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PLANT GROWTH PROMOTION AND INDUCTION OF DEFENSE ENZYME IN TOMATO AGAINST WILT AND ROT PATHOGEN BY COINOCULATION OF *METHYLOBACTERIUM RADIOTOLERANS* AND *GLOMUS* SP.

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ABSTRACT

Tomato (Lycopersicon esculentum Mill.) is affected by several diseases and mostly caused by soil borne pathogens and controlled usually by applying huge quantity of expensive inorganic chemicals that pollutes the environment. Hence, suitable microbial consortium needs to be exploited for effective management of root diseases of tomato. Among the eight isolates taken, the isolate PPFG-1 exhibited maximum production of growth hormones and its 16S rDNA sequence based phylogenetic analysis revealed that the isolate more closely related to Methylobacterium radiotolerans. The coinoculation of M. radiotolerans PPFG-1 with Glomus sp. was found to be best among other treatments by increasing the germination percentage (96.67%), disease reduction over control (96.67%) and seedling vigour index (4141.34) in nursery. It also improved growth parameters by increasing shoot length , root length and disease reduction over control (95.83%) at the time of harvest. And the coinoculation increased induced systematic resistance activity by increase in chitinase, peroxidase, phenylalanine ammonia lyase, β-1,3 glucanase, poly phenol oxidase and phenolics on 5th day after treatment than uninoculated control and achieved higher yield . Hence, the finding that Methylobacterium and Arbuscular mycorrhizal (AM) fungi increases defense enzymes along with plant growth promotion when compared with existing recommended Pseudomonas fluorescens and Trichoderma viride application and this new microbial consortium will facilitate effective soilborne diseases management in sustainable agriculture.

I. INTRODUCTION

In the world market, tomato (*Lycopersicon esculentum* Mill.) is placed in the third place because its volume of production. In addition, it is the vegetable more consumed around the world. This vegetable crop reaches high price in the international market during some seasons (De Curtis *et al*., 2010). Prevention and control of this severe disease has been based on different strategies, mainly on the use of synthetic agrochemicals which had not been successful to eradicate the pathogen, causing pathogen resistance to this kind of pesticides (Siddiqui and Akhtar, 2009). Based on that background and the severe environmental problems caused by synthetic agrochemicals around the world, novel organic and biological options have been evaluated in order to prevent and control diverse plant pathogens, given that these options are more ecofriendly (Baniasadi *et al*., 2009). On the other hand, excessive utilization of synthetic fertilizers results in high production costs and pollution of soils and water (Kachari and Korla, 2009; Lana, 2008). In the last two decades, one of the study areas that are positively impacting sustainable agriculture is the application of biofertilizers and other organic compounds (Farahvash *et al*., 2010; Haghighi *et al*., 2010) Most of the biofertilizers are based on bacteria and fungi that are living in a symbiotic way with plants; this is a positive way to organic fertilization of different crops (Pathak and Godika, 2010; Al-Taweil *et al*., 2009).

The consistent success of the *Methylobacterium* plant association relies on methylotrophy, the ability to utilize the one-carbon compound methanol emitted by plants. However, the efficiency of *Methylobacterium* in plant growth promotion could be better exploited and thus has attracted increasing interest in recent years (Jeounghyun *et al*., 2006). Induced systemic resistance activity in groundnut, rice and tomato against rot pathogens and *Pseudomonas syringae* pv. *tomato* in response to methylotrophic bacteria suggests the possibility that pink-pigmented facultative methylotrophic bacteria might be used as a means of biological disease control (Madhaiyan *et al*., 2004; Madhaiyan *et al*., 2006; Indira Gadhi *et al*., 2008).

Arbuscular Mycorrhizae Fungi (AMF) are symbiotic associations, formed between plants and soil fungi that play an essential role in plant growth, plant protection and soil quality. This association is beneficial to plants in manyfold, since it increases the area of rhizosphere for water and nutrient absorption by plants, decreases disease susceptibility, increases tolerance to adverse environmental conditions and biomass production of plants (Michel, 1989). Linderman, 1995 has studied the effect of AMF on reduction of root rot diseases caused by fungi viz., *Pythium*, *Pytophthora*, *Fusarium*, *Verticillium* and *Rhizoctonia*.

The beneficial effect of plant growth promoting bacteria (PGB) and AM fungi have been mainly studied separately (Artursson *et al*., 2006). Only recently consortium concepts have emerged to study the synergistic effects of the microorganisms with respect to their combined beneficial effects on plant (Medina *et al*., 2003).

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A major biotechnology goal for sustainable agriculture is to use the combined inoculation of selected rhizosphere microorganism to reduce chemical inputs.

Kim *et al*. (2010) reported coinoculation of *Methylobacterium* and AM fungi in tomato stimulated the plant growth and increased the yield. To date none of the studies have been reported the disease reduction by co-inoculation of *Methylobacterium* and AM fungi. Accordingly, the present study was aimed to investigate the co-inouclation of *Methylobacterium* and AM fungi on induction of defense enzyme and suppression of root rot pathogens incidence of tomato under *in vitro* and field conditions.

2. MATERIAL AND METHODS

Bacterial strains, pathogens, culture conditions, and plant material

Pink pigmented facultative methylotrophs (PPFM's) were isolated from phylloplane of healthy tomato plants as described previously (Whittenbery *et al.,* 1970). Unless otherwise stated, PPFMs were grown for 72 h on AMS medium. Plant Pathogens like *Fusarium* oxysporum f. sp. *lycopersici* KACC 40032, *Sclerotium rolfsii* KACC 40957, *Ralstonia solanacearum* KACC 10694, *Pythium ultimum* KACC 40705 were obtained from Korean Agricultural Culture Collection, Suwon, Republic of Korea. Then *Rhizoctonia solani* MTCC 4633 and *Macrophomina phaseolina* were obtained from Microbial Type Culture Collection, Chandigarh, India and Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu. All the fungal pathogens were grown and maintained on potato dextrose agar (PDA) before use. Tomato cultivar Co-3, which is highly susceptible to both wilt and rots, was obtained from the Department of vegetables at Tamil Nadu Agricultural University, Coimbatore, India.

Morphological and biochemical characterization of Methylotrophs

The following morphological tests *viz*., colony colour, cell shape, Gram reaction, cyst formation, accumulation of poly ß-hydroxy butyrate granules and motility were carried out as described previously (Gerhardt *et al*. 1981). Methyl red test, Voges proskauer, nitrate reduction, catalase, indole production and starch hydrolysis were performed (Smibert and Krieg, 1994). All the methylobacterial isolates were tested for utilization of various carbon (ethanol, fructose, D- glucose, glycerol, methanol, sucrose, and acetate) at 0.5% level in AMS broth as described by Green and Bousifield (1982). The growth was observed after 14 days of incubation at 30ºC.

Plant hormones assay

Polysaccharide production was determined by (Dubois *et al*., 1951; Nelson, 1944). IAA was estimated using Salper's reagent (Ivanova *et al*., 2001). Extraction of gibberellins (Tien *et al*., 1979) and Spectrophotometric estimation of gibberellins (GA_3) was performed as per procedure followed by Mahadevan and Sridhar, (1982).

Antagonistic activity

The antifungal and antibacterial efficacies of *Methylobacterium* were tested by dual culture technique (Dennis and Webster, 1971) using PDA medium with obtained plant pathogens.

