Misan Journal of Agricultural and Environmental Sciences

RESEARCH ARTICLE

Screening of *Trichoderma* isolate effective in controlling lettuce drop disease caused by *Sclerotinia sclerotiorum*

Ali Athafah Tomah [1]* and Zahraa Majed Hadi [2]

[1] *Plant Protection, College of Agriculture, University of Misan, AL-amarah 62001, Iraq*

[2] *Misan Governorate, AL-amarah 62001, Iraq*

ABSTRACT

Lettuce drop disease caused by *Sclerotinia sclerotiorum* is an epidemic plant disease, and there are urgent economic incentives to control it in ways consistent with the sustainable approach. The study aimed to isolate *Trichoderma* species from the healthy rhizosphere of lettuce and identify and characterize the most effective isolate against fungal growth and sclerotia. Six isolates of *Trichoderma* were obtained, while the isolate QLZ-2 showed increased efficiency in its opposition to the development of *S. sclerotiorum* in the laboratory. The isolate QLZ-2 was identified as *Trichoderma asperellum* by morphological and microscopic diagnosis. The laboratory experiment revealed that the isolate QLZ-2 spreads on the surface of sclerotia during the fungal mycoparasitism process in the soil of the dishes. Further, the pot experiment showed that splashing the conidial suspension of the isolate QLZ-2 reduced the symptoms of lettuce fall disease. The severity of lettuce drop disease was reduced by 14.79% compared severity of disease in the water-only spray treatment, which had a 100%.

Keywords: *Trichoderma;* Antagonism; Sclerotia; Lettuce drop disease

Corresponding Author:
Ali Athafah Tomah, Plant Protection Department, College of Agriculture, University of Misan, AL-amarah 62001, Iraq.
E-mail: ali_athafah@uomisan.edu.iq

Received: 12/11/2024
Accepted: 22/01/2025
Published: 31/03/2025
DOI: https://doi.org/10.64333/MJAE.25.1.1

INTRODUCTION

Lettuce (*Lactuca sativa* L.) which belongs to the Asteraceae family is one of the important winter vegetable crops grown in Iraq and the world due to its high nutritional value which rarely changes or even loses its consumption. Local varieties and most foreign varieties grown in Iraq belong to the elongated head lettuce group. This group is the richest in nutritional value and lettuce is ranked 26th in the nutritional value list of vegetable and fruit crops (Ryder, 1999). The Agricultural Statistics Directorate reported (2021) that the areas planted with lettuce in Iraq are about 16,571 dunums, with a production quantity of 37,809 tons. Many biological factors limit the growth and production of lettuce, including infection with soil-borne and airborne pathogens. Phytopathogenic of *Sclerotinia sclerotiorum*, a fungus of the family Sclerotiniaceae is the most important and common causative of fungal diseases that cause major problems in lettuce fields (Chen *et al*. 2016). *S. sclerotiorum* is a soil-borne pathogen that infects lettuce plants in the early stages of plant life in the crown area and causes Lettuce drop disease (Chitrampalam *et al*., 200). The infected plants are not marketable because their leaves will rot very quickly once infected (Boland and Hall 1994). The pathogen spends the winter as sclerotia in plant debris or the soil for more than 10 years (Clarkson *et al*., 2004). The sclerotia play a major role in producing the pollen of the fungus *S. sclerotiorum* and are the primary means of keeping the fungus alive in harsh environmental conditions (Michael *et al*., 2009). In terms of epidemiology, the presence of 2-3 sclerotia of *S. sclerotiorum* per m^2 in the soil is capable of causing an infection of 60-90% (Kobayashi and Suzui, 1972). The wide host range of this pathogen has made crop rotation ineffective and negative because

the succession of susceptible crops to *S. sclerotiorum* has been responsible for the continued increase in white mold incidence (Hossain *et al*. 2023).

On the other hand, there are no lettuce cultivars resistant to *S. sclerotiorum* and they are relied upon for control. Fungicides are commonly used as a means of mitigating crop yield losses. However, over-reliance on broad-spectrum fungicides poses a serious risk to the environment and farmer health, and has accelerated the selection of S. sclerotiorum-resistant strains (Kumar *et al*. 2022).

The role of biological control agents is a fact, as they are complementary in some cases to chemical control due to the presence of antagonistic fungi, which play the most important role. Biocontrol based on fungi has wide acceptance due to its control over plant diseases, and the species belonging to the genus *Trichoderma* are the focus of many researchers who have contributed to biological control through the use of fungi (Pandya and Saraf, 2010).

Trichoderma strains have been applied worldwide in the management of a large number of plant diseases (Al-Malki, 2014). At present, a variety of mechanisms including direct and indirect mechanisms have been reported to be involved in the biological control of *Trichoderma* strains against various plant pathogens (Al-Tharwani *et al*. 2023). Mycoparasitism has been proposed to be the main direct mechanism (Harman, 2006; Tomah *et al*., 2024).

