botanicals must be tested, iv) suitable method of mass production and delivery system which support more number of propagules and long shelf life, easy to prepare and adopt must be selected, v) mass produced bioagents should be applied at right quantity (the initial inoculum level of bioagents should be more than the inoculum level of the pathogen) at the right place (at the soil around the rhizosphere) at the right time (before planting or at the time of planting and also at 2nd and 4th month after planting as booster application) and at the appropriate physiological state, vi) mass production and delivery system should be compatible with the production system of banana, vii) application of bioagents with other organic amendments which can support the survival and multiplication of bio-agents and vii) integration of biological control with other cultural or agronomic practices so that the Fusarium wilt disease can be controlled effectively.

12. References


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Plant pathology is an applied science that deals with the nature, causes and control of plant diseases in agriculture and forestry. The vital role of plant pathology in attaining food security and food safety for the world cannot be overemphasized.

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