



Received: 27-03-2025
Accepted: 07-05-2025

ISSN: 2583-049X

Evaluation of the Antagonistic Potential of *Trichoderma atroviride* and *Trichoderma harzianum* against Fusarium Wilt of Banana (FWB), under *in vitro* conditions

¹ Wariebi Alaric Browne, ² Mwajita Mwashasha, ³ Kavoo Agnes

^{1,2,3} Jomo Kenyatta University of Agriculture and Technology (JKUAT), P. O. Box 62000-00200, Nairobi, Kenya

¹ Ministry of Education, Republic of Liberia – Oldest Congo Town, P.O Box 1000-10, Monrovia, Liberia

Corresponding Author: Wariebi Alaric Browne

Abstract

Banana (*Musa spp.*) is the most valued and traded fruit worldwide and is a major food security crop and source of incomes for millions of people in the global south. *Fusarium oxysporum* f.sp. *cubense* (*Foc*), the causal agent of Fusarium wilt of banana (FWB) is the most prominent threat and devastating disease of banana, posing a serious threat to global banana trade. *Trichoderma* species are renowned biocontrol agents of many phytopathogens, showing encouraging results *in vitro*. This study was conducted to assess the antagonistic and biocontrol potentials and mechanisms of mycoparasitism of *Trichoderma atroviride* and *Trichoderma harzianum* against *Foc*. The Dual culture technique and culture filtrates were used to evaluate the efficacy of both *Trichoderma* species against *Foc*. Mycoparasitism of *Trichoderma* against *Foc* were

determined by observing a mixture of both fungal species taken from a 14-days old dual culture. The mixture was observed under a simple light microscope. The means of both *in vitro* tests was analysed in GenStat software, 14th edition ($p < 0.05$). Dual culture and culture filtrates of *T. atroviride* inhibited the mycelial growth of *Foc* by 60.56% and 65.33%, respectively. Consequently, *T. harzianum* exhibited 64.21% and 61.75% inhibition of *Foc*. As per Bell's Degree of Antagonism, both *Trichoderma* species were highly antagonistic against *Foc* after seven days. Both species demonstrated coiling, lysis, penetration and winding as mycoparasitism. The results show that *T. atroviride* is a promising biocontrol agent and can be used alongside *T. harzianum* in the management of Fusarium wilt of banana.

Keywords: Dual culture, *Fusarium oxysporum* f.sp. *cubense* (*Foc*), Fusarium Wilt of Banana (FWB), Mycoparasitism, *Trichoderma* species, *Trichoderma atroviride*

1. Introduction

Bananas and plantains (*Musa spp.*) singularly referred to as bananas, are the most popular and most produced fruits in the world with 179.3 million tonnes ^[1], and ranked sixth in food crop value ^[2]. There are several groups (and subgroups) and varieties of banana ^[3]. They are high in nutritional fibre, potassium, calcium, magnesium, and vitamins A, B6, and C, they are also exceptionally rich in carbohydrates ^[4,5]. Whether eaten cooked or fresh, bananas are essential component of the daily diet of millions of peoples in the global south, particularly in Africa where 21 kg of the fruit is eaten per capita ^[5]. Half of the Ugandan population consumed banana as their primary meal ^[6]. Dual-purpose bananas that can be cooked or utilized as dessert are commonly grown across Kenya ^[7]. Bananas are also used to wrap and cover food, make ropes and mats, thatch houses, and the leaves and trunks are used to feed livestock in eastern and central Kenya ^[8].

Approximately 15% of global banana production reached the international markets, while 85% are used for consumption and sold on local markets ^[5]; that 15% resulted in 52 million USD as per ^[2] reports. According to a ^[9] podcast, the livelihood of more than 400 million people in 125 countries is dependent on banana production. Banana is also an important source of income for poor, small-scale farmers in Uganda ^[6] and Kenya ^[7]. Banana is the foremost fruit and makes up 33.44% of importance value, 16% of all horticultural produce and 48.76% of fruit production in Kenya ^[10].

Agro-biological constraints, pests and diseases have been documented to be the major causes of banana yield losses and low productivity at both pre- and post-harvest levels ^[4]. Panama disease or Fusarium wilt of banana (FWB) is the prominent threat

and the most destructive of all diseases that attack banana. It is caused by the soil-dwelling, ubiquitous, ascomycete, hyphomycete and saprophytic fungi *Fusarium oxysporum* f. sp. *cubense* (*Foc*)^[11]. *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is a member of the *Fusarium oxysporum* species complex (FOSC)^[12], which are known producers of mycotoxins in food crops and pose significant danger to the lives of humans and animals^[13]. The disease has several cycles annually^[14] due to its chlamydospores, the paramount components of the pathogen's survival, overwintering for about 30 years in the soil^[15] or on alternative hosts until the suitable hosts and favourable conditions are available^[14].

Fusarium wilt of banana was first reported and described by Dr. Joseph Bancroft in Australia though the disease may have originated in Southeast Asia^[16]. *Foc* is known to exist as four strains or "races"^[17]. *Foc* Race 1 additionally impacts bananas of genome AAB and ABB^[18]. Race 2 has impacts on ensets and other ABB cooking bananas. Race 3 has negative impacts on most banana cultivars^[18], but occasionally affects the Gros Michel variety and *Heliconia* species^[19]. Presently, Cavendish banana is severely threatened by *Foc* Race 4, particularly Tropical Race 4 (*Foc*TR4). *Foc*TR4 has its origin in Taiwan, from where it has spread across the globe to Africa (Mozambique), Australia, the Middle East (Israel, Jordan, Lebanon, Oman, and Pakistan), South and Southeast Asia (India, Laos, Myanmar, Pakistan, Thailand and Vietnam) and Latin America (Colombia, Costa Rica, Ecuador and Venezuela)^[11, 20, 22, 21].

Infected asymptomatic planting materials are the primary ways that *Foc* spread internationally, nationally and locally^[16, 21]. Irrigation and drainage water, plant debris, dusts and contaminated soil particles clinging to any object, including agricultural implements, shoes, clothes, animals, and automobiles are also important ways the fungus spreads^[16, 23]. External FWB symptoms are manifested about two to five months after planting and infection of the roots^[24]. *Foc* damages the vascular tissues of banana plants, resulting in the eponymous wilting, chlorosis, formation of a "skirt" by collapsed leaves, splitting at the bottom of the banana trunks, xylem and internal rhizome discoloration, and subsequently plant death^[16, 12].

