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Research Article

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The antagonistic potential of *Trichoderma harzianum* against barley leaf-stripe disease

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Abstract

Drechslera graminea is the causal agent of leaf-stripe disease in barley leading to substantial crop losses. The current work aimed to investigate the antagonistic activity of *Trichoderma harzianum* against *D. graminea*. Pathogen was isolated from the naturally infected barely leaves and molecularly identified. The identified isolate was deposited in GenBank with an accession number of OR827023. *In vitro* dual culture assay showed a decrease in *D. graminea* radial growth with a growth inhibition percentage of 77.8 %. *T. harzianum* culture filtrate (TCF) (30×10^6 spore/ ml) was efficiently applied in the greenhouse causing a significant reduction in final disease severity (FDS %) by 77 %, compared to the positive control. Moreover, application of TCF against the natural infection in the open field reduced FDS, the area under the disease progress curve (AUDPC), and the average coefficient of infection (ACI). Furthermore, TCF significantly improved yield components compared to the untreated control. To assess the plant response on a molecular basis, the protein profile was analyzed showing a wide range of variability. Five polymorphic bands were recognized; mainly 66, 48, 35, 29, and 16 kDa. The band with a molecular weight of 66 kDa had completely disappeared in the negative control, but was observed in the positive control and upon application of TCF. Meanwhile, the bands with molecular weights of 35, 29, and 16 kDa were recorded only in the positive control but were completely vanished in the negative control and upon application of TCF. Additionally, upon application of TCF under the infection stress, phenylalanine ammonia lyase (*PAL*) mRNA transcript accumulation had increased significantly in a way that exceeded two-fold of the non-infected control. Finally, it was concluded that *T. harzianum* was able to reduce leaf-stripe infection damage and promoted the barley-induced resistance in the susceptible cultivars; with the improvement of vegetative growth parameters, which may be a real prospect for leaf-stripe disease management in the future.

Keywords: *Drechslera graminea*, Bioagent, SDS-PAGE, PAL transcripts, Barely

1. Introduction

Barley (*Hordeum vulgare* L.) crop is ranked in the 4th place as a worldwide strategic cereal crop after maize, rice, and wheat. It is widely distributed and cultivated in more than 100 countries in Europe, Asia and America for human consumption, animal feed, malting process, and food industry in many countries (Giraldo *et al*., 2019).

World Barley Production will be 142.224 million metric tons (USDA. 2024) According to the Egyptian Ministry of Agriculture and Land Reclamation (2020/2021 c), about 53.3 thousand feddans in Egypt were cultivated with barley producing 87.6 thousand tons with an output of 1.6 tons/ fed. Barley has different forms such as six-rowed form, which contains the essential micronutrients and provides an excellent substituent for enriching the human diet in the developing countries. Moreover, it contains other components such as β-glucan; a cell-wall polysaccharide, which has a cholesterol-lowering effect and supports the human body to avoid chronic diseases (Zeng *et al*., 2020). Foodstuffs with hulless barley have an increased content of β–glucans compared to the products with pure wheat flour (Narwal *et al*., 2017). Raj *et al*., (2023) revealed that different types of hulless barley contain higher protein content and lower ash content in the crude fiber than the hulled types, and the hulled barley of two -rows is mostly used for malt manufacture.

Barley plants are exposed to several fungal pathogens such as leaf stripe disease caused by *D. graminea*. The teleomorph stage of this pathogen is *Pyrenophora graminea* and its synonym is *Helminthosporium gramineum*. Leaf stripe disease has been recognized as a seed-born disease and caused substantial economic yield losses for barley crops, which in turn needs the development of serious programs to manage this disease (Biselli *et al*., 2010).

Barely leaf-stripe fungal pathogen survives in the outer layers of the infected grains under cooling humid conditions with soil temperature below 15 °C. Through coleoptiles, the pathogen penetrates the grains and grows systemically inside the plant body producing toxins. The produced toxins cause cell death and discoloration of the leaf tissue causing the appearance of striped lesions. Consequently, fungal conidia are produced on the leaves surface and then move to spikes of healthy plants by air currents and/ or splashing rain. Then the conidia develop and give the mycelial growth under humid conditions. The produced mycelium begins to grow between the hulls and kernels and consequently may penetrate the embryo. The fungal pathogen remains latent on or inside the dry grains till the grain sprouts. Following that, the fungus begins its active growth forming a sheath around the first seedling leaf, spread to the next leaf, and continues until all of the leaves become infected (Yuceler *et al*., 2022).

In Egypt, leaf stripe causal agent was primarily isolated by Mehiar *et al*., (1976) and the pathogenicity test was performed by inoculating the isolated fungus in both tested attached and detached leaves. Hussien *et al*., (2009) tested four different fungicides, including Tetraconazole, Diniconazole, Teboconazole and Semiconazole, in controlling fungal leaf-stripe disease as seed treatment and they found that Tetraconazole displayed the best efficacy with the highest average of spike weight and grain yield/ m^2. Mahmoud *et al*., (2020) tested two biocontrol agents in managing the leaf stripe disease and they demonstrated that the fungal bio-agent (*T. harzianum*) was more effective than the bacterial bio-agent (*Bacillus subtilis*) in obstructing the growth of *D. graminea* through the dual culture method.

