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RESEARCH ARTICLE

Investigation on the effect of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria against wilt caused by *Fusarium oxysporum* f. sp. *cubense* in banana

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Abstract

Fusarium wilt of banana is a significant limitation to banana production in many countries around the globe, which is caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). This research was intended to determine the good performance of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) as sustainable biocontrol agents against Foc. Disease prevalence was recorded to be 10 and 48 % across large areas of Trichy district as indicated by a field survey done in major banana-producing regions. Microbial analysis in the rhizosphere resulted in habitation of two predominant AM fungal species, *Acaulospora laevis* and *Glomus intraradices*, along with several isolates of PGPR. *Bacillus tequilensis* was found to have better plant growth-promoting properties and strong antagonistic activity against Foc under *in vitro* conditions in comparison to the other strains of the PGPR. *In vivo* analysis showed that the combined inoculation of AMF and PGPR produced significant improvements in plant growth parameters such as the plant height and the number of leaves in comparison to the uninoculated control group. It is important to note that the combined treatment (*A. laevis* + *B. tequilensis*) significantly lowered disease incidence, in comparison to control plants. Biochemical tests also showed improved activity of defence activities like peroxidase, polyphenol oxidase, catalase and superoxide dismutase as well as improved phenolic content, which suggested systemic induced resistance. The research determines that the combination of AMF and PGPR is an effective and environmentally friendly approach of controlling the Fusarium wilt disease in banana.

Keywords: arbuscular mycorrhizal fungi; *Bacillus tequilensis*; banana; Fusarium wilt; plant growth-promoting rhizobacteria

Introduction

Banana (*Musa* spp.) is one of the most important fruit crops cultivated globally due to its high nutritional value, economic significance and role in food security. It serves both as a staple food and a dessert fruit for millions of people owing to its rich carbohydrate content and calorific value (67–137 kcal per 100 g fresh weight) (1). India is the largest producer of banana, contributing approximately 37.30 % of global production, thereby playing a pivotal role in sustaining agricultural economies and livelihoods. Despite its importance, banana production is severely constrained by several biotic stresses, among which Fusarium wilt, commonly known as Panama disease, remains the most devastating (2).

Fusarium wilt of banana is caused by the soil-borne fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (Foc), which is regarded as one of the most destructive diseases affecting banana cultivation worldwide. The disease was among the first to spread globally in the early twentieth century and continues to threaten banana production systems (3, 4). The pathogen infects plants through the root system and colonises the vascular tissues, leading to internal discoloration, disruption of water transport, progressive leaf wilting and eventual plant death. The absence of visible symptoms in fruits further complicates early detection and management (5–7). Moreover, the persistence of chlamydospores in soil for prolonged periods makes eradication extremely difficult. Conventional management strategies, including chemical fungicides and cultural practices, have shown limited effectiveness and may pose environmental and health risks, thereby necessitating the development of sustainable and eco-friendly alternatives.

In this context, biological control using beneficial microorganisms has emerged as a promising approach for managing soil-borne pathogens. Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms that form mutualistic associations with the roots of most terrestrial plants (8, 9). In this symbiotic relationship, the plant supplies carbon to the fungus, while the fungus enhances nutrient acquisition, particularly phosphorus,

through an extensive extraradical hyphal network. Beyond nutrient uptake, AMF play a crucial role in improving plant tolerance to biotic and abiotic stresses by modulating physiological and biochemical processes. Mycorrhizal colonization has been associated with structural modifications such as cell wall thickening and improved vascular development, which contribute to enhanced resistance against root pathogens.

Plant growth-promoting rhizobacteria (PGPR) represent another group of beneficial microorganisms that colonise the rhizosphere and promote plant growth through diverse mechanisms (10, 11). These include the production of phytohormones such as indole-3-acetic acid (IAA), solubilisation of essential nutrients and synthesis of siderophores and antibiotics that suppress pathogenic microorganisms (12, 13). Plant growth-promoting rhizobacteria are also known to induce systemic resistance (ISR) in plants by activating defence-related pathways, thereby enhancing the plant's ability to withstand pathogen attack. Under field conditions, certain PGPR strains, particularly *Pseudomonas* spp., have been reported to reduce Fusarium wilt incidence by up to 79 %, highlighting their significant biocontrol potential (14).

Several studies have demonstrated that AMF can independently reduce the severity of diseases caused by soil-borne pathogens, including fungi and nematodes (15). This reduction is attributed to both morphological and physiological changes in the host plant, such as increased accumulation of phenolic compounds, enhanced phosphorus uptake and activation of defence-related enzymes (16). Additionally, AMF colonisation has been shown to trigger biochemical responses, including increased activity of peroxidase, polyphenol oxidase and other antioxidant enzymes, which contribute to the suppression of pathogen development (17). The effectiveness of AMF in disease management has been reported in several crops, including tomato, chickpea and banana, where species such as *Glomus intraradices* and *Acaulospora laevis* significantly reduced disease incidence (18). The combined application of AMF and PGPR has gained increasing attention due to their synergistic interactions in the rhizosphere. Such interactions enhance plant growth, nutrient uptake and resistance to pathogens more effectively than individual inoculations. Dual inoculation has been reported to significantly improve shoot and root growth, dry matter accumulation and yield parameters in various crops (19). The synergistic effect is attributed to the complementary mechanisms of AMF and PGPR, including improved nutrient acquisition, enhanced root colonisation and increased production of bioactive compounds that inhibit pathogen growth.

Among PGPR, *Bacillus tequilensis*, a gram-positive, spore-forming bacterium, has been identified as a potent biocontrol agent against several plant pathogens (20). This bacterium exhibits strong antagonistic activity through the production of antibiotics, siderophores and lytic enzymes, as well as the induction of systemic resistance in plants. In addition to its biocontrol properties, *B. tequilensis* enhances plant growth by producing IAA, solubilising phosphate and improving nutrient uptake. It also plays a crucial role in activating antioxidant defence systems, including superoxide dismutase, catalase and peroxidase, thereby reducing oxidative stress during pathogen infection. Furthermore, increased accumulation of phenolic compounds and flavonoids in treated plants contributes to enhanced resistance against pathogens (21). Despite the growing body of evidence supporting the individual roles of AMF and PGPR in disease management, there is limited information on their combined efficacy under field conditions, particularly in the context of Fusarium wilt of banana. Understanding the synergistic interactions between these beneficial microorganisms is essential for developing effective bioinoculant formulations and integrated disease management strategies (22).

The present study is significant in addressing the persistent challenge of Fusarium wilt in banana by developing a sustainable and eco-friendly biocontrol strategy as an alternative to conventional chemical methods, which are often ineffective against soil-borne pathogens and pose environmental risks. This work is novel in its integrative approach, combining field survey, microbial isolation, *in vitro* screening and *in vivo* validation to evaluate the synergistic interaction between AMF and PGPR. Unlike previous studies that have largely focused on individual bioinoculants, this research demonstrates the enhanced efficacy of a microbial consortium, particularly *Acaulospora laevis* and *Bacillus tequilensis*, in suppressing *Fusarium oxysporum* f. sp. *cubense* while simultaneously improving plant growth, yield and physiological performance. The study further provides mechanistic insights into induced systemic resistance through elevated activities of defence-related enzymes such as peroxidase, polyphenol oxidase, catalase and superoxide dismutase, along with increased phenolic accumulation. Importantly, the validation of these effects under field conditions highlights the practical applicability and scalability of the proposed bioinoculant strategy. Overall, this work contributes a novel and robust framework for the development of microbial consortia for sustainable disease management in banana cultivation.

Materials and Methods

Study location

The current study was conducted in the laboratory and in the field. The laboratory experiments transpired at the Department of Fruit Science and Horticultural College and Research Institute of Women and Tiruchirappalli, Tamil Nadu, India. Further experimental research was done at the Department of Natural Resource Management, Horticultural College and Research Institute, Periyakulam, Tamil Nadu Agricultural University (TNAU), India, in terms of microbial analysis and biochemical research. To determine the *in vivo* efficacy of bioinoculants in the presence of natural disease pressure, field experiments were carried out in an infested (sick) Fusarium wilt a plot at Melmangalam, Theni district, Tamil Nadu, India.

Survey and sample collection

Eight major banana growing blocks of the district Tiruchirappalli in the Tamil Nadu state was selected where a systematic survey was carried out namely Anthanallur, Lalgudi, Manichanallur, Nambiyaiyur, Thiruverumbur, Manachanallur, Musiri and Uppiliyapuram of the district. Some of the popular cultivars, such as Poovan, Ney Poovan, Nendran, Karpooravalli and Rasthali were chosen and subjected to examination for Fusarium wilt symptoms. Symptomatic plants were those with progressive leaf yellowing, wilting, pseudostem splitting and discoloration of the internal vessels. Disease incidence was expressed as per cent of the number of plants that were symptomatic to the number of plants surveyed at each site.

Root, rhizome, pseudostem tissues were sampled from infected banana plantations showing symptoms and rhizosphere soils were collected from corresponding root zones for the isolation of the pathogen. Soil around the roots (rhizosphere) was taken from the root active zone about 15 to 20 cm deep with sterile soil sampling tools and placed in separate sterile polythene bags.

Rhizosphere soils of banana, rice, maize, green gram, mango, moringa, tomato, brinjal, onion, cocoa and other locally grown crops were sampled to make indigenous AM fungal assessment and isolate PGPR in selected locations in Tiruchirappalli and Theni districts. Soil that was in close contact with the roots was collected at a depth of 15–20 cm and taken to the lab under sterile conditions for physicochemical analysis, determination of the AM fungal infective propagules and establishment of trap culture, as well as AM fungal spore isolation and PGPR isolation.

Isolation of pathogen (*Fusarium oxysporum* f. sp. *cubense*)

Fusarium oxysporum f. sp. *cubense* (Foc) is a pathogen that was obtained in infected banana plants that were taken in the field survey. Pathogen isolation was done on diseased plant tissues especially those that had characteristic vascular discolorations in the rhizome and root segments. The samples were well washed with running tap water to remove the soil particles that adhered to it and then surface sterilisation using 0.1 % mercuric chloride solution was done and then thoroughly rinsed with sterile distilled water to remove surface contaminants.

Streptomycin was added to Potato Dextrose Agar (PDA) medium to eliminate bacterial contamination and small areas (5–10 mm) of infected tissue were aseptically removed and placed on the media. The plates were incubated in 5–7 days at 28 ± 2 °C. The emergent fungi with typical morphological appearances of those of *Fusarium* spp. including white to pinkish mycelium growth were sub -cultured several times to obtain purified cultures. The cultures that were purified were kept in PDA slants at a refrigeration of 4 °C to proceed with the experimental work (23).

Isolation and characterisation of arbuscular mycorrhizal fungi

This was done by isolating arbuscular mycorrhizal fungi (AMF) in rhizosphere soil samples obtained in the survey of various crops fields (where the fungi were isolated) and their isolation, quantification and characterisation was done using the standard microbiological and microscopic methodologies. The obtained soil samples were air-dried, sieved and subjected to physico-chemical analysis, whereby the soil pH was established by using a soil-to-water suspension (1:2.5 ratio) with a digital pH meter and the electrical conductivity (EC) was performed by the use of a conductivity meter and presented in dS m^{-1}. The most probable number (MPN) technique was used to estimate the number of propagules using ragi (*Eleusine coracana*) as host plant with serially diluted soil samples inoculated in growth media and root colonization evaluated to determine the number of propagules using values of MPN index. Multiplication of native AMF was carried out by setting up the trap cultures with maize (*Zea mays* L.) as the host plant by combining rhizosphere soil with sterilised sand and soil at a (1 : 1 ratio) under controlled conditions through periodic incubation of the cultures with the Hoagland nutrient solution, after 90 days, roots and soil were harvested to analyse further. The spores of AM fungi in trap culture soils were harvested through the wet sieving and decantation technique by subjecting soil suspensions through successive sieves (180, 106 and 45 µm) and the resulting spores were observed and counted under a stereoscopic microscope and the numbers were expressed as spores per 100 g of soil. The isolated spores were placed on glass slides with polyvinyl alcohol-lactoglycerol (PVLG) and Melzer`s reagent and identified by morphological observations such as spore size, shape, colour, wall structure and hyphe attachment using the standard taxonomic keys and showed the major presence of genera, including *Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora* and *Sclerocystis*, among others (24).

Soil physico-chemical analysis

Rhizosphere soil samples used in the study were sampled at various sites and air dried at room temperature and crushed gently by a sieve of 2 mm sieve to eliminate the presence of stones, plant waste and other impurities. The processed soil samples were kept in clean labelled containers and subjected to physico-chemical analysis in accordance with standard procedures. The pH of soil was established by making a soil suspension of 1:2.5 (w/v) in distilled water, the mixture was thoroughly stirred and left to stabilise after 30 min. A digital calibrated pH meter was then used to measure the pH of the suspension. Using a conductivity meter the electrical conductivity (EC) being used as an indicator of the level of soluble salts in the soil, the conductivity meter was measured in the same soil extract in dS m^{-1}.

