AMERICAN UNIVERSITY OF BEIRUT

SURVEY AND DEVELOPMENT OF AN INTEGRATED DISEASE MANAGEMENT STRATEGY FOR BANANA PANAMA WILT DISEASE IN LEBANON

by
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ABSTRACT
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Panama wilt disease, caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4), is a significant threat to banana production worldwide, leading to severe yield losses in both commercial and local banana varieties. The pathogen was first identified in Lebanon in 2013. Despite its global impact, there has been limited practical research on the spread and management of banana Fusarium wilt in Lebanon. This study had three primary objectives: (1) to determine the distribution and significance of the disease in South Lebanon, (2) to evaluate the antagonistic activity of selected biocontrol agents, and (3) to assess an integrated management approach to mitigate the disease. First, a survey was conducted across 87 sites in South Lebanon to determine the current spread of the disease. A total of 260 plant samples, including both asymptomatic and symptomatic plants exhibiting wilt symptoms, were collected. The results indicated that 30% of the fields were infested with *Foc* TR4, demonstrating the widespread occurrence of the disease in the coastal area. Second, laboratory tests using a dual culture assay were performed to evaluate the antagonistic activity of seven *Trichoderma* spp. strains against *Foc* TR4. The results revealed that all *Trichoderma* isolates significantly reduced the mycelial growth of the pathogen, with *T. viride* strain T24 showing the highest inhibition, reducing growth by 74%. Third, an integrated management approach was evaluated through both pot experiments with artificially inoculated soil and a large-scale field trial. The field experiment, conducted in South Lebanon using a completely randomized design with four replications, involved four banana varieties: Baladi, Canarian, Grand Nain, and GCTCV2 18. The former two varieties were subjected to five treatments: a control, *Trichoderma harzianum*, *Bacillus amyloliquefaciens* (Novo Treat®), *Pythium oligandrum* (Polyversum®), and a combination of *Bacillus subtilis* + *Pseudomonas putida* (Fulzyme®). Disease severity assessments were based on both external and internal disease symptoms. Among all varieties tested, GCTCV 218 demonstrated the highest tolerance to *Foc* TR4, showing the lowest disease severity index (33%) and the highest bunch weight (31 kg). In both the Baladi and Canarian varieties, *T. harzianum* significantly reduced the *Foc* TR4 disease index, decreased endophytic *Fusarium* infections, and enhanced yield. In the pot experiment, *T. harzianum* also significantly reduced the disease index compared to the control treatment for both varieties. Fulzyme® was identified as the second most effective treatment. Given the widespread presence of the disease in South Lebanon, an integrated management approach is recommended to contain further spread and minimize damage in infested orchards. Strict sanitation measures should be implemented within and between fields. The use of tolerant or resistant varieties like GCTC V218 is advisable, along with an integrated crop management approach, including the use of biocontrol agents such as *Trichoderma*, *B. subtilis,* and *P. putida*. Considering the microclimate and edaphic variations between different regions, further local studies on the management of the disease are recommended.

TABLE OF CONTENTS

ILLUSTRATIONS

Figure

TABLES

Table

ABBREVIATIONS

°C	Degree Celsius
g	Gram(s)
Kg	Kilogram(s)
L	Liter(s)
bp:	base pair
mL	Milliliter(s)
mm	Millimeters
DNA	Deoxyribonucleic acid
mg	Milligram(s)
%	Percent of a hundred
m^2	square meter
a.i.	Active ingredient
ANOVA	Analysis of Variance
FAO	Food and Agriculture Organization
IPM	Integrated Pest Management
PDA	Potato Dextrose Agar
WA	Water agar
Foc	*Fusarium oxysporum* f. sp. *cubense*
TR4	Tropical Race 4
SR4	Subtropical Race 4
VCG	Vegetative compatibility group
GCTCV	Giant cavendish tissue culture variant
etc	et cetera

f. sp.	formae speciales
F/For	Forward
Spp.	Species
T. harzianum	*Trichoderma harzianum*
B. subtilis	*Bacillus subtilis*
P. putida	*Pseudomonas putida*
P. oligandrum	*Pythium oligandrum*
B. amyloliquefaciens	*Bacillus amyloliquefaciens*
T. viride	*Trichoderma viride*
DSI	Disease Severity Index
LDSI	Leaf Disease Severity Index
RDSI	Rhizome Disease Severity Index
pH	potential of hydrogen
NGS	Next Generation Sequencing
BLASTn	Nucleotide Basic Local Alignment Search Tool
INIBAP	The International Network for the Improvement of Banana and Plantain
LAMP	Loop-mediated isothermal amplification
BBTV	Banana bunchy top virus
GDP	Gross Domestic Product
SPSS	Statistical Package for the Social Sciences
PCR	Polymerase Chain Reaction
qPCR	Quantitative PCR
DAI	Days After Inoculation

CHAPTER 1

INTRODUCTION

Bananas are a fundamental part of many diets across the globe, symbolizing more than just a popular fruit; they are an essential food source for millions. With the global population surpassing 8 billion, addressing hunger and ensuring food security have become increasingly critical challenges. In 2019, UNICEF reported that about 820 million people were experiencing chronic hunger (FAO et al., 2019). This vast number of hungry individuals places tremendous pressure on global food production systems, which must expand by at least 70% to meet the increasing demand (FAO, 2017). To achieve this growth, various measures are necessary, including expanding agricultural land, enhancing production techniques, increasing productivity, and improving the efficiency of supply chains. However, these approaches carry potential adverse effects, such as deforestation, soil degradation, water scarcity, pollution, greenhouse gas emissions, and challenges associated with monoculture, collectively contributing to global climate change.

Climate change poses significant challenges to agriculture globally, altering temperature and precipitation patterns, which directly impact crop growth, water availability, and pest dynamics (IPCC, 2019). These changes can lead to reduced crop yields, increased vulnerability to extreme weather events such as droughts and floods and shifts in pest and disease distribution. Adapting agriculture to climate change requires implementing resilient practices such as drought-resistant crop varieties, integrated pest management (IPM), improved water management techniques, agroforestry to enhance carbon sequestration, and soil conservation methods. Integrated pest management is a crucial approach that combines biological, cultural, physical, and chemical tools in a way that minimizes economic, health, and environmental risks.

Improper pest management of plant-related diseases play a critical role in agriculture, frequently causing substantial reductions in crop yields and overall productivity. These diseases can affect various parts of the plant, including roots, stems, leaves, and fruits, leading to symptoms such as wilting, discoloration, lesions, and stunted growth. The impact of plant diseases extends beyond mere yield losses. They can also affect crop quality, making produce unsuitable for market or consumption. According to the Food and Agriculture Organization of the United Nations (FAO), pests are responsible for a staggering 20-40% loss in global crop production each year, with insects being the primary culprits, followed by diseases and weeds (FAO, 2020). Similarly, pests in agricultural fields contribute significantly to increased production costs. Beyond reducing crop yields and quality, pests necessitate extensive control measures involving pesticides, traps, and other interventions. These measures incur additional expenses for farmers, ranging from purchasing pesticides to labor costs associated with pest monitoring and management. Moreover, pests that cause substantial economic damage can lead to prolonged periods of reduced productivity, further exacerbating financial strain on farmers. In some cases, pest outbreaks may require urgent responses such as emergency spraying or crop rotations, all of which add logistical complexity and cost to agricultural operations.

As the global population grows and climate change poses increasingly complex challenges to food security, the role of staple crops in ensuring reliable food supplies has never been more crucial. Bananas, among these staple crops, stand out for their multifaceted importance. Beyond their nutritional richness—providing essential vitamins such as vitamin C and B6, minerals like potassium, and dietary fiber—bananas play a pivotal economic role globally. Bananas are a staple food for millions of people across the world, playing a crucial role in food security. Their widespread consumption in tropical and subtropical regions highlights their nutritional significance, often serving as a primary food source and contributing to overall dietary diversity and health. According to the FAO, bananas are the fourth most important food crop in the world after rice, wheat, and maize in terms of gross production value. In many developing countries, bananas serve as a primary food source and a significant component of daily diets. They are particularly vital in regions where food scarcity and malnutrition are prevalent. Moreover, bananas are relatively easy to grow, harvest, and transport, making them an accessible and cost-effective food option for millions.

Globally, bananas are cultivated on approximately 5.6 million hectares, with an annual production of about 150 million metric tons (FAOSTAT, 2021). Major producers include India, China, the Philippines, and various countries in Central and South America. India alone accounts for more than 26% of the world's banana production (FAOSTAT, 2021), highlighting its significant role in global agriculture. Economically, bananas are among the most traded commodities worldwide, supporting millions of livelihoods from small-scale farmers to large plantation operations (FAO, 2020). This economic significance extends beyond local markets, with major exporting countries such as Ecuador, Costa Rica, and the Philippines playing crucial roles in global trade networks (FAO, 2020). This extensive trade network not only underscores the economic importance of bananas but also ensures a steady supply of this essential fruit worldwide, thereby contributing significantly to global food security. Panama wilt disease of banana caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is considered the most devastating and extensively disseminated disease in banana-producing regions worldwide and has been placed on the quarantine list of many countries (Ploetz et al., 2015a & b; Siamak and Zheng, 2018). However, in 1967, a more aggressive *Foc* race strain was identified and called Tropical Race 4 (TR4) (Su et al., 1986). *Foc* TR4 has a devastating impact on Cavendish banana plants, leading to significant economic losses. The annual financial burden of TR4 is estimated to reach approximately USD 121 million in Indonesia, USD 253 million in Taiwan, and USD 14 million in Malaysia (Aquino et al., 2013).

In Lebanon, Bananas play a crucial role by serving as a vital agricultural commodity, particularly in regions with favorable coastal climates. The cultivation of bananas, concentrated in southern areas like Tyre and Sidon districts, forms a significant part of Lebanon's agricultural sector. This sector not only provides livelihoods for thousands of families, but also contributes substantially to the local economy through employment and trade. Bananas are an essential component of the Lebanese diet, offering a nutritious and accessible food option that supports food security. Their local production helps reduce dependence on imports, which is especially important given economic challenges and disruptions in trade. Furthermore, as a key export crop, bananas contribute to Lebanon's economic stability by generating revenue and strengthening regional food security through exports to neighboring countries.

Having explored the vital role of bananas in Lebanon's agricultural and economic framework, it is important to address the ongoing challenges faced by banana cultivation. *Foc* TR4 is indeed a significant threat to bananas worldwide, particularly to the Cavendish variety, which is the most exported and consumed type (FAOSTAT

2021). This disease which poses a serious threat to banana production worldwide, was first reported in Lebanon in 2014 (Garcia-Bastidas et al., 2014). The spread of *Foc* TR4 results in reduced harvests and, in severe cases, complete crop loss, posing significant economic challenges and threatening global food security where bananas are a dietary staple (Ploetz et al., 2015a & b). Efforts are underway to develop resistant banana varieties, but progress has been hindered by the complex genetics involved in breeding (Dita et al., 2018). Meanwhile, Integrated Disease Management strategies and stringent containment measures are advised, including regular surveys to monitor disease presence and detect outbreaks early, enabling prompt intervention to minimize crop losses.

While some farmers resort to chemical pesticides to control *Foc* TR4, these methods often prove ineffective and can degrade the environment and soil quality (FAO, 2020). In contrast, biological control agents such as parasitic fungi and bacteria offer promising alternatives, demonstrating effectiveness against *Foc* TR4 while promoting sustainable and eco-friendly agricultural practices (Benhamou et al., 2012; Bakker et al., 2018).

The scope of this thesis project is to setup a management strategy for the eradication or containment of the disease in Lebanon. The specific objectives of this study are as follows:

1. To investigate the extent of *Foc* TR4 spread in South Lebanon.
2. To examine the antagonistic activity of seven *Trichoderma* strains against *Foc* TR4 in vitro.
3. To assess the tolerance or susceptibility of four banana varieties to *Foc* TR4 and to evaluate the efficacy of four biocontrol agents in both field and pot

experiments, with the aim of developing a locally adapted integrated disease management strategy.

CHAPTER 2

LITERATURE REVIEW: BANANA CULTIVATION, DISEASES AND THEIR MANAGEMENT

2.1. Origin of Banana

Bananas have been cultivated for centuries, originating in Southeast Asia around 7,000-10,000 years ago. Historical evidence suggests initial domestication in Indonesia, Malaysia, and the Philippines. The earliest evidence of banana cultivation in Africa dates to 2,500 BCE (Perrier et al., 2011). Bananas spread to the Pacific Islands around 3,500 years ago and reached the Caribbean via Spanish traders in the 16th century (Simmonds, 1962). There are over 1,000 varieties of banana worldwide, resulting due to traditional domestication processes and hybridization between wild species, *Musa acuminata* and *Musa balbisiana*. Each variety offers unique taste and texture, showcasing the diversity of this globally cherished fruit (Heslop-Harrison & Schwarzacher, 2007). Despite the wide diversity of banana varieties, varieties within the Cavendish banana have emerged as the most widely cultivated and consumed type worldwide. Its dominance in the market highlights the remarkable journey and continued significance of this ancient and versatile fruit.

2.2. Genomic Constitution of Banana

Bananas are categorized into assorted groups in view of their genomic complement, referring to the number and association of sets of chromosomes which they contain. The genomic classification of bananas is quite complex and is crucial for understanding their breeding and cultivation. Most cultivated bananas are the result of both interspecific and intraspecific hybridizations between two diploid wild species: *Musa acuminata* and

Musa balbisiana (Simmonds and Shepherd, 1955). These species have a diploid chromosome number of 2n = 2x = 22, with *M. acuminata* possessing the AA genome and *M. balbisiana* the BB genome. The fruit of these diploid *Musa* spp. contain seeds, are low in starch, and have only a small amount of fleshy pith, which makes them unsuitable for consumption or commercial cultivation. ***Musa acuminata*** is typically represented by the "A" genome, while ***Musa balbisiana*** is denoted by the "B" genome. Depending on the combination and number of these genomes, bananas are categorized into different genomic groups such as AA, AB, BB, AAA, AAB, ABB, and so forth (Heslop-Harrison & Schwarzacher, 2007). Each of these groups signifies a different set of genetic traits, influencing characteristics like taste, texture, disease resistance, and suitability for various climates (Perrier et al., 2011). Simmonds (1962) classified the genomic divisions of bananas as follows:

- **AAA Group:** Includes commercially important desert bananas like Cavendish, Gros Michel, and Dwarf Cavendish varieties, all with three sets of chromosomes.
- **AAB Group:** Predominantly desert bananas with some cooking types; Silk and Pisang Mas are notable AAB varieties, possessing two sets from the A genome and one from the B genome.
- **ABB Group:** Mainly cooking varieties, such as Fe'i bananas, with one set from the A genome and two sets from the B genome.
- **AB Group:** Like the ABB group but primarily associated with cooking bananas, possessing one set from each genome.
- **AA Group:** Comprises wild bananas like *Musa acuminata*, which are crucial for breeding modern banana varieties.

- **BB Group**: BB diploid bananas are typically wild types or varieties used in breeding programs rather than commercial production, as they often possess characteristics like seediness, which is not desirable in edible bananas. Edible diploid varieties of the species do not exist.

2.3. Morphology of Banana Plants

Bananas (*Musa* spp.) exhibit a unique and complex morphology that distinguishes them from other fruit-bearing plants. As members of the family *Musaceae*, bananas are large herbaceous perennials, characterized by their substantial pseudostem, expansive leaves, and distinctive inflorescence. This section delves into the detailed morphological features of banana plants, including their root system, pseudostem, leaves, inflorescence, and fruit (Fig. 1).

2.3.1. Root System

The banana root system is adventitious, primarily consisting of fibrous roots that develop from the base of the pseudostem. These roots are typically shallow, spreading horizontally to cover a large surface area, usually within the top 60 centimeters of soil, but they can extend vertically to depths of 1.5 meters or more depending on soil conditions (Robinson & Saúco, 2010). The roots are crucial for anchoring the plant and facilitating the uptake of water and nutrients. The primary roots branch out into secondary and tertiary roots, forming a dense network that enhances the plant’s absorptive capacity (Price, 1995).

2.3.2. Pseudostem

The pseudostem of a banana plant is a distinctive feature, often mistaken for a true stem. The mature pseudostem comprises overlapping leaf sheaths rather than wood (Heslop-Harrison & Schwarzacher, 2011). This pseudostem can reach heights of 2 to 9 meters, depending on the variety and growing conditions (Robinson & Saúco, 2010). Unlike woody stems, the pseudostem is herbaceous and filled with sap, contributing to the plant's rapid growth. The pseudostem is cylindrical and hollow in the center, and its diameter varies with the plant's age and size, typically ranging from 10 to 30 centimeters (Simmonds, 1962).

2.3.3. Leaves

Banana leaves are among the largest in the plant kingdom, with some ranging from 1.27 m^2 to 2.8 m^2, with a total leaf area of up to 25 m^2 (Stover & Simmonds in Karamura & Karamura, 1995). Each leaf consists of a petiole, a midrib, and a lamina or leaf blade. The petiole is a long, sturdy stalk that attaches the leaf to the pseudostem, while the midrib runs the length of the leaf, providing structural support. The leaf blade is large, broad, and elliptical, with a smooth texture and parallel venation. The leaves emerge in a spiral arrangement from the center of the pseudostem, forming a rosette pattern (Simmonds, 1962). Typically, the stem supports 10 to 15 functional leaves, with a new leaf generated every 7 to 10 days (Purseglove, 1975). Each banana plant produces 30 to 40 leaves during its lifecycle, which plays a critical role in photosynthesis and transpiration (Robinson & Saúco, 2010).

2.3.4. Rhizome

The banana plant's true stem, known as a tuberous rhizome, is mostly underground and is crucial for the plant's growth and propagation. Unlike typical rhizomes, the banana rhizome has limited horizontal growth. This structure should not be confused with a corm, which is a vertical, compact stem, although the terms rhizome and corm are sometimes used interchangeably in literature. The mature rhizome, usually about 300 mm in diameter, is essential for nutrient storage, particularly before flowering when it holds around 45% of the plant's total dry matter (Robinson & Saúco, 2010). The rhizome is internally structured with a central cylinder and cortex, surrounded by starchy parenchyma tissue. During its growth phase, the rhizome produces leaves from a central apical meristem and vegetative buds that develop into suckers. These suckers are critical for plant propagation. After the aerial parts of the plant are harvested, the rhizome continues the propagation process by producing new suckers, with up to 15 suckers developing in a specific pattern around the parental rhizome (Robinson & Saúco, 2010).

2.3.5. Inflorescence

The inflorescence of a banana plant, commonly known as a banana flower or blossom, emerges from the apex of the pseudostem. It consists of a central axis called the rachis, from which clusters of flowers develop in a spiral fashion (Robinson & Saúco, 2010). The inflorescence is initially enclosed in a large, purple brace that opens as the flowers mature. The banana flower is divided into female, male, and neuter flowers, with female flowers located at the base, capable of developing into fruits, while the male flowers, located at the tip, are primarily involved in pollination (Simmonds, 1962). Female flowers are larger and possess a superior ovary, which, upon fertilization, swells and matures into the banana fruit. Male flowers are smaller, with reduced reproductive structures. The development of the inflorescence is a key phase in the banana lifecycle, leading to fruit formation and maturation (Stover & Simmonds, 1987).

2.3.6. Fruit

The banana fruit is a berry, botanically termed a simple, fleshy fruit with multiple seeds embedded within its flesh (Simmonds, 1962). However, most cultivated bananas are seedless and propagate vegetatively due to their triploid nature, which prevents the formation of viable seeds (Simmonds, 1962). The fruit develops from the inferior ovary of the female flower and consists of a peel or rind, which encloses the edible pulp. The banana peel is typically thick, ranging from yellow to green in color, and contains numerous phytochemicals that protect the fruit from environmental stressors and pests (Stover & Simmonds, 1987). The pulp is rich in starch, sugars, vitamins, and minerals, making bananas a nutritious food source. Bananas are arranged in clusters called hands, each containing 10 to 20 individual fruits known as fingers, which are attached to the main stem or peduncle (Robinson & Saúco, 2010).

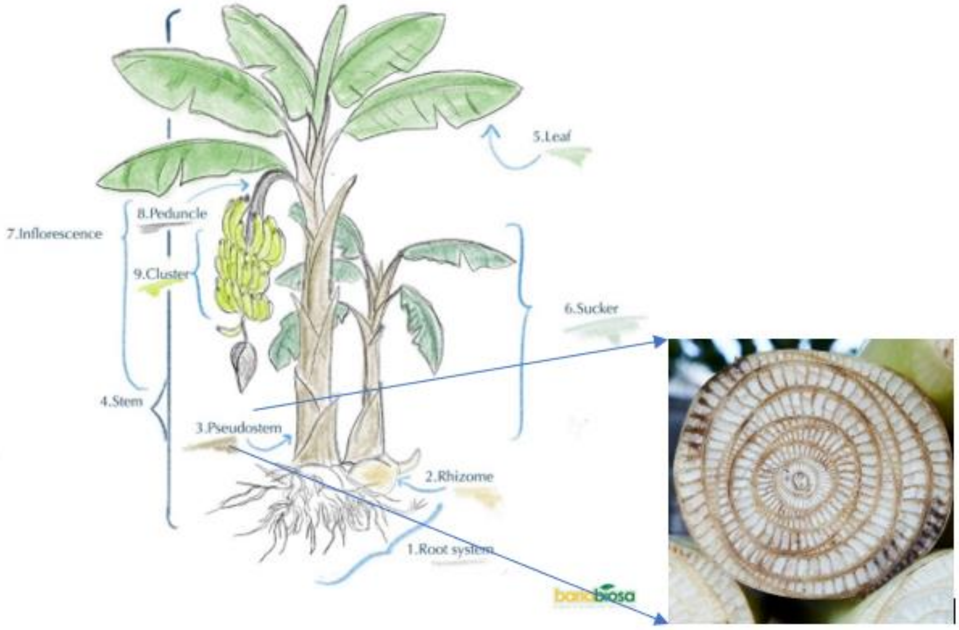

Figure 1. A. Morphology of a Banana Plant. This diagram illustrates the key features of banana morphology, including the pseudostem, leaves, inflorescence, and fruit structure.

2.4. Uses of the Banana Plant

Bananas (*Musa* spp.) are a versatile staple food in many tropical and subtropical regions, not only valued for their delightful taste but also esteemed for their nutritional richness and versatility. They are consumed fresh, making them a convenient and energy-dense fruit that supplies essential nutrients such as potassium, vitamin C, and dietary fiber, which are crucial for maintaining overall health and preventing conditions like hypertension and digestive issues (Marriott et al., 1981). Bananas also undergo various processing methods that expand their utility in food products. For instance, they are transformed into dried chips, which are a popular snack, and into purees and flours that are key ingredients in bakery items, snacks, and baby foods, catering to diverse dietary needs and preferences (Aurore et al., 2009). The versatility of bananas extends to the production of alcoholic beverages in various cultures, where they are used to create banana beer and wine. These beverages leverage the fruit's natural sugars through fermentation processes, contributing to local economies and cultural traditions while offering unique flavors and nutritional benefits. Such applications highlight the economic significance of bananas beyond their nutritional value.

The by-products of banana cultivation are remarkably valuable and diverse in their applications. For example, banana peels, often regarded as waste, are rich in antioxidants and are being explored for their potential in enhancing animal feed, which can improve livestock health and productivity. The peels are also investigated for their use in bioactive compounds and as a raw material for environmentally friendly packaging solutions (Emaga et al., 2007). Banana leaves, another by-product, are traditionally used as biodegradable plates and wrappers in many cultures, offering a sustainable alternative to single-use plastics. They are valued for their large size, flexibility, and natural wax coating, which makes them an ideal material for food packaging and serving (Emaga et al., 2007). This practice not only reduces waste but also embodies a holistic approach to utilizing the entire plant. In addition to these applications, the fibrous parts of the banana plant are employed in various traditional and industrial uses. The fibers are utilized in the production of textiles, paper, and biodegradable composites, demonstrating the banana plant's potential in contributing to sustainable industrial practices. Medicinally, banana fibers have been used in traditional remedies for treating a range of ailments due to their anti-inflammatory and antimicrobial properties (Morton, 1987).

2.5. Significance of Bananas

2.5.1. Economic Impact and Export

Banana cultivation provides a primary source of income for smallholder farmers and agricultural laborers across diverse geographic regions, particularly in countries within Asia, Latin America, and Africa. For many of these individuals, banana farming is not only a livelihood but a means of survival. In countries like India and Ecuador, which are among the top banana producers, the fruit plays a pivotal role in the national economy, supporting millions of jobs directly and indirectly linked to cultivation, processing, and export activities. Bananas are also a major export commodity, with significant economic implications for producing countries. For instance, in Ecuador, the largest exporter of bananas, the industry contributes substantially to the national GDP (Gross Domestic Product), providing crucial foreign exchange earnings and sustaining rural economies (FAO, 2021). The global banana trade is characterized by a complex supply chain that includes production, transportation, and distribution networks, which are vital for delivering this staple fruit to international markets. According to the FAO, bananas account for a significant portion of the agricultural export revenues in many producing countries, highlighting their economic importance on a global scale (FAO, 2021). FAO reveals a staggering global production number of 135 million metric tons in 2020, with much of it originating from Asia and Latin America (FAO, 2021).

