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Isolation Identification and Biocontrol of *Fusarium Oxysporum* Affecting *Zamioculcas Zamiifolia*

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Abstract

This study was conducted to isolate and identify the *Fusarium oxysporum* fungi, the causal agent of wilt in the ornamental plant *Zambia Zamioculcas Zamiifolia*, in Basra Governorate. The study involved morphological and molecular identification of the fungus, testing its pathogenicity on Zambia plants, and identifying the most effective biological control agents against it. These agents included the fungi *Trichoderma viride*, *T. longibrachiatum,* an aqueous extract of Moringa oleifera leaves, and an alcoholic extract of M.oleifera leaves. The results showed that F. oxysporum caused a severe infection rate85.5%, Invitro T. viride exhibited 93.7% inhibition of the pathogen, and T. *longibrachiatum* 96.2%. The aqueous extract of *M.oleifera* leaves showed 56.2% inhibition against the pathogen. The results also indicated the superiority of the alcoholic extract of *M.oleifera* leaves, which demonstrated 98.7% inhibition against the pathogen. *In-vivo* experiments the pots experiment, the aqueous extract gave the best results fordisease resistance, reached 33.3%, while the alcoholic extract reached 62.5%. Treatments using the fungi *T. longibrachiatum* and *T. viride* were similar in reducing the severity of infection, at 41.2% and 41.6% respectively.

Keywords: *Fusarium Oxysporum, Zambia plant, Trichoderma viride, T. longibrachiatum and Moringa oleifera leaves*

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عزل وتشخيص فطر Fusarium oxysporum من نبات Zamioculcas zamiifolia واختبار مقاومته الحيوية باستخدام بعض عوامل المكافحة الحيوية

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الخلاصة:

أُجريت هذه الدراسة لعزل وتحديد فطر *Fusarium oxysporum* المسبب لذبول نبات الزينة زامبيا *Zamoiculcas zamiifolia* في محافظة البصرة. شملت الدراسة التحديد المورفولوجي والجزيئي للفطر، واختبار قدرته على إحداث المرض في نباتات زامبيا، وتحديد أكثر عوامل المكافحة البيولوجية فعالية ضده.
تضمنت هذه العوامل فطريات Trichoderma viride ، و *T. longibrachiatum* ، ومستخلص مائي لأوراق *Moringa oleifera* ومستخلص كحولي لأوراق المورينغا. أظهرت النتائج أن F .oxysporum تسبب في معدل إصابة شديد بلغ 85.5%، بينما أظهر T.viride في المختبر تثبيطًا بنسبة 93.7% للفطر، و *T.longibrachiatum* بنسبة 96.2%. أما المستخلص المائي لأوراق المورينغا فقد أظهر تثبيطًا بنسبة 56.2% للفطر. أشارت النتائج أيضًا إلى تفوق المستخلص الكحولي لأوراق المورينغا، حيث أظهر تثبيطًا بنسبة 98.7% ضد العامل الممرض. في التجارب الحيوية، وتحديدًا في تجارب الأصص، حقق المستخلص المائي أفضل النتائج في مقاومة المرض، بنسبة 33.3%، بينما بلغت نسبة التثبيط للمستخلص الكحولي 62.5%. وكانت المعالجات باستخدام فطريات *T. longibrachiatum* و T. viride متقاربة في تقليل شدة الإصابة، بنسبة 41.2% و41.6% على التوالي.

الكلمات المفتاحية :الفيوزاريوم أوكسيسبوروم، نبات زامبيا، الترايكوديرما فيريدي، الترايكوديرما لونجيبراكياتوم، أوراق المورينغا أوليفيرا..

