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HARNESSING ANTAGONISTIC POTENTIAL OF NATIVE BANANA ENDOPHYTES AGAINST *Fusarium oxysporum* f. sp. *Cubense* CAUSING FUSARIUM WILT: AN *IN VITRO* APPROACH

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ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) has emerged as the most destructive disease of banana, compromising yield and quality. The ineffectiveness of prevalent control measures raises pressing need to perceive alternative strategies, which has paved way towards exploration of endophytes as a promising biocontrol tool chiefly contributed by their increased ability to survive in plant endosphere, occupy pathogen niches and channelized cross talk with plants. This study evaluated native bacterial and fungal endophytes isolated from four banana cultivars- Red Banana (AAA), Grand Naine (AAA), Malbhog (AAB) and Kothia (ABB), for their in vitro biocontrol potential against Foc race 1. A total of 120 endophytes (90 bacterial, 30 fungal) were isolated from pseudostem, root, and rhizome tissues across selective media. Based on morpho-cultural characterization, 55 bacterial and 13 fungal isolates were selected for further evaluation. The endophytic abundance and diversity varied significantly across cultivars and tissues. Cultivar Kothia yielded the highest isolate count (30 bacterial, 3 fungal), followed by Malbhog, Red Banana, and Grand Naine. Tissue-wise, roots exhibited the greatest proportion of isolates (41%), followed by rhizomes and pseudostems. In vitro dual-culture bioassays demonstrated significant pathogen inhibition by the bacterial and fungal endophytes. The root-derived endophytic fungal isolate KERF-1 (cv. Kothia) exhibited the highest mycelial growth inhibition at 77.37%, followed by rhizome-derived G9ERhF-1 (cv. Grand Naine) at 60.15%. Bacterial endophytic isolate KERB-1 (cv. Kothia) showed the highest antagonistic efficacy overall, exhibiting 82.09% mycelial growth inhibition of *Foc*, followed by isolates KERB-5 and KERhB-11 with 80.37% and 80.15% inhibition, respectively. The superior mycelial suppression of *Foc* by these bacterial and fungal endophytes place them as potential frontline biocontrol agents, emphasizing the importance of host and tissue specific endophytes in disease management and providing a strong baseline for formulating eco-friendly, microbial-based strategies to sustainably combat Fusarium wilt in banana cultivation.

Key words : Banana, Fusarium wilt, Endophytes, Biological control.

Introduction

Banana is one of the most important fruit crops playing a critical role in global food security chiefly due to its high genetic diversity, availability throughout the year, affordability, nutritional contents, therapeutic values and economic significance. Banana is grown across 130 countries, primarily in the tropical and subtropical regions of the world, covering an area of 5.33 million hectares and annual global production of 124.98 million tonnes (Thangavelu *et al*., 2025). India is the largest producer of banana in the world with nearly 37.6 million tonnes of banana harvested annually from an area of 0.94 million ha (FAO, 2024). However, banana production is challenged by several biotic and abiotic stresses that significantly affect yield and quality of production. In the history of plant pathology, Fusarium wilt of banana (FWB), also called Panama wilt, caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* that wiped

out the Gros Michel variety in Central America during 1950s is considered as one of the most destructive diseases. Even today, it is one of the most destructive diseases of banana that greatly affects banana production, particularly the small and marginal farmers. Besides the persistence of the pathogen as chlamydospores in the soil even in absence of host, colonization of vascular tissues of the plant and rapidly spreading nature through contaminated soil, water, and intercultural tools, the inefficacy of existing management strategies including phytochemicals and resistant cultivars, make it very difficult and challenging to eradicate once an area is infected. (Dita *et al*., 2018; Wang *et al*., 2023). In recent scientific approaches, the study of plant-microbe interaction with respect to combating such phytopathogens and their resulting crop losses to ensure sustainable agriculture and food security, has taken the center stage. In order to checkmate these threats, research on exploration of potential endophytic microorganisms, the silent residents of the plants, have gained importance as biocontrol arsenals against the devastating plant pathogens. "*Endophytes are a class of microorganisms that survive inside the plant tissues without eliciting the disease symptoms or injury to the host and can be isolated from the surface sterilized plant organs or internal plant tissues*" (Rabee *et al*., 2024). The endophytes offer a great advantage as biocontrol agent against vascular pathogen by occupying the same ecological niche as the pathogen and acting as a "second microbial layer of plant defence" (Dubey *et al*., 2020).

