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Biological Control of Tomato soil borne fungi by *Aspergillus niger* and *Trichoderma harzianum*

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I. Abstract

The current study aimed to assess *Aspergillus niger* and *Trichoderma harzianum* antagonistic potential against major soil-borne pathogen fungi in vitro, such as *Fusarium oxysporum*, *Alternaria solani*, *Pythium aphanidermatum*, and *Sclerotinia sclerotiorum*. *A. niger* and *T. harzianum* fungal inoculants prepared on millet seeds were used to inoculate the sterilized soil, which was made using gypsum-based soil and sterilised with polyethylene and formalin. To assess the impact of the fungus on plant growth, Super Queen tomato seedlings of comparable height were cultivated in the sterilized pots with three replicates per treatment. The present study demonstrated that *T. harzianum* highly effective on the vegetative length and root length as compared with control, while the lowest vegetative and root length were in the treated with pathogenic fungus. Furthermore the combination *T. harzianum* with pathogen fungus highly increased vegetative and root length. These results show that *T. harzianum* has strong antagonistic activity against *F. oxysporum* and greatly reduces its harmful effects. This leads to better vegetative growth and biomass accumulation in tomato plants grown in a greenhouse. In general, *T. harzianum* was the best biocontrol agent tested in this study.

Keywords : *Biological Control, soil borne fungi, Aspergillus niger and Trichoderma harzianum.*

I. Introduction

The tomato (*Solanum lycopersicum*) is a vital crop for both commercial horticulture and home gardening, owing to its extensive consumption and nutritional significance(1, 2). Nonetheless, many fungal diseases significantly impact tomato cultivation, resulting in substantial yield loss and quality degradation. These fungal pathogens include *Fusarium solani,* , *Alternaria solani*, *F. oxysporum*, and *Rhizoctonia solani* (3). While chemical management of tomato infections is often effective, numerous fungicides provide environmental hazards, facilitate the emergence of resistant fungal strains, and create issues about food safety and human health(4). Consequently, there is significant interest in the biological management of tomato diseases. This encompasses numerous studies on *Trichoderma harzianum*, a helpful fungus acknowledged for its capacity to manage plant diseases through mycoparasitism, the synthesis of hydrolytic enzymes, and the enhancement of plant defenses (5).

Trichoderma spp. are soil fungi categorized in the phylum Ascomycota and the family Hypocreaceae. The Trichoderma genus comprises around 500 species commonly found in soil, associated with plant roots, and in degraded organic matter as saprophytes(6). Trichoderma species are swiftly multiplying fungus distinguished by hyaline phialides, septate hyphae, and conidiophores that often display arboreal arrangements; their mature conidia are typically yellow to green in color (7, 8).. Numerous Trichoderma species are extensively utilized in agriculture due to their advantageous interactions with plants and capacity to antagonise diverse plant diseases, rendering these fungi significant biotechnological assets for sustainable agriculture(9-11).

T. harzianum is recognised as a biocontrol agent effective against numerous plant infections, utilised in the formulation of innovative biological fungicides in agriculture. Besides safeguarding crops, it is well acknowledged for enhancing nutrient availability, improving plant resilience to abiotic stresses like salinity and drought, and facilitating advantageous microbial interactions in the rhizosphere, thereby highlighting the efficacy of plant growth-promoting fungi (12). Before its application in the field to mitigate the dissemination of phytopathogens, a comprehensive understanding of their properties and mechanisms is essential. Moreover, further investigation is necessary to enhance the efficacy and safety of these fungi. The current work aimed to evaluate the antagonistic potential of *Aspergillus niger* and *T. harzianum* against significant soil-borne phytopathogenic fungi in vitro, including *Fusarium oxysporum*, *Alternaria solani*, *Pythium aphanidermatum*, and *Sclerotinia sclerotiorum*. The study aimed to evaluate the efficacy of these biocontrol agents in reducing disease incidence and suppressing the proliferation of several pathogenic fungi associated with tomato plants. The impact of the fungus *T. hazarianum* and *A. niger* on several vegetative and root growth parameters of tomato seedlings in sterile soil.

