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# Integrated disease management components for Fusarium wilt of banana in India: Effects of organic amendments, flooding, and paddy rotation on disease severity

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The banana Fusarium wilt pathogen, *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is a major threat to banana production globally. In India, *Foc* race 1 and tropical race 4 (TR4) are reported from multiple banana-growing states where it causes severe yield losses. To develop integrated approaches, in the present study the effect of organic amendments, water logging and paddy rice cultivation on Fusarium wilt development was assessed. Cavendish cv. Grand Nain (AAA) bananas were grown in small plots and pots in soils with organic amendments. Of the organic amendments, groundnut and gingelly cakes applied at 300 g per plant fully suppressed Fusarium wilt caused by *Foc* TR4, with an internal wilt disease score of 0 on a 0–5 scale, while groundnut and neem cake strongly suppressed the disease, with disease scores of 0.3–0.33 at the same concentration in plants infected with *Foc* race 1. Plant growth parameters (height, girth, number of leaves, and leaf area) were also significantly increased for plants treated with groundnut cake (*Foc* TR4-inoculated plants) and neem cake (*Foc* race 1-inoculated plants). The neem cake application significantly increased the fungal (up to  $7 \times 10^{10}$  cfu/g), bacterial (up to  $33 \times 10^{10}$  cfu/g of soil) and actinomycetes (up to  $4 \times 10^6$  cfu/g) numbers in soil compared to soil without the organic amendment. *In vitro* evaluation of 49 bacterial and 14 fungal isolates identified six *Bacillus* and five *Trichoderma* spp. as highly suppressive to *Foc* TR4, with enhanced protease and cellulase activities and IAA production, indicating both pathogen suppression and plant growth-promoting mechanisms. Waterlogging and paddy rice cultivation under micro-plot conditions for 4 months reduced Fusarium wilt (TR4) severity to score of 2.2 and 1.13, respectively, on a 0–5 rating scale, compared with the control treatment, which recorded severity scores of 3.4 and 3.5, respectively. qPCR analysis revealed that pot soils amended with groundnut cake reduced *Foc*

TR4 and race 1 DNA by 90.6% and 81.2%, respectively, while mustard and gingelly cakes reduced *Foc* TR4 by 86.9% and 84.5%, respectively, and neem cake reduced *Foc* race 1 by 85.1%. The waterlogging for 4 months and paddy rice cultivation in micro plots resulted in even greater reductions of *Foc* TR4 DNA by 98.1% and 97.8%, respectively. These results suggest that organic amendments, flooding, and paddy rice cultivation could be effective strategies for managing Fusarium wilt of banana in the field.

#### KEYWORDS

banana, Fusarium wilt, integrated disease management (IDM), organic amendments, paddy rice cultivation, qPCR, water stagnation

## 1 Introduction

Bananas (*Musa* spp.) are one of the most widely cultivated and consumed fruits globally. Known for their rich nutritional profile and versatility, bananas are a staple in the diets of millions and are a key cash crop for many farmers. According to [FAO Report, 2024](#), the production of bananas worldwide is valued at around 10 billion USD. Among the wide range of varieties grown, the Cavendish (AAA) bananas are the most widely traded and account for approximately 50% of global production, with an estimated annual output of 50 million tonnes ([Staver et al., 2020](#)). In India, bananas rank first among all fresh fruits in terms of production, with a total output of 36.61 million tonnes cultivated on 0.996 million hectares ([IndiaStat, 2019](#)). In India, the major banana-producing states include Andhra Pradesh (5.68 million tonnes), Maharashtra (4.91 million tonnes), Tamil Nadu (3.95 million tonnes), Gujarat (3.97 million tonnes), Uttar Pradesh (3.39 million tonnes), Karnataka (2.43 million tonnes), Madhya Pradesh (2.22 million tonnes), Bihar (1.96 million tonnes), and West Bengal (1.20 million tonnes) ([Ministry of Agriculture and Farmers Welfare, Government of India, 2022](#)). The major banana varieties cultivated across India include Grand Nain (AAA), Robusta (AAA), Dwarf Cavendish (AAA), Red Banana (AAA), Ney Poovan (AB), Nendran (Plantain, AAB), Poovan (Mysuru, AAB), Rasthali (Silk, AAB), Karpuravalli (Pisang Awak, ABB), and Monthan (Cooking banana, ABB). Among these, the Cavendish group (AAA) comprising Grand Nain, Robusta, and Dwarf Cavendish dominates production, particularly in the subtropical states of Gujarat, Maharashtra, Madhya Pradesh, West Bengal, and Bihar, where it is predominantly cultivated as a monoculture crop ([Thangavelu et al., 2024](#)).

Banana farmers globally are facing various production constraints. Among these, Fusarium wilt, caused by the soilborne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*), poses a serious threat to production ([Thangavelu et al., 2020](#)). *Foc* is found in most banana-producing countries and can survive as chlamydospores in soil for decades ([Moore et al., 1995](#)). Once a susceptible banana plant is infected, typical symptoms develop that include internal rhizome and vascular discoloration, followed by leaf yellowing and ultimately plant death ([Stover, 1962](#)). *Foc* populations are traditionally classified into races based on host specificity and into vegetative compatibility groups (VCGs) reflecting genetic diversity ([Mostert et al., 2017](#)). Of these, *Foc* TR4 (VCG 01213/16) is

considered the most destructive due to its wide host range ([Ploetz, 2015](#); [Dita et al., 2018](#)). *Foc* TR4 was first found in Indonesia and Malaysia in the early 1990s and has since been reported from 24 countries in Asia, Australia, Europe, the Middle East, South America and Southern Africa ([Viljoen et al., 2020](#)). It is predicted that *Foc* TR4 could affect 1.7 million hectares of bananas by 2040 ([Scheerer et al., 2016](#)), reducing production by 2% between 2019 and 2028, with 240,000 job losses and a 3.2% increase in retail prices ([FAO, 2019](#)). In India, besides *Foc* TR4, virulent form of *Foc* race 1 infecting Cavendish is also widely distributed ([Thangavelu et al., 2025](#)).

Managing banana Fusarium wilt without resistant germplasm is considered difficult ([Dita et al., 2018](#)). Nevertheless, management approaches that enhance soil health and suppress the pathogen, such as crop rotation, the use of cover crops, the application of organic amendments and biofertilisers have been shown to suppress Fusarium wilt development and increase banana productivity ([Pattison et al., 2014b](#); [Rames et al., 2018](#); [Fu et al., 2016](#); [Huang et al., 2012](#); [Thangavelu et al., 2003, 2004](#)). Currently, organic farming is gaining popularity due to adverse effects of chemicals ([Mie et al., 2017](#)). In organic farming, soil amendments cover a range of materials such as animal manure, composts, high N-containing products ([Bonanomi et al., 2007](#)) and plant-waste residues ([Yogev et al., 2006](#)). Soilborne pathogens that had been effectively managed with organic amendments include *Gaeumannomyces graminis* f. sp. *tritici*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Thielaviopsis basicola*, *Verticillium dahliae*, species of *Fusarium*, *Phytophthora*, *Pythium* and *Sclerotium*. Plant-waste residues effectively mitigated diseases caused by four distinct *formae speciales* of *F. oxysporum*: *basilici*, *melonis*, *radicis-cucumerinum*, and *radicis-lycopersici* ([Bonanomi et al., 2007](#)). The addition of organic amendments could influence soil microbiota, which may suppress disease by soil fungistasis ([De Corato, 2020](#)). Manure can further change the structure of the soil, introducing allochthonous microorganisms, and alter the survival of both plant-beneficial and soil-borne pathogens ([Tang et al., 2023](#); [Todorovi'c et al., 2023](#)).

In ancient agriculture, flooding was a pre-planting strategy for the suppression of soil-borne pathogens by lowering O<sub>2</sub>, elevating CO<sub>2</sub>, and creating microbial changes that generate toxic compounds to pathogens ([Bruehl, 1987](#)). Studies conducted in the 1940s have shown that various diseases, including Fusarium wilts and diseases caused by nematodes, can be effectively controlled through the creation of an anaerobic environment ([Thurston,](#)

2019). In the 1950s, flood fallowing was used to eradicate *Foc* from the pathogen infested soils in Latin America (Stover, 1962). The survival of the fungus in water can decrease over time, particularly if it sinks to the bottom and when water is not stirred (Stover, 1979; Ullah et al., 2021). Flooding has allowed Latin American producers to regrow bananas in flooded fields, but 2 years later losses increased again to levels that prevented further production (Stover, 1962). The technique is routinely used in Asian countries by rotating crops with paddy to decrease soil-borne plant pathogens (Kelman and Cook, 1977). Katan (2000) indicated that vegetables grown in soils flooded by rain for several weeks in winter and spring were healthier than vegetables grown in dry fields. In addition, rotation of cotton with paddy effectively reduced *V. dahliae* and *F. oxysporum* f. sp. *vasinfectum* inoculum, and controlled diseases of cotton in California and China (Cook and Duniway, 1981; Pullman and De Vay, 1981).