Each measurement was done thrice to enhance accuracy and reproducibility and the mean values were recorded. The experiments on these parameters gave information on the environment of soil specifically its suitability to microbial activity and colonisation. The soil pH determines the availability of nutrients and the microbial diversity and EC indicate the salinity status which may influence the growth and distribution of arbuscular mycorrhizal fungi (AMF). The data obtained were correlated to identify the association between soil characteristics and AMF occurrence and infective propagule density in the rhizosphere.

Estimation of infective propagules by most probable number (MPN) technique

Most probable number (MPN) technique was used to estimate the density of the infective propagules of arbuscular mycorrhizal fungi (AMF) in the rhizosphere soil using ragi (*Eleusine coracana*) as a host plant. The samples of the rhizosphere soils were sequentially diluted with the sterilised soil-sand mixture to get a series of the dilutions that were subsequently loaded into sterile containers or tubes. Ragi surface-sterilised seeds were planted in both dilution sets and kept under controlled conditions by regular watering and provision of nutrients.

On average 45 days of growth the seedlings were then very gently uprooted and a root sample was taken and it was then processed to determine AM colonisation. Using standard procedures, the roots were cleared and stained and the presence or absence of AM fungal colonisation was recorded under a microscope. According to the positive and the negative tubes in subsequent dilutions, the value of MPN index was calculated using the standard MPN tables.

The count of propagules of the infective type that are present in 1 g of soil was determined by multiplying the value of the MPN index by the dilution factor and expressed as the number of infective propagules per 100 g of soil. This method allowed a quantitative assessment of viable AMF propagules which were in the soil and was used as a measure of inoculum potential of the rhizosphere (25).

Trap culture and spore extraction

Trap cultures were formed on native arbuscular mycorrhizal fungal (AMF) propagules in the rhizosphere soils using as a host plant, maize (*Z. mays*). The gathered soil samples were adequately mixed in a 1 : 1 ratio to proportion with sterilised sand and soil to facilitate aeration and root growth and the mixture was packed into sterile plastic pots. Maize seeds that were surface sterilised were planted in each pot and cultivated in a greenhouse under normal watering. The nutrient solution by Son et al. was applied at intervals with the aim of supplying plants and also reducing any outside interference of microbes (26). The cultures of the traps were held over a span of around 90 days to enable sufficient colonisation of the roots and sporulating of the AM fungi. Plants were also checked periodically during this period with regard to growth and AM colonisation. The plants were uprooted at the end of the growth period and the roots and rhizosphere soil were collected and further analysed.

The wet sieving and decantation technique was used in extraction of AM fungal spores to the trap culture soil. In short, a specified amount of soil was put into water and stirred it and then left it to be settled over a few seconds to allow the settling of the heavy particles. The suspension was then filtered using an order of sieves of different sizes (180 µm, 106 µm and 45 µm). The residues left behind on both sieves were collected individually, washed lightly to get rid of the fine materials and then put into petri dishes. The spores that were extracted were then observed under a stereoscopic microscope and their number could be counted and used to further identify and characterise AM fungi.

Morphological identification of arbuscular mycorrhizal fungi

Isolated AMF spores that were isolated in trap culture soils were placed under thorough morphological characterisation to identify their taxonomy. The intact and healthy spores were then selected, under the stereoscopic microscope and by use of a fine needle and then immobilised on clean glass slides by use of polyvinyl alcohol lactoglycerol (PVLG) as well as Melzer reagent. To mount the slides, the fixed ones were pressed lightly with a coverslip to distribute the contents of the spores so that structural features could be observed. The spores were inspected under a compound microscope and identification was done using standard taxonomic keys by observing morphological features of the spores including size, shape, colour, surface ornamentation, the number and nature of spore wall layers and the type of hyphal attachment (subtending hypha). Other properties like, the presence of germinal walls, septa and response to Melzer reagent were also taken account in order to identify it correctly. The AMF isolates were also categorised into various genera based on these morphological characteristics with a majority of them being *Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora* and *Sclerocystis*. These prevalent genera were identified that gave information on the diversity and distribution of AM fungi in the rhizosphere soils and their possible application in growth promotion and disease suppression of plants (27).

Isolation and characterisation of plant growth-promoting rhizobacteria

Isolation of plant growth-promoting rhizobacteria

Rhizosphere soil samples of different crop fields were collected using serial dilution and plating technique and rhizobacteria that induced plant growth were isolated. About 1 g of the freshly collected soil was transferred into 9 mL of sterile distilled water aseptically and mixed thoroughly to create a homogeneous suspension. Serial dilutions were made up to 10^{-6} dilution and 0.1 mL of suitable dilutions (10^{-4}, 10^{-5} and 10^{-6}) was spread onto sterile Petri plates containing the King medium of B agar, a selective medium of fluorescent pseudomonads and other PGPR. Inoculated plates were incubated at 28 ± 2 °C, in aseptic conditions, 24–48 hr. The morphologically different colonies depending on morphological characteristics were chosen after incubation and sub-cultured using streak plate technique to gain pure cultures. The confirmed isolates were stored in short-term in nutrient agar slides at 4 °C and stored in long-term in stocks of glycerol (20 %) at -80 °C.

Biochemical characterisation of PGPR isolates

A series of biochemical tests to identify the preliminary identification and functional characterisation of the purified bacterial isolates were done according to standard microbiological procedures. To distinguish Gram-positive and Gram-negative bacteria by cell wall components, crystal violet, iodine, alcohol and safranin were used to complete Gram staining. The degree of starch hydrolysis was determined by inoculating the isolates on starch agar plates and incubating them 28 ± 2 °C for 48 hr of incubation after which they were flooded with the iodine solution. A clear halo around the colonies showed that the enzyme amylase is present and the starch hydrolysis is positive. Nutrient gelatin medium was used to measure gelatinase activity whereby, liquid gelatin after incubation and refrigeration at 4 °C served to indicate the presence of gelatinase enzyme. The production of acids and gases was measured by using lactose broth that had phenol red as a pH indicator and Durham tubes in the detection of gases. An acid production was detected by a change of colour red to yellow whereas a gas production was confirmed by presence of gas bubbles in the tube of Durham. The production of pigments was measured by growing the isolates in King`s B broth and the emergence of the specific pigments with the naked eye or the UV light. These biochemical tests gave information on the metabolic potential and taxonomic position of the PGPR isolates.

Indole Acetic Acid (IAA) production assay

Quantitative quantification of the production of Indole-3-Acetic Acid (IAA) which is a major phytohormone that promotes growth in plants was done by using culture filtrates of the isolates of the PGPR. King's B broth with L-tryptophan (100 µg mL^{-1}) as precursor was grown at 28 ± 2 °C in the dark to avoid photodegradation of IAA. Then, after incubation, the cultures were centrifuged at 5000 rpm during 15 min to get cell-free supernatants. The reagent of Salkowski (ferric chloride and perchloric acid) was added to the supernatant and blending to a pink to reddish colour was an indication of IAA production. The colour strength was obtained by spectrophotometric at 530 nm and the IAA concentration was determined using a standard curve that was prepared with known levels of IAA. This test allowed to find effective isolates of PGPR with high plant growth-promoting characteristics.

Siderophore and hydrogen cyanide (HCN) production

The antimicrobial activity of the isolates of the antagonistic plants was assessed qualitatively in terms of the siderophore and hydrogen cyanide (HCN) production. Siderophore production was determined by the use of Chrome Azurol S (CAS) agar medium which is a medium that serves as a reflector of iron-chelating compounds. The plates inoculated were incubated at 28 ± 2 °C for 48–72 hr and the development of orange or yellow halo around the bacterial colonies was a positive indication of the production of siderophore, which meant that the isolates had the ability to sequester the iron and

suppress the growth of pathogens. The glycine-amended medium method was used to determine the production of HCN. Nutrient agar with glycine was streaked with PCR isolates and a filter paper infused with picric acid solution was put in the lid of petri dish. The plates were closed and incubated at 28 ± 2 °C for 48 hr. The colour of the filter paper changing to light brown, brown or reddish-brown was a sign of the production of HCN. HCN and siderophores production indicates that the isolates of the *P. aeruginosa* could prevent pathogenic microorganisms by the actions of antibiosis and competition.

in vitro antagonistic assay

In vitro selection of antagonistic potential of the selected isolates of PGPR against *Fusarium oxysporum* f. sp. *cubense* (Foc) was done by the dual culture technique. Cultures of Foc were actively grown on day 5–7-day-old colonies on Potato Dextrose Agar (PDA) medium. A pathogen mycelial disc of size of about 8 mm was cut aseptically with a sterile cork borer and laid at one side of a sterile Petri plate with PDA medium with distance of around 1 cm above the side. The identified isolates of the PGPR were streaked at the opposite part of the plate, parallel to the fungal disc and maintained the same distance to enable the bacterial isolate and fungal pathogen to interact. Only the fungal pathogen without bacterial inoculation control plates were also kept as a control. The plates were incubated under aseptic conditions at 28 ± 2 °C and 4–5 days.

Measurement of radial growth of the fungal mycelium was done on the treated plates and in the control plates after incubation. The zone of inhibition measured in millimeters was used to measure the degree of inhibition by measuring the distance between the bacterial streak and fungal colony. The rate of growth inhibition of the mycelia was determined as a percentage by the formula:

$$\text{Percentage inhibition} = \frac{C - T}{C} \times 100$$

where C is the radial growth of the pathogen in the control plate and T is the radial growth of the pathogen in the presence of the isolates of the PGPR. In total 28 different morphologically different bacterial isolates were recovered from the collected rhizosphere soils. The isolates were initially differentiated according to colony colour, form, margin, elevation, surface texture and pigmentation. Isolates with possible iron-competition mediated antagonistic activity were then identified by preliminary screening for siderophore production. A total of five isolates with good functional properties were chosen for further biochemical characterisation.

The selected isolates were tested for gram reaction, acid production, gas production, pigment production, hydrolysis of starch, gelatinase activity and growth at 4 °C and production of IAA. The two best candidates, based on their combined plant growth promoting and biocontrol properties, were chosen for antagonistic testing in dual culture against Foc: TBPGR3 and CPGR1. After comparing pathogen radial growth and inhibition-zone development, CPGR1 was chosen for molecular identity and evaluation in the field.

Molecular identification of the CPGR1 clone

The bacterial isolate CPGR1 was sent to Yazhl Genomics, Coimbatore, Tamil Nadu, India for molecular identification using 16S rRNA gene sequence. The nucleotide sequence provided by the service provider was compared to known sequences in GenBank database of NCBI using the BLASTn algorithm. The isolate was characterised on the basis of its sequence similarity and phylogenetic placement with reference strains from the closely related *Bacillus* species and assigned to the species *Bacillus tequilensis*. Isolate was related to other species of *Bacillus* as shown in Fig. 1a–c.

Field experiment (*In vivo* study)

Experimental design

This field experiment was carried out on an infected (sick) banana plot with *Fusarium* f. sp. *cubense* (Foc) under natural disease pressure in order to determine how effective the bioinoculants of interest were in controlling *Fusarium* f. sp. *cubense* (Foc). The experiment has been formatted in a Randomised Block Design (RBD) comprised of seven treatments and three repetitions. Treatment plots were approximately 4 x 4 m and had four plants with a distance of 2 m x 2 m. The pits (45 × 45 × 45 cm) were filled with a blend of field soil and farmyard manure (FYM) and healthy banana suckers (cv. Chakka) were planted.

The chemical-control treatment (T1) was a combination of sequential applications of carbendazim and propiconazole. Healthy sword suckers were dipped in 0.2 % of carbendazim solution before planting. The plants were sprayed with 0.1 % carbendazim at 30 days after planting. Five months after planting, the soil around the pseudostem was alternately treated with 0.2 % carbendazim and 0.1 % propiconazole every two months.

Inoculation and crop management

The bioinoculants were used at planting and later after 45 days after planting (DAP). In the case of individual treatments AM fungal inoculum (about 250 g per plant comprising of about ~35 spores g^{-1} soil) and PGPR inoculum (*Bacillus tequilensis*, about ~1.4 × 10^8 CFU g^{-1}) were used in the area around the root zone. When combining treatments, each inoculant was applied in a reduced proportional amount to achieve a consistent rate of application.

The basal dose of fertilisers such as super phosphate (300 g per plant) was applied to all plants and split application of urea (110 g per plant) and murate of potash (80 g per plant) was done at regular intervals. The standard agronomic practices were observed during the course of the experiment with irrigation being done using a drip system. Plants were being observed to the growth parameters, signs of diseases and general health in order to determine the efficacy of the treatments in the field.

Growth and disease assessment

Growth parameters

The parameters of plant growth were measured at intervals within the period of crop growth to check the impact of various treatments on the performance of the plants. The height of the plants was measured between the base of the pseudostem and the tip of the highest leaf in centimeters. The count of functional leaves per plant was done manually at every stage of observation. Measurements such as yield attributes, such as pseudostem girth, bunch weight, the number of hands per bunch and fingers per hand, were taken at harvest. These variables were employed to evaluate the general growth and productivity of banana plants in various treatment conditions.

Disease incidence (%)

Incidences of disease were determined after intervals depending on the frequency of plants exhibiting common symptoms of Fusarium wilt including yellowing of leaves, wilting and vascular discoloration. The incidence of the disease was computed using the formula:



Fig. 1a. *In vivo* screening of bioinoculants against *F. oxysporum cubense* in Banana.



Fig. 1b. Effect of combined inoculation of AMF and PGPR on growth of banana.