Biocontrol agents (BCAs) have been used in the management of phytopathogens, including fusarial pathogens and *Foc*. The use of BCAs is a sustainable environmental practice^[25]. Species of the filamentous, mycotrophic, ubiquitous, global, avirulent, opportunistic and fast-growing, prolific producers of spores and green conidia fungal genus *Trichoderma*^[26] are employed as BCAs. They are used as antagonists for most phytopathogens because of mechanisms including techniques which includes mycoparasitism, antibiosis, competition, and generation of systemic resistance in host plants^[25, 27]. The genus is also renowned for its plant growth promoting potential^[26]. Mycoparasitism, defined as a direct attack of one fungus on another, is an extremely complicated process that encompasses a series of phases, such as recognition, attack, and eventual penetration and death of the host fungus^[28, 27]. It is one of the biocontrol mechanisms carried out by *Trichoderma* species against phytopathogenic fungi.

Trichoderma harzianum, *T. viride*, *T. reesei* and other unidentified *Trichoderma* species have been experimented with in the management of FWB^[29, 30, 31].

The antagonistic and mycoparasitic behaviours of *Trichoderma atroviride* towards *Foc* has not been evaluated. Therefore, this study was conducted to examine the response of two *Trichoderma* species, *Trichoderma atroviride* and *Trichoderma harzianum*, against *Foc*, *in vitro*, and to examine their mechanism of mycoparasitism of the fusarial pathogen.

2. Materials and Methods

2.1 Biocontrol agents and phytopathogenic fungal strains

Fusarium oxysporum f. sp. *cubense*, used in this study was isolated from the rhizosphere of bananas as per the procedure described by^[32]. *Trichoderma atroviride* (Accession number: MH398583.1) and *Trichoderma harzianum* (Accession number: MK913350.1) isolated and identified by^[33] were utilized as the BCAs and their antagonistic ability against *Foc* was evaluated. The pathogen was cultured through serial dilution (10⁶). Subsequently, fungal strains were grown on recently prepared Potato Dextrose Agar media (HiMedia; composition: Potatoes (200g/l), Dextrose (20g / l), and Agar (15 g / l) supplemented with streptomycin. The isolates were cultured on 90 mm petri dishes and kept in an incubator for seven days at 25 °C prior to use.

2.2 *In vitro* determination of the efficacy of *Trichoderma* species against *Foc*

All experiments of Section 2.2 and 2.3 were conducted in triplicates in Completely Randomized Design (CRD) and repeated thrice. Plates were stored in complete darkness at 25 °C in an incubator for the duration of the experiments.

The Dual Culture technique as described by^[34] was used to test for efficacy of antagonism of *Trichoderma atroviride* and *Trichoderma harzianum* against *Foc*, *in vitro*. All fungi were used after an incubation period of seven (7) days. A sterilized corkborer was utilized to dissect 5 mm-diameter mycelia of *Foc*, which was placed in the middle of 90 mm petri dishes containing PDA media to be used as controls. Mycelial plugs (5 millimetres in diameter) of *Foc* were cut off and placed 10 mm from the edge of a 90 mm petri dishes and 5 mm mycelial plugs of *Trichoderma atroviride* and *Trichoderma harzianum* were also cut and positioned proportionally opposite from *Foc*. Individual fungus was considered as treatment. All petri dishes containing only *Foc* and those containing both the pathogen and the antagonists were placed in an incubator at 25 °C until the pathogen filled the control plates. Pathogen growth of both the control and those in the treatment plates were monitored during the incubation period. After the incubation period, five measurements of the mycelial growth of the pathogen were taken with Vernier callipers, and percentage inhibition over the control was calculated utilizing: $L = [(C - T)/C] \times 100$ ^[35]; where L = percent inhibition of mycelial growth; C = radial growth measurement of pathogen in control; T = radial growth measurement of pathogen in the presence of antagonists.

Table 1: Degree of Antagonism^[36]

Class	Characteristics
1	<i>Trichoderma</i> completely overgrew the pathogen and covered the entire medium surface
2	<i>Trichoderma</i> overgrew at least $\frac{2}{3}$ of the medium surface
3	<i>Trichoderma</i> and the pathogen each colonized approximately $\frac{1}{2}$ of the medium surface
4	The pathogen colonized at least $\frac{2}{3}$ of the medium surface
5	The pathogen completely overgrew the <i>Trichoderma</i> and covered the entire medium surface

2.3 Effects of culture filtrates (CFs) of *Trichoderma* species on *Foc*

The effect of the CFs of *Trichoderma atroviride* and *Trichoderma harzianum* on *Foc* was evaluated using the protocol described by^[37] and^[27], with some modifications. Individual 20 mycelial plugs (5 mm in diameter) each of *Trichoderma atroviride* and *Trichoderma harzianum* were dissected and inoculated afloat into separate 100 ml Potato Dextrose Broth (PDB, HiMedia) in 250 ml and 150 ml conical flasks, respectively, and placed on a rotary shaking incubator (150 rpm) at 25 °C. Following eight days of incubation, the resulting culture broth was filtered employing a double layer cheesecloth, then filtered through a Whatman filter paper No. 1 and finally refiltered using Millipore membrane filters (0.45 μ) for the removal of mycelial mats and production of purified culture filtrates. To attain a final concentration of 20% for the culture filtrates, four ml of each *Trichoderma* species' culture filtrates were added to individual sterile petri plates (90 mm), and then 16 ml of PDA was poured in promptly. The control plates were freed of culture filtrates of the *Trichoderma* species. Five (5) \mm-diameter mycelial discs of *Foc* were placed in the middle of PDA plates of both the treatments and control. All plates were incubated at 25 °C and checked every 24 hours until the pathogen covered the control plates. Using Vernier callipers, *Foc*'s mycelial diameters in the control and treated plates were measured. The mycelial percent inhibition rate was computed using this formula: % inhibition = (A – B) / A x 100 (27); where: A is the diameter of the mycelial growth of the pathogen in the control plates; B is the diameter of the mycelial growth of the pathogen in the treatment plates.

2.4 Mycoparasitism of *Trichoderma* species versus *Foc*

The mycoparasitic activities of *T. atroviride* and *T. harzianum* against *Foc* was evaluated using this protocol: A sterilized inoculating needle was used to pick from above the pathogen a mixture of one of the *Trichoderma* sp. and *Foc* hyphae from a 14-days old dual culture setup as depicted in Section 2.2. This mixture was placed on a microscope slide and stained with two drops of lactophenol cotton blue and covered with a slip. The slide was observed under a light microscope (OPTIKA Microscopes, Italy) at 40x magnification.

2.5 Data Analysis

The recorded data from both antagonistic tests (dual culture assay and culture filtrates) were subjected to statistical analysis in GenStat, 14th Edition (<http://www.biometris.wur.nl/uk/Software/Genstat+Procedures.htm>), with the data on dual culture assay been arcsine transformed before undergoing analysis. Both sets of data were subjected to a one-way analysis of variance (ANOVA), and means were compared using Tukey's Honest Significant Difference (HSD) test. The means of all replicates \pm

standard deviation was considered statistically significant at $p \leq 0.05$.