The study aims to isolate from the root zone of intact lettuce and identify the isolates of *Trichoderma* active against the *Sclerotinia sclerotium* test its mycoparasitism on sclerotia and verify its ability to suppress the disease of drop disease in lettuce in a pot experiment.

MATERIALS AND METHODS

Pathogenic *S. sclerotiorum*

A plant pathogenic *S. sclerotiorum* strain obtained from the Plant Pathology lab, College of Agriculture, University of Misan, Iraq. The plant pathogenic was re-cultured in PSA media, and stored at 4 °C until used.

Soil Sample Collection

This study was conducted in the Plant Pathology lab, College of Agriculture, University of Misan, during the seasons 2023-2024. Soil specimens were accumulated from the lettuce rhizosphere in six different farms of Misan Governorate, namely; Al-Kahla, Ali Al-Gharbi, Al-Amara, Qalaat Saleh, Al-Maymouna, and Al-Majar Al-Kabir, respectively. Farms (2-4) were identified in each area. The samples were brought to the laboratory after being packed in plastic bags and stored in the 5°C until use.

Isolation of *Trichoderma* from soil

The soil-dilution method was utilized to isolate *Trichoderma*. The soil 1 g (airily dried) was transferred to a 10 ml test tube containing 9 ml of sterile distilled water, and shaken well until it became homogeneous to obtain a 1/10 dilution. Then 1 ml was transferred from the previous dilution to 9 ml of sterile distilled water, to get 1/100. The same process was repeated until we reached the 7th dilution. The 1 ml of the 7th dilution was spread onto the surface of a plate containing the previously prepared solidified PSA medium containing 5% Chloramphenicol. The plates were incubated at 25 °C for 7 days until they appeared as colonies. The fungi were purified based on the shape and color of the colonies by transferring the terminal part of the hyphae tips to a PSA medium and incubating at 25°C for 4 days. After colonies were formed, the purified plates were kept in the refrigerator until subsequent tests were conducted.

Antagonistic efficacy examination of *Trichoderma* isolates

The culture dual method was used to study the antagonistic ability of *Trichoderma* isolates against *S. sclerotiorum* in petri dishes containing sterile PSA medium (Demirci *et al*., 2011).

The center of the first half of the dish was inoculated by a 0.5 cm disk possessed from the *Trichoderma* colony. The center of the second half of the dish was inoculated by a 0.5 cm disk taken from the edge 5-day-old of *S. sclerotiorum*. The plates inoculated by pathogenic without *Trichoderma* were prepared as a control treatment. The plates were incubated at 20 ± 2 °C with three replicates for each *Trichoderma* isolate. The diameters of the colonies were measured after a seven-day incubation period, after which the degree of antagonism was estimated according to the Bell *et al*. (1982) scale, which consists of five degrees:[(1) The *Trichoderma* covers the entire plate; (2) *Trichoderma* covers 3/4 of the area of the plate; (3) *Trichoderma* and the plant-pathogenic each cover half of the area of the plate; (4) pathogenic fungus covers 3/4 of the area of the plate; (5) pathogenic fungus covers the entire plate].

Filtrates efficacy examination of *Trichoderma* isolates

The culture filtrates of *Trichoderma* isolates were prepared by preparing 250 ml glass flasks containing 100 ml of sterile PSB liquid medium. Each flask was inoculated with four discs from the edge of a 5-day-old Trichoderma colony in PSA medium. The flasks were incubated at a temperature of 25 ± 2 °C for 14 days, taking into account shaking the flasks twice daily. After the incubation period, the biomass of *Trichoderma* isolates was separated using Whatman No. 1 filter papers.

The filtrates were sterilized by a Millipore filter (0.22 µm) equipped by Sartorius Stedim Biotech - Germany Pump. Filtrates were tested at a rate of 10% in 9 cm diameter plates, through 1 ml of the *Trichoderma*-nominate of each isolate was jumbled with 9 ml of the PSA-runny medium before the solidification under sterile conditions. The centers of plates were inoculated discs with a diameter of 0.5 cm taken from the edge of a 5-day-old *S. sclerotiorum*. Each treatment was repeated three times with the presence of the control treatment of plates inoculated by the pathogenic and not treated with any filtrate. The plates were incubated at 25 ± 2 °C for 4 days. To examine the effectiveness of *Trichoderma* isolate filtrates in inhibiting the growth of the pathogen, the diameter of colonies was measured. The results were recorded and the percentage of inhibition (PI) was calculated using the following equation; PI=[(Growth rate of pathogen colonies in control treatment - Growth rate of pathogen colonies in filtrate treatment)/Growth rate of pathogen colonies in control treatment]×100 (Al-Kaabi *et al*., 2010).