Although the fungicides are common tools for managing the fungal infections; however, they have

many predictable undesirable effects on the environment, economy, and public health. Therefore, safe alternatives are urgently required for controlling the phytopathogenic fungi. *Trichoderma* sp. is a well-known endophytic fungus, which is widely distributed, and proved to have antimicrobial activity. Ghorbanpour *et al*., (2018) informed that *T. harzianum* can effectively compete with the other microorganisms for space and nutrient requirements; mainly carbon sources. Moreover, *Trichoderma* spp. can produce volatile organic compounds (VOCs) (Oszako *et al*., 2021; Rajani *et al*., 2021) and/ or mucolytic enzymes that reduce the radial growth of the phytopathogenic fungi (Stracquadanio *et al*., 2020). In addition, *Trichoderma* crude extract contains a reliable number of secondary metabolites that may have antimicrobial competency and have been used to evaluate the efficacy of this fungus for manipulation in biological control and manufacture (Li *et al*., 2018). Furthermore, *Trichoderma* elicits the local or systemic defense responses of the host plant as well as improves the plant development and growth (Guzmán-Guzmán *et al*., 2023).

As barley plants respond to *D. graminea* infection, different defense-related mechanisms have been activated. These mechanisms include the appearance of new proteins that are recognized as pathogenesis-related proteins (PRPs) (Dinglasan *et al*., 2019). In addition, the defense-related genes; such as phenylalanine ammonia-lyase (*PAL*), which is the key enzyme involved in the phenylpropanoid pathway during the host-pathogen interaction, were overexpressed under the infection stress (Cynthia *et al*., 2015). Quantitative polymerase chain reaction (qPCR) is regarded as an actual method for determining the relative expression level of *PAL* gene after infection (Zhang *et al*., 2017).

The objective of this work was to highlight the impact of the eco-friendly alternative to the synthetic fungicides; mainly *T. harzianum* bioagent, to manage the barely fungal leaf stripe disease, improve the plant induced resistance, and consequently leads to the production of high-quality crop yield.

2. Materials and methods

2.1. Barley grains, biocontrol agent, and the fungicide

Barley grains of Egyptian susceptible cultivars: *Giza 2000* and *Giza 126* were kindly provided by the Barley Research Section, Field Crop Institute, Agriculture Research Center (ARC), Giza, Egypt. *T. harzianum* isolate was kindly obtained from the culture collection of Mycology Research and Diseases Survey Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. The obtained isolate was deposited in NCBI GenBank with an accession number of (OL454813). The Opus (12.5 % SC*) fungicide (*i.e*., active ingredient Epoxiconazole) at a recommended dose of 0.75 ml/ l was provided by BASF Co., the largest chemical company in Germany.

2.2. Isolation and morphological identification of the pathogenic fungus

The pathogen was isolated from infected barley leaves showed typical symptoms of leaf stripe disease in naturally infected area in Giza Experimental Station, Agricultural Research Centre (ARC). Infected leaves were cut into small pieces (5 mm) and sterilized using 2 % sodium hypochlorite solution (NaOCl) for 2-3 min. and then washed well with sterile distilled H_2O and placed into Potato Dextrose agar (PDA) medium. The Petri plates were incubated at 20± 2 °C for seven d in dark with daily follow-up (Wahiba and Fadhila, 2021). To obtain pure culture, a hyphal tip of the targeted pathogen was removed using sterile capillary glass tube and put on to the solid PDA plate. Incubate the plates at 20± 2 °C for 5-7 d (Mahmoud *et al*., 2020). The developing fungal pathogen was identified and characterized in Barley Disease Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt, based on the mycelial growth pattern, morphological, and microscopic characteristics (Mahmoud *et al*., 2020).

2.3. Pathogenicity test

Barley grains were surface sterilized using 2 % NaOCl for 5 min. and washed several times with sterile dist. H_2O. Then grains were put onto a half part of 7-day old pathogen growth culture in a Petri plate and the other fungal growth half was flipped over the grains in a way of placing grains between two films of the pathogen growth culture as a modified sandwich technique according to Benkorteby-Lyazidi *et al*., (2018). The prepared plates were sealed well and incubated at 4 °C for 72 h in the dark. For the control treatment, the grains were placed similarly between two halves of the PDA medium lacking the tested fungus. Then the inoculated and un-inoculated grains were taken carefully using sterile forceps and planted into small plastic pots (10 cm diam., three grains/ pot) containing disinfested soil (Sand: Petmos at 1:1 v/v) for 14 d, according to the modified method of Wahiba and Fadhila, (2021). Symptoms were followed up in the emerged seedling leaves and the pathogen was re-isolated, and cultured on a PDA growth medium to fulfill Koch's, postulates. The pathogenicity test was carried out for the two barley cultivars.

2.4. Molecular identification of the isolated fungus and phylogenetic analysis

DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used in the isolation of fungal genomic DNA. ITS-rRNA region was amplified using two primers: *ITS1* (5′ TCC GTA GGT GAA CCT GCGG 3′) and *ITS4* (5′TCC TCC GCT TAT TGA TATGC 3′) (White *et al*., 1990). Polymerase chain reaction (PCR) was performed with a reaction mixture of 50 µl consisting of 1.5 mM of $MgCl_2$, 1× PCR buffer, 15 pmol of each primer, 1U of Taq polymerase enzyme, 200 mM of each dNTP, and 2 µl of the DNA template. The thermocycler program for amplification of the ITS region was: 30 cycles at 95 °C for 3 min. as an initial denaturation followed by 40 cycles of denaturation at 95 °C for 30 sec. Annealing at 68 °C for 45 sec. and extension at 72 °C for 90 sec. were performed. A final extension was made at 72 °C for 8 min. PCR reactions were conducted at the Plant Pathology Research Institute, ARC, Giza, Egypt.