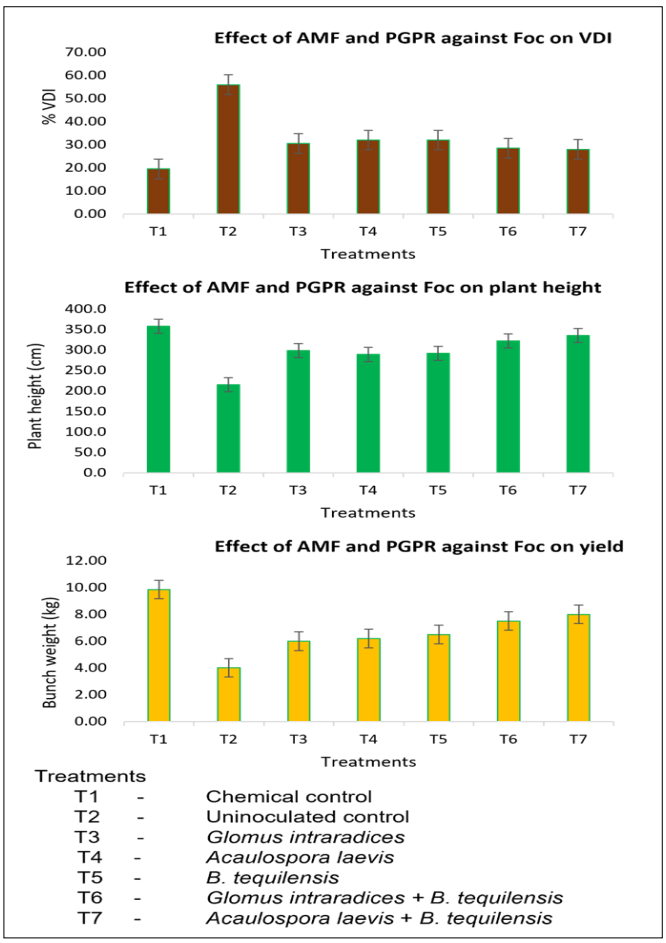


Fig. 1c. Evaluation of growth and yield attributes against Foc incidence.

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

This measure was a quantitative one of the efficacy of various treatments in inhibiting Fusarium wilt in the field.

Vascular discoloration index (VDI)

The level of internal infection was evaluated with the help of the vascular discoloration index (VDI) which was calculated by cutting the pseudostem and rhizome longitudinally to determine the level of vascular discoloration. The discoloration was graded on a scale of 1 to 6, where 1 = no discoloration and 6 = complete vascular discoloration. The VDI was obtained by use of the following formula:

$$\text{VDI (\%)} = \frac{\Sigma\ (\text{disease grades})}{\text{Total number of plants} \times \text{maximum grade}} \times 100$$

The VDI was a good indicator of disease severity and internal tissue damage to supplement external symptom based disease incidence assessment.

Biochemical analysis

The induction of the defence-related analyses. Fresh samples of enzymes and metabolites in banana plants undergoing various treatments were performed through biochemical the pseudostem/ root were taken at a certain time, homogenised under cold conditions and subjected to an enzyme assay under established procedures. All the enzyme activities were expressed in the fresh weight basis and measured spectrophotometrically.

Peroxidase (PO)

The peroxidase activity was ascertained by the involvement of pyrogallol as a donor of hydrogen and hydrogen peroxide (H_2O_2) as a substrate. Homogenate Fresh plant tissue (1 g) was homogenised in 0.1 M phosphate buffer (pH 7.0) at 4 °C and centrifuged at 16000 rpm during 15 min. The source of enzyme was taken to be the supernatant. The reaction mixture was prepared in pyrogallol, enzyme extract and H_2O_2 and change in absorbance per minute of min^{-1} g^{-1} and fresh weight at 430 nm at 30 s intervals measured till 3 min (28).

Polyphenol oxidase (PPO)

The estimation of polyphenol oxidase activity was done with the use of catechol as the substrate. 1 g of fresh plant tissue was homogenised with 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16000 rpm/15 min at 4 °C. Enzyme extract was added to the reaction mixture with catechol and absorbance of the reaction mixture was increased as a result of the formation of quinones at 495 nm which were monitored at periodic intervals. Change in absorbance min^{-1} g^{-1} fresh weight was taken as PPO activity.

Catalase (CAT)

The activity of catalase was calculated by assessing the breakdown of hydrogen peroxide (H_2O_2). The tissue (1 g) was put into the homogeniser and the homogenate was centrifuged at 16000 rpm in 0.1 M phosphate buffer (pH 7.0) in 15 min. Enzyme extract and a known concentration of H_2O_2 were used to prepare the reaction mixture and the mixture incubated at a constant period. Sulfuric acid (H_2SO_4) was used to end the reaction and the remaining H_2O_2 was titrated in the presence of 0.01 N potassium permanganate ($KMnO_4$). The catalase activity was in the form of µmol of H_2O_2 broken down per minute divided by the fresh weight of the sample min^{-1} g^{-1}.

Superoxide dismutase (SOD)

The activity of superoxide dismutases was determined in terms of the inhibitory effect on the photochemical reduction of nitro blue tetrazolium (NBT). Homogenisation, 1 g of fresh tissue was homogenised in 0.05 M phosphate buffer (pH 7.8) and centrifuged at 16000 rpm and at 4 °C 15 min. In the reaction mixture, there was methionine, NBT, EDTA, riboflavin and enzyme extract. The tubes were kept under light exposure of 15 min and the absorbance was recorded at 560 nm. The SOD activity to generate half-inhibition of the NBT reduction was considered as one unit of SOD activity and measured in the enzyme units mg^{-1} protein (29).

Total phenol content

The Folin-Ciocalteu method was used to estimate the total phenolic content. A homogenate of fresh plant tissue (1 g) was prepared in 80 % methanol and centrifuged at a rate of 10000 rpm in 20 min. Supernatant was then evaporated and dried followed by filling in distilled water. Folin-Ciocalteu reagent was added to an aliquot of the extract, then sodium carbonate solution and incubated at room temperature. The developed strength of the blue colour was spectrophotometrically assessed at 725 nm. Phenolic content was determined by use of catechol as a standard and converted to µg catechol equivalents g^{-1} fresh weight.

Statistical analysis

Experimental data was analysed statistically through analysis of variance (ANOVA) to establish significance of treatment effect on all experimental data collected in the field and laboratory. The importance of treatment means differences was assessed at 5 % probability level ($p \leq 0.05$). In case any important differences were noted the least significant difference (LSD) test was used to carry out mean separation.

Before analysis, the arcsine-transformed percentage data including disease incidence and the log-transformed microbial population data were normalised by taking a log to achieve an equal spread of variance. All statistical interpretations were done as per the standard procedures (30). The data were presented in form of mean ± standard error and critical difference (CD) values were calculated accordingly to compare the results of the treatments.

Results and Discussion

The current research examined the utility of AMF and PGPR toward Panama wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) using a combination of survey, *in vitro* and *in vivo* studies. To evaluate the performance of various bioinoculants in the presence of natural disease pressure, a field experiment was carried out in a diseased (sick) plot of Fusarium wilt at Melmangalam, in the Theni district. These investigations yielded the following results which are discussed below.

Survey of disease incidence

An organised survey was done on eight large blocks of banana cultivation in the Tiruchirappalli district by surveying eight blocks and the findings showed a lot of difference in the area under cultivation, distribution of cultivars and Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) (Table 1). The surveyed areas revealed that Thottiyam had the largest area under banana plantation (2500 ha) which was accompanied by Lalgudi (1800

ha) and Anthanallur (1600 ha). Poovan, Ney Poovan and Nendran constituted the major cultivars in these regions and Karpooravalli and Rasthali represented the minor cultivars.

In the surveyed blocks, the disease incidence differed significantly falling between 10 % and 48 % (Table 1). It was highest at Manachanallur (48 %) and Uppiliyapuram (38 %), Thottiyam (34 %), Musiri (32 %) and Thiruverumbur (31 %) respectively. Anthanallur (25 %) and Manikandam (22 %) had moderate levels of incidence and the lowest level of incidence was registered at Lalgudi (10 %).

The symptoms of Fusarium wilt were always found in all the places, where the banana plants showed signs of being targeted by the fungi in terms of the pseudostem, leaves and the rhizosphere soil. Typical appearances were yellowing, wilting and discoloration of the internal vascularity. The prevalence of the disease in all the regions surveyed shows the endemic status of the disease in the Trichy district. The infected plant and soil samples were obtained from the Foc incident fields, especially in the most infected ones like Manachanallur, where the samples were further isolated and characterised (Fig. 1a, b).

Soil properties and pathogen isolation

The physico-chemical characteristics of soils in the rhizosphere of the banana-growing areas were examined to comprehend how it affects the prevalence and activity of pathogens and microbes. The findings showed that the pH of soil was 6.5 to 7.8 indicating average to slightly alkaline soils with EC values of less than 1 dS m^{-1}. These are the basic soil conditions that are usually deemed to be conducive to the survival and growth of soil-borne microorganisms such as *Fusarium oxysporum* f. sp. *cubense*. The evidence of slightly alkaline soils can also help to support the pathogen presence in the rhizosphere hence affecting the disease occurrence on the surveyed sites. A causal organism was isolated using banana plant samples that had wilt symptoms typical of an infected plant. Disease pseudostem tissues were also inoculated on potato dextrose agar (PDA) medium in small sections that were surface sterilised and inoculated under aseptic conditions. The plates were incubated using inoculated plates and fungal colonies that had characteristic morphological characteristics of *Fusarium oxysporum* were observed. The pathogen was further purified by repeated subculturing to get pure cultures (31). The cultures that were purified had normal colony morphology with mycelial growth of cottony white to pale pink which is typical of the *Fusarium* genus. These cultures were kept under the controlled laboratory conditions in order to do *in vitro* and *in vivo* experiments (Fig. 2).

Arbuscular mycorrhizal fungal diversity and screening

The MPN technique and trap culture method were used to avert the AMF diversity and distribution in the various rhizosphere soils (Fig. 2a). The findings showed that, there were varying AM fungal populations in the different crop rhizospheres, with a great difference in the density of infective propagules and species composition (Table 2). The MPN analysis showed that the number of propagules was between 0.120 ± 0.001 and 0.570 ± 0.002 x 10^2 propagules 100 g soil of the infective propagule (IP). The greatest IP count was in tomato rhizosphere (0.570 ± 0.002), then banana (0.540 ± 0.006) and moringa (0.470 ± 0.005) whilst the lowest was registered in brinjal (0.120 ± 0.001) and maize (0.140 ± 0.002) (Table 2). The soils were mostly neutral to slightly alkaline (pH 7.4–8.8) and low electrical conductivity (0.07–0.25 dS m^{-1}), in which AM fungi could survive and be active (32).

Table 2 shows that the most common AM fungal genera found in the various rhizospheres were: *Acaulospora*, *Glomus*, *Sclerocystis*, *Scutellospora* and *Gigaspora*. The most common of these are *Acaulospora laevis* which was widespread in banana rhizosphere and *Glomus intraradices* that was predominant in tomato and *Glomus mosseae* that was found in moringa. Crop-specific associations of AM fungi were also observed in other species like *Scutellospora* sp., *Sclerocystis* sp. and *Gigaspora* sp. which were found in particular crop rhizospheres. The sporulation potential and the colonisation efficiency of AM fungi was further identified by trap culture studies (Table 2). Root colonisation values were 81 % to 93 % with the highest colonisation value of onion (93 %) then moringa (91 %) and mango (89 %) with tomato being relatively lower (81 %). Conversely, tomato rhizosphere

Table 1. Area under banana cultivation in Trichy district and the percentage disease incidence of Foc

Sl. No	Block name	Area (ha)	Banana cultivars	Major/minor	Disease incidence %
1.	Anthanallur	1600	Nendran & Ney Poovan	Major	25
			Poovan	Minor	
2.	Lalgudi	1800	Poovan & Ney Poovan	Major	10
			Nendran & Karpooravalli	Minor	
3.	Manikandam	280	Nendran & Ney Poovan	Major	22
			-	Minor	
4.	Thiruverumbur	210	Nendran & Ney Poovan	Major	31
			-	Minor	
5.	Manachanallur	750	Nendran, Ney Poovan & Poovan, Karpooravalli	Major	48
				Minor	
6.	Musiri	750	Poovan & Ney Poovan	Major	32
			-	Minor	
7.	Thottiyam	2500	Poovan, Ney Poovan, Karpooravalli & Rasthali	Major	34
			-	Minor	
8.	Uppiliyapuram	100	Karpooravalli & Poovan	Major	38
			-	Minor	
	TOTAL	7990 ha			

Table 2. Combined assessment of AM fungal infective propagules, trap-culture colonisation, spore load, occurrence and morphological identification