The benefits that organic amendments, flooding and paddy rice cultivation offer to the management of soilborne pathogens, including *F. oxysporum*, provided the rationale for their use in the present study on banana Fusarium wilt caused by *Foc* TR4 and race 1. The objectives of this study, therefore, were to i) evaluate organic amendments for the suppression of Fusarium wilt disease as well as their impact on plant growth promotion, ii) study the likely mechanism involved in suppression of the Fusarium wilt, and to iii) assess the role of waterlogging and paddy rice cultivation in suppressing Fusarium wilt and lowering of *Foc* inoculum levels in the soil.

## 2 Materials and methods

### 2.1 Effect of organic amendments on Fusarium wilt disease (*Foc* TR4 and *Foc* race 1)

Three-month-old tissue culture-derived plants of the Cavendish cv. Grand Nain (AAA) were planted in 30L pots that were filled with a potting mixture consisting of red soil (lateritic soil), sand, and farmyard manure at a 1:1:1 ratio. The pots were prepared and left for 10 days before planting one banana plant per pot and the different organic amendments were added. Eight different organic amendments were applied at three different doses of 100, 200 and 300 g per plant. The amendments included neem cake [NC, contains 5.2% N, 1.0% P<sub>2</sub>O<sub>5</sub>, 1.4% K<sub>2</sub>O], groundnut cake [GNC, contains 7.3% N, 1.5% P<sub>2</sub>O<sub>5</sub>, 1.3% K<sub>2</sub>O], castor cake [CC, contains 4% N, 1% P<sub>2</sub>O<sub>5</sub>, 1% K<sub>2</sub>O], gingelly (sesame) cake [GC, contains 6.2% N, 2.0% P<sub>2</sub>O<sub>5</sub>, 1.2% K<sub>2</sub>O], mustard cake [MC, contains 4.8% N, 2.0% P<sub>2</sub>O<sub>5</sub>, 1.3% K<sub>2</sub>O], vermicompost [VER, contains 2–3% N, 1.55–2.55% P<sub>2</sub>O<sub>5</sub>, 1.8–2.2% K<sub>2</sub>O], rice husk ash [ASH, contains 1.0% P<sub>2</sub>O<sub>5</sub>, 1.0% K<sub>2</sub>O, 92.8% SiO<sub>2</sub>, 2.6% Na<sub>2</sub>O, 0.4% CaO, 0.31% Fe<sub>2</sub>O<sub>3</sub>, 0.2% MgO] and thoroughly decomposed farmyard manure [FYM, contains 0.5% N, 0.2% P<sub>2</sub>O<sub>5</sub>, 0.5% K<sub>2</sub>O] and they were sourced from local market. The amendments were applied by removing the surface soil around each plant, depositing the amendment, and covering it with the removed soil.

A week after application, about 30 g of sorghum grain colonised with either *Foc* TR4 (VCG 01213/16) or *Foc* race 1 (VCG 0125)

containing a minimum 10<sup>6</sup> CFU/g was buried around each plant. Two control treatments were also included in the experiment. The first included plants treated with *Foc* TR4 or *Foc* race 1 alone, without any organic amendments, and the other involved neither fungal inoculum nor organic amendments. Each treatment was replicated five times, with each replicate consisting of a single plant grown in an individual pot, and the pots were arranged in a completely randomised block design. Six months post inoculation plant height, pseudostem girth, number of leaves and leaf area were recorded. The plants were then taken out of the soil and disease development was assessed based on a rhizome discolouration index using a 0–5 rating scale (Zuo et al., 2018). Rhizosphere soil samples were also collected to enumerate microbial populations and quantify *Foc* TR4 (VCG 01213/16) and race 1 (VCG 0125) levels using qPCR assay. The experiment was repeated twice.

### 2.2 Enumeration of microbial population and screening *in vitro* against *Foc* TR4

Microbial numbers of fungi, bacteria and actinomycetes present in the rhizosphere soil of banana plants, across the eight treatments were recorded on potato dextrose agar (PDA), nutrient agar (NA) and actinomycetes isolation agar (AIA). Briefly, 1 g of rhizosphere soil with organic amendment was air dried and transferred to a sterile 250-ml conical flask containing 100 ml of sterile distilled water. After shaking for 15 min in a shaker at 120 rpm, the soil solution was serially diluted up to 10<sup>-11</sup> using sterile distilled water. Then 1 ml from each dilution was plated out onto PDA, NA and AIA to count fungi, bacteria and actinomycetes, respectively (Lapage et al., 1970; Collee et al., 1996; Downes and Ito, 2001).

### 2.3 *In vitro* screening of microbes against *Foc* TR4

Representative isolates of fungi, bacteria, and actinomycetes were picked randomly based on morphological characters and identified to the genus level based on their morphological and biochemical characteristics (Akande et al., 2019) and utilized for the following further screening against *Foc* TR4.

#### 2.3.1 Dual culture assay

Indigenous soil bacterial and fungal isolates from different treatments were screened for antagonism against *Foc* using a dual culture assay. A 10 mm mycelial disc from a 7-day-old *Foc* culture was placed on PDA plates. Bacterial isolates were streaked on one side after 2 days, while in antagonistic isolates, a 2 mm mycelial disc was placed directly opposite *Foc*. Plates were incubated at 25–28 °C for 7 days. *Foc* alone served as control, with three replicates per treatment. Mycelial growth inhibition (%) was calculated following Dennis and Webster (1971).

#### 2.3.2 Spore germination assay

Bacterial isolates were grown in nutrient broth for 48 h and fungal isolates in PDB for 7 days. Cell-free culture filtrates were obtained by filtering in muslin cloth followed by centrifugation.

In cavity slides, 30  $\mu\text{L}$  of *Foc* conidial suspension ( $4 \times 10^6$  spores  $\text{ml}^{-1}$ ) was mixed with 70  $\mu\text{L}$  of culture filtrate and incubated at 25 °C. Sterile water served as control. Spore germination was recorded at 24 h intervals up to 96 h, and per cent germination was calculated with three replicates (CSFT, 1943).

### 2.3.3 Agar well diffusion assay

PDA plates were seeded with 0.1 ml of *Foc*-TR4 spore suspension ( $10^5$  spores  $\text{ml}^{-1}$ ). Wells (8 mm) were filled with 200  $\mu\text{L}$  of bacterial (48 h) or fungal (7 days) culture filtrates. Sterile broth served as negative control. Plates were incubated at 25 °C for 7 days, and antifungal activity was assessed by measuring inhibition zones (Mehmood et al., 1999).

### 2.3.4 Protease production assay

Protease activity of soil bacterial isolates was assessed by streaking on skim milk agar and incubating at 28 °C for 2 days. Fungal isolates were tested by placing a 2 mm mycelial disc on skim milk agar and incubating at 25 °C for 7 days. Protease production was indicated by a clear zone around the colonies (Smibert et al., 1994).

### 2.3.5 Cellulase production assay

Cellulase activity was determined by streaking bacterial isolates on Carboxymethyl Cellulose (CMC) agar and incubating at 28 °C for 2 days. Fungal isolates were screened by placing a 2 mm mycelial disc on CMC agar and incubating at 25 °C for 7 days. Enzyme activity was indicated by clear zones around the colonies (Dashtban et al., 2009).

### 2.3.6 Indole acetic acid (IAA) production

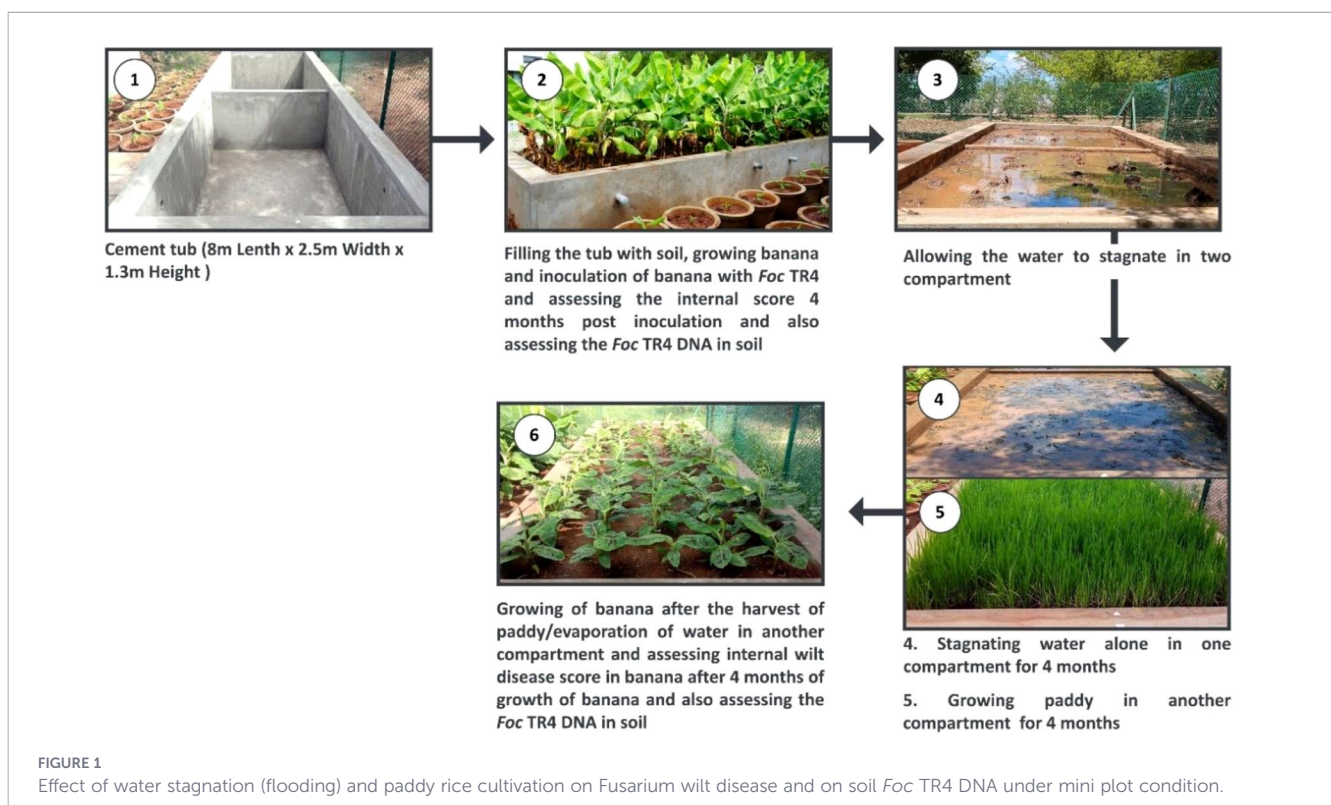
IAA production by bacterial and fungal antagonists was detected following Brick et al. (1991). Culture filtrates (48 h for bacteria and 7 days for fungi) were obtained by centrifugation at 10,000 rpm for 10 min. 2 ml of supernatant was mixed with orthophosphoric acid and Salkowski reagent; development of a pink colour indicated IAA production.