Sequencing

DNA sequences were generated through sequencing the amplified PCR products in both directions by the ABI Prism 3130 ×*l* Genetic analyzer using the same primers *ITS1* (5′ TCC GTA GGT GAA CCT GCGG 3′) and *ITS4* (5′TCC TCC GCT TAT TGA TATGC 3′) (White *et al*., 1990). The process of sequencing was performed in Macrogen Corp., Korea, accessed, and has been deposited in NCBI-GenBank using the BLAST function. The sequence was then aligned and subjected to phylogenetic analyses. The phylogenetic tree was performed using the maximum Neighbor-joining method of Saitou and Nei, (1987). MEGA11 software was used to calculate the average nucleotide distinctiveness between the isolated fungal strain and the strictly related reference strains (Tamura *et al*., 2021).

2.5. *In vitro* antagonistic potential

The antagonistic effect of *T. harzianum* against *D. graminea* was evaluated *in vitro* using the dual culture method (Khan *et al*., 2020). A mycelial disc (0.5 cm diam.) of a 7-day-old PDA pathogen culture was cut using a sterile cork borer, placed at 1 cm away from the edge of the Petri plate (9 cm), and a disc of the bioagent culture with the same size was placed in the opposite side of the same plate. Plates were incubated at 20± 2 °C for 7 d. For control, a disc of *D. graminea* strain was placed peripherally in a PDA plate. When the pathogen growth reached the entire surface of the plate in the control treatment, the percentage of growth inhibition (PGI) was determined using the following equation of Miyashira *et al*., (2010):

$$PGI\ (\%) = [(C - T)/C \times 100)]$$

Where; PGI= Percent of growth inhibition (%); C= Pathogen radial growth (Control); T= Pathogen radial growth in dual culture (Test).

2.6. *In vivo* experiments

2.6.1. Grain soaking

Grain soaking method was suggested by Rocha *et al*., (2019) as a tool for delivering beneficial microbes to agriculture crops. For treatment with *T. harzianum* bioagent, the *Giza 126* and *Giza 2000* cultivar grains were immersed in a TCF of 30×10^6 spores/ ml for 60 min., in the presence of Arabic gum (50 % w/v) (Abdulkerim *et al*., 2023) to assure the adhesion of *Trichoderm*a spores on the grain surface. The fungicide (Opus) was applied as a seed treatment. The treated grains were left to air dry before inoculation with the pathogen.

2.6.2. Inoculation of barley grains

The treated barley grains were inoculated with the fungal pathogen according to the method conducted by Benkorteby-Lyazidi *et al*., (2018), as mentioned earlier in the pathogenicity test.

2.6.3. Greenhouse experiments

All greenhouse experiments were conducted at the Plant Pathology Research Institute, ARC, Giza, Egypt. Pots (25 cm in diam.) were filled with the soil (sand & clay at 1:2 v/v) and the barely grains were used as ten grains per pot with five replicates for each treatment. Four experimental treatments were arranged as a complete experimental block design as follows: the first treatment (T_1) involved sowing of the pathogen inoculated grains that were soaked in TCF of 30×10^6 spores/ ml. The second treatment (T_2) involved the application of fungicide only at 12.5 %. The third treatment (T_3) acted as a positive control where the grains were inoculated with the pathogen only, while the fourth treatment (T_4) represented the negative control grains that were not inoculated with the pathogen and non-treated with the bioagent. Development of disease symptoms on the seedling leaves were expected to appear after 12-15 d. The growing leaves were used for further estimation of the plant defense response on a molecular basis, including the protein profile analysis and assessment of PAL transcripts accumulation.

2.7. Disease assessment

Disease severity of the infected barley plants was recorded and estimated as a grade (0-5) represented in Table (1) using a formula adopted by Szczepanek *et al*., (2020) as follows:

Disease severity (%) =

$$\sum (NPC \times CR) / (NIP \times MSC) \times 100$$

Where: NPC = No. of plants in each class rate; CR = Class rate; NIP = No. of tested plants; MSC = Maximum severity class rate.

2.8. Field experiment

To evaluate the potential of *T. harzianum* (TCF) and a chemical fungicide (OPUS) treatments against the leaf-stripe natural infection, field experiments referring to Mahmoud *et al*., (2020) were conducted at Giza Experimental Station, ARC, at latitude: 30˚ 01' 16.99" N and longitude: 31˚ 12' 30.01" E. A field trial was performed for the two susceptible Egyptian barley cultivars, *Giza 126* and *Giza 2000* during two successive winter growing seasons, mainly 2021-2022 and 2022-2023. Grains were sown in a randomized complete block design with three replicates. Each plot consists of five rows in total area of 3 m^2. Barley grains were sown in the two sides of the row as 50 grain/ row.

Conferring to the Ministry of Agriculture guidelines, all traditional cultural preparations were applied at the proper time. Foliar spray treatments were individually applied twice with the previously mentioned recommended doses; mainly 30×10^6 spores/ ml and 0.75 ml/ l for TCF and the fungicide, respectively. Firstly, at the heading stage (70 d after planting) at the start of the infection, and the second time after 10 d depending on the recorded disease severity. To keep the plots weed-free, three manual weeding were performed.

