Plant Archives Vol. 16 No. 1, 2016 pp. 51-59 *ISSN 0972-5210*

BIOLOGICAL CONTROL OF FUSARIUM WILT DISEASE IN BANANA WITH EMPHASIS ON *TRICHODERMA* SPP. AND *PSEUDOMONAS* SPP.

Y. Pushpavathi*, S. N. Dash, N. Madhavi and D. Deepika

Orissa University of Agriculture and Technology, Bhubaneswar - 751 003 (Orissa), India.

Abstract

Banana is the most widely consumed, exported fruit in the world and it is the staple food for for millions of people in the developing countries of tropics. 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. Since, the discovery of Fusarium wilt of banana, though various control strategies like soil fumigation, crop rotation, flood –fallowing and organic amendments, have been evolved and attempted, yet, the disease could not be controlled effectively except by planting of resistant cultivars. Planting of resistant varieties also cannot be implemented because of consumer preference. 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. *Trichoderma* spp., 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. *Pseudomonas* spp., possess diverse mechanisms of actions towards phytopathogens including the production of a wide range of antagonistic metabolites and capable of inducing a systemic resistance to pathogens.

Key words : Fusarium wilt, biological control, *Trichoderma* spp., *Pseudomonas* spp.

Introduction

Banana and plantains, the major staple food crop for more than 400 million people in the developing countries of tropics (Molina and Valmayor, 1999). It is grown in more than 130 countries across the world. In India, there has been a significant increase in terms of area, production and productivity in the last two decades. India is the largest producer of banana in the world, producing 29.22 million tonnes from an area of 0.821 million hectares with a productivity of 34.2 $MTha^{-1}$ (NHB, 2015). Although, India accounts for only 11.1 per cent area, it has 32.6 per cent of world production. Thus, banana has emerged as one of the important fruits, which is in the easy reach of common man. It is envisaged that the demand is ever increasing and 50 million tonnes of banana will be needed to meet the domestic demand in 2050.

Banana cultivation continued to face several pests and diseases problems, which have affected the production and productivity. 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 and 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).

***Author for correspondence :** E-mail: anjali5rap@gmail.com

Prevalance of fusarium wilt in banana

Panama disease or Fusariumwilt is a lethal disorder of banana (Ploetz, 1994) and is considered as one of the most destructive diseases of banana industry. The disease was first identified by a medical practitioner Dr. Joseph Bancroft at Eagle Farm, near Brisbane, Australia in the variety Sugar (AAB-Silk) in 1874 (Bancroft, 1876). Subsequently, the disease was noticed in export plantations as early as 1890 in Costa Rica and Panama and then in Tropical America and Africa (Stover, 1962 a). Now, the disease has been reported from all the banana growing regions of the world except Papua New Guinea, the South Pacific Islands and some of the countries bordering Mediterranean Sea (Moore *et al*., 1995). In Asia, the first report of occurrence of wilt was reported in Bengal by Basu in 1911 (Basu, 1911). He reported the disease in the variety Kathali. However, at that time, the most profitable variety 'Martaman' was practically exterminated (Stover, 1962b). Then the disease was found by Reinking (1934) between 1925 and 1927 in Gros Michel and Silk (Rasthali) in Malaysia, Myanmar, Sri Lanka, Indonesia and Thailand.

Symptoms of *Fusarium* wilt

The disease symptoms usually become more evident at the time of flowering. 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 and 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.

Purplish brown discolouration of the vascular bundles, which can be seen in cross section of the corm and pseudostem is the typical internal symptom. In the corm, the discolouration appears as collection of tiny dots. The severity of the disease depends on host susceptibility, fungal virulence and environmental conditions such as rainfall and temperature. In highly susceptible cultivars, under the conditions of water stress or water logging, the entire foliage may become yellow, growth is ceased and there will not be emergence of bunch and finally the whole plant may collapse. The disease also spreads to suckers and the internal symptoms can be seen even after one or two months of emergence of suckers (Moore *et al*., 1995).

