



Effectiveness of *Trichoderma asperellum* as a biocontrol agent against eggplant damping-off disease caused by *Fusarium proliferatum*

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Abstract

In 2025, a severe case of eggplant damping-off disease was reported in nurseries of Maysan Governorate, Iraq. In light of this, the causative agent was isolated, tested for pathogenicity, and identified morphologically and molecularly. The isolate *Trichoderma asperellum* HZA84 was tested against the phytopathogenic *in vitro* and evaluated for its effectiveness in controlling damping-off disease in soil trays. Six fungal genera were identified, with isolate RAM3 being the most frequent at 44%. RAM3 was identified as *Fusarium* spp. morphologically and by amplifying the ITS gene region as *Fusarium proliferatum*. The antagonistic isolate HZA84 demonstrated high antagonistic efficacy against the pathogen, of 100% after 10 days. The filtrate of the antagonistic HZA84 isolate showed an inhibitory efficacy of 87.71% at a dilution of 30%. Addition of isolate HZA84 with the isolate RAM3 in soil trays also led to an increase in the seed germination and a decrease in the eggplant seedling damping-off to 57.22% and 42.85%, respectively, compared to 21.44% and 81.03% in the treatment of the isolate RAM3 alone. Furthermore, the addition of the antagonistic isolate HZA84 with RAM3 improved seedling growth parameters, exceeding those reduced with RAM3 alone.

Keywords: Antagonistic; Biocontrol; ITS; *Fusarium proliferatum*.

1. Introduction

Eggplant (*Solanum melongena* L.) is a staple vegetable in many Asian and global cuisines. In Iraq, it is a near-constant food source. However, its cultivation faces several challenges, including the damping-off of seedling during the early stages of growth. Several fungal species have been implicated in causing damping-off diseases, including the genera *Rhizoctonia* and *Fusarium*, as well as Oomycetes such as species from the genera *Pythium* and *Phytophthora* (Das, 2021; Talukder et al., 2024). These species collectively cause 80-100% of nursery

vegetable seedlings to die (Agrius, 2005). Thus, damping-off diseases are considered among the most significant biotic stressors for farmers, requiring seed reseeded in seedling production (Lamichani et al., 2017). Some species of *Fusarium* have been documented as the most dangerous pathogens on horticultural crops, with infection occurring on the plant at all stages of its growth (Li et al., 2025). The *Fusarium* sp. exhibits high growth rates and a high number of reproductive propagules when suitable biotic conditions are present, most importantly, temperature and decaying organic matter (Garcia et al., 2025). Traditional agricultural



methods have been used to control diseases caused by *Fusarium*, but its lifestyle and wide host range have rendered crop rotation ineffective (Marburger et al., 2015). Furthermore, the emergence of new resistant *Fusarium* strains has led to the breakdown of resistance in selected and hybrid varieties (Dumbai et al., 2025).

In addition to the health and environmental risks posed by overuse, the development of resistance in *Fusarium* spp. to chemical pesticides makes it difficult to control and spread (Jayawardana et al., 2024). The challenges of plant disease management have called for the development of innovative and environmentally viable ideas for controlling plant diseases. Researchers have been inspired to select soil-borne microorganisms that exhibit a distinctive antagonism against plant pathogens (Villavicencio-Vásquez et al., 2025). Among the most important antagonistic organisms for plant pathogens are *Trichoderma* species. Some *Trichoderma* spp. has been utilized, due to their unique living and nutritional characteristics, to combat various plant diseases. These characteristics include competing with pathogens for space and nutrients, secreting fungal compounds (some of which have been classified as antifungal), and parasitizing plant pathogenic fungi (Yao et al., 2023). In light of this, the study focused on isolating and identifying the fungus causing eggplant seedling damping-off, and controlling the disease in a bio- and eco-friendly manner *in vitro* and in soil trays.

Materials and methods

2.1. Isolation of eggplant seedling damping-off fungi

Fungi associated with damping-off disease were isolated from dead eggplant seedlings in trays obtained from various vegetable nurseries in Maysan Governorate. The 0.5 cm section of infected seedlings was immersed in sodium hypochlorite solution (2% commercial solution) for 3–5 minutes, then dried on sterile filter paper, washed with sterile distilled water, and dried again. Five sterile sections were placed in 8.5 cm dishes containing 10 mL of PDA medium supplemented with ampicillin (200 mg/L) and rifampicin (10 mg/L) as antibiotics (Abo-Elyousr et al., 2022). The dishes were incubated at 27°C for 5–7 days, and the developing fungal colonies were monitored. The genus frequency of isolates was calculated using the equation: Frequency% = (Number of isolates of one genus / Total number of isolated genera) × 100. The isolates were purified by taking a small portion of the colony tip hyphae and re-cultured in new dishes containing PDA, and kept at 4°C until use.