Table 1: Grade of disease severity depending on disease progression in the greenhouse (Tanwar *et al*., 2022)

Grade	Disease progress	Severity/ Index
0	No disease	0
1	Leaves with more than four white stripes	1-20
2	Leaves with white and yellow stripes	21-40
3	Leaves with dark yellow to light brown stripes	41-60
4	Leaves with dark brown to black stripes and loose normal green color	61-90
5	Leaves drying with stripes running through the leaf	91-100

Plants were selected randomly in each replication and disease symptoms were recorded on the leaves using a 0-5 grading scale as mentioned earlier in Table (1) during two successive growing seasons. The disease severity was then calculated by using the following formula adopted by Tanwar *et al*., (2022):

Disease severity (%) =

(Sum of all numerical ratings)/ (Total number of leaves observed × maximum disease rating) ×100

2.8.1. Area under disease progress curve (AUDPC)

The AUDPC was calculated using a simple formula used by Draz *et al*., (2015) as follows:

$AUDPC = D\ [1/2\ (Y_1 + Y_K) + (Y_2 + Y_3 + \ldots\ldots\ldots + Y_{(K-1)})]$

Where: D= Time intervals in days;

$Y_1 + Y_K$ = sum of the first and last disease scores;

$Y_2 + Y_3 + \ldots\ldots\ldots + Y_{K-1}$ = sum of all in-between disease scores.

2.8.2. Average coefficient of infection (ACI)

The average coefficient of infection (ACI) was calculated according to Pathan and Park, (2006) by multiplying disease severity (DS) and constant values of infection type (IT), R= resistant, MR= moderately resistant, M= moderately resistant to moderately susceptible, MS= moderately susceptible and S= susceptible. The constant values for infection types were used based on; R = 0.2, MR = 0.4, M = 0.6, MS = 0.8, and S = 1.0.

The efficacy (%) = $[(D_2-D_1)/ D_2] \times 100$

Where: D_1 = Value of disease component in treated plant; D_2 = Value of disease component in the untreated plant (control) (Hussien *et al*., 2009).

2.8.3. Yield components

At the maturity stage, the plant height, grain number per spike, grain weight per spike, and the weight of thousand grains (thousand kernel weight) (TKW) were determined from the harvested plants and the collected grains. The increase over control was assessed according to the equation stated by Hafez and Abou El Hassan, (2015) as follows:

Increase in yield components (%) = (Treatment-Control)/ Control ×100

Where; Treatment refers to the application of TCF and fungicide; Control refers to the untreated plants

2.9. Molecular changes in the barley leaves

Protein SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) and real time quantitative polymerase chain reaction (RT-qPCR) were performed for barley seedling leaves obtained from the greenhouse experiments to assess the plant defense response under the infection stress (positive control) and disease management using the *T. harzianum* culture filtrate, compared to the healthy plants (negative control).

2.9.1. Protein gel electrophoresis (SDS-PAGE)

About 1g of barley leaf tissue was ground in 2 ml of the extraction buffer (1 × of buffer soluble protein containing 6.0 ml of 1 M tris-hydroxy methyl amino- methane, pH 8.8, 20.0 ml of 10 % SDS (sodium dodecyl sulphate), 10 ml glycerol, 0.8 ml of 0.25 M EDTA (ethylene diamine tetra acetic acid), and dist. water up to 100 ml. Shake for 2 h (leave at 4 °C overnight) and then centrifuge for 20 min. at 1000 rpm at 4 °C. Transfer the supernatant to a new tube and keep it at -20 °C for subsequent use. SDS–PAGE of the protein polypeptides was performed according to Laemmili, (1970).

Staking gel preparation

Staking gel (the upper layer) consists of 1.99 ml of acrylamide stock (30.0 g acrylamide + 0.8 g bis-acrylamide and dist. H_2O up to 100 ml) and a staking gel buffer, pH 6.8 (6.05 g of Tris-base + 30 ml dist. H_2O, adjust pH to 6.8, and complete with H_2O up to 50 ml), add 11 ml of dist. H_2O, and then filter. Add 0.18 ml of 10 % SDS to the filtrate; 10 % of 0.25 g ammonium persulphate in 2 ml dist. H_2O and 30.0 µl of TEMED (N, N,N',N'-Tetramethylethylenediamine).

Resolving gel preparation

The resolving gel (the lower layer) consists of 23.7 ml of acrylamide stock and 12.3 ml of resolving gel buffer, pH 8.8 (18.17g of Tris-base + 80 ml dist. H2O, 13.5 ml of concentrated HCl, adjust to pH 8.8, and complete with H_2O up to 100 ml), add 12.0 ml of dist. H_2O, and then filter. Add 0.5 ml of 10 % SDS; 0.37 ml of 10 % ammonium persulphate and 30 µl of TEMED. Pour the resolving gel directly, and add isopropanol (25 %). Discard the isopropanol, add the staking gel layer, placed the comb directly, and then leave the gel to polymerize. After gel polymerization, add 10 µl of bromophenol blue and load the samples (20 µl sample extract/ well) using 10 microliter protein marker of (98, 75, 66, 54, 48, 35, 29, 25, 16, 11, and 5 kDa) (Pharmacia, USA). Start the run at 100 volts for 20 min., and then raise the voltage gradually to 150 volts after the sample migrates into the resolving gel. Continue the migration until bromophenol reaches the end of the gel.

