



CO-INOCULATION OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) AND THEIR MYCORRHIZAE HELPER BACTERIA (MHB) EFFECTIVELY SUPPRESSES FUSARIUM WILT IN BANANA

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Abstract

In the field of biocontrol, several plant growth practices have been in use but of varying efficiency. Integrated disease management in banana has been largely hampered by Fusarium wilt disease causing severe loss across the world. *Glomus* spp. has been regarded as an effective control agent of several plant diseases. Moreover, Mycorrhizal helper bacteria synergistically enhance the bioactivity of *Glomus* spp. In the present study, a total of 25 isolates of AMF and 29 isolates of MHB were evaluated for abatement of Fusarium wilt disease (*Foc* -VCG 0124) in *cv.* Grand Naine both individually and in combination. Among the treatments, *Glomus etunicatum* (KPV) + *P. aeruginosa* (Ge-A + Ge-B) and *Glomus mossae* (TPV) + *Pseudomonas* sp. (Gm-A) showed significant inhibition of Fusarium wilt with an augmented plant growth. Nevertheless, the results were also corroborated statistically prominent with increased root parameters. AMF in combination with their helper bacterium could act as a prospective mode in biocontrol agents for the management of Fusarium wilt disease in banana. Furthermore, this way of combinations could be provocatively used and rationalized for most of the field crops infected with Fusarium wilt.

Key words : Arbuscular mycorrhizal fungi, Mycorrhizae helper bacterium, *Glomus* spp, biocontrol, plant growth promoting bacteria.

Introduction

Banana and plantations face a plethora of infectious threats worldwide, among which *Fusarium oxysporum* f. sp. *cubense* occurs predominantly (Moore *et al.*, 2001). About 80-90% of yield loss in banana plantations of India is prevalently attributed to Fusarium infections (Mustaffa and Thangavelu *et al.*, 2009). Pathogenicity of *Foc* can be elaborated as entry through roots, blockage of vascular system, plant wilt finally leading to mortality of the whole plant. The onset of characteristic wilting symptoms occur after 5-6 months of planting and symptoms get expressed externally and internally (Wardlaw *et al.*, 1961 and Stover *et al.*, 1962). Moreover, the fungus can survive in soils for up to 30 years as chlamydospores in the soil (Ploetz *et al.*, 2000).

Although several strategies have been reported for combating the infection, cost effective management measures are still scantily available. Presently available management strategies encompass crop rotation with

rice, soil fumigation (Herbert and Marx, 1990) and fungicides (Lakshmanan *et al.*, 1987). Nonetheless, efficient strategies in arresting the disease till recent past have been by planting of resistant cultivars (Moore *et al.*, 1999). Consumer preference limitations hinder the growing of resistant varieties (Viljoen *et al.*, 2002). In this circumstance, usage of antagonists that protect and promote plant growth by colonization and multiplication in duality of locations comprising rhizosphere and plant could act as a provocative alternate in management of Fusarium wilt of banana.

Ample amount of research have been reported for the use of Arbuscular mycorrhizal (AM) fungi in reducing the incidence of plant root diseases caused by pathogens (Filion *et al.*, 1999). Utilization of AMF in plant disease control has been well documented worldwide. *Glomus fasciculatum* and *Gigaspora margarita* applications profoundly decrease root rot diseases caused by *Fusarium oxysporum* in Asparagus (Matsubara *et al.*, 2001). *Glomus clarum* was reported to reduce the root necrosis caused by *Rhizoctonia solani* in cowpea (Abdel-

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Fattah and Shabana, 2002) and *Glomus mossae* systemically reduces infection rate caused by *Gaeumannomyces graminis* in Barley (Khaosaad *et al.*, 2007). Further, AMF have been described to lessen the severity of disease caused by *Foc* and *Cylindrocladium spathiphylli* (Jaizme-Vega *et al.*, 1998 and Declerck *et al.*, 2002).

Mycorrhizae Helper Bacterium (MHB) harbouring the cytoplasm of many AMF spores (Cruz *et al.*, 2004 and Lumini *et al.*, 2007) have exemplary resources in potentiating biocontrol of a wide variety of plant pathogens. Among which, *Paenibacillus* spp. is a renowned MHB possessing extensive bioactive potentials against diverse fungal pathogens including *Phytophthora infestans*, *Fusarium oxysporum* and *Rhizoctonia solani* (Budi *et al.*, 1999). AM fungi-associated bacteria from the genus *Paenibacillus* spp. also was found to habitat potent bioactives in hampering *Pythium* infections that is the causative agent of damping-off in cucumber (Li *et al.*, 2007).

In the present study, an attempt was made to assess the active potentials of four different AM fungi and its MHB both individually and in combination for the effective suppression of *Fusarium* wilt disease in banana.

Materials and Methods

Isolation and maintenance of fungal pathogen

The casual agents of *Fusarium* wilt disease, *Foc* was isolated from a naturally wilt affected banana plant (*cv.* Grand Naine). The wilt affected samples were surface sterilized using 0.1% mercuric chloride and placed aseptically on potato dextrose agar (PDA) medium amended with streptomycin sulphate ($100\mu\text{g ml}^{-1}$) and incubated at 25°C for 7 days. Finally, the purified single spore culture was maintained in PDA slants at 25°C . Also the filter paper culture of pure *Foc* culture was prepared and stored at 4°C .