Molecular characterization and phylogenetic analysis

Molecular characterization of PPFG-1 alone was performed because, it consistently produced higher amount of plant growth hormones. Genomic DNA from the methylobacterial isolate PPFG-1 was isolated using the standard protocol of hexadecyl-trimethyl ammonium bromide (CTAB) method given by Melody (1997). The 16S rDNA nucleotide sequences were identified by PCR direct sequencing using the fluorescent dye terminator method with ABI Prism equipment and a Bigdye terminator cycle. The products were then purified using a Millipore-montage kit, and finally run in an automatic ABI3730XL capillary DNA sequencer (50 cm capillary). The complete 16S rDNA gene sequence was aligned and the affiliations deduced using BLAST analysis. The closest known relative of the new isolate was determined by comparing the sequences of closely related strains retrieved from EzTaxon server (http://www.eztaxon.org/; Chun *et al.,* 2007). The sequence of the new isolate and those retrieved from the public databases were initially aligned using the Clustal W Program (Thompson *et al.,* 1994). The method of Jukes and Cantor (1969) was used to calculate evolutionary distances; phylogenetic dendrogram was constructed using the neighbour-joining method (Saitou and Nei, 1987), and tree topologies evaluated by performing a bootstrap analysis of 1000 data set using MEGA 4.1. The evolutionary tree for the dataset was inferred using the neighbour-joining method. The 16S rDNA gene sequence of the isolate PPFG-1 (*Methylobacterium radiotolerans*) was deposited in the GenBank database under accession no. JN 047544.

***Isolation, identification and maintenance of Glomus* sp.**

The rhizosphere soil samples collected from maize plants were thoroughly mixed to form composite sample. From the composite sample, 100-250 g soil was sampled to isolate *Glomus* sp. spores by wet sieving and

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decanting technique (Gerdemann and Nicolson, 1963). The identification of *Glomus* sp. spores was done according to the description available in web site (http://www.invam.caf.wvu.edu). The identified *Glomus* sp. spores were picked up through capillary tube and it was transferred in to the plastic cup containing 3 to 4 days germinated roots of maize crop under aseptic condition. The root samples were stained to assess the AMF infection percentage in roots following the method of Phillips and Hayman (1970). The *Glomus* sp. was trapped by soil trap culture method (Murakoshi *et al*., 1998) and multiplied with maize cv., CO-1 as host plant in the pots containing sterilized soil. The pots were maintained in a green house for 3 months to develop the *Glomus* sp. inoculum. The propagules of the hyphae and spores of *Glomus* sp. present in the maize root and the associated rhizosphere soil served as inoculums source contained 200 spores $50g^{-1}$ of soil.

Glass house experiment

A Pot culture experiment in tomato (*Lycopersici esculantum*) CO 3 with eleven treatments and three replications was conducted to assess the disease recution by coincoulation of AM fungi and methylotrophs. The experiment was carried out in completely randomized block design with the following treatments: T_1 – *Glomus* sp.; T_2 – *Glomus* sp. + *Methylobacterium radiotolerans* PPFG -1; T_3 – *Fusarium oxysporum* f. sp. *lycopersici* (wilt pathogen); T_4 – *Macrophomina phaseolina* + *Sclerotium rolfsii* (root rot pathogen); T_5 – T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp; T_6 – T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp. ;T_7 – T_4 + *Methylobacterium radiotolerans* PPFG -1 + *Glomus* sp.; T_8 – T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp; T_9 – T_3 + *Pseudomonas fluorescens* Pf1 + *Trichoderma viride* ;T_{10} – T_4 + *Pseudomonas fluorescens* Pf1 + *Trichoderma viride*; T_{11} – Control; (T_1 to T_{10} – Received FYM + Azophos treatment).

The sandy loam soil was passed through a 4mm sieve and mixed along with farmyard manure in 2:1 proportion and filled in pots of 28x10cm size @ 5 kg pot^{-1}. The seeds were surface sterilized with 0.1per cent $HgCl_2$ for 3 min and washed three times successively in sterile distilled water and the sterilized seeds were sown (30 seeds per polybag). Surface sterilized seeds were imbibed with 15 ml of screened PPFG-1inoculam (10^9 cells ml^{-1} diluted at 1:1 ratio with sterile distilled water) for 6 h and shade dried for 30min before sowing. After 30 days 2 to 3 plants were transplanted in each pot.

Standard inoculum of PPFG (10^9 cells ml^{-1} of liquid culture) was diluted at 1:1 ratio with sterile water and sprayed on leaf at 6 PM in the evening to have uniform wetting. Foliar spray was given at 30, 60, 90 days after sowing. Standard inoculum of methylobacterial culture (diluted 1:100 ratio with sterile water) was mixed with lignite till 40 per cent water holding capacity and pH of the lignite was adjusted to neutral with $CaCO_3$. Then the lignite along with the methylobacterial culture was applied to the soil at the rate of 2 kg ha^{-1}. The *Glomus* sp. inoculum of 50g was spread 2.5cm below the soil surface at the time of sowing. Twenty grams of *Glomus* sp. inoculum is applied per seedling at the time of planting.

Talc based *Trichoderma* and pseudomonas was inoculated to the seeds at the rate of 4 g and 10g kg^{-1} of seeds (obtained from Department of Plant Pathology, Agricultural College and Research Institute, Madurai). Soil application of talc based *Trichoderma* and *Pseudomonas* was done with 2.5 kg/ha after mixing with 50 kg of farmyard manure. Soil application was done by mixing 4 kg^{-1} ha of Azophos (obtained from Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai).

The fungal pathogens were multiplied in sand – maize medium (Riker and Riker, 1993). The pathogens multiplied on sand maize medium were applied at a rate of 10 g kg^{-1} of soil to one month old tomato seedlings. Three replications (three pots per replication) were maintained and the pots were arranged in a randomized manner.

Under pot culture, plant growth biometric observations *viz*., shoot length, root length, plant dry weight, per cent disease incidence, germination percentage were recorded on 30, 60, 90 days after sowing. Seedling vigor index (SVI) was calculated as % germination × seedling length (shoot length + root length) in cm. (Baki and Anderson, 1973).

Evaluation of pathogenecity related proteins under pot culture conditions

In foliar application, 25 ml of liquid culture of *Methylobacterium* sp. (1×10^9 CFU ml^{-1} of culture) per pot was sprayed 40 days after planting and inoculated with root disease causing pathogens of tomato. Leaves were collected from tomato plants on 0, 1, 3, 5, and 7 days after challenge inoculation with pathogens, the seedlings were evaluated for various PR-proteins *viz*, chitinase, peroxidase, phenylalanine ammonia lyase (PAL), β-1,3 glucanase, polyphenol oxidase (PPO) and phenolics PAL activity was measured following the method of (Dickerson *et al*., 1984). β-1, 3-glucanase activity was assayed by the laminarin-dinitrosalicylic acid method (Pan *et al*., 1991), peroxidase (Hammerschmidt *et al*., 1982), polyphenol oxidase (Mayer *et al*., 1965) and phenolic compounds by (Swain and Hillis, 1959).

Fruit yield

Fruit yield per plant was recorded from the harvested fruit. The total number of fruits produced by the plant was counted and fruit yield was expressed in number plant^{-1}.

Statistical analysis

Statistical analyses of the data were done based on the methods given by Gomez and Gomez, 1984.

3. RESULTS

Isolation of Methylobacterium in the phylloplane of plants

Eight Methylotrophic bacteria were isolated from phylloplane of healthy tomato plants. They were identified based on the characteristics pink pigmented bacterial colonies having a diameter of 1-10 mm in AMS agar media. These isolates were further confirmed by the characteristics pink pigmented surface ring and thin pellicle indicating the aerobic nature in ammonium mineral salts (AMS) liquid media with methanol as sole source of carbon and energy. All the isolates were rod shaped and stained Gram negative. The isolates PPFC-1, PPFCF, PPFE, and PPFS were dark pink while the isolates PPFC-2 and PPFG-1 were light pink and the isolates PPFG-2 and PPFT were fairly pink and pinkish yellow respectively. The colony size varied from 0.3 to 2 mm. All the isolates accumulated PHB granules within the cells. All the isolates show motility in character. None of the isolates exhibited cyst formation. All the isolates tested were negative for methyl red, Voges-Proskauer, nitrate reduction, and indole production tests. The isolates exhibited pink pigmentation to varying degree in AMS agar for 7days. But all the isolates tested showed positive for catalase test and starch hydrolysis. All the isolates utilized glycerol, ethanol and methanol as carbon sources. Glucose was the preferred carbon source for all the methylobacterial isolates except PPFC-1, PPFT and PPFE. Mannitol was utilized as carbon sources by all the methylobacterial isolates except PPFCF. Acetate was utilized as carbon sources by all the methylobacterial isolates except PPFCG-2 and PPFE.