Panama disease diversity

The causal organism of the disease is *Fusarium oxysporum* f. sp. *cubense*. The pathogen was first isolated by Smith (1910) and the cause of the disease was proved by Brandes (1919). It produces abundant oval-ellipsoid microconidia from simple shot-lateral phialidic conidiophores and later, cluster of typical fusoid three to five macroconidia. In culture, the fungus produces a reddish pigment and as it becomes old, globosechlamydospores are produced. These characteristics readily separate it from other similar species such as *Fusarium solani* and *Fusarium moniliforme*, which also produce abundant microspores (Booth, 1971).

So far, four races of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) have been reported (Moore *et al*., 1995) based on their pathogenicity to different banana cultivars.

Race –1: It occurs throughout the world. It attacks cultivars like Silk (AAB) and Pome (AAB) groups.

Race –2: It also widely distributed in the entire banana growing regions. Pathogenic to Bluggoe, Monthan and other closely related cooking bananas.

Race –3: It occurs in Honduras, Costa Rica and Australia. Pathogenic to *Heliconia* spp.

Race –4: It occurs in most of the banana growing regions like Canary Islands, Taiwan, Australia, South Africa, Malaysia, Brazil etc. Not reported in India. It attacks Cavendish group of banana (AAA) and also race 1 and race 2 susceptible varieties.

Survival of *Fusarium* wilt

The pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is a facultative parasite capable of saprophytic growth and it would be classified as a root inhabiting fungus. Soil populations are unevenly distributed and decline rapidly in the absence 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 infested plant debris or in the roots of alternative hosts. It also survives in the roots of several species of common grasses and weed species such as *Paspalum, Panicum, Ixophorus* and *Commelina* which are the non-symptomatic hosts of the pathogen (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 populations tend to be higher and survive longer in light texture soils than in heavy alkaline soils. Certain crop

residues may stimulate antagonistic microflora and reduce survival of the pathogen (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 soil, which contains more microbial population, the pathogen development is suppressed and this type of soil has been reported in Central America, Canary Islands, Australia and South Africa (Moore *et al*., 1995).

Biological control

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 different species of *Trichoderma*, *Pseudomonas*, *Streptomyces*, non pathogenic *Fusarium* (np*Fo*) 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). Pushpavathi *et al*. (2015) reported that, Sucker treatment before planting with biocontrol agents *Trichoderma viride* and *Pseudomonas fluorescens* and soil drenching with same biocontrol agents twice at 30 and 180 DAP as booster application, effectively reduced the fusarium disease incidence and intensity thereby increasing the yield.

The details on the effect of these biocontrol agents *Trichoderma* spp. and *Pseudomonas* spp. in controlling Fusarium wilt disease of banana are discussed in detail hereunder.

***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, ϒ-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 Musataffa (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 Phenylalanine 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.**

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 and 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 and Powell, 1994) and capable of inducing a systemic resistance to pathogens (van Loon *et al*., 1998 and 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 green house 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 DPAG 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

Table 1 : List of bio-control agents used in the management of Fusarium wilt disease of banana with their mode of action.