Isolation and identification of AM fungal spores from banana rhizospheric soil

AMF spores were isolated by wet sieving and decantation method (Gerdemann and Nicolson, 1963). One hundred gram of banana rhizospheric soil sample was dispersed in one litre of water and the suspension was decanted through a series of sieves (of sizes 180, 106 and 45μ) arranged descendingly. The residues in the sieves were washed into the beakers and the sievates filtered through filter paper. Each filter paper was then spread on a Petridish and scanned under a dissection microscope at 40X magnification for the isolation of AM fungal spores. Each individual spores showing different

spore morphology were then transferred using a wet needle and mounted in polyvinyl alcohol-lactoglycerol on a glass slide for identification (Schenck and Perez, 1990).

Isolation of MHB from AM fungal spores

About 20-25 spores were taken and surface sterilized using 3% chloramine-T and washed with sterile distilled water for 3 times. The last wash was streaked on to Kings B medium to check contamination. MHB were isolated by crushing the 20 numbers of surface sterilized AMF spores in 1.5 ml eppendorf tubes using micropestle. From this, 1ml of supernatant was taken and serially diluted up to 10^{-5} dilutions and from each of these dilutions such as 10^{-3} , 10^{-4} and 10^{-5} , 0.1 ml was taken and evenly spread on the Petri plates containing sterilized King's B agar medium. Then, the plates were incubated at room temperature ($25\pm 2^{\circ}\text{C}$) for 2 days and observed for bacterial colonies. A single bacterial colony was then purified by streaking individually on to plates containing King's B agar medium and glycerol stock of each MHB bacterial isolate was maintained at -80°C .

Assessment of AM colonization in roots

The quantification of root colonization of VAM was done by using Tryphan blue staining method (Phillips and Hayman, 1970). After staining, the root bits were transferred to a clean glass slide (10-15 bits slide⁻¹) using dissection needles and few drops of water was added over the root bits and fixed with a cover slip. Then the root bits were observed under a bright field microscope for the presence of AM colonization.

The level of colonization was calculated using the formula:

$$\text{AMF colonization (\%)} = \frac{\text{Number of root bits with AMF colonization}}{\text{Total number of root bits examined}} \times 100$$

In vitro screening of MHB isolates against *Foc* (VCG-0124) by spore germination assay

The individual MHB isolates was inoculated in to the conical flasks containing King's B broth and incubated at 28°C in a rotary shaker (120 rpm) for 48 h. The sample was centrifuged at 10,000 rpm for 10 min at 4°C to obtain a cell free liquid. $30\mu\text{l}$ of conidial suspension (4×10^6 spores ml^{-1}) of *Foc* pathogen and $70\mu\text{l}$ of culture filtrate of respective MHB isolate were placed in individual cavity slides and the cavity slide was kept in Petri dishes on a glass bridge chamber and incubated at 25°C . The spore suspension in sterile distilled water alone served as control. The germination of spores was observed for up to 96 h at 24 h interval and the percent germination of spores

was calculated. Three replications were maintained to obtain an average per cent germination (CSFT, 1943).

Phenotypic and biochemical characterization of MHB isolates

Each MHB isolates was characterized phenotypically (colony morphology) and biochemically [Gram reaction, Capsule staining, Motility, Indole formation, Methyl red (MR), Voges–Proskauer (VP) test, Triple sugar iron test, Starch and gelatin hydrolysis catalase test)]. The results were recorded and analyzed according to Bergey's manual of determinative bacteriology (Kreig and Holt *et al.*, 1994).

Molecular identification of MHB isolates

Bacterial genomic DNA extraction from pure bacterial cultures was carried out (Leach *et al.*, 1992). Nearly full-length 16S rRNA gene of MHB isolates were amplified by a 50 µL PCR reaction mixture containing 50 ng of DNA template, 1X PCR buffer, 20 pmol of each primer (FD1-5'-AGT TTG ATC CTG GCT CA-3', RP2, -5'-ACG GCT ACC TTG TTA CGA CTT-3'), 200 µM of each dNTPs and 1.5 U of Taq DNA polymerase (Sigma). After initial denaturation at 94°C for 5 min, each thermal cycling was as follows: denaturation at 94°C for 1 min, annealing at 52°C for 45 sec and elongation at 72°C for 1 min. At the end of 30 cycles, the final extension step was at 72°C for 8 min. The resulted PCR products were electrophoretically separated in 1.5% agarose gel at 80 volts for 1 h. The band of approximately 1500 bp size observed under UV trans illuminator was excised from an agarose gel and purified using Gen elute (Sigma laboratories, USA) kit and each of them were sequenced. Both forward and reverse sequences were then assembled within Bioedit and compared to sequences already available in GenBank database using BLAST algorithm (Altschul *et al.*, 1990).

Molecular identification of AM fungi

Total genomic DNA was extracted from the AMF spores belong to different soil samples separately (Husband *et al.*, 2002). For this, approximately 100 spores were crushed with a miniature pestle in microcentrifuge tubes containing 40 µl nuclease free water and 13 µl of 100mM Tris/HCl (pH 8.0) was added to the crushed spores. The spore suspension was vortexed vigorously and heated at 95°C for 5 min. The sample was brought to ice for 5 minutes and then centrifuged at 10,000 rpm for 2 min. The supernatant was frozen at -20°C and was used as template for PCR (Jacquot *et al.*, 2000).