2.2. Pathogenicity Testing in Seedling Trays

Pathogenicity was carried out for the RAM3 isolate using the method described by Migliorini et al. (2018). Conidia were harvested from a 10-day-old colony of the pathogenic fungus by adding 9 ml of sterile distilled water supplemented with 0.05% Tween 20. The plate was shaken well, and the suspension was then centrifuged at 2000 rpm for 5 min. A conidia suspension

of the pathogen with a concentration of 1.5×10^6 conidia/mL was prepared using a Hymenocytometer slide. Nursery soil and peat moss (1:2 w/w) were mixed and sterilized at 121°C and 15 psi/in² for 1 h. The sterilized soil was placed in the seedling trays, inoculated with 1 ml of fungal suspension, and the trays were covered with a layer of perforated polyethylene for three-days pre-planting. The sterilized eggplant seeds were sown in trays (1 seed/hole), moved to the growth chamber, and watered with sterilized water until seedling emergence. The trial was designed with three replicates with four holes. After 20 days from planting, the percentage of damping-off was calculated according to the equation: damping-off % = [(Number of damping-off seedlings / Total of germinated seedlings)] × 100.

2.3. Morphological Identification

The pathogenic RAM3 isolate was examined morphologically using the methodology described by Suwannarach et al. (2024). Colony characteristics, including morphology and pigmentation, were examined on Potato Dextrose Agar (PDA). Measurements were performed on at least 10 specimens for each anatomical structure (Microconidia, Macroconidia, and Chlamydospores) using a compound light microscope with a 5-megapixel camera. The identification of the fungal species was confirmed based on the morphological and microscopic characteristics, as described by Leslie and Summerell (2006).

2.4. Molecular Identification

The RAM3 isolate biomass was produced by re-cultivating it in potato dextrose broth (PD broth) at 25°C for 10 days. Biomass was harvested and ground in liquid nitrogen. Genomic Total DNA was extracted using a protocol described by Zhang and Li (2009). The rDNA ITS was amplified using the automated thermal cycler (Eppendorf AG, Germany) by primer pairs ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The sequences in both directions for the gene were obtained from the Sangon Biotech Company Limited (Shanghai, China). The sequence of the ITS gene was verified as queries in GenBank database searches using the Blastn algorithm. The sequences of different *Fusarium* spp. were downloaded from the NCBI site <https://blast.ncbi.nlm.nih.gov/Blast.cgi>, and the *Trichoderma harzianum* was used as an out-group. The MEGA 7.0.26 software tool was used to align all obtained sequences and phylogenetic tree construction via the test neighbor-joining (NJ) with the most appropriate models, such as maximum composite likelihood, p-distance matrices under partial deletion of alignment gaps, and missing data applied. Tree topology confidence limits were explored in non-parametric bootstrap analyses of 1000 replicates. The sequence of the gene was deposited in the GenBank database.

2.5. Antagonism test *Trichoderma* HZA84 isolate

T. asperellum HZA84 (OR888903.1) was obtained from the Plant Pathology Laboratory, Plant Protection Dept.

An antagonistic test was performed using a dual culture technique as described by Tomah et al. (2024). The mycelia-discs 0.5 cm (taken from both 7-day-old *Trichoderma* and pathogen colony) were opposite-placed 1 cm from the dish edge in the petri dish containing 10 ml of PDA. Also, a mycelia-disc 0.5 cm of a pathogenic fungus was placed on one side, 1 cm from the edge, as a control, and the dishes were kept at 25°C. The antagonistic potential of the *Trichoderma* isolate was determined when the radial growth of the pathogen reached the dish edge in the control. Five-point antagonistic scale proposed by Bell et al. (1982) was followed, with some modifications, as follows: (1) *Trichoderma* colony covers all dish; (2) *Trichoderma* colony covers two-thirds of the dish; (3) *Trichoderma* colony covers half the dish; (4) *Trichoderma* colony covers one-third of the dish; (5) *Trichoderma* colony covers less than one-third of the dish. Furthermore, the percentage inhibition (PI) of the pathogenic fungus was calculated using the formula mentioned in Muthukumar et al. (2011): $PI\% = [(T_0 - T_1)/T_0] \times 100$, where T_0 is the average of radial growth of the pathogen in the control, and T_1 is the average of radial growth of the pathogen in *Trichoderma* treatment.