### 2.3.7 Phosphate solubilisation

Bacterial isolates were screened on Pikovskaya's agar by streaking, while fungal isolates were tested using a 2 mm mycelial disc. Plates were incubated at 28 °C, and phosphate solubilisation was indicated by clear zones around colonies after 3 days (Guar, 1990).

## 2.4 Effect of flooding on Fusarium wilt (*Foc* TR4)

The experiment was conducted under microplot conditions, and 30 tissue culture (TC) plants (cv. Grand Nain) were planted in the plot and each plant was considered as an individual biological replicate. A cement tub with a size of 8 m length x 2.5 m width x 1.3 m height was constructed and divided into two equal compartments (Figure 1). One of the compartments was used to determine the effect of flooding on Fusarium wilt, and the other to determine the effect of paddy on the disease. To study the effect of flooding, a compartment was filled with a mixture of red soil, sand and FYM at equal proportion. Subsequently, 1.5 kg of sorghum seed colonised with *Foc* TR4 was applied as inoculum to the subsurface soil of one



of the compartments and mixed thoroughly. The soil was then watered to saturation level.

After 4 days, 30 tissue culture-derived Cavendish banana cv. Grand Nain plants were planted at a spacing of 30 x 30 cm. Three months after planting, all 30 plants were gently removed from the soil and rated for inner rhizome discoloration and scored on 0–5 scale (0 = healthy and 5 = >75% of the rhizome discoloured). After the disposal of the banana plants, the same cement compartment was filled with water (Figure 1) for 4 months. At 4 months, the water in the tank was allowed to evaporate naturally. Once the soil was no longer water saturated, 30 tissue culture-derived Grand Nain plants were planted, and normal agronomical practices were applied. Four months after planting, the banana plants were gently removed and the internal rhizome discoloration scored on 0–5 scale, as described earlier. In addition, representative soil samples were collected (before and after flooding) for the quantification of *Foc* DNA present in the soil using qPCR with *Foc* TR4-specific primers (Thangavelu et al., 2022).

## 2.5 Effect of paddy cultivation on Fusarium wilt disease (*Foc* TR4)

In the second compartment of the cement tub, the effect of paddy rice cultivation in reducing *Foc* inoculum level was studied. The same methods as described above were applied. However, instead of maintaining water stagnation [i.e., flooding] for 4 months, paddy was grown for a period of 4 months. Subsequently, 30 tissue culture-derived Grand Nain plants were grown for 4 months. At 4 months, the banana plants were gently removed of the soil and assessed for internal wilt disease score based on discoloration in the rhizome tissues using a 0–5 disease scale as described by Zuo et al. (2018) (Figure 1). From the soil collected before and after the paddy cultivation, the *Foc* TR4 DNA was also assessed.

## 2.6 *Foc* races-DNA quantification (qPCR assay)

### 2.6.1 Soil DNA isolation

From the pot and tub, about 3 g of subsurface soil was taken from different places of the same treatment, pooled and total soil DNA was isolated using the method adopted by Yeates et al. (1998) with some modifications. DNA was extracted by first adding 3 ml of extraction buffer [100 mM Tris-HCl [pH 8.0] and 100 mM sodium EDTA [pH

8.0], 1.5 M NaCl) to the soil, along with an equal volume of glass beads, and by vigorously vortexing the mixture for 1 min. Subsequently, 3  $\mu$ l of  $\beta$ -mercaptoethanol and 10% sodium dodecyl sulphate were added, mixed, and the sample incubated at 65 °C for 1 hr. After centrifugation at 6,000 rpm for 10 min, the supernatant was transferred to fresh tubes containing a half-volume of polyethylene glycol (30%) and sodium chloride (1.6 M) in a ratio of 1:2 and incubated at -20 °C overnight. The sample was then centrifuged (10,000 rpm for 10 min) and the partially purified nucleic acid pellet was resuspended with 200  $\mu$ l of TE buffer (10 mM Tris HCl, 1 mM sodium EDTA, pH 8.0). An equal amount of chloroform: isoamyl alcohol (24:1v/v) was added and mixed gently and centrifuged at 10,000 rpm for 5 min at 4 °C. The top aqueous layer was transferred to a fresh tube and 2.5 volumes of 70% absolute ethanol, and 0.1 volume of 5 M potassium acetate, were added. This was incubated at -20 °C for 2 hrs and centrifuged at 10–000 rpm for 15 min at 4 °C. The DNA pellet was then washed with 200  $\mu$ l of 70% ethanol, air-dried and dissolved in 1 x TE buffer (10 mM Tris-HCl, pH 8; 0.1 mM EDTA) for further analysis. The total soil DNA obtained was checked for its purity and quantified at 260/280 nm using Nanodrop LITE (Thermo Scientific, Waltham, MA, USA).

### 2.6.2 *Foc* DNA quantification by qPCR analysis

The total soil DNA extracted (25 ng/ $\mu$ l) from the organic-amendment soil, waterlogged and paddy rice-grown soils were quantified by qPCR using the *Foc* TR4-specific primers *Foc* TR4F and *Foc* TR4R and the *Foc* race 1-specific primers *Foc* R1F and *Foc* R1R (Thangavelu et al., 2022) (Table 1). In order to determine the concentration of *Foc* TR4 and *Foc* race 1 in the soil, standard DNA isolated from known *Foc* TR4 and *Foc* race 1 isolates was used at different concentrations, ranging from 0.001 to 50 ng  $\mu$ L<sup>-1</sup>. A non-template control was included to determine specificity. qPCR was performed using SYBR Green mix (Thermo Fisher scientific™) to amplify the target DNA with the respective markers. The PCR reaction mixture of 10  $\mu$ L contained 0.5  $\mu$ M  $\mu$ L<sup>-1</sup> each of the forward and reverse primers, SYBR Green mix (5.0  $\mu$ L), ultra-pure water (1.0  $\mu$ L) and 25 ng  $\mu$ L<sup>-1</sup> of the target DNA extract. The qPCR analysis was performed in a Step One plus Real-Time PCR System (Applied Biosystems™) using the following conditions: Initial denaturation at 95 °C for 5 min, followed by 40 cycles of amplification at 95 °C for 30 s, annealing at 65 °C *Foc* TR4 (62°C for *Foc* race 1) for 45 s and 72 °C for 45 s, and a final elongation at 72 °C for 5 min. A melt curve analysis was also carried out to assess amplification specificity.

TABLE 1 Primers used to quantify DNA of *Foc* TR4 and race 1 inoculum in soil samples.

Primer name	Gene ID	Primer sequence (5' to 3')	Length (bp)	Annealing conditions	Reference
<i>Foc</i> TR4F <i>Foc</i> TR4R	KX434998.1 (SIX1a)	TGATTTGCCGTGGAATGACA TGGTCTTGACACGACCCA	250 bp	65°C for 30 s 30 cycles	Thangavelu et al., 2022
<i>Foc</i> R1F <i>Foc</i> R1R	XM_018394505.1 (Hypothetical protein)	TACCTCCTTGGTCGACAGGT CAGACTTCCAACGTCCTCGGT	320 bp	62 °C for 30s 30 cycles	

## 3 Results

### 3.1 Effect of organic amendments on Fusarium wilt disease (*Foc* TR4) and plant growth

Organic amendments at 200 and 300 g per plant (except Ash and FYM) significantly suppressed Fusarium wilt severity in Cavendish banana cv. Grand Nain based on internal rhizome discoloration ( $P \leq 0.05$ ), with suppression levels ranging from 23.3 to 100% compared with *Foc* TR4-inoculated control plants (Table 2). Complete disease suppression (disease score = 0) was observed in banana plants treated with gingelly and groundnut cakes at 300 g per plant, whereas the *Foc* TR4-inoculated control plants recorded an average disease severity score of 4.3 (Table 2 and Figure 2).