The total genomic DNA was amplified using nested PCR using the primer pairs NS1, NS4 and AML1, AML2

which were designed based on the small subunit of the nuclear ribosomal DNA (18S rDNA). The 20 µl PCR reaction mixture contained DNA template 50 ng, 1X PCR buffer, 0.2 mM of each dNTPs, 1 µM of each primers, 1.5 mM MgCl₂ and 2U of Taq DNA polymerase. PCR amplification was performed in an automated thermal cycler (Eppendorf, USA) with a program of initial denaturation at 95°C for 1 min, 34 cycles consisting of 94°C for 1 min (denaturation), 51°C for 1 min (annealing), 72°C for 1 min (primer extension) and 72°C for 10 min (final extension) and the PCR product was stored at -20°C (Kowalchuk *et al.*, 2002). The PCR amplified 18S rRNA was checked in 1% agarose gel for its presence and then purified by using GenElute PCR Clean-up kit (Sigma, USA) and sent for sequencing. Both forward and reverse sequences were then assembled with Bioedit and compared to sequences already available in GenBank database using BLAST algorithm (Altschul *et al.*, 1990).

Multiplication of AMF spores and MHB under *in vivo* condition

The AMF spores were isolated and mass multiplied initially using onion as host culture in funnel containing sand and red soil in the ratio of 1:1 (Morton *et al.*, 1988). These isolates were then further purified and mass multiplied using maize as a host source. The MHB isolates were mass produced in King's B broth as stated in section 2.5.

Individual and Combined evaluation of AMF and MHB isolates against virulent strain of *Foc* race-1 (VCG 0124) under pot culture condition

Equal sized mud pots were taken and filled with sterilized sand and red soil in the ratio of 1:1 and planted with tissue cultured banana plant *cv.* Grand Naine. The mass multiplied AMF and MHB isolates were applied individually as well as in different combinations in each pot @ 250 gm plant⁻¹ of AMF containing 80 spores 100 g⁻¹ of soil and 15 ml of MHB containing 10⁸ cells ml⁻¹. After 30 days of application, the sand maize culture of *Foc* was inoculated @ 50 gm plant⁻¹. A total of five replicates were maintained for each treatment and *Foc* alone inoculated plants were taken as untreated control.

After six months of inoculation of *Foc*, the observations on the plant growth parameters (plant height, girth, number of leaves, leaf area and number of roots) and also the internal score of Fusarium Wilt Disease (vascular discoloration) was calculated by using 1-6 grade (Orjeda *et al.*, 1998). (Grade1-corm completely clean, no vascular discoloration, 2- isolated points of discoloration in vascular tissue, 3-discoloration of up to one-third of vascular tissue, 4-discoloration of below one-third and

two-thirds of vascular tissue, 5-discoloration of greater than two-thirds of vascular tissue. 6-total discoloration of vascular tissue).

Statistical analysis

The experiments were conducted with five replicates and for the data of all the experiments the standard deviations (\pm) were computed using Microsoft Excel 2010 (Windows 8 Edition, Microsoft Corporation, USA). One-way analysis of variance and comparison of means based on the Duncan's multiple range test ($P < 0.05$) were used to determine the significant differences between the treatments. Paired T-test was used to analyze significant differences between initial and final results of a particular treatment. All statistical tests were evaluated at 95% confidence level. Statistical analyses were carried out with SPSS statistical package version 21 from SPSS Inc., Chicago, IL, USA (Griffith *et al.*, 2010).

Results

Isolation and characterization of AMF isolates

Totally 25 isolates of AMF were obtained from different rhizospheric banana soil samples collected from different varieties grown in Tamil Nadu, Maharashtra and Tripura states. Based on the morphological characters of the spores, these AMF isolates were identified as *Acaulospora* spp. (*Acaulospora* sp., *A. foveata* and *A. capsicula*), *Glomus* spp. (*G. aggregatum*, *G. viscosum*, *G. etunicatum*, *G. clarum*, *G. geosporum*, *G. mossae* and *G. claroideum*) and *Scutellospora* spp. (*S. calospora* and *S. heterogama*). Based on the root infection studies, the AMF isolates *viz.* *Acaulospora* sp. (LPV), *Glomus etunicatum* (KPV), *Glomus geosporum* (C4V) and *Glomus mossae* (TPV), which recorded 60-80% per cent infection in the roots of banana *cv.* Grand Naine (AAA) were alone selected for further studies. Besides, these four strains of AMF were further confirmed by sequencing their 18s-rDNA region.

Isolation and characterization of MHB strains

Totally 27 isolates of MHB were isolated from four different AMF isolates *viz.*, *Acaulospora* sp. (LPV), *Glomus etunicatum* (KPV), *Glomus geosporum* (C4V) and *Glomus mossae* (TPV). The phenotypical and biochemical characterization of these 27 isolates of MHB isolates revealed the presence of 10 different genera of bacteria *viz.*, *Enterobacter* spp., *Citrobacter* spp., *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Brevibacillus* spp., *Klebsiella* spp., *Rhizobium* spp., *Azotobacter* spp. and *Rhodococcus* spp.