Despite growing interest in endophyte-based biopesticides, comprehensive studies examining how endophytic microbial diversity and biocontrol potential vary across distinct host cultivars and anatomical plant organs remain limited (Souza *et al*., 2014; Chaudhary *et al*., 2022). Therefore, the present study was undertaken to isolate native bacterial and fungal endophytes from four distinct banana cultivars, namely, Red Banana (AAA), Grand Naine (AAA), Malbhog (AAB) and Kothia (ABB). Furthermore, this study aimed to evaluate the antagonistic efficacy of these native endophytes against *Fusarium oxysporum* f.sp. *cubense* (*Foc*), to thereby identify potent candidate strains for the future development of sustainable bio-fungicidal formulations.

Materials and Methods

Procurement and maintenance of the pathogen

The pathogen, *Fusarium oxysporum* f.sp. *cubense (Foc)* used in this investigation was procured from the Fruit Pathology Laboratory, Department of Plant Pathology and Nematology, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India. The culture was purified by single spore (Choi *et al*., 1999) and hyphal tip (Tuite, 1969) methods on potato dextrose agar (PDA) plates. The culture was maintained by subculturing every 30 days on potato dextrose agar slants and subsequently refrigerating at 4°C. Cultural and morphological studies were carried out according to the standard protocols. After 7-8 days of incubation, the colony characteristics, including colony colour, growth pattern, pigmentation, and margin, were examined and noted. The morphological characteristics including macroconidia, microconidia and chlamydospores, were observed using a light microscope.

Isolation of endophytic fungi and bacteria from different banana cultivars

Healthy eight-month-old banana plants of four popular cultivars from different genomic groups: Red Banana and Grand Naine (AAA), Malbhog (AAB) and Kothia (ABB) were selected for collection of samples from root, rhizome, and pseudostem (Plate 1). Different locations of ICAR-All India Coordinated Research Project (AICRP) on Fruits, Horticultural Research Farm, Dr RPCAU, Pusa, Samastipur was chosen for the collection of the samples. The samples were brought to the Fruit Pathology Laboratory, Department of Plant Pathology and Nematology, and processed within 4-5 hours of collection. The root, rhizome and pseudostem were washed thoroughly under running tap water to remove any dirt and debris, followed by air drying for few minutes. The subsequent steps of isolation of endophytes from the collected samples were carried out inside the laminar air flow in accordance with the protocol described by Panda *et al.* (2019) with few modifications. Each air-dried sample was cut into four to five-centimetres pieces with a sterile scalpel, washed in 1% Tween20 TM solution for 30 minutes and rinsed with sterile distilled water. The samples were surface sterilized first by treating them with 4% sodium hypochlorite (NaOCl) for 20 minutes, followed by two washes with sterile distilled water to remove surface sterilant. The samples were then immersed in 70% ethanol for 30 seconds, followed by eight rinses in sterile distilled water, and blot drying. An aliquot of 1ml from last wash (8^{th} wash) was spread plated on suitable media as a sterility check and incubated at 26±1°C for 4 days. Two replicates of sterility check were maintained for each sample.

The surface sterilized tissues were macerated with 8 ml of sterile 0.1% peptone solution (1 g peptone and 8.5 g NaCl in 1000ml sterile distilled water) using a sterile

mortar and pestle. The macerate was allowed to stand for 30 minutes. One milliliter of the supernatant was pipetted out of this homogenate and serially diluted (10^{-1} to 10^{-8}). For each dilution, 0.1 ml was spread plated on Potato Dextrose Agar (PDA) and Trichoderma selective media for fungi; Nutrient Agar (NA) and Luria Bertani Agar for bacteria and incubated at 26±1°C. Three technical replicates were maintained. Based on varied cultural characteristics, distinct representative colonies of each morphotype were selected and streaked on NA and PDA for bacteria and fungi, respectively. The isolates were studied for their cultural characteristics. Pure cultures of the isolated bacterial and fungal endophytes were maintained in slants and stored at 4^0C.