II. Methods

Soil Preparation and Sterilization

Gypsum soil sourced from a field in the Samarra district was utilised. The material was cleaned and sifted to eliminate plant detritus and weeds. The soil was hydrated and sterilised using a 5% concentration of commercial (37%) formalin solution. The item was thereafter encased in polyethylene for seven days and aired for three days to eliminate formalin vapours. The soil was subsequently compacted into 1 kg perforated plastic pots.

Preparation of the Fungal Inoculum

All fungi were sourced from the laboratory of the Plant Protection Department at Tikrit University's College of Agriculture. Local millet seeds (*Panicum miliaceum* L.) were utilised to prepare the fungal inocula and inoculate the seeds with the specified fungus. The seeds were meticulously rinsed multiple times with water to eliminate dirt and contaminants, then buried in water overnight. Superfluous water was eliminated with gauze, and the seeds were apportioned into 50g segments for each 250ml flask. Fifteen millilitres of distilled water were introduced to hydrate the seeds, which were subsequently sterilized in an electric arc furnace at 121°C and one atmosphere of pressure for one hour. The flasks were subsequently inoculated by introducing five discs (each with a diameter of 0.5 cm) of agar infused with 7-day-old fungus. The flasks were thereafter incubated at a temperature of 25±2°C for 10 days, with the flasks being shaken every 3 days to ensure even distribution of the fungal inoculum across all seeds and to prevent clumping(13).

Soil inoculation with fungi:

The inoculum of each fungus, *A. niger* and *T. hazarianum*, was incorporated into the sterilized soil at a concentration of 1% (relative to the fungus-bearing millet seeds). Thirty grams of fungal inoculum on millet seeds were incorporated into three kilograms of sterilized soil. The soil inoculated with fungi was partitioned into three replicates, with 1 kilogram of fungus-inoculated soil allocated to each pot. The procedure was reiterated for all chosen fungus, encompassing pathogenic varieties. The soil was subsequently lightly wet, and the pot openings were covered with polyethylene film to preserve moisture. The pots were allowed to sit for a week to facilitate the dissemination of fungi within the soil.

Experimental Treatments

On March 1, 2021, the seedlings were relocated to plastic pots and allocated according to a Complete Randomized Design (CRD), with four Super Queen tomato seedlings of comparable height (5-7 cm) put in each pot, and three pots designated per treatment. The trial comprised the subsequent treatments, as detailed in Table (1):

Table (1) Experimental coefficients and their symbols

NO.	abbreviation	**Treatments**
1	Control	Sterilizer soil + sterilized millet seeds+
2	AN	Sterilizer soil + millet seeds colonized by *Aspergillus niger*
3	TH	Sterilizer soil + millet seeds colonized by *T.hazarianum*
4	AS	Sterilizer soil + millet seeds inoculated with the pathogen *Alternaria solani*
5	FO	Sterilizer soil + millet seeds inoculated with the pathogen *F.oxysporium*
6	FS	Sterilizer soil + millet seeds inoculated with the pathoge *F.solani*
7	PA	Sterilizer soil + millet seeds inoculated with the pathogen *P.aphandermatium*
8	SS	Sterilizer soil + millet seeds inoculated with the pathogen *S.sclerotiorum*
9	ANAS	Sterilizer soil + millet seeds inoculated with *A.niger +A.solani*
10	ANFO	Sterilizer soil + millet seeds inoculated with *A.niger +F.oxysporium*
11	ANFS	Sterilizer soil + millet seeds inoculated with *A.niger +F.solani*
12	ANPA	Sterilizer soil + millet seeds inoculated *A.niger +P.aphandermatium*
13	ANSS	Sterilizer soil + millet seeds inoculated with *A.niger + S.sclerotiorum*
14	THAS	Sterilizer soil + millet seeds inoculated with *T.hazarianum +A.solani*
15	THFO	Sterilizer soil + millet seeds inoculated with. *hazarianum +F.oxysporium*
16	THFS	Sterilizer soil + millet seeds inoculated with *T.hazarianum +F.solani*
17	THPA	Sterilizer soil + millet seeds inoculated with T.hazarianum + P.aphandermatium
18	THSS	Sterilizer soil + millet seeds inoculated with *T.hazarianum+S.sclerotiorum*

Statistical Analysis:

The results were statistically analyzed using the MINITAB statistical software, version 17. Analysis of Variance (ANOVA) was used, and the arithmetic means of the different treatments were compared using Duncan's multiple range test at probability levels of 0.05 and 0.01 (Al-Rawi, 2000).