The effect of the eight organic amendments on plant height, girth, number of leaves and leaf area showed significant improvements ( $P \leq 0.05$ ) relative to the *Foc* TR4-inoculated plants. The highest increases in plant height (158.9%), pseudostem girth (147.8%), number of leaves (41.3%) and leaf area (315.5%) were observed in plants treated with 300 g of groundnut cake (GNC) (Table 2).

### 3.2 Effect of organic amendments on Fusarium wilt disease (*Foc* race 1) and plant growth

In general, the application of organic amendments at 200 and 300 g per plant, significantly suppressed the Fusarium wilt caused by *Foc* race 1 in banana cv. Grand Nain ( $P \leq 0.05$ ), with disease reductions of 33.3% and 93.3%, respectively, compared with the *Foc* race 1-inoculated control plants (Table 3). The highest suppression of Fusarium wilt indicated by a significantly lower internal disease score of 0.3 was observed in plants treated with 300 g of neem cake per plant, followed by those treated with 300 g of groundnut cake, which recorded an internal wilt disease score of 0.33. In contrast, the *Foc* race 1-inoculated control plants recorded a significantly higher disease severity score of 4.5 (Table 3 and Figure 3). All eight organic amendments significantly ( $P \leq 0.05$ ) enhanced plant growth parameters, including plant height, pseudostem girth, total number leaves, and leaf area. The application of 300 g of neem cake per plant showed the highest increase in plant height (100.3%), girth (50.6%), total number of leaves (25%) and leaf area (164.9%) as compared to *Foc* race 1-inoculated control plants as determined by Duncan's Multiple Range Test (DMRT) at  $P \leq 0.05$  (Table 3).

### 3.3 Effect of organic amendments on the microbial population in the soil

The application of organic amendments significantly increased the microbial population of fungi, bacteria and actinomycetes as compared to the controls (Table 4). Differences among treatments were tested using analysis of variance (ANOVA) and were considered statistically significant at  $P \leq 0.05$ , as indicated by the critical difference (CD) at the 5% level. Among the treatments, groundnut

cake applied at 300 g per plant recorded the highest significant ( $P \leq 0.05$ ) increase in bacterial ( $33 \times 10^{10}$  cfu g<sup>-1</sup>) and actinomycete ( $4 \times 10^6$  cfu g<sup>-1</sup>) populations compared with the positive (*Foc* TR4-inoculated) control (bacteria:  $11 \times 10^{10}$  cfu g<sup>-1</sup>; actinomycetes:  $1 \times 10^6$  cfu g<sup>-1</sup>) and the non-inoculated control (bacteria:  $9 \times 10^{10}$  cfu g<sup>-1</sup>; actinomycetes:  $1 \times 10^6$  cfu g<sup>-1</sup>). Gingelly oil cake applied at 300 g per plant also resulted in a significant ( $P \leq 0.05$ ) increase in microbial populations, with bacterial and actinomycete counts of  $28 \times 10^{10}$  and  $4 \times 10^6$  cfu g<sup>-1</sup>, respectively. The highest fungal population was recorded in FYM applied at 300 g per plant ( $7 \times 10^{10}$  cfu g<sup>-1</sup>), followed by neem cake, gingelly cake, mustard cake, and ash applied at 300 g per plant ( $5 \times 10^{10}$  cfu g<sup>-1</sup>) (Table 4).

Under organic amendments + *Foc*-race 1 inoculation, the FYM applied at 300 per plant resulted in the highest fungal ( $8 \times 10^{10}$  cfu g<sup>-1</sup>) and actinomycetes ( $4 \times 10^6$  cfu g<sup>-1</sup>) populations. However, these effects were statistically comparable ( $P \leq 0.05$ ) to those observed with FYM at 200 per plant for fungal populations, and neem cake (300 per plant) and groundnut cake (300 per plant) for actinomycete populations. With respect to bacterial populations, gingelly cake applied at 300 per plant produced the highest significant ( $P \leq 0.05$ ) increase ( $42 \times 10^{10}$  cfu g<sup>-1</sup>), followed by castor cake applied at 300 per plant ( $36 \times 10^{10}$  cfu g<sup>-1</sup>) (Table 4).

### 3.4 *In vitro* screening of microbes from soils with organic amendments for the suppression of *Foc* TR4

A total of 49 bacterial and 14 fungal isolates obtained from soil treated with organic amendments were evaluated for the suppression of *Foc* TR4 *in vitro*. Of these, *Trichoderma* sp. F13 exhibited the highest growth inhibition (83.0%) of *Foc* TR4, while *Trichoderma* sp. F6 suppressed its spore germination most (26.6%). In addition, *Trichoderma* sp. F13 and *Bacillus* sp. B6 recorded the largest zone of inhibition (3.1 cm) in the agar well diffusion assay.

Analysis of hydrolytic enzyme production revealed that three bacterial isolates (*Bacillus* sp. B2, B17, and B22) and three fungal isolates (*Trichoderma* spp. F5, F6, and F10) tested positive for protease activity. Similarly, *Bacillus* isolates B6, B17, and B22, along with *Trichoderma* spp. isolates F6, F9, and F13, demonstrated cellulase production. In terms of IAA production, four *Bacillus* strains (B2, B12, B22, and B23) and three *Trichoderma* isolates (F5, F6, and F13) exhibited the ability to produce this growth hormone (Table 5).

### 3.5 Effect of soil organic amendments on *Foc* DNA

The specific primers for *Foc* R1 and TR4 were evaluated using the SYBR Green qPCR method, and exponential amplification of PCR products was observed at each cycle. For *Foc* R1, Ct values of 16.7, 19.5, 22.2, 24.8, and 28.3 corresponded to DNA concentrations of 50.0, 5.0, 0.5, 0.05, and 0.005 ng, respectively, with an amplification efficiency exceeding 90% (slope  $-3.32$ ,  $R^2$  0.996). For *Foc* TR4, Ct values of 15.7, 20.9, 25.7, 26.6, and 28.9 corresponded to 10.0, 1.0, 0.1, 0.01, and 0.001 ng DNA, with an efficiency of 105% (slope  $-3.20$ ,  $R^2$  0.92). No cross-hybridization or fluorescence signals above the baseline were observed for either primer set. In general, the application of organic amendments

TABLE 2 Effect of organic amendments on various growth parameters and Fusarium wilt disease (*Foc* TR4) under pot culture condition.