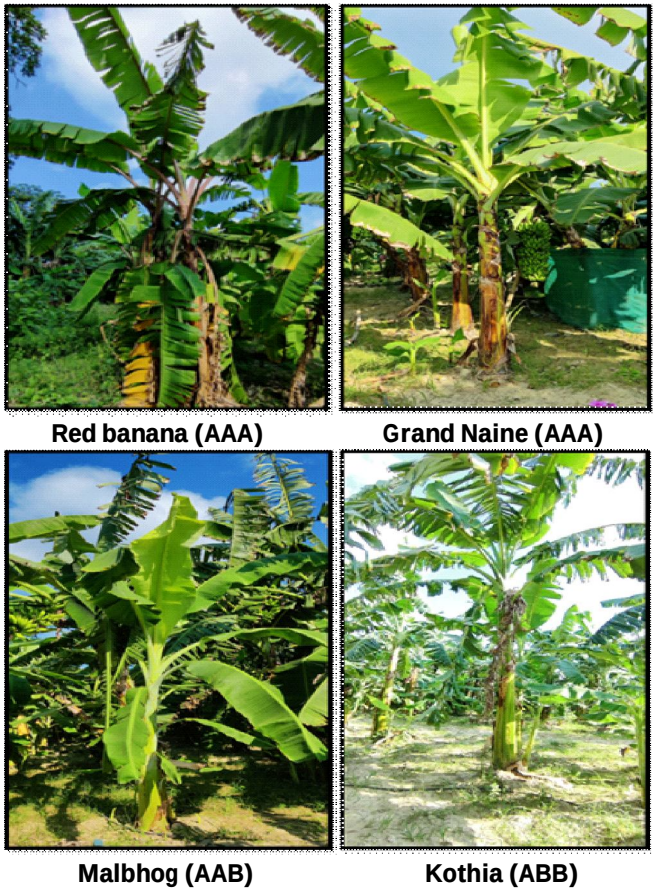


Plate 1 : Photographic represenation of the different banana cultivars selected for isolation of endophytes.

In vitro efficacy of fungal endophytes of banana against *Fusarium oxysporum* f.sp. *cubense*

The *in vitro* assessment of antagonistic potential of the isolated fungal endophytes against *Foc* was done by employing dual culture method (Morton and Stroube, 1955). On a 90 mm PDA plate, 8 mm mycelial discs of the test pathogen from a 7-day old culture and a fungal endophyte isolate from an actively growing culture were aseptically placed 60 mm apart opposite each other and incubated at 28± 1^0C. Three replications were maintained for each isolate. Plates without endophytic fungi served as control. The radial growth of the pathogen (mm) on control plates and radial growth of the pathogen (mm) on dual culture plates was recorded at equal intervals, beginning from 72 hours after incubation to full growth of the pathogen in the control. the corresponding per cent inhibition of mycelium over control (PI) was calculated as per the formula given by Vincent (1947), as follows:

$$PI = \frac{C - T}{C} \times 100$$

Where, PI = Per cent inhibition of mycelium over control (%)

C = Radial growth of the pathogen in control plate (mm)

T = Radial growth of the pathogen in dual culture (mm)

In vitro efficacy of bacterial endophytes of banana against *Fusarium oxysporum* f.sp. *cubense*

The bacterial endophytic isolates were subjected to a preliminary screening against *Foc* race 1, according to Utkhede and Rahe (1983). Agar disc (8 mm) of the test pathogen from a 7- day-old culture was placed at the center of the 90 mm PDA plate. Four different bacterial isolates were streaked on the four sides of the Petri plate opposite each other, forming a square around the test pathogen, 1 cm away from the periphery, and incubated. Uninoculated plate with only the test pathogen was maintained as a control. The inhibition of mycelium was recorded for each bacterial isolate. The bacterial isolates which showed antagonistic activity in the preliminary screening were selected for further study. The selected bacterial isolates were then screened individually against *Foc* using dual culture assay as described in the previous section.

Data analysis

The statistical design completely randomized design (CRD) was implemented for all the *in vitro* experiments of the present study. Data were obtained from three biological replications and expressed as the mean ± standard deviation. All the data obtained in the present study were statistically analysed using SPSS 21.0 software. To compute the difference in the mean value of each isolate, one-way ANOVA was employed. Duncan's multiple range test was used to determine the significance of the difference among all treatments ($P < 0.05$).