Production of polysaccharides and plant growth hormones by methylobacterial isolates

All the isolates secreted polysaccharides in very negligible amount. The content varied from 0.148mg of glucose ml-1 of culture filtrate in PPFC-1 to 1.632mg of glucose ml-1 of culture filtrate in PPFG-1. All the isolates tested produced the plant growth hormone indole acetic acid (IAA) and gibberellic acid (GA) under in vitro conditions. The maximum IAA was recorded in isolate PPFG-1 8.874µg ml-1 of culture filtrate. The maximum GA content (104.62µg ml-1) was recorded in PPFG-1.

Bacterial identification by16S rDNA sequence analysis

An almost complete 16S rDNA gene sequence of PPFG-1 was obtained (1381 bp). The 16S rDNA sequence based phylogenetic analysis of PPFG-1 revealed that the isolate more closely related to *Methylobacterium radiotolerans* and exhibited 99.93% similarity with type strain of *Methylobacterium radiotolerans* JCM^{T} 2831.

Evolutionary tree based on 16S rDNA with neighbor-joining method, strain PPFG-1 formed a stable clade with Methylobacterium radiotolerans JCMT 2831, which was supported by strong bootstrap value (52%).

Biocontrol effect of Methylobacterium radiotolerans PPFG-1 with Glomus sp. against root pathogens of tomato (CO 3) grown under pot culture conditions

Germination of the tomato seed was influenced due to combined inoculation of Methylobacterium radiotolerans PPFG-1 and Glomus sp. which showed highest germination of 96.67%. Glomus sp. with Methylobacterium radiotolerans PPFG-1 treated plants showed higher shoot and root lengths (35.70 and 7.41 cm plant-1). Seedling vigour index was higher 4141.34 when Glomus sp. was inoculated along with Methylobacterium radiotolerans PPFG-1 and registered lower disease incidence (3.33%) in nursery.

Glomus sp. with Methylobacterium radiotolerans PPFG-1 inoculation registered a higher shoot and root lengths (78.8 and 27.1 cm plant-1) and root lengths (27.1cm plant-1), dry weight (37.6g) and minimum disease incidence (14%) at 90 days of plant under pot culture condition.

Induction of systematic resistance in tomato under pot culture conditions

The plants challenge inoculated with wilt pathogens (*Fusarium oxysporum* f. sp. *lycopersici*, *Ralstonia solanacearum*) with *Methylobacterium radiotolerans* PPFG-1 along with *Glomus* sp. at fifth day after application recorded significantly higher Peroxidase (1.662 absorbance units min^{-1} g^{-1} of fresh weight), PPO (1.55absorbance units min^{-1} g^{-1} of fresh weight), PALase (139.05 µg trans-cinnamic acid min^{-1} g^{-1} of fresh weight), chitinase (3.498 nmol of N-acetylglucosamine equivalents released min^{-1} mg^{-1} of fresh weight, phenol content (0.428 µg phenol g^{-1} of fresh weight) and β-1, 3-glucanase content (95.58 nmol of reducing substances min^{-1} mg^{-1} of fresh weight).

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Yield of tomato (CO 3) grown under pot culture conditions

Total fruit yield per plant was maximum 162.8 g fruits plant^{-1} with 13.98 fruits plant^{-1} in plants inoculated with wilt pathogens (*Fusarium oxysporum* f. sp. *lycopersici*, *Ralstonia solanacearum*) with *Methylobacterium radiotolerans* PPFG-1 and *Glomus* sp. Plants challenge inoculated with wilt pathogens (*Fusarium oxysporum* f. sp. *lycopersici*, *Ralstonia solanacearum*) with *Pseudomonas fluorescens* Pf1 and *Trichoderma viride* registered 155.03 g fruits plant^{-1} with 11.43 fruits plant^{-1}. The minimum yield 110.76 g fruits plant^{-1} with 6.33 fruits plant^{-1} was found in uninoculated plants

4. DISCUSSION

A major biotechnology goal for sustainable agriculture is to use the microbial consortium of selected rhizosphere microorganisms to reduce inputs of chemical fertilizers. Further research on in-depth understanding of the cooperative microbial interactions will facilitate the successful application of *Methylobacterium*-AM fungi products in biotechnology. The majority of studies showed that elevated CO_2 had a positive influence on the abundance of arbuscular and ectomycorrhizal fungi. The numerous studies indicated that plant growth-promoting microorganisms positively affected plants subjected to drought stress. In the last few years, it has become evident, moreover, that some plant associated bacteria elicit a plant response and thereby induce systemic tolerance (Compant *et al*., 2010).

Kim *et al.* (2010) in a greenhouse experiment to examine the effects of inoculation with two *Methylobacterium oryzae* strains (CBMB20 and CBMB110) and a consortium of three arbuscular mycorrhizal (AM) fungi on the growth of red pepper (*Capsicum annum* L.,) reported a perfect mutualism among CBMB110-AMF resulting in improved macro- and micronutrient uptake along with higher chlorophyll content in red pepper. The pink pigmented facultative methylotrophs (PPFMs) are a group of non pathogenic phyllosphere microbes ubiquitously distributed on plants. Although they are not well known, these bacteria are co evolved, interacting partners in plant metabolism. They utilize the oxidized and substituted derivatives of methanol as sources of carbon and energy in addition to various multi-carbon compounds (Fall, 1996). They do not merely colonize the plants but are symbiotically related to them. Successfully co existence of PPFMs with plants is largely due to their ability to assimilate methanol which is a plant exometabolite (Holland and Polaco, 1994). They provide the plant with growth promoting substances, vitamins and enhance their resistance to various stresses. Unlike several other species of epiphytic bacteria, the strains of *Methylobacterium extorquens, M. mesophilicum* and *M. fujisawaense* do not catalyze ice nucleation. Hence, they do not increase frost injury to plants. These results indicate that the coexistence of PPFMB and plants is mutually beneficial and the bacteria can be protective for plants (Romanovskaya *et al*., 2001).

AMF are known to form a symbiotic association with a great majority of crop plants. It is well documented that these AMF enhance the plant growth through increased uptake of nutrients and alleviating various plant stresses (John *et al*., 1994; Tarfadar, 1995; Tohamy *et al*., 1999; Subramanian *et al*., 2001; Matsubara *et al*., 2004; Jalaluddin *et al*., 2008). More than 80% of the land plants form association with AM fungi (Smith *et al*., 2003). The mycorrhizal fungi are capable of producing morphological, physiological and biochemical modifications in the root cells that increase plant tolerance to root diseases (Mangala Rai, 2005).

PPFMs are easily identified by their pink colour, which distinguishes them from other unrelated methylotrophic organisms that may normally be encountered on plant phyllosphere (Corpe and Basile, 1982). All the methylobacterial isolates obtained during the study exhibited pink coloured smooth colony morphology with varying intensities of pink colour ranging from intense pink colour to light pink colour. In static liquid media, the isolates formed a pink surface ring or pellicle.