Sl. No.	Rhizosphere/ culture	pH	EC (dS m^{-1})	Infective propagules (×10^2 per 100 g soil)	Root colonization in maize roots (%)	Spore load (No. per 100 g soil)	Predominant AM fungus / tentative identification	Occurrence (%)	Morphological characteristics
1	Banana	7.7 ± 0.50	0.10 ± 0.01	0.540 ± 0.006	83 ± 4.79	545 ± 3.4	*Acaulospora laevis*	85	Spores globose to subglobose and pale yellow. The spore wall consisted of three layers (L1, L2 and L3): an outer hyaline layer (L1) and two light-yellow membranous layers (L2 and L3). Mature spores possessed a two-layered germinal wall (GW1 and GW2), although a single-layered germinal wall was occasionally observed.
2	Mango	7.6 ± 0.50	0.25 ± 0.02	0.330 ± 0.004	89 ± 5.14	360 ± 2.7	*Scutellospora* sp.	54	Spores were milky white and globose. The spore wall consisted of two closely adherent layers: a smooth, hyaline outer layer (SW1) and a laminated yellow inner layer (SW2). Two germinal walls (GW1 and GW2) were observed. GW1 comprised two firmly attached layers, whereas GW2 contained a flexible hyaline layer that stained dull red and a
3	Moringa	8.5 ± 0.49	0.23 ± 0.01	0.470 ± 0.005	91 ± 5.25	320 ± 1.4	*Glomus mosseae*	35	Spores were globose and golden yellow to brown. Funnel-shaped subtending hyphae and a curved septum separating the spore from the subtending hypha were the characteristic features.
4	Greengram	8.8 ± 0.51	0.18 ± 0.01	0.200 ± 0.002	—	—	*Sclerocystis* sp.	42	Sporocarps were globose to subglobose, brown to blackish brown and lacked a peridium. They contained one to four monohyphal stalks. Chlamydospores were brown to dark brown and developed asynchronously. Club-shaped vesicles or young spores were frequently observed together with mature spores.
5	Maize	8.5 ± 0.55	0.21 ± 0.01	0.140 ± 0.002	—	—	*Glomus geosporum*	65	Spores were reddish brown, smooth-walled and associated with long, tube-like hyaline subtending hyphae. Three firmly adherent wall layers (L1, L2 and L3) were observed. L1 was a hyaline, granular and sloughing layer; L2 was a rigid laminated orange-brown layer; and L3 was semi-rigid and yellow to orange-brown. A recurved septum developed in the
6	Tomato	7.5 ± 0.50	0.07 ± 0.01	0.570 ± 0.002	81 ± 4.68	620 ± 3.4	*Glomus intraradices*	75	Spores were brown, occurred singly and were borne on swollen subtending hyphae.
7	Brinjal	7.8 ± 0.51	0.10 ± 0.01	0.120 ± 0.001	—	—	*Gigaspora* sp.	25	Spores were dark brown, subglobose and possessed two wall layers. Bulbous, stout subtending hyphae with tapered ends were characteristic of the genus.
8	Onion	7.4 ± 0.40	0.25 ± 0.02	0.266 ± 0.002	93 ± 5.37	280 ± 1.1	*Glomus* sp.	40	Spores were globose, with cylindrical-to-flared and constricted subtending hyphae. Three wall layers (L1, L2 and L3) were observed. The mucilaginous L1 layer was prominent in juvenile spores but became indistinct near the mature spore surface. The germ tube emerged through the lumen of the subtending hypha, either near the septum or

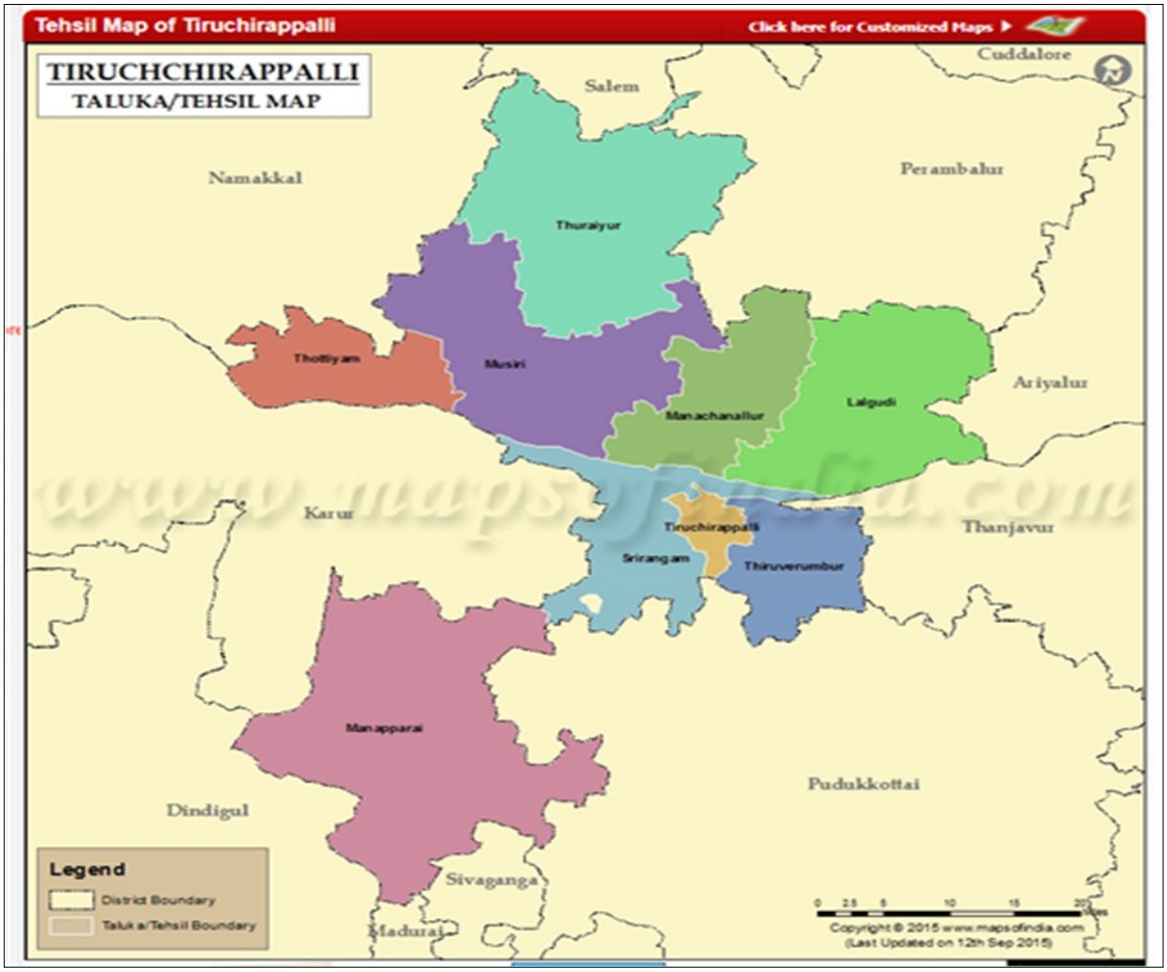


Fig. 2a. Survey for Foc infected banana plantations in and around Trichy [Source : https://www.mapsofindia.com/maps/tamilnadu/tehsil/tiruchirappalli.html].

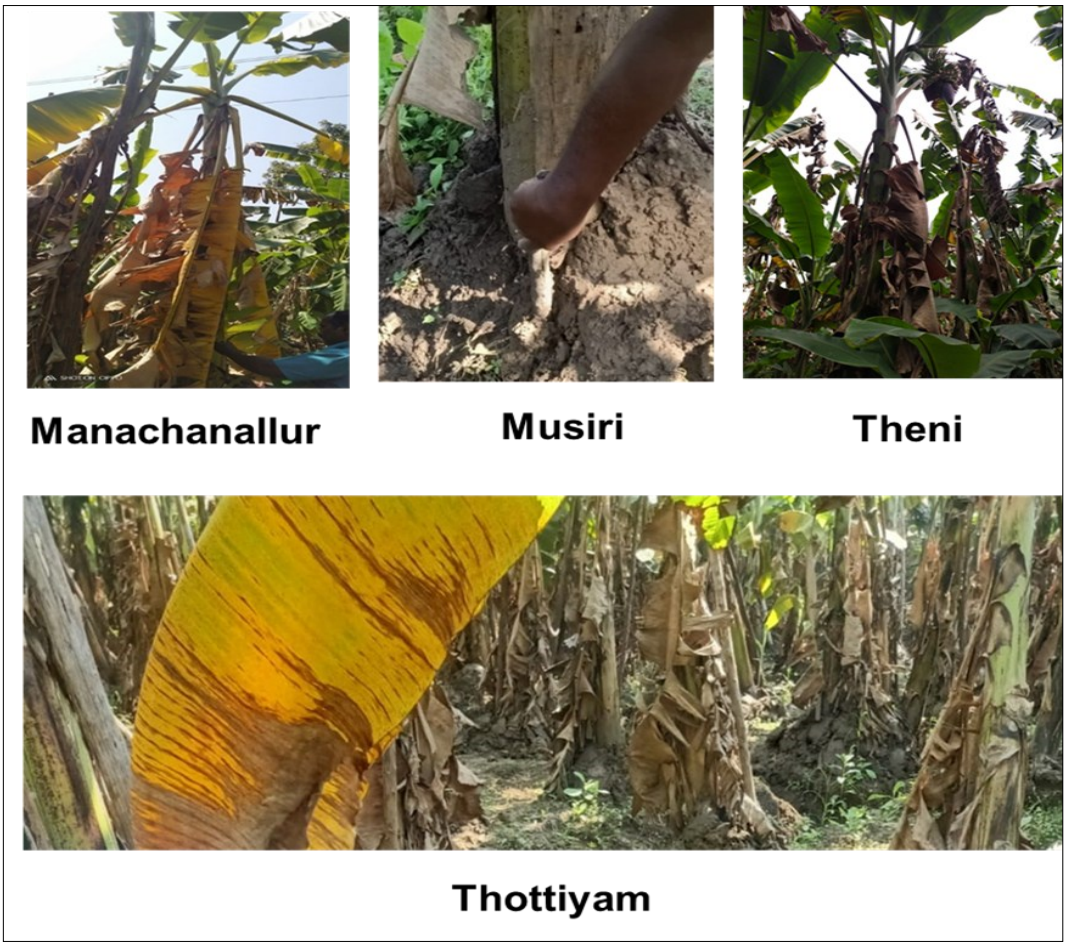


Fig. 2b. Banana plantations wilted due to Foc incidence.

(620 ± 3.4 spores/100 g of soil), banana (545 ± 3.4) had the highest spore load with onion (280 ± 1.1) having the lowest spore load (Table 2). These findings reveal that the density of spores and colonization of roots are not necessarily directly related and could have an impact on the compatibility of the hosts and environmental factors.

Morphological identification of AM fungal spores demonstrated that there are five major genera, which are *Glomus*, *Acaulospora*, *Scutellospora*, *Sclerocystis* and *Gigaspora* (Fig. 2b). The most common isolates were a variety of *Glomus* species known as; *Glomus intraradices*, *Glomus mosseae* and *Glomus geosporum*. Twenty-eight morphologically different bacterial isolates were recovered from the rhizospheres sampled. Five isolates (individually insert verified isolate codes as listed in Table 3) were selected for preliminary functional screening and were subsequently selected for biochemical characterisation. Shortlisted isolates showed variation in Gram and gelatinase activity and production of IAA. TBPGR3 and CPGR1 had the highest IAA concentration, 4.20 µg mL^{-1} and 4.00 µg mL^{-1} respectively. All isolates were positive for starch hydrolysis and positive for gelatinase activity with CPGR1 also being positive for HCN production. From the functional characteristics of both, TBPGR3 and CPGR1 were selected for the dual-culture antagonistic assay against Foc.

PGPR isolation and characterisation

Soil samples were collected from the crop rhizospheres of different locations and viz., Manikandam, Manachanallur, Musiri, Thottiyam and Theni (Table 3). The bacterial isolation of these soils resulted diversified plant growth-promoting rhizobacteriae (PGPR). There was a great range of diversity in the morphology of the colonies; the isolates differed in colour (white, yellow, straw, fluorescent), texture (smooth, slimy, rough), elevation (flat, raised) and margin (entire, irregular). Morphological heterogeneity is a characteristic of the rhizosphere ecosystem that indicates the abundant microbial diversity. Interestingly, the presence of fluorescent and pigmented colonies was observed in some of the isolates. Characterisation and screening of these isolates for their biocontrol potential revealed that, five isolates (TBPGR 1, TBPGR 2, TBPGR 3, TBPGR 4 and CPGR 1) had the ability to produce siderophores, while CPGR 1 had the ability to produce of hydrogen cyanide (HCN), expressing its significant antagonism (Fig. 3).

Further biochemical characterisation of these isolates described they were Gram-positive bacteria of the genus *Bacillus* (TBPGR 2, TBPGR 3 and CPGR 1), while TBPGR 1 was Gram-negative. All the isolates were positive for acid and gas production indicating active metabolism of carbohydrates. Enzyme tests indicated that all isolates were positive to starch hydrolysis implying the expression of extracellular amylase enzymes. It was found that gelatinase was active in TBPGR 3 and CPGR 1 hence could degrade protein substrates, which could contribute to their antagonistic activity. No growth or pigment production of any of the isolates at 4 °C indicated adaptation to mesophilic conditions found in rhizosphere habitats.