In vitro screening of MHB isolates against *Foc* (VCG-0124)

The *in vitro* bioassay study conducted for 27 MHB isolates belonging to 10 different genera indicated that nine out of 27 isolates of MHB isolates recorded 100% inhibition of spore germination of *Foc*. These *Foc* effective MHB isolates belong to *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Pseudomonas* spp. and these were confirmed by sequencing 16S rRNA.

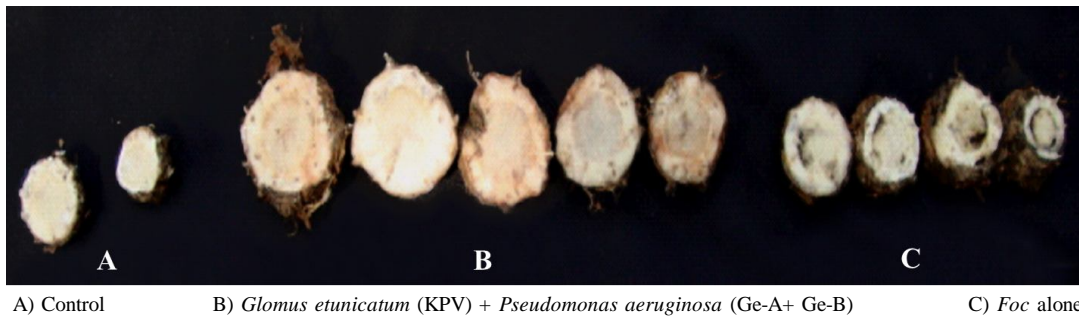
In vivo evaluation of MHB for their ability to help respective AMF for root infection in banana

The result of *in vivo* studies indicated that among different AMF tested for their ability to infect the roots of banana *cv.* Grand Naine, the AMF isolates *Acaulospora* sp. (LPV), *Glomus etunicatum* (KPV), *Glomus geosporum* (C4V) and *Glomus mossae* (TPV) recorded 100% root infection in the presence of its MHB *viz.*, *Bacillus subtilis* (AS), *Pseudomonas aeruginosa* (Ge-A), *Pseudomonas aeruginosa* (Ge-B), *Pseudomonas aeruginosa* (Gg-A) and *Pseudomonas* sp. (Gm-A), *Pseudomonas* sp. (Gm-B) compared to other isolates of AMF and MHB isolates (table 1).

In vivo evaluation of AMF, MHB and AMF + MHB against virulent strain of *Foc* race-1 (VCG 0124)

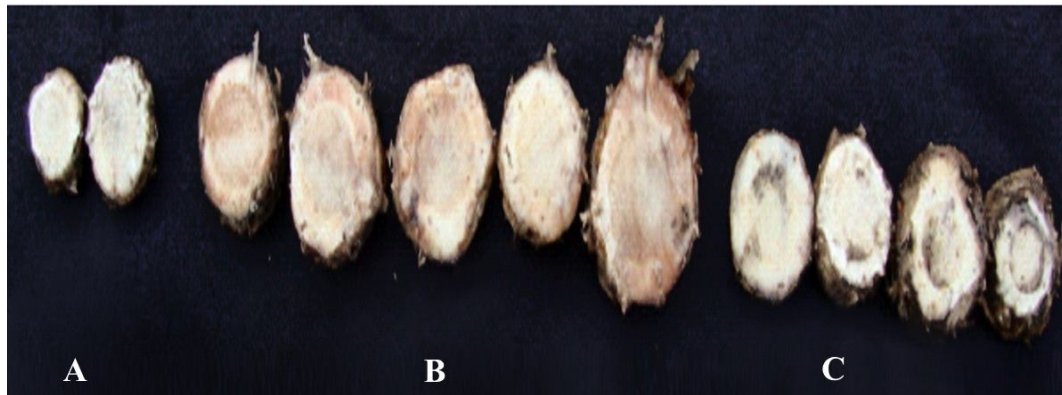
Among the four isolates of AMF evaluated individually, the application of *Glomus geosporum* (C4V) recorded lowest wilt disease score of 1.4 followed by the isolate *Glomus mossae* (TPV), which has recorded a wilt disease score of 2.0 whereas *Foc* alone inoculated plants recorded a wilt disease score of 4.0. Besides, these AMF isolates also enhanced plant growth parameters *viz.*, plant height (up to 136.8%), girth (up to 211.1%), number of leaves (up to 50.0%) leaf area (up to 632.3%), total roots (up to 334.7%) and root biomass (up to 584.6%) compared to *Foc* alone-inoculated control plants (table 2).

In the case of individual application of nine different MHB isolates, the isolate *Pseudomonas aeruginosa* (Gg-A) had significantly ($p > 0.05$) lowered wilt disease score (1.2) followed by the other two isolates *viz.*, *Pseudomonas* spp. (Gm-A) and *Pseudomonas* spp. (Gm-B), which have recorded a wilt disease score of 1.4 and 1.8 respectively when compared to *Foc* alone inoculated plants (4.0). Moreover, these isolates have also increased the plant growth parameters such as plant height (up to 39.8%), girth (up to 88.8%), number of leaves (up to 20.8%) leaf area (up to 125.0%), number of roots (up to 121.1%) and root biomass (up to 81.3%) compared to *Foc* alone-inoculated control plants (table 3).