All the methylobacterial isolates tested negative for methyl red and Voges-Proskauer test, nitrate reduction test, indole production and positive for starch hydrolysis and catalase activity. The above facts with respect to the PPFMs were established by Ivanova *et al.* (2000; 2001). All isolates showed positive for starch hydrolysis. Methylotrophic bacteria are capable of growing on C_1 compounds such as formate, formaldehyde, and methanol as sole source of carbon and energy. They can also grow on a wide range of multi carbon growth substrates thus making them facultatively methylotrophic (Lidstrom, 1992). In the present study, the PPFM isolates were tested for utilization of the 6 carbon compounds and they showed wide variability in carbon utilization pattern. This test confirmed their facultatively methylotrophic nature.

Many methylotrophs can synthesize exopolysaccharides (Large and Bamforth, 1988) which play an important part in the formation of bacterial colonies on the surface of host plants. In the study, the polysaccharide content of the isolates varied from 0.148mg of glucose ml^{-1} of culture filtrate in PPFC1 to 1.632mg of glucose ml^{-1} of culture filtrate in PPFG1.

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According to the observation of Oleskin *et al*. (2000) a microbial cell (capsules and extracellular slime) merge to form a bipolymeric matrix composed of acidic polysaccharides and glycosyl phosphate containing bipolymers, such as teichoic acid and glycoprotein. Structurally, the matrix represents a system of microtubules intended for the movement of cells and the transport of various substances. The matrix also performs a protective function, preventing constituent cells and the whole colony form dehydration, extreme temperatures, the action of hydrolytic enzymes, UV radiation, etc. Some polysaccharide and peptide components of the matrix can act as cryo-, thermo-, and xeroprotectants (Trotsenko *et al*., 2001).

It has been shown that many aerobic methylotrophic bacteria were able to synthesize IAA (Doronina *et al,* 2001; Ivanova *et al,* 2001). In particular, various indole compounds were detected in the culture liquids of 37 methylotrophic bacteria belonging to different taxa (Ivanova *et al.,* 2001). In the present study, all the 8 isolates tested were found to produce IAA. The various isolates showed wide variation in the IAA content (8.874 µg ml^{-1} to 3.724 µg ml^{-1}). In the present study to estimate the production of GA by PPFMs it was found that all the 8 isolates tested produce gibberellic acid under *in vitro* conditions. The content varied from 12.76µg ml^{-1} to 104.62µg ml^{-1}.

Moreover, conventional methods of bacterial detection are limited by the time and labour involved in analyzing a large number of samples. Hence, molecular tool like PCR amplification of 16S rDNA sequences enables identification and discrimination of bacteria (Drancourt, 2000). It is also highly reliable as it provides unambiguous data even for rare isolates, which are difficult to identify with the phenotypic identification schemes. The potential of sequencing and comparison of 16S rDNA in the identification and discrimination of bacterial species has been well documented by various researchers (Kolbert *et al*., 1999 and Vela *et al*., 2002). In the present study, the isolate PPFG-1 exhibited 99.93% homology with type strain of *Methylobacterium radiotolerans.* Hence, it is concluded that PPFG-1 is another strain of *Methylobacterium radiotolerans* which has plant growth promoting activity.

Methylobacterium influence seed germination and seedling growth by producing plant growth regulators, cytokinins, and auxins which was previously reported by Dileepkumar and Dube, (1992). Thomson *et al.* (1996) who reported that the mycorrhizal tomato seedlings exhibited significantly high germination rate, shoot length, root length and dry matter than non-mycorrhizal plants. Beside, significant increase in seedling vigor would have occurred by better synthesis of auxins (Bharathi *et al.,* 2004). Seed imbibition of groundnut seeds with *Methylobacterium* sp. increased the germination percentage and seedling vigour (Madhaiyan *et al.,* 2006).

Satter and Khanam (2006) observed that the AM fungal inoculation helped to produce healthy and vigorous chilli seedlings with faster growth. Rice seeds were inoculated with *Methylobacterium* sp. strain PPFM-Os-07 and seed germination was evaluated in terms of morphometric measurements, seedling growth, rate of germination (RG), and seedling vigour index (SVI). The induction of pathogenesis-related proteins (PR-proteins) in rice plants inoculated with methylotrophic bacteria by seed imbibition or foliar spray (Madhaiyan *et al.,* 2004).

In present study *Methylobacterium radiotolerans* PPFG-1 along with *Glomus* sp. had highest germination increased the germination percentage showing higher germination of tomato seeds (96.67%) and seedling vigour index (4141.34) in nursery. Damping off of chilli incited by *Pythium aphanidermatum* is responsible for 90 percent mortality of seedlings both in nursery and field (Satter and Khanam, 2006). Arbuscular Mycorrhizal Fungi (AMF) effectively suppressed damping – off of cucumber (Rosendhal and Rosendhal, 1990) and pepper (Odebode *et al*., 2001).

In this study, *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. treated plants with challenge inoculation of wilt pathogen recorded the lowest value of root disease incidence (3.33%) in nursery. This result suggested that co-inoculation was effective in root disease control in tomato nursery.

Madhaiyan *et al.* (2006) reported that the percent disease index (PDI) value observed in *Methylobacterium* treated plants with challenge inoculation of pathogens was 16.5%, whereas PDIs in the pathogen treated plants were as high as 96.1% for *Aspergillus niger* and 94.8% for *Sclerotium rolfsii.*

The percent disease reduction recorded for seed imbibition alone and for combined applications of seed imbibition and phyllosphere spray were 17.8% and 23.5% in Rice seeds inoculated with *Methylobacterium* sp. strain PPFM-Os-07. The bacteria also significantly reduced the sheath blight incidence when applied as either bacterial culture through seed imbibition and/or phyllosphere spray. The percent disease reduction recorded for seed imbibition alone and for combined applications of seed imbibition and phyllosphere spray were 17.8% and 23.5% (Madhaiyan *et al.,* 2004).

The phenomenon of AMF protecting plants from root pathogens is known from studies involving root-infecting pathogens *eg., Phytophthora parasitica* or *Fusarium* sp., root-invading nematodes (Dodd, 2000) of horticultural and agricultural species such as tomato (*Lycopersicum esculentum* Mill.) and alfalfa (*Medicago sativa* L.) (Dehne and Schonbeck, 1979). *G. mosseae* induced local and systemic resistance to *P. parasitica* and was effective in reducing symptoms produced by this pathogen (Maria *et al*., 2002).

In this study, *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. treated plants with challenge inoculation of wilt pathogen under pot culture condition and *Methylobacterium radiotolerans* PPFG-1 applied as seedling root dip, foliar spray and soil application along with *Glomus* sp. under field condition recorded the lowest root disease incidence.

The treatment *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. under pot culture condition and *Methylobacterium radiotolerans* PPFG-1 applied as seedling root dip, foliar spray and soil application along with *Glomus* sp. under field condition recorded the highest dry weight compared to all other treatment. It was also previously reported that the dual inoculation of AM fungi with other beneficial microorganisms increased dry matter yields (Antunes and Cardoso, 1991; Boby *et al*., 2008; Medina *et al.,* 2003; Ortas *et al.,* 2002). *Methylobacterium* inoculation promoted seed germination and plant growth. Increased plant height, number of tillers, plant biomass, and grain yield were observed sixty-day-old rice plants grown in pots were challenge inoculated with *Rhizoctonia solani* strain TNAU-01. Increased plant height, number of tillers, plant biomass, and grain yield were observed. The average yield increases for seed imbibition and phyllosphere spray were, respectively, 22.1% and 24.3% greater than control (Madhaiyan *et al.,* 2004).

Madhaiyan *et al*. (2006) reported that under pot-culture conditions, in *Methylobacterium* sp. seed treated groundnut plants challenge-inoculated with *A. niger/*S. *rolfsii* through foliar sprays on day 30, the activities of enzymes PO, PAL, and β-1,3-glucanase increased constantly from 24 to 72 hours, after which decreased activity was noted. Five isozymes of polyphenol oxidase and PO could be detected in Methylobacterium-treated plants challenged with *A. niger* and *S. rolfsii*.