Identification of the isolate QLZ-2

Morphological characterization was performed on the more active *Trichoderma* isolate which was grown on two media (PSA and water Agar) for five days at a temperature of 25 °C and exposed to a lighting period of about 8 hours daily (Jaklitsch, 2009). The asexual microstructures (conidiophores, phialides, conidia, and chlamydospores) were observed using a compound light microscope and photographed using a camera. The isolate was identified using the symbols and descriptions of Barnett and Hunter (1972) for fungal genera and species and compared with closely related species according to taxonomic characteristics as reported by (Rifai, 1969).

Efficacy examination of QLZ-2 isolate against sclerotia

To confirm the antagonistic role of QLZ-2 isolate in the degradation of sclerotia of *S. sclerotiorum* on the surface of sterile soil in *in vitro* dishes according to the method described by Soylu *et al*. (2007). Sclerotia were prepared sterile (70% absolute ethanol 99% concentration for 2 min, sterile water 5 min, and air-drying 30 min). Ten sterile sclerotia were randomly distributed on 9 cm Petri dishes containing 20 g of sterile soil (soil was sterilized in two periods at 121 °C and 1.5 kg pressure for 2 h each period). The surface of the plates containing soil and conidia was splashed with 1 ml of the conidia of the QLZ-2 at a concentricity of $2.4 \times {}^{6}10$ conidia while the other plates were splashed with 1 ml of sterile water-distilled as a treat-control. The plates were kept in an incubator at 25°C for 7 days. The growth of the isolate QLZ-2 and the colonization of conidia were monitored daily and photographed.

Evaluation of a QLZ-2 isolate in suppression of lettuce drop disease.

The highly effective isolate QLZ-2 protects lettuce plants from infection by *S. sclerotiorum* causing lettuce drop disease was tested in the soil of the College of Agriculture, Department of Plant Protection, in the winter season of 2023-2024. Local variety lettuce seedlings (45 days old) were prepared. Seedlings were transferred onto the plastic pot containing 2 kg of soil mixed with peat moss and sandy soil at a ratio of (1:1), watered, and left for 5 days for the plants to settle in the pots. Five sclerotia were added onto the pots-surface soil and around the stem of the lettuce plant. The experiment was carried out with three treatments included; (1) Pots with sclerotia were sprayed with a suspension of conidia of the isolate QLZ-2 ($2.4 \times {}^{6}10$/ conidia). (2) Pots with sclerotia were sprayed with sterile distilled water. (3) Pots with no-sclerotia were splashed with sterile water-distilled only. The experiment was carried out three times and repeated three times for each treatment. The plants were watered regularly. The severity of infection was calculated after 21 days of spray conidia of isolate QLZ-2 according to the scale mentioned by Macioszek *et al* (2023) as follows: [(0)No symptoms; (1) Small necroses on the stem; (2) Wilted leaf base, necroses on leaves and stem; (3) Necroses on leaves and stems, marginal leaves wilted and brown; (4) Wilted, rotten, and discolored leaves, rotten stem, and (5) Decoyed, dead plant]. The disease severity (DS) was filtered using the formula that was mentioned by Tomah *et al* (2024).

Statistical Analysis

Laboratory and pot experiments were carried out according to Completely Randomized Design (CRD). The results were analyzed according to SAS version 9.2.0. (Cary Institute, North Carolina, USA). The means were compared according to the Least Significant Difference (LSD) method at a probability level of 0.05.

RESULTS AND DISCUSSION

***Trichoderma* Isolates**

The results of the dilution method used to isolate *Trichoderma* from soil samples showed several fungal isolates for each sample obtained. The genera *Trichoderma* were identified after purification based on the shape and color of the growing colonies and the color of the green or greenish-yellow conidia formed. The isolation results in Table (1) show that 19 Trichoderma isolates which selected based on the differences in the shape of the growing colonies. In light of this, six isolates were selected and given different symbols according to the name of the site and sequence of the isolate (AMZ-3, AMZ-5, KAZ-1, QLZ-2, MIZ-3, and AGZ-4). Several previous literatures have indicated the possibility of obtaining *Trichoderma* isolates from the zone-root of many uninfected plants as chili, tomatoes, watermelon, cherry, and aubergine (Xue *et al*., 2021; Korkom and Yildiz, 2022).