2.5.2. Agricultural Advancements

The banana industry has been a catalyst for significant agricultural advancements, particularly in the areas of cultivation practices and sustainability. The challenges posed by pests, diseases, and environmental factors have driven innovation in banana farming techniques. For example, the widespread threat of Black Sigatoka (*Mycosphaerella fijiensis*) and Fusarium wilt has prompted extensive research into disease-resistant banana varieties and sustainable farming practices (Ploetz, 2006). Sustainable banana cultivation techniques have become a focal point for many producing countries, aimed at enhancing productivity while minimizing environmental impact. Practices such as integrated pest management (IPM), organic farming, and the use of biodegradable materials for packing and transportation are being increasingly adopted (Unal et al., 2022; Wahome et al., 2021; Waseem et al., 2020). These practices not only help in maintaining soil health and reducing chemical usage but also contribute to the long-term viability of banana farming. The industry has heavily invested in research and development to create disease-resistant banana varieties through traditional breeding, tissue culture, and genetic engineering. These techniques ensure the production of disease-free plants that maintain high quality and resistance, reducing the need for chemical pesticides and thus minimizing environmental impact. By lowering the dependency on chemical treatments, these practices also contribute to the sustainability of banana farming, preserving soil health and protecting beneficial organisms within the ecosystem.

2.5.3. Social and Cultural Significance of Banana

Beyond their economic and agricultural contributions, bananas hold significant social and cultural value in many producing regions. They are a staple food for millions of people, providing essential nutrients and serving as a key component of local diets. In many cultures, bananas are not only a source of sustenance but also play a role in traditional practices and ceremonies, reflecting their deep-rooted cultural importance

(Simmonds, 1966). Culturally, bananas are woven into the rituals, traditions, and ceremonies of many societies. For instance, in several Asian and African cultures, bananas are offered during religious ceremonies or used in symbolic rituals to mark important life events, such as weddings or harvest festivals. The banana plant itself is sometimes revered as a symbol of fertility, prosperity, and longevity, reflecting its deep-rooted cultural importance. Moreover, the cultivation and trade of bananas have historically shaped social structures and community life in producing regions. The industry provides employment and sustenance for countless families, fostering a sense of identity and continuity within these communities. Thus, bananas are not only a critical food source but also a cultural touchstone that connects people to their heritage and traditions, highlighting their enduring significance in the lives of those who cultivate and consume them (Simmonds, 1966).

2.6. Commercial Propagation of Banana

Banana propagation primarily relies on vegetative methods due to the sterility of seeds in most cultivated varieties, which are often triploid and unable to produce viable seeds (Stover & Simmonds, 1987). The predominant vegetative techniques include the use of rhizome "bits" and suckers. Rhizome "bits" are small sections of the underground stem that, when planted, develop into new banana plants. Suckers are shoots that arise from the base of the parent plant's corm and can be separated and replanted to grow independently. These methods are widely used by farmers because they ensure that the new plants are genetically identical to the parent, thereby maintaining desirable traits such as disease resistance, fruit quality, and yield consistency (Robinson & Saúco, 2010).

In addition to traditional vegetative propagation, tissue culture has become an indispensable tool in modern banana propagation. This advanced technique involves the isolation of meristematic tissue, typically from the shoot tip or other actively growing parts of the plant. The tissue is then cultivated in a sterile, nutrient-rich medium under controlled environmental conditions (Cronauer & Krikorian, 1984). Tissue culture offers several advantages over conventional methods. It facilitates the rapid multiplication of large quantities of disease-free planting material, which is crucial for managing and preventing the spread of diseases such as Fusarium wilt and *Banana bunchy top virus* (BBTV). Furthermore, tissue culture plays a critical role in the conservation and propagation of rare and endangered banana varieties, which might be difficult or impossible to preserve through traditional means. This technique allows for the ex-situ preservation of genetic diversity, which is vital for breeding programs aimed at developing new varieties with improved traits such as resistance to biotic and abiotic stresses (Al-Khayri et al., 2018)

Recent advancements in tissue culture techniques, such as somatic embryogenesis, have further enhanced the efficiency and effectiveness of banana propagation. Somatic embryogenesis involves the development of embryos from somatic or non-reproductive cells, providing an alternative route for mass propagation. This method not only increases the propagation rate but also helps in producing plants that are genetically uniform and true-to-type.

2.7. Banana Cultivation in Lebanon

Banana cultivation in Lebanon began in the mid-1940s, initially in the southern regions and later expanding to the coast of Jbeil (Younes, 2022). The subtropical climate of the coastal areas offered optimal growing conditions, leading to a concentration of cultivation in these regions. Long rains occur between November and January, while short rains are observed in October, March, and April, with a dry period from May to October. Humidity levels range from 60% to 85%. Temperatures vary from a minimum of 8 °C to a maximum of 35 °C.

The region's soil is predominantly clayey, with a significant organic matter content and poor to medium drainage. The southern coastal zone features expandable clay brown/black soils with moderate organic matter content (1.2-2.8%). The soil is non saline and can be slightly calcareous or non-calcareous, with a slightly basic pH value (7.5-8) and a low to moderate cation exchange capacity (UNEP-MAP, UNESCO-IHP, CNRS Lebanon, 2015).

Due to the favorable climate and the adoption of both open field and net house cultivation methods, bananas are available year-round in Lebanon. Planting occurs twice a year: from mid-March to mid-April and again in July and August. Banana production is limited to coastal areas below 150 meters above sea level. Typically, bananas bear fruit 6 to 12 months after planting, depending on the transplanting time, variety, and growing conditions. The mother plant produces only one fruit cluster and is cut after harvesting to encourage new sucker growth.

New cultivation techniques, including protected net houses, were introduced in the early 1990s (Younes, 2022). This development led to the importation of new varieties from research centers in Spain, South Africa, and Latin America. The most cultivated varieties in Lebanon are the local dwarf Cavendish and the Grand Nain (Chalak & Sabra, 2007). Bananas' relative tolerance to cooler climates makes them an attractive alternative to traditional citrus cultivation. In 2019, an estimated 4,092 hectares were dedicated to banana cultivation, producing 156,506 tons with an average annual growth rate of 2.54% (Selina Wamucii, 2020). However, banana production in Lebanon faces increasing challenges from both biotic and abiotic factors.

Biotic threats include viruses, bacteria, nematodes, and fungi, all of which significantly impact banana plantations. Uddin et al. (1973) reported that parasitic nematodes lead to decreased growth and yield, causing stunted growth, yellowing leaves, and root decay, which results in smaller feeder roots. Pinese and Piper (1994) found that spider mites damage the fragile leaf cells of nursery banana seedlings, reducing overall leaf performance. Abiotic factors also pose risks, with extreme temperatures, particularly frost, potentially reducing yields due to plant death or damage. Drought conditions cause moisture stress, limiting water availability for plant growth and metabolism, which negatively affects banana quality. Additionally, insufficient soil nutrients can lead to deficiencies, such as yellowing leaves, adversely impacting plant health and crop productivity.

2.8. Major Global Banana Diseases

Banana production around the world is under constant threat from several major diseases. One of the most significant diseases is Black Sigatoka, a leaf spot disease, also known as black leaf streak, is a devastating fungal disease that affects banana plants. It is caused by the fungus *Pseudocercospora fijiensis* (formerly *Mycosphaerella fijiensis*). This disease primarily attacks the leaves of banana plants, which are crucial for photosynthesis and, consequently, the plant's overall health and fruit production. The disease starts as small, dark spots on the undersides of the youngest leaves. These spots enlarge and merge, forming streaks that turn black, hence the name "Black Sigatoka"

(Jones, 2000). As the disease progresses, the affected leaves begin to die prematurely, significantly reducing the plant's ability to photosynthesize. This leads to reduced fruit size, lower yields, and poor fruit quality. In severe cases, the entire plant can be defoliated, making it highly susceptible to other stresses and diseases (Lima et al., 2017). Black Sigatoka spreads through spores that are carried by wind, rain, irrigation water, and even by humans and machinery moving through the plantations. High humidity and temperatures, common in many banana-growing regions, favor the development and spread of the disease (Bennett et al, 2003).

Black Sigatoka affects banana-growing regions all over the world. Black Sigatoka, which originated in Asia during the late twentieth century, has now fully spread across the banana-growing regions of Latin America and the Caribbean. (Bebber, 2019). In Africa, the disease is widespread in East African nations like Uganda, Kenya, Tanzania, and Rwanda, as well as in West African countries such as Nigeria and Ghana, and Central African countries like Cameroon (Yonow et al., 2019; *Black Sigatoka Disease of Banana and Plantain Is Spreading in the Americas*, 1998). In Asia, countries such as the Philippines, Indonesia, Malaysia, Thailand, and India face significant challenges from Black Sigatoka, affecting both smallholder farms and large plantations (Jones, 2000). In Oceania, the disease is a major problem in Pacific Island nations such as Fiji, Papua New Guinea, and Samoa, impacting both commercial and subsistence banana production (Fullerton & Olsen, 1995).

Banana bunchy top virus (BBTV) is another devastating viral disease that affects banana plants worldwide. It is caused by the *Banana bunchy top virus,* classified within the genus *Babuvirus* in the family Nanoviridae. The disease is characterized by distinct symptoms where infected plants display stunted growth, with new leaves emerging narrower and shorter than normal. One of the hallmark signs of BBTV is the "bunchy top" appearance of the leaves, where they bunch tightly at the top of the pseudostem (Tennant et al., 2018). This virus is primarily transmitted by the banana aphid (*Pentalonia nigronervosa*), which feeds on the sap of infected plants and spreads the virus to healthy ones during feeding (Jekayinoluwa et al., 2020). BBTV-infected plants often suffer from reduced fruit production and quality, leading to significant economic losses in affected regions. Controlling BBTV involves strategies such as the removal and destruction of infected plants to prevent further spread, along with efforts to manage aphid populations to reduce transmission.

Root-knot nematodes also pose a serious threat to banana cultivation, particularly nematodes from the genus *Meloidogyne*, causing considerable damage to banana plants. These nematodes are microscopic roundworms that invade the root system, leading to the formation of characteristic galls or knots on the roots. The most common species affecting bananas include *Meloidogyne incognita, Meloidogyne javanica*, and *Meloidogyne arenaria* (Luquini et al., 2019). The life cycle of root-knot nematodes begins with the eggs hatching into larvae, which then invade the root tissues. Once inside, they induce the plant cells to form giant cells and galls, disrupting the plant's vascular system. This disruption severely hampers the plant's ability to absorb water and nutrients from the soil, leading to symptoms such as stunted growth, chlorosis (yellowing of the leaves), wilting, and overall reduced vigor (Moens et al., 2009). Root-knot nematodes can significantly reduce banana yields. The galled roots are less effective in anchoring the plant, making it more susceptible to toppling, especially under adverse weather conditions. Additionally, the galls provide entry points for secondary pathogens, further complicating the plant's health (Gowen et al., 2005).

Radopholus similis, commonly known as the burrowing nematode, is one of the most destructive nematodes affecting banana crops worldwide. It invades the roots and rhizomes of the banana plant, causing extensive damage by tunneling through the root tissues. This damage disrupts water and nutrient uptake, leading to stunted growth, yellowing of leaves, and reduced fruit production. Over time, the weakened root system causes the plant to become unstable and susceptible to falling over, a condition commonly referred to as "toppling disease" (Gowen et al., 2005). The nematode's ability to reproduce rapidly and its capacity for wide dispersal through infected planting materials exacerbate the issue, making *R. similis* a serious threat in many banana-producing regions. In many cases, once *R. similis* infests a plantation, it becomes challenging to eliminate, leading to significant yield losses and increased production costs (Quénéhervé, 2008).

Moko disease and Anthracnose also pose challenges to banana cultivation, though their impact is less severe. Moko disease, caused by the bacterium *Ralstonia solanacearum* race 2, affects the vascular system of banana plants, leading to wilting, yellowing, and eventual plant death (Sequeira, 1998). It spreads through soil, water, and contaminated tools, making control challenging. Anthracnose, caused by the fungus *Colletotrichum musae*, primarily affects banana fruit, leading to black spots and rot, which can be especially problematic post-harvest (Jeger et al., 1995). Managing these diseases involves sanitation measures, use of resistant varieties, and careful handling and storage practices to minimize spread and damage (Ploetz, 2003).

Fusarium wilt, also known as Panama disease, is currently the most devastating and feared diseases affecting banana production globally. This disease warrants special attention due to its devastating impact on banana production. Given its significance, a more detailed examination of *Foc* TR4 will be addressed in the following section.

2.9. Fusarium Wilt in Bananas

Fusarium wilt of bananas, also known as Panama disease, is one of the most recent and significant banana pathogens. It is a severe soil-borne fungal disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), which primarily targets the vascular system of the banana plant. This pathogen disrupts the plant's ability to transport water and nutrients, resulting in symptoms such as wilting and yellowing of the leaves, which eventually lead to the plant's collapse and death (Ploetz, 2015b). Fusarium wilt manifests in different forms, classified into several races based on their pathogenicity and the specific banana cultivars they infect. The most notable of these are Race 1, Race 2, Tropical Race 4 (TR4), and Subtropical Race 4.

Race 1 was historically responsible for the destruction of the Gros Michel variety, which dominated global banana production until the mid-20th century. Race 2 affects cooking bananas and specific varieties used in East Africa. However, Tropical Race 4 (TR4) is the most alarming, as it has a wide host range, including the widely cultivated Cavendish variety, which had previously been resistant to earlier races. TR4's virulence and adaptability have made it a significant threat to global banana production.

The disease spreads efficiently through contaminated soil, water, infected plant material, and farming equipment (Stover, 1962). Once established in a plantation, Fusarium wilt is nearly impossible to eradicate due to the pathogen's persistence in the soil for decades, even in the absence of host plants. This tenacity has forced the abandonment of entire plantations, resulting in significant economic losses for banana-producing regions.

The global spread of Fusarium wilt, particularly TR4, has heightened concerns within the banana industry, given its potential to devastate banana production worldwide. The economic impact is profound, affecting not only large-scale commercial operations but also smallholder farmers who rely on banana cultivation as a primary source of income. The challenge posed by Fusarium wilt underscores the need for integrated disease management strategies, including strict quarantine measures, effective sanitation practices, and ongoing research into resistant banana cultivars to protect this vital crop from further devastation.

2.9.1 Historical Impact and Spread of Fusarium Wilt

Banana Panama disease has been a major destructive force in banana agriculture for over a century. The first documented instance was in Australia on Gros Michel bananas (Bancroft, 1876; Ashby, 1913). The disease caused significant losses in the 1950s in Central and South America, leading to the devastation of approximately 40,000 hectares of Gros Michel banana plantations (Stover, 1962). *Foc* Race 2 later affected Bluggoe variety bananas in Latin America (Ploetz, 2006). Although race 3 causes Fusarium wilt in *Heliconia* spp., its effect on banana plants is unclear and it is no longer considered part of the *Fusarium* genus (Ploetz 2005). To manage *Foc* Race 1, Gros Michel was replaced by Cavendish varieties, which exhibited greater resistance to Fusarium wilt.

However, in 1967, a new strain of the disease, *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (TR4), was discovered in Taiwan (Su et al., 1986). This strain spread rapidly, causing significant concern for the global banana industry. Ploetz (2005) estimated that around 80% of Cavendish bananas are at potential risk from TR4, which continues to spread unless effectively contained. Recent studies by Ordonez et al. (2015) indicated that TR4 has affected as much as 100,000 hectares of land, severely impacting small-scale farmers' livelihoods.

2.9.2. Global Distribution of Fusarium Wilt

The geographical distribution of Fusarium wilt of banana, caused by *Fusarium oxysporum* f. sp. *cubense*, reflects its significant global impact on banana cultivation. The widespread occurrence of Fusarium wilt, particularly races 1 and 2, has extended its reach to numerous territories worldwide (ProMusa, 2022). The disease is now present in almost all banana-growing regions (Fig. 2). Subtropical Race 4 (SR4) has been identified in regions such as Taiwan, the Canary Islands, South Africa, and southern Brazil (Ploetz & Pegg, 2000; Perez-Vicente, 2004). Tropical Race 4 (TR4), which affects banana varieties of the Cavendish group, has been reported in the Northern Territory of Australia, as well as in the tropical regions of Southeast Asia, including Taiwan (Ploetz & Pegg, 2000), China (Qi, 2001), India (Damodaran et al., 2019), Indonesia, Malaysia (Herrera et al., 2023), and the Philippines (Gotor et al., 2021). Furthermore, TR4 was officially reported for the first time in the Middle East in Jordan and Lebanon in 2013 (García-Bastidas et al., 2014), followed by northern Mozambique (Ordoñez et al., 2016). In Lebanon, yellowing of leaves and internal vascular discoloration in the pseudostem of Cavendish plants were first observed in the Mansouri and Berghliyeh regions in October 2013 (Ordoñez et al., 2016). Other countries, including Venezuela, Colombia, Peru, Turkey, Palestine, Pakistan, Vietnam, Laos, Myanmar, Thailand, and Mayotte, have also reported the presence of *Foc* TR4.

Notably, TR4 was observed in a banana exhibition in Cornwall, UK, in 2009 and reappeared in 2015 (ProMusa, 2022).

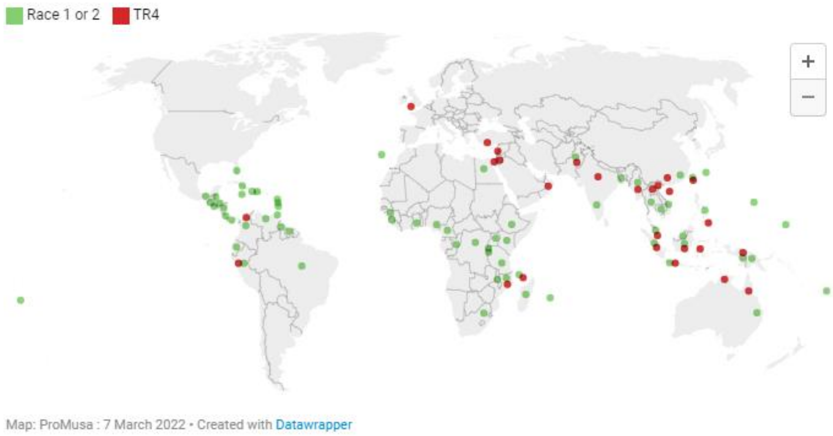

Figure 2. Global geographical distribution of *Foc* races (ProMusa, 2022).

2.10. *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4

2.10.1 Taxonomy

Fusarium oxysporum f. sp. *cubense* Tropical Race 4 is a eukaryotic fungus belonging to the *Fusarium* genus within the Nectriaceae family, Hypocreales order, Hypocreomycetidae subclass, Sordariomycetes class, Pezizomycotina subphylum, and Ascomycota phylum (Fourie et al., 2011). *Fusarium oxysporum* is divided into various formae speciales or "races," with four identified races (1-4). Race 4 is further subdivided into two subgroups: Tropical Race 4 (TR4), which affects Cavendish varieties in both tropical and subtropical regions, and Subtropical Race 4, which poses a threat to banana fields in subtropical regions under certain biotic or abiotic conditions. The taxonomy of formae speciales (f. sp.) emerged from the specialization of pathogenicity to plant genera and families (Snyder and Hansen, 1940). At first, it was thought that formae speciales was unique to a single host, therefore, names like betae, callistephi, apii, mori, and roughly 60 others were derived from the host. Because of this early understanding of extremely specific pathogenicity, several formae speciales merely races of formae speciales that have been described in other hosts have been established. Smith in 1908 discovered the first isolate of the fungus from sick banana plants in Cuba in 1908, and he gave the species the name f.sp. *cubense* (Smith, 1910). Ashby (1913) provided the first in-depth description of the causal agent in culture, and Brandes (1919) confirmed Koch postulates in the variety Bluggoe as well as in Gros Michel (AAA) and Manzano (AAB). *Fusarium cubense* was identified as a subspecies of the virtually ubiquitous *Fusarium oxysporum*. Snyder and Hansen (1940) developed the formae speciales system, all species of the complex *Fusarium oxysporum* that produced wilt symptoms in *Musa* spp. were renamed as *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Phylogenetic studies reveal that *Foc* TR4 is an asexual polyphyletic fungus with various strains due to convergent evolution (Ploetz, 2006; Fourie et al., 2011). Strains of *Fusarium oxysporum* are also categorized into Vegetative Compatibility Groups (VCGs), with at least 24 VCGs identified in recent classifications (Mostert et al., 2022).

2.10.2. Lifecycle

Fusarium oxysporum f. sp. *cubense* is known for its ability to overwinter in soil and plant debris for up to two decades, primarily as chlamydospores. Other forms, such as microconidia and macroconidia, are also observed. Microconidia are oval or reniform, while macroconidia are sickle-shaped with thin walls. In special structures called sporodochia and which are orange, macroconidia and microconidia generates. Chlamydospores are round with thick walls, formed either internally within macroconidia or terminally on aging mycelium (Dita et al., 2018). Germination is initiated upon encountering susceptible root tissues or host/non-host root exudates, which trigger the dormancy of *Foc* TR4 structures.

Primary infection occurs in the root area, progressing to lateral roots and eventually leading to systemic rhizome infection (Dita et al., 2018) (Fig. 3). The fungus develops penetrates to the plant's xylem vessels, causing discoloration and blockage, which disrupts nutrient and water transport and results in wilting during the flowering stage due to insufficient supply (Garcia-Bastidas, 2022).

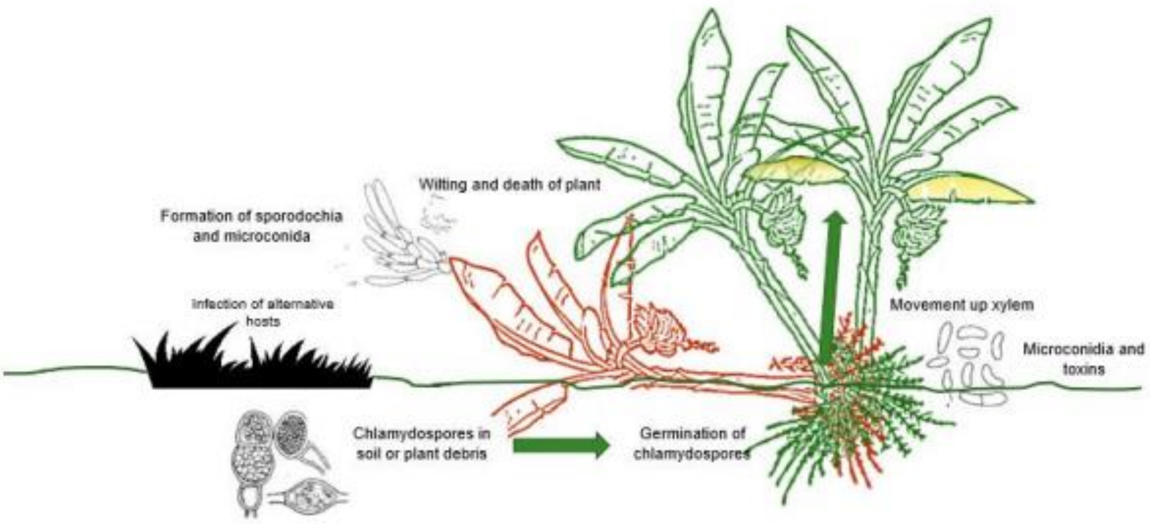

Figure 3. *Fusarium oxysporum* f. sp. *cubense* lifecycle and disease process (Crozier, 2023)

2.10.3. Epidemiology

The spread of *Foc* TR4 is facilitated when healthy plant roots meet those of infected plants, chlamydospores or conidia. Primary transmission occurs through contaminated planting materials, such as suckers or tissue culture specimens, which often show no visible symptoms (Ploetz, 2015b). This makes detection and control challenging (Dita et al., 2018). Once introduced to a new plantation, the pathogen can rapidly spread through the soil, affecting all host plants in its path (Ploetz et al., 2015a & b).

Foc TR4 does not spread via banana fruits but can disperse undetected through fruit crown vasculature (DAFF, 2004). The pathogen can also move with contaminated soil particles or plant debris, aided by strong winds. Such dispersal has been observed when massive gusts propel contaminated soil from infested areas over large distances (Dita et al., 2018). Additionally, water sources such as rivers and dams can facilitate contamination, especially during flooding or irrigation (Su et al., 1986).

Meldrum et al. (2013) found evidence suggesting that *Foc* TR4 could be associated with the exoskeletons of the banana weevil borer (*Cosmopolites sordidus*), indicating a potential vector role for this insect, though further research is needed to confirm this, particularly regarding the airborne larvae infecting above-ground organs.

