International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

http://ijpsat.ijsht-journals.org
Vol. 29 No. 1 October 2021, pp.01-10

Fusarium wilt and Southern Blight Diseases of Tomatoes: Trichoderma spp. as Antagonists of the Causative Organisms

Olusola, E. Ayodeji[1*]., Ayodele, M. Ajayi[1]., David, B. Olufolaji[2]., Ganiyu, F. Hassan[3].

[1,2&3]Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, P M B, 704, Akure, Ondo State, Nigeria.

Abstract – Fusarium wilt (Fusarium oxysporum f. sp. lycopersici) and southern blight (Sclerotium rolfsii) are two of the soil borne diseases limiting tomato cultivation globally and are difficult to manage. This study evaluated biocontrol agents, Trichoderma harzianum and T. viride for antagonistic efficacy against these pathogens in vitro. The dual culture technique was adopted using two approaches; namely, pre-inoculation of Trichoderma spp. into culture medium at 24 and 48 hours ahead of the pathogen and simultaneous inoculation of Trichoderma spp. and pathogen. The Trichoderma spp. were evaluated singly against each pathogen. The sole culture of each pathogen was the control. Each treatment was replicated thrice and laid out in a completely randomized design. Inoculated Petri plates were incubated at 27º C ± 2º C. Data collected were subjected to statistical analysis and mean separation using Minitab software. Results showed that the inhibition of the mycelial growth of both pathogens by the biocontrol agents were days dependent. Furthermore, prophylactic evaluations gave significantly higher inhibition of the mycelial growth of the pathogens as against therapeutic evaluation. Additionally, F. oxysporum was more susceptible to antagonism by both biocontrol agents as higher percentages of mycelial growth inhibition were recorded for it in all treatments. Finally, T. harzianum was a better antagonist of the two pathogens, recording 84.62% and 69.01% inhibition of mycelial growth of F. oxysporum and S. rolfsii, respectively at 9 days after inoculation. Trichoderma spp. maybe exploited as biocontrol agents for the management of these pathogens.

Keywords – Fusarium wilt, southern blight, Fusarium oxysporum, Sclerotium rolfsii, Trichoderma spp. biocontrol agents.

I. INTRODUCTION

Tomato, *Solanum lycopersicum* L. is a vegetable crop belonging to the Solanaceae family. [1]. Its importance is associated with its nutritional, economic and health benefits. [2, 3]. The crop is a widely grown vegetable in most parts of the world and it ranks as the second most important vegetable, next to potato. [4]. Nigeria is a major producer of tomatoes in West Africa. [5].

Reference [6] reported that fungal pathogens are particularly damaging as they cause losses in significant proportions. *Fusarium oxysporum* and *Sclerotium rolfsii*, the causal agents of Fusarium wilt and southern blight diseases ,respectively are known to cause significant losses in the yield of the crop [7], [8]. In Nigeria, these diseases ranked among the most common and most devastating, making large scale cultivation of the crop very difficult, especially in the more humid southern part of the country [9], [10], [6].

Studies have shown that both *F. oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii* are soil-borne. [11]. Reference [12] reported that sclerotia from *S. rolfsii* can remain viable in the soil for many years, serving as the primary source of inoculum. Fusarium wilt is a vascular disease that causes wilt in tomato plants, accompanied by yellowing of leaves from the lower part of the stem which progresses rapidly to the apex. Massive and rapid defoliation and death of infected tomatoes are some of the other symptoms [11],

Corresponding Author: Olusola, E. Ayodeji

[13]. Southern blight disease, on the other hand, is characterized by a mass of whitish mycelium that grows on the surfaces of infected tomatoes. Destruction of the roots, leaves and flowers of infected plants and massive fruit rots are some of the other symptoms of the disease [14], [15]. Both diseases have been reported to bring about significant annual yield loss in tomatoes and other economically important vegetable crop like cucumber and watermelon [16], [8].

The use of synthetic chemicals and resistant cultivars are the most popular management options for these diseases. Concern about human health is at the top of the chart, while pollution of the environmental and the development of resistant strains of pathogens are some of the other issues. [13]. It is worthy of note also that acceptability, cost and availability are some factors militating against the adoption of resistant cultivars by peasant farmers in most developing countries. New races of the target pathogen may also arise, causing resistance to break down. [11].

It is therefore imperative to adopt options that are environment friendly and sustainable in the management of these diseases. Biological control with antagonistic micro-organisms is one such option. Reference [17] pointed out that fungi in the genus *Trichoderma* have been known for their ability to act as biocontrol agents against plant pathogenic organisms. Their principal mechanisms for control are known to include mycoparasitism, antibiosis, and competition for nutrient and space. They produce several antimicrobial metabolites and other compounds that play important roles in differentiation, symbiosis, metal transport, and stimulation or inhibition of spore formation. [18].