1. Introduction

Zamioculcas Zamiifolia, commonly known as the *Zambian* plant, is a prominent species in the *Araceae* family, belonging to the *Aroideae subfamily*, which includes several tropical genera. *Zamiifolia* is classified within the *Zamioculcadeae* tribe, a small tribe containing only two genera, Zamioculcas and *Gonatopus*, which share similar morphological and anatomical characteristics reflecting their evolutionary origin. This taxonomic position highlights the features that distinguish *Zamiifolia* from other plants in the family. (Hetterscheid and Boyce, 2020) Z. zamiifolia is a perennial evergreen plant native to tropical East Africa, particularly Tanzania, Kenya, Mozambique, and Zanzibar, where it grows in savannas and dry forests with fluctuating climatic conditions (Mayo et al., 1997). Its thick fleshy rhizomes enable it to store water and carbohydrates, enhancing its resilience in poor soils and under water stress (Huxley et al., 2022). The plant has gained global importance as one of the most tolerant shade plants, in addition to its ability to absorb volatile organic compounds (VOCs) such as benzene and toluene, thus contributing to improved indoor air quality (Kim et al., 2020). Studies have also indicated its role in promoting psychological well-being and regulating humidity in indoor spaces (Pennisi et al., 2021). Thanks to its ease of propagation and low maintenance requirements, it has become a popular houseplant in the interior design of offices, healthcare facilities, and educational institutions worldwide (Pourhassan et al., 2023). Zambia plant is susceptible to several diseases. López-López et al. (2021) reported root infections caused by Pythium spp. and Fusarium spp. on Z. zamiifolia in a study on tropical ornamental plant pathogens, while Rohde et al. (2020) documented the isolation of Pythium ultimum from the rotting roots of Zambia plants in humid indoor environments. Choi et al. (2019) also observed infections caused by Rhizoctonia solani AG-4 in potted Zambia plants, resulting in basal spots and stem rot. The Fungal Planet series published by Crous et al. (2012–2022) indicated the presence of multiple isolates of Fusarium and Pythium on plants of the Araceae family, including Z. zamiifolia.
Recently, Thokchom et al. (2025) documented the first case of stem rot in Z. zamiifolia in India, caused by the fungus Agroathelia rolfsii. Infected plants exhibited marked rot at the base of the stem and rhizome. The research team confirmed the causal agent through laboratory isolation and completion of Koch rules, as well as molecular diagnosis using ITS sequencing. This report adds to the known diseases affecting Z. zamiifolia and confirms its susceptibility to pathogenic fungi, particularly in warm and humid conditions. Greece, caused by the bacterium Pectobacterium Brasiliense (Pagoulatou et al., 2024). Severe infections were observed, characterized by water-soaked spots and a foul-smelling, moist rot on the petioles and rhizomes. Researchers confirmed the identity of the causative bacteria based on morphological characteristics and biochemical tests, as well as molecular diagnosis using the specific primers BR1f/L1R and MLSA analysis. These infections are often associated with inappropriate agricultural practices such as over-irrigation and poor drainage. Vascular wilt is one of the most devastating plant diseases affecting annuals and perennial woody plants. These diseases are generally

caused by soil-borne bacteria, fungi, and molds that infect the roots and invade the water-conducting xylem vessels, where they multiply and interfere with the transport of water and minerals. As a result, the leaves wilt, the entire plant weakens, and it may eventually die. Symptoms include yellowing of the leaves, stunted growth, discoloration and death of the plant's vascular tissue (Velarde-Félix et al., 2018; Sun et al., 2019).
The fungus F. oxysporum is an imperfect fungus belonging to the phylum Ascomycota, class Sordariomycetes, order Hypocreale, and family Nectriaceae. It is characterized by spore formation and causes vascular wilt diseases. F. oxysporum is one of the most important soil fungi and is a facultative parasite, parasitizing organic matter in the soil in the presence of a plant host; this was demonstrated by Batson et al. (2020). This fungus has a greater tendency to parasitize living tissue than to live saprophytically on plant debris. Its dormant chlamydospores are retained in the soil for extended periods, and under favorable environmental conditions, the mycelium grows, causing root rot (Anon, 2011).
Lahuf (2019) and Jaber (2020) reported that the pathogenic fungus Fusarium spp. causes tissue death due to its parasitic nature and the profuse growth of mycelium in vascular tissues. This prevents the supply of water and salts to leaves. The fungus also secretes enzymes that damage and decompose plant cell walls, allowing it to penetrate root and seed tissues. In the 19th century, Fusarium infection of ornamental plants increased (Ortu et al., 2015). Fusarium oxysporum is the most common species of the genus Fusarium causing wilt in many plants. Numerous fungi cause diseases in ornamental plants, but the most concerning pathogens worldwide are those transmitted through the soil, such as F. oxysporum (O'Donnell et al., 2009). This fungus causes other symptoms, including root and crown rot, which are associated with the rot (Gullino et al., 2012). Several species of this fungus are responsible for wilting and root rot in various ornamental plant species (Dean et al., 2012). Vascular wilting, root rot, and crown rot occur during the seedling storage period until marketing (Brayford, 1996). Cultivating disease-resistant varieties of ornamental plants is considered the best way to combat these diseases; however, this is a costly and difficult process to achieve (Lecomte et al., 2016). This study aimed to identify and diagnose the fungus causing wilt in F. oxysporum and explore the possibility of biological control, given the limited research on *Zamioculcas Zamiifolia*.

2. Materials and Methods

2.1. Sample Collection

Samples of the Zambian plant (*Z. zamiifolia*) were collected from nurseries in Basra Governorate. These samples showed signs of wilting, including yellowing and discoloration of the vascular tissue five samples were taken from each nursery. They were transported to the laboratory and refrigerated at 4°C until use.