2.10.4. Symptoms

Banana Fusarium wilt appears externally as either yellow leaf syndrome or green leaf syndrome (Pérez-Vicente, 2004). The most obvious indicator of Fusarium wilt in bananas is yellow leaf syndrome (Fig. 4). This symptom can be mistaken for potassium deficiency due to the yellowing along the edges of older leaves, especially dry and cold conditions. The progression of leaf yellowing starts from older leaves towards younger ones. A "skirt" of dead leaves surrounds the pseudostem, bending at the petiole, usually near to the midrib. Over time, these leaves gradually collapse, bending at their petioles near the midrib. Unlike the yellow leaf syndrome typically associated with Fusarium wilt, in the green leaf syndrome variation, affected leaves tend to retain their green coloration until the petioles bend and detach. This creates a distinct visual contrast compared to the usual yellowing symptoms observed in other affected plants. Younger leaves typically display symptoms last, and frequently continue to stand unnaturally upright, giving bristle-like appearance. In the base of the plant, the pseudo stem eventually separates longitudinally. The fruits show no signs of any symptoms. Fusarium wilt-infected susceptible banana plants rarely make a full recovery. While it that could happen, but the growth is poor, and the mother plant makes numerous infected suckers before it eventually dies.

Vascular discoloration is an internal symptom characterized by the yellowing of vascular tissue in the root and rhizome. This discoloration develops leading to the formation of continuous yellow, red, or brownish strands in the pseudostem (Fig. 4). Fungus develops in the tissues around the xylem as the plant dies and produces many chlamydospores, which persist in the soil after the plant decomposes. Although these plants are still asymptomatic in the field, *Foc* TR4 colonizes and survives in secondary host roots, including those connected to some weed species and banana species. In Bananas, there are no distinctions in the symptoms amongst the various *Foc* races. Hence, it is impossible to distinguish between races only on symptoms caused by disease (Bragard et al., 2022).

Figure 4. Symptomatic banana plant infected with *Foc* TR4. A: Reddish to brown discoloration of the vascular system B: Collapsed older leaves hanging down the pseudostem. C: Yellow leaf syndrome. D: split of the pseudostem.

2.10.5. Diagnosis

Diagnosing Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 involves a combination of field observations and advanced laboratory techniques. Field symptoms typically include leaf discoloration, wilting, and yellowing of the lower leaves, which can initially suggest a *Foc* TR4 infection (Clark & McKechnie, 2020). However, these symptoms are not exclusive to *Foc* TR4 and can be mimicked by other banana diseases, such as Black Sigatoka or bacterial wilt, making field diagnosis challenging and often unreliable.

To confirm the presence of *Foc* TR4, several advanced diagnostic methods are employed. Molecular techniques, such as Polymerase Chain Reaction (PCR), are highly effective for detecting the pathogen's genetic material in soil, plant tissues, and water samples. PCR allows for early detection even before symptoms become apparent (Dita et al., 2018). Additionally, genetic sequencing can provide precise identification of the pathogen's race, distinguishing TR4 from other *Fusarium* races (Clark & McKechnie, 2020).

Biological methods include isolating and culturing the pathogen from infected plant tissues. Although these methods can confirm the presence of the pathogen, they are time-consuming and require specialized laboratory facilities (Ploetz, 2015a). Morphological analysis of fungal colonies grown in the lab can also aid in identification but is less definitive compared to molecular techniques.

Recent advancements in technology have introduced remote sensing methods, such as drone management, camera monitoring, and satellite imaging, which facilitate large-scale surveillance and early detection of potential outbreaks. These technologies can identify stress patterns in crops that might indicate the presence of *Foc* TR4. However, their reliability in diagnosing specific fungal diseases is still under evaluation and they are often used in conjunction with more traditional methods (Su et al., 1986).

2.10.5.1. Morphological Diagnosis

Morphological diagnosis involves observing fungal cultures macroscopically and microscopically. On potato-dextrose-agar (PDA) medium, colonies have a variable morphology. Mycelia can be hairy to cottony, spaced or abundant and variable from white, salmon, to pale violet (Fig. 5). Black to violet sclerotia can be produced in some isolates. *Fusarium oxysporum* commonly produces pale violet to dark red color pigments in PDA (Pérez-Vicente et al., 2003). Some isolates mutate quickly from pionnotal (with abundant greasy or brilliant conidia aggregates) to a flat humid mycelium of white-pale yellowish to peach color on a PDA culture (Ploetz, 1990).

2.10.5.1.1. Cultures on PDA

On Potato Dextrose Agar (PDA), *Foc* TR4 typically exhibits distinctive morphological characteristics. Initially, the fungus grows as a white, fluffy mycelium, which gradually turns pinkish or purple over time due to the production of pigments. The colony may also develop a cottony or woolly texture. As the culture matures, concentric rings of mycelial growth may be visible, and the pigment can intensify, often creating a darker purple or violet hue at the center of the colony. Additionally, the underside of the culture plate often appears reddish-purple as the pigment diffuses through the medium. The production of spores, such as macroconidia and microconidia, can also be observed under a microscope as the colony develops.

2.10.5.1.2. Microconidia

According to the *Fusarium* manual (Leslie & Summerell, 2006), microconidia (5-16 × 2.4-3.5 µm), usually without septa, can be oval, elliptic to kidney shaped and developed abundantly in false heads in short monophialides (Fig. 5). They are commonly observed in false heads within the aerial mycelium above the substrate surface. Produced abundantly by conidiogenous cells (single-celled stalks), microconidia play a crucial role in fungal dispersal and colonization (Leslie & Summerell, 2006). Due to their minute size and tremendous abundance, microconidia play a crucial role in fungal biology specifically enabling organisms to disperse into new environments for colonization purposes more effectively than before achievable.

2.10.5.1.3. Macroconidia

Foc TR4 isolates often produce pale orange sporodochia, although some cases exhibit low numbers or an absence of these structures. Macroconidia (27-55 x 3.3-5.5 μm) have thin walls, curved cone-shaped apical cells, and either rounded or sharply defined basal cells with three septa (Fig. 5). They are typically abundant in sporodochia and on agar surfaces (Leslie & Summerell, 2006).

2.10.5.1.4. Chlamydospores

According to the *Fusarium* manual (Leslie & Summerell, 2006), chlamydospores (7-11 μm diameter), are specialized structures formed by fungi in response to environmental stress or to survive dormancy. These structures, which can be found within two to four weeks on specific media, may occur singly, in pairs, clusters, or form short chains (Fig. 5). They exhibit different wall shapes (smooth or rough) and serve multiple functions, including resistance to harsh conditions and providing diagnostic information based on their size, shape, and other morphological features.

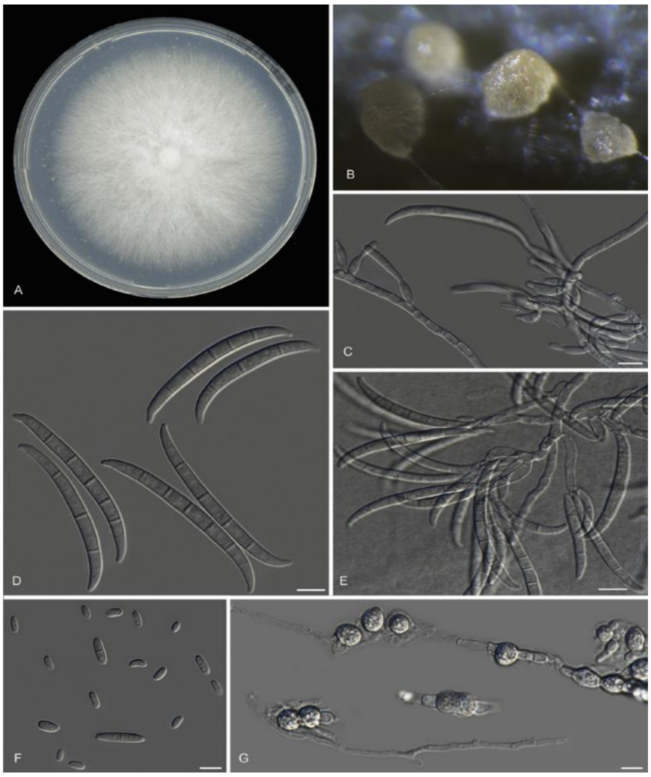

Figure 5. A. Culture of *Foc* TR4 grown on PDA. B. Sporodochia on carnation leaves. C. Monophialides with first conidia being formed. D. Falcate-shaped macroconidia. E. Branched conidiophores. F. Elliptical microconidia. G. Thick-walled chlamydospores.

2.10.5.2. Vegetative Compatibility Groups

The identification of different strains of filamentous fungi, such *Foc* TR4, is achievable using an approach known as Vegetative Compatibility Group testing (VCG). This method is founded on the capability of fungal strains to fuse with each other and spawn heterokaryons; cells possessing two or more genetically separate nuclei (Ploetz, 2015b). VCGs serve as a tool for classifying and characterizing various fungal strands to learn their genetic likenesses and differences versus others strain types. Regarding *Foc* TR4, it belongs to vegetative compatibility group 01213 or within the classification complex VCG01213/16 (Mostert et al., 2022). Even though 24 distinctive VCGs have already been recognized for this type of fungus species; mutations could arise that lead to novel additional groups which may yet remain undisclosed at present time (Mostert et al., 2022).

2.10.5.3. Molecular Diagnosis

Polymerase Chain Reaction (PCR) is a powerful molecular tool that has revolutionized the field of molecular biology by enabling the amplification and detection of specific DNA sequences. This technique has been widely adopted for the diagnosis of fungal diseases, especially *Foc* TR4 (Li et al., 2019). Different PCR-based methods such as conventional PCR, nested PCR, real-time PCR, and loop-mediated isothermal amplification (LAMP) can be employed to target distinct regions within the genome of *Foc* TR4.

Conventional or basic PCR involves using sets of primers (Table 1) to selectively amplify target DNA fragments from a sample, which can then be separated according to size through a gel electrophoresis process (Zhang et al., 2019). However, this approach may not always yield reliable results due to its low sensitivity and specificity levels. Nested PCR was developed for situations that require higher sensitivity and specificity as it uses two rounds involving outer and inner primer pairs. These primers identify larger genomic regions in first-round amplifications followed by pinpointing smaller populations in second-round amplification, increasing efficiency several-fold.

Table 1: Primer pairs reported for the specific detection of *Foc* TR4 in PCR assays.

Amplicon size	**Primers**	**Reference**	**Notes**
463	Forward 5’CACGTTTAAGGTGCCATGAGAG Reverse 5’ CGCACGCCAGGACTGCCTCGTGA	Dita et al., 2010, LINK	Primers we used for PCR amplification
884	Forward 5’CGGATTTTGAGCTTTCGACAAG Reverse 5’ GGAGGGCTTTAGAGCGCAAC	Widinugraheni et al., 2018 LINK	
1400	Forward 5’CGCCAGGACTGCCTCGTGA Reverse 5’ GCCAGAGTGAAGGGGAAT	Bentley et al., 2001 LINK	

Real-time PCR, also referred to as quantitative PCR (qPCR), is another precise technique employed in diagnosing *Foc* TR4 (Wang et al., 2019). Fluorescent probes link with the outputs of the process of amplification and, using specialized equipment, this level of fluorescence can be measured swiftly. This method additionally has the capacity to differentiate between different species or strains that are closely related. LAMP PCR is an alternative modern form of polymerase chain reaction which amplifies DNA amidst isothermal conditions, eliminating the requirement for a thermal cycler (Li et al., 2019). The amplified products can be visualized by color change or turbidity, making it a simple and cost-effective method for *Foc* TR4 diagnosis. Additionally, Next Generation Sequencing (NGS) represents a high-throughput method for detecting and accurately determining *Foc* TR4 within banana plants (Cai et al., 2020). Through sequencing millions of fragments from one sample, NGS provides strategies for exploring the complexity among demographic genetics associated with *Foc* TR4 samples.

2.10.6. Economic Importance

2.10.6.1. Direct losses

Fusarium oxysporum f. sp. *cubense* Tropical Race 4 has caused substantial direct losses in the banana industry, primarily through severe reductions in crop yields and increased production costs. A 2019 study published in the journal Plant Pathology found that *Foc* TR4The annual economic impact of TR4 has been estimated at approximately USD 121 million in Indonesia, USD 253 million in Taiwan, and USD 14 million in Malaysia (Aquino et al. ,2013). Farmers are compelled to invest heavily in disease management strategies, including the cultivation of resistant banana varieties, soil treatments, and enhanced biosecurity measures to prevent the spread of the pathogen. These increased costs, combined with the lost income from reduced yields, create a significant economic strain on banana producers. The significant reduction or complete loss of income thrust many into poverty, increasing issues of food insecurity and even prompting migration in search of more stable opportunities.

2.10.6.2. Increased Costs of Production

To combat the spread of *Foc* TR4, farmers are compelled to adopt costly biosecurity measures such as disinfecting tools, restricting movement between fields, and implementing quarantine zones. These practices, while necessary to contain the disease, increase operational costs and reduce efficiency. Additionally, the need to shift to more resistant, but often less commercially viable, banana varieties require substantial investment in research and development, as well as in the propagation and distribution of these new varieties. Furthermore, the long-term economic impact of *Foc* TR4 extends to the supply chain, where the reduced availability of bananas can lead to higher prices for consumers and potential disruptions in global trade. For instance, countries that rely heavily on banana exports face the prospect of diminished export revenues and increased costs in managing the disease across extensive plantations. The economic burden also includes increased labor costs due to the need for more intensive management practices and the potential for job losses in regions where banana farming is a primary source of employment.

2.10.6.3. Yield Losses

In addition to increased production costs, *Foc* TR4 has also been found to cause sever yield losses depending on the severity of infection. Infected plants show stunted growth, chlorosis, and premature death, which can result in reduced yields and lower quality fruits. Furthermore, this pathogen's notable ability to persist over many years in soil means that banana producers have difficulty in managing infested land in a sustainable manner. Growers might find crop rotation as a solution; however, it can further increase production costs and reduce profits for growers (Ploetz et al., 2015a & b).

2.10.6.4. Indirect Losses

2.10.6.4.1 Trade Restrictions

The detection of *Foc* TR4 in a region can have major consequences, imposing restrictions on market access and impacting export opportunities. Several governments have put phytosanitary regulations in place to avoid the introduction and spread of the disease. For example, China ceased shipments from the Philippines due to *Foc* TR4, which constituted an immense financial hit against banana growers in that country (Reuters, 2020).

2.10.6.4.2. Environmental Impacts

The use of chemical fungicides and other control methods to combat *Foc* TR4, a fungus plaguing banana plantations, can have unintended negative side-effects. Specifically, pollutants from the pesticides used in banana cultivation can infiltrate water sources through runoff and leaching, leading to contamination of rivers, lakes, and groundwater. These chemicals can have detrimental effects on aquatic ecosystems, particularly harming fish and other aquatic organisms. The toxic substances can disrupt the reproductive systems of fish, reduce their populations, and alter the overall biodiversity within these aquatic habitats (Brock et al., 2013). Additionally, the accumulation of these pollutants can lead to bioaccumulation in the food chain, affecting not only aquatic life but also birds and mammals that rely on these water bodies for food and habitat.

Finally, relying solely on chemical treatments for pest control poses the risk of developing resistance in the target fungi, which leads to increased costs and reduced efficacy over time (Dita et al., 2010). As pests evolve and adapt, they can become resistant to commonly used fungicides, necessitating the use of higher doses or more potent chemicals, both of which can be more expensive and less effective. This escalating cycle not only strains financial resources but also exacerbates environmental damage due to the higher volumes and increased toxicity of chemicals applied.

One sustainable alternative to reduce reliance on chemical pest management is integrated pest management (IPM). IPM employs a comprehensive approach, combining multiple strategies to manage pests effectively and sustainably. These strategies include cultural modifications, such as crop rotation and the use of resistant varieties, which can disrupt pest life cycles and reduce their prevalence. Biological controls, such as introducing natural predators or beneficial microorganisms, help keep pest populations in check without harmful chemicals. Chemical controls are still a component of IPM but are used judiciously and as a last resort. By targeting specific pests and applying chemicals only, when necessary, IPM minimizes the environmental harm associated with excessive pesticide use. This multifaceted approach not only preserves the effectiveness of chemical treatments by reducing the pressure for resistance development, but also promotes a healthier ecosystem by maintaining biodiversity and reducing pollution. Moreover, IPM practices can be tailored to specific crops and regions, making them highly adaptable and effective in various agricultural settings. Implementing IPM requires thorough knowledge and monitoring of pest populations, understanding the interactions between different pest control methods, and ongoing education for farmers and agricultural workers. Despite these challenges, the long-term benefits of IPM, including improved crop yields, reduced environmental impact, and sustainable pest management, make it a vital strategy for modern agriculture (Akutse et al., 2019).

2.10.6.4.3. Economic Impacts Beyond the Agricultural Sector

Banana production is a cornerstone of the economies in multiple nations, and an outbreak of Fusarium wilt Tropical Race 4 can have serious economic repercussions. In The Philippines, where the banana industry is a major source of employment and foreign currency income, the advent of *Foc* TR4 resulted in significant redundancies and millions lost in exports (Gallardo et al., 2018). The economic impacts extend beyond direct agricultural losses. It has also been noted that contaminated areas could lead to higher bills spent by healthcare organizations, given they are at greater risk from environmental detriments while treating patients connected with plant diseases like *Foc* TR4 (Fitt et al., 2018). Finally, research devoted to controlling and managing such maladies may draw resources away from other places, thus adding another burden onto an already strained system. These additional costs were observed by Ordonez et al. (2015).

In addition to these immediate impacts, there are long-term economic considerations. The disruption of the banana supply chain can lead to increased prices for consumers, which can, in turn, affect food security in regions heavily reliant on bananas as a staple food. Higher prices may also lead to reduced consumption, impacting the nutritional status of vulnerable populations. The economic instability caused by *Foc* TR4 can also discourage investment in affected regions. Potential investors may be wary of the risks associated with agricultural ventures in areas prone to such devastating diseases, leading to reduced economic development opportunities. This reluctance to invest can stifle innovation and growth, perpetuating a cycle of economic hardship. Moreover, the labor market can be significantly impacted. Job losses in the banana industry can lead to increased unemployment rates, exacerbating poverty levels in affected regions. The loss of income for workers and their families can have a ripple effect, reducing spending power and negatively impacting local economies. The social implications of these economic impacts cannot be ignored. Communities dependent on banana production may experience increased social tension and instability because of economic hardship. This can lead to migration, as individuals and families seek better opportunities elsewhere, further depleting the labor force and economic potential of affected areas.

2.11. Management strategies of *Fusarium oxysporum* f. sp. *cubense* TR4

2.11.1. Prevention

The prevention of *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4 on bananas is essential to maintain the health and productivity of banana crop production. Preventing Fusarium wilt of banana, caused by *Foc* TR4 necessitates a comprehensive, integrated approach involving multiple strategies to effectively manage and mitigate the disease. Key strategies include maintaining strict field hygiene and sanitation, implementing crop rotation and fallowing practices, enhancing soil health through organic matter and specific amendments, developing resistant varieties, and utilizing biological control methods.

One of the primary methods involves maintaining strict field hygiene and sanitation. This includes thorough cleaning and disinfection of farm equipment, tools, and footwear to prevent the transfer of contaminated soil between fields. Ensuring the use of pathogen-free planting material and avoiding the movement of soil, water, and plant debris from infected areas are critical steps in minimizing disease spread (Dita et al., 2018; Ordonez et al., 2015).

Implementing crop rotation and fallowing practices can significantly reduce the pathogen load in the soil. Rotating bananas with non-host crops such as rice, legumes, and grasses can help diminish *Foc* TR4 populations. Allowing fields to lie fallow for several years also reduces the pathogen's viability in the soil (Ploetz, 2015a). Enhancing soil health by incorporating organic matter like compost or manure improves microbial diversity, which can suppress *Foc* TR4. Soil amendments such as biochar and lime can alter soil pH and microbial communities, creating an unfavorable environment for the pathogen (Garcia-Bastidas et al., 2019).

An integrated disease management (IDM) approach, which combines cultural practices and soil health management, provides a synergistic effect, reducing the overall spread of *Foc* TR4. Regular monitoring and early detection of disease symptoms, combined with rapid response measures, are essential components of an effective IDM program. Educating farmers on the importance of disease prevention and the proper implementation of management strategies is crucial for the success of IDM. Extension services and training programs can help disseminate knowledge and promote best practices among banana growers, ensuring a coordinated and effective response to Fusarium wilt (Cook et al., 2015).

2.11.2. Chemical Control

Chemical control of *Foc* TR4 in banana cultivation presents a significant challenge due to the pathogen's ability to persist in the soil for extended periods and its resistance to many chemical treatments. Traditional fungicides have limited efficacy against *Foc* TR4 because the pathogen resides deep within the plant's vascular system, making it difficult for chemicals to reach the infected tissues (Ploetz, 2015a). Recent research has explored the use of systemic fungicides that can be absorbed and transported throughout the plant to target the pathogen internally. However, the success of these treatments has been inconsistent, and there are concerns about the environmental impact and potential development of resistance (Dita et al., 2018). Moreover, the soil-borne nature of *Foc* TR4 necessitates soil fumigation techniques, which, although effective, are often costly and pose risks to soil health and non-target organisms (Ordoñez et al., 2016). Despite these efforts, the development of reliable chemical control methods remains an ongoing area of research, highlighting the need for innovative approaches to protect global banana production from this devastating disease.

2.11.3. Biological Control

Biological control methods involve the use of beneficial microorganisms that suppress *Foc* TR4 through competition, antibiosis, and induced systemic resistance in banana plants. The integration of biologically based control measures into the management of Fusarium wilt Tropical Race 4 is gaining traction due to their eco-friendly nature and promising effectiveness. Numerous types of microorganisms, such as bacteria, fungi, and actinomycetes have been identified as biological control agents (BCAs) that have shown promise in controlling this pathogen (Wang et al., 2023). *Bacillus* species specifically has proven useful in managing TR4 due to its ability to produce antifungal compounds--including lipopeptides and polyketides--which interrupt the cell walls of pathogens, limiting their growth capacity (Xiang et al., 2023). Additionally, strains of *Pseudomonas* spp. and *Trichoderma* spp. can trigger a systemic response within banana plants which makes them more prosperous against infection like TR4 by priming up the plant's natural defense mechanisms, so it responds quickly when attacked (Hermosa et al., 2012; Ploetz, 2015a). Implementing biofertilizers packed with beneficial microorganisms can upgrade soil’s structure while also enhancing vegetation health. Arbuscular mycorrhizal fungi have been shown to enhance resources uptake between plants and soil while also amplifying resistance towards numerous kinds of pathogenic organisms (Bonfante & Genre 2010). As an extra precautionary measure some less virulent strains of *Fusarium oxysporum* might be added through a procedure referred to as "*Fusarium* competition" to lessen the influence caused by TR4 whatever nutrients or colonization sites may exist (Alabouvette et al., 2009). Still one major struggle found when using BCAs is viewing how well they survive specific environmental conditions therefore diligence must be placed on researching strategies for understanding soil microbiome dynamics along with devising suitable bioformulations that will guarantee long lasting BCAs performance levels (Weller 2007).

2.11.3.1. *Trichoderma* spp.

Trichoderma harzianum, a species within the *Trichoderma* genus, has emerged as a main component in the field of biological control due to its remarkable ability to combat a wide range of phytopathogens. The genus *Trichoderma*, first described in the 18th century, encompasses several species known for their rapid growth, prolific sporulation, and robust environmental adaptability. *Trichoderma* spp., belonging to the Hypocreaceae family and including more than 254 species (Bissett et al., 2015) (other sources claim over 370 species such as those mentioned by Sánchez Montesinos et al. (2021) and Sun et al. (2022), is a fungus widely used in agriculture for managing plant diseases and enhancing crop productivity. *Trichoderma harzianum*, which reproduces asexually, falls into a group characterized by its branching patterns of conidiophores which possess side branches and small smooth conidia. This classification was established by Rifai in 1969 based on the phialides of the fungus.

Different species within the genus can produce a range of pigments, which can vary in color from greenish yellow, to reddish. However, it is worth noting that some of these pigments can also be colorless. 70 years ago, Weindling (1932) proposed the idea of utilizing *Trichoderma* species as a method of control being the first to demonstrate their combat against bacterial pathogens present in soil. The historical background of biocontrol traces back to ancient agricultural practices where natural enemies of pests were employed to protect crops. However, it wasn't until the mid-20th century that scientific exploration into microbial biocontrol agents gained momentum. *Trichoderma harzianum's* journey into biocontrol began with its identification as an antagonist to other fungi. Over the years, extensive research has elucidated its multiple mechanisms of action, making it one of the most studied and utilized fungal biocontrol agents. The development of commercial formulations containing *T. harzianum* spores and mycelial preparations has facilitated its widespread application in various cropping systems.

The fungus primarily colonizes the rhizosphere, establishing a symbiotic relationship with plant roots. This colonization results in the secretion of a range of enzymes, antibiotics, and secondary metabolites that inhibit the growth of pathogenic fungi and induce systemic resistance in plants (Benítez et al., 2004). *Trichoderma harzianum's* efficacy is not confined to its direct antagonistic actions against pathogens. It also plays a pivotal role in promoting plant growth and enhancing soil health. By colonizing plant roots, *T. harzianum* establishes a beneficial symbiotic relationship that stimulates root development, improves nutrient uptake, and enhances overall plant vigor. This multifaceted interaction underscores its potential not only as a biocontrol agent but also as a biofertilizer, contributing to integrated pest and nutrient management strategies. *Trichoderma* cultures tend to grow when cultivated under temperatures ranging from 25- 30°C. They struggle to thrive at temperatures exceeding 35°C.