This study evaluated two *Trichoderma* spp. for antagonistic properties against the causal pathogens of fusarium wilt and southern blight diseases of tomatoes. The aim is to encourage the use of bio-control option for the management of these diseases. The objectives were to; i. evaluate *T. harzianum* and *T. viride* for antagonistic properties against *F. oxysporum* and *S. rolfsii*, ii. Determine the most suitable method of application of the biocontrol agents.

II. Materials And Methods

2.1. Study location

This study was carried out at the Pathology Laboratory of the Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure (longitude 5°06′ E to 5°38′ E and between latitude 7°07′N to 7°37′N).

2.2. Preparation of Culture medium

Potato Dextrose Agar-PDA (3.9 g) was dissolved in 100 ml of sterile water in a conical flask and cotton plugged. PDA and other glass wares were sterilized in an autoclave at 121° C for 15minutes. Amendment of the sterilized medium, to inhibit bacterial growth, was with Streptomycin antibiotics (0.2 grams). Sterilized PDA poured into Petri-dishes when it cooled to 45° C.

2.3. Isolation of F. oxysporum and S. rolfsii.

Tomato leaves expressing symptom of fusarium wilt were obtained from the Teaching and Research Farm (TRF) of the Federal University of Technology, Akure. Small segments, about 3 x 5 mm, from the advancing region of the infected tissue were obtained with a sterile scalpel, surface sterilized, rinsed in three changes of sterile distilled water and inoculated on the prepared growth medium in Petri-dishes. Infected tomato fruits containing the mycelia of *Sclerotium rolfsii* was obtained from a vegetable market, “Shasha” at the outskirt of Akure. A sterile needle was used to tease the threadlike mycelia of the pathogen on the infected fruit. The mycelia were then placed on PDA in Petri-dishes and incubated at 28 ± 2° C for 48 hours, after which sub-culturing was carried and pure cultures of the pathogens were obtained.

2.4. Isolation of Trichoderma species

Two strains of *Trichoderma* spp. were isolated from a dead log and moist garden soil. Small splints (about 3 x 4 mm) were obtained from a dead and decaying log at TRF. The splints were inoculated directly on the PDA growth medium prepared as described previously. Small segments of moist garden soil were picked with sterile forceps and sprinkled directly on PDA. Both cultures were incubated for 24 hours, followed by two sets of sub culturing to obtain pure cultures of colonies that were observed in the first isolation.

2.5. Identification of Isolated Pathogens and Trichoderma species

The tentative identification of isolated pathogens was based on their physical, growth characteristics and microscopic observation, following illustrations in standard texts [19], [20]. The identification of *Trichoderma* spp. was based on their morphological and growth characteristics, colour and microscopic observation, with reference to standard texts and report on the morphological and molecular sequence on both organisms [21], [22]. Pathogenicity tests were also carried out by infecting healthy tomato plants (Hausa scissor variety) with 10^6 spore/ml of each of the isolated pathogens.

2.6. Evaluation of T. harzianum and T. viride species for antagonistic efficacy against F. oxysporum and S. rolfsii.

The dual culture method was adopted in a PDA growth medium for evaluation of Trichoderma species for antagonistic efficacy against F. oxysporum and S. rolfsii. The first method involved the inoculation of pathogen and *Trichoderma* at the same time (therapeutic). A 5 mm agar disc each from pathogen and *Trichoderma* were placed at opposite ends in a Petri-dish at the same time. In the second method, *Trichoderma* was introduced into the PDA growth medium at 24 and 48 hours before the introduction of the pathogen (prophylactic). The two *Trichoderma* spp. were evaluated separately against each pathogen using the therapeutic and prophylactic methods. The controls were the sole culture of each pathogen. A 5 mm agar disc of each pathogen was inoculated separately at one end of a Petri-dish at the same time with the dual cultures. Each treatment was replicated thrice and laid out for incubation using the completely randomized design (CRD). The treatments evaluated were;

(i). Simultaneous inoculation of *T. harzianum* and *F. oxysporum* (Therapeutic)

(ii). Inoculation of *T. harzianum* 24 hours ahead *of F. oxysporum* (Prophylactic)

(iii). Inoculation of *T. harzianum* 48 hours ahead *of F. oxysporum* (Prophylactic)

(iv). Simultaneous inoculation of *T. harzianum* and *S. rolfsii* (Therapeutic)

(v). Inoculation of *T. harzianum* 24 hours ahead *of S. rolfsii* (Prophylactic)

(vi). Inoculation of *T. harzianum* 48 hours ahead *of S. rolfsii* (Prophylactic)