2.6. Filtrate test of *Trichoderma* HZA84 isolate

The culture filtrate of the tested *Trichoderma* isolate was followed by the method of Tomah et al. (2020). Flasks of 250 ml containing 100 ml of PD broth were inoculated with five mycelium-discs 0.5 cm taken from the five-day-old *Trichoderma* colony and incubated at 150 rpm in $25 \pm 2^\circ\text{C}$ for 14 days. The culture filtrate was purified using Whatman No. 1 filter paper and then centrifuged at 3000 rpm for 20 min, and then sterilized using a 0.22 μm filter (Hawash Scientific Co., Ltd., Shaanxi, China). Culture filtrate was mixed with PDA medium to obtain 0%, 10%, 30%, and 50% dilutions. 10 ml of each dilution was poured into 8.5 cm petri dishes and allowed to solidify. The dishes were inoculated with a mycelium-disc 0.5 cm of a 5-day-old colony of the pathogen. The 0% dilution was considered the control. The trial was repeated with three dishes for each dilution, and the dishes were incubated at 25°C. The percentage inhibition (PI %) of pathogenic fungal growth was calculated using the following formula: $PI\% = [(S_0 - S_1)/S_0] \times 100$, where S_0 is the radial growth of the pathogenic fungus in the control treatment, and S_1 is the radial growth of the pathogenic fungus in the treatment with culture filtrates.

2.7. Efficacy evaluation of *T. asperellum* HZA84 in reducing eggplant seedling damping-off caused by *F. proliferatum* RAM3 in a growth chamber.

The efficacy of the antagonistic *T. asperellum* HZA84 in reducing damping-off disease in eggplant seedlings was assessed in trays under controlled growth chamber conditions. The growing soil was prepared by mixing beach soil with peat moss at a 1:2 (w:w) ratio and sterilized twice at 121°C and 15 psi/in² for 2 h. The sterilized soil was distributed in plastic trays (28 holes

per tray). The planting holes in the trays were inoculated with 1 ml of conidia suspension of the pathogenic at a concentration of 1.5×10^6 conidia/ml and 1 ml at a concentration of 5×10^6 of *T. asperellum* HZA84 (Okorley et al., 2024). The tray's holes were also inoculated with 1 ml (1.5×10^6 conidia/ml) of the RAM3 isolate conidia suspension only as a control. The trays were then covered with a layer of perforated polyethylene to retain moisture for three days before planting at lab temperature. Three days post-inoculated the eggplant seeds were surface-sterilized with a 1% sodium hypochlorite solution for 2 minutes, then rinsed twice in sterile distilled water and dried on sterile filter paper. The sterilized eggplant seeds were then planted in the treated trays. The trial trays were transferred to a growth chamber, and the growth conditions were set at 25°C, relative humidity of 70-80%, and a light period of 12 h (50 to 60 kLux m⁻²). After 21 days of planting, the percentage of seed germination and seedling damping-off disease were calculated, as well as the lengths of the seedlings and the fresh and dry weight of the seedlings.

2.8. Statistical Analysis

All trial units were implemented with 4-replicates. Excel was used to extract the confidence (\pm) among the replicates of the means. SAS software version (9.1.3) was used to analyze the data with a completely randomized design (CRD). A 5% significance level was used in the least significant difference (LSD) test among the means.

3. Results and Discussions

3.1. Frequency of Damping-off fungi isolates

The results showed clear differences in the frequency of fungal genera isolated from eggplant seedlings infected with damping-off disease (Table 1). The genus *Fusarium* sp., which forms a reddish-pink colony (isolate RAM3), recorded the highest frequency at 44%, followed by the genus *Fusarium* sp., which forms a white colony (isolate RAM4) at 21%. The frequencies of genera *Aspergillus* sp. and *Rhizoctonia* sp. were 10% and 11%, respectively. The lowest frequencies were 9% and 5% for the genera *Alternaria* sp. and *Cladosporium* sp., respectively. Nursery soils are a nutritious environment for many plant pathogens due to the availability of moisture and decomposing organic matter (Longjam et al., 2024). The *Fusarium* sp. infects many plant crops and causes damping-off, such as cotton and tomatoes (Zaki et al., 2021; Chiquito-Contreras et al., 2026).