In the present investigation under pot culture condition induced systematic resistance activity in treatment *Fusarium oxysporum* f. sp. *lycopersici*, *Ralstonia solanacearum, Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. inoculated plants was increasing from 1st day after challenge inoculation with wilt pathogens and maximum was recorded on 5th day after challenge inoculation tomato plants and same result was noted under field conditions in treatment *Methylobacterium radiotolerans* PPFG-1 applied as seedling root dip, foliar spray and soil application along with *Glomus* sp.

Inoculation of *Azospirillum*, Phosphobacteria and *Methylobacterium* has resulted in increased rhizosphere population of the inoculants, drymatter production and yield of cotton under field condition (Raja and Sundaram, 2006). Hence due to the compatibility in the present study all the treatments were given Azophos and FYM.

In the present study the number of fruits per plant and total yield also increased substantially in the treatment *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. under pot culture condition and *Methylobacterium radiotolerans* PPFG-1 applied as seedling root dip, foliar spray and soil application along with *Glomus* sp. under field condition (30.53 yield ton ha^{-1}). PPFM inoculation has resulted in increased seedling vigour, dry matter production and yield of cotton under field condition (Raja and Sundaram, 2006). The increase in yield parameters is because of the several factors such as release of growth promoting substances like IAA, GA, and proliferation of beneficial organisms in the rhizosphere, in addition to nitrogen fixation and P transformation. Holland, (1997) reported the beneficial effects of application of methylotrophic bacteria through seed imbibition and phyllosphere spray in increasing soybean plant growth and productivity.

Like little farmers, PPFM bacteria play an important role in seed germination and seedling establishment. Holland (1997) reported that PPFMs could be used as seed inoculum or in seed coatings designed to enhance germinability, storability, or vigour of seeds. Enhanced plant growth was observed in treatments involving both seed imbibition and phyllosphere spray compared to either treatment alone or to control. *Methylobacterium* sp. strain PPFM-Os-07 treated plants showed increased numbers of tillers and height when compared to untreated control in rice (Madhaiyan *et al*., 2004).

At present recommendation of *Pseudomonas fluorescens* and *Trichoderma viride* was in practice for root diseases of tomato but the performance of coinoculants *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. was much better than those usual practices. The present study clearly indicated that co-inoculation of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. to tomato plants under pot culture was found to be

comparatively best treatment in terms of yield increase and less disease incidence than that of plants co-inoculated with *Pseudomonas fluorescens* Pf1 and *Trichoderma viride*. Also, combination of *Trichoderma viride* with *Methylobacterium radiotolerans* PPFG-1 was found to be best in plant growth promotion compared with co-inoculation of *Pseudomonas fluorescens* Pf1 and *Trichoderma viride* under field condition. Hence, increase in growth, reduction in disease incidence and higher yield in the co-inoculation of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. may be due to several factors such as increase of growth promoting substances like IAA, GA, ISR and proliferation of beneficial organisms in the rhizosphere. The role and contributions of *Glomus* sp. symbiosis to plant growth and biocontrol potential may be increased in association with selected *Methylobacterium* sp. Wilt disease incidence was lower when compared to root rot disease in treatments challenge inoculated with wilt pathogens along with *Methylobacterium radiotolerans* PPFG-1 and *Glomus* sp. in nursery and pot culture condition. Hence, this consortium will be more effective in controlling root diseases of tomato especially wilt pathogens.

Till to date no reports were found on inoculation of PPFM with AM fungi, induces defense enzymes in plants. The above findings evidenced that the inoculation of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. improved plant growth along with induction of defense enzymes of tomato. Further research in understanding the cooperative microbial interactions will facilitate the successful application of microbial consortium based products for effective disease management with nutrient transformation.

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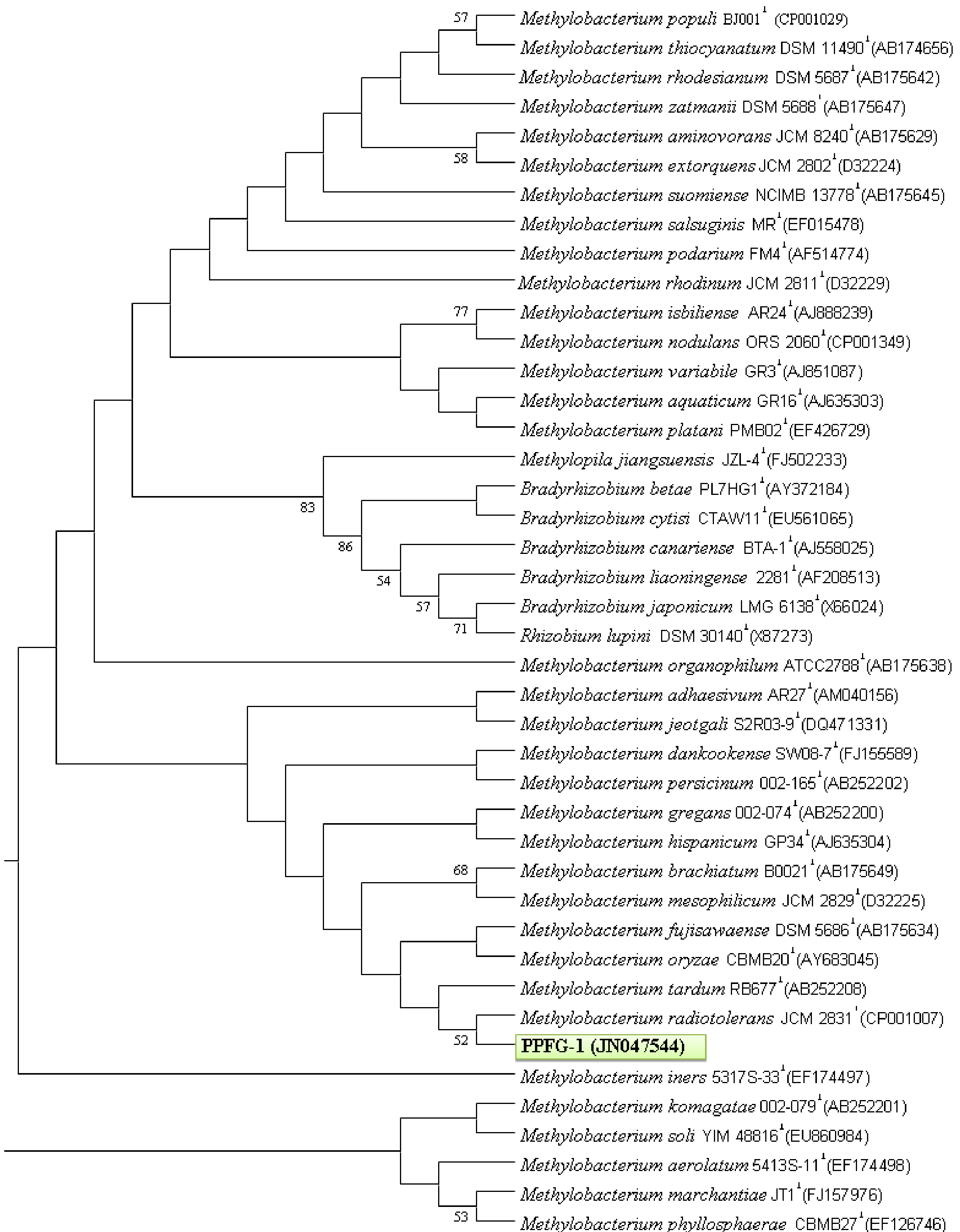

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rDNA sequence of methylobacterial isolate PPFG-1

Table 1. Morphological and biochemical characterization of methylobacterial isolates

Sl. No	Isolates	Size (mm)	Shape of colony	Cells shape	Pink pigmentation	Glucose	Mannitol	Acetate
1.	PPFC-1	1	Slimy	Rod	Dark pink	-	+	+
2.	PPFC-2	1.5	Slimy	Rod	Light pink	+	+	+
3.	PPFG-1	0.4	Slimy	Rod	Light pink	+	-	+
4.	PPFG-2	0.3	Slimy	Rod	Fair pink	+	+	-

5.	PPFCF	1.9	Slimy	Rod	Dark pink	+	-	+
6.	PPFT	2	Slimy	Rod	pinkish yellow	-	+	+
7.	PPFE	2	Slimy	Rod	Dark pink	-	+	-
8.	PPFS	1.5	Slimy	Rod	Dark pink	+	+	+

All are Gram Negative, motile , accumulated PHB granules and did not produce cyst. All isolates are positive for catalase and starch hydrolysis and indole production and negative for methyl red, Voges-Proskauer, and nitrate reduction . all isolates assimilated the methanol, ethanol and glycerol.