(vii). Simultaneous inoculation of *T. viride* and *F. oxysporum* (Therapeutic)

(viii). Inoculation of *T. viride* 24 hours ahead *of F. oxysporum* (Prophylactic)

(ix). Inoculation of *T. viride* 48 hours ahead *of F. oxysporum* (Prophylactic)

(x). Simultaneous inoculation of *T. viride* and *S. rolfsii* (Therapeutic)

(xi). Inoculation of *T. viride* 24 hours ahead *of S. rolfsii* (Prophylactic)

(xii). Inoculation of *T. viride* 48 hours ahead *of S. rolfsii* (Prophylactic)

(xiii). Sole cultures of F. oxysporum and S. rolfsii (Controls)

2.7. Data collection and Statistical analysis

The mycelial growth of *Trichoderma* spp. and pathogens in the sole and dual cultures were measured, at three days intervals. The values obtained were converted to percentage inhibition of the mycelial growth of pathogens by the *Trichoderma* spp. using equation 1.

$$img = \frac{mc - mt}{mc} x\ 100 \qquad \text{(Equation 1)}$$

Where;

img = inhibition of the mycelial growth of the pathogen by *Trichoderma* spp.

mc = mycelial growth in control

mt = mycelial growth of pathogen in treatment

The measurement of the mycelial growth in all treatments continued until the sole cultures, controls, filled up their respective growth medium. Data collected were subjected to analysis of variance (ANOVA) and mean separation using MINITAB, version 17, software.

III. Results

3.1. Identification of F. oxysporum and S. rolfsii

Morphologically, *F. oxysporum* appeared cottony and fluffy, with a tint of pink colour on the PDA growth medium (Figure 1a). Microscopically, the hyphae had cross walls, while the conidia appeared hyaline and shaped like a small canoe. Generally, the growth rate was slow, as it took about nine days for the sole cultures to fill up the Petri-dishes. *Sclerotium rolfsii* on the other hand had a rapid growth. The mycelium was white/cream coloured and ramify profusely, like a network of plant roots, from the inoculum source towards the edge of the Petri-dish (Figure 1b). Microscopically, the mycelium was also hyaline and septate. No spore or conidia was observed, but a characteristic hook-like structure was seen at some points in the mycelium. At about one week of growth, a certain brown structure, sclerotia were seen on the mycelium.

3.2. Identification of T. harzianum and T. viride

Trichoderma harzianum and *T. viride* grew very fast and looked very similar morphologically at the first few days of growth. As the culture got older, however, the differences became more obvious. Morphologically, *T. harzianum* had a little tint of blue colour in a one-week-old culture, which was absent in *T. viride*. Both were however green in colour. Additionally, the PDA culture medium assumed a dull yellow colouration on the reverse side of Petri-dish in *T. harzianum* culture, while it was colourless in *T. viride* (Figure 1c and 1d). Microscopically, differences were observed in the structure of phialide and conidia of the two *Trichoderma* spp. While spherical shaped conidia were seen in *T. viride* while that of *T. harzianum* was ovoid [23].

Fig 1. Pure culture of (a). *Fusarium oxysporum,* (b). *Sclerotiu rolfsii,* (c). *Trichoderma harzianum,* (d). *Trichoderma viride*

3.3. Inhibition of the mycelial growth of F. oxysporum by T. harzianum (%)

The observed trend was an increasing inhibition percentage of the mycelial growth of *F. oxysporum* by *T. harzianum* with the increasing number of days after inoculation in all treatments (Figure 2). Significant differences ($P \leq 0.05$) were observed in the mycelial growth inhibition of *F. oxysporum* across treatments and days after inoculation. On the third day after inoculation, the 48 hours prophylactic inoculation of *T. harzianum* ahead of *F. oxysporum* recorded a 45.23% inhibition of the mycelial growth of the pathogen. This was the highest value and it was significantly different from the other two treatments (Figure 2). On the 6th day after inoculation, the therapeutic treatment recorded the lowest percentage of mycelial growth inhibition of the pathogen, 38.92% (Figure 2 and Figure 3a). On the other hand, however, the 48 hours prophylactic evaluation had 84.62% mycelial growth inhibition of the pathogen on the 9th day after inoculation (Figure 2 and Figure 3b). This was the highest value.