2.2. Isolation and Identification of the Pathogen

The stems and tubers of *Z. zamiifolia* plants were cut into small pieces (2-5 mm) and surface-sterilized with a 10% sodium hypochlorite solution for two minutes. They were then washed with 70% ethyl alcohol for one minute, followed by a final rinse with distilled water for one minute on sterile filter paper until dry. Sterile Petri dishes containing sterile PDA culture medium were prepared, and four pieces of infected stems or tubers were cultured in each of ten dishes. The dishes were incubated at 25 ± 2°C for seven days. The fungal isolates were then purified by taking the edges of the colonies individually and re-cultured in Petri dishes containing PDA culture medium. The fungal colonies were cultured in these dishes for purification and identification and incubated for seven days at 25 ± 2°C. The fungal isolate *F. oxysporum* was identified morphologically and under a microscope. Dr. Yahya Ashour Saleh, Plant Protection Department, College of Agriculture, University of Basrah (Leslie and Summerell 2006). Genomic diagnosis of the fungus *F. oxysporum* was performed by extracting genomic DNA using the Yeast Genomic Miniprep Kit from MesGen Biotechnology (Cat. No. MYG4850). Extraction was carried out according to the manufacturer's instructions. The area was diagnosed Translation Elongation Factor1-

alpha (TEF) is a single copy gene, used primers forward 5'ATGGGTAAGGAAGACAAGAC Reverse 5'GGAAGTACCAGTGACATGTT using the following program.

Table (1) DNA amplification for the diagnosis of the fungus *F. oxysporum* using PCR technique

PCR steps	NO. cycle	℃	time
Initial denaturation	1	97	
Denaturation	35	96	1 min
Annealing		50	1min
Extension		72	1min
Final extension	1	72	10 min

2.3. Preparation of inoculum for the pathogenic fungus *F. oxysporum* and the antagonistic fungi *T. longibrachiatum* and *T. viride*.

The antagonistic fungal isolates *T. longibrachiatum* and *T.viride* were obtained from the Bioresistance Laboratory in the Plant Protection Department, College of Agriculture, University of Basrah and identified by Dr. Yahya Ashour Saleh. Local millet seeds were prepared and soaked for 12 hours. They were then drained, excess water was removed, and they were left to dry for 10 minutes before being distributed into 250 ml glass flasks. The flasks were tightly sealed with cotton plugs and sterilized in an autoclave at 121°C and 15 bar/in² pressure. After cooling, the flasks were inoculated with 5 day old tablets of the pathogenic fungus *F.oxysporum*, for a total of two flasks. Other flasks were inoculated with 5-day-old tablets of the growing colony of *T.viride*, for a total of two flasks. The same method was used for *T.longibrachiatum*. The flasks were then incubated for 14 days at 25 ± 2°C, with the flasks being shaken every three days to ensure uniform growth of the fungal inoculum (Dewan,1989).

2.4. Preparation of the aqueous extract of *Moringa oleifera* leaves

50 g of dried *Moringa oleifera* leaf powder was added to 500 ml of boiled distilled water and left to infuse for 12 hours. The extract was then filtered through clean cheesecloth and further filtered through filter paper until a clear extract was obtained (Al-Mansour, 1995). The aqueous extract was then sterilized in a microwave for 60 seconds.

2.5. Preparation of the alcoholic extract of *Moringa oleifera* leaves

The alcoholic extract of *M. oleifera* leaves was prepared using 50 g of powder. It was placed inside a thimble in a Soxhlet extractor, and 500 ml of 95% ethanol was added to the boiling flask. The extractor was then stabilized with the cooling system activated. The extraction process was carried out using a soxhlet pparatus for 6–8 hours at the boiling point of ethanol (78°C). After cooling the apparatus, the liquid alcoholic extract was collected in a clean flask (Al-Mansour, 1995), and then left to partially evaporate the solvent at room temperature (25 ± 2°C) for 24 hours to obtain a more concentrated extract.

2.6. Antibiotic Test between the Pathogenic Fungi *F. oxysporum*, *T. longibrachiatum*, and *T. viride*

The experiment was conducted using the Dual Culture Technique. A disc of *T.viride* was cultured from one edge of a Petri dish containing sterile PDA culture medium, and a disc of the pathogenic fungus *F. oxysporum* was cultured from the other end. Three replicates were performed. Similarly, a disc of *T. longibrachiatum* was cultured with the pathogenic fungus *F. oxysporum*, also with three replicates. A control treatment of the pathogenic fungus alone was included (Dennis and Webster,

1971). The dishes were incubated at 25 ± 2°C for 7 days. Afterward, specific readings were taken, and the antagonism ratio was calculated using the Bell et al. scale (1982).

Grade 1: Complete spread of the antagonistic fungus on the dish.