CPGR1 and TBPGR3 limited mycelial growth of Foc compared to an inoculation of the pathogen alone in a dual-culture assay. CPGR1 showed the highest antagonistic response with a pathogen inhibition zone of 21.3 mm and combined activity of siderophore, HCN, IAA and extracellular enzymes while TBPGR3 showed the lowest with 18.0 mm. CPGR1, which showed the highest antagonistic response, was molecularly identified and further evaluated in the field.

In vitro antagonistic activity

The antagonistic ability of identified isolates of PGPR against *Fusarium oxysporum* f. sp. *cubense* (Foc) was tested under the dual plate technique. There was a considerable fungal growth inhibition in presence of the antagonistic bacteria than compared to the uninoculated control (Table 4; Fig. 3a). CPGR 1 had the strongest antagonistic activity with a minimum mycelial growth of 32.0 mm and the largest inhibition zone of 21.3 mm, which can be expressed as 61.0 percent inhibition compared to control. TBPGR 3 on the other hand exhibited moderate antagonistic behaviour with a mycelial growth of 38.7 mm, an inhibition zone of 18.0 mm and a 52.9 % inhibition. The highest fungal growth was observed in the control treatment (82.0 mm) and no inhibition was witnessed (Table 4).

The best isolate, CPGR1, was chosen for 16S rRNA gene sequencing for its strong antagonistic activity versus Foc. The sequence obtained was compared against other sequences in the GenBank of the NCBI database using BLASTn and was found to be closest to *Bacillus tequilensis*. This identification was also corroborated using its phylogenetic relationship with reference *Bacillus* sequences included in the analysis (Fig. 3b). Hence, CPGR1 was tentatively classified as *B. tequilensis* and subsequently tested in the field.

In vivo evaluation of bioinoculants

Growth and yield attributes

The response to the inoculation of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal (AM) fungi was compared at various periods of banana crop development. The treatments showed significant differences concerning plant height and the number of leaves after 60 and 120 days after planting (DAP) (Table 4; Fig. 4). When DAP was 60, the number of leaves per plant was between 3.0 and 5.0 with the highest recorded in treatment T1, T5, T6 and T7 (5.0 leaves $plant^{-1}$) and lowest in treatment T2 (3.0 leaves). On the same note, the height of the plants was between 70.0 cm (T2) to 95.0 cm (T7) which showed better vegetative development when bioinoculant was applied. At 120 days after planting, the highest number of leaves and plant height was recorded in T7 (*Acaulospora laevis* + *Bacillus tequilensis*) (11 per plant and 267.0 cm respectively) and T6 (*Glomus intraradices* + *B. tequilensis*) (10.0 per plant and 263.0 cm respectively). On the contrary, the growth was the lowest, 186.0 cm in the T2 (uninoculated control), which is a strong indication of severe growth inhibition in the presence of pathogen stress (Table 4).

During the harvesting stage, there was a similar trend in growth and yield attributes (Table 4; Fig 4 a, b). The highest plant height (358.20 cm), girth (51.22 cm) and bunch weight (9.85 kg $plant^{-1}$) were measured in the chemical control (T1). But out of the bioinoculant treatments, T7 was better in terms of plant height of 335.50 cm, girth of 47.38 cm and bunch weight of 8.00 kg $plant^{-1}$ and then T6 was with plant height of 322.50 cm, girth of 44.75 cm and bunch weight of 7.50 kg $plant^{-1}$. Single inoculations with T3 (*Glomus intraradices*), T4 (*Acaulospora laevis*) and T5 (*Bacillus tequilensis*) demonstrated moderate increases in growth and yield parameters in relation to uninoculated control but did not do as well as combined treatments. The highest parameter values were obtained in the uninoculated control (T2) that was recorded in plant height (215.12 cm), girth (27.70 cm) and bunch

Table 3. Morphological, biochemical and *in vitro* antagonistic screening of PGPR isolates recovered from different rhizosphere soil samples

Sl. No.	Sampling location	PGPR isolate	Colony morphology	Gr	AP	GP	Pig	SH	Gel	G4	IAA (µg mL^{-1} broth)	Foc mycelial growth (mm)	Inhibition zone (mm)	Inhibition over control (%)
1	Manigandam	RPGR 1	Straw-coloured, round, entire, smooth and flat	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
2	Manigandam	MzPGR 1	White, smooth, tiny and slightly raised	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
3	Manigandam	VPGR 1	Milky white, slimy, entire, raised and tiny	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
4	Manigandam	VPGR 2	Transparent, smooth, entire and slightly raised	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
5	Manigandam	VPGR 3	White, slimy, entire and raised	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
6	Manigandam	GPGR 1	Semi-transparent, slightly raised and slimy	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
7	Manigandam	MoPGR 1	Transparent white, smooth, round, entire and raised	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
8	Manigandam	MnPGR 1	Milky white, glistening, round and entire	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
9	Manigandam	OPGR 1	Yellow, irregular and flat	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
10	Manigandam	OPGR 2	White, irregular, flat and transparent	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
11	Manigandam	OPGR 3	Straw-coloured, round, entire, large and raised	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
12	Manachanallur	BPGR 1	Straw-coloured, transparent, smooth and raised	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
13	Manachanallur	BPGR 2	Pure white, entire, rough and slightly raised	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
14	Manachanallur	BPGR 3	Pinkish-orange, round, entire and tiny	–	+	+	–	+	+	–	1.96	NT	NT	NT
15	Manachanallur	BPGR 4	Fluorescent green, round, entire, slightly raised and glistening	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
16	Musiri	MBPGR 1	Creamy white, slimy, irregular, raised and smooth	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
17	Musiri	MBPGR 2	White, entire, flat, smooth and punctiform	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
18	Musiri	MBPGR 3	Off-white, entire, raised and smooth	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
19	Musiri	MBPGR 4	Transparent white, pale and irregular	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
20	Musiri	MBPGR 5	Yellowish or fluorescent, flat, irregular and smooth	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
21	Musiri	MBPGR 6	Pale yellow, entire, smooth and punctiform	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
22	Musiri	MBPGR 7	Whitish, irregular, flat and rough	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
23	Thottiyam	TBPGR 1	Straw-coloured, punctiform, raised and entire	–	+	+	–	+	–	–	1.24	NT	NT	NT
24	Thottiyam	TBPGR 2	Yellow, glistening, raised and entire	+	+	+	–	+	–	–	2.55	NT	NT	NT
25	Thottiyam	TBPGR 3	Yellowish, shiny, entire and flat	+	+	+	–	+	+	–	4.20	38.7	18.0	52.8[1]
26	Thottiyam	TBPGR 4	Creamy white, irregular and flat	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
27	Thottiyam	TBPGR 5	Pale white, irregular and flat	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
28	Theni	CPGR 1	Whitish, irregular, flat and rough	+	+	+	–	+	+	–	4.00	32.0	21.3	61.0[1]
—	Control	Foc alone	Not applicable	NA	NA	NA	NA	NA	NA	NA	NA	82.0	—	0.0

* Values represent mean of three replications

Table 4. Effects of individual and combined AMF–PGPR inoculation on banana growth, yield and Fusarium wilt development at different crop-growth stages

Treatment	Leaves plant^{-1} at 60 DAP	Leaves plant^{-1} at 120 DAP	Plant height at 60 DAP (cm)	Plant height at 120 DAP (cm)	Plant height at harvest (cm)	Pseudostem girth at harvest (cm)	Bunch weight plant^{-1} (kg)	Disease incidence at 60 DAP (%)	Disease incidence at 120 DAP (%)	VDI at harvest (%)
T1 – Chemical control	5.0 ± 0.09	8.6 ± 0.15	88.0 ± 1.52	244.0 ± 4.23	358.20 ± 2.07^{a}	51.22 ± 0.30^{a}	9.85 ± 0.06^{a}	35.0 ± 0.61	38.0 ± 0.66	19.5 ± 0.11^{a}
T2 – Uninoculated control (pathogen only)	3.0 ± 0.05	6.0 ± 0.10	70.0 ± 1.21	186.0 ± 3.22	215.12 ± 1.24^{g}	27.70 ± 0.16^{g}	4.01 ± 0.02^{g}	97.3 ± 1.18	98.3 ± 1.19	56.0 ± 0.32^{g}
T3 – *Glomus intraradices*	4.0 ± 0.07	7.0 ± 0.12	85.0 ± 1.47	255.0 ± 4.42	298.50 ± 1.72^{d}	37.50 ± 0.22^{e}	6.00 ± 0.03^{f}	47.0 ± 0.81	63.0 ± 1.09	32.0 ± 0.18^{f}
T4 – *Acaulospora laevis*	4.0 ± 0.07	8.0 ± 0.14	89.0 ± 1.54	245.0 ± 4.24	289.25 ± 1.67^{f}	36.30 ± 0.21^{f}	6.20 ± 0.04^{e}	48.0 ± 0.83	65.0 ± 1.13	32.0 ± 0.18^{e}
T5 – *Bacillus tequilensis*	5.0 ± 0.09	8.0 ± 0.14	90.0 ± 1.56	257.0 ± 4.45	292.10 ± 1.69^{e}	38.13 ± 0.22^{d}	6.50 ± 0.04^{d}	45.0 ± 0.78	59.0 ± 1.02	30.5 ± 0.18^{d}
T6 – *G. intraradices* + *B. tequilensis*	5.0 ± 0.09	10.0 ± 0.17	93.0 ± 1.61	263.0 ± 4.56	322.50 ± 1.86^{c}	44.75 ± 0.26^{c}	7.50 ± 0.04^{c}	42.0 ± 0.73	56.0 ± 0.97	28.5 ± 0.16^{c}
T7 – *A. laevis* + *B. tequilensis*	5.0 ± 0.09	11.0 ± 0.19	95.0 ± 1.65	267.0 ± 4.62	335.50 ± 1.94^{b}	47.38 ± 0.27^{b}	8.00 ± 0.05^{b}	42.0 ± 0.73	51.0 ± 0.88	28.0 ± 0.16^{b}
Mean	4.40	8.40	87.1	245.3	301.60	40.40	6.90	50.9	61.5	32.40
CD (P = 0.05)	0.055	0.095	0.445	1.453	0.833	0.126	0.053	0.614	0.687	0.223

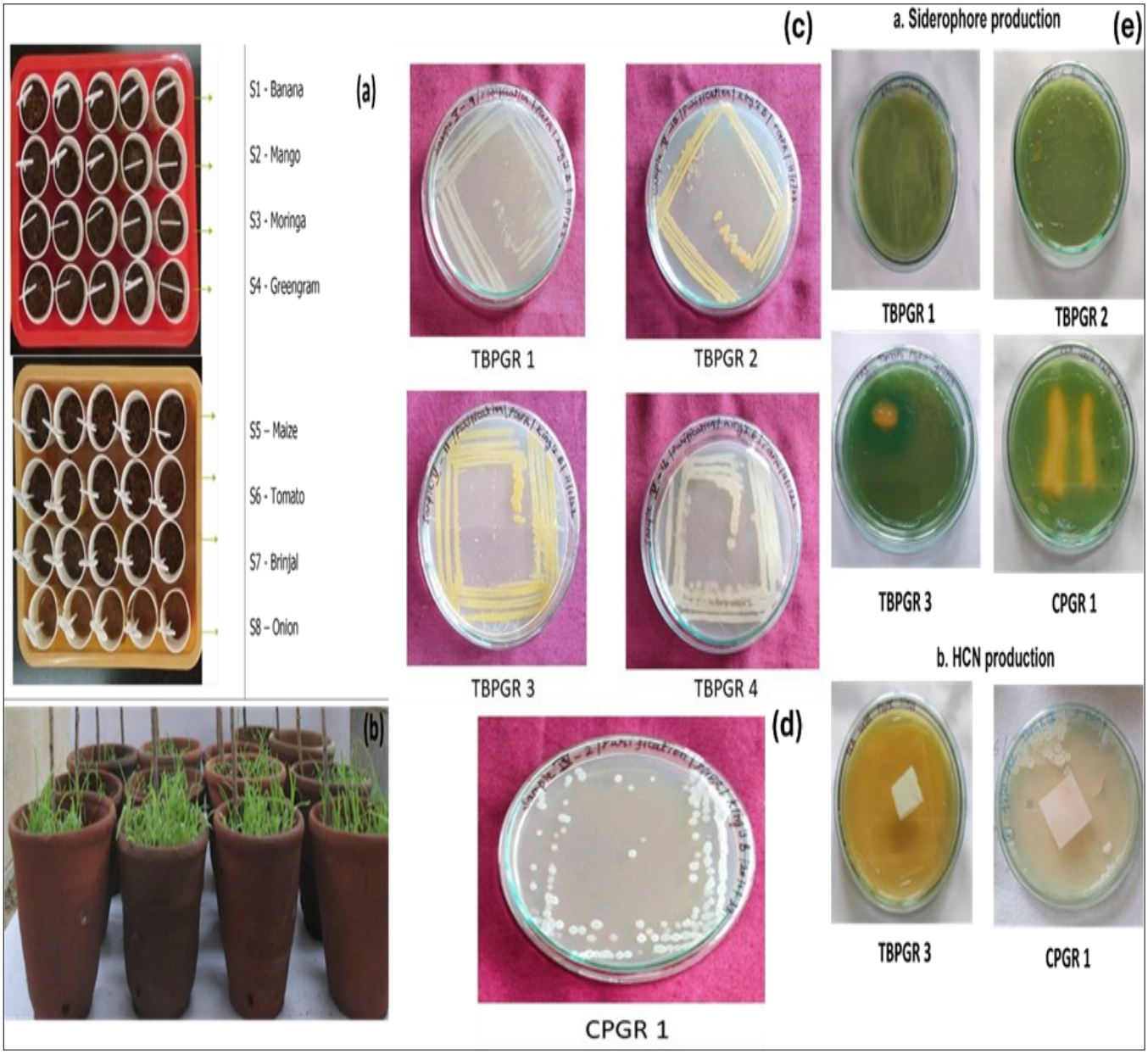


Fig. 3. Isolation, multiplication and characterization of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria from collected rhizosphere soils. (a) Most probable number (MPN) technique used to estimate the total infective propagule density of AM fungi in soil samples; (b) soil trap-culture technique used for multiplication of indigenous AM fungal spores; (c) representative PGPR isolates TBPGR1–TBPGR4 recovered from rhizosphere soils of the Thottiyam block; (d) PGPR isolate CPGR1 recovered from a rhizosphere soil sample collected in Theni; and (e) representative biochemical and plant growth-promoting characterisation assays of the selected PGPR isolates.

