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Understanding and Biocontrol Management of Panama Disease in Banana Cultivation

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ABSTRACT

Panama disease, caused by *Fusarium oxysporum* is one of the most devastating diseases affecting banana cultivation worldwide. The spread of this soil-borne pathogen has severely threatened banana production, particularly the Cavendish variety, leading to significant economic losses. Traditional control methods, such as chemical fungicides, have proven ineffective and unsustainable, prompting the need for innovative, environmentally friendly management strategies. This article explores sustainable management practices for the control of Panama disease, focusing on integrated approaches that combine biological, cultural and agronomic methods. Biological control, involving the use of antagonistic microorganisms such as *Trichoderma* species, offers promising results in suppressing *Fusarium* through competition and parasitism. Crop rotation, resistant banana cultivars, and the use of organic amendments are also highlighted as effective strategies to reduce pathogen inoculum levels in the soil. Moreover, soil health management, including improved drainage and soil aeration, can mitigate the spread of the disease by enhancing microbial diversity and reducing the pathogen's survival capacity. This study reveals that *Trichoderma harzianum* and *Pseudomonas fluorescens* are potential biocontrol agents against isolated pathogens viz *Fusarium* spp. and *Alternaria* spp. The essential oils were also tested against isolated Panama disease causing pathogens and they have also proved effective. In conclusion, sustainable management practices that integrate biological control, resistant cultivars, and soil health optimization offer a holistic approach to managing Panama disease. Such strategies not only reduce the reliance on harmful chemicals but also contribute to the long-term resilience of banana production systems, promoting environmental sustainability and food security in banana-growing regions.

Keywords Panama disease, *Fusarium oxysporum*, Biological control, Sustainable agriculture, Soil health, Banana cultivation.

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INTRODUCTION

Panama disease, caused by *Fusarium oxysporum*, is one of the most destructive diseases affecting banana (*Musa* spp.) production worldwide. First identified in Panama in the early 20th century, the disease has since spread globally, threatening banana plantations, especially those of the Cavendish cultivar, which accounts for over 90% of global banana exports (Ploetz 2006, Simmonds 2013). The pathogen infects the vascular tissues of the banana plant, causing wilting, yellowing of leaves, and eventually plant death. Due to the persistent nature of *Fusarium*, the disease is difficult to control, and its spread is accelerated by the global movement of infected plant material, soil, and water (Stover & Simmonds 1987). Historically, the management of Panama disease relied on the use of chemical fungicides and soil fumigation, but these methods have proven to be ineffective over time. Furthermore, the persistence of *Fusarium* in the soil, even in the absence of banana crops, makes chemical control unsustainable. The failure of traditional approaches, combined with environmental and health concerns associated with chemical use, has prompted the exploration of more sustainable management practices (Pushpavathi *et al.* 2016). Recent research emphasizes integrated management strategies that combine biological control, cultural practices, and soil health management (Bubici *et al.* 2017). Biological control agents, such as *Trichoderma* spp. and other soil microbes, have shown promise in suppressing *Fusarium* through mechanisms like competition, antibiosis, and mycoparasitism (Diksha *et al.* 2020). Crop rotation with non-host plants and the introduction of disease-resistant banana cultivars are also being investigated as ways to reduce inoculum pressure and enhance plant resilience (Berg and Smalla 2009). Also, improving soil health through organic amendments, better drainage, and mulching has been found to create unfavorable conditions for *Fusarium*, thereby reducing disease incidence (Wang *et al.* 2019). The integration of these approaches into a holistic management strategy not only helps control Panama disease but also promotes long-term sustainability in banana farming. As global banana production faces increasing pressure from Panama disease and other challenges such as climate change and market demands, the development of effective and eco-friendly disease management strategies is critical to ensuring food security and environmental sustainability.

Biological control: Biological control has gained attention as a promising alternative to chemical fungicides for managing Panama disease. Several studies have demonstrated the potential of *Trichoderma* spp., *Pseudomonas* and *Bacillus* species in inhibiting *Fusarium* growth through mechanisms such as competition for nutrients, production of antibiotics and direct parasitism (Nayak *et al.* 2020). These biocontrol agents can be applied directly to the soil or as seed treatments to reduce initial pathogen load.

Resistant cultivars: The development of disease-resistant banana cultivars has been a key focus in Panama disease management. Some research has identified genetic resistance in wild *Musa* species and their hybrids, with ongoing breeding programs aiming to introduce these traits into commercial banana varieties (Wang *et al.* 2019). However, achieving durable resistance remains challenging due to the genetic diversity of *Fusarium* and the possibility of the pathogen evolving to overcome resistance.