Table 2. *In vitro* production of polysaccharide, indole acetic acid and gibberellins in AMS broth by methylobacterial isolates 10 days after incubation.

Sl. No	Isolates	Polysaccharide production (mg of glucose ml^{-1})	Indole acetic acid (µg ml^{-1})	Gibberellins (µg ml^{-1})
1.	PPFC-1	0.148 ± 0.003^{f}	4.539 ± 0.091^{f}	61.83 ± 1.237^{f}
2.	PPFC-2	0.155 ± 0.003^{g}	3.724 ± 0.075^{g}	85.39 ± 1.708^{d}
3.	PPFG-1	1.632 ± 0.033^{a}	8.874 ± 0.178^{a}	104.62 ± 2.093^{a}
4.	PPFG-2	0.265 ± 0.005^{d}	6.578 ± 0.132^{d}	12.76 ± 0.255^{g}
5.	PPFCF	1.568 ± 0.031^{b}	8.526 ± 0.171^{b}	99.51 ± 1.990^{b}
6.	PPFT	0.274 ± 0.006^{e}	5.722 ± 0.114^{e}	89.86 ± 1.797^{c}
7.	PPFE	0.784 ± 0.016^{f}	4.361 ± 0.087^{f}	64.36 ± 1.287^{f}
8.	PPFS	0.816 ± 0.016^{c}	6.844 ± 0.137^{c}	70.14 ± 1.403^{e}

Table 3. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on growth of tomato seedlings (CO 3) in nursery (15 DAS)

S.No.	Treatments	Shoot length (cm)	Root length (cm)
1.	*Glomus* sp.	26.82 ± 0.54^{c}	4.59 ± 0.092
2.	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	35.70 ± 0.71^{a}	7.41± 0.15
3.	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	19.53 ± 0.39^{g}	4.86 ± 0.097
4.	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	14.28 ± 0.29^{h}	3.92 ± 0.078
5.	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	30.98 ± 0.62^{b}	7.14 ± 0.14
6.	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	27.13 ± 0.55^{c}	5.11 ± 0.10
7.	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	26.72 ± 0.53^{cd}	4.54 ± 0.09
8.	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	25.92 ± 0.52^{d}	4.28 ± 0.085
9.	T_3 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	24.46 ± 0.49^{e}	4.29 ± 0.058
10.	T_4 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	26.48 ± 0.53^{cd}	3.24 ± 0.064
11.	Control	22.52 ± 0.51^{f}	2.94 ± 0.058
	Mean	25.50	4.76
	Sed	0.05	0.01
	CD (*P*=0.05)	0.10	0.02

DAS: Days after sowing

Table 3. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on germination percentage and seedling vigour index of tomato seedlings (CO 3) in nursery (15 DAS)

Sl.No.	Treatments	Germination Percentage	Seedling vigour index
1.	*Glomus* sp.	66.67 ± 1.33^{e}	2094.11 ± 41.9^{e}
2.	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	96.67 ± 1.93^{a}	4141.34 ± 82.8^{a}
3.	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	40.00 ± 0.8^{i}	975.60 ± 19.5^{i}
4.	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	36.67 ± 0.73^{j}	667.39 ± 13.3^{j}
5.	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	86.67 ± 1.73^{b}	3327.26 ± 66.5^{b}
6.	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	73.33 ± 1.46^{d}	2364.16 ± 47.2^{d}
7.	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	66.67 ± 1.33^{e}	2084.10 ± 41.6^{e}
8.	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	83.33 ± 1.67^{c}	2516.57 ± 50.3^{c}
9.	T_3 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	56.67 ± 1.13^{g}	1629.26 ± 32.6^{g}
10.	T_4 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	63.33 ± 1.26^{f}	1882.17 ± 37.6^{f}
11.	Control	50.00 ± 1^{h}	1273 ± 25.46^{h}
	Mean	65.46	2086.82
	Sed	0.31	8.24
	CD (*P*=0.05)	0.65	17.19

DAS: Days after sowing

Table 4. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on disease incidence in tomato seedlings (CO 3) grown in nursery (15 DAS)

Sl. No	Treatments	Disease incidence (%)
1.	*Glomus* sp.	46.67 ± 0.93^{h}
2.	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	3.33 ± 0.06^{a}
3.	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	93.33 ± 1.86^{j}
4	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	96.67 ± 1.93^{k}
5.	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	13.33 ± 0.27^{b}
6.	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	26.67 ± 0.53^{d}
7.	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	16.67 ± 0.33^{c}
8.	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	33.33 ± 0.67^{e}
9.	T_3 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	43.33 ± 0.87^{g}
10.	T_4 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	36.67 ± 0.73^{f}
11.	Control	50.00 ± 1^{i}
	Mean	41.82
	Sed	0.39
	CD (*P*=0.05)	0.81

DAS: Days after sowing

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Table 5. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on shoot length and root length of tomato (CO 3) grown under pot culture condition

S.No	Treatments	Shoot length (cm plant $^{-1}$)				Root length (cm plant $^{-1}$)			
		DAS				DAS			
		30	60	90	Mean	30	60	90	Mean
1	*Glomus* sp.	42.4 ± 0.84b^{c}	69.3 ± 1.39^{ab}	73.2 ± 1.46^{bc}	61.63	13.8 ± 0.28^{e}	17.1 ± 0.34^{e}	20.2 ± 0.40^{e}	17.03
2	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	45.1 ± 0.90^{a}	71.5 ± 1.43^{a}	78.8 ± 1.58^{a}	65.13	18.8 ± 0.38^{a}	23.2 ± 0.46^{a}	27.1 ± 0.54^{a}	23.03
3	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	24.5 ± 0.49^{g}	53.7 ± 1.07^{e}	54.3 ± 1.09^{g}	44.17	11.3 ± 0.23^{f}	15.9 ± 0.32^{f}	17.4 ± 0.35^{f}	14.87
4	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	25.9 ± 0.52^{f}	56.5 ± 1.074^{d}	57.4 ± 1.15^{f}	46.60	10.4 ± 0.21^{g}	14.2 ± 0.284^{g}	17.8 ± 0.36^{f}	14.13
5	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	43.4 ± 0.87^{b}	70.3 ± 1.13^{ab}	72.2 ± 1.4^{cd}	61.97	18.4 ± 0.37^{a}	22.0 ± 0.44^{a}	26.5 ± 0.53^{a}	22.30
6	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	42.5 ± 0.85^{bc}	68.3 ± 1.41^{b}	70.8 ± 1.42^{d}	60.53	17.6 ± 0.35^{b}	21.8 ± 0.44^{b}	25.1 ± 0.50^{b}	21.50
7	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	43.2 ± 0.86^{b}	70.4 ± 1.37^{ab}	74.5 ± 1.49^{b}	62.70	16.0 ± 0.32^{c}	20.7 ± 0.41^{c}	22.5 ± 0.45^{c}	19.73
8	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	41.4 ± 0.83^{cd}	71.2 ± 1.41^{a}	72.4 ± 1.45^{bcd}	61.67	15.64 ± 0.31^{c}	19.24 ± 0.39^{d}	22.38 ± 0.48^{cd}	19.09
9	T_3 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	40.5 ± 0.81^{d}	69.3 ± 1.42^{ab}	71.1 ± 1.3^{cd}	60.30	14.5 ± 0.29^{d}	18.2 ± 0.36^{c}	21.8 ± 0.44^{d}	18.17
10	T_4 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	40.44 ± 0.81^{d}	64.96 ± 1.39^{c}	67.94 ± 1.0^{e}	57.78	13.6 ± 0.27^{e}	17.4 ± 0.35^{e}	20.3 ± 0.41^{e}	17.10
11	Control	30.8 ± 0.62^{e}	44.4 ± 1.3^{f}	50.4 ± 1.01^{h}	41.87	10.5 ± 0.21^{f}	12.1 ± 0.24^{g}	14.0 0.28^{g}	12.20
	Mean	37.51	64.16	69.42	57.03	14.59	18.35	21.37	18.10