Grade 2: Coverage of three-quarters of the dish with the antagonistic fungus. Level 3 – Half of the plate is covered with both the antagonistic fungus and the pathogen.

Level 4 – Three-quarters of the plate is covered with the pathogen.

Level 5 – The pathogen is completely spread across the plate. Biological control is considered effective if it shows a level of antagonism of 1 or 2. The biological agent is considered effective if the antagonism is level 1 or 2.

2.7. Biological antagonism test with aqueous and alcoholic extracts of *Moringa* spp. leaves

The aqueous extract of *Moringa oliefera* leaves was taken and mixed with PDA culture medium at a rate of 1 ml per dish. A rolling motion was used to ensure homogeneity of the extract with the culture medium, and the dishes were allowed to solidify. Similarly, the alcoholic extract was taken at a rate of 1 ml per dish, mixed with the culture medium, and allowed to solidify. Each dish was then inoculated with a disc of *F. oxysporum* fungal colony, with three replicates for each treatment (aqueous and alcoholic). A control treatment of the pathogenic fungus alone was also performed with three replicates. The percentage of inhibition was then calculated. The method for determining the percentage of fungal growth inhibition (Nwankiti and Gwa, 2018) was used, applying Abbott's equation (1925) to measure the percentage of inhibition. Growth rate in control

Growth rate in treatment = Percentage of inhibition/ Growth rate in control x 100

2.8. Pathogenicity test of *F. oxysporum*

Hypotheses were made on plants *Z. zamiifolia* was not infected, as the peat moss soil was sterilized in an Autoclave device and then distributed in pots weighing 1 kg each. The fungal inoculum loaded onto millet seeds for the pathogenic fungus was added at a rate of 10 g/pot and a light watering was given. The pots were covered with transparent nylon and left for 18 hours to allow the fungus to grow. After that, tubers of Zambia plants that were not infected were planted and left for 30 days with a light watering every 7 days to monitor the growth and development of the pathogen and the occurrence of infection. After the infection appeared, the pathogenic fungus was re-isolated from the infected plants, and the diagnosis was confirmed as the same pathogen *F. oxysporum.*

Pot Biocontrol Experiment

A pot biocontrol experiment was conducted on Zambian plants to determine the effect of biocontrol agents on the pathogenic fungus *F. oxysporum* and their effectiveness in reducing the severity of vascular wilt disease. Sterile peat moss was prepared and distributed in 1kg pots, and the following treatments were carried out:

1- **Treatment of the Pathogenic Fungus Alone**

10 g of the inoculum carrying the millet seeds of the pathogenic fungus *F. oxysporum* was added to each pot and left for 48 hours to ensure the growth of the pathogen.

2- Treatment of the Pathogenic Fungus + Antagonist Fungus (*T. viride*)

Add 5g of the antagonist fungus inoculum loaded onto millet seeds per pot and leave for 48 hours. Then add 5g of the pathogenic fungus inoculum per pot, cover the pots with clear plastic wrap, and leave for another 48 hours. Afterward, plant Zambia plants and leave them for 45 days, watering every 7 days.

3- Treatment of the Pathogenic Fungus + Antagonist Fungus (*T. longibrachiatum*): Performed as described in the previous section.

11-Data analysis

The data was analyzed using a Genestate program at $P<0.01$ *invitro* and $P<0.05$ for pot experiments

3. Results and Discussion

3.1. Isolation and Identification of the Pathogenic Fungus:

The isolation and identification results showed that the isolated fungus was *F.oxysporum*. Morphological examination revealed that the fungus grew as a dense, spreading mycelium that covered the plate within 5–7 days at 25°C. This growth pattern was clearly noted by Leslie and Summerell (2006). The mycelium in all isolates started out bright white at the margin, then developed a distinctive color gradient towards the center from pale pink to pinkish-purple and sometimes dark purple, with a cottony to floccose appearance consistent with the description given by Sandoval-Denis and Crous (2018) of the characteristics of colonies belonging to the fungus *F. oxysporum*. Some isolates were characterized by a raised central protrusion of aerial mycelium due to high enzymatic activity and the production of conidia, a common feature reported by Lombard et al. (2019).