Table (1) *Trichoderma* isolates obtained the selected isolates, and isolates name

Administrative unit	Samples No.	Isolations No.	Selected isolates	Isolates Name
Ali al-Gharbi	1	7	1	AGZ-4
Amara	4	6	2	AMZ-3 AMZ-5
Al-Maymouna	2	3	1	MIZ-3
Qalaat Saleh	2	2	1	QLZ-2
al-Kahla	2	1	1	KAZ-1
Al-Majar Al-Kabir	5	0	0	--
Total	16	19	6	

Antagonismal efficacy of *Trichoderma* isolates against *S. sclerotiorum*

The results of the Antagonism trial showed the different abilities of the selected *Trichoderma* isolates in their antagonizing against *S. sclerotiorum*. Where isolate QLZ-2 showed a high antagonistic efficacy (Figure 1). The results of the statistical analysis showed significant differences between the capacity of the tested isolates-*Trichoderma* to antagonize the pathogen (Figure 2). The highest percentage of antagonism to the growth of the pathogenic fungus was recorded in the treatment of isolate QLZ-2, reaching 100%, while it was followed by isolate AMZ-5 and isolate AGZ-4, in which the percentage of antagonism reached 79.17% and 76.57%, respectively. The results also showed that the lowest percentage of antagonism was in isolates AMZ-3, KAZ-1, and MIZ-3, where the percentage of antagonism reached 50.26, 50.39, and 49.52%, respectively. The above results show that according to the Bell scale scores, the isolate QLZ-2 was considered to have an antagonistic efficacy of (1), while the antagonistic efficacy of both AMZ-5 and AGZ-4 was considered to be (2), while the antagonistic efficacy of the isolates AMZ-3, KAZ-1, and MIZ-3 was (3). In light of the above results, the isolate QLZ-2, which had an antagonistic efficacy of (1), and the isolates AMZ-5 and AGZ-4, which had an antagonistic efficacy of (2), can be considered successful biocontrol agents in the antagonistic experiment. *Trichoderma* are killing causative agents of plant diseases by exerting multiple mechanisms, ex, physical-parasitism, antibiotics release, and the earning of nutrients (Daghir *et al*., 2020; Tyśkiewicz *et al*., 2022; Manzar *et al*., 2022).

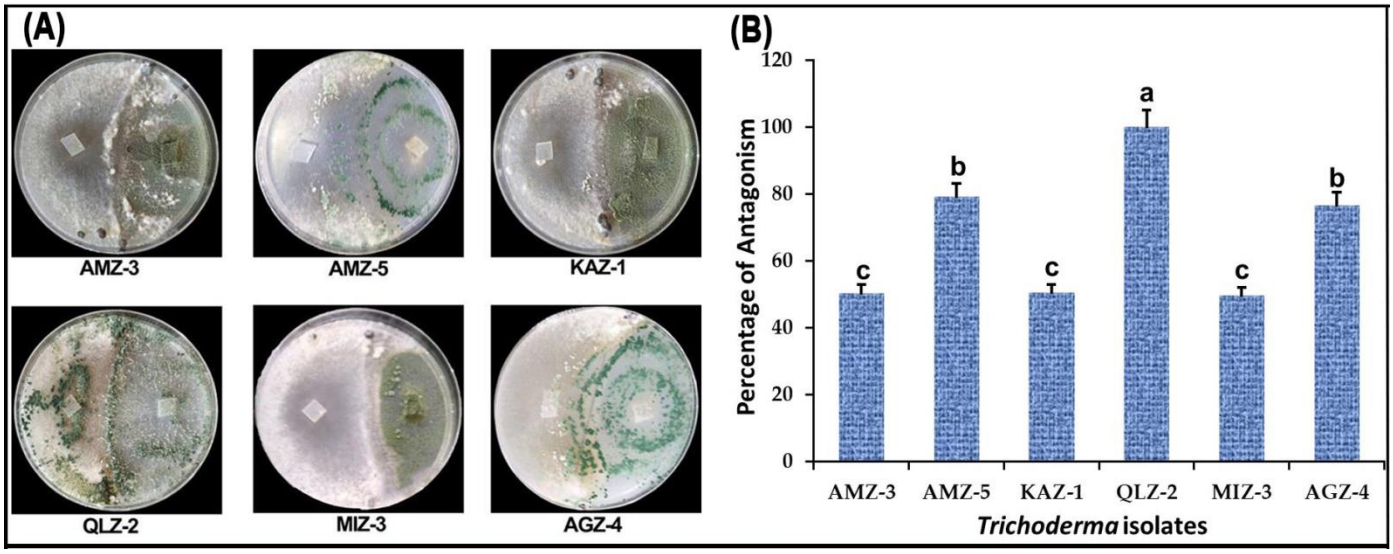

Figure (1) Antagonism efficacy assay to *Trichoderma* isolates, (A) Antagonism efficacy of *Trichoderma* isolates against the *S. sclerotiorum* in PDA plates. (B) Percentage of antagonism efficacy of *Trichoderma* isolates against *S. sclerotiorum*.

Filtrates efficacy of *Trichoderma* isolates against *S. sclerotiorum*

The filtrates impact of isolates of *Trichoderma* selected against pathogen were tested to confirm which isolates have the highest inhibition efficiency. The results of the filtrate (10%) trial of the isolate filtrates (Figure 3) showed that the isolate QLZ-2 recorded in first place in inhibition efficiency, as the inhibition rate reached 48.23%. While the inhibition rate in the other selected isolates reached 0.0%. These differences in the effect among *Trichoderma* isolates may likely be due to the difference in their sources and areas of isolation and their species (Tomah *et al*., 2020a and 2023). In light of these results, the isolate QLZ-2 was selected in the following tests.