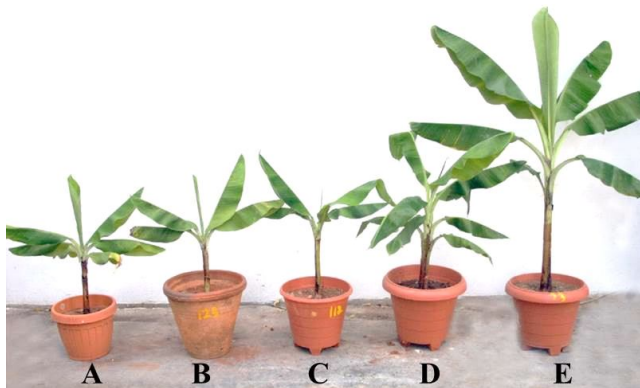
A) Control B) *Glomus etunicatum* (KPV) + *Pseudomonas aeruginosa* (Ge-A+ Ge-B) C) *Foc* alone

Fig. 1 : Combined effect of *Glomus etunicatum* (KPV) + *Pseudomonas aeruginosa* (Ge-A + Ge-B) on Fusarium wilt disease in banana plant cv. Grand Naine (AAA).



A) Control B) *Glomus mossae* (TPV) + *Pseudomonas* sp. (Gm-A) C) *Foc* alone

Fig. 2 : Combined effect of *Glomus mossae* (TPV) + *Pseudomonas* sp. (Gm-A) on Fusarium wilt disease in banana plant cv. Grand Naine (AAA).



A) *Foc* alone B) Control C) *Pseudomonas aeruginosa* (Gg-A), D) *Pseudomonas aeruginosa* (Gg-B), E) *Glomus etunicatum* (KPV) + *Pseudomonas aeruginosa* (Ge-A+ Ge-B).

Fig. 3a : Effect of combined application of AMF and its MHB isolates on growth parameters in banana plant cv. Grand Naine (AAA).

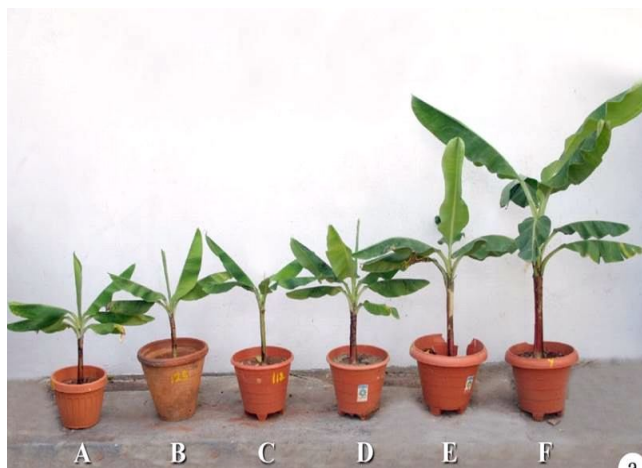


A) *Foc* alone B) Control C) *Pseudomonas aeruginosa* (Gg-A), D) *Glomus etunicatum* (KPV) , E) *Pseudomonas aeruginosa* (Ge-A & Ge-B) F) *Glomus etunicatum* (KPV) + *Pseudomonas aeruginosa* (Ge-A+Ge-B).

Fig. 3b : Effect of combined application of AMF and its MHB isolates on root parameter of banana plant cv. Grand Naine (AAA).

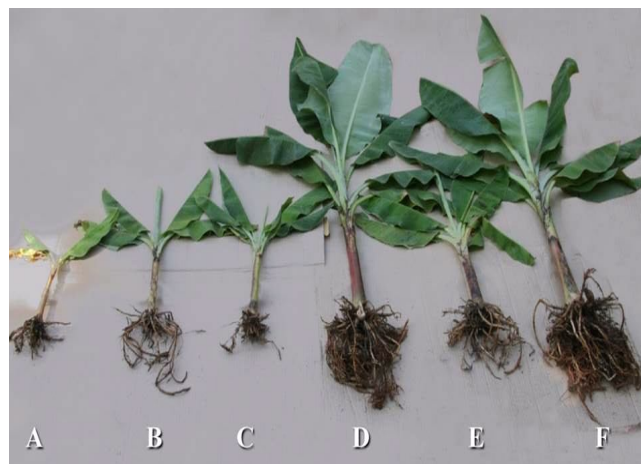
Further, among different combinations of AMF + MHB evaluated, the combined application of *Glomus etunicatum* (KPV) + *P. aeruginosa* (Ge-A + Ge-B) and *Glomus mossae* (TPV) + *Pseudomonas* sp. (Gm-A) have recorded 100% reduction of Fusarium wilt disease (wilt disease score 1.0- healthy) (figs. 1 & 2) compared to *Foc* alone inoculated control plants.

Furthermore, these combinations also recorded significant increase in plant growth parameters such as plant height (up to 157.2%), girth (up to 218.5%), number of leaves (up to 54.16%), leaf area (602.0%), number of roots (up to 357.8%) and root biomass (692.3%) compared to *Foc* alone inoculated control plants (table 4) (figs. 3a-3b) & (figs. 4a-4b).



A) *Foc* alone B) Control C) *Pseudomonas aeruginosa* (Gg-A), D) *Glomus mossae* (TPV) a, E) *Pseudomonas* sp. (Gm-A), F) *Glomus mossae* (TPV) + *Pseudomonas* sp. (Gm-A).

Fig. 4a : Effect of combined application of AMF and its MHB isolates on growth parameters in banana plant on cv. Grand Naine (AAA).