S. no.	Name of biocontrol agents	Mode of action	References
1.	*Trichoderma viride*	Induction of defense related enzymes, production of antibiotics	Thangavelu and Mustaffa (2010)
2.	*Pseudomonas* spp.	Production of volatiles (2-Pentane 3-methyl, methanethil and 3-undecene, antibiotics DAPG and Siderophore production.	Ting *et al.* (2011)
3.	*Pseudomonas aeruginosa*	Production of antibiotics (2,4-Diacetyl Phloroglucinol	Saravanan and Muthusamy (2006)
4.	*P. fluorescens*	Competition for space, cell wall appositions lining the cortical cell wall	Sukhada *et al.* (2004)
5.	*Bacillus* spp.	Antibiotics, induction of defense related enzymes such as Peroxidase and Polyphenol oxidase.	Sukhada *et al.* (2004)
6.	*Streptomyces violaceusniger*	Production of Antibiotics	Getha *et al.* (2005)
7.	*Streptomyces violaceusniger*	Production of Antibiotics	Getha and Vikineswary (2002)
8.	Non-pathogenic Fusarium plant growth promotion	Plant growth promotion	Ting *et al.* (2009)
9.	*Serratia* sp	Plant growth promotion	Ting *et al.* (2008)
10.	*F. oxysporum*	Plant growth promotion	Ting *et al.* (2008)
11.	γ-Proteobacteria	Increase in Polyphenol oxidase, Peroxidase, Superoxide dismutase	Jie *et al.* (2009)
12.	*P. fluorescens*	Induction of defense related enzymes such as Peroxidase & Polyphenol oxidase	Akila *et al.* (2011)
13.	*Bacillus subtilis*	Induction of defense related enzymes such as Peroxidase & Polyphenol oxidase	Akila *et al.* (2011)
14.	*Trichoderma viride*	Production of antibiotics	Pushpavathi *et al.* (2015)
15.	*P. fluorescens*	Production of metabolites and antibiotics	Pushpavathi *et al.* (2015)

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).

Conclusion

Although, several biocontrol agents have been tried against Fusarium wilt disease, still this lethal disease could not be controlled completely. Most of the bioagents tested against Fusarium wilt of banana have not yet registered and reached the end users ie. banana growers, Because most of the biocontrol experiments were conducted either under lab condition or green house conditions and only in few cases, field experiments were conducted. 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 viii) integration of biological control with other cultural or agronomic practices so that the Fusarium wilt disease can be controlled effectively.

References

Alabouvette, C., P. Lemanceau and C. Steinberg (1993). Recent advances in the biological control of *Fusarium* wilts. *Pesticides Science*, **37** : 365–373.

Akila, R., L. Rajendran, S. Harish, K. Saveetha, T. Raguchander and R. Samiyappan (2011). Combined application of botanical formulations and biocontrol agents for the management of *Fusarium oxysporum* f. sp. *cubense* (Foc) causing Fusarium wilt in banana. *Biological Control*, **57** : 175–183.

Amsellem, Z., N. K. Zidack, P. C. Quimby Jr. and J. Gressel (1999). Long-term dry preservation of viable mycelia of two mycoherbicidal organisms. *Crop Protection*, **18** : 643–649.

Anjaiah, V., P. Cornelis and N. Koedam (2003). Effect of genotype and root colonization in biological control of *Fusarium* wilts in pigeonpea and chickpea by *Pseudomonas aeruginosa* PNA1. *Canadian Journal of Microbiology*, **49** : 85–91.

Ayyadurai, N., P. Ravindra Naik, M. Sreehari Rao, R. Sunish Kumar, S. K. Samrat, M. Manohar and N. Sakthivel (2006). Isolation and characterization of a novel banana rhizosphere bacterium as fungal antagonist and microbial adjuvant in micropropagation of banana. *Journal of Applied Microbiology*, **100** : 926–937.

Bancroft, J. (1876). Report of the board appointed to enquire into the cause of disease affecting livestock and plants. In: *Votes and Proceedings Queensland*, pp: 1011-1038.

Basu, S. K. (1911). Report on the banana disease of Chinsurah. *Quart. J. Dep. Agric. Bengal*, **4** : 196-198.

Booth, C. (1971). The Genus *Fusarium*. Commonwealth Mycol. Inst. Kew, UK, pp: 237.

Bastasa, G. N. and A. A. Baliad (2005). Biological control of *Fusarium* wilt of abaca (*Fusarium oxysporum*) with *Trichoderma* and yeast. *Philippine Journal of Crop Science* (PJCS), **30(2)** : 29-37.

Benhamou, N. and I. Chet (1993). Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani* : Ultrastructure and gold cytochemistry of the mycoparasitic process. *Phytopathology*, **83** : 1062-1071.