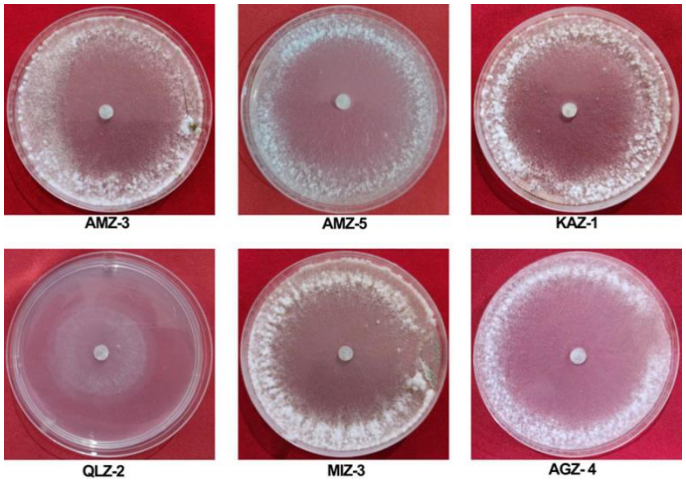

Figure (3) Effect of filtrates of *Trichoderma* isolates against *S. sclerotiorum*

Microscopic characterization of *Trichoderma* QLZ-2

The microscopic observation results of *Trichoderma* isolate QLZ-2 showed dense conidia, branched conidiophores, ampullate phialides, and slightly spherical conidia with yellow-green pigmentation (Figure 4A). Most of the conidiophores were symmetrically formed along the branches and main axis (Figure 4B). The branching patterns of conidiophores were broad, at an angle of approximately 90° (Figure 4C). Phialides were characteristically elongated, variably lageniform (bowling-shaped), and could be ampullate in the dense region (Figure 4D).

At the end of the phialides or the subterminal cell of the conidiophores, the conidiophores were spherical to sub-spherical (ovoid) and the conidiophores were light to dark green under light microscopy (Figure 4E). The chlamydospores formation was observed to have a terminal spherical shape (Figure 4F). Based on the morphological and microscopic characteristics mentioned below, the QLZ-2 is identified as *T. asperellum* agreed with those reported for *T. asperellum* (Rifai, 1969; Jaklitsch, 2009).

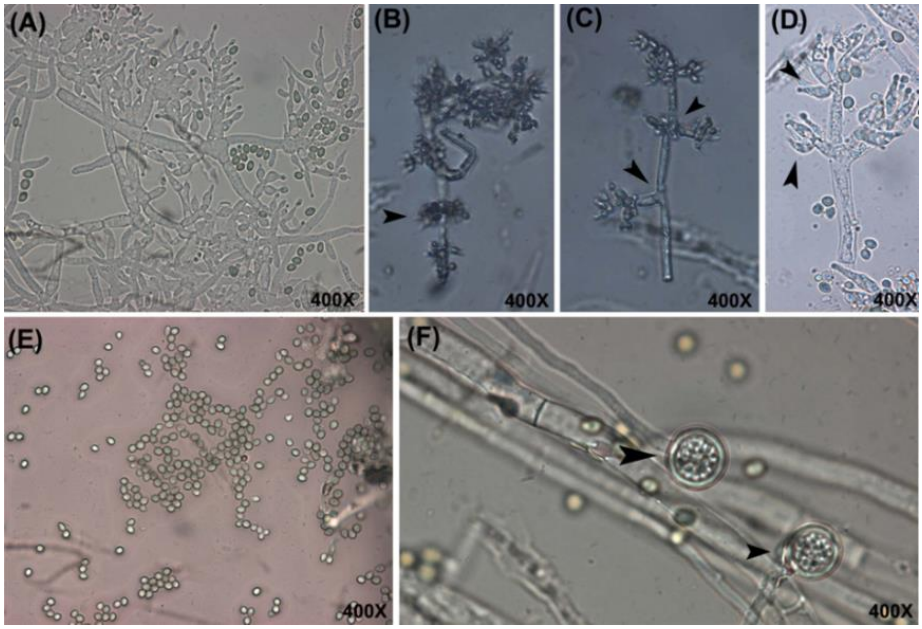

Figure (4) Microscopic characteristics of *Trichoderma* isolate QLZ-2. (A), the dense morphology of conidiophores and Phialides. (B, C, and D), Characteristics of the conidiophore and the species-specific traits. (E), the green spherical and sub-spherical conidia. (F), The terminally formed chlamydospores.