3. Results

3.1 Biocontrol agents and phytopathogenic fungal strains

All fungal species were cultured and subcultured on PDA and incubated at 25 °C. The pathogen, *Foc*, appeared to be a slow grower with hairy, pinkish (and on the reverse, pink) mycelium. Microscopically, the macroconidia looked straight, thin walled, with four to five septa; while the microconidia were oval and kidney shaped (Fig 1). The description of the pathogen matched that of^[32] and^[38]. *Trichoderma atroviride* initially appeared greenish-white before the formation of conidia and the characteristically green colour of the spores became visible. It looked dull on the reverse (Fig 2). *T. atroviride* also gave out a distinct coconut odour. *Trichoderma harzianum* produced a spacious, vast concentric ring, with white mycelium, that later turned green. The strain appeared yellowish on the reverse (Fig 3). Both *Trichoderma* species had green, globose conidia and grew quickly.

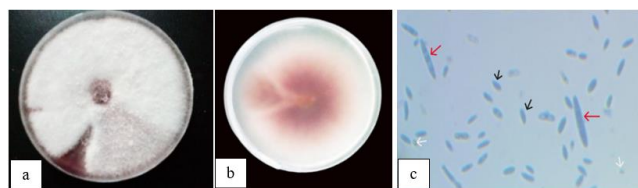


Fig 1: Morphological and microscopic characteristics of *Foc*: (a) Front, (b) reverse and (c) macroconidia (red arrow) microconidia (black arrow), and globose chlamydospores (white arrow)

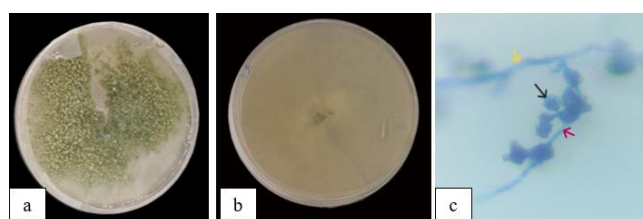


Fig 2: Morphological and microscopic characteristics of *Trichoderma atroviride*: (a) Front, (b) reverse; (c) conidia (black arrow), branch conidiophore (red arrow) and hyphae (yellow arrow)

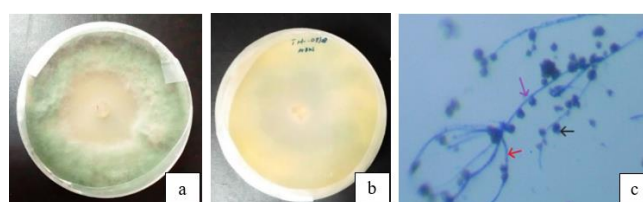


Fig 3: Morphological and microscopic characteristics of *Trichoderma harzianum*: (a) Front, (b) reverse; (c) hyphae (purple arrow), conidia (black arrow) and branched conidiophore (red arrow).

3.2 In vitro determination of the efficacy of *Trichoderma* species against *Foc*

The results revealed that both *Trichoderma* species, *T. atroviride* and *T. harzianum* significantly ($p < 0.05$) reduced the growth of *Foc* in the treatment plates when compared to the control plates. The mycelial growth in the control plates was averaged at 83.83 mm. *Trichoderma harzianum* inhibited the pathogen mycelial growth by 64.23% (30 mm), while *T. atroviride* reduced the growth of *Foc* by 60.56%

(33.06 mm) (Table 2). This was most likely triggered by competition for the limited nutrients and space. All results were highly significant ($p < 0.001$). The *Trichoderma* species completely overgrew the pathogen, including on both sides and displayed a clear zone of inhibition (Fig 4). Due to the difference in the growth rates of the pathogen and the BCAs, culture filtrates of both *Trichoderma* species were used to conduct a second antibiosis test with the aim of attaining uniform growth period.

Table 2: Dual Culture Assay of *Trichoderma* species vs *Foc*

Treatments	Mycelial growth of <i>Foc</i> (mm)*	Percent inhibition of radial mycelial growth (%)	Antagonistic classification ⁺	
			Day 7	Day 14
<i>Foc</i> + <i>T. atroviride</i>	33.06 ± 0.737 (35.06 ± 0.479) ^a	60.56 (47.23)	2	1
<i>Foc</i> + <i>T. harzianum</i>	30.00 ± 3.901 (33.12 ± 2.355) ^a	64.21 (50.15)	2	1
<i>Fusarium oxysporum</i> f.sp <i>cubense</i> (<i>Foc</i>)	83.83 ± 1.086 (66.44 ± 0.704) ^b			

* Mean of three replicates ± standard deviation. Results in parentheses are arcsine transformed.

^{a, b} Means with the same superscript are not significantly different from one another.

+ (36)'s Degree of Antagonism

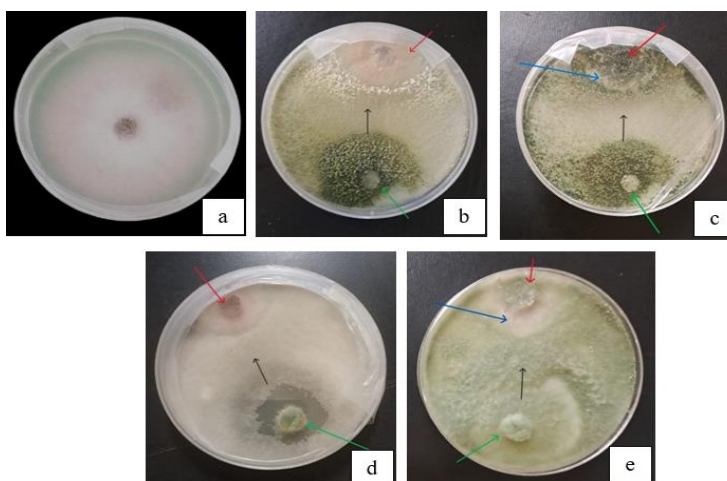


Fig 4: Dual culture assay of *Foc* vs *Trichoderma* species: (a) Control, *Foc*; (b) *Trichoderma atroviride* vs *Foc* after seven days; (c) and after 14 days; (d) *Trichoderma harzianum* vs *Foc* after seven days; (e) and after 14 days. The red arrow indicates *Foc*; green arrow indicates the *Trichoderma* species. The blue arrow points out the zone of inhibition and the black arrow shows the direction of growth of the *Trichoderma* species.

3.3 Effects of culture filtrates of *Trichoderma* species on *Foc*

The experiment with the culture filtrates (CFs) of *Trichoderma* species against *Foc* revealed significant suppression ($p < 0.05$) of the pathogen by the BCAs. Purified

CFs of *T. atroviride* recorded mycelial inhibitory rate of 65.33% (23.58 mm), while *T. harzianum* produced an inhibitory rate of 61.75% (26.02 mm) (Table 3). The average mycelial growth in the control plates was recorded as 68.02 mm.