DAS: Days after sowing

	Shoot length		Root length	
	Sed	**CD (P=0.05)**	**SEd**	**CD (P=0.05)**
T	0.28	0.55	0.09	0.18
R	0.15	0.29	0.05	0.09
TxR	0.48	0.96	0.15	0.30

Table 6. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on disease incidence and dry weight of tomato (CO 3) grown under pot culture condition

S.No	Treatments	Disease incidence (%)				Dry weight (g plant $^{-1}$)			
		DAS				DAS			
		30	60	90	Mean	30	60	90	Mean
1	*Glomus* sp.	16.0 ± 0.32^{e}	20.7 ± 0.41^{g}	22.5 ± 0.45^{e}	19.73	14.4 ± 0.29^{f}	28.7 ± 0.57^{d}	33.2 ± 0.66^{cd}	25.43
2	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	10.5 ± 0.21^{a}	12.1± 0.24^{a}	14.0 ± 0.28^{a}	12.20	17.4 ± 0.35^{a}	34.0 ± 0.68^{a}	37.6 ± 0.75^{a}	29.67
3	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	18.4 ± 0.37^{g}	22.0 ± 0.44^{h}	26.5 ± 0.53^{g}	22.30	14.2 ± 0.28^{f}	29.1 ± 0.58^{d}	33.9 ± 0.68^{c}	25.73
4	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	18.8 ± 0.38^{g}	23.2 ± 0.46^{i}	27.1 ± 0.54^{g}	23.03	14.1 ± 0.28^{f}	26.9 ± 0.54^{e}	31.4 ± 0.63^{e}	24.13
5	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	10.4 ± 0.21^{a}	14.2 ± 0.28^{b}	17.8 ± 0.36^{b}	14.13	16.3 ± 0.33^{b}	32.9 ± 0.66^{b}	36.2 ± 0.72^{b}	28.47
6	T_3 + *Methylobacterium* sp. PPFCF +	11.3 ± 0.23^{b}	15.9 ± 0.32^{c}	17.4 ± 0.35^{b}	14.87	15.4 ± 0.31^{c}	31.7 ± 0.63^{c}	35.8 ± 0.72^{b}	27.63

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	Glomus sp.								
7	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	13.6 ± 0.27^{c}	17.4 ± 0.35^{d}	20.3 ± 0.41^{c}	17.10	15.65 ± 0.31^{c}	32.98 ± 0.66^{b}	36.34 ± 0.73^{b}	28.32
8	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	13.8 ± 0.28^{c}	17.1 ± 0.34^{d}	20.2 ± 0.40^{c}	17.03	14.94 ± 0.3^{d}	31.56 ± 0.63^{c}	35.23 ± 0.70^{b}	27.24
9	T_3 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	15.64 ± 0.313^{e}	19.24 ± 0.38^{f}	22.38 ± 0.45^{d}	19.09	*14.66 ± 0.29*^{de}	29.38 ± 0.59^{d}	33.46 ± 0.67^{cd}	25.83
10	T_4 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	14.5 ± 0.29^{d}	18.2 ± 0.36^{e}	21.8 ± 0.44^{d}	18.17	13.43 ± 0.27^{g}	27.45 ± 0.55^{e}	32.54 ± 0.65^{de}	24.47
11	Control	17.6 ± 0.35^{f}	21.8 ± 0.43^{h}	25.1 ± 0.5^{f}	21.50	11.1 ± 0.22^{h}	24.4 ± 0.49^{f}	28.4 ± 0.57^{f}	21.30
	Mean	14.59	18.35	21.37	18.10	14.69	29.92	34.01	26.20

DAS: Days after sowing

	Disease incidence		**Dry weight**	
	Sed	**CD (*P*=0.05)**	**SEd**	**CD (*P*=0.05)**
T	0.18	0.36	0.13	0.26
R	0.09	0.18	0.07	0.14
TxR	0.30	0.61	0.23	0.45

Table 7. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on per plant fruit yield of tomato (CO 3) under pot culture condition

S. No	**Treatments**	**Number of fruits (plant $^{-1}$)**	**Total fruit yield (g plant^{-1})**
1.	*Glomus* sp.	10.34 ± 0.21^{f}	147.40 ± 2.95^{e}
2.	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	15.21 ± 0.30^{a}	158.61 ± 3.17^{abc}
3	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	5.12 ± 0.10^{h}	95.34 ± 1.91^{g}
4.	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	4.23 ± 0.09^{i}	86.92 ± 1.74^{h}
5.	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	13.98 ± 0.28^{b}	162.85 ± 3.26^{a}
6.	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	13.33 ± 0.27^{c}	160.56 ± 3.21^{ab}
7.	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	12.88 ± 0.26^{d}	159.43 ± 3.19^{ab}
8.	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	12.67 ± 0.25^{d}	157.34 ± 3.15^{bc}
9.	T_3 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	11.43 ± 0.23^{e}	155.03 ± 3.1^{cd}
10.	T_4 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	11.21 ± 0.22^{e}	150.65 ± 3.01^{de}
11.	Control	6.33 ± 0.13^{g}	110.76 ± 2.22^{f}
	Grand Mean	10.61	140.44
	SED	0.18	2.34
	CD (*P*=0.05)	0.38	4.84

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Table 8. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on induction of peroxidase (PO) activity of tomato (CO 3) grown under pot culture condition

S. No	Treatments	PO (change in absorbance units min^{-1} g^{-1} of fresh weight)					Mean
		DAA					
		0	1	3	5	7	
1.	*Glomus* sp.	0.519	0.725	0.793	0.983	0.748	0.75
2.	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	0.558	0.754	0.826	0.995	0.842	0.80
3.	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	0.195	0.137	0.134	0.472	0.157	0.22
4.	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	0.146	0.178	1.169	0.436	0.193	0.42
5.	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	0.755	0.976	0.987	1.662	1.356	1.15
6.	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	0.744	0.948	1.964	1.597	1.334	1.32
7	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	0.663	0.918	1.872	1.295	1.198	1.19
8	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	0.637	0.881	0.864	1.246	1.128	0.95
9	T3 + *Pseudomonas fluorescens* Pf1+ *Trichoderma viride*	0.632	0.724	0.469	0.5834	0.948	0.67
10	T4 + *Pseudomonas fluorescens* + *Trichoderma viride*	0.607	0.695	0.458	0.869	0.914	0.71
11	Control	0.336	0.438	0.173	0.534	0.392	0.37
	Mean	0.53	0.67	0.88	0.97	0.84	0.78

DAA: Days after pathogen and treatment application

	SEd	CD (*P*=0.05)
T	0.007	0.013
R	0.004	0.009
TxR	0.015	0.029

Table 9. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on induction of poly phenol oxidase (PPO) activity of tomato (CO 3) grown under pot culture condition