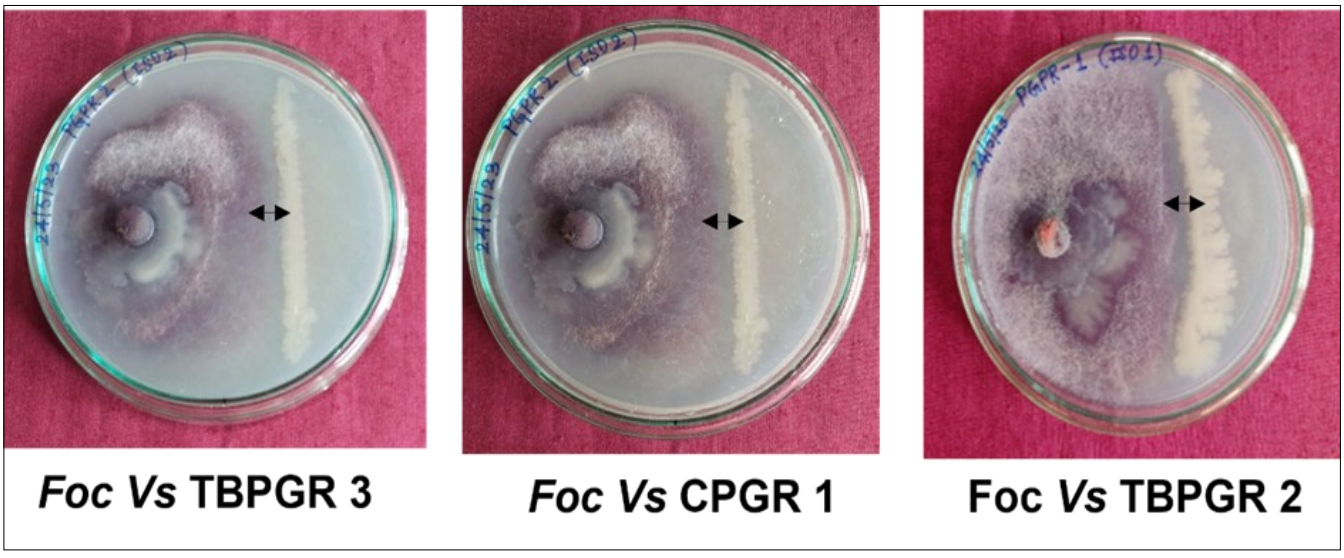


Fig. 4a. Antagonistic potential testing of PGPR against Foc under dual plate technique.

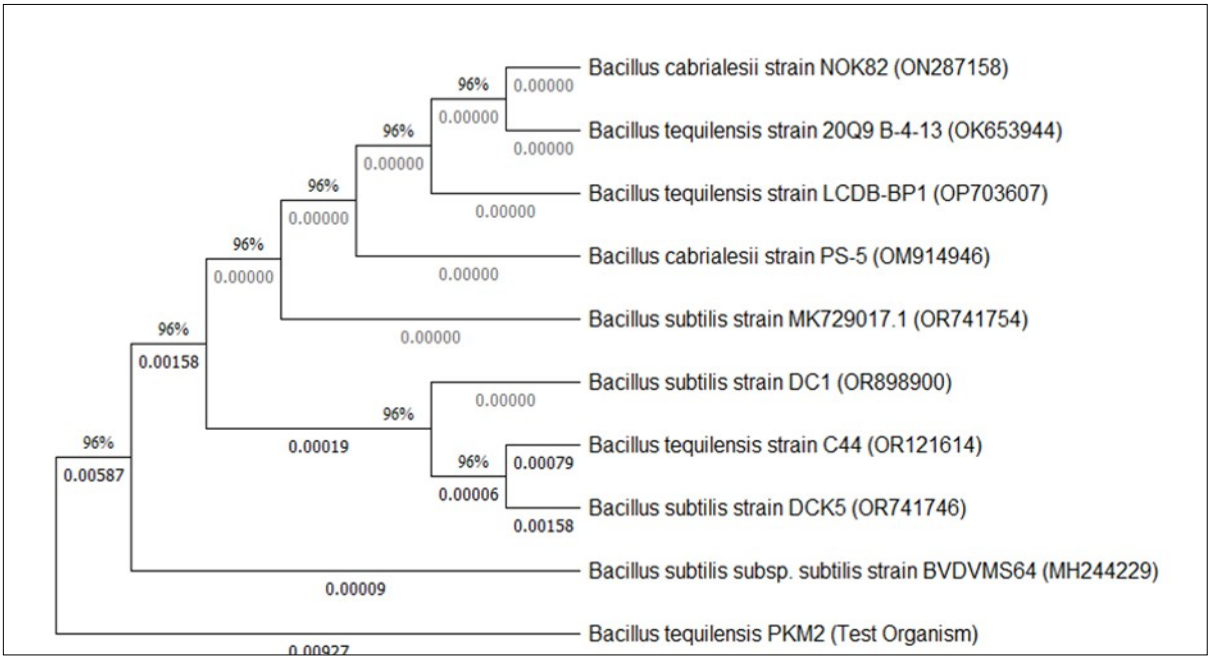


Fig. 4b. Phylogenetic tree representing genetic relatedness of *Bacillus* sp.

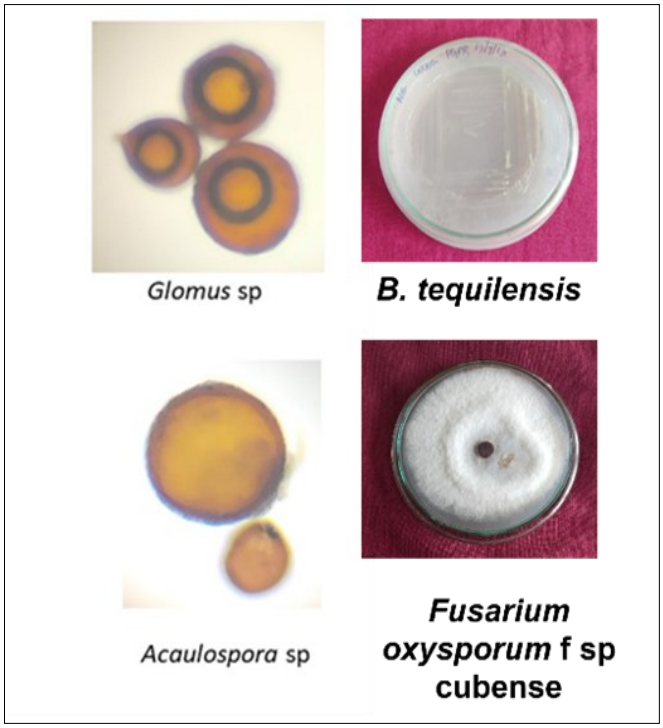


Fig. 4c. Bio inoculants screened for the field experiment.

weight (4.01 kg/plant^{-1}) and indicate the negative influence of Fusarium wilt on the growth of plants (33). The improved growth and yield of combined treatments suggest a synergistic relationship between AM fungi and the presence of the PGPR that can be explained by the fact that it helps to improve nutrient uptake and root development, as well as physiological efficiency of the plants in the stressful environment.

Disease incidence

Individual and combined inoculation of AM fungi and PGPR on the incidence of Fusarium wilt at 60 and 120 days after planting (DAP) were evaluated and the treatments were found to differ significantly (Table 4). At 60 DAP, the disease incidence was between 35.0 and 97.3 %. The chemical control (T1) had the lowest incidence of 35.0 % followed by the combined inoculated treatments, T6 (*Glomus intraradices* + *Bacillus tequilensis*) and T7 (*Acaulospora laevis* + *B. tequilensis*) with 42.0 and 42.0 percent incidence respectively. Individual treatments had relatively less incidence (45.0 % in T5 and 47.0 % in T3 and 48.0 % in T4). Conversely, the disease incidence was the highest (97.3 %) in the uninoculated control (T2), which showed extreme evidence of the pathogen infection in the untreated conditions.

At 120 DAP, the same trend was noted with the disease incidence range of 38.0 - 98.3 %. The incidence was again the lowest in chemical control (38.0 %) while among the bioinoculant treatments T7 recorded the lowest (51.0 %). Single inoculated treatments of AM fungi (T3 and T4) had slightly higher values of incidence at 63.0 and 65.0 respectively. The control (T2) that was uninoculated had the highest percentage of disease incidence of 98.3 % (Table 4). As per the results, it is evident that the inoculation of AM fungi in combination with PGPR was more efficient to prevent disease occurrence compared to the sole inoculation. It was noted that the combined treatment has a high biocontrol potential as the incidence of the disease was reduced by about 50 % in T7 relative to the uninoculated control. Combined treatments were found to perform better, which implies the existence of the synergistic effect with AM fungi and PGPR and therefore, the increased ability to suppress the pathogen in fields (34).

Vascular discoloration index (VDI)

Vascular discoloration index (VDI), representing the degree of internal tissue damage inflicted by *Fusarium oxysporum* f. sp. *cubense*, demonstrated great differences across treatments (Table 4; Fig. 5). There was a significant decrease in VDI with all bioinoculant treatments over the uninoculated control and this supported the idea of pathogen colonization suppression in the vascular tissues.

The lowest VDI was achieved with the chemical control (T1) (19.5 %), followed by the combined treatments T7 (*Acaulospora laevis* + *Bacillus tequilensis*) (28.0 %) and T6 (*Glomus intraradices* + *B. tequilensis*) (28.5 %). Single treatments including T5 (*Bacillus tequilensis*), T3 (*Glomus intraradices*) and T4 (*Acaulospora laevis*) had mediocre decreases in VDI that differed between 30.5 % and 32.0 %. On the contrary, the VDI (56.0 %) of the uninoculated control (T2) was the highest, which implies that the vascular discoloration was severe and internal infection was

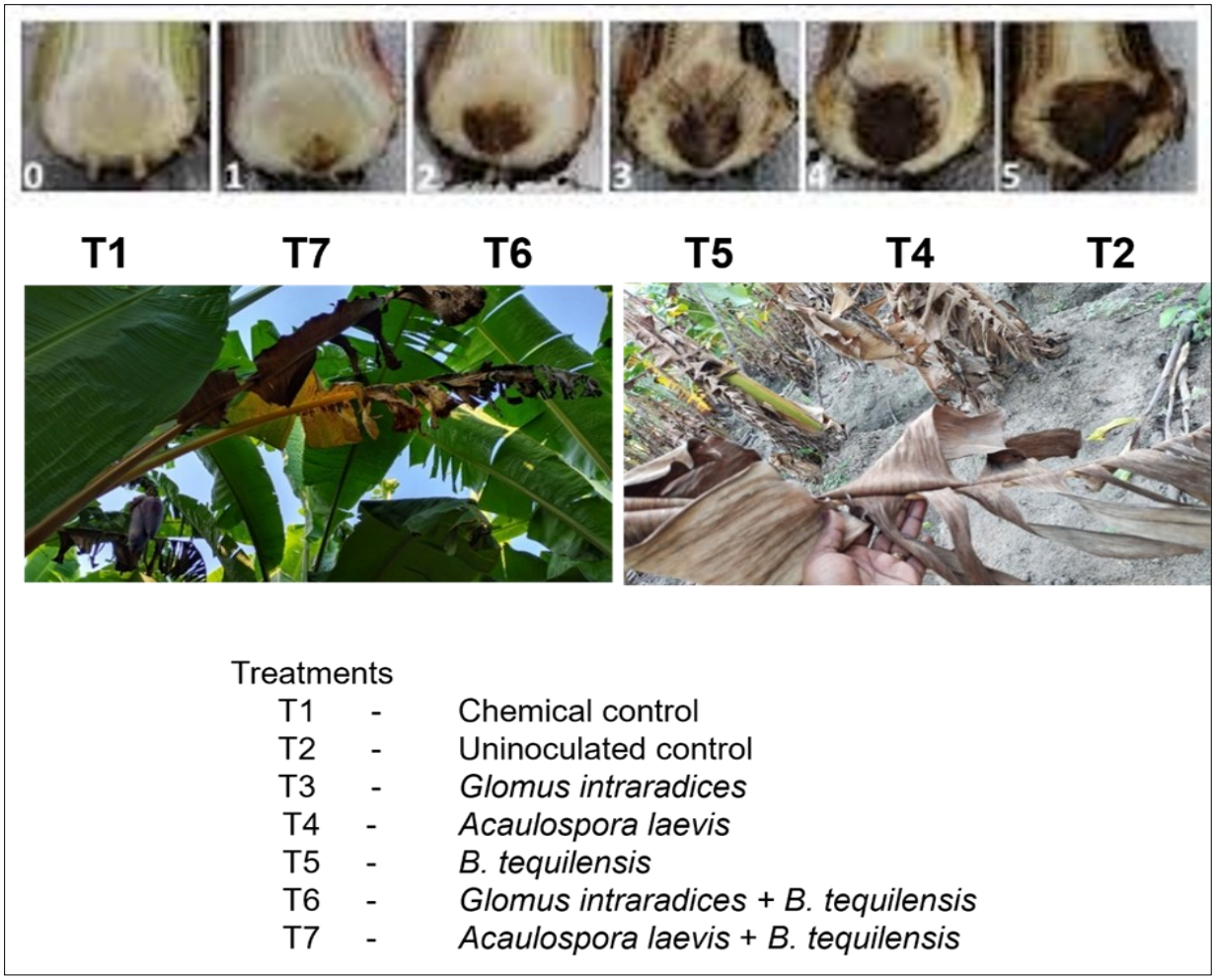


Fig. 5. Assessment of Vascular Discoloration Index (VDI) due to Foc incidence in the pseudostem of Banana.

widespread (Table 4).