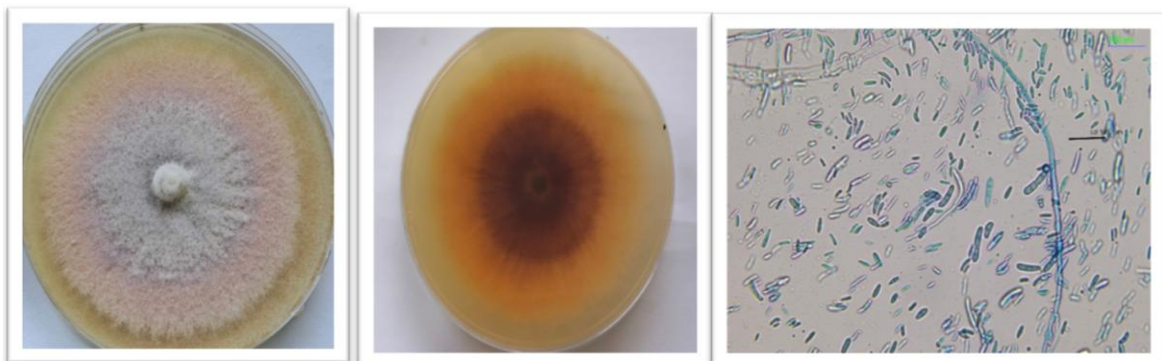

Figure (1) Pathogenic fungus *F. oxysporum* isolated from Zambia wilt-infected plants. A_ Pure culture of the fungus. B_ Fungal spores under 40X magnification

From the back of the plate, the growth medium showed discoloration with radially diffused fungal pigments ranging from pale yellow to orange, and in some isolates, it may reach crimson or dark purple, reflecting the fungus's ability to produce carotenoid and fusarean pigments diffused in the medium, a characteristic accurately described by Sandoval-Denis and Crous (2018) as a distinctive marker for the

diagnosis of *F. oxysporum*. Concentric color rings were also observed, showing successive growth periods, with a smooth, regular colony margin with a clear radial extension, consistent with the characteristics documented by Leslie and Summerell (2006). Microscopically, the isolates produce oval to elliptical microconidia with no or one septum on short phialides, as well as spindle-shaped macroconidia with 3–5 septums, with single or double chlamydospores formed in the mycelium, features confirmed by Lombard et al. (2019) in their detailed study of the morphological differences of *F.oxysporum* isolates. Genetic identification of the fungus confirmed the diagnosis as *F.oxysporum*, and the isolate was registered in the GenBank of Japan (DDBJ) under number LC904884.

3.2. Dual culture with the fungi *T. longibrachiatum* and *T. viride*

The results of the Dual culture between the pathogenic fungus *F. oxysporum* and the two *T. longibrachiatum* and *T. viride* showed that the antagonism ratio on the Bell et al. scale (1982) was as follows Table (2):

Table 2- Antagonism score on the Bell scale for the Dual culture *T. longibrachiatum* and *T. viride* against the pathogenic fungus *F. oxysporum*

Pathogenic fungus + Biological fungus	ell Value	Contradiction
Pathogenic fungus + *T.longibrachiatum*	1.3	contradiction
Pathogenic fungus + *T.longibrachiatum*	1.5	contradiction

These results demonstrate the potent effect of the antagonistic fungus on the growth of the pathogenic fungus. This effect may be attributed to the fact that the antagonistic fungus possesses several mechanisms that enable it to inhibit pathogenic growth. For example, *T. longibrachiatum* secretes cell wall-degrading enzymes such as chitinase, β-1,3-glucanase, and protease, which weaken the basic structures of pathogenic fungal cells and lead to their destruction (Zhang, 2021). It is also characterized by its rapid growth and high adaptability to the root environment, allowing it to compete with pathogens for nutrients and vital sites, preventing them from obtaining their essential growth requirements (Li et al., 2022). Furthermore, it contributes to stimulating plant immunity by activating jasmonic acid (JA) and salicylic acid (SA) pathways, increasing the production of defensive compounds (phytoalexins) and enhancing the activity of defensive enzymes such as peroxidase and polyphenol oxidase (El-Khallal, 2021).

The fungus *T. viride* secretes cell wall-degrading enzymes such as chitinases, β-1,3-glucanases, and proteases, which target structural cell wall components like chitin and glucans, leading to weakening or death of pathogenic cells (Sultana, 2022). *T.viride* also directly attacks pathogenic hyphae by wrapping around them and secreting degrading enzymes that penetrate the cell wall and cause cell lysis, a key characteristic of *Trichoderma* as a natural antifungal agent (Gupta, 2023). Furthermore, *T. viride* enhances plant immunity by stimulating the jasmonic acid (JA) and salicylic acid (SA) pathways, leading to the activation of defense genes and increased production of infection-resistant enzymes such as peroxidase and polyphenol oxidase (Elsharkawy, 2021). This aligns with several studies that have shown both *T. viride* and *T. Longibrachiatum* possess a high capacity to inhibit *F. oxysporum* and reduce the severity of infection through enzymatic degradation, competition, and the production of fungal-resistant secondary compounds (El-Sharkawy et al., 2021; Zhang et al., 2020).

