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EFFECT OF APPLICATION METHODS OF *Trichoderma asperellum* ON THE CONTROL OF WHITE MOLD DISEASE (*Sclerotium rolfsii*) OF TOMATO (*Solanum lycopersicum MILL*)

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Abstract
A greenhouse study at the Federal University of Technology, Akure's screen house and laboratory of the Department of Crop, Soil, and Pest Management assessed the effectiveness of *Trichoderma asperellum* under three application methods on growth and control of a tomato white mold disease caused by *Sclerotium rolfsii*. Dual culture techniques under the prophylactic method recorded 60% inhibition of the mycelia growth of *S. rolfsii* when *T. asperellum* was inoculated twenty-four hours before the introduction of *S. rolfsii.* This was much greater than the 20% mycelial inhibition reported by curative and simultaneous application methods, in which *S. rolfsii* was introduced first and concurrently with the pathogenic fungus. *Trichoderma asperellum* at 10^5 spores/ml *was tested as a* seed treatment agent, prophylactic, and curative (Foliar) application in relation to application time. Seeds moistened with *T. asperellum* for five days were transferred into a plastic experiment pot filled with sterilized soil as a seed treatment, whereas the prophylactic method involved foliar application *of T. asperellum on healthy* tomato seedlings for two weeks, followed by covering the experimental pots with transparent polythene for twenty-four hours before the introduction of *S. rolfsii* suspension. The curative application involved the initial application of *S. rolfsii* suspension on soil containing two weeks of healthy tomato seedlings and then covered with transparent polythene for twenty-four hours before the introduction of *T. asperellum* on the soil surface. The results show that tomato yield increased significantly with seed treatment, while prophylactic application had the greatest antifungal effects with the lowest incidence and severity values.
Key words: *Trichoderma asperellum*, seed treatment, prophylactic, curative.

Introduction

White mold disease, caused by the fungus *Sclerotium rolfsii,* poses a significant threat to global tomato production. This soilborne pathogen infects tomato plants, leading to damping-off of nursery seedlings and crown rot in adult plants (Abd-Elghany *et al.*, 2021). Tomatoes, as a common crop in home gardens, are indeed vulnerable to various diseases that can lead to significant annual yield losses. Factors such as diseases caused by pathogens, insects, or nutritional deficiencies have a substantial impact on tomato crops. Studies have shown that these

losses can reach up to 100% in extreme cases (Chaudhary *et al.*, 2022). Among the major economically important diseases are damping off, wilt blight, leaf spots and white mold which are triggered by several pathogens such as *Pythium aphanidermatum, Fusarium spp.*, *lycopersici*, *Rhizoctonia solani*, Phytophthora infestans and *Scelerotium rolfsii. Fusarium oxysporum*, *Sclerotium rolfsii*, and *Phytophthora infestans* have also been identified as causes of damping-off disease in tomato plants (Elshahawy *et al.*, 2018)

In recent years, biological control agents have become more popular as an environmentally friendly substitute for chemical pesticides in managing plant diseases. *Trichoderma asperellum* has shown promise in treating *Phytophthora infestans, Alternaria alternate* f. sp. lycopersici, *Septoria lycopersici,* and *Sclerotuim rolfsii.* Studies indicate that this fungus exhibits various mechanisms aiding disease control, including antagonism, competition, mycoparasitism, antibiosis, and the induction of plant defense responses. Notably, *Trichoderma asperellum* demonstrates potential as a biological control agent against white mold disease (Si *et al.*, 2022).

Research has extensively highlighted *Trichoderma asperellum*'s efficacy against soil-borne fungal plant pathogens like *Sclerotinia spp.*, *Fusarium oxysporum spp.*, *lycopersici,* and *Rhizoctonia solani*, (Si *et al.*, 2022). Moreover, it shows characteristics ideal for biocontrol, such as extensive colonization of root surfaces, enzyme production that degrades pathogenic fungi, and the induction of systemic resistance in plants.