Table1. Frequency% of fungal genera isolated from eggplant seedlings infected with damping-off disease.

Isolated Fungi	Frequency %	Isolates Code
<i>Aspergillus</i> sp.	10.00	RAM1
<i>Rhizoctonia</i> sp.	11.00	RAM2
<i>Fusarium</i> sp. reddish-pink colony	44.00	RAM3

<i>Fusarium</i> sp. White colony	21.00	RAM4
<i>Alternaria</i> sp.	9.00	RAM5
<i>Cladosporium</i> sp.	5.00	RAM6
Total	100	

3.2. Pathogenicity of RAM3 Isolate

Pathogenicity testing of the most frequent RAM3 isolate was conducted on eggplant seedlings under nursery conditions. The pathogenicity results (Fig.1) showed that the conidia suspension of isolate RAM3 was capable of infecting eggplant seedlings with damping-off disease to 78%. This pathogen is highly virulent in eggplant seedlings. *Fusarium* spp. cause young seedling rot, ranging from 5% to 80% in vegetable seedlings (Lamichani et al., 2017). *Fusarium* also causes damping-off of 30% to 80% in various crops in India (Gopi, et al., 2012; Singh et al., 2022).



Fig. 1. Pathogenicity test of isolate RAM3 on eggplant seedlings under nursery conditions

3.3. Morphological Identification of RAM3

The morphological characteristics of the RAM3 isolate showed that it forms a colony that is initially white, and then turns reddish-pink in the center due to the secretion of a pink pigment on PDA at 25°C for 10 days (Fig. 2A). The RAM3 isolate produces the macroconidia, which are sickle-shaped and pointed with 3-5 septa, and microconidia, which are elongated oval with 0-1 septa (Fig.2B). It also produces single interstitial chlamydsopores on PDA (Fig.2C). The RAM3 isolate, with its white colony and pink center is consistent with the *Fusarium proliferatum* mentioned in a study by (Arias et al., 2011). The size of the mac and microconidia was also found to be similar to that of identified *F. proliferatum* in a study by Zhang et al. (2013). Furthermore, Chang et al. (2015) reported that *F. proliferatum* produces single interstitial chlamydsopores in PDA.

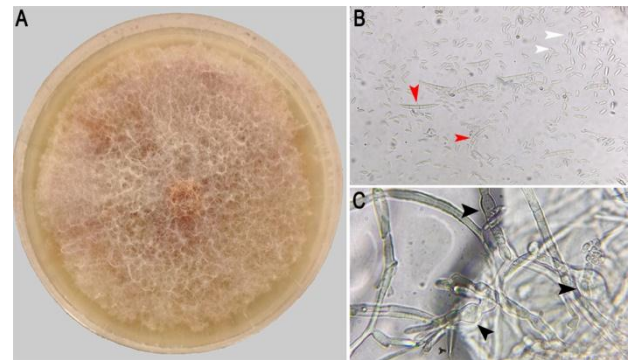


Fig. 2. Morphological characteristics of the RAM3 isolate. (A) The RAM3 isolate colony growing on PDA, (B) Mac and Microconidia red and white arrowheads, (C) Chlamydsopores black arrowheads

3.4. Sequence Analysis and Phylogenetic Tree

The DNA sequencing findings of the pathogenic RAM3 yielded a 578-base-pair sequence using the ITS primer, consistent with those obtained by Quazi et al. (2013). BLAST data analysis revealed that the RAM3 isolate, obtained using ITS primer amplification, shares 99% identity with the *Fusarium proliferatum* (m2) (MW405882.1) isolated from maize in Egypt.

Phylogenetic tree analysis of the ITS region sequence confirmed that RAM3 belongs to the species of *F. proliferatum* with 100% (Fig.3). Based on morphological characteristics and phylogenetic tree analysis, our results indicate that the pathogenic RAM3 isolate is indeed *F. proliferatum*. Isolate of RAM3 has been deposited in GenBank under the accession number PZ335594.1. To the best of our knowledge, this is the first study to identify *F. proliferatum* as the causative of eggplant seedling damping-off disease in Iraq.