Efficacy of QLZ-2 isolate in sclerotia-degradation

The antagonism method of QLZ-2 on sclerotia body, the surfaces of the trial-plates and their soil and sclerotia were splashed with the conidial of isolate-QLZ-2. Visual inspection of the trial-plates displayed that isolate-QLZ-2 rapidly-grew and resulted white mycelial on parts of the sclerotia surface. After that, QLZ-2 isolate grew densely and produced abundant mycelium as well as numerous green conidia on all sclerotia surfaces (Figure 5A), compared to the completely healthy sclerotia in the control treatment sprayed with sterile water only, where the pathogen growth gradually appeared (Figure 5B). Mycoparasitism is the main qualifications that *Trichoderma* directs in his nutritional lifestyle towards other fungal species (Sood *et al*., 2020; Tomah *et al*., 2020b; Tomah *et al*., 2023). The mechanism of mycoparasitism has been linked within the control strategies adopted by *Trichoderma* species such as *T. asperellum*, *T. virens* and *T. koninigii* in their aggressive path towards sclerotia (Silva *et al*., 2022 and Geraldine *et al*., 2013).

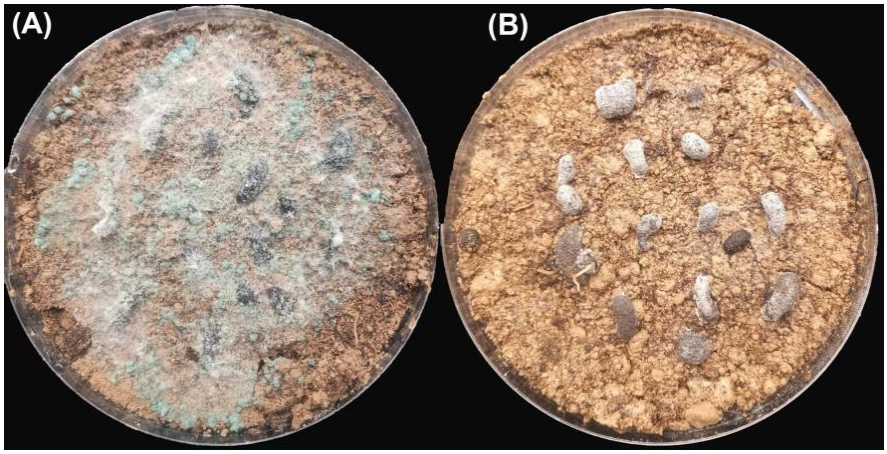

Figure (5) The antagonism behavior of QLZ-2 isolate on sclerotia. (A) Spraying with 1 ml of the conidial suspension of QLZ-2 isolate. (B) Spraying with sterile distilled water.

Efficacy of QLZ-2 isolate in suppressing lettuce drop disease in pot trial

The efficiency of *Trichoderma* isolate QLZ-2, which is inhibitory to *S. sclerotiorum*, was evaluated in laboratory experiments in controlling lettuce drop disease in pot experiments. Based on the results in Figure (6A), the pots with sclerotia and splashed with water-sterile exhibited an severity increase in the lettuce drop-disease. Symptoms of necrosis showed in the leaves-basal and up-stem then developed into severely wilted-brown in lettuce leaves until the plants died completely of 21 days post-inoculation., The results of visual interrogations exhibited that splashing with conidia suspension of *Trichoderma* isolate QLZ-2 significantly suppressed lettuce drop disease. Slight necrosis and wilting were found at the bases of a few leaves and the symptoms did not increase significantly as the experiment continued until 21 days later. On the other hand, infection with the pathogen did not appear in the control treatment (distilled water only without sclerotia). Statistical analysis of disease severity in Figure (6B) showed that the severity of disease-lettuce fall in trial pots with arteriosclerosis splashed with 1 ml of water-sterile was 100%. Spraying the suspension-conidia of *Trichoderma* isolate QLZ-2 on pots containing sclerotia reduced the severity of lettuce drop disease to 14.79%. While 0.0% was the control treatment. Based on the results obtained, *Trichoderma* QLZ-2 succeeded in the reducing the development of lettuce drop disease. A previous study has shown that sclerotia germination is associated with the occurrence of primary infection and plant-death (Garrabrandt *et al*., 1983). Therefore, *Trichoderma* can degrade sclerotia during its parasitism, reducing the incidence of diseases caused by S. sclerotiorum (Silva *et al*., 2022). Therefore, *T. asperellum* QLZ-2 can grow and parasitize on the surface of the sclerotia, secreting sclerotiolytic enzymes, which inhibits myceliogenic germination and

thus reduces the incidence of disease on lettuce plants.