T. No	Treatment	Height (cm) and % increase	Girth (cm) and % increase	Total leaves and % increase	Leaf area and % increase	Rhizome wilt rating and % reduction
T1	NC-100 g+ <i>Foc</i>	76.5 <sup>bcd</sup> (105.6)	23.0 <sup>cde</sup> (103.5)	10.7 <sup>ab</sup> (33.8)	1938.8 <sup>abcde</sup> (150.0)	3.50 <sup>abc</sup> (18.6)
T2	NC-200 g+ <i>Foc</i>	85.0 <sup>ab</sup> (128.5)	23.3 <sup>bcde</sup> (106.2)	10.7 <sup>ab</sup> (33.8)	2238.9 <sup>abcde</sup> (188.7)	2.00 <sup>def</sup> (53.5)
T3	NC -300 g+ <i>Foc</i>	87.3 <sup>ab</sup> (134.7)	27.0 <sup>ab</sup> (138.9)	11.5 <sup>a</sup> (41.3)	2573.1 <sup>abcde</sup> (231.8)	1.50 <sup>ef</sup> (65.1)
T4	GNC -100 g+ <i>Foc</i>	91.0 <sup>ab</sup> (144.6)	27.5 <sup>ab</sup> (143.3)	10.0 <sup>cde</sup> (25.0)	2738.4 <sup>gh</sup> (253.2)	2.67 <sup>cd</sup> (37.9)
T5	GNC-200 g+ <i>Foc</i>	94.5 <sup>a</sup> (154.0)	27.7 <sup>ab</sup> (145.1)	10.3 <sup>bcd</sup> (28.8)	2918.6 <sup>ab</sup> (276.4)	1.33 <sup>f</sup> (69.1)
T6	GNC -300 g+ <i>Foc</i>	96.3 <sup>a</sup> (158.9)	28.0 <sup>a</sup> (147.8)	11.0 <sup>a</sup> (37.5)	3222.0 <sup>a</sup> (315.5)	0.00 <sup>g</sup> (100.0)
T7	CC-100 g+ <i>Foc</i>	78.5 <sup>bc</sup> (111.0)	23.5 <sup>bcde</sup> (108.0)	9.0 <sup>efg</sup> (12.5)	2493.0 <sup>abcde</sup> (221.5)	4.00 <sup>ab</sup> (7.0)
T8	CC -200 g + <i>Foc</i>	88.0 <sup>ab</sup> (136.6)	24.3 <sup>bcd</sup> (115.0)	9.3 <sup>efg</sup> (16.3)	2589.6 <sup>abcd</sup> (234.0)	2.67 <sup>cd</sup> (37.9)
T9	CC-300 g+ <i>Foc</i>	89.0 <sup>ab</sup> (139.2)	25.7 <sup>abc</sup> (127.4)	10.0 <sup>cde</sup> (25.0)	2748.0 <sup>bc</sup> (254.4)	0.33 <sup>g</sup> (92.3)
T10	GC-100 g+ <i>Foc</i>	54.7 <sup>efg</sup> (47.0)	15.7 <sup>def</sup> (38.9)	8.3 <sup>gh</sup> (3.8)	1369.1 <sup>bcde</sup> (76.6)	3.67 <sup>ad</sup> (14.7)
T11	GC -200 g+ <i>Foc</i>	64.3 <sup>cde</sup> (72.8)	19.3 <sup>def</sup> (70.8)	10.3 <sup>bcd</sup> (28.8)	1846.9 <sup>abcde</sup> (138.2)	2.33 <sup>de</sup> (45.8)
T12	GC -300 g+ <i>Foc</i>	77.7 <sup>cd</sup> (108.9)	21.3 <sup>de</sup> (88.5)	10.7 <sup>ab</sup> (33.8)	2005.6 <sup>abcde</sup> (158.7)	0.00 <sup>g</sup> (100.0)
T13	MC-100 g+ <i>Foc</i>	64.0 <sup>cde</sup> (72.0)	18.5 <sup>def</sup> (63.7)	10.0 <sup>cde</sup> (25.0)	1552.5 <sup>bcde</sup> (100.2)	3.30 <sup>bc</sup> (23.3)
T14	MC-200 g+ <i>Foc</i>	64.6 <sup>cde</sup> (73.7)	21.3 <sup>de</sup> (88.49)	10.3 <sup>bcd</sup> (28.8)	2010.0 <sup>abcde</sup> (159.2)	2.6 <sup>cd</sup> (39.5)
T15	MC-300 g+ <i>Foc</i>	78.0 <sup>bc</sup> (109.7)	24.3 <sup>bcd</sup> (115.5)	10.6 <sup>bc</sup> (32.5)	2232.0 <sup>abcde</sup> (187.9)	1.6 <sup>ef</sup> (62.8)
T16	FYM -100 g+ <i>Foc</i>	51.2 <sup>efgh</sup> (37.6)	11.6 <sup>gh</sup> (2.7)	9.6 <sup>def</sup> (20.0)	916.2 <sup>gh</sup> (18.2)	4.0 <sup>ab</sup> (7.0)
T17	FYM-200 g+ <i>Foc</i>	54.6 <sup>efg</sup> (46.7)	12.0 <sup>fgh</sup> (6.2)	10.0 <sup>cde</sup> (25.0)	1147.6 <sup>defg</sup> (48.0)	3.6 <sup>ab</sup> (16.3)
T18	FYM-300 g+ <i>Foc</i>	55.2 <sup>efg</sup> (48.4)	12.6 <sup>efg</sup> (11.5)	10.0 <sup>cde</sup> (25.0)	1142.4 <sup>defg</sup> (47.3)	2.60 <sup>cd</sup> (39.5)
T19	VER-100 g+ <i>Foc</i>	50.6 <sup>efgh</sup> (36.0)	12.0 <sup>fgh</sup> (6.2)	9.3 <sup>efg</sup> (16.3)	820.2 <sup>gh</sup> (5.8)	4.0 <sup>ab</sup> (7.0)
T20	VER-200 g+ <i>Foc</i>	58.0 <sup>ef</sup> (55.9)	12.3 <sup>efg</sup> (8.8)	9.0 <sup>efg</sup> (12.5)	822.8 <sup>gh</sup> (6.1)	3.3 <sup>bc</sup> (23.3)
T21	VER-300 g+ <i>Foc</i>	60.0 <sup>ef</sup> (61.3)	13.0 <sup>efg</sup> (15.0)	10.0 <sup>cde</sup> (25.0)	1213.8 <sup>cdefg</sup> (56.5)	2.3 <sup>de</sup> (46.5)
T22	ASH -100 g+ <i>Foc</i>	45.0 <sup>fgh</sup> (21.0)	11.6 <sup>gh</sup> (2.7)	9.0 <sup>efg</sup> (12.5)	966.4 <sup>fgh</sup> (24.6)	3.6 <sup>ab</sup> (16.3)
T23	ASH-200 g+ <i>Foc</i>	51.0 <sup>efgh</sup> (37.1)	12.0 <sup>fgh</sup> (6.2)	9.5 <sup>def</sup> (18.8)	979.2 <sup>fgh</sup> (26.3)	3.6 <sup>ab</sup> (16.3)
T24	ASH-300 g+ <i>Foc</i>	62.0 <sup>de</sup> (66.7)	12.5 <sup>efg</sup> (10.6)	10.0 <sup>cde</sup> (25.0)	1024.2 <sup>efgh</sup> (32.1)	2.3 <sup>de</sup> (46.5)
T25	Control	41.7 <sup>gh</sup> (12.1)	12.3 <sup>efg</sup> (8.8)	10.0 <sup>cde</sup> (25.0)	838.3 <sup>gh</sup> (8.1)	0.0 <sup>g</sup> (100.0)
T26	<i>Foc</i> alone	37.2 <sup>h</sup> (0.0)	11.3 <sup>h</sup> (0.0)	8.0 <sup>b</sup> (0.0)	775.4 <sup>h</sup> (0.0)	4.3 <sup>ab</sup> (0.0)
	CV%	4.02	3.51	0.99	5.31	22.14
	CD (0.05)	4.32	1.09	0.16	152.42	0.91

DMRT: Mean separation was performed using Duncan's Multiple Range Test; means followed by the same letter(s) do not differ significantly at  $P \leq 0.05$ . NC, Neem cake; GNC, Ground nut cake; CC, Castor cake; GC, gingelly cake; FYM, Farmyard manure; MC, Mustard cake; VER, Vermicompost; ASH, Rice husk ash.

significantly reduced *Foc* TR4 and race 1 DNA in soil expressed as pg per 25 ng of total soil DNA. Groundnut cake (50.3 and 98.1 pg) and mustard cake (70.3 and 98.8 pg), when applied at 300 g per plant, significantly reduced the *Foc* DNA of *Foc* race 1 and TR4, respectively, when compared to *Foc* treatment alone (537.7 and 524.3 pg). The percentage reduction of TR4 DNA in the groundnut and mustard cake applied soil was 90.6 and 86.9, respectively, while it was 81.2 and 81.1% for *Foc* race 1, respectively (Supplementary Figures 1 and 2). Interestingly, among the organic amendments, the maximum reduction in *Foc* TR4 DNA was observed in the groundnut cake (300 g/plant) treatment, whereas the maximum reduction in *Foc* race 1 DNA was for neem cake (300 g/plant) (Supplementary Figures 1 and 2). In Principal component analysis (PCA), PC1 and PC2 explained 77.7% and 22.3% of total variance,

respectively. The analysis also showed that groundnut and mustard cakes (300 g/plant) significantly reduced *Foc* inoculum for both *Foc* race 1 and TR4 races. However, neem cake (300 g/plant) was most effective against *Foc* race 1, while gingelly cake (300 g/plant) significantly reduced *Foc* TR4 (Figure 4).

### 3.5.1 Effect of flooding on Fusarium wilt severity and *Foc* DNA in the soil

Flooding of *Foc* TR4-inoculated soil for 4 months reduced internal rhizome discoloration from 3.4 to 2.2. qPCR analysis showed that *Foc* DNA levels, expressed as pg per 25 ng of soil DNA, declined from 5630 pg to 104.5 pg following flooding, representing a 98.14% reduction (Figures 5 and 6).



FIGURE 2

Effect of Groundnut cake on Fusarium wilt disease (*Foc* TR4) under pot culture condition. GNC, Ground nut cake.

TABLE 3 Effect of different organic amendments on various growth parameters and Fusarium wilt disease (*Foc* R1) under pot culture condition.