Cultural practices: Cultural control methods, including crop rotation with non-host plants and the careful management of water and soil, have shown potential in reducing the spread of Panama disease. Rotation with leguminous crops or other non-host species can reduce pathogen inoculum in the soil, while improving soil structure through proper drainage and organic amendments helps to suppress *Fusarium* by enhancing beneficial microbial communities (Nasir *et al.* 2003 and Sijun, Bidabadi *et al.* 2018). Mulching and composting have also been found to improve soil health and reduce pathogen viability.

Soil health management: Improving soil health is a crucial element of sustainable disease management. Studies have shown that organic practices, including the use of composts, biochar, and reduced tillage, can alter the soil microbiome in ways that inhibit the growth of *Fusarium* while promoting beneficial microorganisms (Dita *et al.* 2018, El Bilali *et al.* 2021). These practices also reduce soil erosion and enhance plant nutrition, leading to more resilient

banana plants.

Integrated disease management: The most promising approach for controlling Panama disease lies in an integrated disease management (IDM) strategy that combines biological control, resistant cultivars, and sustainable soil management practices (Khatri-Chhetri *et al.* 2017). According to a study by (Sudha *et al*. 2019), IDM strategies tailored to local conditions, such as the use of biocontrol agents in conjunction with resistant cultivars and improved soil management, offer a holistic solution to Panama disease. While Panama disease remains a formidable challenge to banana production, sustainable management practices that integrate biological control, resistant cultivars, and soil health optimization offer significant promise. These strategies not only help control the spread of *Fusarium* but also promote long-term ecological balance, contributing to the resilience of banana farming in the face of evolving threats.

MATERIALS AND METHODS

Methodology

Isolation of pathogenic fungi from infected banana plant parts

The infected leaves of banana plants were collected from Bakshi Ka Talab area. The sample was brought to laboratory and stored at 4ºC. Potato Dextrose Agar (PDA) media were prepared and sterilized in autoclave at 121ºC temperature & 15 lbs psi pressure along with petridishes and other materials. After sterilization, all materials were transferred to laminar air flow chamber.

Small infected part of leaf cut off and washed properly with sterile water. Then the infected part of leaf was dipped in 0.2% of mercuric chloride solution and properly washed in sterile water. The sterile solidified PDA plates (with antibiotic streptomycin sulfate) were taken and sample was inoculated in the center of the plates with the help of flame sterilized and cooled forceip in aseptic condition under laminar air flow chamber. The inoculated plates were incubated in BOD. Incubator for 72 hrs at 27ºC. After the growth of fungus appeared, the isolated colonies were sub-cultured to obtain pure colonies. Isolated fungus culture plate of banana plant was taken. The isolated fungal colony was inoculated in the center of plate with the help of sterile needle in aseptic condition under laminar air flow chamber. This step is repeated for all the plates. The plates were incubated in BOD. Incubator at 27ºC for 72 hrs.

Identification of fungal isolates through microscopy

Clean glass slide was taken and put a drop of lacto phenol cotton blue stain in the center of slide. Fungus culture plate was taken and some mycelia of fungus was taken and put in lactophenol on center of slide. The mycelia was teared with the help of needle and a coverslip was placed on it. The slides were observed under microscope.

To check antagonistic activity of *Trichoderma* spp. against isolated plant pathogens- *Fusarium* spp. and *Alternaria* spp. (by dual culture method)

6 sterile petri dishes was taken and poured the PDA medium (already prepared) in each petri dishes and kept it for solidification. One poured plate was taken and do subculture of *Fusarium* and marked it as control. Another poured plate were taken and *Trichoderma* and *Fusarium* were inoculated on opposite ends in the same PDA plate in duplicate. The same procedure was applied against *Alternaria.* The plates were incubated in BOD incubator at 27°C for 4 days. The growth of microorganisms were recorded every 24 hrs till 4 days (Nayak *et al.* 2020).

Percentage of mycelia growth inhibition was calculated according to the formula

Calculation

$$MGI\% = (dc - dt) \times 100$$

dc

dc=Fungal colony diameter in control test

dt=Fungal colony diameter in treatments

To check the antimicrobial activity of essential oil against isolated plant pathogens- Fusarium spp. and Alternaria spp.