Carefoot, G. L. and E. R. Sprott (1969). '*Famine on the Wind*.' (Angus and Robertson: London).

Chin-A-Woeng, T. F. C., G. V. Bloemberg, A. J. Vander Bij, K. M. G. M. Vander Drift, J. Schripsema, B. Kroon, R. J. Scheffer and C. Keel (1998). Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis lycopersici*. *Mol Plant Microbe Interact.*, **11** : 1069–1077.

de Freitas, J. R. and J. J. Germida (1991). *Pseudomonas cepacia* and *Pseudomonas putida* as winter wheat inoculants for biocontrol of *Rhizoctonia solani*. *Canadian Journal of Microbiology*, **37** : 780–784.

Dowling, D. N. and F. O'Gara (1994). Metabolites of Pseudomonas involved in the biocontrol of plant disease. *Trends in Biotechnology*, **3** : 121–141.

Dunlap, C., J. J. Crowley, Y. Moënne-Loccoz, D. N. Dowling, F. J. de Bruijn and F. O'Gara (1997). Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. *Microbiology*, **143** : 3921–3931.

Fishal, E. M. M., S. Meon and W. M. Yun (2010). Induction of Tolerance to Fusarium wilt and Defense-Related Mechanisms in the Plantlets of Susceptible Berangan Banana Pre- Inoculated with *Pseudomonas* sp. (UPMP3) and *Burkholderia* sp. (UPMB3). *Agricultural Sciences in China*, **9** : 1140-1149.

Fravel, D., C. Olivain and C. Alabouvette (2003). *Fusarium oxysporum* and its biocontrol. *New Phytologist*., **157** : 493–502.

Getha, K., S. Vikineswary, W. Wong, T. Seki, A. Ward and M. Goodfellow (2005). Evaluation of *Streptomyces* sp. strain G10 for suppression of Fusarium wilt and rhizosphere colonization in pot grown banana plantlets. *Journal of Industrial Microbiology and Biotechnology*, **32** : 24-32.

Gowen, S. (1995). *Bananas and plantains*. Chapman and Hall, London, pp: 612.

Getha, K. and S. Vikineswary (2002). Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f.sp. *cubense* race 4 : Indirect evidence for the role of antibiosis in the antagonistic process. *Journal of Industrial Microbiology & Biotechnology*, **28** : 303 – 310.

Hwang, S. C. (1985). Ecology and control of *Fusarium* wilt of banana. *Plant Protection Bulletin* (Taiwan), **27** : 233-245.

Jie, L., W. Zifeng, C. Lixiang, T. Hongming, I. Patrik, J. Zide and Z. Shining (2009). Artificial inoculation of banana tissue culture plantlets with indigenous endophytes originally derived from native banana plants. *Biological Control*, **51** : 427-434.

Lugtenberg, B. J. J., L. A. de Weger and J. W. Bennett (1991). Microbial stimulation of plant growth and protection from disease. *Current Opinions in Biotechnology*, **2** : 457–464.

Lugtenberg, B. J. J., L. A. de Weger and B. Schippers (1994). Bacterization to protect seed and rhizosphere against disease. *BCPC Monograph*, **57** : 293–302.

Lugtenberg, B. J. J., L. C. Dekkers, M. Bansraj, G. V. Bloemberg, M. Camacho, T. F. C. Chin-AWoeng, C. van den Hondel, L. Kravchenko, I. Kuiper, A. L. Lagopodi, I. Mulders, C. Phoelich, A. Ram, I. Tikhonovich, S. Tuinman, C. Wijffelman and A. Wijfjes (1999b). *Pseudomonas* genes and traits involved in tomato root colonization. In: De Wit PJGM, Bisseling, T, Stiekema, W.J., eds 1999. IC-MPMI

Congress Proceedings: *Biology of plant–microbe interactions*, Vol. **2**. St Paul, MN, USA*: International Society for Molecular Plant–Microbe Interactions*, 324–330.