However, the effectiveness of Trichoderma species as a biocontrol agent depends on several factors, including soil type, environmental conditions, inoculum density, timing of application, and the specific plant-pathogen interaction. Additionally, *Trichoderma asperellum* displays potential as a bioremediation agent for soil, sediment, or water contaminated with heavy metals, (Guzmán-Guzmán *et al.*, 2023).

Various studies have underscored the critical role of the application method in the efficacy of Trichoderma as a biocontrol agent, often more significant than the concentration of the inoculum. Different application techniques impact the mechanisms of bio-control exhibited by the fungus strain, influencing its ability to suppress plant pathogens.

Furthermore, the presence of Trichoderma strains in soil enhances the availability of essential nutrients like phosphorus, iron, and zinc, stimulating root and shoot growth and micronutrient uptake (Zhao *et al.*, 2014). This fungus versatility in metabolite production makes it a compelling option for diverse applications as biocontrol agent. This research aims to uncover the most effective approach to leverage the biocontrol capabilities of *Trichoderma asperellum* in combating white mold disease in tomatoes.

Materials and methods

Place of study and isolation of fungi strains:

The study, conducted at the Federal University of Technology, Akure, focused on isolating the pathogen *Sclerotium rolfsii* from diseased tomato fruits collected at the Akure market in Nigeria. The isolation involved surface sterilization, culturing on Potato Dextrose Agar (PDA), and subsequent sub-culturing to obtain pure cultures of *S. rolfsii.* The isolation method followed techniques described Nevalainen *et al.*, (2014). The antagonistic fungus *Trichoderma asperellum* was isolated from soil samples taken from soil rhizosphere. This fungus was identified as *Trichoderma asperellum* (Lierkf) based on

colony morphology and microscopic observations followed a modified technique described by Williams *et al.* (2003), at the biotechnology and pathological section of the International Institute of Tropical Agriculture (IITA), Ibadan

Preparation of Trichoderma inoculums.

Trichoderma conidia were harvested from a five-day old pure culture by adding 10mL of sterilized distilled water, swirling, and scraping the culture, following a method outlined by Prasad *et al.,* (2022) The resulting conidial suspension was filtered through muslin to eliminate mycelium. Three different concentrations of conidia were prepared and their spore densities adjusted using a haemocytometer slide to create concentrations of $1x10^4$ spores/ml, 1×10^5spores/ml, and 1×10^6 spores/ml. The viability of the conidia was assessed by plating small amounts of the suspension on suitable media to observe further growth, following a method detailed by Denman *et al.,* (2005). The study utilized a conidial suspension of 1×10^5 spores/ml, which showed fungal growth during the viability evaluation.

In-vitro antagonistic test

The *in-vitro* antagonistic test evaluated the activity of *T. asperellum* against *S. rolfsii* using Petri dishes containing PDA, (Evans *et al.,* 2003). Three different inoculation approaches were utilized: Simultaneous inoculation (SIM), Delayed inoculation for prevention (PRV), and Delayed inoculation for curative (CUR) alongside a control (CNTR). Each method involved placing mycelial discs of *T. asperellum* and *S. rolfsii* 8cm apart on the same dish, following specific timelines.

For PRV, *T. asperellum* was introduced twenty-four hours before *S. rolfsii* to prevent infection, while for CUR, *S. rolfsii* was incubated twenty-four hours before the introduction of *T. asperellum* for curing. The plates were incubated at a constant temperature, and the formation of inhibition zones between the isolates was observed and measured at three and six days. Radial growth of mycelia was measured, and the inhibition of mycelial growth was calculated by comparing the pathogen's growth in control plates with its growth in the presence of *Trichoderma asperellum*.

The percentage of inhibition of mycelial growth was calculated using the method of Sivan and Chet, (1989), modified by Shoreh *et al.*, 2010

Inhibition Percentage= (Control Growth (CG) - Treatment Growth (TG)) \Control Growth* 100

CG - Control Growth refers to the radial growth of the pathogen in the absence of *T. asperellum*. - TC-Treatment Growth refers to the radial growth of the pathogen in the presence of *T. asperellum.*

This was done to determine the percentage of mycelial growth inhibition.