Sterile PDA medium were taken and mixed antibiotic in it. *Fusarium* spores were picked up from pure plate and mixed with 1 ml (0.5%) NaCl and then this solution was mixed in PDA medium in aseptic condition under laminar air flow chamber. This seeded agar medium was poured in sterile petridishes and kept for solidification. One poured plate were left as control. Another poured plate was taken and 3 filter paper disc were placed at 3 different ends. 2 µl, 3 µl & 5 µl of concentrated lemon grass oil and 1: 1 (ratio) diluted with dimethyl sulphooxide was dispensed in a plate on 3 different filter discs separately. The same procedure was applied against *Alternaria*. The plates were incubated in BOD incubator at 27°C for 72 hrs. The inhibition % were calculated according to the following formula:

Calculation

$$\text{Inhibition \% of each dilution} = \frac{\text{Diameter of the zone of particular dilution}}{\text{Diameter of the negative control}} \times 100$$

RESULTS

The infected sample was brought to laboratory for isolation of plant pathogen (Fig. 1). The pathogenic fungi were successfully isolated from the infected part of the plant (Fig. 2). The isolated fungi was repeatedly subcultured to obtain pure cultures (Fig. 3). On the basis of morphological characteristics and mycelial structure along with conidia (observed under microscope), *Fusarium* spp. and *Alternaria* spp. were identified (Figs. 4–5). The pure cultures were stored at 4°C for further activities.

Fig. 1. Infected banana plant leaf.

Fig. 2. Isolated plates

The antagonistic activity of *Trichoderma* spp. against *Alternaria* spp and *Fusarium* spp. (Figs. 6 –7) was tested using the dual culture method. Initially, after 1 day, the growth of both pathogens in control and combined petri dishes was similar. However, after 2 days, the growth in the combined dishes was significantly retarded due to enzymes secreted by *Trichoderma* spp. By the third day, *Trichoderma* completely inhibited the growth of both pathogens, demonstrating its strong antagonistic effect.

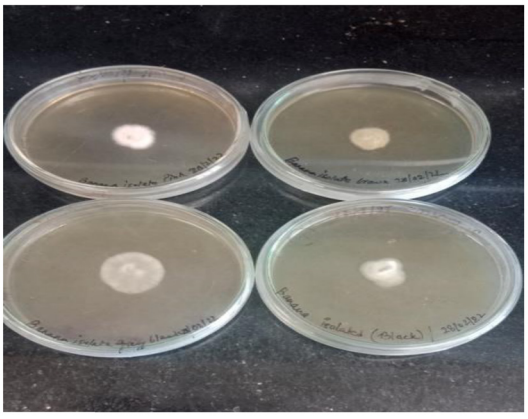


Fig. 3. Sub-cultured plates.

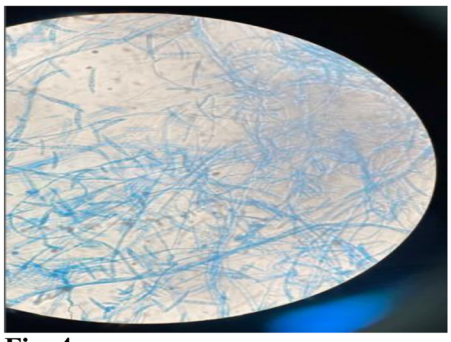


Fig. 4

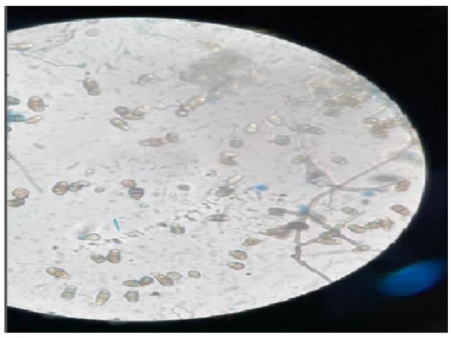


Fig. 5

Fig. 4. *Fusarium* spp. **Fig. 5.** *Alternaria* spp.