Staining and de-staining steps

Load the gel in the staining solution for 2-24 h prepared by dissolving 1.0 g of Coomassie brilliant blue-250 in 455 ml of dist. H_2O, add 455 ml of methanol, 90 ml of acetic acid, mix carefully, and then filter. A staining step is followed by de-staining one where the de-staining solution is prepared by mixing 250 ml of dist. H_2O, 140 ml methanol, 40 ml acetic acid, leave the gel in this solution for 1 h, change this solution, and leave the gel overnight. Repeat changing the de-staining solution until the band appears clearly (Brunelle and Green, 2014). The protein bands were observed by a trans-illuminator and photos were taken to compare between the results depending on the presence or absence of the polypeptide bands. The similarity index was calculated for all the possible pairs of protein types. Protein patterns were clustered using the un-weighted pair group technique of arithmetic means (UPGMA) by the gel documentation system (Uvitec, Cambridge, UK) (Abadi *et al*., 2022).

2.9.2. Assessment of the molecular defense-related mechanism of PAL mRNA transcripts

The molecular defense-related mechanism was assessed by determining the phenylalanine

ammonia-lyase (*PAL*) gene expression in healthy and infected leaves (Kohler *et al*., 2002) of barely seedlings after 7-10 d of cultivation in the greenhouse. RNA was isolated using Thermo-Scientific Gene-JET RNA purification kits (0731, 0732). To confirm RNA purity and concentration, a Nano-Drop spectrophotometer (ND 2000c, Thermo-Fisher-Scientific, Wilmington, DE, USA) was used. The cDNA was synthesized using 1 µg RNA using the Thermo-Scientific Revert-Aid First Strand cDNA synthesis kit (1621, 1622) following the standard protocol of the manufacturer. With the specific primers: 5'-CCATTGATGAAGCCAAAGCAAG -3' and 5'-ATGAGTGGGTTATCGTTGACGG-3' (Arabi *et al*., 2020) and using the M×3000P (Stratagene, CA, USA) qPCR system, quantitative amplifications of the PAL gene were performed. Results were normalized to those of actin as endogenous control using the primers 5'-GCCGTGCTTTCCCTCTATG-3' and 5'-GCTTCTCCTTGATGTCCCTTA -3'. The thermal cycler program had initial denaturation at 95°C for 10 min., followed by 40 two-step cycles of amplification (95 °C for 15 sec. and 60 °C for 60 sec.).

2.10. Statistical analyses

Data were analyzed using a one-way analysis of variance (ANOVA). Tukey–Kramer test for multiple comparisons with $p \leq 0.05$ level of probability as the significance level. The statistical software MINITAB (Minitab® 19.2020.1 version, Minitab Inc., PA, USA) was used. Very strong correlation at $p \leq 0.01$ was used.

3. Results

3.1. Molecular identification of the isolated fungus and pathogenicity test

The fungal isolate obtained from the naturally infected barley leaves produced typical long pale or yellow stripes in the emerging seedling leaves, which were converted to darker after 10 d of grain inoculation as the fungus sporulated on the leaf surface compared to the healthy control leaves (Fig. 1). The created phylogenetic tree of the tested fungus exhibited high alignment with the earlier identification of *Pyrenophora graminea* and deposited in a GenBank with an accession number of OR827023 (Fig. 2).

3.2. *In-vitro* antagonistic activity of *T. harzianum*

The *in vitro* dual culture result of *T. harzianum* and the pathogenic isolate of *D. graminea* showed that the antagonistic fungus limited the radial mycelial growth of *D. graminea*, recording percentage of growth inhibition (PGI) of 77.8 % within 7 d of incubation as shown in Fig. (3C).

3.3. Greenhouse experiments

As referenced to the scale in Table (1), the results recorded in Table (2) revealed that the use of *T. harzianum* culture filtrate and the fungicide had reduced the disease severity by 77 % and 88 %; respectively, compared to the positive control.

3.4. Disease progress and yield components under field conditions

The obtained data presented in Tables (3 and 4) displayed a significant decrease in the final disease severity (FDS %), ACI, and AUDPC, with the application of TCF and fungicide (Opus) for the two barley cultivars, *Giza 126* and *Giza 2000*. In addition, the yield components including plant height, grains number per spike, grains weight per spike, and thousand kernel weight, were also affected by *T. harzianum* (TCF) and the fungicide treatments, where they were increased significantly compared to the untreated control as shown in Tables (5 and 6). These obtained results were confirmed through two successive growing seasons with a strong correlation (Table 7). Moreover, the vegetative growth parameters were significantly affected with the change in the final disease severity (FDS) through the two seasons, with a strong correlation as shown in Table (8).

Fig. 1: Disease symptoms recorded in the emerged barley seedling leaves (*Giza 126*). Where; A: a healthy control leaf, B: typical long yellow stripes in an infected leaf