Trichoderma harzianum employs several mechanisms to suppress plant pathogens. Mycoparasitism is a key mechanism, where *T. harzianum* parasitizes other fungi by coiling around the host hyphae and secreting cell wall-degrading enzymes such as chitinases, glucanases, and proteases. These enzymes break down the cell walls of pathogens, leading to their destruction (Benítez et al., 2004). Additionally, *T. harzianum* competes with pathogens for space and nutrients, thereby inhibiting their growth. This competitive advantage is bolstered by *T. harzianum's* rapid colonization and prolific sporulation. Antibiosis, another critical mechanism, involves the production of secondary metabolites with antimicrobial properties. *Trichoderma harzianum* produces a variety of antibiotics, including trichodermin, gliotoxin, and viridin, which inhibit the growth of pathogenic fungi and bacteria (Harman, 2006). These compounds disrupt cellular processes in pathogens, providing an additional layer of defense against infections. Moreover, *T. harzianum* enhances plant resistance to diseases through the induction of systemic resistance (ISR). Upon colonization of plant roots, *T. harzianum* triggers the plant's immune responses, leading to the activation of defense-related genes and the production of defensive compounds such as phytoalexins and pathogenesis-related proteins (Shoresh et al., 2010). This priming effect enables plants to respond more swiftly and robustly to subsequent pathogen attacks.

It has been widely believed that *Trichoderma* species are effective in controlling *Fusarium oxysporum* fungi, primarily through the establishment of physical barriers on the root surface that prevent fungal colonization. These barriers are created as *Trichoderma* aggressively colonizes the rhizosphere, forming a protective shield that inhibits the entry and spread of pathogens like *Fusarium*. Moreover, *Trichoderma* species produce a range of secondary metabolites, including hydrolytic enzymes, antibiotics, and antifungal compounds, which act directly against *Fusarium* toxins, thereby reducing the pathogen's virulence. In addition to these mechanisms, *Trichoderma* has demonstrated the capacity to colonize both soil and plant surfaces, offering further protection against infections such as *Foc* TR4, the causative agent of Panama disease in bananas. Numerous studies have highlighted the antagonistic effects of *Trichoderma* species against pathogens like Fusarium wilt in bananas. Research has shown that inoculation with specific *Trichoderma* species, or combinations of different species, can create a protective zone around the application site. This zone, located at a distance from the site of inoculation, has been observed to slow the progression of *Foc* TR4 compared to control treatments without *Trichoderma*. Such findings provide strong evidence of the antagonistic effects offered by various *Trichoderma* spp. against pathogenic microorganisms, including *Foc* TR4, reinforcing the potential of *Trichoderma* as a critical component in the integrated management of banana diseases (Harman et al., 2004; Verma et al., 2007; Hermosa et al., 2012).

2.11.3.2. *Bacillus* spp.

Bacillus subtilis, a Gram-positive, rod-shaped bacterium, is widely recognized for its role as a biological control agent in agriculture. This bacterium is facultatively aerobic, forming durable endospores that allow it to withstand extreme environmental conditions, including heat, desiccation, and radiation (Harwood & Archibald, 1990). *Bacillus subtilis* is motile due to peritrichous flagella, which enables it to navigate through soil and colonize plant roots efficiently (Kearns, 2010). Taxonomically, *Bacillus subtilis* belongs to the phylum Firmicutes, class Bacilli, order Bacillales, and family Bacillaceae. The species was first described by Ferdinand Cohn in 1872, and it has since become one of the best-studied bacterial species due to its importance in biotechnology and agriculture (Earl et al., 2008). The strain 168 of *B. subtilis* has been extensively used in research due to its well-characterized genome (Barbe et al., 2009).

Morphologically, *B. subtilis* cells are typically 4–10 µm in length and 0.25–1.0 µm in diameter. They can form biofilms on plant roots, enhancing their persistence and efficacy as a biocontrol agent (Vlamakis et al., 2013). The bacterium is known for its ability to secrete a wide array of extracellular enzymes, including proteases, lipases, and cellulases, which contribute to its competitive edge in the rhizosphere (Schallmey et al., 2004). *B. subtilis* is renowned for its ability to produce a variety of secondary metabolites, including antibiotics like bacitracin, and antifungal compounds, which play crucial roles in its biocontrol activity (Stein, 2005). Its genetic tractability and robust stress response mechanisms make it an ideal candidate for use in biotechnological applications and as a model organism for studying bacterial physiology and genetics (Kunst et al., 1997).

A recent greenhouse experiment conducted in China by Fan et al. (2021) evaluated two strains of *Bacillus*: *Bacillus amyloliquefaciens* and *B. subtilis* both originating from banana fields to assess their effect on banana *Foc* TR4. The study found that *B. amyloliquefaciens* showed a control effect of 82.6% while *B. subtilis* exhibited a higher control effect of 85.6%. Another experiment conducted under field and greenhouse conditions showed that incorporating *Bacillus* strain W19 enriched with organic fertilizer enhanced banana root colonization and plant growth while effectively suppressing Fusarium wilt according to Wang et al., (2016).

In a study conducted by Tian et al. (2021), they employed DNA sequencing methods to acquire the sequence of a *B. amyloliquefaciens* strain found in banana root (GKT04). Through an analysis of transcriptomic data, they discovered that *Bacillus amyloliquefaciens* exhibited increased production of difficidin, bacillibactin, and bacilysin while inhibiting *Foc* TR4. It has been reported that *Bacillus licheniformis* exhibits an antagonistic impact against *Foc* TR4 with a substantial inhibition rate of 77.9% in a dual plate screening procedure (Yadav et al., 2021).

2.11.3.3. *Pseudomonas* spp.

Pseudomonas spp. are a diverse group of Gram-negative, rod-shaped bacteria known for their metabolic versatility and ability to thrive in various environmental conditions (Palleroni, 2010). It includes a diverse group of over one hundred species (Mulet et al., 2010; Loper et al., 2012; Hesse et al., 2018). These bacteria are aerobic, although some species can grow anaerobically using nitrate as an electron acceptor (Iglewski, 1996). *Pseudomonas* spp. are motile due to the presence of polar flagella, which allows them to move toward favorable environments through chemotaxis (Kearns, 2010). Many strains from this group naturally occur in the plant rhizosphere and/or phyllosphere, where they often act as symbiotic organisms. These bacteria are characterized by their ability to effectively colonize plant surfaces, internal tissues, and/or pathogenic structures. They can also produce compounds that suppress phytopathogens and adapt to utilize specific nutrients found in their habitats. One of the defining features of *Pseudomonas* spp. is their ability to produce a wide range of secondary metabolites, including antibiotics, siderophores, and enzymes. These compounds play crucial roles in their ecological interactions, particularly in their capacity to suppress plant pathogens and promote plant growth (Raaijmakers et al., 2002). *Pseudomonas fluorescens* and *Pseudomonas putida* are two well-studied species that are frequently used as biological control agents due to their effectiveness in protecting plants against diseases (Haas & Défago, 2005).

In 1988, researchers Sivamani and Gnanamanickam conducted a study to explore the potential of using strains of *Pseudomonas fluorescens*, which were isolated from sources of rice root (Pfrl3), peanut (Pfgn), banana (Pfb), black gram leaves (Pfbg), citrus (Pfcp), and cotton (Pfco) to suppress Fusarium wilt disease. The results revealed that a strain isolated from citrus plants (Pfcp) exhibited the greatest inhibitory activity towards *Foc* TR4 mycelial growth. As a result, this strain was selected for applying it to wild banana seedlings belonging to the *Musa balbisiana* species. The seedlings treated with Pfcp demonstrated reduced symptoms of wilting and internal discoloration, improved root development. and an overall increase in plant height under controlled greenhouse conditions.

Furthermore, a research study conducted by Bubici and his colleagues, in 2019 discovered that *Pseudomonas* spp. can reduce Fusarium wilt in bananas by 77%. This conclusion was drawn after analyzing five studies. In another investigation by Lv et al. (2023), the effectiveness of P8 was successfully demonstrated, along with either S25 or S36 strains of *Pseudomonas*, in suppressing the spread of *Foc* TR4 within soil. This positive outcome was observed both in laboratory settings and controlled greenhouse environments. It is worth noting that these strains were extracted from the rhizosphere and roots of banana plantlets. Furthermore, this combination had an impact on the composition of soil microbiota while also enhancing microorganisms' activity.

2.11.3.4. *Pythium* spp.

Pythium spp. are a group of oomycetes, often referred to as water molds, which are distinguished from true fungi by their unique cell wall composition and reproductive structures. Unlike fungi, the cell walls of *Pythium* spp. contain cellulose and β-glucans rather than chitin (Judelson & Blanco, 2005). These organisms are primarily found in aquatic environments and soil, where they play a significant role in the decomposition of organic matter and nutrient cycling (Van West, 2006). *Pythium* spp. are well-known plant pathogens, causing diseases such as damping-off, root rot, and seed rot, which can lead to significant losses in agricultural production (Martin & Loper, 1999). However, certain strains of *Pythium* have been explored for their potential as biological control agents against other pathogenic fungi and oomycetes. This antagonistic activity is primarily attributed to their ability to compete for nutrients, produce antimicrobial compounds, and induce systemic resistance in plants (Benhamou et al., 1997).

Currently there isn't literature or research exploring the use of *Pythium* spp. to control *Foc* TR4 on banana plants. This lack of knowledge can be attributed to the limited understanding of how *Pythium* spp. and *Foc* TR4 interact with each other as their effects on banana plants. Recent studies have shown results when using strains of *Pythium* spp. as biocontrol agents against plant pathogenic fungi. Although more research is needed to grasp how *Pythium* spp. can be used as a biocontrol agent for Fusarium wilt of banana, initial findings are promising enough to warrant investigation.

CHAPTER 3

MATERIALS AND METHODS

3.1. Survey on the spread of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4) in South Lebanon.

3.1.1. Description of the study area

South Lebanon is the most suitable and productive region for banana farming in Lebanon. Banana cultivation is primarily concentrated in the coastal plains of South Lebanon, where the climate is particularly favorable for this crop. The region receives around 900 mm of rainfall annually, and its mild winter climate allows for the successful growth of tropical fruits like bananas, as well as vegetables and greenhouse crops. In 2019, approximately 4,092 hectares in Lebanon were dedicated to banana cultivation, resulting in a yield of about 156,506 tons. Salim Mrad explains that the banana industry in South Lebanon has also seen steady growth, with an average annual increase of 2.5%, reflecting the crop's increasing economic significance in the region (Mrad S., personal communication, 2022), according to the Citrus & Banana Union in South Lebanon.

3.1.2. Sampling

The survey to determine the status of the diffusion of banana Panama wilt disease caused by *Foc* TR4 in South Lebanon was carried out during the period of January-April 2023. Field surveys and sample collection activities were conducted both in large and small banana plantations where symptoms of Fusarium wilt were reported or observed. The survey focused on Tyre district and a part of Sidon district encompassing 87 fields within 22 villages known for their banana cultivation (Fig. 6) During this survey, one to three symptomatic banana plants were sampled per field. The sampling consisted of the collection of bananas pseudostem tissues from banana plants that showed characteristic *Foc* TR4 symptoms of leaf yellowing as described by Viljoen et al. (2017). The symptoms that were used to identify the samples included yellow discoloration that turns brown to red in severe cases, wilting starting with aged leaves at the base, longitudinal splitting at the base, and collapsing of leaves forming a skirt round the pseudostem (Fig. 7).

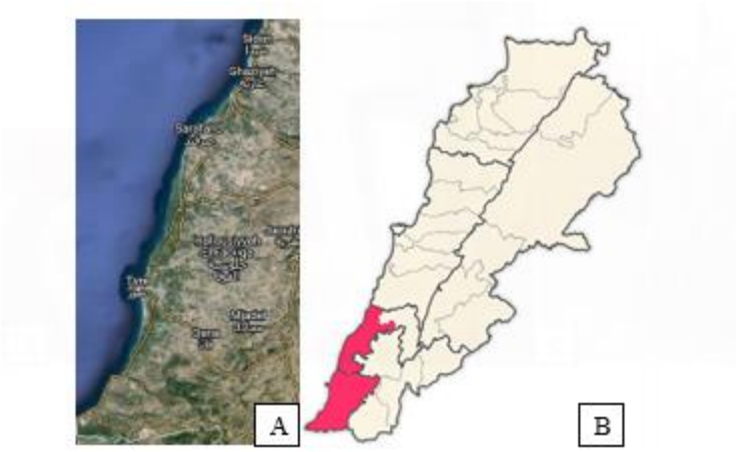

Figure 6. Satellite map of Tyre and Sidon districts (A). Lebanon map highlighting Tyre and Sidon districts (B).

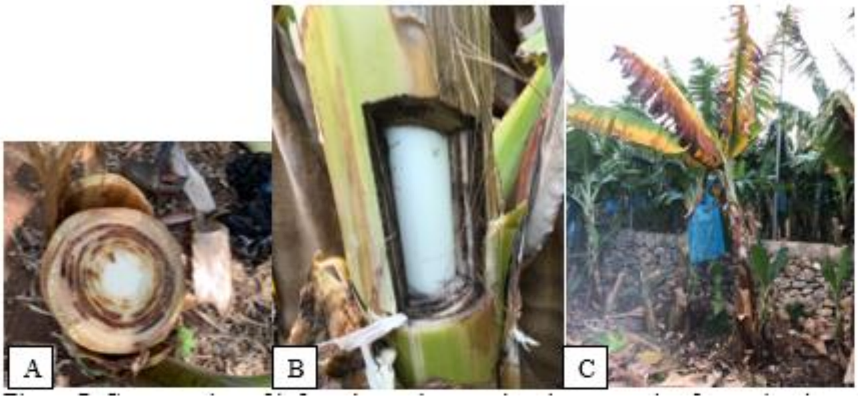

Figure 7. Cross section of infected pseudostem showing necrosis of vascular tissue (A); Rectangular cut in the pseudostem (B); Typical external symptoms *Foc* TR4 in an infected Cavendish banana plant (C).

Field sample collection data were mapped into GOOGLE MYMAPS platform and saved as a KMZ file and collated into spread sheets. Farmers were also asked about the presence of Fusarium wilt in their banana fields, the constraints this disease and other biotic factors have caused to their banana production and which disease mitigation and control measures were applied. Table 2 summarizes the locations visited during the survey, detailing the number of banana sites inspected and number of samples collected at each. Multiple sites were visited within each location to ensure comprehensive coverage.

Table 2. List of visited locations, number of visited fields, number of taken samples, and number of symptomatic samples. Symptomatic plants showed external and/or internal symptoms.

Country	**Location**	**# of fields**	**# of samples**	**# of fields tested positive**	**# of symptomatic samples**
Lebanon	Naqoura	3	10	0	0
	Iskandarouna	3	9	2	4
	Mansouri	7	22	4	7
	El Qlaileh	4	12	2	6
	Maaliyeh	3	12	2	5
	Taybeh	4	13	1	1
	Rachideyeh	1	2	0	0
	Bourj El Shemaleh	7	18	3	5
	Tyre	1	4	0	0
	Abbasieh	7	21	2	5
	Borghliyeh	6	21	2	4
	Qasmieyeh	4	11	0	0
	Mazraat El Yahodieh	7	20	4	9
	Borj Rahal	4	13	1	2
	Matareyet El Shouamr	3	9	1	1
	Kfar Bedda	1	3	0	0
	Adloun	6	18	0	0
	Ansarieh	2	5	0	0
	Sarafand	7	16	2	4
	Aaqbiyeh	2	6	0	0
	Addousieh	3	10	0	0

	Ain baal	1	3	0	0
	Bazourieh	1	2	0	0
TOTAL		87	260	26	

3.1.3. Detection of Foc TR4 in collected samples

3.1.3.1. Media preparation:

Several culture media were used in the various steps of the diagnosis. Potato dextrose agar (PDA) medium was prepared according to the manufacturer instructions (Fig. 8). Water agar (WA) medium was prepared at a concentration of 2.5% agar. All media were autoclaved for 15 min at 121˚C before pouring them in Petri dishes. To reduce the bacterial contamination during isolating the fungus, PDA was acidified to pH 5.5 by adding lactic acid, at a final concentration of 500 mg/ L after autoclaving, when the temperature of the medium reached 55˚C.

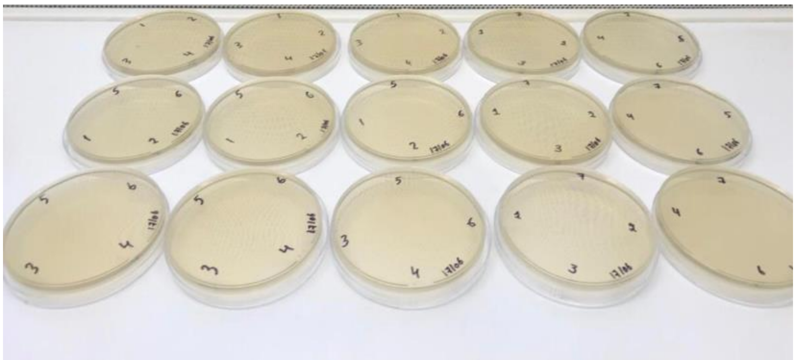

Figure 8. Medium poured into 90 mm Petri dishes.

3.1.3.2. Fungal isolation:

The isolation of *Foc* TR4 from clear and discolored pseudostem samples was conducted at the Plant Protection Laboratory in Beirut, at the Department of Agriculture in the American University of Beirut. In the laboratory, the isolation from plant tissues started by the surface decontamination of pieces of internal plant tissues by dipping them in Clorox 1% (1.5 min) followed by ethanol 70% (30sec), then washing them in sterile distilled water for few seconds and letting them dry on a sterile filter paper, using a sterile forceps and under the laminar flow hood. For each sample, 5 pieces (5 x 5 mm) were placed on each plate containing PDA acidified with lactic acid. The culture plates were sealed with parafilm and incubated at 24˚C for 5-7 days in the dark (Fig. 18). When fungal growth was observed after incubation, a mycelium plug was transferred into new PDA medium plates and incubated at 24 ˚C for 5-7 days at dark to obtain a pure colony of the fungus (Fig. 9).

The identification of Fusarium spp. was based on the morphological characteristics of the colony and the shape, and size of the conidia and their arrangement on the conidiophores, through preparation of slides in lactophenol blue and observations on a binocular microscope. However, since morphological characteristics alone are not sufficient to accurately identify Foc TR4, molecular tests were performed to confirm its presence as elaborated below.

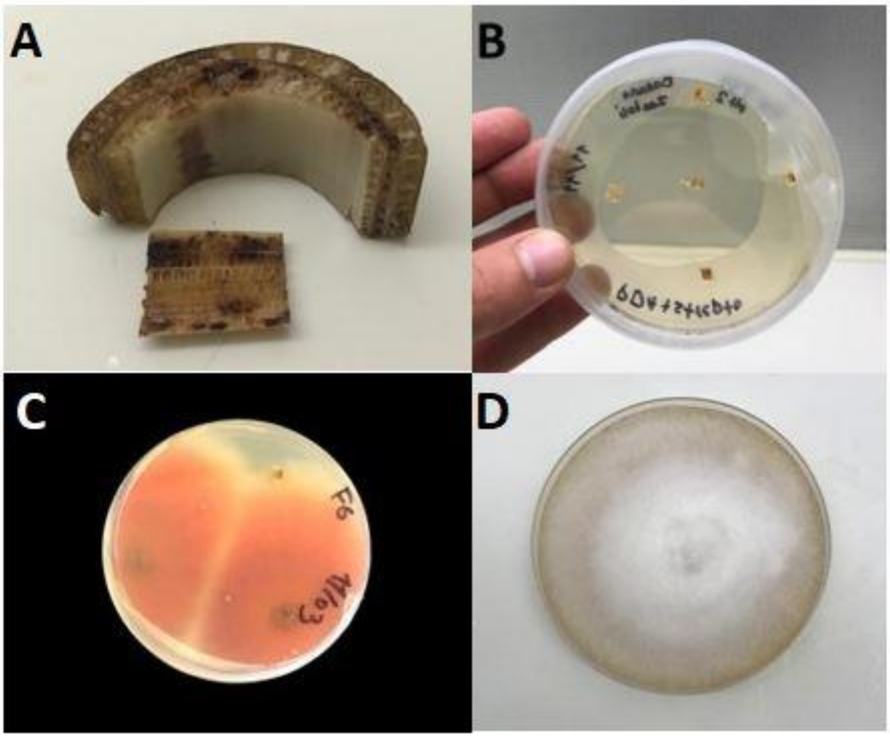

Figure 9. (A) Parts of the pseudostem sample taken for isolation; (B) Pieces placed on the PDA + Lactic Acid plate; (C) Fungal colonies observed after incubation; (D) Pure *Foc* TR4 colony grown on PDA medium

3.1.3.3. Single spore isolates

Single spore isolates were prepared for the molecular characterization. This was carried out by collecting conidia in sterile distilled water and spreading at low density on Water Agar (WA) plates (Fig. 10). After 24 hours of incubation at 24±1 ºC in the dark, 2-3 germinated conidia per isolate were singularly collected, using a binocular stereoscope, and aseptically transferred to PDA plates and incubated at 24 ºC for 3-4 days before storage in PDA tubes at 4 ºC.

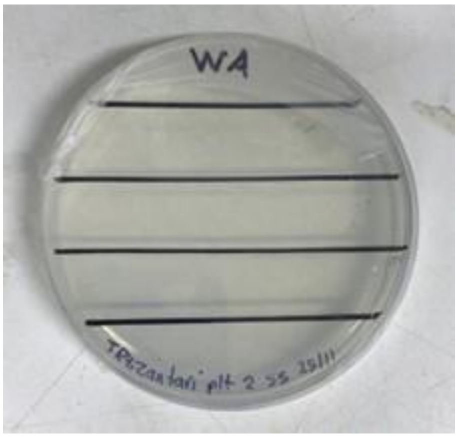

Figure 10. Preparation of a single spore isolate from a WA plates.

3.1.3.4. Genomic DNA extraction:

The DNA extraction and the purification were done according to (Murray & Thompson, 1980). First, 100 mg of fungal mycelium of each isolate were harvested by carefully scraping it off the PDA plates with sterile scalpel blades, and depositing it into separate, labeled, sterile 1.5 mL micro-centrifuge tubes. The micro-centrifuge tubes were then immersed in liquid nitrogen and grinded well to get a light green fine powder. 800 µl of C-TAB buffer and mercaptoethanol (20 µl for 1 ml CTAB) were prepared. The entire mixture is held at 60 ºC for 20 min and then added to the crushed tissues with liquid nitrogen. During incubation, the mixture was briefly vortexed several times. After incubation, 600 µl of isoamyl alcohol: chloroform (1:24) were added and the mixtures was vortexed vigorously and centrifuged at 10,000 rpm for 5 min. The supernatant (upper phase) was then transferred to a clean microcentrifuge tube, and an equal volume of ice cold iso-propanol was added; the mixture was then placed at -80 ºC for 20 min. The mixture was then centrifuged at speed 14,000 rpm for 8 min and the aqueous phase (upper layer) was discarded. The pellet was rinsed with 75 % ethanol, centrifuged at 14,000 rpm for 5 min, air dried and re-suspended in 100 µl of DEPC water (Fig. 11).

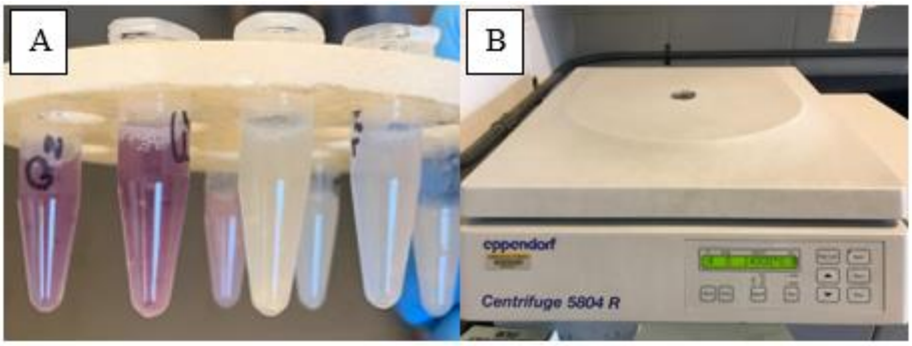

Figure 11. Mixture prepared for DNA extraction in Eppendorf tubes (A); Centrifuge machine (B).

3.1.3.5. Amplification with *Foc* TR4 specific primers

The specific primers FocTR4-F/FocTR4-R (Dita et al., 2010) were used in PCR reactions to detect the race of the pathogen. PCR reactions were carried out using Taq PCR Master Mix (Qiagen, USA). The specific mixtures of this reaction contained a concentration of 1X ready Master Mix, 1.5 mM of MgCl2, 0.2 µM forward primer: FocTR4-F (5'- CACGTTTAAGGTGCCATGAGAG-3'), 0.2 µM reverse primer: FocTR4-R (5'- CGCACGCCAGGACTGCCTCGTGA-3') and 25 ng of DNA. Each reaction volume was made up to 25 µl with sterile nuclease free water.