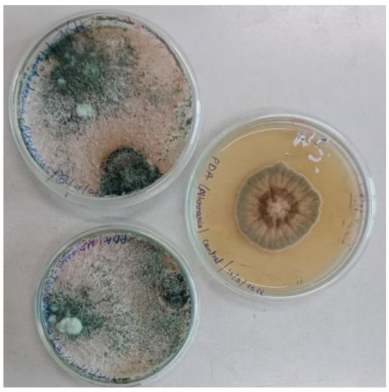


Fig. 6

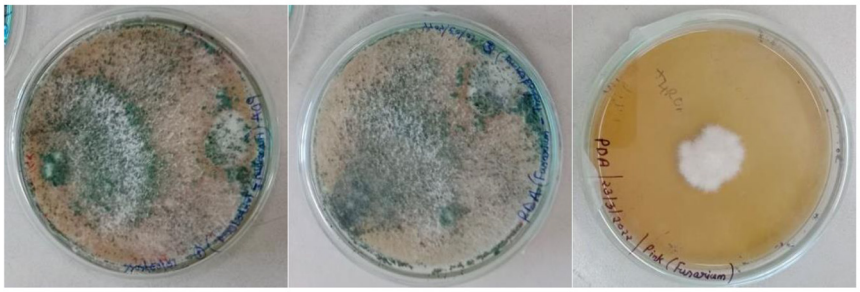


Fig. 7

Fig. 6. *Trichoderma* against *Alternaria* spp. **Fig. 7.** *Trichoderma* against *Fusarium* spp.

$$\text{On day 2 MGI\%} = \frac{(2.20 - 1.03) \times 100}{2.20}$$

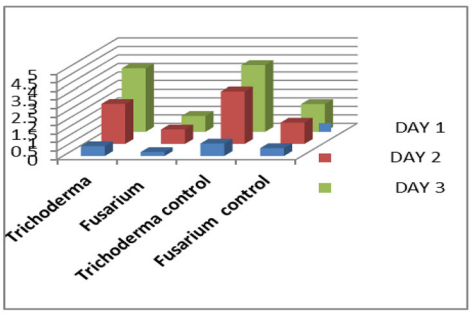
$$= 53.18\% \text{ inhibition}$$

$$\text{On day 3 MGI\%} = \frac{(2.46 - 1.13) \times 100}{2.46}$$

$$= 54.06\% \text{ inhibition}$$

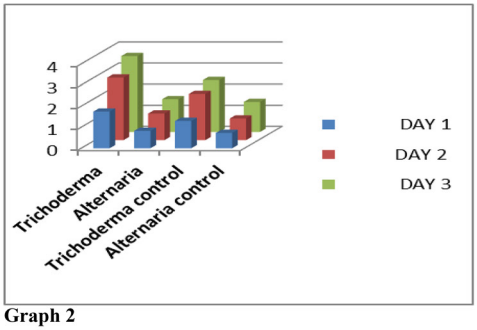
Trichoderma v/s *Fusarium* spp. and *Alternaria* spp.

The antagonistic effect of *Trichoderma* spp. against *Fusarium* and *Alternaria* spp. was evaluated using the dual culture method (Graphs 1–2). Initially, after 1 day, there was no significant difference in the growth of *Fusarium* in both the control and combined petri dishes. However, after 2 days, the growth of *Fusar-*



Graph 1

Graph 1. *Trichoderma* v/s *Fusarium* spp.



Graph 2

Graph 2. *Trichoderma* v/s *Alternaria* spp.

ium in the combined dishes was noticeably reduced due to the enzymes secreted by *Trichoderma* spp. By day 3, *Trichoderma* completely inhibited the growth of *Fusarium*, showing a strong antagonistic activity. This result highlights *Trichoderma* spp. as an effective biocontrol agent against *Fusarium* spp.

Observation and result of Antifungal activity of *Pseudomonas fluorescens* against *Fusarium*

Dual culture method

The antifungal activity of *Pseudomonas fluorescens* against *Fusarium* and *Alternaria* was evaluated using the dual culture method (Figs. 8 a–b, Figs. 9 a–b). After incubating the cultures, significant inhibition of *Fusarium* growth was observed in the presence of *Pseudomonas fluorescens*. The bacterium formed a clear zone of inhibition around its growth, indicating the production of antifungal compounds. This result demonstrates the potential of *Pseudomonas fluorescens* as an effective biocontrol agent against *Fusarium*.