3.4. Inhibition of the mycelial growth of S. rolfsii by T. harzianum (%)

In a similar pattern to what was observed on the inhibition of the mycelial growth of *F. oxysporum* by *T. harzianum*, the inhibition of the mycelial growth of *S. rolfsii* increased with increasing days after inoculation. It was observed, however, that the inhibition percentages were lower against *S. rolfsii* as compared to *F. oxysporum* in the three treatments (Figure 4). Significant differences ($P \leq 0.05$) on the inhibition of the growth of S, rolfsii across treatments and days after inoculation. On the third day after inoculation, 42.88% of mycelial growth inhibition was recorded in 48 hours of prophylactic treatment. This value was the highest at this time, and it was significantly different from the other treatments. On the 6th day after inoculation, the therapeutic and 24

hours prophylactic evaluations recorded statistically similar values, 39.23% and 37.27% respectively, of mycelial growth inhibitions against *S. rolfsii* (Figure 4 and Figure 5a) On the 9th day after inoculation, 48 hours prophylactic evaluation recorded 69.01% inhibition (Figure 4 and Figure 5b). This value was significantly higher than the other two treatments. The therapeutic evaluation recorded the lowest value of inhibition, 64.13%.

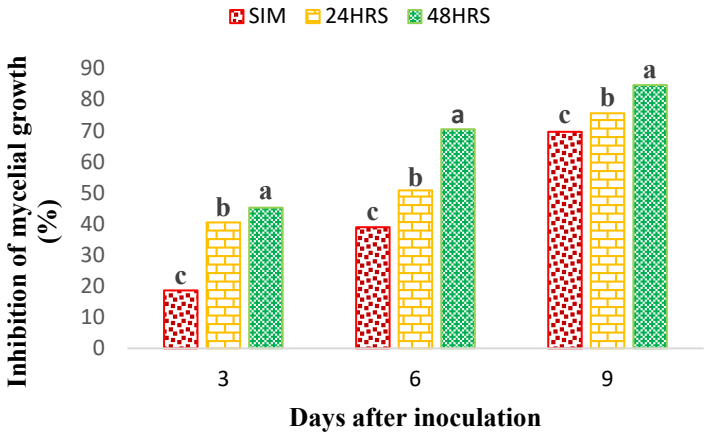

Fig 2: Inhibition of the mycelial growth of *F. oxysporum* by *T. harzianum* (%)

Bar columns with the same pattern and letter(s) are not significantly different by Tukey's test at 5% level of probability.

Legend:

SIM: Simultaneous inoculation of *T. harzianum* and *F. oxysporum*

24HRS: Inoculation of *T. harzianum* 24hrs ahead of *F. oxysporum*.

48hrs: Inoculation of *T. harzianum* 48hrs ahead of *F. oxysporum.*

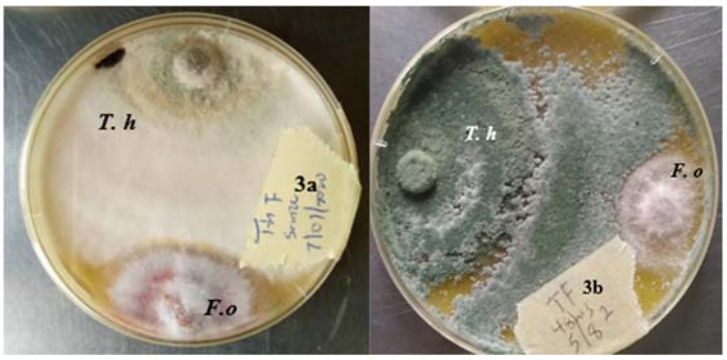

Fig 3: (a). Therapeutic evaluation of *T. harzianum* against *F. oxysporum* (six days after inoculation), (b). Prophylactic (48 hours) evaluation of *T. harzianum* against *F. oxysporum* (nine days after inoculation)

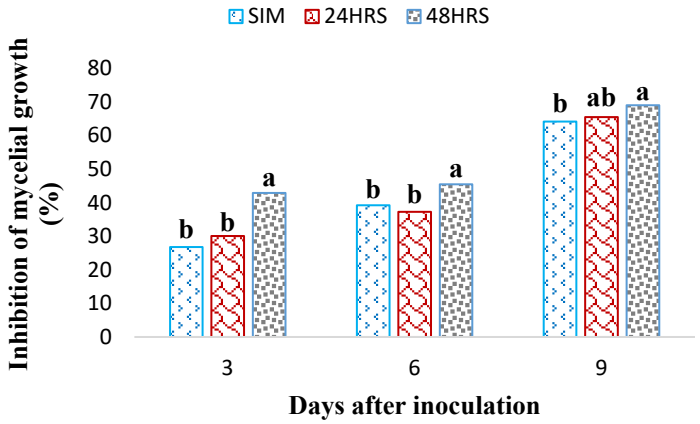

Fig 4: Inhibition of the mycelial growth of *S. rolfsii* by *T. harzianum* (%)

Bar columns with the same pattern and letter(s) are not significantly different by Tukey's test at 5% level of probability.