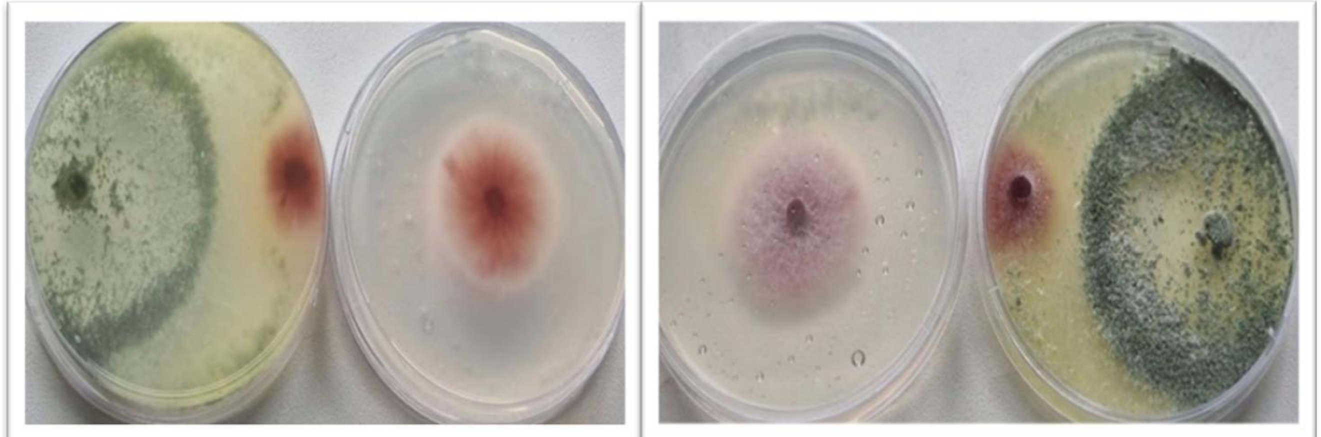

Figure (2): Fungal antagonism *F. oxysporum* with the pathogenic fungus *T. longibrachiatum*

Figure (3) Antagonism of the biotic fungus *F. oxysporum* with the pathogenic fungus. *T. viride*

3.3. Bio-antagonism experiment with aqueous and alcoholic extracts of *Moringa oleifera* leaves

The results of this experiment showed the efficiency of the alcoholic Moringa leaf extract in inhibiting the growth of the pathogenic fungus by 98.7%, with highly significant differences compared to the aqueous extract, which had an inhibition rate of 56.2%, and which also significantly outperformed the control treatment. This indicates the efficiency of the alcoholic extract and the concentration of the active ingredient in it, and this is consistent with what a number of researchers have found. The study by Ogunyemi et al (2021). showed that *M. oleifera* phenolic compounds such as Quercetin and Kaempferol bind to the components of the fungus's cell wall, especially chitin and glucans, which leads to increased permeability and the occurrence of cracks and damage in *F. oxysporum* hyphae (Ogunyemi et al., 2021). As demonstrated by Nwachukwu et al. (2022), the alcoholic extract rich in flavonoids and saponins disrupts the fungal plasma membrane by altering its permeability and leaking cellular contents (electrolytes, proteins, and amino acids), leading to fungal cell death.

Other study indicated that the active compounds in moringa extract inhibit key fungal enzymes such as cellulase, pectinase, and laccase, which are essential for penetrating plant tissues and sustaining the fungal life cycle. This inhibition, therefore, weakens the pathogen's ability to spread (Al-Snafi, 2020). Furthermore, Sharma et al. (2023) revealed that the alcoholic extract inhibits gene expression associated

with membrane component synthesis, such as ergosterol biosynthesis genes, and disrupts the MAPK pathway, which is linked to fungal growth, division, and reproduction, significantly reducing the fungus's adaptability and growth potential. As Gondwal et al. demonstrated, some Moringa compounds, such as Gallic acid and Vitamin C, contribute to the generation of free radicals (ROS) within fungal cells, leading to damage to DNA, proteins, and the cell membrane, and subsequently inducing apoptosis-like cell death processes (Gondwal et al., 2021). Hassan and El-Sayed (2020) also demonstrated the ability of an aqueous extract of Moringa leaves to reduce wilting severity in ornamental plants, including Philodendron, by inhibiting mycelial growth and suppressing fungal spore formation, thus reducing the spread of the pathogen *F.oxysporum*.