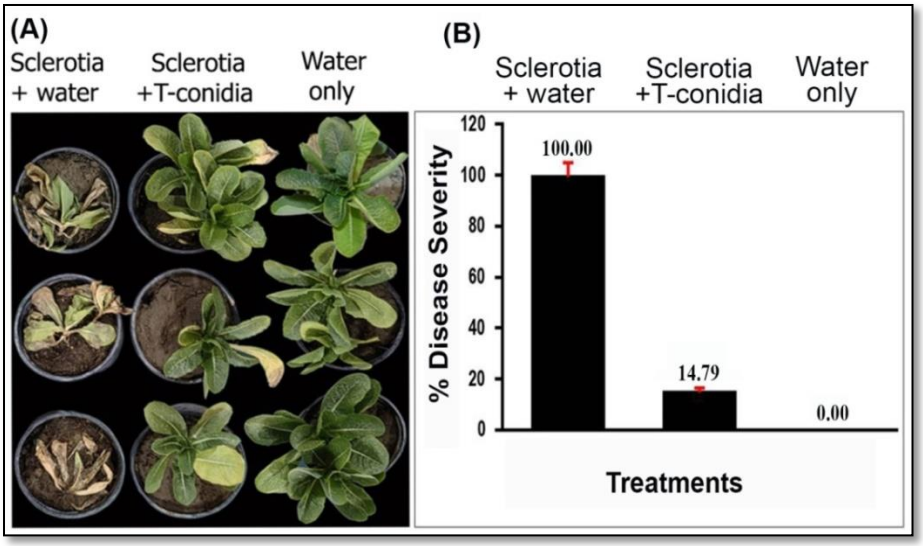

Figure (6) Effect of *Trichoderma* isolate QLZ-2 on suppressing lettuce seedling drop disease. (A) Disease symptoms of lettuce seedlings inoculated and not inoculated with sclerotia that were sprayed with T-conidia, and sprayed with sterile water as a control. (B) Statistical significance of the lettuce drop severity-disease inoculated with sclerotia and splashed with conidia of the isolate QLZ-2 and with sterile water as a control.

CONCLUSIONS

Trichoderma isolate QLZ-2, which was morphologically identified as *Trichoderma asperellum*, was able to inhibit the growth of a Plant pathologic *S. sclerotiorum* in antagonism and filtrates trials, in addition to being able to mycoparasitic the sclerotia of *S. sclerotiorum*. The *Trichoderma* QLZ-2 suppresses the development of lettuce drop disease caused by *S. sclerotiorum* through its parasitism of sclerotia in pot experiments. The present project is unique in highlighting the mycoparasitic of *T. asperellum*-activity in destroying sclerotia and its subsequent properties in suppressing lettuce drop disease, thus adopting biological methods using biocontrol agents is an effective and sustainable approach to control plant pathogens in an environmentally friendly approach.

Disclaimer: None

Conflict of interest:

Funding disclosure: None

REFERENCES

Al-Malki, A.A.T (2014). Effect aqueous extract of *Xanthium strumarium* L. and *Trichoderma viride* against *Rhizctonia solani*. International Journal of Botany and Research 4:1–16

Al-Tharwani ,W.S; Mohammadali, M.T and Abd al-Ridha, I.M (2023). Evaluation of the efficiency of *Trichoderma harzianum* and *Azotobacter chroococcum* in biodegradation of glyphosate and measurement of its residual in soil by using HPLC. Misan Journal of Academic Studies 22:46–54.

Boland, G.J and Hall, R (1994). Index of plant hosts of *Sclerotinia sclerotiorum*. Canadian Journal of Plant Pathology 16:93–108.

Chen, X.; Pizzatti, C.; Bonaldi, M.; Saracchi, M.; Erlacher, A.; Kunova, A.; Berg, G. and Cortesi, P (2016). Biological control of lettuce drop and host plant colonization by rhizospheric and endophytic *streptomycetes*. Frontiers in Microbiology 7:196457.

Chitrampalam, P.; Figuli, P.J.; Matheron, M.E.; Subbarao, K.V., and Pryor, B.M. (2007). Biocontrol of lettuce drop caused by *Sclerotinia sclerotiorum* and *S. minor* in desert agroecosystems. Plant Disease 92; 1625-1634.

Daghir, G.M.; Tomah, A.A.; and Alyaseri, I.I. (2020). First record the fungus *Bipolaris australiensis* as a cause of leaf blight date palm in Misan and attempt to control it in in vitro condition. Plant Archives, 20(2): 966-970.

Directorate of Agricultural Statistics (2021). Crop and Vegetable Production Report for the year 2021. Iraqi Ministry of Planning for the month of April 2021.

Garrabrandt, L.E.; Johnston, S.A. and Peterson, J.L. (1983). Tan Sclerotia of *Sclerotinia sclerotiorum* from Lettuce. Mycologia 75:451-456.

Geraldine, A.; Lopes, F.; Costa, C.D; Barbosa, E.; Rodrigues, A.; Ulhoa, C. M. L.J. (2013). Cell wall-degrading enzymes and parasitism of sclerotia are key factors on field biocontrol of white mold by *Trichoderma* spp. Biological Control 67:308-316.

Harman, G. E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology, 96: 190-194.