Screenhouse (*In vivo*) test of antagonism

Thirty healthy tomato seeds were distributed among three Petri dishes, lined with sterile filter paper, and consistently moistened with *T. asperellum* concentrations of 10^5 spore/ml to apply a seed bio-priming method until sprouting occurred. For the preventive and curative approaches, another thirty healthy seeds were moistened with sterile distilled water until germination. These seedlings were then transplanted into experimental pots containing sterilized soil.

After two weeks, different methods were employed for *S. rolfsii* application. For the seed bio-priming method, a suspension of *S. rolfsii* was applied to the initially moistened seeds. In the preventive method, a suspension of 10^5 spores/ml of *T. asperellum* was foliarly applied per plant stand twenty-four hours prior to *S. rolfsii* application. In the curative method, a suspension of *S. rolfsii*

was applied using foliar application methods 24hours before the surface soil application of 10^5 spores/ml of *T. asperellum*.

The Control treatments is a healthy tomato plants without inoculation of *S. rolfsii* and, consequently, no disease control methods were applied. Additionally, a standard control involved the use of chemical fungicides (Mancozeb) applied prophylactically through foliar spray twenty-four hours after pathogen inoculation. Each treatment was replicated three times, resulting in thirty-six experimental pots arranged in a Completely Randomized Design (CRD). All experimental pots were covered with transparent polythene bags intermittently for twenty-four hours before the introduction of the respective organisms.

Data collection

Data were analysis on growth parameters, disease incidence and severity and analyzed using ANOVA Minitab17vs, and statistical means separated by Tukey's test.

Results

Colony description

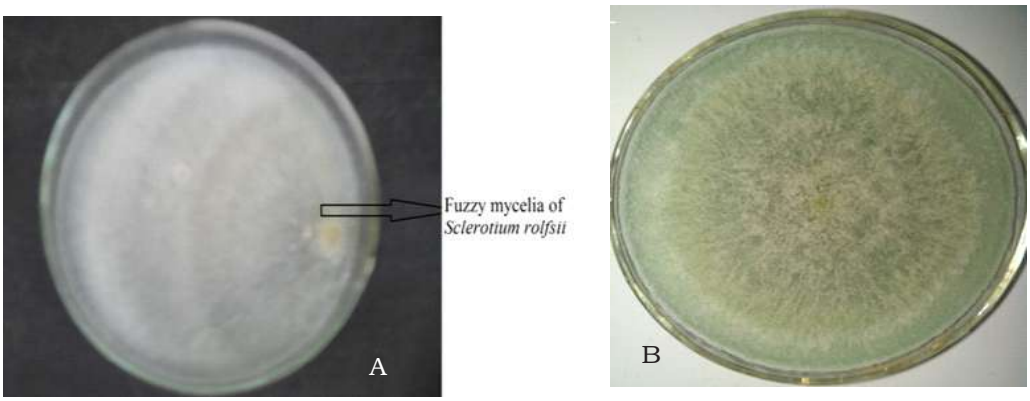


Plate 1: A - fuzzy white mycelia mass of pathogenic fungus (*S. rolfsii*) on PDA media.
B: watery white colony to dark/dull green with compact conidiophores forming undulating concentric rings of *T. asperellum* on PDA media