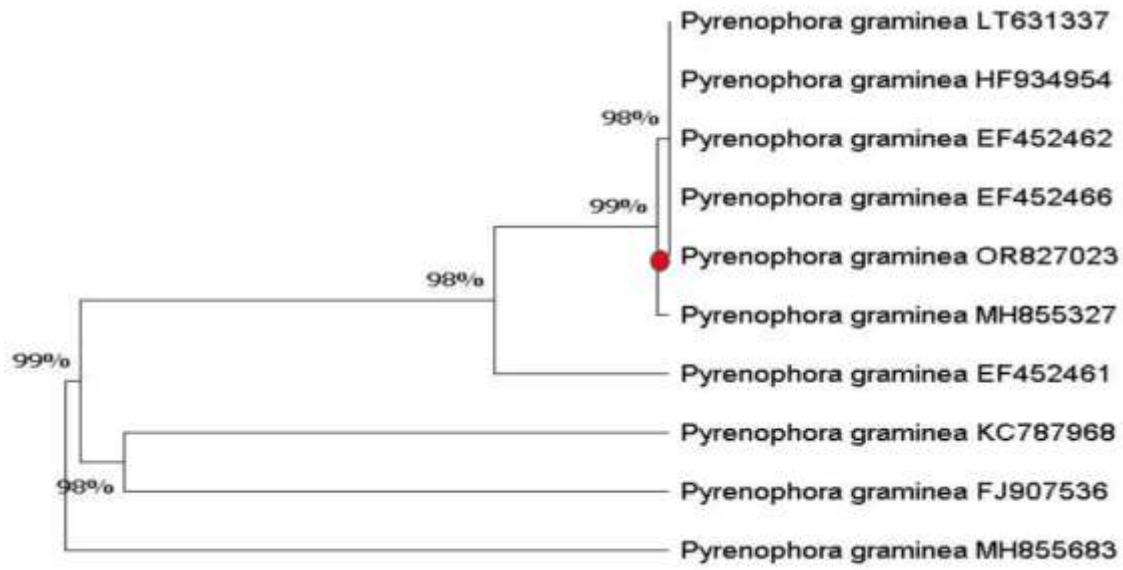

Fig. 2: Phylogenetic tree constructed using partial sequences of the ITS, showing the location of *Pyrenophora graminea* (OR827023), represented by a red dot, in relation to the other related sequences obtained from GenBank

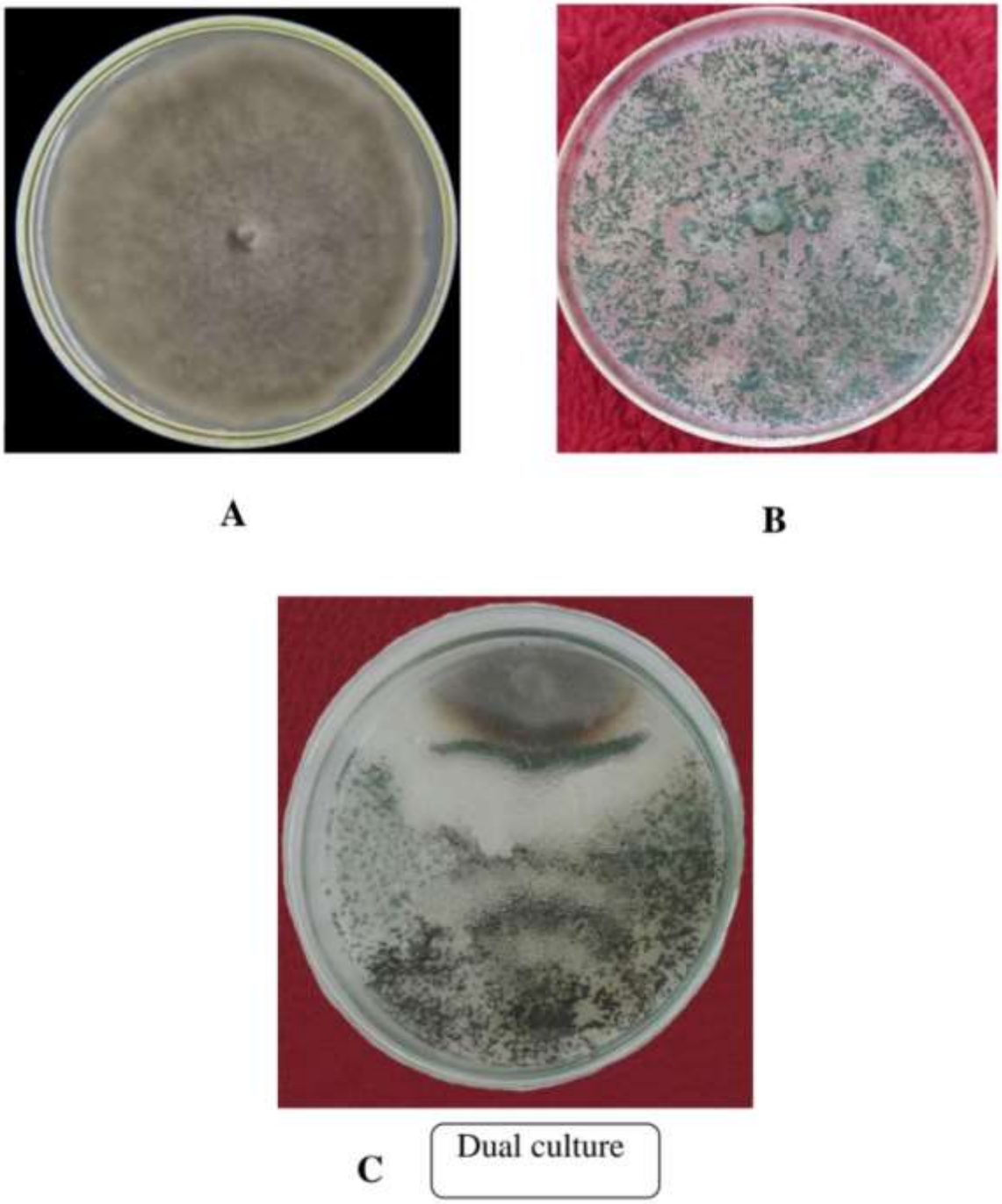

Fig. 3: Growth culture pattern of *T. harzianum* and *D. graminea* growing individually and in dual culture. Where; (A): growth of *D. graminea* on PDA for 7 d; (B): growth of *T. harzianum* on PDA for 7 d; (C): Antagonistic activity in the dual culture of *T. harzianum* (at the bottom) and *D. graminea* (at the top)