Amplifications were carried out in a BIORAD C1000 thermal cycler (Fig. 12) using the following conditions: an initial denaturation of 5 minutes at 95 ºC, followed by 30 cycles of 1 minute at 95 ºC (denaturation), 1 minute at 60 ºC (annealing), 3 minutes at 72 ºC (extension) and a final extension step of 10 minutes at 72 ºC.

Figure 12. C1000 Thermal cycler for DNA amplification (BIORAD, USA).

3.1.3.6. Electrophoresis

Following the amplification, 10µl of each PCR product were loaded on 1.5% agarose gel (1.5 g of agarose in 100ml TAE (Tris-Acetate-EDTA) buffer 1X) after mixing them with DNA Electrophoresis Sample Loading Dye, one well was loaded with a standard ladder (Promega, BenchTop 1kb DNA Ladder). Then electrophoresis was run in TAE 1X (Tris-Acetate-EDTA) buffer at 110 volts for 45 minutes. Finally, the gel was placed on a transilluminator, and the bands were observed under the UV lights.

3.1.3.7. DNA Sequencing

Following agarose gel electrophoresis, the PCR amplified products (amplicons) were purified using the QIA quick Gel Extraction Kit (Qiagen, Germany) according to manufacturer’s recommendations. Sequencing of the amplicons was conducted in both directions (forward and reverse). Samples were sequenced at the “Sequencing unit of molecular and cellular biology lab” at the American University of Beirut using the Sanger dideoxy sequencing reactions. The sequences were subjected to BLASTn analysis (http://www.ncbi.nlm.nih.gov/BLAST/I)

3.2. Evaluation of the in-vitro antagonistic potential of different *Trichoderma* strains *Foc* TR4.

3.2.1. Fungal Isolates

The experiment used a *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 isolate from Lebanon, along with six *Trichoderma viride* strains (T3, T16, T24, T26, T42, and T4) kindly provided by Dr. Thaer Yassine (FAO), and *Trichoderma harzianum*.

3.2.2. Dual Culture Bioassay

In-vitro experiments were designed to evaluate the antagonistic activity of seven *Trichoderma* species against *Foc* TR4. The experiments were conducted in bicultures, on an agar medium (potato dextrose agar, PDA) following the procedure described by Błaszczyk et al. (2017). A 5-mm-diameter PDA plug from the growing mycelium edges of 7-day old *Foc* TR4 culture, isolated from an infested field in South Lebanon, was removed using a cork borer and placed at 2 cm from the edge of 9 -cm diameter plates containing freshly prepared PDA medium (Fig. 13). After 72 hours, 5-mm mycelial plugs of each of the seven *Trichoderma* strains were removed from the growing edges of a four-day old culture using a cork borer (5mm). Each plug was placed 2 cm from the opposite edge of the plates having *Foc* TR4 (Fig. 13). This was performed to compensate for the faster growth of *Trichoderma* species compared to *Foc* TR4.

Control plates containing fungal plugs only were also prepared. All plates were sealed with Parafilm and incubated in the dark at 25 ºC for seven days.

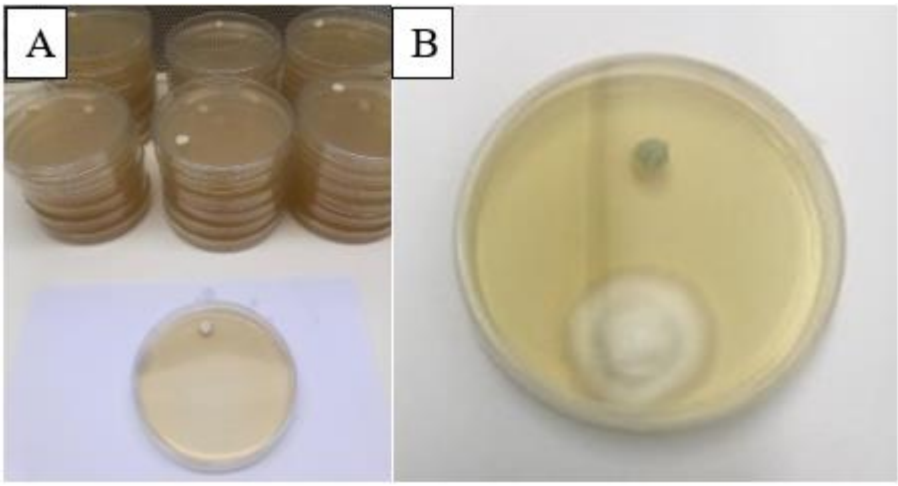

Figure 13. Culture of *Fusarium oxysporum* f. sp. *cubense* TR4 (A); Dual culture plates of a newly added *Trichoderma harzianum* plug (Top) and 72 hours old *Foc* TR4 culture (Bottom) (B).

3.2.3. Measurement of radial mycelial growth

Radial fungal colony growth of both species in the direction of the opposite colony was measured. The inhibitory effect of *Trichoderma* isolates on *Foc* TR4 growth was assessed by estimating the percentage (%) reduction in pathogen growth in the presence of the antagonist, according to the following formula:

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

where **L** stands for the percentage inhibition of radial mycelial growth, **C** for the radial growth of the pathogen in the control, and **T** is radial growth of the pathogen in the presence of a *Trichoderma* strain (Edington et al. 1971). Readings of the *Fusarium* mycelial growth was recorded after 10 days from inoculation. Macroscopic morphological observations indicating an interaction between species were recorded: Formation of a hyphal barrier (thickened mycelial growth between cultures), development of an inhibition zone, and overlapping growth.

3.2.4. Experimental Design

The experiment was done in an incubator at the plant pathology lab in the Department of agriculture, at AUB. The incubator temperature was maintained at an average temperature of 20 ± 4°C. All treatments were replicated three times (3 plates); each containing both *Foc* TR4 culture and one of the *Trichoderma* isolates.

3.2.5. Statistical Analyses

A one-way ANOVA was performed to analyze the antagonistic activity of *Trichoderma* against *Fusarium*. Post hoc testing with Tukey's HSD was performed to identify statistically significant differences between groups. All reported p-values were based on two-sided tests at a 95% confidence level.

3.3. Field trial for evaluation of the reaction of four different banana varieties to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 and the antagonistic activity of four biocontrol products.

3.3.1. Field location

A large-scale trial was conducted in a 3750 m^2 field located in Borj Rahal, South Lebanon (Fig 14) at an attitude of 20 m above sea level during October 2022-March 2024. This region lies at the heart of South Lebanon's banana plantations. The soil test analysis revealed that the soil is loamy clay, characterized by good organic matter content (4.4 %) and a pH value of 8.2.

Figure 14.Satellite image showing boundaries of the field in Borj Rahal, South Lebanon (33.315678366861256, 35.265299270346624) where banana plants were transplanted for the field experiment.

3.3.2. Pre-transplanting

Previously grown banana plants were uprooted, and the field was ploughed using mounted plough and smoothened using mounted pulveriser twice to ensure proper soil structure. A total of 650 holes were drilled using a tractor mounted post hole digger. Five kilograms of well fermented compost (Doubaline ®; C/N 16, 50% OM, 20% moisture, NPK>4%, Highly composted) were manually distributed into each hole. A drip irrigation system was used for irrigation. The diameter of the irrigation laterals was 16mm, 1mm wall thickness, 4 L/hour drippers with 40 cm spacing between drippers. Two laterals for each row were installed. The submain line was 50 mm diameter installed through the middle of the field. Banana plants were transplanted in a single row arrangement with a spacing of three meters between rows and two meters within rows (Fig. 15). The cultural practices were carried out in accordance with the standard commercial practices used in South Lebanon.

Figure 15. Preparation of the land in the field experiment area for transplanting banana plants. The image shows the ploughed field, irrigation driplines, holes, and banana seedlings ready for transplanting.

3.3.3. Treatments and Experimental Design

Biological control products were applied in the afternoon of the day before planting on 08/10/2022. Four sanitized barrels each of 200 liters volume were filled with well irrigation water. Each product was diluted with the needed amount of water according to the application rate (Table 3). Two liters of the diluted products were manually applied to each banana planting hole (Fig. 16). Control treatments were irrigated same amount of water. The following day (on 09/10/2022), banana plants were transplanted into the field according to a predetermined experimental design. The varieties selected in this trial included two reference varieties, GCTCV 218, reported to be *Foc* TR4 resistant/tolerant (Hwang & Ko, 2004), and Grand Nain, normally grown in greenhouses or under nets, reported for potential tolerance to *Foc* TR4. The two other varieties are Baladi (local) and a Canarian variety, which are believed to be susceptible to *Foc* TR4 and planted on a large scale in South Lebanon. The seedlings of the four banana varieties were provided by a nursery with ISO 9001 accreditation for production of tissue culture banana seedlings. Once received the seedlings were potted into 3 L pots with sterilized potting media and placed in a greenhouse for four weeks. Plants were then moved into shade net house to harden off for four weeks before transplanting into the field.

Figure 16. Application of biological treatments one day before transplanting.

The field trial followed a randomized complete block design based on the INIBAP Technical guidelines. All treatments were distributed across four independent replicate plots. The four plots were separated and surrounded by border plants to prevent cross-contamination and minimize the spread of disease or treatment effects between plots (Fig. 17). These border plants functioned as a physical barrier, ensuring that biocontrol agents applied within a specific plot remained confined to that area. Additionally, the use of border plants helped to reduce the influence of environmental factors, such as wind or water runoff, which could otherwise carry pathogens or treatments from one plot to another. This setup allowed for more accurate comparisons between treatments and ensured the integrity of the experimental design. Each replicate included eight plants. The outer plants, 1 and 8, functioned as added border plants, while the internal plants (2–7) were sampled for data collection. Plants were then irrigated for 4 hours through the irrigation system.

The experiment involved a total of 650 banana plants, of which 384 were included in the trial and the others served as border plants. The trial was divided into two main overlapping assessments:

1. Evaluation of Antagonistic Activity:
   - Treatments: Five different treatments were applied: The Control, *Trichoderma harzianum*, *Pythium oligandrum* (Polyversum®*)*, *Bacillus subtilis* & *Pseudomonas putida* (Fulzyme®) and *Bacillus amyloliquefaciens FD777* (Novo Treat®).
   - Varieties: Two banana varieties were used: Baladi & Canarian varieties.
   - Replicates: Each treatment was replicated four times (four independent blocks).
   - Plants per Treatment: Eight plants were used per treatment per replicate.
   - Total Plants: Five treatments × Two varieties × Four replicates × Eight plants/treatment=320 plants

2. Evaluation of Resistant Varieties:
   - Varieties: Two additional varieties were assessed: Grand Nain, suspected to be somewhat tolerant & GCTCV 218 specially imported for its reported tolerance.
   - Treatments: One treatment: the Control.

- Replicates: Each treatment was replicated four times.
- Plants per Treatment: Eight plants were used per treatment per replicate.
- Total Plants: Two varieties × One treatments × Four replicates × Eight plants/treatment=64

Table 3: Summary table of the application rates of biological control agents and fertilizers, with their corresponding application dates.

Soil Managment		**Application**	**Timing**
General	Compost	5 kg per plant in each planting hole	At planting: 09/10/2022
	Startup fertilization	100 N, 18 K2O, 27 MgO, 53 CaO, 20 S, 5.8 Zn and 0.8 B (in kg ha−1 year−1)	During the first 7 weeks, divided over weekly applications.
	Basic fertizlation	105 P2O5, 610 K2O, 4.0 CaO, 60 S, 9.2 Zn and 3.2 B (in kg ha−1 year−1)	After week 8, divided over 17 3-weekly applications per year
Treatments	*B. subtilis + P. putida* (Fulzyme®)	12g per plant 2 x 10^{10} CFU/g	**5 applications of each during the trial:** 1 day before planting 6 weeks after planting 16 weeks after planting 20 weeks after planting 26 weeks after planting
	Trichoderma harzianum	150g per plant 1 x 10^{7} spore/g	
	Bacillus amyloliquefaciens FD777 (Novo Treat®)	12.5ml per plant 7.0 x 10^{9} CFU/ml	
	Pythium oligandrum (Polyversum®)	0.5g per plant 1 x 10^{6} oospores /g	
	Control	-	-

Figure 17. Experimental design of the field experiment in Borj Rahal, South Lebanon. The field experiment treatments were distributed in a Randomized Complete Block Design (RCBD).

3.3.4. Post transplanting

Weed removal was done manually by hand every two to three weeks; no herbicides were used. No pesticides or fungicides were sprayed or chemigated. Fertilization program was followed according to the soil test analysis done and was similar to the fertigation practices adopted by farmers based on the recommendations of consultants (Table 3). The normal banana cultural practices and irrigation schedules were followed (Fig. 18).

Figure 18. Image of field experiment showing banana plants transplanted and irrigation system installed.

3.3.5. Assesment criteria

3.3.5.1. External symptoms

Banana plants were to be assessed based on *Foc* TR4 external symptoms using the Leaf Disease Severity Index reported by Ssali et al. (2013):

$$= \frac{score\ in\ the\ scale * frequency}{total\ number\ of\ plants * maximum\ class\ in\ the\ sclae} * 100$$

Usually, external symptoms are noted as leaf yellowing (chlorosis) and wilting on the oldest leaves.

3.3.5.2. Internal symptoms

Internal symptoms were assessed at harvest, the lower portion of the pseudostem was cut and examined for internal symptoms of *Foc* TR4, seen as vascular browning. If no symptoms were noted, additional cuts were made at lower levels. All plants in the field were cut and samples were collected.

Pseudostem tissue samples were isolated then decontaminated by dipping them in Clorox 1% (1.5 min) followed by ethanol 70% (30sec), then rinsed in sterile distilled water and then dried on dry sterile filter paper. The surface sterilized sample tissues were cut into small pieces (5mm x 5mm) and placed onto plates containing Potato

Dextrose Agar (PDA) media acidified with lactic acid. The culture plates were sealed with parafilm, incubated at 25 ◦C for 7 days and inspected for mycelial growth.

3.3.5.2.1. Molecular identification

DNA extraction from fungal mycelium, amplification with *Foc* TR4 specific primers, and gel electrophoresis were done as described in sections 3.1.3.2 - 3.1.3.6

3.3.5.3. Bunch Weight Measurement

To determine the effects of biocontrol agents on growth performance and yield of banana plants, the average bunch weight was calculated. Comparisons were made between the four *Musa* spp. varieties and between the different biocontrol treatments, including *Trichoderma harzianum, Pythium oligandrum (*Polyversum®*), Bacillus subtilis & Pseudomonas putida (*Fulzyme®) *and Bacillus amyloliquefaciens FD777 (*Novo Treat®) within Baladi and Canarian varieties, and their respective controls (Fig.29). The bunch stalk was cut above the first hand and immediately below the last hand. Three plants per treatment per block were inspected; a total of 12 bunch weights were recorded for each treatment. In total, 120 plants were inspected for bunch weight measurement.

3.3.6. Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 27 for Windows. Descriptive statistics, including means and standard deviations, were reported for the variables of interest. For data obtained from the banana field trial, a two-way ANOVA was conducted to evaluate the effects of treatment and variety on the bunch weight of bananas. Additionally, a one-way ANOVA was performed to compare the bunch weight across different banana varieties without considering the treatments in the aim of determining which variety produced the largest bunch weight.

3.4. Pot experiment for evaluation of the reaction of four different banana varieties to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 and the antagonistic activity of four biocontrol products.

3.4.1. Site location

The pot experiment was conducted within a 320 meters square greenhouse at a farm found in Abbasieh, South Lebanon (33.283719988013274, 35.27655532054951) (Fig. 19).

Figure 19. Satellite image showing location of the greenhouses for the pot experiment in Abbasieh, South Lebanon (33.28378781905533, 35.27646495400656).

3.4.2. Millet Seed Inoculum Preparation

Millet seeds were prepared by first filling autoclave breathable bags with 250 g of millet seeds. 250 mL of distilled water were added, and the seeds were allowed to soak overnight. The following morning, all excess water was drained off. The millet seeds were then sterilized by autoclaving at 121°C for 20 minutes. After sterilization, the bags were shaken to loosen the grains, and a small sample was collected and plated onto culture media to ensure sterility. For inoculation, a local *Foc* TR4 isolate was cultured on potato dextrose agar (PDA) and incubated at 25°C for 5–7 days. From the growing culture, 10 mycelial plugs, each 0.5 cm in diameter, were cut from the margins and transferred to the flasks or bottles containing the sterilized millet seeds. As soon as white fungal growth appeared on the surface of the seeds, the bags were shaken to distribute the fungus evenly and prevent clumping (Fig. 20). The fungus was further incubated to colonize the millet seeds for 14 days, with shaking every second day to ensure thorough colonization. To confirm that the millet seeds were colonized exclusively by *Foc* TR4, some seeds were plated onto PDA. The colonized millet seeds were stored in lab refrigerator until used (Jankowicz-Cieslak et al., 2022).

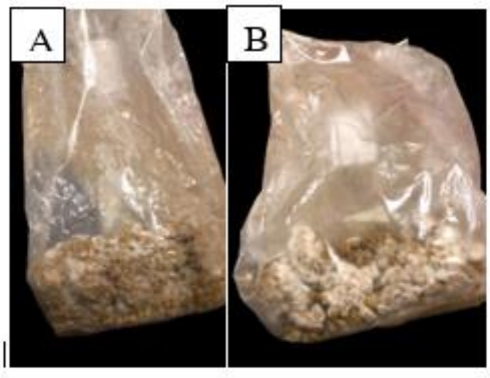

Figure 20. Preparation of *Fusarium oxysporum* f. sp. *cubense* TR4 inoculum on autoclaved millet seed (A); White fungal growth on the surface of the millet seeds two weeks post-inoculation (B).

3.4.3. Pre-transplanting

Tissue culture banana seedlings were grown in a greenhouse in polyethylene bags and adequate cultural growing conditions followed until they reached the transplanting stage. Pots were filled with a potting mixture composed of 70% soil, 20% peat substrate, 10% compost, with a pH of 7. All pots, except those of the negative control treatments were inoculated with 50 g of *Foc* TR4 colonized millet seeds three days before transplanting the banana seedlings (Smith et al. 2018). All pots were watered to saturation and placed in a greenhouse that received sunlight each day with fluctuating day temperatures of 30–35 ºC and night temperatures of 20–24 ºC.

A surface drip irrigation system was installed to deliver the needed amount of water. The diameter of the dripper irrigation laterals used was 16mm, 1mm wall thickness and equipped with 8 L/hour flow rate emitters. The submain line was 40mm diameter installed in the middle of the greenhouse.

3.4.4. Experimental Design and Treatments

Banana seedlings were transplanted into 20L plastic pots and clearly marked and mapped (Fig. 21).

Figure 21. Greenhouse pot trial for evaluation of the reaction of four different banana varieties to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 and evaluation of the antagonistic activity of four biocontrol products

The study was conducted to evaluate the antagonistic activity of four biological control products and to evaluate the reaction of two resistant banana varieties. The experiment used a completely randomized block design (Fig. 22). The experiment involved a total of 240 potted banana plants, divided into two main overlapping assessments:

- Evaluation of Antagonistic Activity:
    - Treatments: Six different treatments were applied: Positive control (artificially inoculated with *Foc* TR4), Negative control, *Trichoderma harzianum*, *Pythium oligandrum* (Polyversum®*)*, *Bacillus subtilis* & *Pseudomonas putida* (Fulzyme®) and *Bacillus amyloliquefaciens FD777* (Novo Treat®).
    - Varieties: Two banana varieties were used: Baladi & Canarian varieties.
    - Replicates: Each treatment was replicated three times.
    - Plants per Treatment: Five plants were used per treatment per replicate.
    - Total Plants:

      6 treatments×2 varieties×3 replicates×5 plants/treatment=180 plants

- Evaluation of Resistant Varieties:
    - Varieties: Two additional banana varieties were evaluated for their tolerance to *Foc* TR4: Grand Nain & GCTCV 218.
    - Treatments: Two different treatments were applied: Artificially inoculated with *Foc* TR4 (positive control) or not inoculated (negative control)
    - Replicates: Each treatment was replicated three times.
    - Plants per Treatment: Five plants were used per treatment per replicate.
    - Total Plants: 2 varieties × 2 treatments × 3 replicates × 5 plants/treatment = 60.

Table 4 summarizes the treatments and varieties combination. Biological control treatments were applied four times during the growing period.

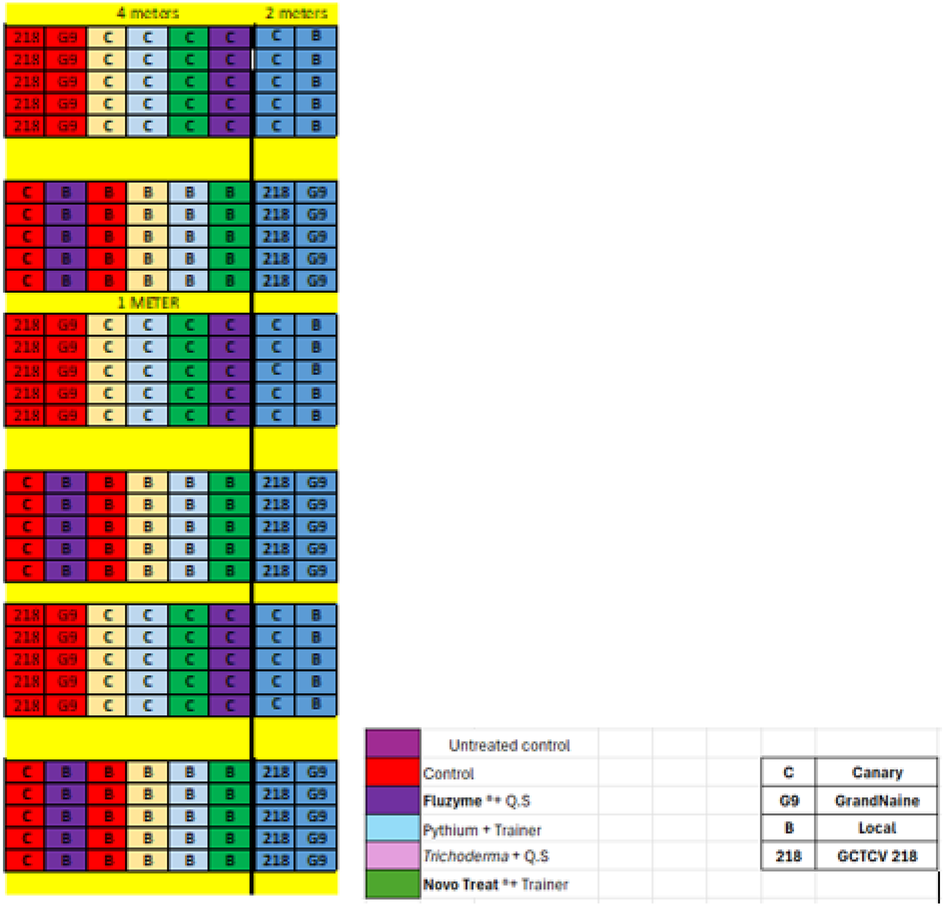

Figure 22. Experimental design of the pot experiment in Abbasieh, South Lebanon. The pot experiment treatments were distributed in a Randomized Complete Block Design (RCBD).

Table 4. Summary table of the treatments used within the pot experiment.

Number	**Treatment**	
	Variety	**Biocontrol agent**
1	Grand Nain	Positive control
2	Grand Nain	Negative control
3	CCTCV 218	Positive control
4	CCTCV 218	Negative control

5	Baladi	Positive control
6	Baladi	Negative control
7	Baladi	*Trichoderma harzianum*
8	Baladi	Fulzyme®
9	Baladi	*Pythium oligandrum* Polyversum®
10	Baladi	Novo Treat®
11	Canarian	Positive control
12	Canarian	Negative control
13	Canarian	*Trichoderma harzianum*
14	Canarian	Fulzyme®
15	Canarian	*Pythium oligandrum.*
16	Canarian	Novo Treat®

3.4.5. Assessment criteria

Disease severity recordings for Leaf Disease Severity Index (LDSI) was conducted 30-, 60- and 90- days after inoculation (DAI). Disease evaluation was based on visual inspection of symptom development of chlorosis on leaves. A disease scoring system was used to calculate the Leaf Disease Severity Index (Viljoen et al., 2017) observed in each treatment within the experiment. LDSI was classified according to the following grading scale (Table 5 and Fig. 23).:

Table 5. Leaf Disease Severity Index scores for the severity of symptoms induced by *Foc* TR4 on banana plants (Viljoen et al., 2017).

Disease Score	**REMARKS**
0	No streaking or yellowing of leaves. Healthy
1	Slight streaking or yellowing of lower leaves.
2	Streaking or yellowing of two or three leaves.

3	Streaking/ yellowing on more than three leaves.
4	Extensive streaking/ yellowing of most or all the leaves.

After recording severity score, the overall Leaf Disease Severity Index (LDSI) for each treatment were calculated following method of (Ssali et al., 2013):

$$= \frac{score\ in\ the\ scale * frequency}{total\ number\ of\ plants * maximum\ class\ in\ the\ sclae} * 100$$

Ninety (90) days after *Foc* TR4 inoculation, plants were uprooted and removed from the pots. Rhizomes were cut for internal disease assessment. Rhizome infection symptoms are characterized: diseased plants will have a very characteristic yellow to dark-brown discoloration of the rhizome. Discoloration of the Rhizome was recorded using a six-point rating scale (Viljoen et al., 2017) with a zero score for the first class for no disease presence (Table 6 and Fig. 23).