Results and Discussion

Morphocultural characterization of *Fusarium oxysporum* f.sp. *cubense*

The pathogen, *Fusarium oxysporum* f.sp. *cubense (Foc)* formed profuse hairy or cottony growth of white mycelium on PDA media with purple to violet pigmentation. The pathogen formed falcate or sickle shaped macroconidia with 2-3 septa which were borne on sparsely branched elongated conidiophores when observed under compound microscope (100X) (Plate 2). The cultural and morphological study of the pathogen as

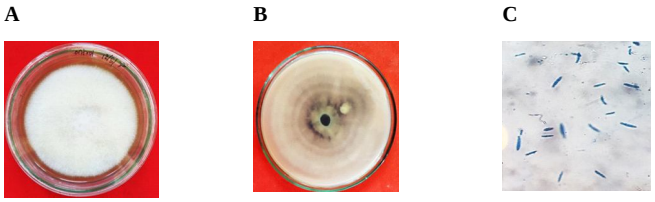


Plate 2 : Cultural and morphological characteristics of the pathogen *Fusarium oxysporum* f.sp. *cubense*. (A-B) Front and back view of *Foc* race 1; (C) Macroconidia of *Foc* race 1 (100X).

observed in the present study has been corroborated by the detailed work done by Pérez-Vicente *et al*. (2004), Shukla and Singh (2019), Damodaran *et al*. (2019) and Thangavelu *et al*. (2019). Also, Prajapati (2024) from Fruit Pathology Laboratory previously molecularly characterised the pathogen strain using internal transcribed spacer region (ITS) region, *Secreted in xylem* (SIX9) genes, and sequence characterized amplified region (SCAR) markers, confirming its identity as *Fusarium oxysporum* f.sp. *cubense* race 1. Molecular identification based on IGS and ITS is an effective approach for identification of the phytopathogen races (Jegan *et al*., 2021). However, recent approaches include molecular identification and comprehensive analysis of variation between *Foc* races based on the sequence of small effector proteins, SIX (Secreted in Xylem) genes that are unique for each race of *Foc*, contributing to their virulence (Ahmad *et al*., 2020; Kaliapan *et al*., 2024).

Isolation of endophytic fungi and bacteria from different banana cultivars

A total of 120 endophytes, 90 bacterial and 30 fungal, were isolated from the pseudostem, root and rhizome of four different cultivars of banana, namely, Red banana (AAA), Grand Naine (AAA), Malbhog (AAB) and Kothia (ABB), on Luria Bertini Agar, Nutrient Agar and Trichoderma Selective Media, Potato Dextrose Agar, respectively. The cultivars were selected on the basis of their popularity in Bihar, unique morphological character and degree of susceptibility towards Fusarium wilt. These represent culturable members of the native endophytic community associated with the banana cultivars. The native endophytic strains are often considered advantageous over the exogenous strains in terms of amplification of their positive effect as biological control agents, contributed by their better adaptability to ecological niches similar to phytopathogens, survival rate, reproductive ability and resistance to biotic and abiotic stresses (Li *et al*., 2023; Gan *et al*., 2024). Screening based on the differences in cultural characteristics yielded 55 bacterial and 13 fungal endophytes, which were maintained for further studies. Similar results of bacteria as a dominant group among endophytes has been suggested in previous study by Muthukumar *et al.* (2017).