Lugtenberg, B. J. J., L. V. Kravchenko and M. Simons (1999a). Tomato seed and root exudates sugars: composition, utilization by *Pseudomonas* bio-control strains and role in rhizosphere colonization. *Environmental Microbiology*, **1** : 439–446.

Lakshmanan, P., P. Selvaraj and S. Mohan (1987). Efficiency of different methods for the control of Panama disease. *Trop. Pest Manage*., **33** : 373–376.

Larkin, R. and D. Fravel (1998). Efficacy of various fungal and bacterial biocontrol organisms for the control of Fusarium wilt of tomato. *Plant Disease*, **82** : 1022-1028.

Lemanceau, P. and C. Alabouvette (1991). Biological control of *Fusarium* diseases by fluorescent *Pseudomonas* and non-pathogenic *Fusarium*. *Crop Protection*, **10** : 279 286.

Molina, A. B. and R.V. Valmayor (1999). Banana production system in South East Asia. In : *Bananas and Food security*. C. Pica, E. Fource, E. A. Frision (eds). INIBAP, Montpellier, France. P. 423-436.

Moore, N. Y., S. Bentley, K. G. Pegg and D. R. Jones (1995). *Fusarium* wilt of banana. *Musa* disease fact sheet. INBAP, Montpellier, France, 5:4.

Moore, N. Y., K. G. Pegg, S. Bentley and L. J. Smith (1999). Fusarium wilt of banana : global problems and perspectives. In: Molina, A.B., Masdek, N.H.N. Liew, K.W, (eds). Banana Fusarium Wilt Management: Towards Sustainable Cultivation. Proceedings of the International Workshop on Banana Fusarium Wilt Disease. Kuala Lumpur, Malaysia: INIBAP, 11–30.

Nel, B., C. Steinberg, N. Labuschagne and A. Viljoen (2006). The potential of non-pathogenic *Fusarium oxysporum* and other biological control organisms for suppressing fusarium wilt of banana. *Plant Pathology*, **55** : 217–223.

NHB (2015). *Indian Horticulture Database, 2015*. National Horticulture Board, Gurgaon, Haryana, India.

Ploetz, R. C. (2005). Panama disease, an old enemy rears its ugly head: Parts 1 and 2. In: *Plant Health Progress*, APSnet: Online doi:10.1094/PHP-2005-1221-01-RV.

Ploetz, R. C. and K. G. Pegg (1997). *Fusarium* wilt of banana and Wallace's line : Was the disease originally restricted to his Indo-Malayan region? *Australasian Plant Pathology*, **26** : 239-249.

Ploetz, R. C. and K. G. Pegg (2000). Fusarium wilt. Pages 143-159 In: *Diseases of Banana, Abacá and Enset*. D. R. Jones, ed. CABI Publishing, Wallingford, UK.

Ploetz, R. C. (1994). *Fusarium* wilt and IMTP Phase ll. In : The improvement and testing of Musa : A Global Partnership. D. R. Jones (ed.), INIBAP. Montpellier, France.

Pushpavathi, Y., S. N. Dash, M. K. Mishra and V. Triveni (2015). Management of Fusarium wilt of banana under coastal Odisha conditions. *International Journal of Farm Science*, **5(4)** : 241-247.

Papavizas, G. C. (1985). Trichoderma and Gliocladium : biology, ecology and potential for biocontrol. *Annu Rev Phytopathol*., **23** : 23–54.

Pieterse, C. M. J., J. A. van Pelt, S. C. M. van Wees, J. Ton, K. M. Leon-Kloosterziel, J. J. B. Keurentjes, B. W. M. Verhagen, M. van Knoester, dSI, P. A. H. M. Bakkerand L. C. van Loon (2001). Rhizobacteria-mediated induced systemic resistance : triggering, signalling and expression. *European Journal of Plant Pathology*, **107** : 51–61.