The much-reduced VDI in the combined inoculations, then compared to individual inoculations indicates that there is synergistic interaction between AM fungi and PGPR in lowering the distribution of the pathogen in plant tissues. The combined bioinoculant treatments showed a significant decrease in the vascular damage although chemical control had the lowest VDI and at the same time, enhanced plant growth and yield. These results prove that AM fungi integration with PGPR is an effective mitigation of plant internal disease severity and resistance against Fusarium wilt.

Defence-enzyme activity and mechanisms of induced resistance

Challenge with *Fusarium oxysporum* f. sp. *cubense* significantly influenced the activities of the defence-related enzymes peroxidase (PO), polyphenol oxidase (PPO), catalase (CAT) and superoxide dismutase (SOD), as well as the total phenolic content of banana plants inoculated with AM fungi and PGPR (Tables 5). The greater activities recorded in the bioinoculant treatments indicate that microbial inoculation enhanced both the inducible defence response and the capacity of the plants to regulate pathogen-associated oxidative stress.

PO activity increased significantly in all inoculated treatments relative to the uninoculated control at 60, 75 and 90 days after planting (DAP) (Table 5). The maximum activity was recorded at 75 DAP. Treatment T7, consisting of *Acaulospora laevis* + *Bacillus tequilensis*, recorded the highest PO activity of 2.67 ΔOD min^{-1} g^{-1}, followed by T5 and T6 with activities of 2.30 and 2.24 ΔOD min^{-1} g^{-1} respectively. The uninoculated control recorded the lowest activity of 0.84 ΔOD min^{-1} g^{-1}. The increased PO activity may have contributed to disease suppression by promoting the oxidation of phenolic compounds, cross-linking of cell-wall proteins and peroxidase-mediated lignification. These structural changes can strengthen root tissues and restrict the penetration and vascular spread of the pathogen.

A comparable response was observed for PPO. At 75 DAP, T7 recorded the highest PPO activity of 0.405 ΔOD min^{-1} g^{-1}, followed by T6 and T5 with activities of 0.367 and 0.221 ΔOD min^{-1} g^{-1} respectively. The uninoculated control recorded only 0.126 ΔOD min^{-1} g^{-1}. The PPO catalyses the oxidation of phenolic substrates to quinones, which are highly reactive compounds that may directly inhibit pathogen enzymes and fungal growth. Quinones can also polymerise with cellular proteins and cell-wall components, thereby producing a physical and biochemical barrier against pathogen invasion. Increased PO and PPO activities have similarly been associated with enhanced Fusarium wilt resistance in banana plants treated with biological control microorganisms (35, 36).

CAT activity also increased markedly in the combined-inoculation treatments (Table 5). At 75 DAP, T7 recorded the highest activity of 1.430, followed by T6 and T5 with activities of 1.150 and 0.770 respectively, whereas the uninoculated control recorded 0.350 in the assay units reported in Table 5. CAT is an important component of the antioxidant system because it decomposes excess hydrogen peroxide into water and molecular oxygen. This function prevents uncontrolled oxidative damage to membranes,

Table 5. Effect of individual and combined AMF–PGPR inoculation on defence-related enzyme activities and total phenolic content in banana plants challenged with *Fusarium oxysporum* f. sp. *cubense*

Treatment	PO 60 DAP	PO 75 DAP	PO 90 DAP	PPO 60 DAP	PPO 75 DAP	PPO 90 DAP	CAT 60 DAP	CAT 75 DAP	CAT 90 DAP	SOD 60 DAP	SOD 75 DAP	SOD 90 DAP	Total phenols 60 DAP	Total phenols 75 DAP	Total phenols 90 DAP
T1 – Chemical control	0.40	1.58	1.36	0.032	0.134	1.047	0.160	0.360	0.270	33.2	91.2	67.0	0.825	0.920	0.745
T2 – Uninoculated control	0.32	0.84	0.94	0.022	0.126	1.021	0.130	0.350	0.210	30.6	65.5	50.0	0.590	0.820	0.460
T3 – *Glomus intraradices*	0.48	1.58	1.02	0.043	0.150	1.156	0.250	0.520	0.330	49.3	113.0	95.5	0.850	0.900	0.690
T4 – *Acaulospora laevis*	0.56	1.66	1.24	0.045	0.227	1.311	0.270	0.640	0.410	57.4	121.6	110.0	0.920	1.275	0.795
T5 – *Bacillus tequilensis*	0.76	2.30	1.76	0.084	0.221	1.390	0.280	0.770	0.460	82.2	140.6	121.1	0.950	1.225	0.830
T6 – *G. intraradices* + *B. tequilensis*	0.90	2.24	1.40	0.052	0.367	1.475	0.200	1.150	0.550	61.6	161.1	128.8	0.995	1.340	0.665
T7 – *A. laevis* + *B. tequilensis*	0.96	2.67	0.82	0.020	0.405	2.204	0.360	1.430	0.650	124.2	170.9	142.2	1.075	1.460	0.855
SEd	0.02	0.04	0.03	0.001	0.012	0.024	0.005	0.014	0.007	1.37	2.61	1.91	0.03	0.04	0.02
CD (P = 0.05)	0.05	0.10	0.08	0.003	0.025	0.051	0.010	0.020	0.015	2.91	5.43	3.06	0.06	0.08	0.05

proteins and other cellular components during pathogen infection.

The SOD response followed a similar pattern. Treatment T7 recorded the highest activity of 170.9, followed by T6 and T5 with activities of 161.1 and 140.6 respectively, while the uninoculated control recorded the lowest activity of 65.5 in the assay units presented in Table 5. The SOD constitutes an early enzymatic defence against oxidative stress by converting the highly reactive superoxide radical into hydrogen peroxide. The resulting hydrogen peroxide may subsequently be removed by CAT and PO or maintained transiently at controlled concentrations as a signalling molecule. Thus, the coordinated increase in SOD, CAT and PO indicates more efficient regulation of reactive oxygen species in the inoculated plants rather than the indiscriminate elimination of all reactive oxygen species.

This coordinated antioxidant response is important because reactive oxygen species have a dual role during plant–pathogen interactions. Excessive accumulation causes lipid peroxidation, protein oxidation and cellular injury, whereas a controlled oxidative burst functions as a signal for defence-gene activation, antimicrobial responses and cell-wall reinforcement. In the present study, the simultaneous elevation of SOD, CAT and PO in T6 and T7 suggests that combined inoculation enabled the plants to generate and regulate defence-associated oxidative signals more effectively than the uninoculated control. Nevertheless, confirmation that the higher antioxidant activities reduced cellular injury would require supporting measurements such as hydrogen peroxide concentration, malondialdehyde content, electrolyte leakage or membrane-stability index.

Total phenolic content was also significantly increased by the bioinoculant treatments (Table 4). The maximum concentration occurred at 75 DAP, with T7 recording 1.460, followed by T6 and T4 with 1.340 and 1.275 respectively, in the units reported in Table 4. The uninoculated control recorded a lower value of 0.820. Phenolic compounds are central components of plant defence because they can function as antimicrobial metabolites, antioxidants and precursors of lignin and other structural polymers. Their accumulation also provides substrates for PO- and PPO-mediated oxidation. Therefore, the simultaneous increase in phenolic content, PO activity and PPO activity indicates coordinated activation of phenylpropanoid-associated biochemical and structural defences.

The stronger response produced by T7 can be explained by the complementary mechanisms of AMF- and PGPR-mediated defence priming. Root colonisation by AMF can induce mycorrhiza-induced resistance by modifying host nutrition, root metabolism, hormone signalling and defence-gene responsiveness. In tomato challenged with *F. oxysporum*, colonisation by *Rhizophagus irregularis* reduced disease severity and increased the expression of the jasmonate-biosynthesis genes AOC and LOXD, together with higher PPO and PAL activities. The absence of equivalent protection in a jasmonate-defective tomato mutant demonstrated that AMF-mediated resistance in that system was strongly associated with JA signalling.

The PGPR component may complement this response through root colonisation and the release of microbe-associated molecular patterns, siderophores, lytic enzymes, lipopeptides and other eliciting metabolites. Recognition of these microbial signals can prime jasmonic acid- and ethylene-associated induced systemic resistance. In banana, treatment with endophytic *B. subtilis* TR21

enhanced resistance to *F. oxysporum* f. sp. *cubense* by upregulating jasmonate- and brassinosteroid-biosynthesis pathways, demonstrating that beneficial Bacillus can induce hormone-associated defence reprogramming in banana.

The involvement of *B. tequilensis* in defence induction is further supported by research showing that *B. tequilensis* PKDN31 and *B. licheniformis* PKDL10 colonised roots, produced siderophores and lytic enzymes and enhanced PO, PPO, PAL, chitinase, β-1,3-glucanase and total phenolic accumulation in tomato plants challenged with *F. oxysporum*. Although the host plant and *Fusarium forma specialis* differed from those used in the present study, the results provide mechanistic support for the ability of *B. tequilensis* to function as an inducer of systemic resistance.

Under combined inoculation, AMF may improve phosphorus and micronutrient acquisition, root growth and physiological condition, while *B. tequilensis* supplies additional defence elicitors and antagonistic metabolites. Better plant nutritional status can support the energetic and metabolic costs of synthesising antioxidant enzymes, phenolic compounds, lignin and other defence products. In return, PGPR-mediated changes in root development and nutrient mobilisation may improve AMF colonisation and activity. Experiments involving AMF and endophytic Bacillus in Fusarium-infected tomato demonstrated that combined application produced greater disease reduction than individual inoculation under both pot and field conditions. Other AMF–PGPR studies have similarly associated dual inoculation with improved nutrient acquisition and increased resistance to soil-borne pathogens.

The maximum activities of PO, PPO, CAT and SOD and the highest phenolic accumulation at 75 DAP indicate that the biochemical defence response reached its highest measured level at this sampling stage. This temporal peak may correspond to active colonization by the bioinoculants, increasing pathogen pressure or intensified plant–microorganism signalling. The subsequent decline at 90 DAP may reflect successful restriction of the pathogen, physiological acclimation, enzyme turnover or redistribution of resources from active defence to plant growth. However, because pathogen abundance, hormone concentrations and defence-gene expression were not measured at the same intervals, these explanations should be regarded as mechanistic interpretations rather than directly demonstrated processes.

Overall, the greater PO, PPO, CAT and SOD activities and phenolic accumulation in T7 indicate that combined inoculation established a more strongly primed defence condition than the uninoculated control and most single-inoculation treatments. The proposed mechanism involves AMF-mediated nutritional and hormonal priming combined with PGPR-mediated elicitation of induced systemic resistance. These processes enhance reactive oxygen species signalling and detoxification, phenolic metabolism, antimicrobial compound formation, lignification and cell-wall reinforcement, thereby creating multiple barriers against the penetration and vascular colonisation of *F. oxysporum* f. sp. *cubense*. Nevertheless, the results should be described as being consistent with a synergistic AMF–PGPR interaction unless a significant AMF × PGPR interaction or a response exceeding the expected additive effect has been statistically demonstrated.

Microbial population dynamics

The rhizobacterial population varied greatly between the treatments and tended to rise with crop development. The highest populations were found in T7 (*A. laevis* + *B. tequilensis*) and T6 (*G. intraradices* + *B. tequilensis*) with 175.50 × 10^7 and 173.40 × 10^7 CFU g^{-1} soil respectively, while the uninoculated control was 24.47 × 10^7 CFU g^{-1} soil. The abundance of AM fungal spores at harvest followed a similar trend as seen at planting, with the T7 and T6 both having 180 spores per 50 g soil, while the uninoculated control had 45 spores per 50 g soil (Table 6).