A considerable variation was observed in the distribution pattern of endophytes among the different cultivars and plant parts in this study. The highest number of endophytes, *i.e.*, 30 bacterial and 3 fungal isolates were obtained from cultivar Kothia, followed by Malbhog, Red banana and Grand Naine (Fig. 1A & B). Also, diversity was observed in the pattern of distribution of endophytes in different sampled plant parts of four banana cultivars. Highest number of endophytes were isolated from the roots, 28 (22 bacterial and 6 fungal) isolates, followed by 23 isolates from rhizome and 17 isolates from pseudostem. Roots showed the highest percent share 41% among the three plant parts taken in the study (Fig. 1C). A plausible explanation for this variation obtained in the present study can be the effects of host genotype, host growth stage, cultural conditions, nutrient status of the host, strain of endophyte, biotic or abiotic stress exposure, defense metabolites and endophytic strain (Beltan-Garcia *et al*., 2021). The seasonal variation in sampling and method of quantification adopted can also affect the diversity of endophytes (Savani, 2019; Beltan-Garcia *et al*., 2021). Previous studies also suggest that endophytic assemblage within a plant is affected by the niche determination and is tissue specific (Hardoim *et al*., 2008; Harrison and Griffin, 2020). The below ground parts are reported to have greater endophytic diversity. Infact, roots are the major habitat and route of colonization for fungal and bacterial endophytes, with great metagenomic diversity (Liu *et al*., 2017; Chaudhary *et al*., 2022).

In vitro efficacy of fungal and bacterial endophytes of banana against *Fusarium oxysporum* f.sp. *cubense* race 1

Among all the endophytic fungi tested against *Foc* race 1, KERF-1 which was isolated from root of cultivar Kothia and isolate G9ERhF-1 from the rhizome of cv. Grand Naine, significantly inhibited the mycelial growth

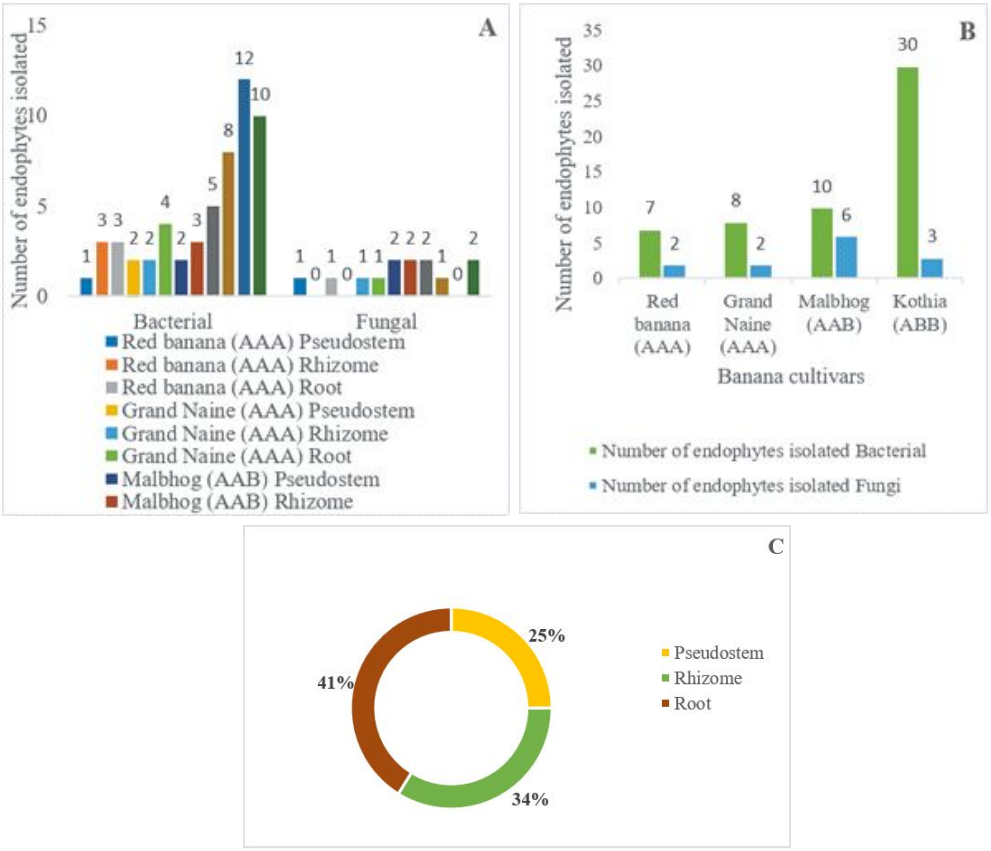
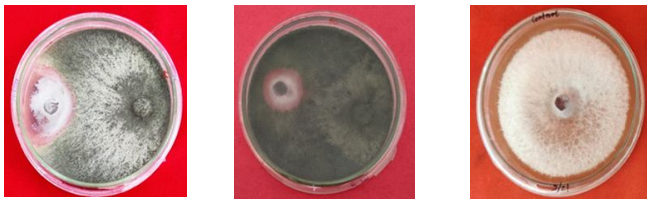