III. Result

Table 2 shows that treatment with the fungus *T. hazarianum* significantly increased the shoot length of tomato seedlings. The shoot length in treatment (TH) reached 25 cm, compared to the control treatment (sterile soil only), which recorded a shoot length of 22.5 cm. Treatment with the fungus *A. niger* alone, without the pathogen, showed a non-significant increase in shoot length, reaching 21 cm. The results also indicated that treatments combining the pathogen with the antagonist showed highly significant differences compared to treatments using only the pathogen. Treatment (THFS) recorded the highest shoot length among all treatments in the experiment, with a significant difference of 19.5 cm, compared to the pathogen treatment. Only the (FS) treatment showed the highest vegetative length (12.2) cm. This was followed by the (THAS) treatment, where the vegetative length reached (19) cm, a significant difference compared to the control treatment (AS), which recorded a vegetative length of (10.6) cm. Next was the (THFO) treatment, with a highly significant difference, reaching a vegetative length of (18.7) cm compared to the (FO) treatment, which recorded a vegetative length of (11.3) cm. The (THSS) treatment followed with a significant difference, reaching a vegetative length of (18.5) cm, showing a significant difference from the (SS) treatment, which recorded a vegetative length of (12.3) cm. The lowest significant difference was observed in the (THPA) treatment, which recorded a vegetative length of (17.9) cm compared to the (PA) treatment, which recorded a vegetative length of (11.4) cm. The results in the table also showed that the treatments combining the pathogenic fungus with the antagonist exhibited significant differences compared to the treatments using only the pathogen. Treatment (ANAS) recorded the highest shoot length among all treatments with *A. niger*, with a significant difference of 17.5 cm, compared to the treatment using only the pathogen, and significantly compared to the control treatment (AS), where the shoot length was

10.6 cm. Treatment (ANSS) followed with a significant difference of 17.4 cm, showing a significant difference from the treatment using only the pathogen (SS), which recorded a shoot length of 12.3 cm. Treatment (ANFS) followed with a shoot length of 17 cm, significantly different from the treatment using only the pathogen (FS), where the shoot length was 12.2 cm. Treatment (ANPA) followed with a significant difference as well, recording a shoot length of (16.3) compared to the diseaser-only treatment (PA), which recorded a shoot length of (11.4). Treatment (ANFO), however, recorded the lowest significant difference, with a shoot length of (16) compared to the diseaser-only treatment (FO), in which the shoot length was.(11.3)

Table (2) Effect of the fungi *T. hazarianum* and *A. niger* on some vegetative length of tomato seedlings in sterile soil

Treatments	**Length of the vegetative system (cm)**
Control	22.5 b
T. hazarianum (TH)	25 a
A.niger (AN)	21 b
A. solani (AS)	10.6 d
F. oxysporium (FO)	11.3 d
F. solani (FS)	12.2 d
P. aphandermatum (PA)	11.4 d
S. sclerotiorum (SS)	12.3 d
T.hazarianum+ A. solani (THAS)	19 bc
T.hazarianum+ F. oxysporium (THFO)	18.7 c
T.hazarianum+ F. solani (THFS)	19.5 bc
T.hazarianum+ P.aphandermatum (THPA)	17.9 c
T.hazarianum+S.sclerotiorum (THSS)	18.5 c
A. solani (ANAS)+ A.niger	17.5 c
A.niger+ F. solani (ANFS)	17 c
A.niger+ P. aphandermatum (ANPA)	16.3 c
A.niger+ S. sclerotiorum (ANSS)	17.4 c
F. oxysporium (ANFO) A.nige	16 c