Legend

SIM: Simultaneous inoculation of T. harzianum and S. rolfsii

24HRS: Inoculation of T. harzianum 24hrs ahead of S. rolfsii.

48HRS: Inoculation of T. harzianum48hrs ahead of S. rolfsii.

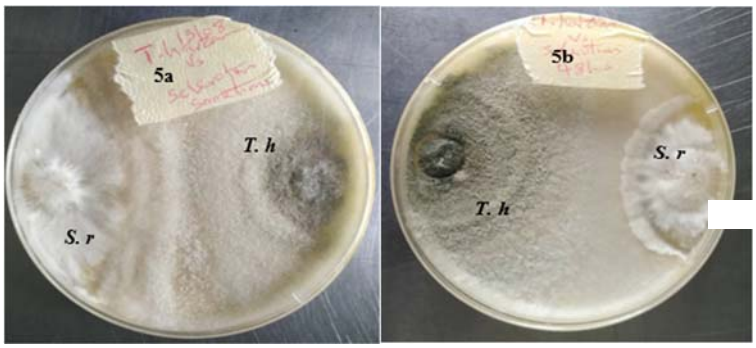

Fig 5: (a). Therapeutic evaluation of *T. harzianum* against *S. rolfsii* (six days after inoculation), (b). Prophylactic evaluation (48 hours) of *T. harzianum* against *S. rolfsii* (Six days after inoculation)

3.5. Inhibition of the mycelial growth of F. oxysporum by T. viride

A trend of increasing percentage inhibition of the mycelial growth of the pathogens with increasing days after inoculation was recorded. *Trichoderma viride* however, had less antagonistic properties against the pathogens when compared to *T. harzianum*. This is because the values of mycelial growth inhibition recorded for it were lower in almost all treatments. Significant differences in the inhibition percentages were however observed among the treatments. On the third day after inoculation, 48 hours of prophylactic treatment recorded 40.12% inhibition of the mycelial growth of *F. oxysporum* (Figure 6). This value was significantly higher than the other treatments. On the 6th day after inoculation, the therapeutic treatment recorded significantly lowest mycelial growth inhibition of 34.72% (Figure 6). A mycelial growth inhibition value of 77.83% was recorded by 48 hours of prophylactic treatment on the 9th day after inoculation. It was statistically the highest value. The therapeutic treatment on, the other hand, produced 53.32% inhibition. It was statistically the lowest (Figure 6).

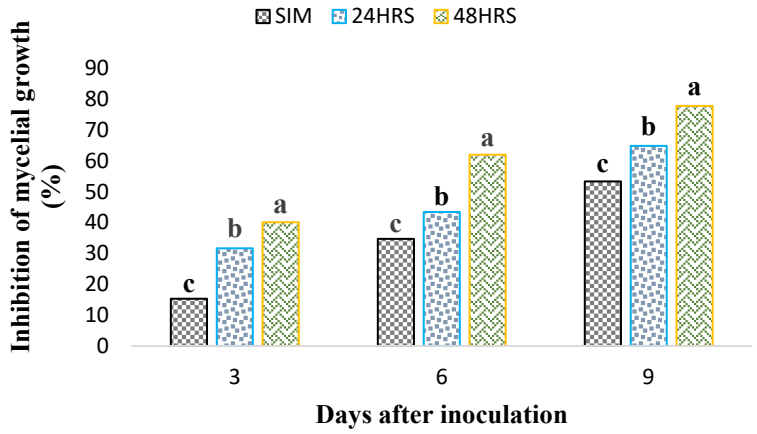

Fig 6: Inhibition of the mycelial growth of *F. oxysporum* by *T. viride* (%)

Bar columns with the same pattern and letter(s) are not significantly (P≤0.05) different by Tukey's test.

Legend

SIM: Simultaneous inoculation of T. Viride and F. oxysporum

24HRS: Inoculation of T. Viride 24hrs ahead of F. oxysporum

48hrs: Inoculation of T. Viride 48hrs ahead of F. oxysporum

3.6. Inhibition of the mycelial growth of S. rolfsii by T. viride

The antagonistic property of *T. viride* against *S. rolfsii* was poor in all treatments, especially on the third day after inoculation. On this day, 9.96% of mycelial growth inhibition was recorded in the therapeutic treatment (Figure 7). It was statistically significant. The 48 hours prophylactic treatment was the most promising, as it consistently produced the highest inhibition percentages on all the days for which data were collected, 27.80%, 42.96%, and 59.78%, on the third, sixth and ninth days after inoculation respectively (Figure 7). These values were statistically the highest on each day the data were collected.