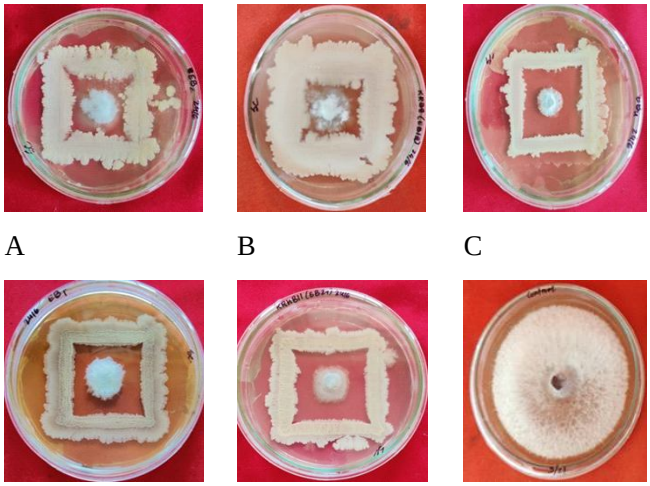
Fig. 1 : Isolation of bacterial and fungal endophytes from different parts of various cultivars of banana. (A-B) Variation in distribution pattern of bacterial and fungal isolates among the four banana cultivars; (C) Variation in distribution pattern of endophytes among three plant parts: pseudostem, root and rhizome.



A **B** **C**

Plate 3 : Antagonistic assay of fungal endophytes against *Foc* race 1. (A) Fungal endophyte isolate G9ERhF-1; (B) Fungal endophyte KERF-1; (C) Control (Unchallenged *Foc* race 1). G9- Grand Naine, K-Kothia, E-Endophytic, Rh- Rhizome, R- Root, F-fungi, Digit- Isolate number.

of the pathogen. The isolate KERF-1 showed highest antagonistic activity against *Foc* race 1 with 77.37% inhibition of mycelium over control, 240 hours after incubation. This was followed by the isolate G9ERhF-1 which showed 60.15% inhibition of mycelium over control. (Table 1; Plate 3). After primary screening, 16 out of 55 bacterial endophytes exhibited antagonistic activity against *Foc* race 1. The secondary screening results showed that 5 isolates *viz.*; RbERB-2, MERB-2, KERhB-11, KERB-1 and KERB-5, significantly inhibited the mycelium growth of the test pathogen. The isolate



A B C

D E F

Plate 4 : Antagonistic assay of bacterial endophytes against *Foc* race 1. (A) isolate RbERB-2; (B) isolate MERB-2; (C) isolate KERB-1; (D) isolate KERB-5; (E) isolate KERhB-11; (F) Control (Unchallenged *Foc* race 1). Rb- Red banana, M- Malbhog, K-Kothia, E-Endophytic, Rh- Rhizome, R- Root, F- fungi, Digit- Isolate number.

KERB-1 showed highest antagonistic activity against *Foc* race 1 with 82.09% inhibition over control, 240 hours

Table 1 : Antifungal activity of fungal endophytes of banana against *Fusarium oxysporum* f.sp. *cubense* Race 1 .

Isolates	G (mm)	PI (%)
RbERF-1	35.90±0.77e	19.32
RbEPF-1	36.37±0.61e	18.26
G9ERF-1	33.23±0.58c	25.32
G9ERhF-1	17.73±0.75b	**60.15**
MERF-1	36.20±0.45e	18.65
MERF-2	39.40±0.79g	11.40
MERhF-1	38.50±0.50fg	13.48
MERhF-2	35.77±0.68de	19.61
MEPF-1	34.73±0.75d	21.95
MEPF-2	39.40±0.79g	11.40
KEPF-1	36.17±0.57e	18.70
KERF-1	10.07±0.40a	**77.37**
KERF-2	37.67±0.76f	15.34
Control	44.50±0.50h	0
SEm ±	0.38	
C.D. (@ 5%)	1.10	

*Means within columns followed by same letter are not significantly different according to Duncan's multiple range test (pd"0.05). Values represent mean of three replicates ± standard deviation. G- Radial growth of the pathogen in mm, PI- Percent inhibition over control (%), Rb- Red Banana, G9- Grand Naine, M- Malbhog, K- Kothia, E- Endophytic, R- Root, Rh- Rhizome, P- Pseudostem, F- Fungi, Digit- Isolate number.