Different letters mean there are significant differences between groups at 0.05

Table (3) shows that treatment with the fungus *T. hazarianum* significantly increased the root length of tomato seedlings. The root length in treatment (TH) reached 11 cm, compared to the control treatment (sterile soil only), which recorded a root length of 9.5 cm. Treatment with the fungus *A. niger* alone, without the pathogen, recorded a root length of 8.3 cm, but did not show any significant increase compared to the control treatment without the fungus. Table 3-4 shows that treatments combining the pathogen with the antagonist exhibited highly significant differences compared to treatments using only the pathogen. Treatment (THFS) recorded the highest shoot length among all treatments in the experiment, with a significant difference, reaching a root length of 7.1 cm, compared to the pathogen-only treatment (FS), where the root length was 3.5 cm. This was followed by the (THAS) treatment, where the total root length reached 8.2 cm, a significant difference compared to the control treatment (AS), where the total root length was 3.4 cm. Next was the (THFO) treatment, with highly significant differences, where the total root length reached 7.1 cm, compared to the (FO) treatment, where the total root length was 4.3 cm. The (THSS) treatment followed with a significant difference, where the total root length reached 8 cm, showing a significant difference from the (SS) treatment, which recorded a total root length of 4.2 cm. The least significant difference was observed in the (THPA) treatment, where the total root length reached 7.3 cm, compared to the (PA) treatment, which recorded a total root length of 3.4 cm. Table (1) shows that the treatments combining the two pathogenic fungi with the antagonist showed significant differences compared to the treatments using only the pathogen. Treatment (ANAS) recorded a root length of 5.1 cm, compared to the control treatment (AS) which recorded a root length of 3.4 cm. Treatment (ANSS) followed with a significant difference, as the root length reached 6.3 cm, showing a significant difference from the treatment using only the pathogen (SS) which recorded a root length of 4.2 cm. Treatment (ANFS) followed with a root length of 6.4 cm, a significant difference compared to the treatment using only the pathogen (FS) which recorded a root length of 3.5 cm. Treatment (ANPA) followed with a significant difference as well, recording a root length of (6.6 cm) compared to the pathogen-only treatment (PA), which recorded a root length of (3.4 cm). Treatment (ANFO) recorded the lowest significant difference, with a root length of (5 cm) compared to the pathogen-only treatment (FO), in which the root length was (4.3 cm).

Table (3): Effect of the fungi *T. hazarianum* and *A. niger* on Root length of tomato seedlings in sterile soil

Treatments	Root length (cm)
Control	9.5 ab
T. hazarianum (TH)	11.0 a
A.niger (AN)	8.3 bc
A. solani (AS)	3.4 e
F. oxysporium (FO)	4.3 de
F. solani (FS)	3.5 e
P. aphandermatum (PA)	3.4 e
S. sclerotiorum (SS)	4.2 de
T.hazarianum+ A. solani (THAS)	8.2 bc
T.hazarianum+ F. oxysporium (THFO)	7.1 bcd
T.hazarianum+ F. solani (THFS)	7.5 bcd
T.hazarianum+ P.aphandermatum (THPA)	7.3 bcd
T.hazarianum+S.sclerotiorum (THSS)	8.0 bc
A. solani (ANAS)+ A.niger	5.1 cde
A.niger+ F. solani (ANFS)	6,4 cde
A.niger+ P. aphandermatum (ANPA)	6.6 cde
A.niger+ S. sclerotiorum (ANSS)	6.3 cde
F. oxysporium (ANFO) A.nige	5.0 cde