Table 3: Effects of Culture filtrates of *Trichoderma* species vs *Foc*

Treatments	Mycelial growth of <i>Foc</i> (mm)*	Percent inhibition of radial mycelial growth (%)
<i>Fusarium oxysporum</i> f.sp <i>cubense</i> (<i>Foc</i>)	68.02 ± 4.871 ^a	
<i>Foc</i> + <i>T. harzianum</i>	26.02 ± 0.611 ^b	61.75
<i>Foc</i> + <i>T. atroviride</i>	23.58 ± 0.616 ^b	65.33

* Mean of three replicates ± standard deviation.

^{a, b} Means with the same superscript are not significantly different from one another.

3.4 Mycoparasitism of *Trichoderma* species versus *Foc*

Both *T. atroviride* and *T. harzianum* demonstrated mycoparasitism versus *Foc*. *T. atroviride* exhibited penetration and winding (Fig 5) as mechanisms of mycoparasitism against the pathogen, whereas *T. harzianum* portrayed its mycoparasitic behaviour against *Foc* in the form of lysis and coiling (Fig 6).

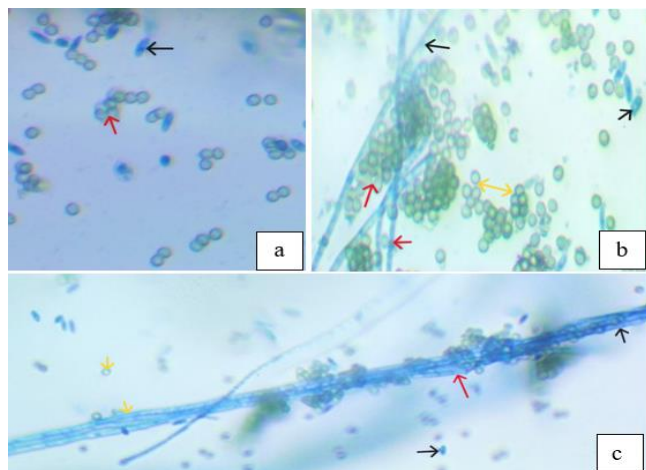


Fig 5: Mycoparasitism of *Trichoderma atroviride* against *Foc*: *Trichoderma atroviride*: (a) interaction with *Foc* (black arrow: microconidia of *Foc*; red arrow: spores of *T. atroviride*); (b) penetration (black arrow: microconidia and hyphae of *Foc*; yellow arrow: spores of *T. atroviride*; red arrow: point of penetration); (c) winding (black arrow: hyphae and conidia of *Foc*; yellow arrow: hyphae and spores of *T. atroviride*; red arrow: point of winding).

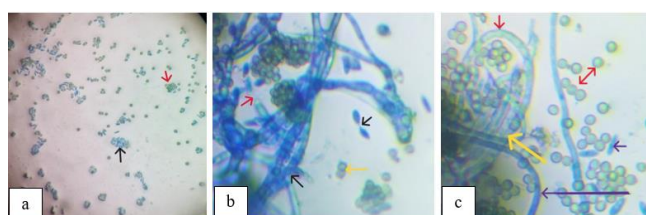


Fig 6: Mycoparasitism of *Trichoderma harzianum* against *Foc*: *Trichoderma harzianum*: (i) interaction with *Foc* (black arrow: microconidia of *Foc*; red arrow: spores of *T. harzianum*); (ii) lysis of *Foc* by *T. harzianum* (black arrow: microconidia and hyphae of *Foc*; yellow arrow: spores of *T. harzianum*; red arrow: lysis); (iii) coiling: yellow arrow; (purple arrow: microconidia and hyphae of *Foc*; red arrow: spores and hyphae of *T. harzianum*)

4. Discussions

4.1 Efficacy of the most abundant beneficial fungal isolates against *Foc*

Assessment of the antagonistic ability of *T. atroviride* and *T. harzianum* against *Foc* under *in vitro* conditions was conducted using the dual culture assay and culture filtrates (CFs) on PDA petri plates at 25 °C. The capability of *Trichoderma* species to inhibit the growth of phytopathogens and conduct mycoparasitism is consequential of the productions of secondary metabolites and non-volatile suppressive compounds. Multiple studies have revealed that *Trichoderma* spp. can suppress phytopathogenic agents and diseases by a variety of methods, including mycoparasitism, production of extracellular enzymes including cellulase, amylase, pectinase, protease, and chitinase, antagonistic chemicals, and induced resistance [28, 39, 40, 41, 42, 43].

[39] and [44] have demonstrated the ability of 6-pentyl-2H-pyran-2-one (one pyrone) (6-PP), a volatile compound

associated with coconut aroma, characteristic of *T. atroviride*, and other aromatic compounds including pyrones, volatile terpenes, and isocyanates, to inhibit the growth of various fungal pathogens *in vitro*, including *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) and *Rhizoctonia solani*. These authors claim that volatile compounds act at a distance, thereby empowering the restricted action of the enzymes and the non-volatile antifungal compounds. The findings of [41] showed that every strain of *T. atroviride* could create enzymes that break down chitin, which is an essential function for a mycoparasite. *Trichoderma atroviride* strains produced soluble antifungal chemical compounds, which suppressed *Fusarium graminearum* more effectively. [42] also credited bioactive secondary metabolites including 6-pentyl-2H-pyran-2-one, quinoline, phenol, 2-(6-hydrazino-3-pyridazinyl), heptadecane, 17-methoxy-4-methyl-d-homo-18-norandrosta, nonadecane, heneicosane, eicosane, dibutyl phthalate, hexadecane and benzene propionic acid for the antifungal prowess of *T. atroviride*. These compounds are documented to have impeded the mycelial growth of various phytopathogenic fungi [45, 46]. [47] isolated 6-pentyl-2H-pyran-2-one (one pyrone) from *T. harzianum* and reported that it reduced the growth of *R. solani* and *Fol* by 69.6% and 31.7%, respectively. They also mentioned that CFs of *T. harzianum* produced many antibiotics, including trichorzianins A and B, trichorzin, etc. The capacity of *Trichoderma* spp. to generate cell wall-degrading enzymes such as b-(1,6)-glucanases, chitinases, and proteases may be culpable for the lysis of the fusarial mycelia [48]. The secondary metabolites and enzymes that are responsible for the antifungal, antimicrobial and plant growth promoting abilities of *Trichoderma* species against various plant pathogens are well documented [39, 48, 49].