Table 2 : Antifungal activity of bacterial endophytes of banana against *Fusarium oxysporum* f.sp. *cubense* Race 1.

Isolates	G (mm)	PI (%)
RbEPB-1	23.97±0.15h	46.14
RbERB-1	18.87±0.32f	57.60
RbERB-2	15.77±0.25d	**64.56**
G9EPB-2	18.10±0.55e	59.32
G9ERhB-1	18.39±0.35ef	58.67
G9ERB-1	17.90±0.36e	59.77
MERhB-1	18.77±0.25f	57.82
MERhB-3	19.93±0.12g	55.20
MERB-2	11.90±0.17c	**73.25**
MERB-4	18.20±0.30e	59.10
KEPB-5	18.40±0.36ef	58.65
KEPB-8	19.83±0.28g	55.43
KERhB-11	08.83±0.28b	**80.15**
KERB-1	07.97±0.06a	**82.09**
KERB-5	08.73±0.25b	**80.37**
KERB-9	25.03±0.35i	43.74
Control	44.50±0.50j	0

Table 2 continued...

Table 2 continued...

Isolates	G (mm)	PI (%)
SEm ±	0.18	—
C.D. (@ 5%)	0.53	—

*Means within columns followed by same letter are not significantly different according to Duncan's multiple range test (pd"0.05). Values represent mean of three replicates ± standard deviation. G- Radial growth of the pathogen at 240 hours after incubation (mm), PI- Percent inhibition over control (%), Rb- Red Banana, G9- Grand Naine, M- Malbhog, K- Kothia, E- Endophytic, R- Root, Rh- Rhizome, P- Pseudostem, B- Bacteria, Digit- Isolate number.

after incubation. Isolates KERB-5 and KERhB-11 also showed relatively high antagonistic activity (80.37% and 80.15% inhibition over control, respectively). Even though, there was no statistically significant difference between the antagonistic activity of KERB-5 and KERhB-11, yet due to the variable plant part origin and pigmentation on PDA plates, both isolates were selected for further studies (Table 2; Plate 4). Taribuka *et al.* (2017) also reported antagonistic potential of fungal endophytes *Trichoderma gamsii*, *Trichoderma harzianum*, *Trichoderma harzianum.swn-1*, *Trichoderma koningiopsis*, *Trichoderma harzianum.swn-2* and *Trichoderma asperellum* against *Foc* with per cent inhibition of mycelium ranging from 49.67- 60.61%. Similar results were obtained by Savani *et al.* (2021), who reported 72.33% inhibition of *Foc* by *Trichoderma reesei*, followed by *Rigidisporus vinctus* (70%) and *Sphingobacterium tabasicoli* (60.55%). Thangavelu and Gopi (2015a) reported 68 to 83% mycelial growth and 98-100% conidial germination inhibition of *Foc* by bacterial endophytes. Similarly, by Munif *et al*. (2020), Xiang *et al.* (2023).

Conclusion

This study highlights the native banana endophytes as effective biocontrol agents against *Fusarium oxysporum* f. sp. *cubense* (*Foc*) race 1. Considerable variation observed among cultivars and plant parts, with cultivar Kothia and root tissues demonstrating the highest endophyte richness and diversity, emphasize on the host and tissue specific advantage of native endophytes over artificially introduced as strong candidates for biological control of a destructive soil-borne, vascular pathogen as *Foc*. Confrontation bioassay identified root-derived bacterial isolate KERB-1 (82.09% inhibition) and fungal isolate KERF-1 (77.37% inhibition) as superior antagonists capable of significantly suppressing *Foc* race 1 mycelial growth *in vitro*. These findings demonstrate the potential of native endophytes as robust candidates for formulating eco-friendly bio-fungicides to sustainably

manage Fusarium wilt in banana crops, strengthened with further *in planta* and robust field testing across affected regions.

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