Different letters mean there are significant differences between groups at 0.05

Table (4) shows that treatment with the fungus T. hazarianum alone without a pathogen showed a significant increase in the wet and dry weight of the vegetative body, reaching 8.37 g for wet weight and 3.37 g for dry weight, compared to the control treatment without a fungus, where the wet weight was 6.56 g and the dry weight was 2.74 g. This was followed by treatment with the fungus A. niger alone without a pathogen, where the weight of the vegetative body also did not show any significant increase compared to the control treatment without a fungus, where the wet weight of the vegetative body for treatment (AN) was 6.11 g and the dry weight was 2.33 g. The treatments combining the two pathogenic fungi with the antagonist showed highly significant differences compared to the treatments using only the pathogen. The (THFS) treatment recorded the highest weight among all treatments in the experiment, with a very clear and significant difference. The weight of the wet and dry vegetative parts reached (5.89 and 2.18 g) respectively, compared to the (FS) treatment using only the pathogen, where the weight of the wet and dry vegetative parts reached (1.45 and 0.81 g) respectively. This was followed by the (THAS) treatment, where the weight of the wet and dry vegetative parts reached (5.13 and 2.31 g) respectively, with a significant difference compared to the control treatment using only the pathogen (AS), where the weight of the wet and dry vegetative parts reached (1.19 and 0.47 g) respectively. Following the treatment (THFO), the weight of the wet and dry vegetative mass reached (5.02 and 2.12 g) respectively, compared to the treatment of the pathogen only (FO), in which the weight of the wet and dry vegetative mass reached (1.55 and 0.20 g) respectively. Following this, treatment (THSS) showed a significant difference in fresh and dry vegetative weight (5.08 and 3 g, respectively), compared to the pathogen-only treatment (SS), which recorded fresh and dry vegetative weights of (1.85 and 0.64 g, respectively). The lowest significant difference was observed in treatment (THPA), where the fresh and dry vegetative weights were (1.85 and 4.17 g, respectively), compared to the pathogen-only treatment (PA), which recorded fresh and dry vegetative weights of (1.77 and 0.50 g, respectively). The results in Table (3-4) show that the treatments combining the two pathogens with the antagonist showed significant differences compared to the pathogen-only treatments. Treatment (ANAS) recorded a fresh and dry vegetative weight of (3.22 and 1 g, respectively), compared to the pathogen-only treatment, which was significantly higher than the control pathogen-only treatment (AS). The wet and dry foliage weights were 1.19 and 0.47 g, respectively. This was followed by the ANSS treatment, which showed a significant difference, with wet and dry foliage weights of 3.33 and 1.30 g, respectively, demonstrating a significant difference from the pathogen-only treatment (SS), which recorded wet and dry foliage weights of 1.85 and 0.64 g, respectively. Next was the ANFS treatment, which recorded wet and dry foliage weights of 4.10 and 1.63 g, respectively, also significantly different from the pathogen-only treatment (FS), where wet and dry foliage weights were 1.45 and 0.81 g, respectively. Treatment (ANPA) followed with a significant difference as well, as it recorded the weight of the wet and dry vegetative body (3.89 and 1.04 g) respectively, compared to the pathogen only treatment (PA), which recorded the weight of the wet and dry vegetative body (1.77 and 0.50 g). As for treatment (ANFO), the weight of the wet and dry vegetative body reached (3.02 and 1.68 g) respectively, compared to the pathogen only treatment (FO), in which the weight of the wet and dry vegetative body reached (1.55 and 0.20 g) respectively.

Table (4): Effect of the fungi T. hazarianum and A. niger on Root length of tomato seedlings in sterile soil

Treatments	Shoot fresh weight (g)	
	Dry	Fresh
Control	2.74 b	6.56 ab
T. hazarianum (TH)	3.37 a	8.37 a
A.niger (AN)	2.33 b	6.11 ab
A. solani (AS)	0.47 gh	1.19 c
F. oxysporium (FO)	0.20 h	1.55 c
F. solani (FS)	0.81 fg	1. 45 c
P. aphandermatum (PA)	0.50 gh	1.77 c
S. sclerotiorum (SS)	0.64 g	1.85 c
T.hazarianum+ A. solani (THAS)	2.31 bc	5.13 ab
T.hazarianum+ F. oxysporium (THFO)	2.12 c	5.02 ab
T.hazarianum+ F. solani (THFS)	2.18 c	5.89 ab
T.hazarianum+ P.aphandermatum (THPA)	1.85 d	4.17 b
T.hazarianum+S.sclerotiorum (THSS)	3.0 ab	5.08 ab
A. solani (ANAS)+ A.niger	1 f	3.22 bc
A.niger+ F. solani (ANFS)	1.63 de	4,10 b
A.niger+ P. aphandermatum (ANPA)	1.04 f	3.89 bc
A.niger+ S. sclerotiorum (ANSS)	1.30 ef	3.33 bc
F. oxysporium (ANFO) A.nige	1.68 de	3.02 bc