A) *Foc* alone B) Control C) *Pseudomonas aeruginosa* (Gg-A), D) *Glomus mossae* (TPV) a, E) *Pseudomonas* sp. (Gm-A), F) *Glomus mossae* (TPV) + *Pseudomonas* sp. (Gm-A).

Fig. 4b : Effect of combined application of AMF and its MHB isolates on root parameter in banana plant on cv. Grand Naine (AAA).

Table 1 : Effect of culture filtrate of Mycorrhizae helper bacterial isolates on the spore germination of *Fusarium* wilt pathogen of banana.

S. no.	Isolate	No. of spores ungerminated	No. of spores germinated	Percentage of spore inhibition
1.	Control	0	42	0
2.	Gc(a)	32	28	96.6
3.	Gs(a)	56	7	16.6
4.	Gg(a)	60	0	100
5.	Gm(a)	60	0	100
6.	As(c)	6	55	10
7.	Gs(c)	3	25	89.2
8.	Ge(d)	60	0	100
9.	Gs(c)	32	28	96.0
10.	Gs(d)	49	15	89
11.	Ge(a)	50	10	6.66
12.	Gg(b)	2	58	86
13.	Ge(a)	60	0	100
14.	Ge(b)	60	0	100
15.	Gc(b)	20	25	44.8
16.	Gs(a)	12	22	35.3
17.	Gc(a)	60	0	100
18.	Gs	4	33	10.8
19.	Gs(e)	21	15	57.8
20.	Gs(f)	4	22	15.5
21.	Gm(b)	60	0	100
22.	Ge(b)	0	41	0
23.	Ge(c)	60	0	100

Table 1 continued...

Table 1 continued...

24.	Gc(c)	6	24	12.5
25.	As(a)	60	0	100
26.	Ge(c)	20	25	44.4
27.	As(b)	12	22	35.3
28.	Gc(b)	19	36	60.9
			CD(P=0.05)	6.15**

Discussion

Root colonizers are well known for beneficiary roles in crop protection (Sharma *et al.*, 1992). Research pertaining to protective effect of AM fungi upon *Foc* wilt infection in banana has been very sparse and only few have been reported (Jaizme-Vega *et al.*, 1998; Sukhada Mohandas *et al.*, 2004; Jefwa *et al.*, 2012). In our current study, an effort was made to develop an eco-friendly approach by using an arbuscular mycorrhizal fungus (AMF) and its helper bacterium (MHB) to control *Fusarium* wilt in banana which is regarded as one of the major limiting factors for banana production worldwide (O'Donnell *et al.*, 1998 and Lin *et al.*, 2009). Arbuscular mycorrhizal fungi (AMF) are key components of soil microbiota and form symbiotic relationships with the roots of most terrestrial plants, improving the nutritional status of their host and protecting it against several soil-borne plant pathogens (Smith *et al.*, 1997; Harrison *et al.*, 1999 and Bi *et al.*, 2007). Several workers have successfully demonstrated the potential of mycorrhizal fungi and also the bacterial strains to control plant pathogenic fungi like *Phytophthora*, *Sclerotinia*, *Rhizoctonia*, *Pythium*, *Verticillium* and *Aphanomyces* (Kegler and Gottwald,

Table 2 : Individual effect of AMF isolates on various growth parameters of banana plant cv. Grand Naine inoculated with Fusarium wilt pathogen (*Foc*-VCG 0124).

AMF isolates	Height (cm)	Girth (cm)	No. of leaves	Leaf area (cm ²)	No. of roots	Biomass (gm)	Wilt disease-internal score (1-6 scale)
<i>Acaulospora</i> sp. (LPV)	41.80±8.17 ^b	13.20±3.56 ^b	5.80±1.30 ^{ab}	590.98±456.49 ^b	60.80±2.78 ^b	6.78±0.82 ^b	2.20±0.45 ^a
<i>Glomus etunicatum</i> (KPV)	51.00±8.60 ^c	16.80±2.59 ^c	7.40±0.55 ^c	973.13±167.84 ^c	76.60±2.07 ^c	9.70±0.45 ^c	2.40±1.67 ^a
<i>Glomus geosporum</i> (C4V)	44.80±1.92 ^{bc}	14.40±2.41 ^{bc}	7.20±0.84 ^c	568.30±110.44 ^b	82.60±5.51 ^d	12.46±2.03 ^d	1.40±0.89 ^a
<i>Glomus mossae</i> (TPV)	48.80±1.79 ^{bc}	16.80±0.45 ^c	6.80±0.45 ^{bc}	795.26±102.55 ^{bc}	72.40±6.39 ^c	9.76±1.25 ^c	2.00±1.00 ^a
<i>Foc</i> alone	20.60±0.89 ^a	5.40±0.55 ^a	4.80±0.45 ^a	132.03±9.65 ^a	19.00±2.92 ^a	1.82±0.15 ^a	4.00±1.23 ^b

Note: Numerical values are given as mean ± standard deviation; column sharing same alphabets are not differ significantly according to DMRT (p>0.05).

Table 3 : Individual effect of MHB isolates on various growth parameters of banana plant cv. Grand Naine inoculated with Fusarium wilt pathogen (*Foc*-VCG 0124).