Hossain, M.M.; Sultana, F.; Li, W.; Tran, L.S.P. and Mostofa, M.G. (2023). *Sclerotinia sclerotiorum* (Lib.) de Bary: insights into the pathogenomic features of a global pathogen. Cells 12:1063.

Jaklitsch, W.M. (2009). European species of Hypocrea Part I. The Green-Spored Species Stud Mycology 63:1–91.

Korkom, Y., and Yildiz, A. (2022). Evaluation of biocontrol potential of native *Trichoderma* isolates against charcoal rot of strawberry. Plant Pathology Journal, 104:671-682.

Kumar, V.; Agrawal, S.; Bhat, S.A.; Américo-Pinheiro, J.H.P.; Shahi, S.K. and Kumar, S. (2022). Environmental impact, health hazards, and plant-microbes synergism in remediation of emerging contaminants. Cleaner Chemical Engineering 2:100030.

Macioszek, V.K.; Marciniak, P. and Kononowicz, A.K. (2023). Impact of *Sclerotinia sclerotiorum* infection on lettuce (*Lactuca sativa* L.) survival and phenolics content—a case study in a horticulture farm in Poland. Pathogens 12:1416.

Manzar, N.; Kashyap, A.S.; Goutam, R.S.; Rajawat, M.V.; Sharma, P.K.; Sharma, S.K.; Singh, H.V. (2022). *Trichoderma*: advent of versatile biocontrol agent, its secrets and insights into mechanism of biocontrol potential. Sustainability 14:12786.

Michael, J. B.; Gardiner, R. B. and Day, A. W. (2009). Melanin Synthesis by *Sclerotinia sclerotiorum*, Mycologia, 101: 296– 304.

Rifai, M. A. A. (1969). Revision of the genus *Trichoderma*. Mycological Papers. Kew, Surrey, UK, Commonwealth Mycological Institute, 116:1-56

Ryder, E. J. (1999). Lettuce Endive and Chichory. C A B I publishing U.K PP: 208.

Silva, L.; Camargo, R.; Moura M. G.; Nunes, P.; Dunlap, C. and Bettiol, W. (2022). Dual functionality of *Trichoderma*: Biocontrol of *Sclerotinia sclerotiorum* and biostimulant of cotton plants. Frontiers in Plant Science 13:983127.

Sood, M.; Kapoor, D.; Kumar, V.; Sheteiwy, M.S.; Ramakrishnan, M.; Landi, M.; Araniti, F.and Sharma, A. (2020). *Trichoderma*: The "Secrets" of a Multitalented Biocontrol Agent. Plants 9:762.

Suzui,T. and Kobayashi,T. (1972). Disspersal of ascospores of *Sclerotinia sclerotiorum* (Lib)de Bary on Kidney bean .cited from ,Schwartz, H.F. and J.R. Steadman. Factors affecting *Sclerotium* populations of, and apothecium production by, *Sclerotinia sclerotiorum*. Phytopathology 68:383- 388.

Tomah, A.A.; Abd Alamer, I.S.; Li, B.; Zhang, J.Z. (2020a). A new species of *Trichoderma* and gliotoxin role: A new observation in enhancing biocontrol potential of *T. virens* against *Phytophthora capsici* on chili pepper. Biological Control, 145, 104261.

Tomah, A.A.; AbdAlamer, I.S.; Li, B.; Zhang, J.Z. (2020b). Mycosynthesis of silver nanoparticles using screened *Trichoderma* isolates and their antifungal activity against *Sclerotinia sclerotiorum*. Nanomaterials,10: 1955.

Tomah, A.A.; Alamer, I.S.A.;Khattak, A.A.; Ahmed, T.; Hatamleh,A.A.; Al-Dosary, M.A.; Ali, H.M.;Wang, D.; Zhang, J.; Xu, L.; *et al*. (2023). Potential of *Trichoderma virens* HZA14 in Controlling *Verticillium* Wilt Disease of Eggplant and Analysis of Its Genes Responsible for Microsclerotial Degradation. Plants, 12, 3761.

Tomah, A.A.; Khattak, A.A.; Aldarraji, M.H. *et al*. (2024). Sclerotia degradation by *Trichoderma*-mycoparasitic; an effective and sustainable trend in the drop lettuce disease control caused by *Sclerotinia sclerotiorum*. Archives of Microbiology 206, 286.

Tyśkiewicz, R.; Nowak, A.; Ozimek, E. and Jaroszuk-Ściseł, J. (2022). *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. International Journal of Molecular Sciences, 93, 85–152.

Xue, M., Wang, R., Zhang, C., Wang, W., Zhang, F., Chen, D., Ren, S., Manman, Z, Hou, J., Liu, T. (2021). Screening and identification of *Trichoderma* strains isolated from natural habitats in China with potential agricultural applications. BioMed Research International, 2021:7913950.