Table 2: Effect of different treatments on the leaf-stripe disease severity of barely seedlings under greenhouse conditions

Treatments	Severity grade (mean± SD)	
	Giza 126	***Giza 2000***
T_1	2 ± 0.19^{b}	2 ± 0.13^{b}
T_2	1 ± 0.12^{c}	1 ± 0.12^{c}
T_3	4 ± 0.23^{a}	4 ± 0.25^{a}
T_4	0 ± 0.00^{d}	0 ± 0.00^{d}

Where; Results are represented as mean of five replicates (± SD). Means of the same parameter with different superscript letters are significantly different (Tukey test at $p \leq 0.05$). T_1: represents *T. harzianum* (30×10^6 spores/ ml) inoculum + pathogen inoculum; T_2: represents treatment with the Opus fungicide only at 12.5 %; T3: Treatment with the pathogen inoculum only (Positive control); T_4: No treatment with the pathogen or the bioagent (Negative control)

Table 3: Effect of foliar spray of *T. harzianum* (TCF) and fungicide (OPUS) on final disease severity (FDS %), average coefficient of infection (ACI), and the area under the disease progress curve (AUDPC) in two barley cultivars *cv. Giza 126* and *cv. Giza 2000* under field conditions during 2021-2022

Barley cultivar	Treatment	Final disease severity (FDS) (%)	Efficacy (%)	ACI	Efficacy (%)	AUDPC	Efficacy (%)
Giza 126	TCF	12.82 ± 1.03^{b}	83.79	4.16 ± 0.36^{b}	94.31	62.95 ± 17.02^{b}	86.72
	Opus	9.12 ± 2.79^{c}	88.47	3.85 ± 0.31^{b}	94.74	51.2 ± 14.89^{c}	89.20
	control	79.11 ± 20.13^{a}	0.00	73.15 ± 18.22^{a}	0.00	474 ± 88.20^{a}	0.00
Giza 2000	TCF	14.65 ± 1.72^{b}	81.89	5.01 ± 0.42^{b}	93.14	61.66 ± 16.35^{b}	87.21
	Opus	9.8 ± 2.85^{c}	87.89	3.21 ± 0.30^{c}	95.61	50.8 ± 15.23^{c}	89.46
	control	80.9 ± 20.56^{a}	0.00	73.05 ± 17.85^{a}	0.00	482 ± 91.08^{a}	0.00

Where; Results are represented as mean± SD for three replicates (n=3). The means of the same parameter with different superscript letters are significantly different (Tukey test at $p \leq 0.05$).

Table 4: Effect of foliar spray of *T. harzianum* (TCF) and fungicide (OPUS) on final disease severity (FDS %), average coefficient of infection (ACI), and area under disease progress curve (AUDPC) in two barley cultivars *cv. Giza 126* and *cv. Giza 2000* under field conditions during 2022-2023

Barley cultivar	Treatment	Final disease severity (FDS) (%)	Efficacy (%)	ACI	Efficacy (%)	AUDPC	Efficacy (%)
Giza 126	TCF	13.21± 1.53[b]	83.46	5.80± 0.76[b]	92.23	65.49± 16.38[b]	86.38
	Opus	9.82± 2.81[c]	87.70	3.40± 0.28[c]	95.45	51.28± 15.51[c]	89.33
	control	79.85± 20.02[a]	0.00	74.65± 16.44[a]	0.00	480.8± 90.12[a]	0.00
Giza 2000	TCF	15.11± 1.79[b]	81.40	5.51± 0.49[b]	92.49	64.68± 17.25[b]	86.82
	Opus	9.32± 2.89[c]	88.53	3.33± 0.35[c]	95.46	51.93± 14.86[c]	89.42
	control	81.25± 21.33[a]	0.00	73.35± 16.92[a]	0.00	490.75± 95.66[a]	0.00

Where; Results are represented as mean± SD for three replicates (n=3). The means of the same parameter with different superscript letters are significantly different (Tukey test at $p \leq 0.05$).

Table 5: Effect of foliar spray of *T. harzianum* (TCF) and fungicide (OPUS) on yield parameters of two barley cultivars *cv. Giza 126* and *cv. Giza 2000* under field conditions during the growing season 2021-2022.

Barley cultivar	Treatment	Plant height (cm)	Increase (%)	Grains number /spike	Increase (%)	Grains weight /Spike (g)	Increase (%)	TKW (g)	Increase (%)
Giza 126	TCF	104.88± 15.65[a]	4.46	43.6± 11.63[a]	3.81	2.12± 0.19[b]	17.13	53.11± 11.87[b]	27.00
	Opus	106.45± 16.22[a]	6.03	44.2± 10.89[a]	5.24	2.25± 0.29[a]	24.31	58.33±12.72[a]	39.48
	control	100.4± 15.31[b]	0.00	42.00± 12.00[b]	0.00	1.81± 0.20[c]	0.00	41.82± 13.33[c]	0.00
Giza 2000	TCF	114.4± 17.22[a]	3.72	57.00± 11.20[a]	4.78	3.32± 0.31[b]	4.40	59.75± 17.49[b]	18
	Opus	116.5± 15.84[a]	5.6	58.00± 10.98[a]	6.62	3.48± 0.41[a]	9.43	64.30± 16.56[a]	26.95
	control	110.3± 16.51[b]	0.00	54.4± 11.30[b]	0.00	3.18± 0.27[c]	0.00	50.65±18.09[c]	0.00

Where; Results are represented as mean± SD for three replicates (n=3). The means of the same parameter with different superscript letters are significantly different (Tukey test at $p \leq 0.05$).