4.1.1 *In vitro* determination of the efficacy of *Trichoderma* species against *Foc* using Dual Culture Assay

Inhibition of the mycelial growth of pathogen using the dual culture assay is frequently employed to examine the biocontrol potential of a beneficial microorganism. The procedure is extensively used because of its convenience and ease of usage. However, its results are often suggestive [41]. In this study, *T. atroviride* and *T. harzianum* inhibited the mycelial growth of *Foc* by 60.56% and 64.21%, respectively, in dual culture plates. The result of *T. atroviride* in this study was more significant than the 38.12% and 50.33% inhibition that was reported by [50] and [51], respectively, who reported antagonistic actions of *Trichoderma* species against *Foc*TR4. [52] disclosed that *T. viride* suppressed the radial growth of *Foc* by 55.11%, which is less than our findings. The findings of *T. atroviride*'s percentage inhibition of radial growth (PIRG) also coincided with the results of [53] and [42] who revealed that the mycelial growth of *Fol* was reduced by *T. atroviride* between 52.37%–70.56% and 46.22%–71.25%, respectively. The result of *T. harzianum* (60.56%) in this study is higher than what was reported by [54] and [55], who both experimented with *T. harzianum* against *Foc* and *Fusarium* spp., respectively. [54] reported a reduction of mycelial growth of 43.6%, while [55] established that *T. harzianum* recorded a reduction of the mycelial growth of *Fusarium* spp. on the scale of 29.56%–53.05%. [27] corroborated the antagonistic efficacy of the *T. harzianum*

strain against *Fusarium proliferatum* and *F. verticillioides*, whose mycelial inhibition rate of 68.38% and 60.64%, respectively, is consistent with our findings. The result of this current study is similar to those of [50] and [56] who reported that *T. harzianum* suppressed the mycelial growth of *Fol* and *Fusarium oxysporum* f. sp. *phaseoli* by 53% and 51.8%–54.6%, correspondingly. Based on (36)'s classification of antagonism, *T. atroviride* and *T. harzianum* were shown to be highly antagonistic against *Foc*, similar to what was reported by [56] and [57]. When *Trichoderma* species operate as antagonists, conspicuous inhibition zones without hyphae interactions indicates that the *Trichoderma* strain emits diffusible, non-volatile suppressive chemical compounds [37].

4.1.2 Effects of Culture Filtrates (CFs) of *Trichoderma* species on *Foc*

Since CFs effectively suppress plant pathogens, their application in agricultural systems is currently being seen as an emerging approach to controlling plant diseases and is receiving considerable attention [31]. In this study, *Trichoderma* CFs were applied *in vitro* and displayed a substantial ($p < 0.001$) inhibition of *Foc*'s mycelial expansion. Our results align with those of [42] who observed 52.2%–77.77% mycelial inhibition rates in their study on the antifungal efficacy of *T. atroviride* CFs against *Fol*. [31] established the percentage of *A. solani* mycelial growth that was suppressed by *T. atroviride* CFs was 57.8%, which is consistent with our findings. The CFs of *T. harzianum* inhibited *Foc* at 61.75% (26.02 mm), higher than the 17.43%–20.59% recorded of the *T. harzianum* CFs against *Fusarium oxysporum* [37]. Our results agree with those of [27], who documented the mycelial inhibition rates of 44.09% and 23.5%, for the antifungal efficacy of CFs of *T. harzianum* strains against *F. oxysporum*. The mycelial growth of *A. solani* was reduced (62.5%) by the CFs of *T. harzianum*, corresponding to a lower mycelial growth [31]. The antifungal efficacy of *Trichoderma* isolate CFs against *F. oxysporum* species was validated by [58], who also suggested that the synthesis of active secondary metabolites accounted for the CFs' strong potency.

4.1.3 Mycoparasitism of *Trichoderma* species against *Foc*

Coiling, penetration, winding and lysis of the pathogen's mycelia were evidence of both *Trichoderma* strains mycoparasitism against *Foc*. *Trichoderma asperellum* utilized winding as a mechanism of mycoparasitism against *F. oxysporum* [59], same was displayed by *T. atroviride* in this study. Unlike this present study where *T. atroviride* displayed mycoparasitism, [60] and [27] revealed that *T. atroviride* and *T. viride*, respectively did not demonstrate any specific mycoparasitic behavior against several phytopathogens. *Trichoderma harzianum* strains are reported to have demonstrated mycoparasitic action against fusarium pathogens. For instance, *T. harzianum* displayed coiling, lysis, and penetration of the mycelia of fusarial and other fungal pathogens [61, 62, 27], which corroborate with our results. Pathogen cells enlarge, swell, shorten, round out, lose their protoplasm, and shatter their cell walls because of *Trichoderma*'s direct invasion or winding of the mycelium [63]. Mycoparasitism is one of several modes of action, including antibiosis, competition for nutrients and space, and modification of the rhizospheres that makes *Trichoderma* species more effective as BCAs [28, 40].

5. Conclusions

Both *Trichoderma* species utilized antibiosis, competition for nutrients and space and mycoparasitism as control of *Foc in vitro*. *Trichoderma atroviride* and *T. harzianum* showed significant reduction in the mycelial growth of *Foc* by both dual culture and CFs and were highly antagonistic against the pathogen according to Bell's scale. They also displayed various mechanisms of mycoparasitism against *Foc*. The production of several secondary metabolites and enzymes are responsible for the antagonistic action of the *Trichoderma* species. Here we report for the first time the use of *Trichoderma atroviride* as a BCA, *in vitro*, against *Fusarium oxysporum* f. sp. *cubense*.

6. Recommendations

These results prove that *Trichoderma atroviride* has the potential to be utilized as an alternative BCA to various chemicals on the markets, or used alongside commercially available *T. harzianum* in the control of Fusarium wilt of banana and for the protection of the environment and small-scale banana producers.

7. Acknowledgement

The authors acknowledge UNIDO – Liberia and the European Union (EU) for funding this research. The authors also acknowledge the teams at the Institute of Biotechnology Research (IBR) and the Horticultural Laboratory of the Jomo Kenyatta University of Agriculture and Technology (JKUAT) for their help and collaboration.

8. References

1. FAOSTAT. Banana Market Review 2023 [Internet]. Rome, Italy: Food and Agriculture Organization of the United Nations. Statistics Division; 2023 p. 1–20. Available from: <https://www.fao.org/faostat/en/#data>
2. FAO. Banana statistical compendium 2017 [Internet]. Rome: Food and Agriculture Organization of the United Nations, 2018, p1-22. Available from: https://www.fao.org/fileadmin/templates/est/COMM_MARKETS_MONITORING/Bananas/Documents/Banana_Statistical_Compndium_2017.pdf
3. Musapedia contributors. Banana cultivar checklist. Musapedia Banana Knowl Compendium [Internet]. 2021 Jun 2 [cited 2024 Oct 14]; Available from: <https://www.promusa.org/Banana+cultivar+checklist>
4. SHEP PLUS, Ministry of Agriculture, Livestock & Fisheries, Agriculture and, Food Authority (Horticultural Crops Directorate (HCD) of the Republic of Kenya, Japan International Cooperation Agency (JICA). Smallholder Horticulture Empowerment & Promotion Project for Local and Up-Scaling (SHEP PLUS) Banana Production. In: Banana Production [Internet]. Kenya; 2019. Available from: https://www.jica.go.jp/Resource/project/english/kenya/015/materials/c8h0vm0000f7o8cj-att/materials_18.pdf
5. IITA. Banana and Plantain (*Musa acuminata* & *balbisiana* hybrids) [Internet]. International Institute of Tropical Agriculture (IITA), 2022. Available from: <https://www.iita.org/cropsnew/banana-plantain/#1620839914105-72dd6f05-477d>
6. Elias O, William T, Georgina K, Wacal C. Distribution and farmers knowledge on Fusarium wilt (Race 1) in cropping systems of Uganda. Afr J Plant Sci [Internet]. 2021 Nov 30 [cited 2024 Aug 16];15(11):277–87.