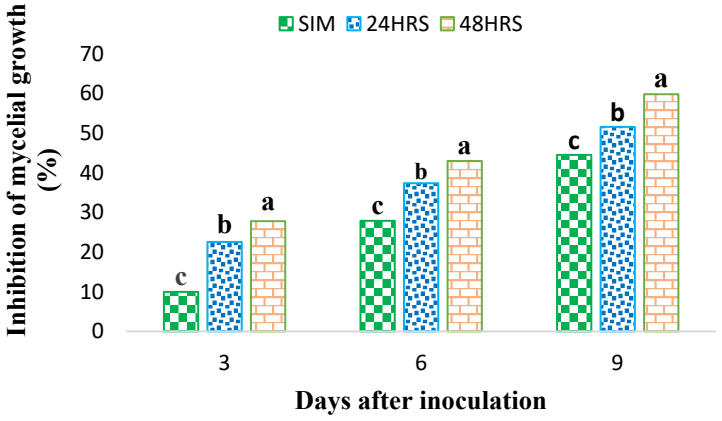

Fig 7: Inhibition of the mycelial growth of *S. rolfsii* by *T. viride* (%)

Bar columns with the same pattern and letter(s) are not significantly (P≤0.05) different by Tukey's test.

Legend

SIM: Simultaneous inoculation of T. Viride and S. rolfsii

24HRS: Inoculation of T. Viride 24 hours ahead of S. rolfsii.

48hrs: Inoculation of T. Viride 48 hours ahead of S. rolfsii.

IV. Discussion

Results from the growth pattern and morphological characteristics of the *F. oxysporum* through macroscopic and microscopic observation conform to that of [24]. Murugan [24] and his team went a step further by conducting molecular analysis of their isolate for characterization. Similarly, the feature that was used in the identification of *S. rolfsii* agreed with that of [25]. Both pathogens were isolated from infected tomato plants (Hausa scissor variety), and their ability to incite the same disease through pathogenicity test by injecting 10^6 spore/ml of each of the isolated pathogens into healthy tomato plants, is a confirmation of the authenticity of the isolates. The features, growth pattern, colour and morphology, that were adopted in the identification of the two *Trichoderma* spp. conforms to the works of [21], [22]. *Trichoderma harzianum* and *T. viride* both showed antagonistic properties against *F. oxysporum* and *S. rolfsii* and inhibited their growth significantly. This is in agreement with the findings of previous studies of [21], [26], [27], [28]. The mechanisms of inhibition of the mycelial growth of fungal pathogen by *Trichoderma* spp. has been explained to be through antibiosis, mycoparasitism, production of toxic metabolites amongst others. [29], [30]. Reference [31] reported that *T. harzianum* produces a large number of peptaibols as its secondary metabolites. These metabolites were earlier confirmed to have antimicrobial properties by [32]. They pointed out that peptaibols inhibit the activity of β-(1, 3) glucan synthase, responsible for the formation of the cell wall in pathogenic fungi. In a similar study on a *Trichoderma* strain, reference [33] obtained the extracellular metabolites from *Trichoderma spirales* cultures filtrates and examined their inhibitory effect against *Phytophthora capsici, Corynespora cassiicola* and *Curvularia aeria*. The results showed that the *Trichoderma* strain completely inhibited the growth of *Phytophthora capsici* and produced metabolites that inhibited the growth of *C. cassiicola* (84.68%) and *C. aeria* (93.03%) respectively. In this study, *Trichoderma harzianum* exhibited a better antagonistic property against both pathogens. This may be due to greater production of toxic metabolites, increased rate of sporulation or mycelial coiling around the mycelium of the pathogen. This view is supported by the findings of [29]. The prophylactic application of both *Trichoderma* spp. had better antagonistic properties than the therapeutic one [34]. This could be because of the presence of toxic metabolites already present in the growth medium, in addition to the deprivation of nutrient medium for growth. The implication is that prophylactic application of *Trichoderma* spp. in the management of Fusarium wilt and southern blight diseases, as seed treatment/priming agent or soil amendment before planting, may be more effective than their application after a tomato plant may have been infected by the pathogens.

V. Conclusion

This study evaluated *T. harzianum* and *T. viride* for antagonistic properties against *F. oxysporum* and *S. rolfsii*. The results obtained showed that both biocontrol agents were able to suppress the growth of both pathogens in vitro, especially when used prophylactically. *Trichoderma harzianum* was however the more effective of the biocontrol agent. Both *Trichoderma* spp. maybe recommended for further work in the formulation of biocontrol agents for the management of the two diseases.

Funding

No funding was received for this work

Competing interest

The authors declare that there is no competing interest in the conduct and publication of this study.