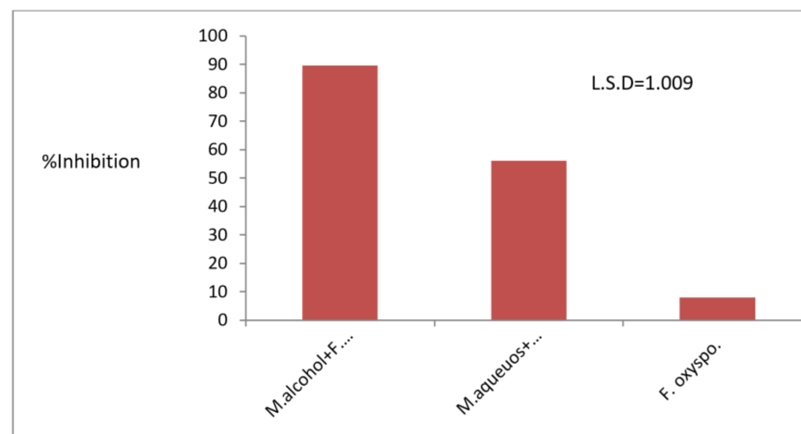

Figure 4 - Inhibition % A. Aqueous extract of Moringa leaves + pathogenic fungus. B. Alcoholic extract of Moringa leaves + pathogenic fungus C. Pathogenic fungus alone

3.4. Pathogenicity test of the fungus *F.oxysporum*

The pathogenicity test results showed that the identified isolate produced positive results in the induction of wilt on Zambia plants approximately two weeks after application, with an infection severity of 85%. This is attributed to the fungus's ability to produce a range of pathogenic factors that contribute to the penetration of plant tissues and disease development. This may be due to *F.oxysporum's* ability to produce cellulase, pectinase, and xylanase enzymes, which are among the most important infection factors, as they break down the cell wall of plant roots and facilitate the entry and spread of hyphae within vascular tissues, consistent with the findings of Mishra et al. (2020). As López-Díaz et al. (2019) demonstrated, the fungus produces toxins such as fusaric acid, which damage plant cell membranes and impair transport in vascular bundles, leading to rapid wilt progression. Di Pietro et al. (2020) showed that *F.oxysporum* forms colonial structures and biofilms within xylem vessels, causing mechanical obstruction and preventing water movement—a key mechanism in the development of vascular wilt. Laurence et al. (2021) confirmed that the SIX genes secreted by the fungus within the vessels contribute to the induction of inflammation and the suppression of plant immunity, facilitating infection and pathogen spread within the roots and stem. Zhou et al. (2022) showed that *F.oxysporum* has the ability to disrupt salicylic acid (SA) and jasmonic acid (JA)-dependent plant immunity pathways, reducing the plant's defensive response and increasing its susceptibility to infection and further disease progression within vascular tissues.

Figure (5) Symptoms of wilt disease on Zambia plants resulting from fungal infection *F.oxysporum*.
A) represents the control treatment without the fungus. B) represents the treatment with the addition of the fungus *F.oxysporum*

3.5. Pot experiment

The experiment showed the efficacy of treatment with the alcoholic extract of Moringa leaves and the aqueous extract against the fungus *F. oxysporum*, followed by treatment with the biofungus *T. longibrachiatum*, and then treatment with the fungus *T.viride*.