S. No	Treatments	PPO (change in absorbance units min^{-1} g^{-1} of fresh weight)					Mean
		DAA					
		0	1	3	5	7	
1.	*Glomus* sp.	0.558	0.627	0.705	0.784	0.607	0.66
2.	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	0.581	0.652	0.734	0.816	0.632	0.68
3.	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	0.151	0.235	0.168	0.365	1.366	0.46
4.	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	0.149	0.292	0.173	0.375	1.283	0.45
5.	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	0.738	0.785	1.152	1.55	0.846	1.01
6.	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	0.675	0.754	1.174	1.489	0.813	0.98

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7	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	0.612	0.765	0.836	0.969	0.663	0.77
8	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	0.568	0.735	0.83	0.931	0.637	0.74
9	T3 + *Pseudomonas fluorescens* + *Trichoderma viride*	0.548	0.683	0.765	0.846	0.652	0.70
10	T4 + *Pseudomonas fluorescens* + *Trichoderma viride*	0.519	0.656	0.735	0.813	0.627	0.67
11	Control	0.411	0.438	0.558	0.676	0.548	0.53
	Mean	0.50	0.60	0.71	0.87	0.79	0.70

DAA: Days after pathogen and treatment application

	SEd	CD (*P*=0.05)
T	0.006	0.011
R	0.004	0.007
TxR	0.012	0.025

Table 10. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on induction of phenyl alanine ammonia lyase (PAL) activity of tomato (CO 3) grown under pot culture condition

S. No	Treatments	PAL (µg *trans*-cinnamic acid $min^{-1}g^{-1}$ fresh weight)					Mean
		DAA					
		0	1	3	5	7	
1.	*Glomus* sp.	55.19	78.25	91.75	112.51	101.16	87.77
2.	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	57.44	81.44	95.52	119.98	112.04	93.28
3.	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	39.22	46.13	48.36	63.68	62.3	51.94
4.	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	37.69	44.32	46.47	61.19	59.85	49.90
5.	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	61.18	88.25	100.39	139.05	122.54	102.28
6.	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	58.72	84.78	96.46	133.87	117.73	98.31
7	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	56.86	84.13	93.36	114.56	97.35	89.25
8	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	57.71	81.04	89.69	112.77	93.23	86.89
9	T3 + *Pseudomonas fluorescens* + *Trichoderma viride*	53.56	77.75	85.39	108.34	88.17	82.64
10	T4 + *Pseudomonas fluorescens* + *Trichoderma viride*	51.45	74.7	83.68	103.55	87.25	80.13
11	Control	42.14	52.46	71.16	87.83	56.88	62.09
	Mean	51.92	72.11	82.02	105.21	90.77	80.41

DAA: Days after pathogen and treatment application

	SEd	CD (*P*=0.05)
T	0.62	1.22
R	0.42	0.82
TxR	1.38	2.73

Table 11. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on induction of chitinase activity of tomato (CO 3) grown under pot culture condition

S. No	Treatments	Chitinase (nmol of N-Acetylglucosamine equivalents released $min^{-1}g^{-1}$ fresh weight)					Mean
		DAA					
		0	1	3	5	7	
1.	*Glomus* sp.	2.646	2.734	2.842	3.108	2.891	2.84
2.	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	2.754	2.845	2.958	3.141	3.009	2.94
3.	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	2.295	2.264	2.56	2.458	2.412	2.40
4.	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	2.205	2.175	2.366	2.361	2.323	2.29
5.	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	3.08	3.202	3.304	3.498	3.366	3.29
6.	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	3.009	3.077	3.233	3.427	3.304	3.21
7	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	2.959	3.115	3.175	3.361	3.121	3.15
8	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	2.891	3.039	3.106	3.213	3.039	3.06
9	T3 + *Pseudomonas fluorescens* + *Trichoderma viride*	2.886	2.947	3.036	3.116	2.92	2.98
10	T4 + *Pseudomonas fluorescens* + *Trichoderma viride*	2.773	2.832	3.009	3.141	2.529	2.86
11	Control	2.548	2.723	2.917	3.087	2.427	2.74
	Mean	2.73	2.81	2.96	3.08	2.85	2.89

DAA: Days after pathogen and treatment application

	SEd	CD (*P*=0.05)
T	0.61	1.22
R	0.42	0.82
TxR	1.38	2.73

Table 12. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on induction of total phenol content of tomato (CO 3) grown under pot culture condition

S. No	Treatments	Total phenol (µg phenol g^{-1} of fresh weight)					Mean
		DAA					
		0	1	3	5	7	
1.	*Glomus* sp.	0.323	0.331	0.352	0.362	0.352	0.34
2.	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	0.336	0.346	0.367	0.377	0.367	0.36
3.	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	0.265	0.295	0.285	0.316	0.284	0.29
4.	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	0.254	0.284	0.274	0.303	0.274	0.28
5.	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	0.377	0.387	0.408	0.428	0.408	0.40

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6.	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	0.362	0.372	0.392	0.411	0.401	0.39
7	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	0.367	0.367	0.387	0.408	0.392	0.38
8	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	0.346	0.352	0.372	0.392	0.386	0.37
9	T3 + *Pseudomonas fluorescens* + *Trichoderma viride*	0.336	0.357	0.367	0.387	0.375	0.36
10	T4 + *Pseudomonas fluorescens* + *Trichoderma viride*	0.323	0.343	0.352	0.382	0.367	0.35
11	Control	0.275	0.284	0.295	0.316	0.295	0.29
	Mean	0.32	0.34	0.35	0.37	0.35	0.35

DAA: Days after pathogen and treatment application

	SEd	**CD (*P*=0.05)**
T	0.003	0.005
R	0.002	0.003
TxR	0.006	0.011

Table 13. Biocontrol effect of *Methylobacterium radiotolerans* PPFG-1 with *Glomus* sp. against root pathogens and their influence on induction of β 1,3 glucanase activity of tomato (CO 3) grown under pot culture condition

S. No	**Treatments**	**β 1,3 glucanase (nmol of reducing substances min^{-1} mg^{-1} of fresh weight)**					**Mean**
		DAA					
		0	**1**	**3**	**5**	**7**	
1.	*Glomus* sp.	79.38	82.62	84.27	85.95	82.58	82.96
2.	*Glomus* sp. + *Methylobacterium radiotolerans* PPFG-1	80.46	82.54	84.11	87.72	84.5	83.87
3.	*Fusarium oxysporum* f. sp. *lycopersici* + *Ralstonia solanacearum*	77.42	80.08	81.68	83.54	80.04	80.55
4.	*Macrophomina phaseolina* + *Rhizoctonia solani* + *Sclerotium rolfsii* + *Pythium ultimum*	77.6	80.1	82.63	84.79	80.76	81.18
5.	T_3 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	88.2	91.8	93.63	95.58	91.75	92.19
6.	T_3 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	86.24	89.76	91.55	93.38	89.71	90.13
7	T_4 + *Methylobacterium radiotolerans* PPFG-1 + *Glomus* sp.	84.28	87.92	89.67	91.46	87.87	88.24
8	T_4 + *Methylobacterium* sp. PPFCF + *Glomus* sp.	83.3	86.7	88.43	90.19	86.66	87.06
9	T3 + *Pseudomonas fluorescens* + *Trichoderma viride*	82.32	85.68	87.39	89.13	85.64	86.03
10	T4 + *Pseudomonas fluorescens* + *Trichoderma viride*	81.34	84.66	86.35	88.07	84.63	85.01
11	Control	79.87	83.16	85.91	87.45	83.05	83.89
	Mean	81.86	85.00	86.87	88.84	85.20	85.55

DAA: Days after pathogen and treatment application

	SEd	**CD (*P*=0.05)**
T	0.63	1.24
R	0.42	0.84
TxR	1.40	2.77