T. No.	Treatments	Height (cm) and % increase	Girth (cm) and % increase	Total leaves and % increase	Leaf area and % increase	Rhizome rating and % reduction
T1	NC-100 g+ <i>Foc</i>	36.3 <sup>gh</sup> (38.1)	11.0 <sup>cd</sup> (34.7)	11.3 <sup>ab</sup> (21.5)	460.8 <sup>ghij</sup> (53.9)	2.33 <sup>cde</sup> (48.1)
T2	NC-200 g+ <i>Foc</i>	48.0 <sup>bcd</sup> (82.5)	11.7 <sup>bc</sup> (42.5)	11.7 <sup>a</sup> (25.0)	650.1 <sup>bcd</sup> (117.1)	1.0 <sup>ghi</sup> (77.8)
T3	NC -300 g+ <i>Foc</i>	52.7 <sup>a</sup> (100.3)	12.3 <sup>ab</sup> (50.6)	11.7 <sup>a</sup> (25.0)	793.3 <sup>a</sup> (164.9)	0.30 <sup>j</sup> (93.3)
T4	GNC -100 g+ <i>Foc</i>	32.0 <sup>ijk</sup> (21.7)	9.0 <sup>gh</sup> (9.8)	10.3 <sup>bcd</sup> (10.8)	308.0 <sup>k</sup> (2.8)	3.33 <sup>b</sup> (25.9)
T5	GNC-200 g+ <i>Foc</i>	33.3 <sup>hij</sup> (26.7)	9.2 <sup>fgh</sup> (11.8)	10.7 <sup>abc</sup> (14.3)	320.0 <sup>k</sup> (6.8)	2.67 <sup>bcd</sup> (40.7)
T6	GNC -300 g+ <i>Foc</i>	48.7 <sup>abc</sup> (85.0)	11.0 <sup>cd</sup> (34.3)	11.3 <sup>ab</sup> (21.5)	496.5 <sup>fghi</sup> (65.8)	0.33 <sup>ij</sup> (92.6)
T7	CC-100 g+ <i>Foc</i>	32.0 <sup>ijk</sup> (21.7)	10.7 <sup>cde</sup> (30.2)	9.7 <sup>cd</sup> (3.6)	347.5 <sup>jk</sup> (16.0)	3.33 <sup>b</sup> (25.9)
T8	CC -200 g + <i>Foc</i>	35.7 <sup>ghi</sup> (35.6)	11.3 <sup>bcd</sup> (38.4)	10.7 <sup>abc</sup> (14.3)	413.9 <sup>hijk</sup> (38.2)	2.00 <sup>def</sup> (55.6)
T9	CC-300 g+ <i>Foc</i>	44.3 <sup>de</sup> (68.6)	12.3 <sup>ab</sup> (50.6)	10.7 <sup>abc</sup> (14.3)	665.3 <sup>abcd</sup> (122.1)	0.67 <sup>hij</sup> (85.2)
T10	GC-100 g+ <i>Foc</i>	36.0 <sup>ghi</sup> (36.9)	10.3 <sup>def</sup> (26.1)	10.7 <sup>abc</sup> (14.3)	326.9 <sup>k</sup> (9.2)	3.33 <sup>b</sup> (25.9)
T11	GC -200 g+ <i>Foc</i>	38.3 <sup>fg</sup> (45.8)	11.3 <sup>bcd</sup> (38.4)	10.7 <sup>abc</sup> (14.3)	485.6 <sup>ghi</sup> (62.1)	1.67 <sup>efg</sup> (63.0)
T12	GC -300 g+ <i>Foc</i>	44.7 <sup>cde</sup> (69.8)	11.3 <sup>bcd</sup> (38.4)	11.0 <sup>ab</sup> (17.9)	592.5 <sup>defg</sup> (97.8)	0.67 <sup>hij</sup> (85.2)
T13	MC-100 g+ <i>Foc</i>	34.3 <sup>ghij</sup> (30.5)	11.0 <sup>cd</sup> (34.3)	10.3 <sup>bcd</sup> (10.8)	398.9 <sup>hijk</sup> (33.2)	4.33 <sup>def</sup> (3.7)
T14	MC-200 g+ <i>Foc</i>	47.0 <sup>bcd</sup> (78.7)	11.3 <sup>bcd</sup> (38.4)	11.0 <sup>ab</sup> (17.9)	624.5 <sup>abcd</sup> (108.5)	2.00 <sup>ghi</sup> (55.6)
T15	MC-300 g+ <i>Foc</i>	49.7 <sup>ab</sup> (88.8)	11.3 <sup>bcd</sup> (38.4)	11.3 <sup>ab</sup> (21.5)	664.8 <sup>cdef</sup> (122.0)	1.00 <sup>ghi</sup> (77.8)
T16	FYM -100 g+ <i>Foc</i>	26.3 <sup>m</sup> (0.1)	9.7 <sup>efg</sup> (18.0)	10.3 <sup>bcd</sup> (10.8)	379.2 <sup>k</sup> (26.6)	4.33 <sup>a</sup> (3.7)
T17	FYM-200 g+ <i>Foc</i>	30.7 <sup>ijkl</sup> (16.6)	11.3 <sup>bcd</sup> (38.4)	11.0 <sup>ab</sup> (17.9)	518.4 <sup>efgh</sup> (73.1)	3.33 <sup>b</sup> (25.9)
T18	FYM-300 g+ <i>Foc</i>	34.3 <sup>ghij</sup> (30.5)	11.7 <sup>bc</sup> (42.5)	11.3 <sup>ab</sup> (21.5)	760.5 <sup>ab</sup> (153.9)	1.33 <sup>fgh</sup> (70.4)
T19	VER-100 g+ <i>Foc</i>	33.0 <sup>hij</sup> (25.5)	11.3 <sup>bcd</sup> (38.4)	10.7 <sup>abc</sup> (14.3)	344.0 <sup>jk</sup> (14.9)	3.33 <sup>b</sup> (25.9)
T20	VER-200 g+ <i>Foc</i>	37.0 <sup>gh</sup> (40.7)	11.5 <sup>bcd</sup> (40.4)	11.0 <sup>ab</sup> (17.9)	555.7 <sup>defg</sup> (85.6)	2.67 <sup>bcd</sup> (40.7)
T21	VER-300 g+ <i>Foc</i>	41.7 <sup>ef</sup> (58.4)	13.3 <sup>a</sup> (62.9)	11.3 <sup>ab</sup> (21.5)	734.7 <sup>abc</sup> (145.3)	1.00 <sup>ghi</sup> (77.8)
T22	ASH -100 g+ <i>Foc</i>	27.3 <sup>lm</sup> (3.9)	11.3 <sup>bcd</sup> (38.4)	11.0 <sup>ab</sup> (17.9)	556.0 <sup>defg</sup> (85.6)	3.00 <sup>bc</sup> (33.3)
T23	ASH-200 g+ <i>Foc</i>	34.0 <sup>hij</sup> (29.3)	11.7 <sup>bc</sup> (42.5)	11.7 <sup>a</sup> (25.0)	634.7 <sup>bcd</sup> (111.9)	3.00 <sup>bc</sup> (33.3)
T24	ASH-300 g+ <i>Foc</i>	42.7 <sup>e</sup> (62.2)	13.3 <sup>a</sup> (62.9)	11.7 <sup>a</sup> (25.0)	739.3 <sup>abc</sup> (147.0)	0.67 <sup>hij</sup> (85.2)

(Continued)

TABLE 3 Continued

T. No.	Treatments	Height (cm) and % increase	Girth (cm) and % increase	Total leaves and % increase	Leaf area and % increase	Rhizome rating and % reduction
T25	Control	28.0 <sup>klm</sup> (6.5)	8.5 <sup>gh</sup> (3.7)	9.7 <sup>cd</sup> (3.6)	306.5 <sup>k</sup> (2.3)	0.00 <sup>j</sup> (100.0)
T26	<i>Foc</i> alone	26.3 <sup>m</sup> (0.0)	8.2 <sup>h</sup> (0.0)	9.3 <sup>d</sup> (0.0)	299.5 <sup>k</sup> (0.0)	4.50 <sup>a</sup> (0.0)
	CV%	6.53	7.05	6.53	12.71	26.15
	CD (0.05)	4.01	1.28	1.16	130.02	0.93

DMRT: Mean separation was performed using Duncan's Multiple Range Test; means followed by the same letter(s) do not differ significantly at  $P \leq 0.05$ . NC, Neem cake; GNC, Ground nut cake; CC, Castor cake; GC, gingelly cake; FYM, Farmyard manure; MC, Mustard cake; VER, Vermicompost; ASH, Rice husk ash.



FIGURE 3

Effect of neem cake on Fusarium wilt disease (*Foc* R1) under pot culture condition. NC, Neem cake.

TABLE 4 Microbial counts in *Foc* TR4- and *Foc* race1 inoculated soil with different organic amendments under pot culture conditions.

T. No.	Treatment	CFU/g of soil					
		Fungi ( $10^{10}$ )		Bacteria ( $10^{10}$ )		Actinomycetes ( $10^6$ )	
		<i>Foc</i> TR4	<i>Foc</i> race1	<i>Foc</i> TR4	<i>Foc</i> race1	<i>Foc</i> TR4	<i>Foc</i> race 1
T1	NC-100 g+ <i>Foc</i>	3	2	16	18	1	1
T2	NC-200 g+ <i>Foc</i>	3	1	25	26	1	2
T3	NC -300 g+ <i>Foc</i>	5	4	28	33	2	4
T4	GNC -100 g+ <i>Foc</i>	2	1	14	16	1	1
T5	GNC-200 g+ <i>Foc</i>	4	3	19	14	2	2
T6	GNC -300 g+ <i>Foc</i>	4	4	33	19	4	4
T7	CC-100 g+ <i>Foc</i>	2	4	20	22	1	1
T8	CC -200 g + <i>Foc</i>	4	2	24	31	1	1
T9	CC-300 g+ <i>Foc</i>	5	4	27	36	2	2
T10	GC-100 g+ <i>Foc</i>	4	3	27	27	1	1
T11	GC -200 g+ <i>Foc</i>	3	4	23	23	2	1
T12	GC -300 g+ <i>Foc</i>	5	6	28	42	4	2
T13	MC-100 g+ <i>Foc</i>	2	5	14	16	1	1
T14	MC-200 g+ <i>Foc</i>	4	3	19	22	2	1
T15	MC-300 g+ <i>Foc</i>	5	6	26	24	1	3

(Continued)