Antifungal activity of *Pseudomonas fluorescens* against *Fusarium*

Percentage of mycelia growth inhibition was calculated according to the formula

$$MGI\% = \frac{(dc - dt) \times 100}{dc}$$

dc=Fungal colony diameter in control test
dt=Fungal colony diameter in treatments

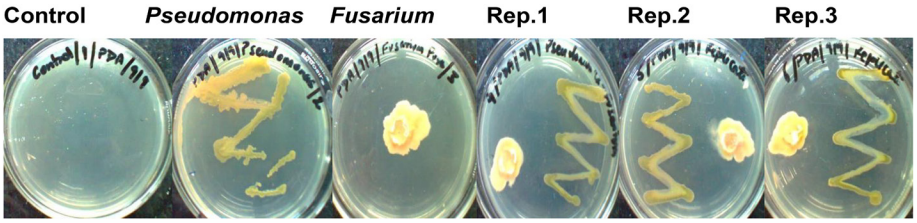


Fig. 8 (a)

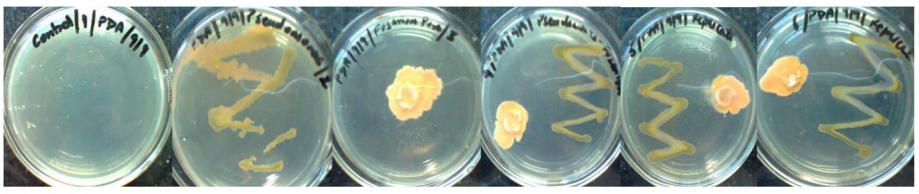


Fig. 8 (b)

Fig. 8. (a) Dual culture method day 3. **Fig. 8**. (b) Dual culture method (dual culture method day 4).

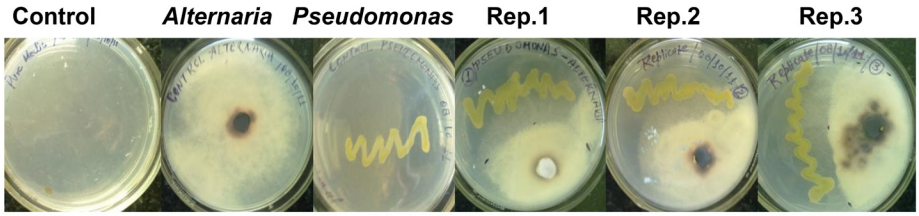


Fig. 9 (a)

Fig.9(b)

Fig. 9 (a) Dual culture method day 3. **Fig. 9** (b) Dual culture method day 4.

$$\text{On day 3 MGI\%} = \frac{(1.23 - 0.70) \times 100}{1.23}$$

$$= 52.33\% \text{ inhibition}$$

$$\text{On day 4 MGI\%} = \frac{(1.43 - 0.82) \times 100}{1.43}$$

$$= 60.33\% \text{ inhibition}$$

Note—Inhibition zone is the mean of triplicateplate.

$$\text{On day 3 MGI\%} = \frac{(3.1 - 2.4) \times 100}{3.1}$$

$$= 22.58\% \text{ inhibition}$$

$$\text{On day 4 MGI\%} = \frac{(3.4 - 2.5) \times 100}{3.4}$$

$$= 26.47\% \text{ inhibition}$$

The results showed strong antagonistic activity of *Pseudomonas fluorescens* against both isolated pathogens.

Antifungal activity of Lemon grass against *Alternaria* and *Fusarium*

The antifungal activity of Lemon grass oil was observed to be most effective at higher concentrations (Table 1). At concentrated levels, Lemon grass oil demonstrated significant inhibition of the growth and development of *Alternaria* and *Fusarium*, two major fungal pathogens (Figs. 10 – 11). The increased concentration led to a more pronounced antifungal effect, suggesting that Lemon grass oil has strong potential as a natural and effective antifungal agent against these pathogens.

Antifungal activity of Eucalyptus oil against *Alternaria* and *Fusarium*

The antifungal activity of Eucalyptus oil was observed to be most effective at higher concentrations (Table 2). At concentrated levels, Lemon grass oil

Table 1. Lemon grass oil inhibition % at each dilution against different fungi.

Sl. No.	Activity of Lemon grass oil against	Dilutions	Inhibition zone diameter (cm)	Diameter of negative control (cm)	Inhibition % at each dilution (%)
A	*Fusarium*				
1		1:1	2.45	7.0	35.0
2		Concentrated	2.72	7.0	38.85
B	*Alternaria*				
1		1:1	2.65	7.0	37.85
2		Concentrated	3.05	7.0	43..57

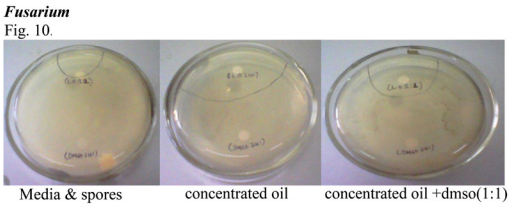


Fig. 10. Media & spores concentrated oil concentrated oil+dmso (1:1). **Fig. 11.** Antifungal activity of Lemon grass oil against *Alternaria.*

Table 2. Eucalyptus oil inhibition % at each dilution against different fungi.