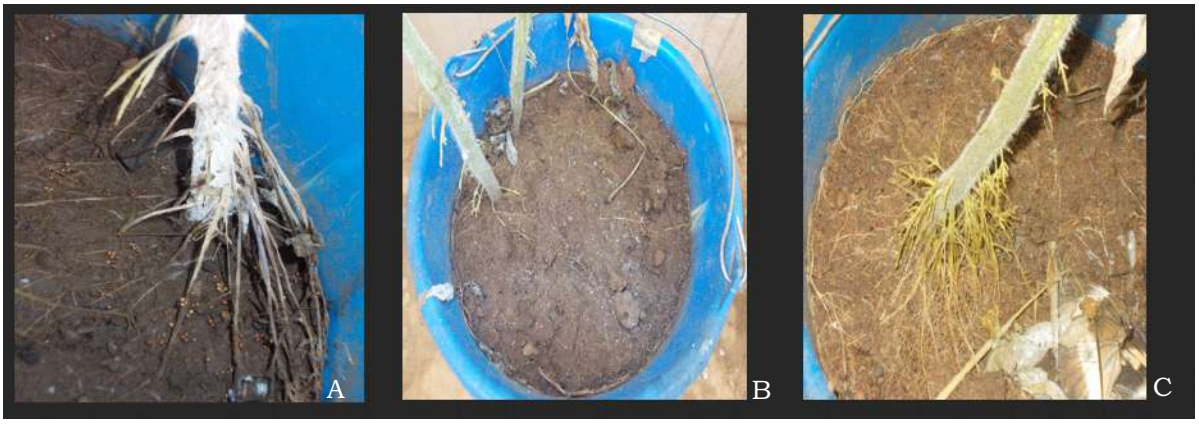


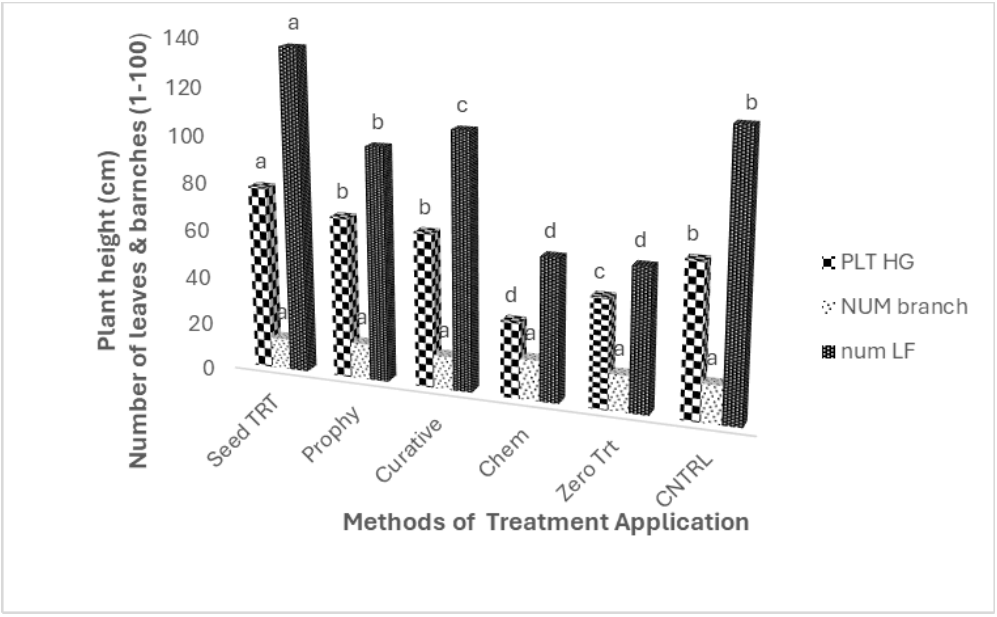
Plate 2. Effects of different treatment methods on root colonization of tomato:
A- Soil surface of tomato plant with no bio- control showing mycelia and sclerotia of *S. rolfsii*.
B- Soil surface of plant treated with Mancozeb (Chemical control)
C- Soil surface of plant treated with *Trichoderma asperellum* showing massive root development with no sign of root infection.

Effects of *T. asperellum* on the germination of tomato seed.

In the seed treatment experiment, the percentage of germination observed at 10^5 spores/ml was 90.2%; at concentrations of 10^6 and 10^4 spores/ml, the corresponding percentages were 86.4% and 86.1%. These values were significantly different from each other. These values were significantly different from each other.

Effects of different treatment methods on growth parameters of Tomato.