References

[1] S. Naika, J. Van Lidt de Jeude, M. De Goffau, M. Hilmi, and B. Van Dam, “Cultivation of tomato. production, processing and marketing,” pp. 92, 2005. Available at: https://www.researchgate.net/publication/331167081_Cultivation_of_tomato_production_processing_and _marketing.

[2] J. K. Willcox, G. L. Catignani, S. Lazarus, “Tomatoes and cardiovascular health,” Critical Reviews in Food Science and Nutrition, vol. 43, pp. 1-18, 2003. DOI: 10.1080/10408690390826437

[3] A. E. Vinha, R. C. Alves, V. P. Sergio, A. Castro, M. B. C. Anabela, and P. P. Olivera, “Effect of Peel and Seed Removal on the Nutritional Value and Anti-oxidant Activity of Tomato (Lycopersicum esculentum. L) Fruit,” Food Science and Technology, vol. 55, pp. 192-202, 2014. DOI: 10.1016/j.lwt.2013.07.016

[4] R. G. Olmstead, L. Bohs, H. A. Migid, E. Santiago-Valentin, V. F. Garcia, and S. M. A. Collier, "Molecular phylogeny of the solanaceae," Taxon, vol. 57, pp. 1159-1181, 2008. DOI: 10.1002/tax.574010

[5] FAOSTAT. Food balance sheet: Tomatoes and products, production and losses. http://www.fao.org/faostat/en/data/FBS. 2013.

[6] M. Nowicki, E. U. Kozik, and M. R. Foolad, "Late blight of Tomato In: Translational genomics for crop breeding," Biotic stress, vol. 1, pp. 241-266, 2013.

[7] P. Kumar, and A. K. Sood, "Control of bacterial wilt of tomato with VAM and bacterial antagonists," Indian Phytopathology, vol. 55, pp. 51-53, 2002.

[8] R. S. Singh, Plant Diseases, (Eighth Ed), Oxford & IBH Publishing Co. Pvt Ltd., New Delhi, 2005, 78.

[9] A.Ayandiji, O. R. Adeniyi, and D. Omidiji, "Determinant post-harvest losses among tomato farmers in Imeko-Afon local government area of Ogun State, Nigeria," Global Journal of Science Frontier Research, vol. 11(5): pp. 22-28, 2011.

[10] M. Ajayi, and G. F. Hassan, "Response of selected tomato (Solanum lycopersicum (L.) cultivars to on-field biotic stress," Journal of Agricultural and Crop Research, Vol. 7(3), pp. 38-46, 2019. Doi: 10.33495/jacr_v7i3.19.110.

[11] Akkopru, and S. Demir, "Biological control of fusarium wilt in tomato caused by Fusarium oxysporum f. sp. lycopersici by AMF Glomus intraradices and some Rhizobacteria," Journal of Phytopathology, vol. 153, pp. 544-550, 2005, http://dx.doi.org/10.1111/j.1439-0434.2005.01018.x

[12] J. B. Ristaino, K. B. Perry, and R. D. Lumsden, "Effect of solarization and Gliocladium virens on Sclerotium rolfsii, soil microbiota, and disease incidence on tomato," Phytopathology, vol. 81, pp. 117-1124, 1991.

[13] M. Moretti, G. Gilardi, M. L. Gullino, and A. Garibaldi, "Biological control potential of Achromobacter xylosoxydans for suppressing fusarium wilt of tomato," International. Journal of Botany, vol. 4, pp. 369–375, 2008. DOI: 10.3923/ijb.2008.369.375

[14] K. Liamngee, Y. H. Zakki, and D. O. Onah, "Sclerotium rolfsii; Causative organism of southern blight, stem rot, white mold and sclerotia rot disease," Annals of Biological Research, vol. 6 (11), pp. 78-89, 2015.

[15] S. K. Dwivedi, and G. Prasad, "Integrated management of Sclerotium rolfsii: an overview," European Journal of Biomedical and Pharmaceutical Sciences, vol. 3(11), pp. 137-146, 2016.

[16] Z. K. Punja, "The biology, ecology, and control of Sclerotium rolfsii," Annual Review of Phytopathology, vol 23, pp. 97–127, 1985. https://doi.org/10.1146/annurev.py.23.090185.000525

[17] G. E. Harman, C. R. Howell, A. Viterbo, I. Chet, and M. Lorito, "Trichoderma species opportunistic, avirulent plant symbionts," Nature Reviews Microbiology, vol. 2(1), pp. 43- 56, 2004. https://doi.org/10.1038/nrmicro797

[18] F. Vinale, K. Sivasithamparam, L. E. Ghisalberti, R. Marra, L. S. Woo, and M. Lorito, "Trichoderma-plant-pathogen interactions," Soil Biology and Biochemistry, vol. 40, pp. 1-10, 2008. https://doi.org/10.1016/j.soilbio.2007.07.002

[19] H. Barnett, and L. Hunter, Illustrated genera of imperfect fungi 4th (ed). American phytopathological society. St. Paul, Minnesota, USA. 1998, 218.