Different letters mean there are significant differences between groups at 0.05

The results in Table (5) show that treatment with the fungus *T. hazarianum* alone, without a pathogen, did not produce a significant increase in root mass. The wet weight was 1.45 g and the dry weight was 0.55 g, compared to the control group, which had a wet weight of 1.35 g and a dry weight of 0.51 g., as shown in Figure (1). This was followed by treatment with the fungus *A. niger* alone, without a pathogen, which also did not show a significant increase in root mass. The wet weight of the root mass was 1.12 g and the dry weight was 0.40 g, as shown in Figure (2). The treatments of mixing the two pathogenic fungi with the antagonist showed highly significant differences compared to the treatments of the pathogen only. For the (THFS) treatment, the wet and dry weights were (0.76 and 0.37 g) respectively, compared to the treatment of the pathogen only (FS), in which the wet and dry weights of the root total were (0.08 and 0.006 g) respectively, as shown in Figure (3). This was followed by the (THAS) treatment, where the wet and dry weights of the root total were (0.88 and 0.26 g) respectively, with a significant difference compared to the control treatment of the pathogen only (AS), where the wet and dry weights of the root total were (0.18 and 0.008 g) respectively. This was followed by the treatment (THFO) with highly significant differences, as the weight of the wet and dry root total was (1.2 and 0.52 g) respectively, compared to the treatment of the pathogen only (FO), in which the weight of the wet and dry root total was recorded as (0.16) and (0.009) g respectively. The THSS treatment followed with a significant difference, recording wet and dry root weights of 1 and 0.40 g, respectively. This showed a significant difference compared to the pathogen-only treatment (SS), which recorded wet and dry root weights of 0.10 and 0.001 g, respectively. The least significant difference was observed in the THPA treatment, which recorded wet and dry root weights of 1.01 and 0.40 g, respectively, compared to the pathogen-only treatment (PA), which recorded wet and dry root weights of 0.06 and 0.005 g, respectively.

The results in Table (3-4) show that the treatments combining the two pathogenic fungi with the antagonist showed significant differences compared to the treatments using only the pathogen. Treatment (ANAS) recorded a wet and dry root weight of 0.63 and 0.03 g, respectively, compared to the treatment using only the pathogen, and then to the control treatment (AS), where the wet and dry root weights were 0.18 and 0.008 g, respectively, as shown in Figure (4). Treatment (ANSS) followed with a significant difference, recording wet and dry root weights of 0.41 and 0.02 g, respectively, thus showing a significant difference from the treatment using only the pathogen (SS), which recorded wet and dry root weights of 0.10 and 0.001 g, respectively. This is followed by the (ANFS) treatment, where the weight of the wet and dry root total was (0.32 and 0.01 g) respectively, with a significant difference compared to the pathogen-only (FS) treatment, in which the weight of the wet and dry root total was (0.08 and 0.006 g) respectively. Treatment (ANPA) followed with a significant difference as well, as it recorded the weight of the wet and dry root total (0.58 and 0.02 g) respectively, compared to the pathogen-only treatment (PA), which recorded the weight of the wet and dry root total (0.06 and 0.005 g) respectively, as shown in Figure (5).. As for treatment (ANFO), it recorded the lowest significant difference, as the weight of the wet and dry root total reached (0.35 and 0.01 g) respectively, compared to the pathogen-only treatment (FO), in which the weight of the wet and dry root total reached (0.16) and (0.009) g respectively.

Table (5) Effect of the fungi *T. hazarianum* and *A. niger* on some vegetative length of tomato seedlings in sterile soil

Treatments	Root weight (g)	
	Dry	Fresh
Control	0.51 a	1.35 a
T. hazarianum (TH)	0.55 a	1.45 a
A.niger (AN)	0.40 b	1.12 ab
A. solani (AS)	0.008 cd	0.18 e
F. oxysporium (FO)	0.009 cd	0.16 e
F. solani (FS)	0.006 d	0.08 f
P. aphandermatum (PA)	0.005 d	0.06 f
S. sclerotiorum (SS)	0.001 d	0.10 c
T.hazarianum+ A. solani (THAS)	0.26 c	0.88 bc
T.hazarianum+ F. oxysporium (THFO)	0.52 a	1.02 ab
T.hazarianum+ F. solani (THFS)	0.37 b	0.76 cd
T.hazarianum+ P.aphandermatum (THPA)	0.40 b	1.01 ab
T.hazarianum+S.sclerotiorum (THSS)	0.40 b	1.0 ab
A. solani (ANAS)+ A.niger	0.03 c	0.63 cd
A.niger+ F. solani (ANFS)	0.01 c	0.32 de
A.niger+ P. aphandermatum (ANPA)	0.02 c	0.58 cd
A.niger+ S. sclerotiorum (ANSS)	0.02 c	0.41 de
F. oxysporium (ANFO) A.nige	0.01 c	0.35 de