- Available from: <https://academicjournals.org/journal/AJPS/article-abstract/D2F2CBA68233>
7. Wahome CN, Maingi JM, Ombori O, Kimiti JM, Njeru EM. Banana Production Trends, Cultivar Diversity, and Tissue Culture Technologies Uptake in Kenya. Serrano M, editor. *Int J Agron* [Internet]. 2021 Feb 16 [cited 2024 Aug 17]; 2021:1-11. Available from: <https://www.hindawi.com/journals/ija/2021/6634046/>
 8. Njau N, Mwangi M, Mbaka J, Gathu R, Muasya R. Biotic constraints to banana production in Eastern and Central Provinces of Kenya. In *Entebbe, Uganda: RUFORUM*, 2010, p267-269. Available from: <https://ir-library.ku.ac.ke/bitstream/handle/123456789/10276/Biotic%20Constraints%20to%20Banana%20Production%20in%20Eastern%20and%20Central%20Provinces%20of%20Kenya.pdf?sequence=3&isAllowed=y>
 9. The Documentary: Going Banana [Internet]. The Documentary. London, UK: The British Broadcasting Corporation; 2024 [cited 2024 Aug 13]. Available from: <https://www.bbc.co.uk/sounds/play/w3ct6x3x>
 10. AFA A and FA. AFA YEAR BOOK OF STATISTICS 2022 [Internet]. Nairobi, Kenya: Agriculture and Food Authority, 2022, 1-141. Available from: https://afa.go.ke/resources/files/a5ea24ba-5fb2-4a62-b4bc-e750c0605ed2_afa-year-book-of-statistics-2022.pdf
 11. Maymon M, Sela N, Shpatz U, Galpaz N, Freeman S. The origin and current situation of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in Israel and the Middle East. *Sci Rep* [Internet]. 2020 Jan 31 [cited 2024 Apr 10]; 10(1):1590. Available from: <https://www.nature.com/articles/s41598-020-58378-9>
 12. Niwas R, Chand G, Nath Gupta R. *Fusarium* Wilt: A Destructive Disease of Banana and Their Sustainable Management. In: Mahyar Mirmajlessi S, editor. *Fusarium: An Overview of the Genus* [Internet]. IntechOpen, 2022 [cited 2024 Apr 11], 1-10. Available from: <https://www.intechopen.com/chapters/79683>
 13. Vismar HF, Shephard GS, Van Der Westhuizen L, Mngqawa P, Bushula-Njah V, Leslie JF. Mycotoxins produced by *Fusarium proliferatum* and *F. pseudonygamai* on maize, sorghum and pearl millet grains *in vitro*. *Int J Food Microbiol* [Internet]. 2019 May [cited 2024 Apr 13]; 296:31-36. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0168160519300418>
 14. Ploetz RC, Pegg KG. Fungal diseases of the root, corm and pseudostem. In: Jones DR, editor. *Diseases of banana, Abacá and Enset*. Wallingford (GBR): CABI, 2000, 143-172.
 15. Wong CKF, Vadamalai G, Saidi NB, Zulperi D. Research Progress, Challenges And Future Perspectives On The Management Of *Fusarium* Wilt Of Banana In Malaysia: A Review. *Malays J Sci* [Internet]. 2019 Aug 28; 38(3):47-66. Available from: <https://mjs.um.edu.my/article/view/15113>
 16. Pegg KG, Coates LM, O'Neill WT, Turner DW. The Epidemiology of *Fusarium* Wilt of Banana. *Front Plant Sci* [Internet]. 2019 Dec 20 [cited 2024 Apr 11]; 10:1395. Available from: <https://www.frontiersin.org/article/10.3389/fpls.2019.01395/full>
 17. Ploetz RC. *Fusarium* Wilt of Banana Is Caused by Several Pathogens Referred to as *Fusarium oxysporum* f. sp. *cubense*. *Phytopathology*® [Internet]. 2006 Jun [cited 2024 Aug 22]; 96(6):653-656. Available from: <https://apsjournals.apsnet.org/doi/10.1094/PHTO-96-0653>
 18. Ploetz RC. Management of *Fusarium* wilt of banana: A review with special reference to tropical race 4. *Crop Prot* [Internet]. 2015 Jul [cited 2024 Apr 11]; 73:7-15. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0261219415000228>
 19. CABI. *Fusarium oxysporum* f.sp. *cubense* (Panama disease of banana). *PlantwisePlus Knowl Bank* [Internet]. 2022 Jan [cited 2022 Jan 7]. Doi: <https://doi.org/10.1079/pwkb.species.24621>
 20. Mmadi M, Azali HA, Mostert D, Robène I, Viljoen A. First Report of *Fusarium* Wilt of Cavendish Bananas Caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 in the Grande Comoros Island. *Plant Dis* [Internet]. 2023 Dec 1 [cited 2025 Mar 31]; 107(12):4029. Available from: <https://apsjournals.apsnet.org/doi/10.1094/PDIS-07-23-1288-PDN>
 21. Dita M, Barquero M, Heck D, Mizubuti ESG, Staver CP. *Fusarium* Wilt of Banana: Current Knowledge on Epidemiology and Research Needs Toward Sustainable Disease Management. *Front Plant Sci* [Internet]. 2018 Oct 19 [cited 2024 Apr 11]; 9:1468. Available from: <https://www.frontiersin.org/article/10.3389/fpls.2018.01468/full>
 22. FAO. *Food Outlook - Biannual Report on Global Food Markets* [Internet]. Rome: Food and Agriculture Organization of the United Nations, Nov 2019, 12-20. Available from: <https://openknowledge.fao.org/server/api/core/bitstream/s/5b53665b-3767-4681-9cad-ebf60d5d1d8e/content>
 23. Musapedia contributors. *Fusarium* wilt of banana. *Musapedia Banana Knowl Compendium* [Internet]. 2022 Mar 9; Available from: https://www.promusa.org/Fusarium+wilt#Modes_of_transmission
 24. Jamil FN, Hashim AM, Yusof MT, Baity N. Association of soil fungal community composition with incidence of *Fusarium* wilt of banana in Malays. *Mycologia*. 2023; 115(2):178-186.
 25. Monte E. Understanding *Trichoderma*: Between biotechnology and microbial ecology. *Int Microbiol Off J Span Soc Microbiol*. 2001 Mar; 4(1):1-4.
 26. Woo SL, Hermosa R, Lorito M, Monte E. *Trichoderma*: a multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. *Nat Rev Microbiol* [Internet]. 2023 May 1; 21(5):312-326. Available from: <https://doi.org/10.1038/s41579-022-00819-5>
 27. Yassin MT, Mostafa AAF, Al-Askar AA, Sayed SRM, Rady AM. Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* strains against some fusarial pathogens causing stalk rot disease of maize, *in vitro*. *J King Saud Univ - Sci* [Internet]. 2021 May [cited 2024 Aug 14]; 33(3):101363. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1018364721000240>
 28. Benítez T, Rincón A, Limón MC, Codón A. Biocontrol mechanism of *Trichoderma* strains. *Int Microbiol Off J*