Treatment Groups	Height (cm)	Girth (cm)	No. of leaves	Leaf area (cm ²)	No of roots	Root bio-mass(gm)	Wilt disease internal score (1-6 scale)
<i>B. subtilis</i> (As)	28.80±3.90 ^b	10.20±1.10 ^a	5.60±0.55 ^a	300.35±58.67 ^c	25.60±2.41 ^{bc}	2.56±0.38 ^b	3.20±1.92 ^{bc}
<i>P. aeruginosa</i> (Ge-A)	23.60±3.65 ^a	8.60±1.14 ^b	5.60±0.55 ^a	165.08±61.81 ^{ab}	27.80±1.79 ^c	3.30±0.51 ^c	2.20±0.84 ^{abc}
<i>P. aeruginosa</i> (Ge-B)	23.00±1.00 ^a	8.80±0.45 ^b	8.80±0.45 ^b	159.41±30.43 ^{ab}	20.60±2.30 ^a	1.76±0.17 ^{bc}	2.00±1.41 ^{ab}
<i>P. aeruginosa</i> (Gg- A)	19.40±2.19 ^a	5.80±0.84 ^a	4.20±1.64 ^a	96.71±34.06 ^a	20.60±3.05 ^a	1.98±0.44 ^a	1.20±0.45 ^a
<i>P. aeruginosa</i> (Gg-B)	20.40±0.89 ^a	6.20±1.10 ^a	5.40±1.14 ^a	122.96±17.88 ^{ab}	21.40±4.83 ^{ab}	2.02±0.68 ^a	2.80±1.79 ^{abc}
<i>Pseudomonas</i> spp. (Gm-A)	19.60±7.06 ^a	5.60±0.89 ^a	5.40±1.67 ^a	124.09±65.05 ^{ab}	34.00±3.39 ^d	2.64±0.42 ^b	1.40±0.89 ^{ab}
<i>Pseudomonas</i> spp. (Gm-B)	21.40±0.55 ^a	5.40±0.89 ^a	5.40±0.89 ^a	171.40±67.77 ^b	40.00±5.96 ^c	2.96±0.17 ^{bc}	1.80±1.30 ^{ab}
<i>Foc</i> alone	20.60±0.89 ^a	5.40±0.55 ^a	4.80±0.45 ^a	132.03±9.65 ^{ab}	19.00±2.92 ^a	1.82±0.15 ^a	4.00±1.23

Note: Numerical values are given as mean ± standard deviation; column sharing same alphabets are not differ significantly according to DMRT (p>0.05).

1998; Harish *et al.*, 2008; Azcon-Aguilar and Barea, 1996; Demir and Akkopru, 2007 and Aysan and Demir, 2009).

Fusarium wilt pathogen and its metabolites might compete with rhizosphere organisms be it AMF or MHB and may inhibit mycorrhizal associations (Liu *et al.*, 1995). Hence, there is a dire need for an alternative in this issue. Co-inoculation protocols efficiently manage plant pathogens and their infectivity are stressed due to competitive inhibition (Schenck *et al.*, 1981; Dehne *et al.*, 1982; Barea *et al.*, 1984; Hornby *et al.*, 1990; Perrin *et al.*, 1990; Reid *et al.*, 1990; Torres-Barrag *et al.*, 1996). Besides, the degree of protection relies on soil and other environmental conditions (Aysan and Demir, 2009). However, affirmative prophylaxis of AMF for efficacious practical application has been cited rarely (Hooker & Jaizme-Vega & Atkinson *et al.*, 1994; Linderman *et al.*, 1994; Lingua *et al.*, 2002; Hao & Christie *et al.*, 2005; Aysan and Demir, 2009). Therefore, the present co-

inoculation experiment would act as a Launchpad for futuristic research in plant protection.

Some cultivable bacteria associated with the spores of *Gigaspora margarita* such as *Paenibacillus polymyxa*, *Janthinobacterium lividum* and other from *Bacillus* genera functioned well to suppress diseases. *Paenibacillus* spp. Strain B2, isolated from the mycorrhizosphere of *Sorghum bicolor* (L) and inoculated with *Glomus mosseae* had antagonistic effect on soil borne pathogens and stimulated mycorrhization (Budi *et al.*, 1999). Similarly (Salem and Dobolyi *et al.*, 2003) have reported the AMF, *Glomus intraradices* and the rhizobacterium, *Pseudomonas fluorescens* Migula, significantly inhibited the development of the disease tomato leaf-specks disease caused by *Pseudomonas syringae* pv. tomato.

In our present study, the efficacy of single and combined applications of the selected AMF and their

Table 4 : Co-inoculation effect of AMF+MHB isolates on various growth parameters of banana plant cv. Grand Naine inoculated with Fusarium wilt pathogen (*Foc*-VCG0124).