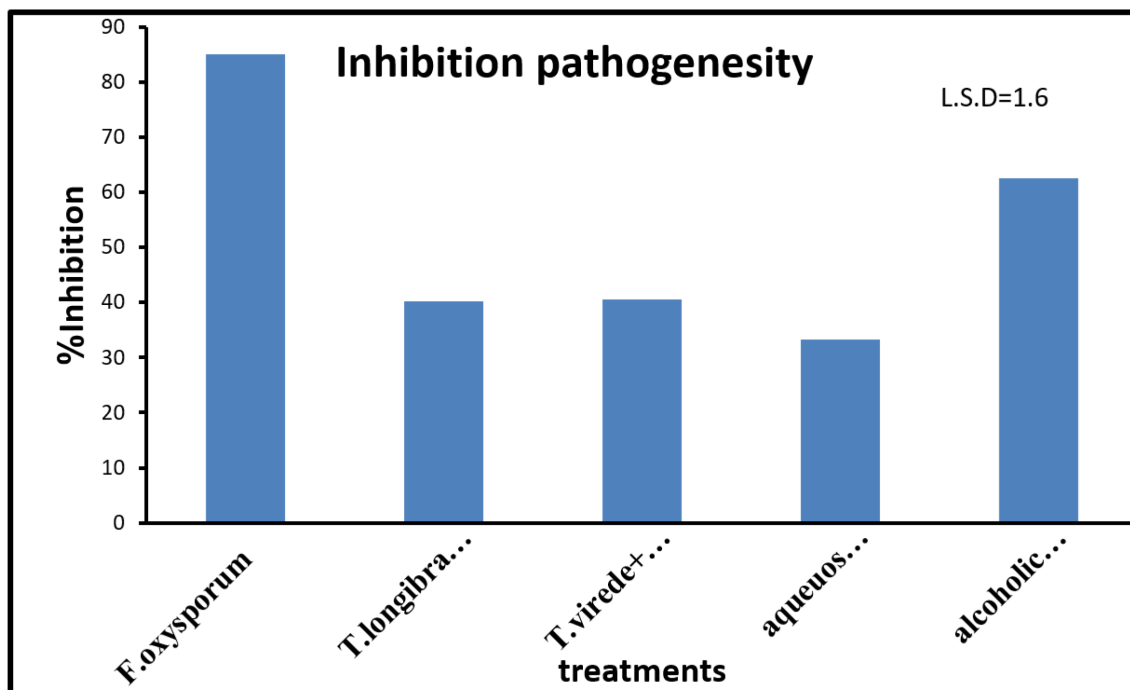

Figure 6 - Effect of biocontrol agents on the fungus *F.oxysporum* pathogenesity in pots

The results of the pot experiment showed a clear disparity in the efficacy of the biological control treatments and plant extracts in reducing the severity of *F. oxysporum* infection on Zambia plants. The equeous extract of Moringa leaves recorded the highest efficacy among all treatments, with an average infection severity of only 33.3%, a significant difference from the other treatments. This was followed

by the alcoholic extract with an average of 62.5%. As for the biological fungi, the two *Trichoderma* treatments showed similar efficacy, with *T. longibrachiatum* recording an average infection rate of 41.2%, while *T.viride* recorded an average of 41.6%, indicating a slight advantage for *T. longibrachiatum*. In contrast, the control treatment infected with *F. oxysporum* recorded the highest infection rate at 85%, confirming the success of the biological control treatments and plant extracts in reducing infection compared to the control.

The high efficacy of the alcoholic extract is attributed to its richness in active compounds such as Quercetin, Kaempferol, flavonoids, and saponins, which have a proven ability to directly damage the fungal cell wall by increasing permeability, causing hyphae collapse, and leaking internal contents, in addition to inhibiting important pathogenic enzymes such as Cellulase, Pectinase, and Laccase (Al-Snafi, 2020; Ogunyemi et al., 2021; Nwachukwu et al., 2022). Recent studies have also shown that alcoholic extracts of Moringa are able to inhibit gene expression associated with ergosterol synthesis and the MAPK pathway, which is essential for Fusarium growth and reproduction (Sharma et al., 2023). The aqueous extract showed good efficacy despite a lower concentration of hydrophobic compounds compared to the alcoholic extract, as its content of water-soluble phenolic compounds contributed to reducing mycelium formation and inhibiting spore production. This is consistent with the results of Hassan & El-Sayed (2020), who recorded a significant reduction in the severity of *F.oxysporum* infection in Dieffenbachia plants using the aqueous extract. Regarding biogenic fungi, *T.longibrachiatum* and *T.Viride* exhibits similar efficacy, however, the slight reduction in infection severity observed in *T.longibrachiatum* treatment may be attributed to its high capacity to secrete fungal cell wall-degrading enzymes such as chitinase, β-1,3-glucanase, and proteases, in addition to producing antifungal compounds like trichodermins, which inhibit fungal growth and division (Kumar et al., 2021; Sultana et al., 2022; Gupta et al., 2023). These findings are consistent with those of Raza et al. (2020), who reported that Trichoderma fungi compete for nutrients, secrete cell wall-degrading enzymes, and stimulate plant immunity by activating the JA and SA pathways, which increase levels of defense enzymes such as peroxidase and polyphenol oxidase, thus contributing to reduced disease progression.

4. Conclusions

In general, these results confirm that the equeous extract of Moringa is the most effective option for reducing infection severity, followed by the alcoholic extract. *Trichoderma* fungi exhibited relatively moderate efficacy, which may be related to the strength of the isolation, the efficiency of root colonization, and variations in microbiological conditions within the pots. The data collectively demonstrate that the isolated *F. oxysporum* possesses clear pathogenic capabilities through the production of cell wall-degrading enzymes and toxins that inhibit vascular transport, resulting in the highest infection severity in the control treatment. In contrast, the biological control treatments and plant extracts demonstrated considerable ability to suppress disease development. The alcoholic extract achieved high levels of inhibition, reflecting its richness in active compounds, while the biological fungi *T .longibrachiatum* and *T. viride* provided a stable antagonistic effect through enzymatic degradation, competition, and stimulation of plant immunity. The results indicate the potential for using plant extracts and biotic agents as effective inputs in reducing vascular wilt in *F .oxysporum* within a recognized biomanagement framework. This aligns with recent studies demonstrating the efficacy of these methods in reducing infection severity and limiting the growth of *F. oxysporum*.

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