Reinking, D. A. (1934). The distribution of banana wilt. *Phillip. J. Sci.*, **53** : 229-243.

Ramakrishnan, T. S. and S. Damodaran (1956). Observation on the wilt disease of banana. *Proc. Indian Acad. Sci*., **43** : 213-22.

Rawal, R. D. (2000). Fungal diseases of banana. Current scenario in India. In: *Banana Improvement, Production and Utilization*. H.P. Singh, K.L. Chadha (eds), National Research Centre for Banana (NRCB), Trichy, India, pp: 23.

Rajappan, K., P. Vidhyasekaran, K. Sethuraman and T. L. Baskaran (2002). Development of powder and capsule formulations of *Pseudomonas fluorescens* strain Pf-1 for the control of banana wilt. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, **109** : 80–87.

Raguchander, T., K. Jayashree and R. Samiyappan (1997). Management of *Fusarium* wilt of banana using antagonistic microorganisms. *Journal of Biological Control*, **11** : 101– 105.

Rhodes, D. J. and K. A. Powell (1994). Biological seed treatments – the development process. *BCPC Monograph*., **57** : 303–310.

Sequiera, L. (1992). Influence of organic amendments on survival of *Fusarium oxysporum* f. sp. *cubence* in the soil. *Phytopathology*, **52** : 976-982.

Sivamani, E. and S. S. Gnanamanickam (1988). Biological control of *Fusarium oxysporum* f.sp. *cubense* in banana by inoculation with *Pseudomonas fluorescens*. *Plant Soil*, **107** : 3- 9.

Stover, R. H. (1962 a). *Fusarium* wilt (Panama disease) of bananas and other *Musa* species. *Phytopathology* No.4, CMI, Kew.

Stover, R. H. (1962 b). *Fusarial* Wilt (Panama Disease) of Bananas and Other *Musa* Species. *Commonwealth Mycological Institute*, Kew, England.

Su, H. J., S. C. Hwang and W. H. Ko (1986). Fusarial wilt of Cavendish bananas in Taiwan. *Plant Disease*, **70** : 814–818.

Sukhada, M., M. Manamohan, R. D. Rawal, S. Chakraborty, H. Sreekantappa, R. Manjula and H. C. Lakshmikantha (2004). Interaction of *Fusarium oxysporum* f.sp. *cubense* with *Pseudomonas fluorescens* precolonized to banana roots. *World Journal of Microbiology & Biotechnology*, **20** :

651–655.

Saravanan, T. and M. Muthusamy (2006). Influence of *Fusarium oxysporum* f. sp. *cubense* (e.f. smith) Snyder and Hansen on 2, 4- diacetylphloroglucinol production by pseudomonas fluorescens migula in banana rhizosphere. *Journal of Plant Protection Research*, **46** : 241-254

Sukhada, M., M. Manamohan, R. D. Rawal, S. Chakraborty, H. Sreekantappa, R. Manjula and H. C. Lakshmikantha (2004). Interaction of *Fusarium oxysporum* f.sp. *cubense* with *Pseudomonas fluorescens* precolonized to banana roots. *World Journal of Microbiology & Biotechnology*, **20** : 651–655.

Sakthivel, N. and S. S. Gnanamanickam (1987). Evaluation of *Pseudomonas fluorescens* for suppression of sheath rot disease and for the enhancement of grain yields in rice (*Oryza sativa* L.). *Applied Environmental Microbiology*, **53** : 2056–2059.

Saravanan, T., M. Muthusamy and T. Marimuthu (2003). Development of integrated approach to manage the Fusarial wilt of banana. *Crop Protection*, **22** : 1117–1123.

Sivan, A. and I. Chet (1986). Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum*. *J. Phytopathol*., **116** : 39–47.

Srinivasan, U., H. J. Staines and A. Bruce (1992). Influence of media type on antagonistic modes of *Trichoderma* spp. against wood decay basidiomycetes. *Mater. Org*., **27** : 301–321.