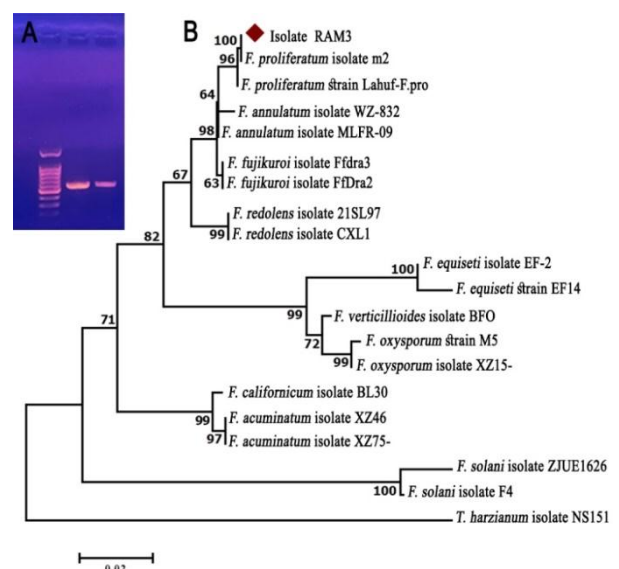


Fig. 3. Sequence Analysis and Phylogenetic Tree. (A) gel electrophoresis. (B) Phylogenetic tree of isolate RAM3 with a group of closely related *Fusarium* species downloaded from GenBank.

3.5. Antagonistic of *T. asperellum* HZA84 against *F. proliferatum*

The results showed that *T. asperellum* HZA84 possesses high antagonistic activity against *F. proliferatum* RAM3 by the double culture test (Fig.4). According to Bell's antagonism score, the antagonism reached a degree (2) at the 7-day double culture test, with the inhibition rate 85% (Fig.4A). The HZA84 isolate continued to expand until it completely covered the pathogen colony to reach a degree (1), at the 10-day double culture test, with the inhibition rate 100% (Fig.4B). In contrast, the *F. proliferatum* RAM3 colony in the control treatment grew uniformly in a radial direction on PDA medium (Fig. 4C). Our results are consistent with the antagonistic ability of *T. asperellum* and its competition for space in PDA against the fungi *Rhizoctonia*, *Pythium*, and *Fusarium* sp. (Mirsam et al., 2023; Vechithran et al., 2025; Christita et al., 2026).

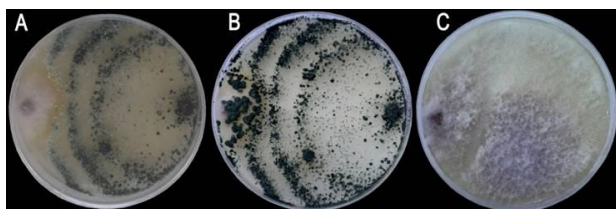


Fig.4. Antagonistic activity test of *T. asperellum* HZA84 isolate against *F. proliferatum* RAM3. (A) At a 7-day double culture test. (B) At a 10-day double culture test. (C) Control treatment of RAM3 Isolate only.

3.6. The efficacy of the *T. asperellum* HZA84 filtrates in inhibiting *F. proliferatum* growth

The filtrate of *T. asperellum* HZA84 showed clear efficacy in inhibiting the growth of *F. proliferatum* colony in PDA. This efficacy increases with increasing filtrating dilution supplemented (Fig.5). The results showed that the antagonistic filtrate of the HZA84 isolate at a dilution of (10%) reduced the growth of the *F. proliferatum*, with a growth rate of 6.04 cm (Fig.5A). A dilution of (20%) resulted in a higher degree of inhibition, reducing the growth to 4.85 cm (Fig.5B). A dilution of (30%) recorded the highest inhibition efficacy, with a growth rate of 1.05 cm (Fig.5C). In contrast, the growth rate in the control treatment at a dilution of 0% was 8.5 cm (Fig.5D). Statistical analysis revealed an inverse relationship between the filtrate dilution and the percentage of inhibition in the growth of the *F. proliferatum* colony, with the inhibition percentage increasing as the filtrate dilution increased. The inhibition percentage in pathogenic fungal growth reached 28.97%, 42.98%, and 87.71% for the filtrate concentrations of 10%, 20%, and 30%, respectively (Table 2). The efficiency of the filtrate of the *T. asperellum* HZA84 isolate in inhibiting pathogen growth is attributed to this isolate's ability to secrete enzymes that degrade the cell walls of pathogenic fungi, such as Chitinase and proteinase, which played a role in the breakdown of the sclerotia of *Sclerotinia sclerotiorum* (Tomah et al., 2024).