Table 6. Rhizome Disease Severity Index (RDSI) for RDSI scores for the severity of symptoms induced by *Foc* TR4 as revealed following cross sections of banana Rhizomes (Viljoen et al., 2017).

Disease Score	**REMARKS**
0	No symptoms
1	Few internal spots
2	<1/3 Discolored tissue
3	1/ 3–2/3 Discolored tissue
4	>2/3 Discolored tissue;
5	Entire inner discoloration.

Thus, samples with slightly different discoloration can have severity scores differing by 33% and samples with the same score may differ by up to 32%. By using a continuous scale that has high reproducibility the precision can be improved. Rhizome Disease Severity Index (RDSI) was also calculated for Rhizome Discoloration based on the same formula above.

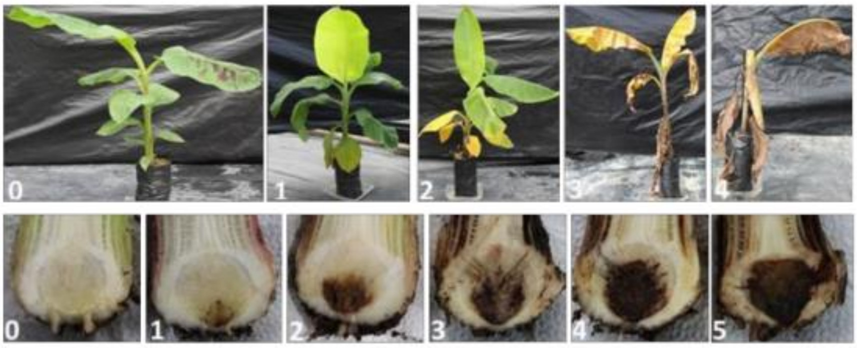

Figure 23. Rating scale for evaluation of external symptoms and internal rhizome discoloration caused by *Foc* TR4. Top photos: External symptoms: 0-no yellowing; 1-Slight streaking or yellowing of lower leaves; 2-Streaking or yellowing of two or three leaves; 3-Streaking/ yellowing on more than three leaves; 4-Extensive streaking/ yellowing of most or all the leaves. Bottom photos: Internal rhizome discoloration: 0- No symptoms; 1- Few internal spots; 2- less than 1/3 Discolored tissue; 3- more than 1/3 Discolored tissue; 4- more than 2/3 Discolored tissue; 5- Entire inner discoloration. (Viljoen et al., 2017).

3.4.6. Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 27 for Windows. LDSI and RDSI were calculated. A two-way ANOVA was then used to assess the effects of variety and treatment on both variables (LDSI & RDSI).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Survey on the spread of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4) in South Lebanon.

4.1.1. Results

The survey conducted across the coastal zone of Tyre and Sidon district revealed the widespread presence of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4. Out of the 87 banana fields inspected (Table 7, Fig. 25), the pathogen was observed in 26 fields, indicating that approximately 30% of the fields had at least one banana plant infected with the pathogen. Molecular diagnosis confirmed the that the disease in Lebanon is caused by *Foc* TR4. The PCR using primer pair forward primer FocTR4-F (5’- CACGTTTAAGGTGCCATGAGAG-3’) and reverse primer FocTR4-R (5’- CGCACGCCAGGACTGCCTCGTGA-3’) showed an amplicon of the expected size of 463bp (Fig. 24). The identification of *Foc* TR4 was confirmed using DNA sequencing and BLASTn analysis. The Lebanese *Foc* TR4 isolates were most closely related to *Foc* TR4 isolates B2-4 (Accession # MN830364.1) from China and P.TH2 (Accession # KP893342.1) from Pakistan, with 99.15 % nucleotide identity. (Appendix)

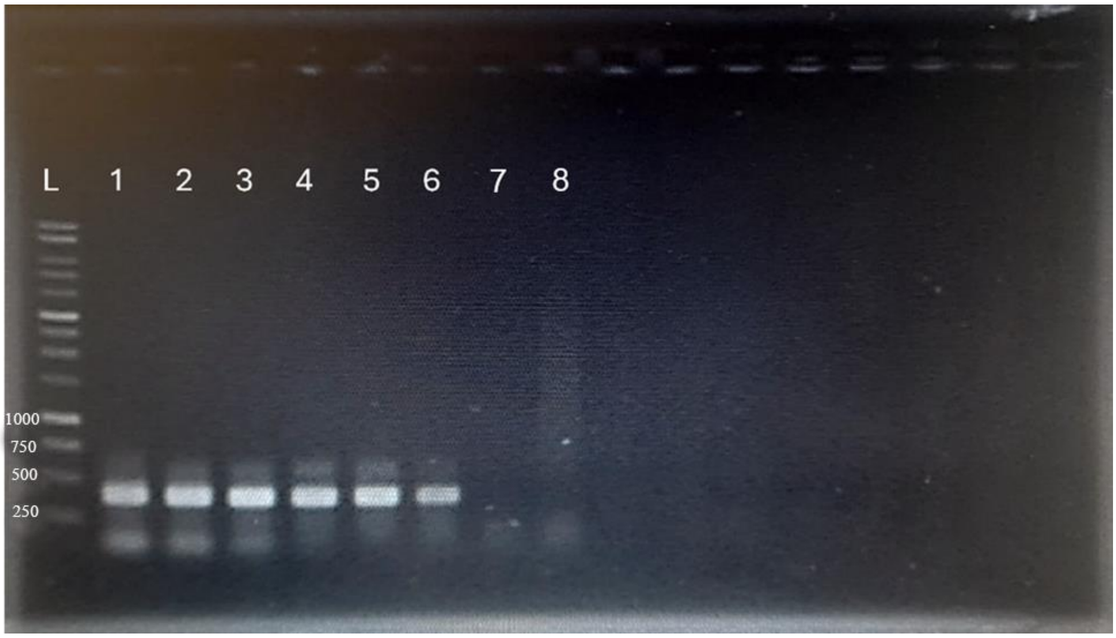

Figure 24. Agarose gel electrophoresis of PCR amplified products obtained from DNA extracts of mycelial culture of *Fusarium* spp. isolated from banana plants from South Lebanon. The specific primer pair FocTR4-F/FocTR4-R was used (Dita et al., 2010). F = 1Kbp ladder (Promega ®); 1-6 = banana samples infected with *Foc* TR4; 7&8= healthy banana plants.

Fusarium wilt was observed in all types of farms, ranging from small-holder farms to large-scale plantations. The distribution of *Foc* TR4 in the banana fields of South Lebanon, reveals distinct patterns of concentration along the coastal regions. Starting from the southernmost point, Iskandarouna, near Naqoura, red circles indicating positive identification of *Foc* TR4 are present, though limited in number. As we move northward towards the areas of El Qlaileh-Mansouri region, the concentration of *Foc* TR4 becomes more pronounced. This central coastal area shows a high concentration of the disease, suggesting that it is a hotspot zone for *Foc* TR4, likely due to previous history of *Foc* TR4 infection, favorable environmental conditions, susceptible banana varieties, or challenges in disease management. Borj El Shemaleh area shows several infected sites. The region between Tyre and Borghliyeh shows both red and blue circles, showing the moderate presence of *Foc* TR4 infection in this area.

Moving North up the area of Mazraat El Yahodieh, *Foc* TR4 shows a significant presence. The pathogen appears well-established in this area, though the spread is not as intense as in the central region. The region between Kfarbedda and Ansarieh reveals a cluster of blue circles, showing fields where banana plants have not been identified as infected, standing out as a healthy zone.

In summary, the distribution pattern of *Foc* TR4 in South Lebanon shows a concentrated presence along the coastal regions, particularly around El Qlaileh, Mansouri and Borghliyeh to Kfarbedda region. This area, along with the affected zone near Tyre, represent the most significant hotspots for the pathogen. In contrast, the southern region near Naqoura shows moderate infestation levels, while the between Kfarbedda and Ansarieh stands out with clusters of healthy, uninfected fields.

Table 7. List of visited locations with number of fields, number of samples, and number of fields with banana plants that tested positive for infection by *Foc* TR4.

Country	Location	# of fields	# of samples	# of fields tested positive
Lebanon	Naqoura	3	10	0
	Iskandarouna	3	9	2
	Mansouri	7	22	4
	El Qlaileh	4	12	2
	Maaliyeh	3	12	2
	Taybeh	4	13	1
	Rachideyeh	1	2	0
	Bourj El Shemaleh	7	18	3
	Tyre	1	4	0
	Abbasieh	7	21	2
	Borghliyeh	6	21	2
	Qasmieyeh	4	11	0
	Mazraat El Yahodieh	7	20	5
	Borj Rahal	4	13	0
	Matareyet ElShouamr	3	9	1
	Kfar Bedda	1	3	0
	Adloun	6	18	0
	Ansarieh	2	5	0
	Sarafand	7	16	2

	Aaqbiyeh	2	6	0
	Addousieh	3	10	0
	Ain baal	1	3	0
	Bazourieh	1	2	0
TOTAL		87	260	26

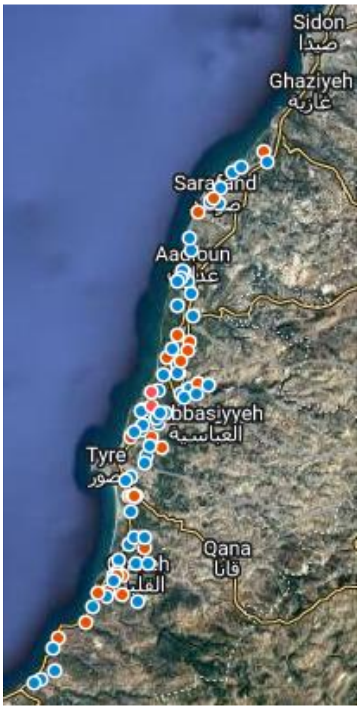

Figure 25. Observations of the spread of *Foc* TR4 in South Lebanon. Blue circles denote healthy fields; red circles denote sites where *Foc* TR4 was positively detected.

The predominant banana varieties grown in these areas were Dwarf Cavendish (Canarian & Baladi) and Grand Nain. Farmers obtained new planting materials from various sources, including their own fields, neighboring farms, and imported tissue culture-derived plantlets. The age of the plantations varied, from as young as one year to ten years old. During the discussions conducted as part of the survey, it was found that while many banana farmers were aware of Fusarium wilt, only few had specific knowledge about its cause, *Foc* TR4 These informed farmers typically obtained their knowledge from the internet, agricultural institutions, consultants, or neighboring farmers. In terms of disease management, most farmers practiced on-spot burning of infected plants and sterilization of tools and equipment to control the spread of the disease. However, the significant movement of people and vehicles within and between farms was identified as a potential contributor to the spread of Fusarium wilt.

The survey also found that some farmers did not implement any pest or disease management practices that may contain or reduce the spread of the disease, which could exacerbate the spread of Fusarium wilt. The cropping systems varied across farms, with most farmers prioritizing economic benefit and diversity of production. The average planting density was 140 plants per 1000 m^2, and bananas were often intercropped with avocados, with some farmers also growing Cherimoya and Papaya. Most banana farms in the region relied on supplementary irrigation rather than rainfall, and good sucker management practices were generally observed.

4.1.2. Discussion

The survey results show a relatively fast spread of Fusarium wilt in banana orchards across Tyre and Sidon, which threatens the sustainability of banana cultivation in South Lebanon. The identification of *Foc* TR4 in 30% of the surveyed fields indicates a significant risk of further spread, particularly given the high movement of people and vehicles between farms, which can facilitate the transmission of the pathogen. The lack of comprehensive knowledge about Fusarium wilt among a significant portion of farmers suggests a need for increased educational outreach and training. While some farmers are aware of basic symptoms like leaf yellowing and stem rot, many do not fully understand the pathogen's lifecycle or the long-term implications of Fusarium wilt. As noted by Dita et al. (2018), effective disease management requires not only awareness but also the implementation of integrated pest management (IPM) strategies that include the use of resistant varieties, proper sanitation, and controlled movement within and between farms.

The relatively lower incidence of Fusarium wilt in certain varieties, such as Grand Nain, could be attributed to inherent genetic resistance, as discussed by Pérez-Vicente et al. (2021). However, reliance on resistant varieties alone is not sufficient, as *Foc* TR4 has shown the ability to overcome resistance over time. Therefore, combining resistant varieties with biocontrol treatments, as demonstrated in the current study, may offer a more sustainable approach to managing Fusarium wilt in South Lebanon. Moreover, the survey highlights the importance of sourcing disease-free planting materials, as contaminated plantlets can introduce *Foc* TR4 into previously uninfected areas. The practice of burning infected plants and sterilizing tools, while beneficial, must be accompanied by stricter quarantine measures to prevent the movement of the pathogen within and between farms. The findings emphasize the need for a coordinated effort to implement robust disease management practices and promote the use of resistant varieties and biocontrol agents. Addressing these challenges is essential for preserving the economic viability of banana production in the region and preventing the further spread of this devastating disease.

In summary, the wide spread of *Foc* TR4 in South Lebanon calls for an official action by the Ministry of agriculture in cooperation with research institution to hold farmer awareness campaigns and to stress on the phytosanitary/sanitation measures to be taken to prevent the further spread of the disease within and between farms. The introduction of resistant/tolerant varieties coupled with the local research on antagonistic organisms, sanitation and cultural practices would lead a way to a successful integrated disease management strategy promoting sustainable banana production in Lebanon.

4.2. Evaluation of the antagonistic potential of different *Trichoderma* strains against *Foc* TR4

4.2.1. Results

The dual culture experiment showed that all six different *T. viride* strains (T24, T26, T16, T43, T42, T3) and *T. harzianum* inhibited the growth of *Foc* TR4. After 10 days of incubation, the pathogen inhibition rate ranged between 74.44% and 56.23%, depending on the *Trichoderma* strain (Fig. 26). The strain T24 was the most efficient, achieving a 74.44% mycelial growth inhibition. The growth inhibition percentages for other strains were as follows: T42 (69.95%), T3 (69.08%), T16 (68.77%), *T. harzianum* (66.12%), T43 (65.61%), and T26 (56.23%) (Table 8). *Trichoderma* strains restricted the growth of *Foc* TR4 and in all cases, it overgrew *Foc* TR4 and sporulating there after 10 days (Fig. 27). The results revealed that growth of the pathogen was more rapid in the control plates than in the dual culture plates in all the treatments. It was also found that *Trichoderma* grew much faster than *Foc* TR4 in all the treatments in dual culture.

Table 8. Mean mycelial growth (cm) of *Foc* TR4 in dual cultures with seven *Trichoderma* strains, 10 days after inoculation. Different letters (a-e) show significantly different values between strains.

Antagonist strain	***Foc* TR4 Mean radial growth (cm)**
T. viride T24	0.72^{a}
T. viride T42	1.56^{b}
T. viride T16	1.67^{b}
T. viride T3	1.69^{bc}
T. harzianum	1.81^{bcd}
T. viride T43	2.36^{cd}
T. viride T26	2.42^{d}
Control	5.47^{e}

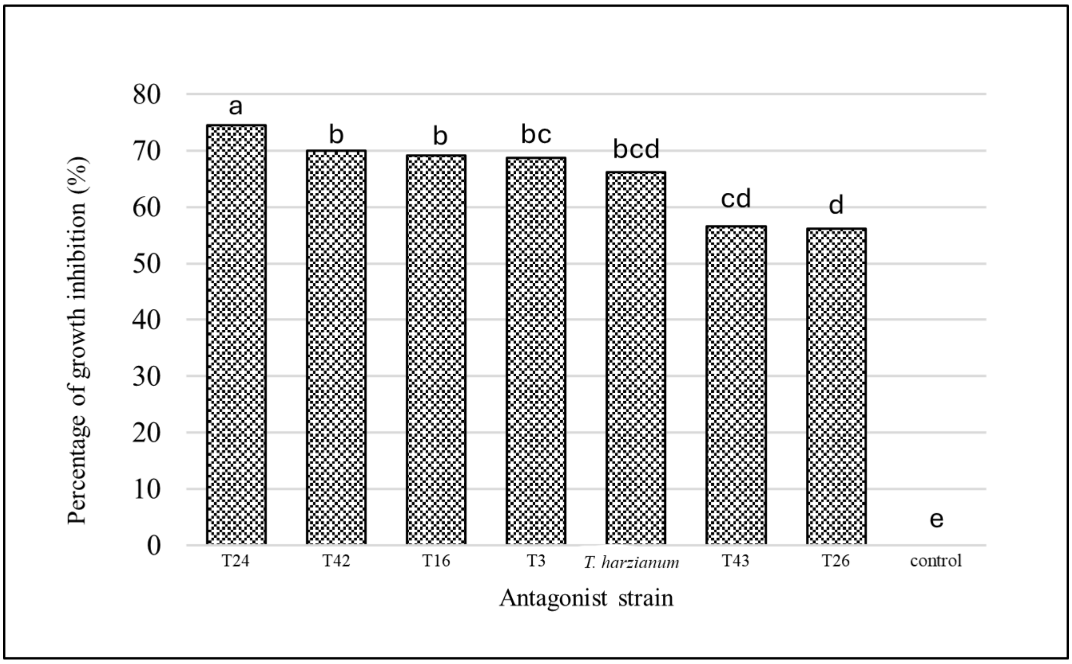

Figure 26. Percentage of growth inhibition of *Foc* TR4 by *Trichoderma* strains (Y axis); Treatments with seven *Trichoderma* strains and control (X axis). Different letters (a-e) show significantly different values within strains.

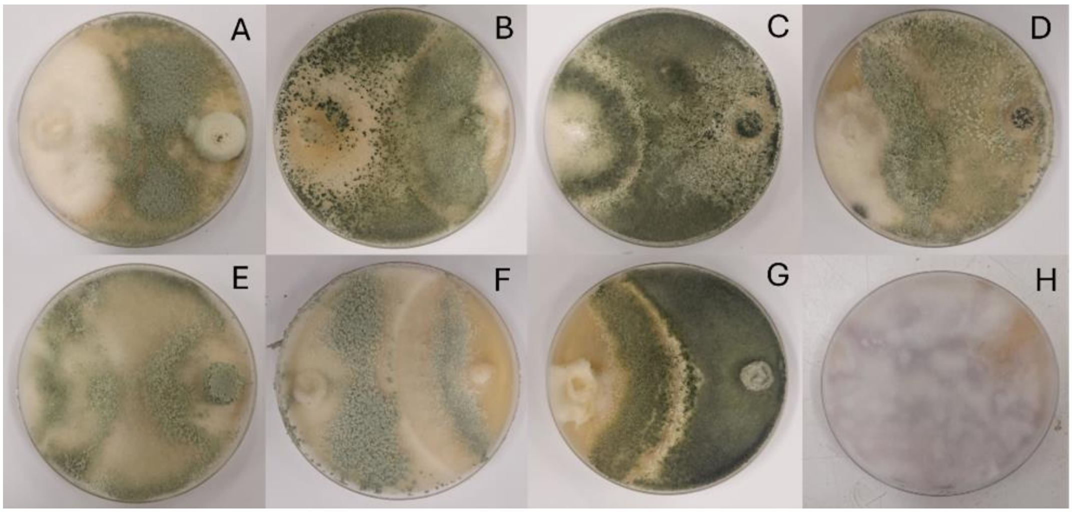

Figure 27. Fungal morphology of the local *Foc* TR4 isoaltes in dual-culture assay on PDA after 10 days of incubation with: A: T26; B: T3; C: T42; D: T43; E: T24; F: *T. harzianum;* G: T16; H: *Foc* TR4 control.

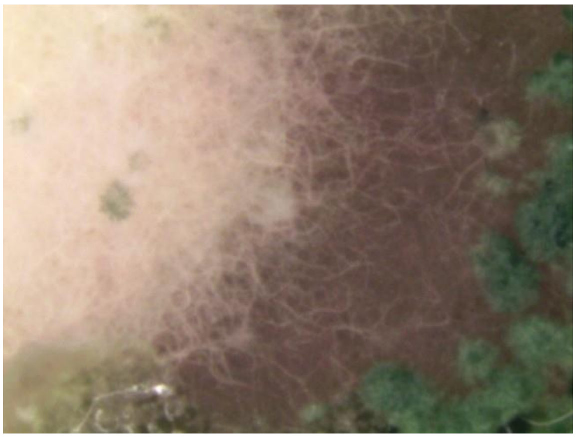

Figure 28. *Trichoderma harzianum* (right) growing on mycelia of *Foc* TR4 (left).

4.2.2. Discussion

The present study evaluated the in-vitro antagonistic potential against *Foc* TR4 of seven *Trichoderma* isolates, including one isolate of *T. harzianum* and six isolates of *T. viride*, using the dual culture plate technique. The results demonstrated significant inhibition of *Foc* TR4 by all tested *Trichoderma* strains, with inhibition rates ranging from 56.23% to 74.44%. However, no inhibition zones were observed in all treatments, indicating that the major mechanism of action is not the secretion of extracellular toxic metabolites but probably by mycoparasitism as suggested by the overgrowth of *Trichoderma* over the mycelium of *Foc* TR4.

4.2.2.1. Antagonistic Properties of *Trichoderma*

The observed antagonistic effect of *Trichoderma* isolates against *Foc* TR4 aligns with previous research highlighting *Trichoderma*'s potential as a biocontrol agent. *Trichoderma* species are known for their ability to inhibit pathogenic fungi through various mechanisms, including competition for nutrients, mycoparasitism, and the production of antibiotics and hydrolytic enzymes (Schirmböck et al., 1994; Elad, 2000).

4.2.2.1.1. *Trichoderma harzianum*

Trichoderma harzianum has been extensively studied for its antagonistic properties against various plant pathogens. In this study, *T. harzianum* achieved a 66.12% inhibition rate against *Foc* TR4. This result is in line with recent research that reported significant inhibition of *F. oxysporum* by *T. harzianum*. For example, a study by Kareem and Al-Araji (2017) demonstrated that *T. harzianum* inhibited the growth of *F. oxysporum* by 89% through the production of lytic enzymes such as chitinases, glucanases and proteases. Another study by Wibowo et al. (2013) found that *T. harzianum* reduced the growth of *F. oxysporum* by 50%. Both studies have identified mechanisms by which *T. harzianum* inhibits fungal pathogen growth, with mycoparasitism being a primary method. Elad (2000), demonstrated that *Trichoderma* species produce chitinases and cellulases, enzymes capable of lysing the cell walls of fungal pathogens. Similarly, Hadar et al. (1979) observed that *T. harzianum* could overgrow colonies of *Rhizoctonia solani,* utilizing the fungal cell wall as a carbon source. Additionally, Sharon et al. (2001) highlighted the roles of glucanase and protease in mycoparasitism. Other proposed mechanisms include antibiosis and competition. In summary, the mechanisms underlying the antagonistic effects of *T. harzianum* include the production of volatile and non-volatile antibiotics, competition for nutrients, mycoparasitism, and the secretion of hydrolytic enzymes (Contreras-Cornejo et al., 2021).

4.2.2.2. *Trichoderma viride*

The six isolates of *Trichoderma viride* tested in this study showed varying degrees of inhibition against *Foc* TR4, with inhibition rates ranging from 56.23% to

74.44%. These findings are consistent with recent studies on *T. viride*'s antagonistic properties. For example, Kumari et al. (2014) reported that *T. viride* inhibited the growth of *F. oxysporum* by 55%, in dual culture. In fact, the author found that management of *F. oxysporum* by *T. viride* was superior compared to two chemical fungicides, namely Mancozeb and Cuprozin. Similarly, upon studying different isolates of *T. viride*, a native Indian isolate was found highly effective against *Foc* TR4 in vitro as well as under natural pot conditions (Shukla et al. 2024). General mechanisms of action of *Trichoderma* species involve efficient colonization of plant roots, where they establish strong interactions with the host plant. They typically colonize root surfaces and may penetrate the epidermis, producing enzymes that degrade the cell walls of pathogenic fungi, thus providing biocontrol benefits. *Trichoderma viride* has been shown to colonize banana roots endophytically, significantly reducing the incidence of Fusarium wilt by up to 93.33%. The mechanisms of action of *T. viride* involve the production of hydrolytic enzymes like chitinases and glucanases, which break down the cell walls of pathogenic fungi, inhibiting their growth and protecting the plant from disease (Sulieman et al., 2023). Thangavelu and Mustaffa (2010) demonstrated that a strain *T. viride* NRCB1 had a crucial role in inducing systemic resistance in plants, offering protection against both soil-borne and foliar pathogens. Upon treatment with ***T. viride*,** there was a significant increase in the activity of defense-related enzymes, such as peroxidase (PO) and phenylalanine ammonia-lyase (PAL), which are essential for reinforcing plant cell walls and synthesizing antimicrobial compounds.