AMF +MHB combinations	Height (cm)	Girth (cm)	Leaves	Leaf area (cm ²)	No. of roots	Root biomass (gm)	Wilt disease internal score (1-6 scale)
<i>Acaulospora</i> sp.(LPV) + <i>B. subtilis</i> (As)	49.40±4.72 ^{cd}	15.60±1.14 ^{bc}	7.40±0.89 ^b	884.52±148.23 ^d	71.20±1.64 ^d	8.64±0.47 ^d	2.60±0.55 ^b
<i>Getunicatum</i> (KPV) + <i>P. aeruginosa</i> (Ge-A)	51.80±2.86 ^{cd}	15.80±1.64 ^c	6.80±0.84 ^b	858.11±108.50 ^d	75.20±3.03 ^c	9.54±0.51 ^d	1.60±0.89 ^{ab}
<i>Getunicatum</i> (KPV) + <i>P. aeruginosa</i> (Ge-B)	53.00±2.00 ^d	17.20±1.79 ^c	6.40±0.89 ^{ab}	931.34±52.65 ^d	85.60±2.88 ^f	12.80±0.73 ^e	2.80±2.05 ^{bc}
<i>Getunicatum</i> (KPV) + <i>P. aeruginosa</i> (Ge-A+Ge-B)	50.40±1.82 ^{cd}	16.00±0.71 ^{bc}	6.80±0.84 ^b	832.36±38.15 ^{cd}	86.60±2.88 ^f	14.14±0.78 ^f	1.00±0.00 ^a
<i>G.geosporum</i> (C4V)+ <i>P. aeruginosa</i> (Gg-A)	43.60±2.19 ^b	14.00±0.71 ^b	6.40±0.55 ^{ab}	637.31±102.00 ^b	57.60±2.70 ^c	7.06±0.92 ^c	2.20±1.10 ^{ab}
<i>G.geosporum</i> C4V + <i>P. aeruginosa</i> (Gg-B)	44.60±3.21 ^b	15.00±1.58 ^b	7.00±0.71 ^b	683.48±67.30 ^b	42.00±4.90 ^b	3.88±0.57 ^b	3.00±0.71 ^{bc}
<i>G. mossae</i> (TPV) + <i>Pseudomonas</i> spp. (Gm-A)	43.60±4.34 ^b	14.20±2.59 ^b	6.80±0.84 ^b	658.37±109.24 ^b	87.00±2.35 ^f	14.42±1.42 ^f	1.00±0.00 ^a
<i>G. mossae</i> (TPV) + <i>Pseudomonas</i> spp. (Gm-B)	48.40±1.82 ^c	15.80±1.30 ^{bc}	6.60±0.55 ^b	735.32±83.14 ^{bc}	69.20±3.03 ^d	8.82±0.38 ^d	1.60±0.55 ^{ab}
<i>Foc</i> alone	20.60±0.89 ^a	5.40±0.55 ^a	5.40±0.55 ^a	132.03±9.65 ^a	19.00±2.92 ^a	1.82±0.15 ^a	4.00±1.22 ^d

Note: Numerical values are given as mean ± standard deviation; column sharing same alphabets are not differ significantly according to DMRT ($p>0.05$).

MHB isolates were evaluated for the management of Fusarium wilt disease under pot culture conditions. In this study, totally four different AMF isolates (LPV, KPV, C4V, TPV), which showed 100% root infection percentage and their MHB isolates *viz.*, *Bacillus subtilis* (AS), *Pseudomonas aeruginosa* (GE), *Pseudomonas aeruginosa* (Gg) and *Pseudomonas* spp. (Gm), which helped their respective AMF for 100% root infection *in vivo* were selected for the suppression of Fusarium wilt disease caused by virulent strain of *Foc* race-1 (VCG 0124).

The result of the study indicated that only the plants treated with AMF + MHB isolates *viz.*, *Glomus etunicatum* (KPV) + *P. aeruginosa* (Ge-A+Ge-B) and *Glomus mossae* (TPV) + *Pseudomonas* sp. (Gm-A) had completely controlled the Fusarium wilt disease compared to individual application of either AMF or MHB. Besides, the combined application of these isolates has also increased the plant growth and root parameters significantly as compared to *Foc* alone inoculated plants. Similarly (Jaizme-Vega *et al.*, 1998) studied the interaction effect of *Glomus intraradices* and *Glomus* spp. and the vascular fungus *Fusarium oxysporum* f. sp. *cubense*, causal agent of Panama disease under greenhouse conditions and the results indicated that both the species of *Glomus* were found to reduce both internal (rhizome necrosis) and external disease symptoms and significantly increased plant growth compared to non-mycorrhizal plants (St-Arnaud and Hamel *et al.*, 1994). Ainhoa Martinez-Medina *et al.* (2011) who evaluated four AMF (*Glomus intraradices*, *Glomus mosseae*, *Glomus claroideum* and *Glomus constrictum*) and the beneficial fungus *Trichoderma harzianum* in a greenhouse nursery and reported that co-inoculation of plants with the AMF and *T. harzianum* produced a more effective control of Fusarium wilt by 60% reduction than each AMF inoculated alone only 25–50% reduction in disease incidence in melon crops. The application of a combined AMF and *Bacillus subtilis* Cohn could reduce the root-rot caused by *Fusarium solani* (Mart.) Sacc. in Geranium (*Pelargonium gravealens* L.) (Wafaa and Abdel, 2001).

Thus, the results accomplished are confirmatory that a single AMF or MHB may not be able to protect banana plants from the disease completely but a mixture of AMF and MHB showed a potential complete suppression of *Fusarium* wilt disease under pot culture condition.

It is also very obvious from this study that the use of AMF in combination with its helper bacterium can be a potential option for the effective management of this *Fusarium* wilt disease in banana rather than using chemical

fungicides. Moreover, this protocol can be rationalized for almost all field crops and as an efficient disease management strategy for the control of wilt diseases of plantation crops.

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