[20] F. M. Dugan, The identification of fungi. An illustrated introduction with keys, glossary and guide to literature. American phytopathological society, St. Paul, Minnesota, USA. 2005, 176 pp.

[21] N. Chakraborty, U. Chakraborty, A. Saha, P. L. Dey, and K. Sumar, "Morphological and molecular characterization of Trichoderma isolates of North Bengal," Journal of Mycology and Plant Pathology, vol. 41(2), pp. 207-214, 2011.

[22] M. M. Shah, and H. Afiya, "Identification and isolation of Trichoderma spp.; their significance in agriculture, human health, industrial and environmental application," p. 12, 2019. Intech Open. DOI: http://dx.doi.org/10.5772/ntechopen.83528.

[23] S. Shaiesta, N. Sahera, and P. A. Sheikh, "Cultural and morphological characterization of Trichoderma spp. Associated with green mold disease of Pleurotus spp. In Kashmir," Research Journal of microbiology, vol. 7, pp. 139-144, 2012.

[24] L. Murugan, N. Krishnan, V. Venkatarvanappa, S. Saha, A. K. Mishra, B. K. Sharma, and A. B. Rai, "Molecular characterization and race identification of Fusarium oxysporum f. sp. lycopersici infecting tomato in India,".3Biotech, vol. 10(486), pp. 1-12, 2020. https://doi.org/10.1007/s13205-020-02475-z

[25] S. Mahadevankumar, V. Yadav, G. S. Tejaswini, and G. R. Janardhana, "Morphological and molecular characterization of Sclerotium rolfsii associated with fruit rot of Cucurbita maxima," European Journal of Plant Pathology, vol. 145, pp. 215-219, 2015. DOI: https://doi.org/10.1007/s10658-015-0818-1.

[26] M. A. H. B. Bhuiyan, M. T. Rahman, and K. A. Bhuiyan, "In vitro screening of fungicides and anatagonists against Sclerotium rolfsii,"

[27] African Journal of Biotechnology, vol. 11(82), pp. 14822-14827, 2012. DOI:10.5897/AJB12.1463. K. Doley, and P. K. Jite, In vitro efficacy of Trichoderma viride against Sclerotium rolfsii and Macrophomina phaseolina," Notulae

[28] Scientia Biologicae, vol. 4(4), p. 39, 2012. DOI: https://doi.org/10.15835/nsb447818
[29] M. Babychanand, and S. Simon, "Efficacy of Trichoderma spp. against Fusarium oxysporum f. sp. lycopersici (FOL) infecting pre-and-post seedling of tomato," Journal of Pharmacognosy and Phytochemistry, vol. 6(4), pp. 616-619, 2017.
[30] R. M. Barakat, F. Al-Mahareeq, and M. I. Al-Maris, "Biological control of Sclerotium rolfsii by using Trichoderma spp. isolates from Palestine," Hebron University Research Journal, vol. 2(2), pp. 27-47, 2001.
[31] Y. Brotman, J. G. Kapuganti, and A. Viterbo, "Trichoderma," Current Biology, vol. 20, pp. 390–391, 2010. DOI:10.1016/J.CUB.2010.02.04
[32] J. Nawrocka, and U. Małolepsza, "Diversity in plant systemic resistance induced by Trichoderma," Biological Control, vol. 67, pp. 149-156, 2013. https://doi.org/10.1016/j.biocontrol.2013.07.005
[33] M. Lorito, V. Farkas, S. Rebuffat, B. Bodo, and C. P. Kubicek, "Cell wall synthesis is a major target of mycoparasitic antagonism by Trichoderma harzianum," Journal of Bacteriology, vol. 178, pp. 6382–6385, 1996. doi: 10.1128/jb.178.21.6382-6385.1996.
[34] Baiyee, C. Pornsuriya, S. I. Ito, and A. Sunpapao, "Trichoderma spirale T76-1 displays biocontrol activity against leaf spot on lettuce (Lactuca sativa L.) caused by Corynespora cassiicola or Curvularia aeria," Biological Control, vol. 129, pp. 195–200, 2019.
[35] Sanchez-Montesinos, M. Santos, A. Moreno-Gavira, T. Marin-Rodulfo, F. J. Gea, and F. Dianez, "Biological control of fungal diseases by Trichoderma aggressivum f. europaeum and its compatibility with fungicides," Journal of fungi, vol. 7, p. 598, 2021. https://doi.org/10.3390/jof7080598