TABLE 4 Continued

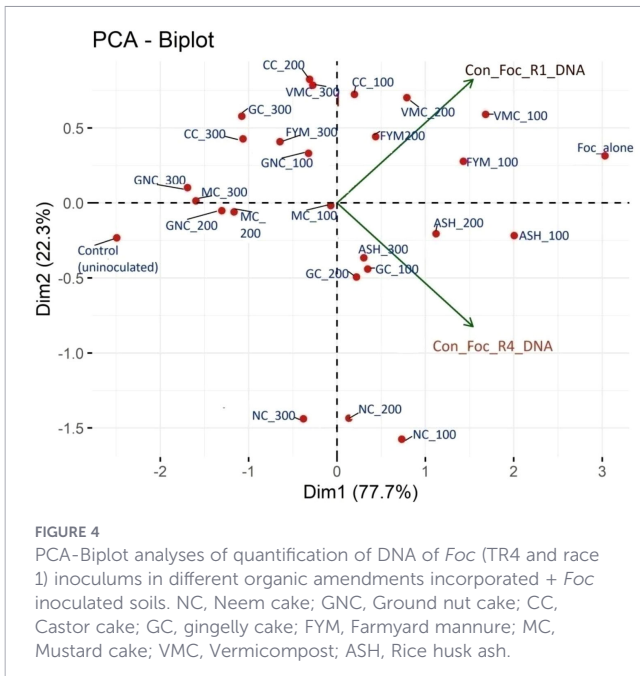
T. No.	Treatment	CFU/g of soil					
		Fungi ( $10^{10}$ )		Bacteria ( $10^{10}$ )		Actinomycetes ( $10^6$ )	
		<i>Foc</i> TR4	<i>Foc</i> race1	<i>Foc</i> TR4	<i>Foc</i> race1	<i>Foc</i> TR4	<i>Foc</i> race 1
T16	FYM -100 g+ <i>Foc</i>	3	3	15	16	3	1
T17	FYM-200 g+ <i>Foc</i>	4	7	17	13	1	3
T18	FYM-300 g+ <i>Foc</i>	7	8	25	21	1	4
T19	VER-100 g+ <i>Foc</i>	2	3	21	23	3	1
T20	VER-200 g+ <i>Foc</i>	4	4	24	26	2	2
T21	VER-300 g+ <i>Foc</i>	3	4	27	34	2	3
T22	ASH -100 g+ <i>Foc</i>	2	2	18	18	3	1
T23	ASH-200 g+ <i>Foc</i>	4	4	20	22	4	1
T24	ASH-300 g+ <i>Foc</i>	5	4	23	24	2	2
T25	<i>Foc</i> Alone	2	2	11	13	1	1
T26	Control	1	2	9	12	1	1
	CV%	17.52	21.24	10.52	14.07	14.71	22.70
	CD (0.05)	1.2	1.63	4.6	6.57	0.57	1.06

Differences among treatments were tested using ANOVA and were considered statistically significant at  $P \leq 0.05$ , as indicated by the critical difference (CD) at the 5% level. NC, Neem cake; GNC, Ground nut cake; CC, Castor cake; GC, gingelly cake; FYM, Farmyard manure; MC, Mustard cake; Ver, Vermicompost; ASH, Rice husk ash; CFU, Colony Forming Unit.

TABLE 5 *In vitro* screening of organic amendments at microbes, obtained from *Foc* TR4 inoculated soils treated with different organic amendments, for their suppressive effects against *Foc*TR4.

Organisms	Percent mycelial growth inhibition (%) (in dual culture assay)	Zone of inhibition (cm) (in agar well diffusion assay)	Per cent spore inhibition	Protease production	Cellulase production	IAA production	Phosphate solubilization
<b>Bacterial isolates</b>							
<i>Bacillus</i> sp. B2	51.1 <sup>bcd</sup>	0.5 <sup>def</sup>	26.7 <sup>a</sup>	+	-	+	-
<i>Bacillus</i> sp. B6	36.2 <sup>d</sup>	3.1 <sup>a</sup>	22.2 <sup>abc</sup>	-	+	-	-
<i>Bacillus</i> sp. B12	36.2 <sup>d</sup>	2.5 <sup>bcd</sup>	23.3 <sup>abc</sup>	-	-	+	-
<i>Bacillus</i> sp. B17	44.7 <sup>cd</sup>	2.8 <sup>abcd</sup>	18.9 <sup>cd</sup>	+	+	-	-
<i>Bacillus</i> sp. B22	53.2 <sup>bcd</sup>	2.9 <sup>abc</sup>	20.0 <sup>cd</sup>	+	+	+	-
<i>Bacillus</i> sp. B23	48.9 <sup>cd</sup>	3.0 <sup>ab</sup>	21.1 <sup>bc</sup>	-	-	+	-
<b>Fungal isolates</b>							
<i>Trichoderma</i> sp. F5	70.2 <sup>abc</sup>	2.4 <sup>bcd</sup>	25.5 <sup>ab</sup>	+	-	+	-
<i>Trichoderma</i> sp. F6	59.4 <sup>abcd</sup>	2.5 <sup>bcd</sup>	26.6 <sup>a</sup>	+	+	+	-
<i>Trichoderma</i> sp. F9	80.9 <sup>a</sup>	2.7 <sup>bcd</sup>	22.2 <sup>abc</sup>	-	+	-	-
<i>Trichoderma</i> sp. F10	76.6 <sup>ab</sup>	2.9 <sup>abc</sup>	23.3 <sup>abc</sup>	+	-	-	-
<i>Trichoderma</i> sp. F13	83.0 <sup>a</sup>	3.1 <sup>a</sup>	15.5 <sup>d</sup>	-	+	+	-

'+' indicates present, '-' indicates absent.



### 3.5.2 Effect of paddy rice cultivation on Fusarium wilt severity and *Foc* DNA in soil

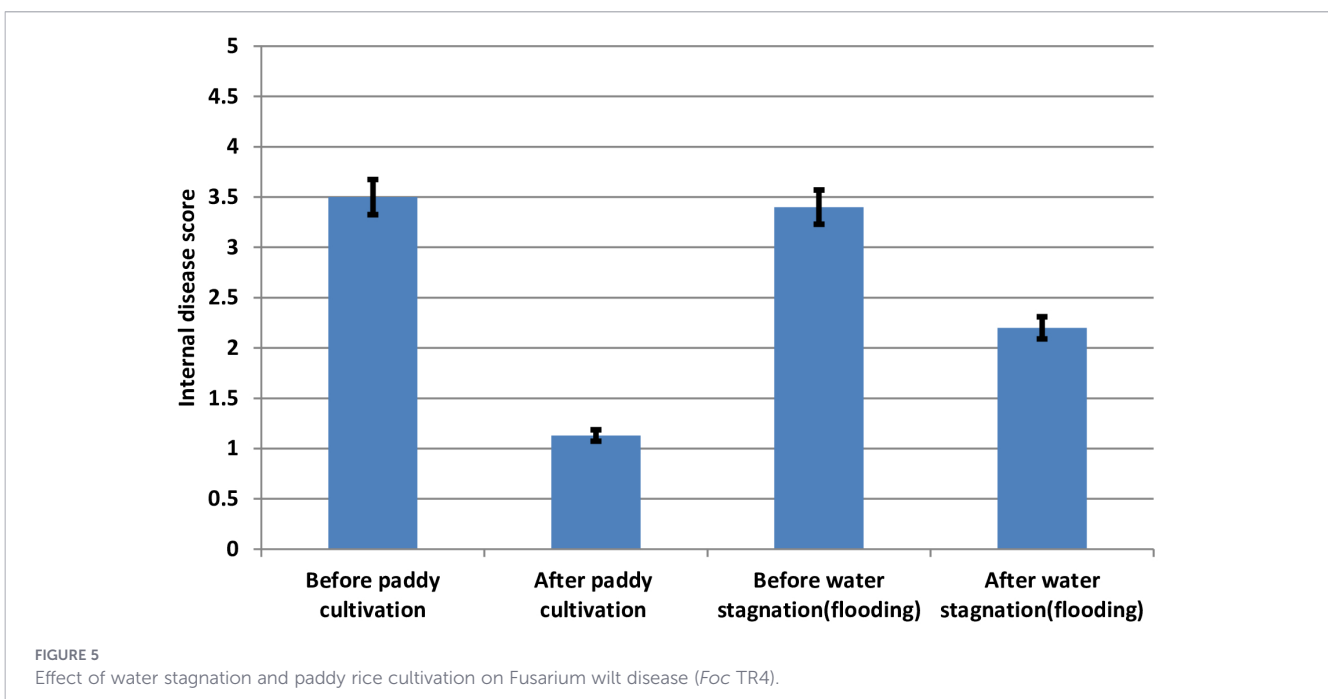
Prior to paddy cultivation, *Foc* TR4 DNA levels in soil were 7995 pg/25 ng, which decreased to 174.2 pg/25 ng after paddy rice cultivation, corresponding to a 97.8% reduction. Internal rhizome discoloration was also reduced from 3.5 before paddy rice cultivation to 1.13 after the paddy cultivation, indicating a 67.7% reduction in Fusarium wilt TR4 severity. These results demonstrate substantial suppression of *Foc* TR4 in soil following paddy rice cultivation (Figures 5 and 6).

## 4 Discussion

Integrated disease management (IDM), which combines multiple approaches to suppress banana Fusarium wilt, is particularly important where resistant cultivars are unavailable or not widely adopted (Viljoen et al., 2020). However, empirical evidence supporting soil-based components of IDM packages remains limited. In this study, cultural practices such as applying organic amendments, flooding of soils, and paddy rice cultivation have shown some value in the management of this devastating disease.