Sl. No.	Activity of Lemon grass oil against	Dilutions	Inhibition zone diameter (cm)	Diameter of negative control (cm)	Inhibition % at each dilution (%)
A	*Fusarium*				
1		1:1	2.45	7.0	35.0
2		Concentrated	2.9	7.0	41.42
B	*Alternaria*				
1		1:1	2.85	7.0	40.71
2		Concentrated	3.0	7.0	42..85

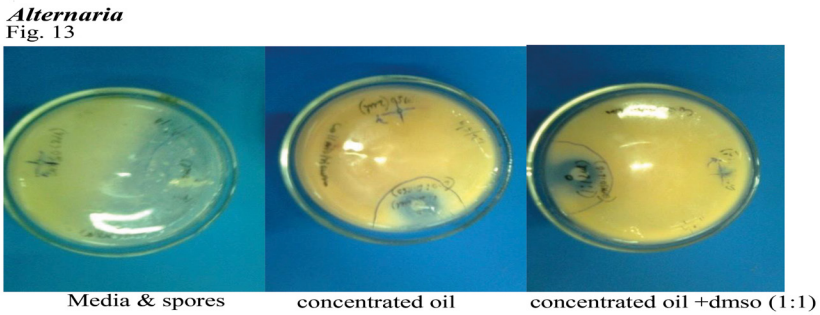


Fig. 12. Media & spores concentrated oil concentrated oil + dmso (1:1). **Fig. 13.** Media & spores concentrated oil concentrated oil +dmso (1:1).

demonstrated significant inhibition of the growth and development of *Alternaria* and *Fusarium*, two major fungal pathogens (Figs. 12 – 13). The increased concentration led to a more pronounced antifungal effect, suggesting that Lemon grass oil has strong potential as a natural and effective antifungal agent against these pathogens.

The antifungal activity of Eucalyptus oil for *Alternaria* and *Fusarium* was observed maximum at concentrated oil. The antifungal activity of Eucalyptus oil was found to be most effective at higher

concentrations. The oil exhibited strong inhibitory effects against both *Alternaria* and *Fusarium*, two common fungal pathogens affecting plants. The results indicated that as the concentration of Eucalyptus oil increased, the growth and spore formation of the pathogens were significantly reduced, highlighting its potential as a natural fungicide. The use of biological control agents, including specific strains of beneficial microbes, was effective in reducing the incidence and severity of Panama disease in banana plants. These agents helped suppress the growth of the *Fusarium* pathogen, which is responsible for the disease, thereby offering a promising alternative to chemical treatments. Cultural practices, such as crop rotation, proper sanitation, and the use of disease-free planting material, were shown to significantly reduce the spread of Panama disease. Implementing these practices helped in maintaining healthier banana plantations and preventing the buildup of the pathogen in the soil. Soil health management practices, including the addition of organic matter, compost, and the use of soil amendments, improved soil microbial diversity and activity. This resulted in a more favorable environment for the growth of beneficial microbes that help suppress *Fusarium* spp. and other pathogens, contributing to overall plant health and reducing the impact of Panama disease.

DISCUSSION

The results demonstrate that sustainable management practices, including biological control and soil health management, offer significant promise in controlling Panama disease in banana plantations. Biological control with *Trichoderma harzianum*, *Pseudomonas fluorescens* was particularly effective in reducing disease severity. Furthermore, cultural practices like crop rotation and organic soil amendments improved soil health and reduced the pathogen's viability, contributing to better plant health and higher yields. The integration of these practices into an IDM framework provides a holistic and sustainable approach to managing Panama disease, reducing dependency on chemical controls and promoting environmental sustainability in banana production. The findings highlight the importance of adopting biocontrol methods and multi-pronged strategies that not only control the pathogen but also enhance overall farm resilience, ensuring the long-term viability of banana farming in Panama disease-affected regions.

CONCLUSION

This study underline the effectiveness of sustainable management practices, combining biological control, cultural practices, and soil health management, in controlling Panama disease and promoting healthier banana production. The use of biopesticides and Botanicals are observed totally effective against the pathogens causing diseases in Banana plants. The antagonists and essential oils can be alternative to the chemical pesticides which are harmful for human health and environment.

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