The combined treatments showed a concurrent increase of rhizobacterial abundance and AM fungal spore count, which is indicative of a beneficial interaction of AMF–PGPR. But the enumeration techniques reported here did not specifically identify the presence of the introduced rhizobacteria and AM fungal spores, so the observations do not conclusively confirm the persistence of the introduced culture. The considerably greater population of the AM spores and the population density of the AM spores in the combined treatments (T6 and T7) are a clear indication of a synergistic relationship between the AM fungi and the PGPR, which results in an increased establishment and dynamism of the microbe in the rhizosphere. The enhanced microbial community due to mixed inoculation indicates the existence of mycorrhiza helper bacteria, which provide AM fungi with colonization and enhanced functioning of the rhizosphere. These findings support the suggestion that dual inoculation is not only effective in promoting plant growth and disease control but also increases the stability and persistence of advantageous microbial communities in the soil.

Microbial population dynamics

The temporal changes in the rhizobacterial population demonstrated that the introduced bioinoculants were able to establish and persist in the banana rhizosphere under field conditions. Although the bacterial population increased between 90 and 210 days after planting in most inoculated treatments, the highest populations were consistently recorded in the dual-inoculation treatments T7 and T6. In contrast, the uninoculated control, T2, maintained a substantially lower culturable bacterial population. This pattern suggests that inoculation provided an initial population of beneficial microorganisms that was subsequently maintained by favourable rhizosphere conditions, including the availability of root-derived carbon compounds, expanding root surfaces and suitable microbial colonisation sites.

The progressive increase in the bacterial population may also be related to changes in banana root development and rhizodeposition during plant growth. As the root system expands, the release of sugars, amino acids, organic acids, phenolic compounds and sloughed root cells can provide substrates that support rhizobacterial multiplication. The comparatively low population in T2 indicates that the native microbial community alone was less able to develop a similarly abundant culturable rhizobacterial population under the experimental conditions. Nevertheless, this result should not be interpreted solely as an effect of pathogen stress because microbial abundance can also be influenced by soil moisture, nutrient availability, plant developmental stage and competition among indigenous microorganisms.

The greater abundance of Bacillus-associated populations in the inoculated treatments is relevant to the biological suppression of banana Fusarium wilt. Fan et al. reported that Bacillus was significantly more abundant in disease-suppressive banana soils than in Fusarium-wilt-affected soils and was negatively correlated

Table 6. Effect of individual and combined AMF–PGPR inoculation on rhizobacterial population and AM fungal spore abundance in the rhizosphere of banana under *Fusarium oxysporum* f. sp. *cubense* incidence

Treatment	Treatment description	Rhizobacterial population at 90 DAP ($\times 10^7$ CFU g^{-1} dry soil)	Rhizobacterial population at 180 DAP ($\times 10^7$ CFU g^{-1} dry soil)	Rhizobacterial population at 210 DAP ($\times 10^7$ CFU g^{-1} dry soil)	AM fungal spore count at harvest (spores 50 g^{-1} soil)
T1	Chemical control	35.45	66.66	108.80	120 ± 6.99
T2	Uninoculated control (pathogen only)	10.22	27.17	24.47	45 ± 5.72
T3	*Glomus intraradices*	38.60	59.03	157.40	90 ± 5.40
T4	*Acaulospora laevis*	40.21	63.70	156.50	110 ± 8.89
T5	*Bacillus tequilensis*	42.15	73.15	169.01	85 ± 4.45
T6	*G. intraradices* + *B. tequilensis*	54.20	78.50	173.40	140 ± 7.62
T7	*A. laevis* + *B. tequilensis*	65.21	82.40	175.50	180 ± 5.08
Mean	—	40.86	64.41	137.87	110.00
SEd	—	0.028	0.035	0.128	0.032[1]
CD (P = 0.05)	—	0.060	0.076	0.275	0.075[1]

with the abundance of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (37). The authors further demonstrated that an isolated *Bacillus velezensis* strain produced substantial control of banana Fusarium wilt under pot conditions. Although the bacterial species and experimental conditions differ from those of the present study, these findings support the association between the persistence of beneficial Bacillus populations and a rhizosphere environment less favourable to the pathogen.

Significant differences were also observed in the AM fungal spore population at harvest. The highest spore density was recorded in T7, followed by T6, whereas the respective single-inoculation treatments and the uninoculated control produced lower values. The greater spore populations in the combined treatments indicate that the presence of *B. tequilensis* was compatible with the inoculated AM fungi and may have created conditions favourable for AM fungal establishment, sporulation, or persistence. Possible mechanisms include phosphate solubilization, production of growth -promoting metabolites, modification of root exudation, improvement of root development, stimulation of fungal spore germination and provision of suitable microsites around AM fungal spores and hyphae.

Comparable responses have been reported in other host–microorganism systems. Combined inoculation with AMF and selected *Pseudomonas* or *Bacillus* strains increased AM fungal root colonisation in tobacco seedlings relative to AMF inoculation alone. In that study, the improvement was associated with changes in rhizosphere microbial composition and enrichment of metabolic pathways related to flavonoid and isoflavonoid biosynthesis, which can participate in signalling during mycorrhizal establishment. These findings provide a plausible biological explanation for the greater AM fungal spore density observed in the dual-inoculation treatments of the present study.

The interaction between AMF and PGPR may be mutually beneficial. PGPR can improve nutrient availability and root growth, thereby increasing the number of potential sites available for AM fungal colonisation. In turn, AM fungal hyphae extend beyond the nutrient-depletion zone surrounding roots and may create additional habitats and nutrient-rich microsites that support bacterial survival. The resulting mycorrhizosphere can therefore contain a larger and more functionally active microbial population than the rhizosphere of uninoculated plants. This interaction may improve nutrient cycling, root health and the ability of beneficial microorganisms to compete with soil-borne pathogens.

However, the effect of dual inoculation is not universally positive and depends on the compatibility of the specific AM fungal species, bacterial strain, host plant, soil properties and environmental conditions. For example, an AMF–Bacillus consortium enhanced phosphorus acquisition by maize under severe water stress, but dual inoculation did not consistently increase AM fungal root colonisation under all moisture conditions. This demonstrates that improved plant performance or nutrient uptake does not necessarily result from an increase in fungal colonization alone. Similarly, co-inoculation of raspberry plants with AMF and spore-associated bacteria improved plant growth and physiological performance, although the bacterial supplementation did not further increase AM fungal root colonisation.

The AM fungal spores detected in T1, T2 and the bacteria-only treatment T5 most likely represent indigenous AM fungal communities already present in the field soil. Therefore, the spore

populations in these treatments should not be interpreted as establishment of the introduced AM fungal inoculants. The relatively high spore population observed in the chemical-control treatment, T1, may reflect the response of native AM fungal populations to the prevailing soil and plant conditions. Its interpretation also depends on the active ingredient, concentration and application frequency of the fungicide because different fungicides can have contrasting effects on non-target soil fungi.

The greater microbial populations in T6 and T7 may have contributed to Fusarium wilt suppression through several complementary processes. These include competition with the pathogen for nutrients and root-colonisation sites, production of antifungal metabolites and hydrolytic enzymes, alteration of rhizosphere nutrient availability, improvement of plant nutritional status and priming of systemic plant defences. In a pathogen-challenged avocado system, combined inoculation with growth-promoting bacteria and AM fungi reduced disease severity more effectively than either microorganism applied alone, although the magnitude of the response varied among the microbial combinations and plant parameters evaluated.

Nevertheless, the bacterial population and AM fungal spore-count data obtained in the present study demonstrate microbial establishment and persistence rather than directly proving the mechanism of disease suppression. Confirmation of the proposed mechanisms would require additional measurements, such as AM fungal root-colonisation percentage, pathogen population or DNA abundance, microbial community profiling, bacterial biofilm formation, antifungal metabolite production and expression of plant defence-related genes.

Overall, the greater rhizobacterial populations and AM fungal spore densities recorded in the combined treatments indicate a positive and compatible interaction between the AM fungi and *B. tequilensis*. These treatments appear to have established a more persistent beneficial microbial community than the single-inoculation and uninoculated treatments. This enhanced microbial persistence may have contributed to improved plant growth and Fusarium wilt suppression by strengthening both direct microbial antagonism and indirect plant-mediated defence responses. However, unless a statistically significant AMF × PGPR interaction was demonstrated, the findings should be described as being "consistent with a synergistic interaction" rather than as definitive proof of synergy.

Discussion

The current research obviously shows that AMF and plant PGPR are effective in managing Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) by means of direct antagonistic action and indirectly by increasing the defence mechanisms in plants. The survey outcomes proved that Fusarium wilt is prevalent in all the major banana producing areas of Trichy district with rate of disease between 10–48 % which proved the endemic nature of the pathogen and necessitated the need to adopt sustainable management practices (38).

The adaptability and ecological importance of AMF is emphasised by the diversity and abundance of AMF in various rhizosphere soils, especially the fact that *Acaulospora laevis* and *Glomus intraradices* are the most abundant. The great root colonisation and spore density observed when the trap culture was used also indicates that they may be effective in association and nutrient acquisition to the root. These results agree with the better results in the field experiment, where AMF inoculation was able to increase the height of the plants and the production of the leaves (39).

Screening and isolation of PGPR showed a huge variation in biochemical characteristics and plant growth-promoting activities. *Bacillus tequilensis* produced more IAA and antagonistic effects against Foc in *in vitro* conditions, hence proving its potential to be used as effective biocontrol agent. The dual culture assay also confirmed that the isolates of the selected PGRPs are effective inhibitors of the growth of fungi and therefore the isolates can inhibit the pathogen by antibiosis and by competition (40).

In field conditions, AMF and PGPR work together produced huge gains in the parameters of plant growth as opposed to single application and control. The *Acaulospora laevis* + *Bacillus tequilensis* treatment always had the best plant height and leaf count, which indicated that there was synergistic interaction between the two microorganisms. Such an increased growth is due to better nutrient uptake, especially phosphorus and generation of growth-enhancing material like phytohormones (41).

The fact that combined treatments also yield reduced disease incidence also serves as a plus in favor of microbial consortia suppressing Fusarium wilt. The minimum level of disease in T7 is the evidence that the combination of AMF and PGPR is essential in inhibiting the establishment and progression of the pathogen. The alleviation of vascular discoloration index (VDI) in combined therapies is also the confirmation of the limitation of pathogen colonisation in the plant tissues; therefore, internal damage is reduced (42).

The positive changes in the work of the enzymes of the defence system, such as peroxidase (PO), polyphenol oxidase (PPO), catalase (CAT) and superoxide dismutase (SOD), as well as in the phenolic content, testify to the mobilisation of induced systemic resistance (ISR) in plants treated. The increased levels of enzymatic activities were found in combined treatments, which indicate that AMF and PGPR jointly activate enhanced levels of defence responses in comparison to single inoculation. The field survey demonstrated substantial spatial variation in Fusarium wilt incidence, ranging from 10 % to 48 % among the surveyed banana-growing blocks. However, AM fungal and PGPR abundance were not consistently determined in the same banana fields in which disease incidence was measured. Therefore, the survey data do not support a direct statistical correlation between indigenous beneficial-microbial populations and Fusarium wilt incidence.

In the inoculation experiment, the combined treatments, particularly T7 and T6, were associated with higher rhizobacterial populations and AM fungal spore abundance than the uninoculated control. These increases occurred together with improved plant growth and lower disease development, suggesting an association between a biologically active rhizosphere and disease suppression. Nevertheless, the enumeration methods measured total rhizobacteria and AM fungal spores and did not distinguish the introduced inoculants from indigenous microorganisms. Future studies should use strain-specific molecular tracking and Foc quantification to confirm inoculant establishment and its direct relationship with disease suppression (43).

Conclusion

The present study demonstrates that combined application of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria has potential as a biologically based strategy for improving banana performance and suppressing Fusarium wilt under field conditions. Among the microbial treatments evaluated, the combination of *Acaulospora laevis* and *Bacillus tequilensis* (T7) produced the most consistent overall response, with improved plant growth and yield-related traits, reduced disease incidence and vascular discoloration, enhanced activities of PO, PPO, CAT and SOD, increased phenolic accumulation and greater establishment of beneficial microbial populations compared with the uninoculated control and individual microbial treatments. The combined treatment of *Glomus intraradices* and *B. tequilensis* (T6) also showed substantial disease-suppressive and plant growth-promoting effects. These findings indicate that the effectiveness of dual inoculation was associated with complementary effects on plant physiological performance, pathogen suppression, defence-related biochemical responses and rhizosphere microbial establishment.

The results have important implications for the development of sustainable Fusarium wilt management strategies. The concurrent improvement in plant performance, reduction in disease development, activation of defence-related biochemical responses and persistence of beneficial microorganisms suggests that compatible AMF–PGPR combinations may provide broader and more stable biological effects than individual inoculants. Such microbial consortia could potentially contribute to integrated disease-management programs by reducing reliance on chemical treatments and improving the biological resilience of banana production systems. However, because the present study was conducted under a limited set of field conditions, broader recommendations regarding large-scale application and commercial use require further validation.

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Authors' contributions

LSP carried out the arbuscular mycorrhizal fungi and PGPR studies, participated in the sequence alignment and drafted the manuscript. VPS carried out the *in vitro* antagonistic activity. KI participated in the sequence alignment. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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