4.2.2.2. Implications for Biocontrol

The results of this study reinforce the potential of *Trichoderma* as an effective biocontrol agent against *Foc* TR4. The ability of *Trichoderma* isolates to rapidly overgrow *Fusarium* mycelium and inhibit its growth through multiple mechanisms makes it a valuable tool in integrated pest management strategies. The findings are particularly relevant in the context of increasing restrictions on chemical pesticides and the need for sustainable agricultural practices (Shukla et al. 2024). While the current study provides valuable insights into the antagonistic potential of *Trichoderma* against *Foc* TR4, further research is needed to explore the efficacy of these biocontrol agents under field conditions and their mechanisms of action. Environmental factors such as temperature, humidity, and soil composition can influence the effectiveness of *Trichoderma* in natural settings. Additionally, investigating the synergistic effects of combining *Trichoderma* with other biocontrol agents or organic amendments could enhance its biocontrol efficacy (Barzman et al., 2020).

4.3. Field trial for evaluation of the reaction of four different banana varieties to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 and evaluation of the antagonistic activity of four biocontrol products

4.3.1. Results

4.3.1.1. Ambient Conditions

Data on temperature and relative humidity in the field trial from October 2022 to October 2023 varied throughout the growing period. The mean temperature ranged between 12.5 °C and 32.5 °C. Relative Humidity ranged between 63% and 78%. These fluctuations in temperature and humidity were crucial for assessing the growth conditions for banana cultivation.

4.3.1.2 Variety Impact on Bunch Weight

The highest bunch weight was observed in GCTCV 218 (30.7 kg) followed by Grand Nain (29.6 kg), with no significant difference between the 2 varieties. Bunch weights within treatments among the Canarian variety were significantly lower compared to the former two varieties ($p = 0.000$), ranging from 27.0 kg for *T. harzianum* to 25.4 kg for control with significant differences observed between treatments. The Baladi variety recorded the lowest bunch weights, ranging from 21.3 kg for *T. harzianum* to 19.1 kg for control with significant differences across treatments ($p = 0.000$). This shows that the variety of bananas plays a critical role in determining the yield obtained. The Control GCTCV 218 and Control Grand Nain varieties consistently outperformed Canarian and Baladi in terms of bunch weight, regardless of the treatment applied (Fig. 29 & Fig. 30).

Figure 29. Bunch weight measurement for the evaluation of four different biocontrol treatments on banana yield.

4.3.1.3. Treatment Impact on Bunch Weight

Among the biocontrol treatments, *Trichoderma harzianum* stood out for its consistent ability to enhance yield across both the Canarian (27.0 kg) and Baladi (21.3 kg) varieties with a significantly higher yields as compared to their respective controls. In the Canarian variety, the control treatment had the lowest yield (25.4 kg), with Fulzyme® giving a slightly higher yield but not significantly different from the control (Fig.30). Similarly, the yields resulting from Polyversum®, and Novo Treat® were not significantly different from the control.

A similar pattern was observed in the Baladi variety, as Fulzyme®, Polyversum and Novo Treat® provided noticeable yield improvements over the control, however, with no significant differences as compared to the control treatment, which had the lowest bunch weight (19.1 kg). These findings suggest that biocontrol agents, in the absence of major diseases may enhance yield. Under our experimental conditions, *T. harzianum* and Fulzyme® showed the highest yield enhancement.

In conclusion: based on the combined analysis of bunch weight, *T. harzianum* and Fulzyme® were the most effective treatments in the Canarian and the Baladi varieties. However, variety has the major influence on bunch weight. The GCTCV 218 and Grand Nain consistently showing the highest bunch weights (Fig.30). This suggests that while biocontrol agents can enhance growth performance, the choice of variety is a critical factor in maximizing yield.

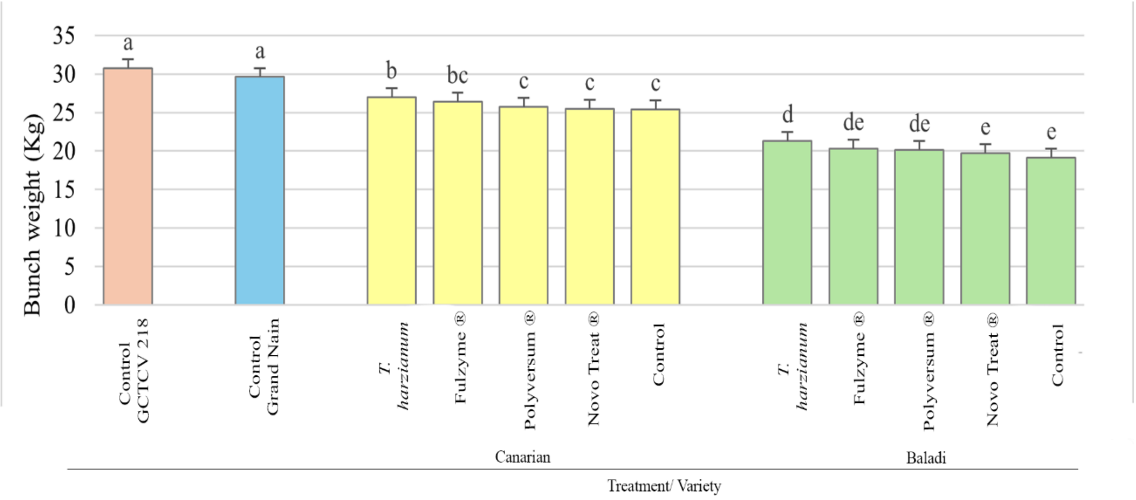

Figure 30. Average banana bunch weight recorded per variety and per treatment: Four varieties were compared. For two varieties, five treatments including four biocontrol products, and the control were evaluated. (a-e) Different letters indicate significantly different values across different treatments based on Tukey's post hoc test.

4.3.1.4. Disease Severity Indices of Panama wilt disease or *Foc* TR4

Eventhough the farmer claimed that his field was infested with *Foc* TR4, no external symptoms were observed on the banana plants in the control as well as in the four treatments, till the end of the field trial. Cross sections of the pseudostem did not reveal the browing of the internal tisse expected upon infection by *Foc* TR4.

Samples were taken from the banana pseudostem to check for the presence of *Fusarium* spp. After the isolation process, PCR detection with specific *Foc* TR4 priemrs was done, it was confirmed that all samples tested negative for *Foc TR4.* This confirms the hypothesis of the presence of an infection by endophytic *Fusarium* spp. was indeed present in some of the asymptomatic samples.

The results in Figure 31 show a clear visualization of the percentage of plates that tested positive for *Fusarium* after isolating samples from the pseudostem. In the Grand Nain and GCTCV 218 varieties, *Fusarium* presence was detected in 50% and 33.33% of the plates respectively.

In the Canarian variety, the control treatment exhibited the highest *Fusarium* incidence, with 100% of the plates testing positive. This suggests that without any biocontrol or chemical intervention, the Canarian variety is highly susceptible to endophytic *Fusarium*. Among the biocontrol treatment, *T. harzianum* showed absence of *Fusarium* (Fig. 31). Fulzyme® and *Pythium oligandrum* treatments also showed moderate levels of *Fusarium* presence at a rate of 66%. Similarly, in Baladi variety, *Fusarium* was not detected in *T. harzianum* treated samples, whereas the control treatment had the highest incidence of 100%, which declined in Fulzyme® treatment, showing a rate of 66% and 33% in Novo Treat® and Polyversum ® (*Pythium oligandrum)* (Fig. 31) .

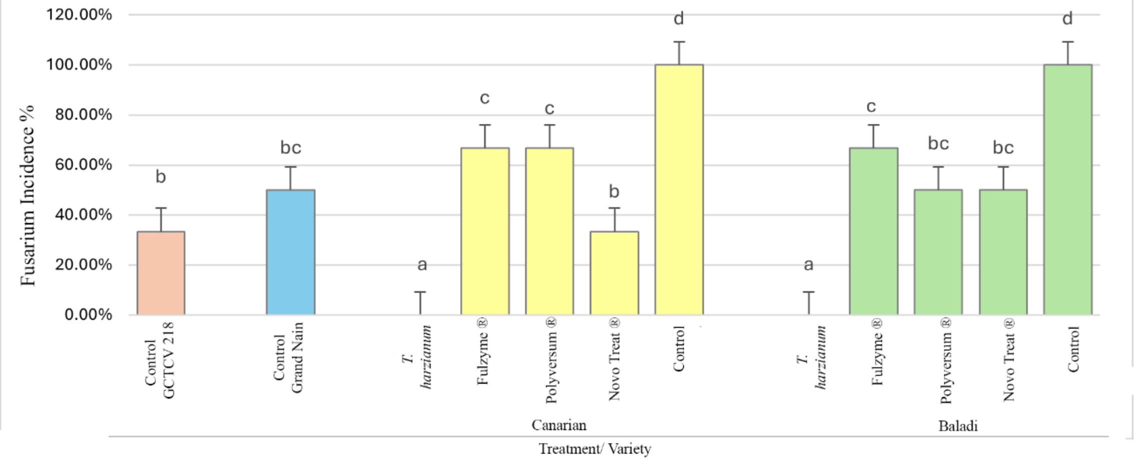

Figure 31. Endophytic *Fusarium* incidence recorded on plates isolated per variety and per treatment: Four varieties were compared. For two varieties, five treatments including four biocontrol products, and the control were evaluated. (a-e) Different letters show significantly different values across different treatments based on Tukey's post hoc test.

In summary, the incidence of infection by endophytic *Fusarium* spp. in the GCTCV 218 and Grand Nain varieties was lower than that observed in the Baladi and Canarian varieties. Treatments having *T. harzianum* showed absence of endophytic

Fusarium spp.; while the other 3 treatments reduced infection, but significantly less than *T. harzianum*.

4.3.2. Discussion

4.3.2.1. Antagonistic activity evaluation

The effectiveness of various biocontrol agents on *Foc* TR4 incidence and severity was not possible in this experiment. However, their effect on banana bunch weight and on the incidence of endophytic *Fusarium* infections was evaluated across different varieties in this study. Despite the absence of external symptoms, the presence of endophytic *Fusarium* was confirmed through laboratory techniques. The discussion below focuses on the performance of each biocontrol agent used in this study, supported by recent research findings.

4.3.2.1.1. *Trichoderma harzianum*

Trichoderma harzianum emerged as the most effective biocontrol agent in this study, significantly reducing the endophytic *Fusarium* incidence to zero in both Canarian and Baladi varieties. The application of *T. harzianum* not only suppressed the fungus but also enhanced bunch weight, particularly in susceptible varieties. *Trichoderma harzianum* was reported to have antagonistic activity against several fungi, including *Fusarium* spp. (Nwankiti et al, 2018). *Trichoderma harzianum* has been shown to promote plant growth and yield, likely through the production of plant growth-promoting substances (Harman et al., 2004). These attributes make *T. harzianum* a promising candidate for integrated pest management (IPM) in banana cultivation.

4.3.2.1.2. Fulzyme® (*Bacillus subtilis* and *Pseudomonas putida*)

Fulzyme®, another biocontrol agent used in this study, demonstrated moderate efficacy in reducing endophytic *Fusarium* incidence and improving bunch weight in the Canarian and Baladi varieties. While it was not as effective as *T. harzianum*, Fulzyme® still managed to decrease *Fusarium* presence by 33% in both varieties. Fulzyme contains beneficial microorganisms, primarily *Bacillus subtilis*, which are known for their antagonistic activity against soil-borne pathogens like *Fusarium* (Kloepper et al., 2004). Additionally, these bacteria can enhance plant health by colonizing the rhizosphere and inducing systemic resistance (Raaijmakers et al., 2010).

4.3.2.1.3. Polyversum® (*Pythium oligandrum*)

Pythium oligandrum, used as a biocontrol agent in this study, showed similar performance to Fulzyme®, reducing endophytic *Fusarium* incidence to 66% and 50% in Canarian and Baladi varieties respectively. *Pythium* spp. are well-known for their role in biological control, particularly through mechanisms such as mycoparasitism and competition for nutrients and space (Martin & Loper, 1999). These oomycetes can effectively suppress soil-borne pathogens by colonizing the root zone and creating a protective barrier that limits pathogen establishment. However, the efficacy of *Pythium* as a biocontrol agent can be influenced by several factors, including soil health, microbial community composition, and environmental conditions (Lumsden et al., 1990). The results of this study align with earlier findings that *Pythium oligandrum* can be an effective biocontrol agent, though its success in managing *Fusarium* and promoting plant growth may not be as pronounced as that of other agents like *Trichoderma* or *Bacillus*. Further research into optimizing *Pythium*-based biocontrol strategies, perhaps through synergistic use with other agents, could enhance its efficacy in banana cultivation.

4.3.2.1.4. Novo Treat® (*Bacillus amyloliquefaciens*)

Novo Treat®, a biocontrol agent used in this study, demonstrated some effectiveness in reducing endophytic *Fusarium* incidence, with 33% and 50% of plates testing positive in Canarian and Baladi varieties respectively. While this is a marked improvement over the control treatment, which exhibited a 100% incidence rate, Novo Treat® was less effective compared to *T. harzianum* and Fulzyme®. However, the relatively lower efficacy observed in this study may suggest that the formulation or application method of Novo Treat® could be optimized for better results. The findings are consistent with previous studies that highlight the potential of such microbial consortia in reducing soil-borne diseases, though the success rate can vary depending on the pathogen and environmental context (Raaijmakers et al., 2002).

4.3.2.2 Resistance Evaluation of Four Banana Varieties to Endophytic *Foc*

The study also revealed differences in the inherent tolerance of the banana varieties to endophytic *Fusarium* infections, with GCTCV 218 and Grand Nain varieties, known for their high yield potential, demonstrating a level of tolerance to endophytic *Fusarium*, with lower incidence rates observed than in the Baladi and Canarian varieties. This may be the first trial where GCTCV 218 was tested locally and showed a high yield potential under the prevalent edaphic and climatic conditions prevailing in Lebanon.

This finding aligns with the work of Pérez-Vicente et al. (2021), who noted that certain banana varieties possess genetic resistance to *Fusarium*, likely due to the presence of resistance (R) genes that trigger defense mechanisms in response to pathogen invasion. While these varieties are not immune, their ability to limit *Fusarium* spread and maintain high yields even under pathogen pressure makes them valuable in breeding programs aimed at developing resistant banana varieties.

The results of this study highlight the importance of selecting effective biocontrol agents for managing Fusarium wilt in banana cultivation. *Trichoderma. harzianum* stood out as the most promising agent, significantly reducing *Fusarium* incidence and enhancing bunch weight across susceptible varieties. While Fulzyme ® and Polyversum ® also showed potential, their effectiveness was somewhat lower, suggesting that further optimization of these treatments is needed. The presence of inherent tolerance in varieties like GCTCV 218 variety further emphasizes the need for integrated approaches that combine biocontrol agents with resistant varieties to achieve sustainable banana production.

4.4. Greenhouse pot trial for evaluation of the reaction of four different banana varieties to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 and evaluation of the antagonistic activity of four biocontrol products.

4.4.1. Results

The pot experiment aimed to evaluate the reaction of four different banana varieties in soil artificially infested with *Foc* TR4 and to assess the antagonistic effects of four biocontrol agents.

Initial analysis of leaf severity and rhizome discoloration indices revealed significant differences between treatments between the 2 varieties, Baladi and Canarian, but there were no significant differences neither between the two varieties nor the interaction between treatments and varieties (Table 9). This suggests that the type of treatment had a notable impact on Leaf Disease Severity Index (LDSI), whereas there was no significant difference between the Canarian and Baladi varieties on LDSI, nor did the combination of treatment and variety ($p_{\text{time}} = 0.000$, $p_{\text{treatment}} = 0.000$, $p_{\text{variety}} = 0.14$, $p_{\text{time*varietty}} = 0.493$, $p_{\text{time*treatment}} = 0.059$, $p_{\text{variety*treatment}} = 0.917$).

4.4.1.1. Leaf Disease Severity Index

4.4.1.1.1. Antagonistic Activity Evaluation

To evaluate the level of tolerance of the imported GCTCV 218 variety to the local *Foc* TR4 isolates, further statistical analysis compared the LDSI among the four varieties, the three widely grown varieties and the GCTCV 218 variety specifically imported for its reported tolerance to *Foc* TR4. This analysis indicated significant differences between the varieties (Table 10), highlighting that the response to *Foc* TR4 varied among the different varieties. Notably, the GCTCV 218 variety exhibited the highest tolerance to the disease, as reflected by the lowest LDSI values compared to other varieties.

Table 9. Mean Leaf Disease Severity Index (LDSI) across different treatments and varieties over time.

Treatment	Leaf Disease Severity Index (Mean)		
	1 Month	2 Months	3 Months
Negative control	0^{a}	0^{a}	0^{a}
Positive control	2.306^{c}	2.5^{c}	2.833^{c}
Fulzyme®	$0.944^{a, b}$	1.667^{bc}	2.5^{c}
Polyversum®	1.556^{bc}	2.222^{c}	2.778^{c}

Trichoderma harzianum	0.778^{ab}	0.944^{ab}	1^{a}
Novo Treat®	1.5^{bc}	1.889^{bc}	2.6^{c}
Variety	Leaf Disease Severity Index (Mean)		
Baladi	1.093	1.426	2
`Canarian	1.278	1.759	2.241
Variable	(*P*-value)		
Treatment	0	0	0
P.-value	0.325	0.189	0.249

$^{a-c}$ Different letters indicate significantly different values across different treatments within the same duration of time according to Tukey's post hoc test.

Table 10. Leaf Disease Severity Indices in positive control treatment across different varieties over time.

Variety	Leaf Disease Severity Index in Positive control (Mean)		
	1 Month	2 Months	3 Months
Baladi	1.778^{a}	2.667^{a}	3^{ab}
`Canarian	2.889^{a}	3^{a}	3.88^{b}
GCTCV 218	2.22^{a}	2^{a}	1.778^{a}
Grand Nain	2.33^{a}	2.5^{a}	2.667^{ab}
P-value	0.33	0.523	0.027

$^{a-c}$ Different letters indicate significantly different values across different treatments within the same duration of time according to Tukey's post hoc test.

- Negative control

Foc TR4 free control plantlets, serving as the negative control in the experiment, consistently exhibited robust health throughout the observation period. There were no signs of leaf chlorosis or rhizome discoloration, which are typical indicators of *Foc* TR4 infection. These plantlets maintained their vigor, showing no external or internal symptoms associated with the disease, thereby confirming the absence of any pathogenic influence (Fig.32).

Figure 32. Symptoms of *Foc* TR4 not appearing in the non inoculated treatments. A: Negative control Baladi, B: Negative control Grand Nain, C: Negative control GCTCV 218, D: Negative control Canarian.

- Baladi Variety

In the Baladi variety, the LDSI values increased over time across all treatments except for *Trichoderma harzianum*. As shown in Figure 33, the negative control consistently exhibited an LDSI of 0%, confirming no infection. The positive control treatment displayed a progressive increase in LDSI, starting from 50% at 30 days and reaching 86% at 90 days. Among the biocontrol treatments, *T. harzianum* demonstrated the highest antagonistic activity with the highest reduction in disease severity, maintaining a value of approximately 25% at 90 days. Fulzyme® and *Pythium oligandrum* (Polyversum®) showed intermediate LDSI values, with Polyversum® having a slightly higher LDSI. *B. amyloliquefaciens* (Novo Treat®) exhibited higher LDSI values compared to the other biocontrol treatments, indicating a lower efficacy (Fig. 33 & Fig. 35).

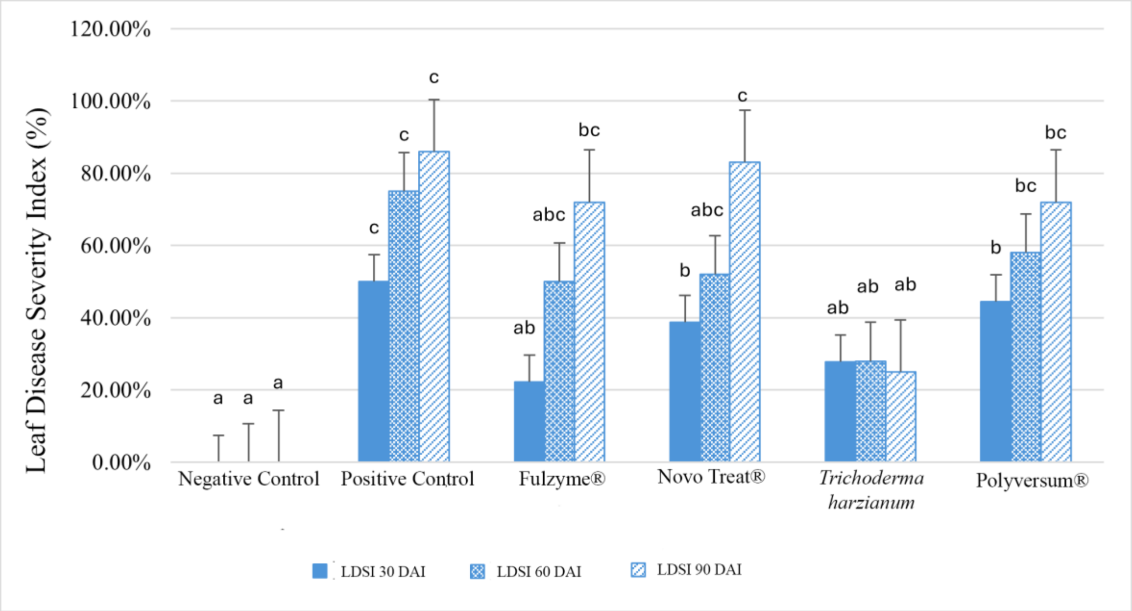

Figure 33. Leaf Disease Severity Index (%) of *Foc* TR4 on Baladi variety across different treatments, 30-, 60- and 90-DAI. . a-c Different letters show significantly different values across different treatments when comparisons are made within the same duration of time according to Tukey's post hoc test.

- Canarian Variety

The Canarian variety followed a similar trend, with increasing LDSI values over time across all treatments. As depicted in Figure 34, the negative control also showed an LDSI of 0%. The positive control treatment recorded the highest LDSI values, starting from 66.67% at 30 days and escalating to 97% at 90 days. *Trichoderma harzianum* again was the most effective biocontrol agent, with LDSI values ranging from 17% at 30 days to 25% at 90 days. Fulzyme® and Polyversum® displayed moderate LDSI values, while Novo Treat® recorded higher values, like the trend observed in the Baladi variety (Fig. 34 & Fig. 35).

Comparing the Baladi and Canarian varieties reveals some similarities and differences. Both varieties showed no infection in the negative control and exhibited increasing LDSI values over time in the positive control and biocontrol treatments.

However, the Canarian variety consistently displayed higher LDSI values in the positive control treatment (66.67% to 97%) compared to the Baladi variety (50% to 86%), indicating a higher susceptibility to *Foc* TR4, even though there were no significant statistical differences. *Trichoderma harzianum* was the most effective biocontrol agent for both varieties, but the Canarian variety showed slightly higher LDSI values even with this treatment. No significant differences were observed between Baladi and Canarian varieties subjected to the biocontrol treatments.

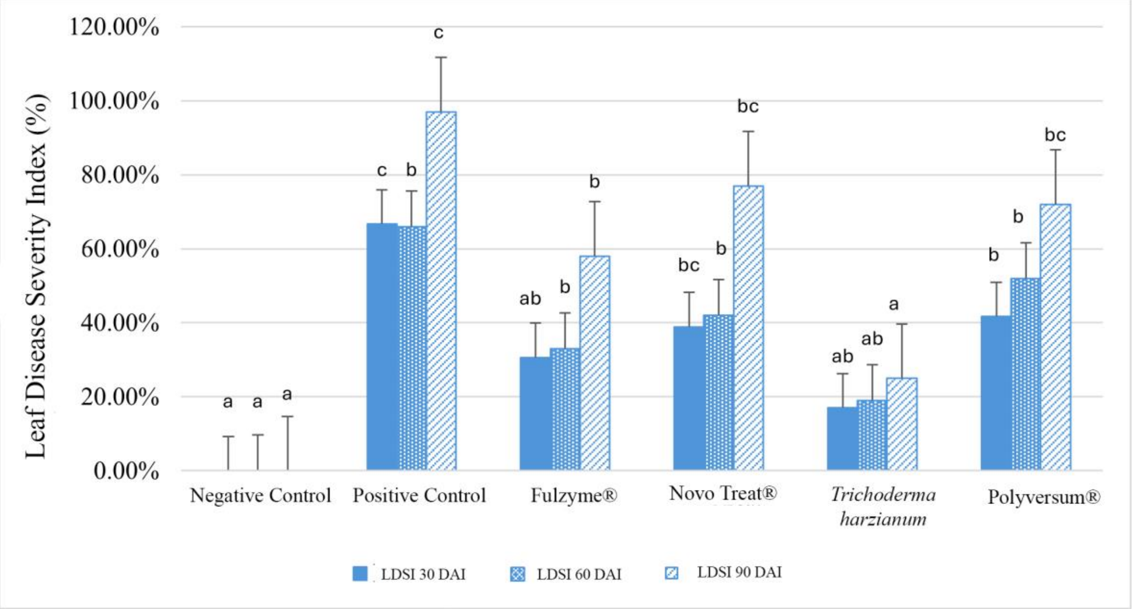

Figure 34. Leaf Disease Severity Index (%) induced by *Foc* TR4 infection of Canarian variety across different treatments, 30-, 60- and 90-DAI. . a-c Different letters show significantly different values across different treatments when comparisons are made within the same duration of time according to Tukey’s post hoc test.