Organic amendments have been used to promote crop growth and yields since ancient times (Zhang et al., 2022), but their application for plant disease management is a more recent approach. Sustaining soil health and strengthening plant resistance to pathogens depend heavily on the efficient management of organic matter. Organic amendments can be selectively administered at different doses and in specific areas, such as disease hotspots, to maximise their impact (Liu et al., 2021). In the current study, organic amendments were applied at 100, 200, and 300 g per plant to assess dose dependent effects. Complete suppression of *Foc* TR4 was achieved in soils applied with gingelly and ground nut cake at 300 g/plant and, in the case of *Foc* race 1, significant suppression was obtained with neem cake and ground nut cake at 300 g/plant.

The 300 g per plant dose consistently resulted in the greatest suppression of the Fusarium wilt and enhanced plant growth, with no observable phytotoxic effects, whereas lower doses were comparatively less effective. This application rate corresponds to 1.0% (w/w) of the pot soil, which falls within the recommended range for oilseed cake amendments. Previous studies have reported effective suppression of Fusarium and improved plant growth through the application of oil cakes at 2% (w/w) (Raj and Kapoor, 1996).



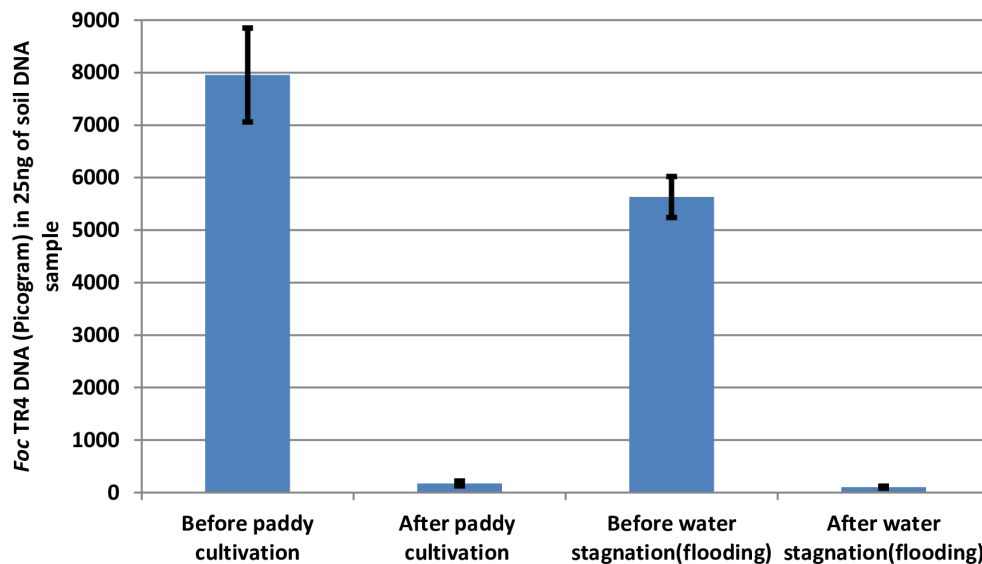


FIGURE 6

Quantification of Foc –TR4 DNA from DNA (25 ng) extracted from the soil of paddy rice cultivation and flooding.

Ehtashamul-Haque et al. (1995) reported that adding oil cakes such as cotton cake and neem cake to the soil significantly reduced fungal root infections, particularly by *Fusarium solani*, *Macrophomina phaseolina*, and *Rhizoctonia solani*. Similarly, Chauhan (1963) observed a significant reduction in redgram wilt incidence following soil amendment with de-oiled cakes of groundnut, sesame, and mustard. Raj and Kapoor (1996) reported a drastic reduction in tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* when soil was amended with cotton cake, mustard cake, sesame cake, and groundnut cake, attributing the disease suppression to a reduction in pathogen propagules.

Of the amendments tested, groundnut and gingelly (sesame) cakes were more effective against *Foc* TR4, whereas neem cake was more effective against *Foc* race 1. This interesting finding may be due to the presence of terpenoids and other volatile compounds released during the hydrolysis of oil cakes and other amendments (Kirkegaard et al., 1993). Additionally, these organic amendments may stimulate microbial activity in the soil, which in turn influences pathogen suppression.

The application of organic amendments not only suppress *Fusarium* wilt but also significantly enhanced plant growth. The organic amendments application increased various plant growth parameters such as plant height, girth, number of leaves and leaf area significantly as compared to *Foc* alone inoculated and non-amended banana plants. In general, the application of organic amendments may directly influence the physical, chemical, and biological characteristics of the soil, and content of nitrogen, phosphorous, potassium, and other nutrients. Hence, the applied amendments likely promoted the development of feeder roots, thereby improving nutrient uptake and overall plant growth (López et al., 2014). Additionally, the organic amendments also significantly increased microbial populations in the plant rhizosphere in this study. *Trichoderma* spp. (five strains) and *Bacillus* spp. (six strains) exhibited strong antagonistic properties against *Foc* TR4 *in vitro*, such as the inhibition of mycelial growth

and spore germination, as well as the production of lytic enzymes. These microbes all have growth-promoting abilities due to the production of IAA.

Organic amendments not only support the proliferation of beneficial microbes known to enhance plant growth and vigour (Castaño et al., 2011; Zhao et al., 2018) but also contribute to the suppression of soil borne pathogens (Punia et al., 2011; Rajik et al., 2011). In this study, qPCR analyses of *Foc* DNA in the soil revealed significant reductions of up to 90.6% for *Foc* TR4 and 85.1% for *Foc* race 1. Notably, groundnut cake (300 g/plant) achieved the highest reduction in *Foc* TR4 DNA, while neem cake (300 g/plant) resulted in the greatest decrease in *Foc* race 1 DNA.

Flooding and paddy rice cultivation reduced *Fusarium* wilt symptoms in the inner rhizome of banana plants in this study. Flooding reduced rhizome discoloration by 35.2% while paddy cultivation, where water stagnation was maintained for 4 months, led to an even greater reduction. In the process, *Foc* inoculum load was decreased by 98.14 and 97.81%, respectively. Flooding for 6 months was used to eradicate *Foc* race 1 from infested banana fields in Latin America in the 1950's (Stover, 1962). Prolonged water stagnation can create anaerobic conditions that reduce the survival of *Foc* (Ullah et al., 2021). However, once banana is replanted in flooded soils, *Fusarium* wilt returned within 2 years. Fan et al. (2021) demonstrated that flooding alone and flooding with paddy straw modified the biological and abiotic properties of soil. Similarly, Wen et al. (2015) further reported that flooding neutralises pathogens by suppressing *F. oxysporum* growth and preventing *Fusarium* wilt. Significant reductions of up to 96% in viable *Foc* populations were observed in organically amended soils under flooded or saturated conditions, where the pathogen load dropped from  $10^4$  cfu  $g^{-1}$  soil to  $10^3$  cfu  $g^{-1}$  soil.

Monocropping increases *Fusarium* wilt incidence as well as severity (Shen et al., 2017) and contributes to biophysical soil health issues such as soil erosion, fertility loss, agrochemical accumulation, and pathogen build-up (Xiong et al., 2015a). In contrast, crop

rotation maintains soil productivity and mitigates soilborne diseases by disrupting the life cycle of pathogens (Krupinsky et al., 2002; Jin et al., 2019). In this study, the rotation of banana with paddy rice suppressed Fusarium wilt under micro-plot conditions by 67.7%. In India, paddy is traditionally rotated with banana cultivation in states such as Tamil Nadu, Kerala, and parts of Uttar Pradesh and Bihar. Field surveys revealed that long-term banana monoculture (10–15 years) in districts like Ayodhya (Uttar Pradesh) and Theni (Tamil Nadu) resulted in severe Fusarium wilt incidence, exceeding 90% (Unpublished data). However, in fields where banana was rotated with paddy rice, Fusarium wilt was significantly lower, which corroborates our findings. When banana ratooning was practiced, however, wilt incidence gradually increased. Therefore further field studies integrating crop rotation, paddy cultivation, organic amendments, biocontrol agents, and host plant resistance are very important to assess their additive or multiplicative effects on Fusarium wilt of banana and such studies eventually would be beneficial for developing an effective IDM strategy.

## 5 Conclusions

In this study, organic amendments such as groundnut, gingelly, and neem cakes applied at 300 g per plant were highly effective in suppressing banana Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* TR4 and race 1 under controlled conditions. Flooding and paddy rice cultivation also significantly reduced the disease severity and soil inoculum levels, supporting their potential role as cultural components of Fusarium wilt management. The integration of organic amendments, flooding or crop rotation, biocontrol agents, and host resistance represents a promising framework for integrated disease management of banana Fusarium wilt. However, field-level validation under diverse agroecological conditions is essential before these strategies can be recommended for large-scale deployment.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

RT: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. ML: Data curation, Supervision, Writing – review & editing. PG: Investigation, Supervision, Writing – review & editing. BN: Data curation, Investigation, Methodology, Writing – review & editing. AV: Writing – review & editing. GB: Writing – review & editing.

TS: Project administration, Supervision, Writing – review & editing. RS: Writing – review & editing.

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## Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2026.1749035/full#supplementary-material>

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