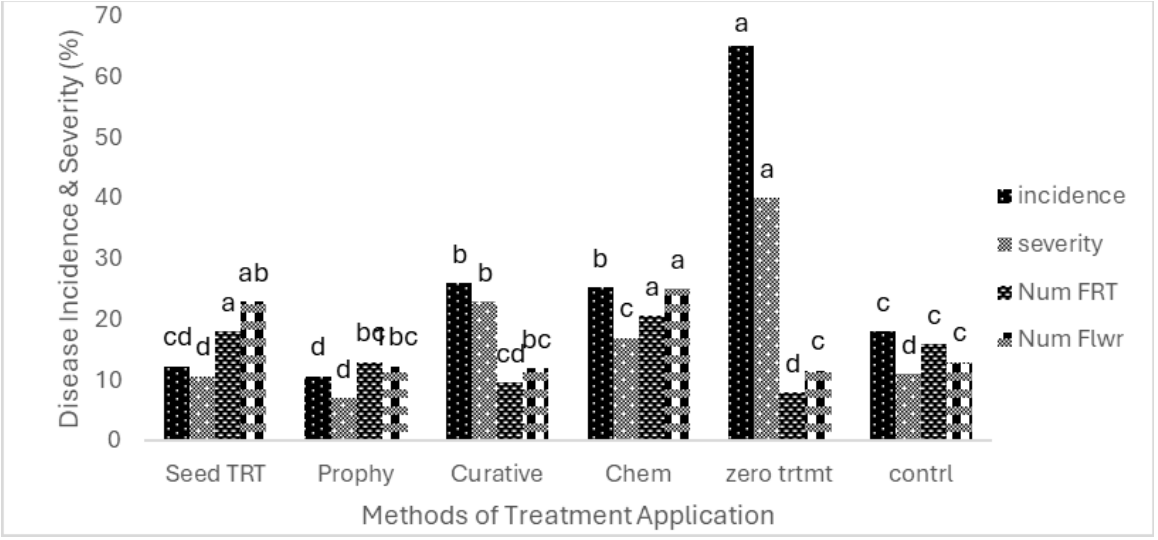
The result obtained for both number of leaves, branches and plant height at five weeks after treatment application are shown in figure 1 below. Treatment with mancozeb and no biocontrol agent (Zero) recorded lower mean value which do not differ significantly in their number of leaves and branches but differs in plant height, while the highest mean value for number of leaves, branches and plant height were recorded in seed treatment method, whereas the prophylatic, curative and control treatment do no differ significantly in the their reported means for all the growth parameters.I t was previously



Means with the same letter (s) are not significantly different from each other according to Tukey's test. ($P \leq 0.05$)

Figure 1: Effects of different application methods of *T. asperellum* on some growth parameter of tomato.

observed that plants treated with Mancozeb and Zero treatement had significantly fewer leaves, branches, and shorter plant height compared to other treatments (Figure 1). However, the Mancozeb-treated plants showed better performance in terms of the number of flowers and fruits produced, which was not significantly different from seed-treated plants that had the highest mean leaf count. While the mean number of flowers do not differ significantly among the treatments, the application methods significantly impacted the number of fruits produced.



Means with the same letter (s) are not significantly different from each other according to Tukey's test. ($P \leq 0.05$)

Figure 2: Effects of different application methods of *T. asperellum* on some growth parameter and white mold disease incidence and severity of Tomato.

Visual observations of diseased tomato plant showed that the initial symptoms of white mold disease caused by *S. rolfsii* manifested in form of elliptical blister (uredia) on the leaves. Partial and irreversible wilting of the plant with visible outgrowth of sclerotia on the stem or soil line (plate 2A) and eventually death of the whole plant was also observed. Plate 2C showed tomato plant on which no bio-control agent was applied revealing development of mycelia and mass sclerotia, while Plate 2A showed treated tomato plant with *T. asperellum*, bearing no symptoms of the disease.

As shown in Figure 2, the highest disease incidence, at 65%, was observed with zero treatment application. Curative treatment and chemical control had disease incidences of 50% and 38%, respectively, with these values being significantly different from each other. The lowest incidence rates, 7% for the prophylactic approach and 10% for seed-treated plants, were noted. While these values did not differ significantly from the control, they were significantly different from those observed in tomato plants treated with Mancozeb.