- Span Soc Microbiol [Internet]. 2004 Sep 15; 7:249-260. Available from: www.im.microbios.org
29. Bubici G, Kaushal M, Prigigallo MI, Gómez-Lama Cabanás C, Mercado-Blanco J. Biological Control Agents Against Fusarium Wilt of Banana. *Front Microbiol* [Internet]. 2019 Apr 5 [cited 2024 Aug 23]; 10(616):1-33. Available from: <https://www.frontiersin.org/article/10.3389/fmicb.2019.00616/full>
 30. Damodaran T, Rajan S, Muthukumar M, Ram Gopal, Yadav K, Kumar S, *et al.* Biological Management of Banana Fusarium Wilt Caused by *Fusarium oxysporum* f. sp. cubense Tropical Race 4 Using Antagonistic Fungal Isolate CSR-T-3 (*Trichoderma reesei*). *Front Microbiol* [Internet]. 2020 Dec 16 [cited 2024 Oct 28]; 11(595845):1-19. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.595845/full>
 31. Imran M, Abo-Elyousr KAM, Mousa MAA, Saad MM. Use of *Trichoderma* culture filtrates as a sustainable approach to mitigate early blight disease of tomato and their influence on plant biomarkers and antioxidants production. *Front Plant Sci* [Internet]. 2023 Jul 17 [cited 2024 Aug 14]; 14:1192818. Available from: <https://www.frontiersin.org/articles/10.3389/fpls.2023.1192818/full>
 32. Pérez-Vicente L, Dita MA, de la Parte EM. Technical Manual Prevention and diagnostic of Fusarium Wilt (Panama disease) of banana caused by *Fusarium oxysporum* f. sp. cubense Tropical Race 4 (TR4). In *West Indies University, Port Spain, Trinidad and Tobago*, 2014, 1-75.
 33. Wariebi AB, Kavoo A, Mwajita M, Wekesa TB. Morphological and Molecular Characterization of Fungal Species Associated with *Fusarium oxysporum* f.sp. cubense (Foc) in Gatundu North, Kenya. *Int J Hortic Sci Technol*, Jan 2025; 12(1):281-296.
 34. Kunova A, Bonaldi M, Saracchi M, Pizzatti C, Chen X, Cortesi P. Selection of *Streptomyces* against soil borne fungal pathogens by a standardized dual culture assay and evaluation of their effects on seed germination and plant growth. *BMC Microbiol* [Internet]. 2016 Dec [cited 2024 Aug 12]; 16(1):272. Available from: <http://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-016-0886-1>
 35. Hajieghrari B, Torabi-Giglou M, Mohammadi MR. Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. *Afr J Biotechnol* [Internet], Apr 17, 2008; 7(8):967-972. Available from: <http://www.academicjournals.org/AJB>
 36. Bell DK, Wells HD, Markham CR. *In vitro* Antagonism of *Trichoderma* Species Against Six Fungal Plant Pathogens. *Phytopathology*. 1982; 72(4):379-382.
 37. Perveen K, Bokhari NA. Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*. *Afr J Microbiol Res* [Internet], Apr 9, 2012; 6(13):3348-3353. Available from: <http://www.academicjournals.org/AJMR>
 38. Leslie JF, Summerell BA. *The fusarium laboratory manual*. 1st ed. Ames (Iowa): Blackwell Publishing, 2006, p388.
 39. Reino JL, Guerrero RF, Hernández-Galán R, Collado IG. Secondary metabolites from species of the biocontrol agent *Trichoderma*. *Phytochem Rev* [Internet]. 2007 Oct 17 [cited 2024 Sep 6]; 7(1):89-123. Available from: <http://link.springer.com/10.1007/s11101-006-9032-2>
 40. Sharma P. Complexity of *Trichoderma*-*Fusarium* interaction and manifestation of biological control. *Aust J Crop Sci*. 2011; 5(8):1027-1038.
 41. Cabrera M, Garmendia G, Rufo C, Pereyra S, Vero S. *Trichoderma atroviride* como controlador biológico de fusariosis de espiga de trigo mediante la reducción del inóculo primario en rastrojo. *Rev TERRA Latinoam* [Internet]. 2020 Jul 28 [cited 2024 Sep 2]; 38(3):629-651. Available from: <http://www.terralatinoamericana.org.mx/index.php/terra/article/view/664>
 42. Yogalakshmi S, Thiruvudainambi S, Kalpana K, Thamizh Vendan K, Oviya R. Antifungal activity of *Trichoderma atroviride* against *Fusarium oxysporum*.f.sp.lycopersici causing wilt disease of tomato. *J Hortic Sci* [Internet]. 2021 Dec 31 [cited 2024 Aug 14]; 16(2):241-250. Available from: <https://jhs.iihr.res.in/index.php/jhs/article/view/1066>
 43. Hegde GM, Vijaykumar KN. Mechanisms of Resistance of *Trichoderma* spp. against Plant Disease Management. *Asia-Pac Biofertilizers Biopestic Inf Platf* [Internet]. 2023 Jul 6; Available from: <https://apbb.fftc.org.tw/article/413>
 44. Garnica-Vergara A, Barrera-Ortiz S, Muñoz-Parra E, Raya-González J, Méndez-Bravo A, Macías-Rodríguez L, *et al.* The volatile 6-pentyl-2H-pyran-2-one from *Trichoderma atroviride* regulates *Arabidopsis thaliana* root morphogenesis via auxin signaling and Ethylene Insensitive 2 functioning. *New Phytol* [Internet]. 2016 Mar [cited 2024 Sep 6]; 209(4):1496-512. Available from: <https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.13725>
 45. Keszler A, Forgacs E, Kotai L, Vizcaino JA, Monte E, Garcia-Acha I. Separation and Identification of Volatile Components in the Fermentation Broth of *Trichoderma atroviride* by Solid-Phase Extraction and Gas Chromatography--Mass Spectrometry. *J Chromatogr Sci* [Internet]. 2000 Oct 1 [cited 2024 Sep 7]; 38(10):421-424. Available from: <https://academic.oup.com/chromsci/article-lookup/doi/10.1093/chromsci/38.10.421>
 46. Jeleń H, Błaszczuk L, Chełkowski J, Rogowicz K, Strakowska J. Formation of 6-n-pentyl-2H-pyran-2-one (6-PAP) and other volatiles by different *Trichoderma* species. *Mycol Prog* [Internet]. 2014 Aug [cited 2024 Sep 7]; 13(3):589-600. Available from: <http://link.springer.com/10.1007/s11557-013-0942-2>
 47. Claydon N, Allan M, Hanson JR, Avent AG. Antifungal alkyl pyrones of *Trichoderma harzianum*. *Trans Br Mycol Soc* [Internet]. 1987 Jun [cited 2024 Sep 9]; 88(4):503-513. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0007153687800347>
 48. Sood M, Kapoor D, Kumar V, Sheteiwiy MS, Ramakrishnan M, Landi M, *et al.* *Trichoderma*: The “Secrets” of a Multitalented Biocontrol Agent. *Plants* [Internet]. 2020 Jun 18 [cited 2024 Sep 9]; 9(6):762.