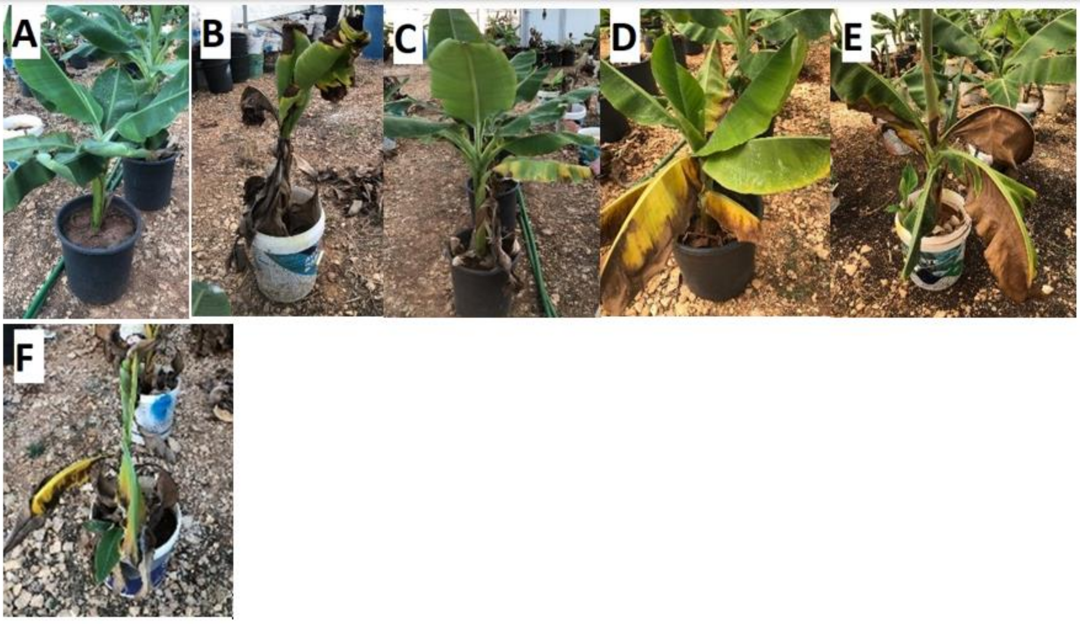

Figure 35. Leaf Chlorosis Symptoms 90DAI caused by *Foc* TR4. A: Negative control, B: Positive control, C: *Trichoderma harzianum*, D: Fulzyme®, E: Polyversum®, F: Novo Treat®.

4.4.1.1.2. Resistance Evaluation of Four Banana Varieties to *Foc* TR4

In the positive control treatment, figure 36 highlight the differences, with Canarian exhibiting the highest LDSI values across all time points (66.67% to 97%), indicating the highest susceptibility. Baladi showed intermediate values (50% to 86%), while Grand Nain displayed slightly lower values (58.3% to 72%). Notably, GCTCV 218 demonstrated the lowest LDSI values (55% to 47%) (Fig. 36), suggesting that it has the highest tolerance to *Foc* TR4 among the tested varieties, even though, there were no statistically significant difference among varieties except when compared to the Canarian variety.

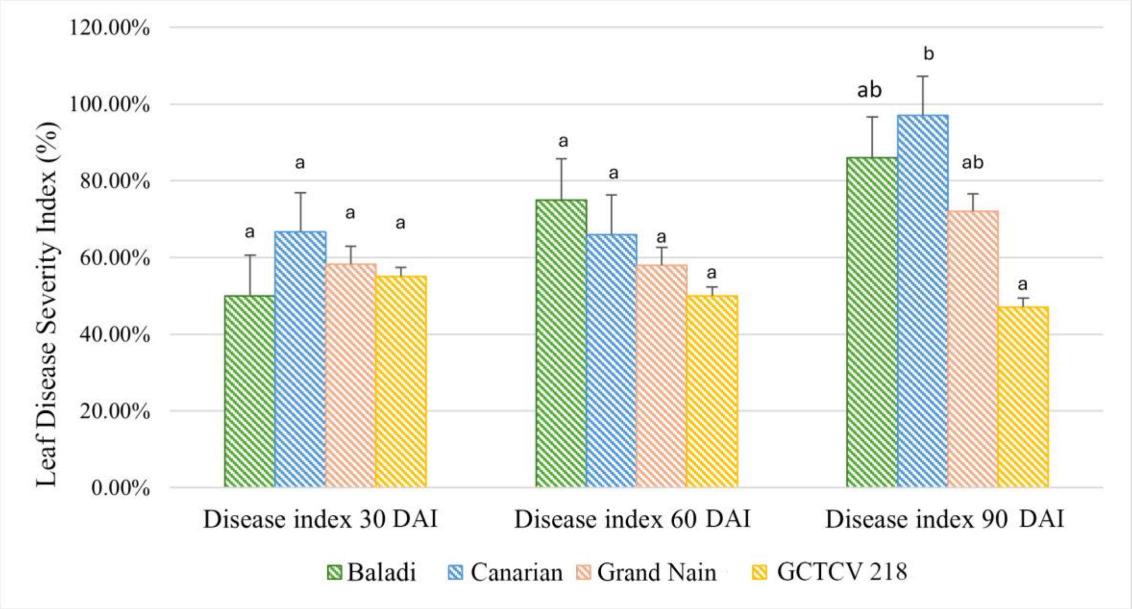

Figure 36. Leaf Disease Severity Index (%) in positive control tretment across Baladi, Canarian, Grand Nain and GCTV 218 varieties 30, 60 and 90 DAI. $^{a-c}$ Different letters indicate significantly different values across different varieties when comparisons are made within the same treatment according to Tukey's post hoc test.

Figure 37. Leaf chlorosis symptoms 90 DAI caused by *Foc* TR4. A: GCTCV 218 positive control: Grand Nain positive control, C: Baladi positive control D: Canarian positive control.

In summary, these results emphasize the potential of *T. harzianum* as a biocontrol agent and the relative resistance of the GCTCV 218 variety, providing important insights for developing integrated disease management strategies for Fusarium wilt in banana cultivation.

4.4.1.2. Rhizome Disease Severity Index

4.4.1.2.1. Antagonistic Activity Evaluation

In the Baladi variety, the negative control consistently exhibited an RDSI of 0% (Fig. 38 and Fig. 39), while the positive control showed the highest RDSI, with significant differences from other treatments. Among the treatments Polyversum® recorded the highest value (67%), followed by Novo Treat® (58%) and Fulzyme® (51%) *T. harzianum* was the most effective biocontrol agent with the lowest RDSI value of 42% (Fig. 38 and Fig. 39).

In the Canarian variety, the negative control exhibited a RDSI of 0% (Fig. 38 and Fig. 39), showing no infection. The positive control treatment displayed an RDSI of 89%. Among the biocontrol treatments, *T. harzianum* demonstrated the highest antagonistic activity with the highest reduction in disease severity, with a value of approximately 47%. Fulzyme® and Novo Treat® showed intermediate RDSI values, with Novo Treat® having a slightly higher RDSI values (Fig. 38 and Fig. 39). *Pythium oligandrum* (Polyversum®) exhibited the highest RDSI values compared to the other biocontrol treatments (71%), indicating a lower efficacy. There was no significant difference between Baladi and Canarian ($p > 0.05$), suggesting similar levels of efficacy for these treatments across both varieties.

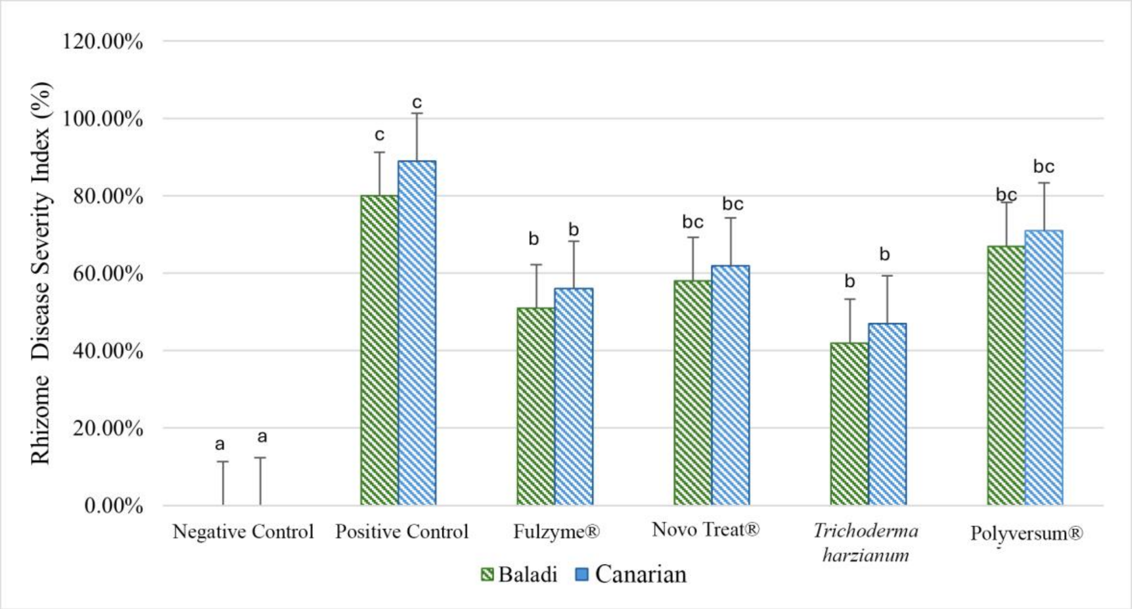

Figure 38. Rhizome Disease Severity Index (%) induced by *Foc* TR4 infections of Baladi and Canarian varieties across different treatments 90 DAI. . $^{a\text{-}c}$ Different letters indicate significantly different values within the same variety when comparisons are made within different treatment according to Tukey's post hoc test.

Figure 39. No Rhizome discoloration in negative control treatments. A: Negative control GCTCV 218, B: Negative control Grand Nain, C: Negative control Canarian, D: Negative control Baladi.

4.4.1.2.2. Resistance Evaluation of Banana Varieties to *Foc* TR4

In the positive control treatment, significant differences were observed among Canarian and GCTCV 218 varieties, and among Canarian and Grand Nain varieties. Figure 40 and Figure 41 highlight these differences, with Canarian exhibiting the highest RDSI values 89% indicating the highest susceptibility. Baladi showed lower value (80%), and Grand Nain displayed a value of 65%. Notably, GCTCV 218 demonstrated the lowest RDSI value of 60%. Suggesting that it has the highest tolerance to *Foc* TR4 among the tested varieties. No significance was observed between Canarian and Baladi varieties.

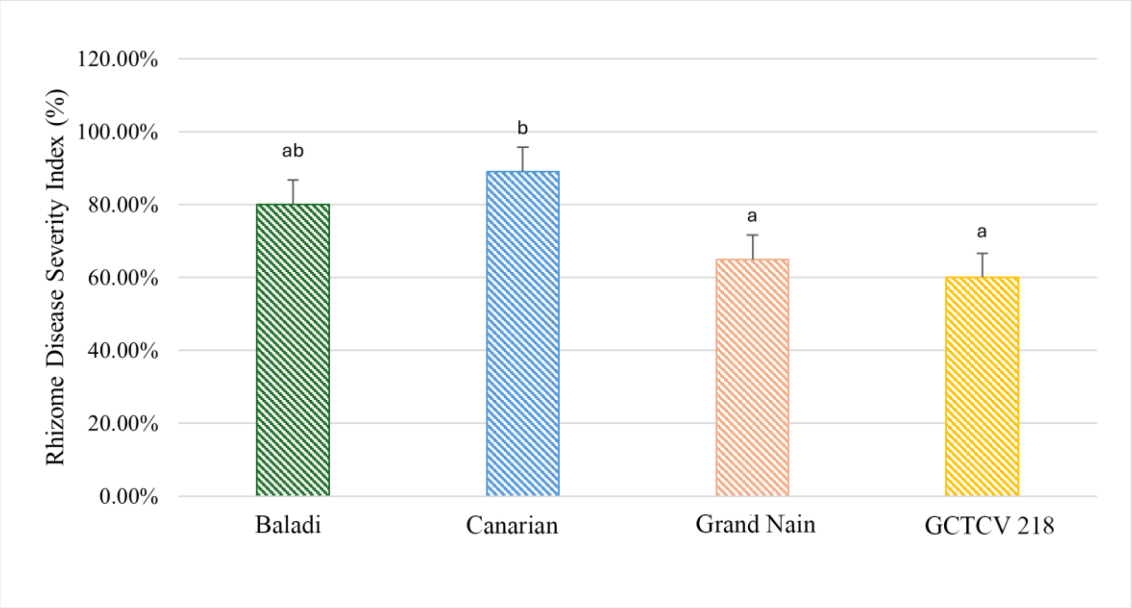

Figure 40. Rhizome Disease Severity Index (%) induced by *Foc* TR4 infections across Baladi, Canarian, Grand Nain and GCTV 218 varieties 90 DAI. a-c Different letters show significantly different values across different varieties when comparisons are made within the same treatment according to Tukey’s post hoc test.

Figure 41. Rhizome discoloration caused by *Foc* TR4. A: Positive control GCTCV 218, B: Positive control Grand Nain, C: Positive control Canarian, D: Positive control Baladi.

4.4.2. Discussion

4.4.2.1. Antagonistic Activity Evaluation

The pot experiment demonstrated significant differences in the Leaf Disease Severity Index (LDSI) among the different biocontrol treatments used against *Foc* TR4 and in the Rhizome Disease Severity Index (RDSI) among the varieties tested. The results showed varying levels of efficacy, with *T. harzianum* consistently showing the lowest values among all varieties, indicating its potential as an effective biocontrol agent. This experiment also proved that GCTV 218 variety is the most tolerant followed by the Grand Nain and Baladi. The Canarian variety was the most susceptible.

4.4.2.1.1. Novo Treat® (*Bacillus amyloliquefaciens*)

Bacillus amyloliquefaciens has been widely studied for its antifungal properties and biocontrol potential. Recent studies have demonstrated its ability to inhibit *F. oxysporum* through multiple mechanisms, including the production of antifungal substances, competition for nutrients, and induction of systemic resistance in plants. For example, Wang et al. (2015) found that *Bacillus amyloliquefaciens* significantly reduced Fusarium wilt severity in banana plants by producing lipopeptides and enhancing plant defense responses. Similarly, Liu et al. (2016) reported the efficacy of *B. amyloliquefaciens* in controlling Fusarium wilt in banana through the production of extracellular enzymes such as chitinase and pectinase, which degrade fungal cell walls. In our experiment, Novo Treat® showed moderate efficacy, that was lower than *Trichoderma harzianum* but higher than *Polyversum* ®,

4.4.2.1.2. Fulzyme® (*Bacillus subtilis* and *Pseudomonas putida*)

Fulzyme®, also demonstrated moderate antagonistic activity against *Foc* TR4. Bacillus. *subtilis* is known for its broad-spectrum antifungal activity, primarily through the production of lipopeptides and antibiotics that disrupt fungal cell membranes. Ho et al. (2015) showed that *B. subtilis* effectively controlled *F. oxysporum* in banana by producing antifungal compounds and inducing systemic resistance. *Pseudomonas fluorescens* has been documented to produce siderophores and other metabolites that inhibit fungal growth and enhance plant defense mechanisms (Afonso et al., 2021). Our results are consistent with previous research that highlights the synergistic effects of these bacteria in controlling Fusarium wilt in bananas.

4.4.2.1.3. *Trichoderma harzianum*

Trichoderma harzianum emerged as the most active biocontrol agent against *Foc* TR4 in our experiment, consistently showing the lowest disease severity indices values across all time points and varieties. *Trichoderma* species are well-known for their mycoparasitic activity, where they directly attack and degrade fungal pathogens through the production of hydrolytic enzymes such as chitinases and glucanases (Harman et al., 2004). Additionally, *Trichoderma* can induce systemic resistance in plants, enhancing their overall defense against a wide range of pathogens. Studies have shown that *T. harzianum* significantly reduces Fusarium wilt severity in banana plants by promoting root colonization and producing antifungal metabolites (Elad, 2000). The high efficacy observed in our study agrees with previous research, reinforcing *T. harzianum*'s role as a potent biocontrol agent.

4.4.2.1.4. Polyversum® (*Pythium oligandrum*)

Pythium spp. showed the highest disease severity indices values among the biocontrol treatments, indicating a lower efficacy in controlling Fusarium wilt. While *Pythium* species are often used as biocontrol agents against root pathogens, their effectiveness against *Fusarium* spp. may be limited due to differences in their mode of action and environmental requirements (Fu et al., 2016). *Pythium* primarily competes with pathogens for space and nutrients but may not produce the same level of antifungal metabolites as *Bacillus* or *Trichoderma* species. This could explain the relatively higher disease severity indices values observed in our experiment.

4.4.2.2. Resistance of Banana Varieties

Among the varieties tested, GCTCV 218 emerged as the most tolerant to *Foc* TR4 infections, as evidenced by its consistently lower disease severity indices values across all time points. This variety showed significant resistance to *Foc* TR4, with disease severity indices values lower than those observed in Baladi, Canarian, and Grand Nain varieties. Previous studies have highlighted the importance of using resistant varieties as a critical component of integrated disease management strategies. For instance, the work by Ghag et al. (2015) emphasizes that breeding and utilizing resistant banana varieties can provide a sustainable solution to manage Fusarium wilt, particularly when combined with effective biocontrol agents.

In conclusion, the findings of the experiment align with recent literature, and show that the integration of tolerant varieties with biocontrol agents may prove beneficial for sustainable banana production. This highlights the need for further research to optimize their application strategies (Wang et al., 2015; Ghag et al., 2015).

CHAPTER 5

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Bananas are cultivated on approximately 5.6 million hectares worldwide, yielding around 150 million metric tons annually (FAOSTAT, 2021). Lebanon contributes to this global production with approximately 165,500 tons each year, supporting both local consumption and exports to neighboring countries such as Syria and Jordan (Selina Wamucii, 2020). However, banana production faces a significant threat from Panama wilt disease, also known as Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4). This fungal strain poses a grave risk to the global banana industry, a crop of immense economic importance. Ordonez et al (2015) has estimated that over 100,000 hectares may be already affected by *Foc* TR4, threatening millions of smallholders worldwide. The presence of *Foc* TR4 in Lebanon, first reported in 2013, raises serious concerns about the sustainability of banana cultivation in the country, necessitating effective management strategies to curb the pathogen’s spread and mitigate its impact.

This study had three main objectives:

1. To investigate the extent of *Foc* TR4 spread in South Lebanon.
2. To examine the antagonistic activity of seven *Trichoderma* strains against *Foc* TR4 in vitro.
3. To assess the tolerance or susceptibility of four banana varieties to *Foc* TR4 and to evaluate the efficacy of four biocontrol agents in both field and pot experiments, with the aim of developing a locally adapted integrated disease management strategy.

A survey conducted across 87 sites in South Lebanon revealed a troubling expansion of *Foc* TR4, with 30% of the fields testing positive for the pathogen. This widespread presence is particularly alarming because *Foc* TR4 is a lethal disease that can persist in the soil for over a decade. The pathogen was first identified in Lebanon around a decade ago, initially confined to the regions of Mansouri and Berghliyeh, affecting only a few hectares. However, the current survey findings indicate significant expansion, demonstrating that the pathogen has not only persisted but has also spread across a much larger area, underscoring the inadequacy of previous containment efforts and the urgent need for more robust management strategies.

Laboratory evaluations using dual culture assays confirmed the efficacy of *Trichoderma* species as biocontrol agents. The *T. viride* strain T24 exhibited the highest antagonistic activity against *Foc* TR4, achieving a 74.44% inhibition rate. Other strains, including T42, T3, and *T. harzianum*, also demonstrated significant inhibition, ranging from 66% to 70%. These findings highlight the potential of *Trichoderma* spp. in integrated pest management strategies, offering a sustainable alternative to chemical fungicides, which are not recommended due to the rapid development of resistant strains.

Field trials and pot experiments revealed that among the four banana varieties tested, GCTCV 218 displayed the highest resistance to *Foc* TR4, with the lowest disease severity index and the highest bunch weight of approximately 31 kg under local edaphic and climatic conditions. This makes GCTCV 218 a valuable option for Lebanon and other regions affected by the pathogen.

Pot experiments further validated the effectiveness of *T. harzianum*, which significantly reduced Fusarium wilt severity. Using a disease severity scale from zero (no symptoms) to four (severe symptoms or plant death), *T. harzianum* reduced the mean disease severity index from 2.83 to 1, demonstrating its potential for incorporation into an integrated disease management strategy. Fulzyme®, a product containing *Bacillus subtilis* + *Pseudomonas putida,* also showed promising results.

The integration of biocontrol agents like *Trichoderma* with resistant varieties such as GCTCV 218 presents a promising approach to managing Fusarium wilt in South Lebanon. However, variability in treatment efficacy across different environmental conditions suggests the need for further research to optimize these strategies for local contexts. Understanding the interactions between biocontrol agents, soil composition, microclimates, and farm management practices will be crucial for ensuring long-term success.

Additionally, the long-term sustainability of biocontrol strategies requires continued research into the persistence of these agents in the soil, their interactions with native microbial communities, and their impact on banana health over multiple growing seasons. Exploring the genetic mechanisms behind GCTCV 218's resistance could also provide insights for breeding new resistant varieties, offering an additional layer of protection against *Foc* TR4.

In conclusion, the findings of this study emphasize the need for an integrated approach to managing Fusarium wilt in South Lebanon. Given the high economic losses caused by this disease, the following actions are recommended:

1. Conduct periodic surveys to monitor the spread of the disease. Utilizing aerial monitoring with drones and AI could significantly reduce the time required.
2. Launch farmer awareness campaigns to educate farmers about the symptoms, epidemiology, and economic impact of the disease, as well as the importance of

sanitation measures to prevent or contain its further spread. Distribute illustrated manuals to farmers.

3. Encourage policymakers and the Ministry of Agriculture to issue internal and external quarantine regulations to contain the spread of the disease and enforce the importation of "disease-free" banana seedlings.
4. Advise farmers to use locally adapted tolerant varieties. This study is the first to report on the locally tried tolerant variety GCTCV 218, which also showed high yield potential. Further trials are recommended to evaluate other promising varieties.
5. Continue research on biocontrol agents like *T. harzianum* and Fulzyme® to optimize application methods and timing, and to explore other potential biocontrol agents.
6. Develop an integrated disease management strategy. Combining biocontrol agents with resistant banana varieties and enforcing strict sanitation measures could mitigate the impact of *Foc* TR4 and protect Lebanon's banana industry.
7. Continued research and collaboration between farmers, researchers, and policymakers will be essential to translate these findings into practical, sustainable solutions for banana production in Lebanon and other regions affected by *Foc* TR4.

APPENDIX

DNA SEQUENCING RESULT OF TWO SAMPLES TAKEN DURING THE CONDUCTED SURVEY IN SOUTH LEBANON

Sample Number	DNA sequence
Sample 3.1	TAGGCACCTCTCCGAGACGACCTCACGGTACCACCGATGTGTTGGTCGGGCTCCTGTGCGGCCGTCCAGGGCGGGATATGTAGAGAATGTGGTGGTGTAGGGTAGGTGGCCAGGTCCAGGGTAGGTTCTCCAAATCCGTCAATCCGGCTTGAATCGGAGGACAGGTCTAGGGTAGGCCAGAGTCGGGTCTAGGGTAGGCAGCTCTAACCCTCGAAGTGGTCTACCCGGTAGTCAACTTCAATCGCCTCTCACGGCCGCCACGGACCTCGCATGACGACGGGACCACCACCATCGGATTTGCCTTGGTCGAAATAGTTGGTATATGCACTTTTGAAAAAATGCGTGCAAAATGGTTTTGAGGTTTGGTGGCCGTGAGTCGATTTTTTTGTTTTCCCATACAATGAATTTTGCGGAAATAAAAAGTGGC
Sample 5.1	TAGGCACCTCTCCGAGACGACCTCAACGGTACCACCGATGTGTTGGTCGGGCTCCTGTGCGGCCGTCCAGGGCGGGATATGTAGAGAATGTGGTGGTGTAGGGTAGGTGGCCAGGTCCAGGGTAGGTTCTCCAAATCCGTCAATCCGGCTTGAATCGGAGGACAGGTCTAGGGTAGGCCAGAGTCGGGTCTAGGGTAGGCAGCTCTAACCCTCGAAGTGGTCTACCCGGTAGTCAACTTCAATCGCCTCTCACGGCCGCCACGGACCTCGCATGACGACGGGACCACCACCATCGGATTTGCCTTGGTCGAAATAGTTGGTATATGCACTTTTGAAAAAATGCGTGCAAAATGGTTTTGTGGTTTGGTGGCCGTGAGTCGATTTTTTTGTTTTCCCATACAAATGAATTTTGCGGAAAATAAAAAGTGGC

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