Discussion

The inhibition and restriction of mycelial growth of *S. rolfsii* at 62.5% and 50% for prophylactic and curative measure could be due to the ability of *T. asperellum* to grow faster in filling up the agar plate and possible secretion of secondary metabolites by *Trichoderma* spp. which antagonized the mycelia of *S. rolfsii.* this agreed with the report by Harman *et al.* (2004), who indicated that bio-controls agent may grow faster or use its food source more efficiently than the pathogen, thereby out crowding the pathogen and taking over the growing surface, and that inhibition could probably also be due to the secretion of extracellular cell-degrading enzymes such as Chitinase B-1, 3-Glucanase, Cellulose and Lectin which could assist in mycoparasitism as proposed by Rodriguez-Kabana (1978).

The significant different in seed germinate on of *T. asperellum* treated seed when compared

to the untreated control correspond with the report by Mukhtar (2008), that seed treatment with *T. harzianum* gave the highest germination index in okra and Bharath *et al.*, (2006), that seeds of watermelon pretreated with *T. viride*, *T. harzianum* and *T. pseudokoningii* inoculant extracts showed increased seed germination rates, seedling vigour and reduced the incidence of seed-borne fungal pathogens compared to the control.

The greenhouse study revealed that treatment with Mancozeb led to production of less profuse vegetative growth in contrast to *T. asperellum* treated plants where there is a visible observation of abundant root colonization, as a result of numerous root network found on the surface of the treated soil. This might be due to the fact that the presence of *T. asperellum* in the tissue of the plant has the effect on increasing vegetative growth of the tomato plant. This is consistent with Vargas *et al.*, (2009), who reported that Trichoderma spp. have the ability to enhance root colonization, coordinate defense mechanisms, increase leaf photosynthesis rates, and act as solute transporters, aiding in the acquisition of root exudates. Similarly, Viterbo and Chet (2006) noted that *T. asperellum* produces a class I hydrophobin known as *T. asperellum* Hydrophobin 1 (TasHyd1), which supports plant root colonization by improving attachment to the root surface and protecting hyphal tips from plant defense compounds.

In the case of disease incidence and severity, both seed treatment and preventive treatments differed significantly in their value over Mancozeb-treated plants and plants on which no bio-control agent was applied. These results agreed with numerous studies on the beneficial impact of *T. asperellum* as reported by Affokpon *et al.* (2011) that the pre-treatment application of two strains of *T. asperellum* in pure culture in an *in vivo* test reduced infection of tomato wilt by more than 50%. Similarly, Bharath *et al.* (2006) reported that in the presence of *Trichoderma* spp., plants frequently have larger roots and higher levels of productivity.

In contrast to the chemical control by Mancozeb, *T. asperellum* reduced disease incidence and severity significantly when applied as seed treatment and prophylactically. Disease incidence and severity values for *T. asperellum*-treated plants were significantly lower than Mancozeb-treated plants. This could be due to the fact that Mancozeb has been reported to be effective in inhibiting the outgrowth of the fungus up to ten weeks after application. In the course of this research work, Mancozeb-treated plants showed signs of infection and outgrowth of the fungus, this finding correlates with other research studies which has shown that Mancozeb is of low soil persistence, with a reported field half-life of 1 to 7days (USEPA 2010), even though it rapidly and spontaneously degrades to ETU (ethylenethiourea) in the presence of water and oxygen, and may persist for longer on the order of 5 to 10 weeks. Based on this study, *T. asperellum* -treated plants appeared to perform better than Mancozeb because no death or outgrowth of the fungus was recorded for *T. asperellum* treated plants.

Conclusion and Recommendation

It was also discovered during this research work that prophylactic application of *T. asperellum* gave the best result and suggested that the use of very high concentration above 10^5 conidia/ml of the control agent might not be desirable. Additionally, the performance of seed treatment method was not significantly different from preventive method on the seedlings with regards to all the parameters investigated. It can therefore be recommended that *Trichoderma asperellum* be used at a concentration 10^5 conidia/ml for

prophylactically or seed treatment as a biocontrol agent in the management of white mold disease of tomato.

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