Thangavelu, R. (2002). Characterization of *Fsarium oxysporum* schlecht. f.sp. *cubense* (e.f. smith) snyd. & hans. and Molecular Approaches for the Management of Fusarium wilt of Banana. *Ph.D. thesis*. Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, 254 pp.

Thangavelu, R., A. Palaniswami, G. Ramakrishnan, D. Sabitha, S. Muthukrishnan and R. Velazhahan (2001). Involvement of Fusaric acid detoxification by *Pseudomonas fluorescens* strain Pf10 in the biological control of Fusarium wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense*. *Journal of Plant Disease and Protection*, **108** : 433 445.

Thangavelu, R., A. Palaniswami and R. Velazhahan (2004). Mass production of *Trichoderma harzianum* for managing *Fusarium* wilt of banana. *Agriculture, Ecosytems and Environmen*., **103** : 259–263.

Thangavelu, R. and M. M. Mustaffa (2010). A Potential isolate of *Trichoderma viride* NRCB1and its mass production for the effective management of *Fusarium* wilt disease in banana. *Tree and Forestry Science and Biotechnology*, **4** (Special issue **2**) : 76-84.

Ting, A. S. Y., S. W. Mah and C. S. Tee (2011). Detection of potential volatile inhibitory compounds produced by endobacteria with biocontrol properties towards *Fusarium oxysporum* f. sp. *cubense* race 4. *World J. Microbiol. Biotechnol*., **27** : 229–235.

Ting, A. S. Y., S. Meon, J. Kadir, S. Son Radu and G. Singh (2008). Endophytic microorganisms as potential growth promoters of banana. *BioControl*, **53** : 541–553.

Ting, A. S. Y., M. Sariah, J. Kadir and S. Gurmit (2009). Field evaluation of Non-pathogenic *Fusarium oxysporum* isolates UPM31P1 and UPM39B3 for the control of *Fusarium* wilt in 'Pisang Berangan' (*Musa*, AAA). In: Proceedings on Banana crop protection for Sustainable Production and Improved Livelihoods (Eds. D. Jones and I. Van den Berg. *Acta Horticulture*, **828** : 139-143.

Thangavelu, R., A. Palaniswami, G. Ramakrishnan, D. Sabitha, S. Muthukrishnan and R. Velazhahan (2001). Involvement of Fusaric acid detoxification by *Pseudomonas fluorescens* strain Pf10 in the biological control of Fusarium wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense*. *Journal of Plant Disease and Protection*, **108** : 433-445.

University of Sydney (2003). Disease management: *Biological control*. http://bugs.bio.usyd.edu.au/plantpathology.

Van loon, L. C., P. A. Bakker and C. M. Pieterse (1998). Systemic resistance induced by rhizospere bacteria. *Annu. Rev. Phytopathol*., **36** : 453-483.

Viljoen, A. (2002). The status of Fusarium wilt (Panama disease) of banana in South Africa. *South African Journal of Science*, **98** : 341–344.

Wardlaw, C. W. (1961). *Banana diseases*, including Plantains and Abaca. Longmans, Green and Co. Ltd, London: 648.

Weber, O. B., R. Celli, C. R. Muniz, O. Aline, A. O. Vitor, F. C. O. Freire, M. Valéria and V. M. Oliveira (2007). Interaction of endophytic diazotrophic bacteria and *Fusarium oxysporum* f. sp. *cubense* on plantlets of banana 'Maça'. *Plant Soil*, **298** : 47–56.

Weindling, R. (1941). Experimental consideration of the mold toxin of *Gliocladium* and *Trichoderma*. *Phytopathology*, **31** : 991-1003.

Weller, D. M., J. M. Raaijmakers, B. B. McSpadden Gardener and L. S. Thomashow (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology*, **40** : 309–48.

Yedidia, I., N. Benhamou, Y. Kapulnik and I. Chet (2000). Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. *Plant Physiol. Biochem*., **38** : 863-873.