- Available from: <https://www.mdpi.com/2223-7747/9/6/762>
49. Guzmán-Guzmán P, Kumar A, De Los Santos-Villalobos S, Parra-Cota FI, Orozco-Mosqueda MaDC, Fadji AE, *et al.* Trichoderma Species: Our Best Fungal Allies in the Biocontrol of Plant Diseases: A Review. *Plants* [Internet]. 2023 Jan 17 [cited 2024 Sep 9]; 12(3):432. Available from: <https://www.mdpi.com/2223-7747/12/3/432>
 50. Sundaramoorthy S, Balabaskar P. Biocontrol efficacy of Trichoderma spp. against wilt of tomato caused by Fusarium oxysporum f. sp. lycopersici. *J Appl Biol Biotechnol* [Internet]. 2013 Oct;1(3):36-40. Available from: <http://www.jabonline.in>
 51. Al-Ani LKT, Albaayit SFA. Antagonistic of some Trichoderma against Fusarium Oxysporum sp. f. cubense Tropical Race 4 (FocTR4). *Eurasia Proc Sci Eng Math EPSTEM*. 2018 Aug 26; 2:35-38.
 52. Kumari A, Kumar R, Kumar H. Efficacy of Fungicides and Trichoderma Viride Against Fusarium Oxysporum F. SP. CUBENSE *In-Vitro*. *The Bioscan*. 2014; 9(3):1355-1358.
 53. Pawloski Schoffen R, Ribeiro A, Oliveira-Junior V, Polonio J, Polli A, Orlandelli R, *et al.* Evaluation of Trichoderma atroviride endophytes with growth-promoting activities on tomato plants and antagonistic action on Fusarium oxysporum. *Ciênc E Nat*. 2020 Jun 29; 42:e47.
 54. Sudantha I. Characterization and virulence of Fusarium oxysporum f. sp. cubense cause wilt disease in banana plants and its biological control using endophytic fungi Trichoderma spp. at West Nusa Tenggara, Indonesia. *IOP Conf Ser Earth Environ Sci*. 2021 Nov 1; 886:012016.
 55. Sánchez-Espinosa AC, Villarruel-Ordaz JL, Maldonado Bonilla LD. Mycoparasitic antagonism of a Trichoderma harzianum strain isolated from banana plants in Oaxaca, Mexico: Novel Trichoderma strain protects against Fusarium. *Biotecnia* [Internet]. 2021 Feb 16 [cited 2024 Aug 14]; 23(1):127-134. Available from: <https://biotecnia.unison.mx/index.php/biotecnia/article/view/1310>
 56. Carvalho DDC, Lobo Junior M, Martins I, Inglis PW, Mello SCM. Biological control of Fusarium oxysporum f. sp. phaseoli by Trichoderma harzianum and its use for common bean seed treatment. *Trop Plant Pathol* [Internet]. 2014 Oct [cited 2024 Aug 14]; 39(5):384-391. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1982-56762014000500005&lng=en&nrm=iso&tlng=en
 57. Napitupulu T. *In vitro* evaluation of Trichoderma harzianum strains for the control of Fusarium oxysporum f.sp. cubense. *Plant Pathol Quar* [Internet]. 2019 [cited 2024 Sep 6]; 9(1):152-159. Available from: http://www.plantpathologyquarantine.org/pdf/PPQ_9_1_13-1.pdf
 58. Marques E, Martins I, Mello SCMD. Antifungal potential of crude extracts of Trichoderma spp. *Biota Neotropica* [Internet]. 2018 Jan [cited 2024 Sep 5]; 18(1):e20170418. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1676-06032018000100503&tlng=en
 59. Andrade Hoyos P, Luna Cruz A, Osorio Hernández E, Molina Gayosso E, Landero Valenzuela N, Barrales Cureño HJ. Antagonismo de Trichoderma spp. vs hongos asociados a la marchitez de chile. *Rev Mex Cienc Agríc* [Internet]. 2019 Sep 23 [cited 2024 Aug 14]; 10(6):1259-1272. Available from: <https://cienciasagricolas.inifap.gob.mx/index.php/agricolas/article/view/1326>
 60. Moreno-Ruiz D, Lichius A, Turrà D, Di Pietro A, Zeilinger S. Chemotropism Assays for Plant Symbiosis and Mycoparasitism Related Compound Screening in Trichoderma atroviride. *Front Microbiol* [Internet]. 2020 Nov 27 [cited 2024 Sep 6]; 11:601251. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.601251/full>
 61. Ojha S, Chatterjee NC. Mycoparasitism of Trichoderma spp. in biocontrol of fusarial wilt of tomato. *Arch Phytopathol Plant Prot* [Internet]. 2011 May [cited 2024 Aug 14]; 44(8):771-782. Available from: <http://www.tandfonline.com/doi/abs/10.1080/03235400903187444>
 62. Larran S, Santamarina Siurana P, Caselles JR, Simon MR, Perello A. *In Vitro* Antagonistic Activity of Trichoderma harzianum against Fusarium sudanense Causing Seedling Blight and Seed Rot on Wheat. *ACS Omega* [Internet]. 2020; 5:23276-23283. Available from: <http://pubs.acs.org/journal/acsodf>
 63. Yao X, Guo H, Zhang K, Zhao M, Ruan J, Chen J. Trichoderma and its role in biological control of plant fungal and nematode disease. *Front Microbiol*. 2023 May 3;14.