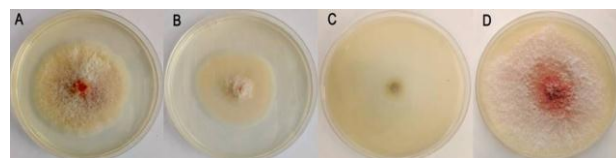


Fig.4. Impact the filtrate of *T. asperellum* HZA84 in inhibition of *F. proliferatum* growth in PDA. (A) filtrate at 10%, (B) filtrate at 20%, (C) filtrate at 30%, (D) filtrate at 0% (control treatment).

Table 2. Effect of *T. asperellum* HZA84 filtrate dilutions in inhibiting growth the *F. proliferatum* in PDA

Filter Dilution%	Colony Growth/cm	Inhibition%
10	6.04±0.03 ^b	28.97±0.43 ^c
20	4.85±0.09 ^c	42.98±1.07 ^b
30	1.05±0.03 ^d	87.71±0.39 ^a
0	8.50±0.00 ^a	

3.7. Efficacy of *T. asperellum* HZA84 in reducing damping-off caused by *F. proliferatum* RAM3 in eggplant seedling

The HZA84 isolate, which showed antagonistic activity against *F. proliferatum* RAM3 *in vitro*, was evaluated for its efficiency in reducing damping-off disease in tray soils. The analysis results in Table 3 showed that adding the antagonistic isolate HZA84 to the tray soils inoculated with the pathogenic RAM3 isolate increased germination % to 57.22% compared with germination% of 21.44 % in tray soils inoculated with the RAM3 isolate alone. The addition of HZA84 isolate also demonstrated a reduction in eggplant seedling damping-off to 42.85%, surpassing the damping-off% of 81.03% rate in the RAM3-only treatment. Furthermore, adding the antagonistic isolate HZA84 to the trays soil inoculated with the RAM3 isolate resulted in a significant increase in the lengths and weights parameters of the shoot and root systems of eggplant seedlings, exceeding the growth parameters of seedlings in the soil of the trays inoculated with the pathogenic RAM3 isolate alone (Table 3). The increased germination rate and reduced seedling damping-off resulting from adding HZA84 to the tray soil are attributed to its competitive ability to prevent pathogen access to seeds and inhibit its growth through its filtrate, which is rich in enzymes that degrade fungal cell walls. The HZA84 isolate was evaluated for its high antagonistic activity against the *Sclerotinia sclerotiorum*, and its hydrolytic enzymes, such as proteases and chitinases, involved in cell wall degradation, were monitored (Tomah et al., 2024). The *T. asperellum* possesses growth-promoting mechanisms such as phosphate breakdown and the secretion of growth regulators, which enhance seedling growth parameters (Sehim et al., 2023).

Table 3. Effect of *T. asperellum* HAZ84 on seed germination, damping-off, and growth parameters of eggplant seedlings under growth chamber conditions

Parameters	Treatments		
	Control	RAM3	HZA84

Seeds Germination%	100.00±0.00 ^a	21.44±0.46 ^c	57.22±1.33 ^b
Damping-off%	00.00±0.00 ^c	81.03±0.16 ^a	42.85±0.33 ^b
Shoot system length/cm	4.43±0.33 ^b	3.05±0.27 ^c	4.46±0.32 ^a
Soft Stem weight/g	0.0521±0.007 ^b	0.0385±0.004 ^c	0.0548±0.005 ^a
Dry Stem weight/g	0.0043±0.015 ^b	0.0033±0.008 ^c	0.0046±0.005 ^a
Root system length/cm	3.41±0.14 ^b	2.79±0.45 ^c	3.45±0.16 ^a
Soft Roots weight/g	0.0043±0.004 ^a	0.0036±0.001 ^b	0.0044±0.001 ^a
Dry Roots weight/g	0.00045±0.01 ^a	0.00039±0.07 ^b	0.00045±0.01 ^a

4. Conclusion

For the first time in Iraq, the *Fusarium proliferatum* RAM3 was isolated and identified morphologically and molecularly as the cause of eggplant damping-off. The antagonistic *Trichoderma asperellum* HZA84 possesses competitive, antagonistic, and inhibitory mechanisms that enable it to suppress the pathogenic fungi *in vitro* and control the seedlings' damping-off in tray soil in an eco-friendly and sustainable manner.

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