Different letters mean there are significant differences between groups at 0.05

Figure (1): A, Control: B, treatment with T.hazarianum

Figure (1): A, Control: B, treatment with A.niger

Figure (3): A, treatment with FS: B, treatment with THFS

Figure (4): A, treatment with AS: B, treatment with ANAS

Figure (5): A, treatment with SS: B, treatment with THSS

Discussion

The existence of advantageous bacteria in the plant microbiome is essential for the advancement of novel biocontrol agents and enhancers of plant growth. Microbiome engineering is an emerging agricultural biotechnology that entails the control or alteration of the plant microbiome to use its advantages in enhancing plant output and resilience to environmental stressors(14-16). Consequently, altering the plant microbiome through plant-associated microbes like Trichoderma spp. may significantly enhance sustainable agriculture practices.

The current investigation concurs with (17) that the effective utilisation of Trichoderma isolates can alleviate Fusarium wilt disease, produced by F. solani in cherry tomato plants, while concurrently enhancing the growth and development of cherry tomatoes.

Numerous investigations have demonstrated that Trichoderma spp. predominantly manage root, shoot, and postharvest diseases through their antagonistic capabilities, which are activated by several biocontrol pathways (18, 19). Benítez et al. .(20) assert that Trichoderma spp. provide indirect biocontrol of fungal soil-borne pathogens by competing for nutrients and space, modifying environmental conditions, promoting plant growth, augmenting plant defence mechanisms, and inducing antibiosis, or direct biocontrol via mycoparasitism. Trichoderma spp. initiate the synthesis of hydrolytic or lytic enzymes during mycoparasitic contacts, including glucanase, chitinase, and protease, which decompose the chitin polymers of the fungal pathogen cell wall (21, 22). Moreover, Trichoderma can synthesise antibiotics or low-molecular-weight diffusible compounds such as tricholin, harzianic acid, peptaibols, viridin, 6-pentyl-pyrone, and heptelidic acid, which inhibit the proliferation of other bacteria (19). The interplay of indirect and direct biocontrol processes is influenced by Trichoderma spp., crop plants, and environmental conditions, including nutrient availability, pH, temperature, and iron content(20). Consequently, Trichoderma spp. may serve as effective biofungicides and alternative agents against soil-borne fungal infections (23).

The current work concurs with (24), which shown that T. harzianum, utilised as a biological agent, hindered the growth of *F. oxysporum* isolates examined in vitro and diminished infection in the screen house. The results indicated that *T. harzianum* suppressed the growth of *F. oxysporum* when introduced into pots with tomato plants. This may happen from the rapid proliferation of the antagonist, leading to conflict for nutrients and space with the pathogen, ultimately causing the antagonist to colonize the pathogen. The study by (25)observed that T. harzianum's method of action involves competing with *F. oxysporum* for nutrients and space, mycoparasitism on the pathogen, and likely the release of antibiotics. The swift proliferation and competition for nutrients and space by the antagonist impeded the growth of *Fusarium oxysporum* infecting tomato seedlings. A comparable study conducted by (26) demonstrated the effectiveness of Trichoderma spp. against (FOL) affecting both pre- and post-seedling stages of tomato plants. Research conducted by (27) revealed that fusarium wilt of tomato, induced by *Fusarium oxysporum* f. sp. lycopersici, is among the most economically significant diseases in principal tomato cultivation areas globally. A study by (28)identified *Fusarium oxysporum* as a severe disease responsible for crop losses ranging from 10% to 50% in certain tomato cultivation regions. Recently, , (29)showed that in a greenhouse experiment, the simultaneous inoculation of five Trichoderma isolates mitigated damping-off caused by P. aphanidermatum and enhanced the survival rate of tomato plants by 74.5%. Ghazanfar et al. (30) demonstrated that the fungus Trichoderma improved seed germination and augmented the plant's systemic resistance, hence facilitating and greatly enhancing plant growth. The fungus *T. harzianum* actively reduces the occurrence of the pathogenic fungus *Fusarium oxysporum* by generating systemic resistance via the enzyme Phenylalanine ammonia-lyase. The data by (31) indicate that the fungus Trichoderma diminished the severity of early blight caused by A. solani by 80%.

II. References

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