Table 6: Effect of foliar spray of *T. harzianum* (TCF) and fungicide (OPUS) on yield parameters of barley cultivars *cv. Giza 126* and *cv. Giza 2000* under field conditions during growing season 2022-2023

Barley cultivar	Treatment	Plant height (cm)	Increase (%)	Grains number /spike	Increase (%)	Grains weight /Spike (g)	Increase (%)	TKW (g)	Increase (%)
Giza 126	TCF	103.72± 15.82[a]	5.49	43.51± 11.65[a]	4.00	2.00± 0.31[b]	12.36	52.15± 11.97[b]	28.13
	Opus	105.82± 17.39[a]	7.63	44.00± 9.89[a]	5.14	2.20± 0.27[a]	23.60	56.85± 18.05[a]	39.68
	control	98.32± 15.41[b]	0.00	41.85± 10.78[b]	0.00	1.78± 0.19[c]	0.00	40.70± 10.87[c]	0.00
Giza 2000	TCF	113.52± 18.22[a]	3.63	56.75± 15.33[a]	5.78	3.30± 0.35[b]	9.63	58.66± 11.48[b]	17.67
	Opus	115.85± 17.68[a]	5.76	58.00± 16.63[a]	8.11	3.43± 0.41[a]	13.95	61.02± 17.39[a]	22.41
	control	109.54± 16.91[b]	0.00	53.65± 19.72[b]	0.00	3.01± 0.28 [c]	0.00	49.85± 13.45[c]	0.00

Where: Results are represented as mean± SD for three replicates (n=3). The means of the same parameter with different superscript letters are significantly different (Tukey test at $p \leq 0.05$).

Table 7: Correlations between the disease parameters and the yield components with different treatments through the two successive seasons for barely plants, *cv. Giza126* and *cv. Giza 2000*

Measured parameter	*cv. Giza 126*	*cv. Giza 2000*
Disease parameters		
(FDS) (%)	0.98**	0.98**
ACI	0.94**	0.96**
AUDPC	0.96**	0.96**
Yield components		
Plant height (cm)	0.96**	0.95**
Grains number/ spike	0.96**	0.97**
Grains weight/ spike (g)	0.97**	0.96**
TKW (g)	0.95**	0.95**

**: Represent significantly strong correlation (Pearson correlation at $p \leq 0.01$).

Table 8: Correlations between the final disease severity and yield components through the two successive seasons for barely plants, *cv*. *Giza126* and *cv*. *Giza 2000*

Measured parameter	(FDS %)	
	cv. Giza 126	***cv. Giza 2000***
Plant height (cm)	0.88**	0.89**
TKW (g)	0.85**	0.84**
Grains number /spike	0.87**	0.85**
Grains weight /spike (g)	0.86**	0.85**

**: Represent significantly strong correlation (Pearson correlation at $p \leq 0.01$).

3.5. Assessment of protein profile (SDS-PAGE)

The protein pattern was performed to assess the plant response against the fungal infection stress in the form of the production of new proteins that are recognized as pathogenesis-related proteins (PRPs). Additionally, this pattern expresses the effect of *T. harzianum* culture filtrate on reduction of infection by altering some PRPs. The protein marker contained 11 bands in a lane named (M) ranging from 98 to 5 kDa. Lanes from 1 to 3 acted as the protein pattern of *cv*. *Giza 126* and lanes from 4 to 6 referred to that of *cv*. *Giza 2000*. Lanes 1 and 6 served as a negative control while lanes 3 and 4 expressed the positive controls. Lanes 2 and 5 were related to the application of TCF treatment (Fig. 4). SDS-PAGE results showed a different electrophoretic banding pattern with different mobilities and intensities. Eight polypeptide bands ranging from 98 to 8.5 kDa were recognized. Three bands (98, 54, and 8.5 kDa) were monomorphic while the other five bands (66, 48, 35, 29, and 16 kDa) were polymorphic. A band with a molecular weight of 66 kDa completely disappeared in lanes 1 and 6, but was observed in lanes 2, 3, 4, and 5. Meanwhile, the bands with molecular weights of 35, 29, and 16 kDa appeared only in lanes 3 and 4; however, they completely vanished in lanes 1, 2, 5, and 6. Dendrogram data analysis based on a similarity matrix showed two main clusters at 60 % level of similarity (Fig. 5). The first cluster included the uninfected leaves of the two cultivars (negative control) that grouped at 98 %level of similarity. The second cluster related to the infected leaves of the two cultivars at an 88 % level of similarity, which were divided into two sub-clusters. The first sub-cluster showed a high degree of similarity between the two cultivars that represented the positive control and grouped at a 98 % level of similarity, while the second sub-cluster related to the application of *T. harzianum* CF on the two cultivars under the fungal infection stress, which were grouped at a 98 % level of similarity.

3.6. Accumulation of PAL transcripts

In the positive control treatment and after 7 d of cultivation, *D. graminea* enhanced significant PAL gene expression in the seedling leaves reaching about 1.5 fold of the corresponding non-infected control (negative control). Moreover, PAL transcript accumulation increased significantly reaching almost 2.5 fold of the corresponding non-infected control with the application of *T. harzianum* culture filtrate. However, after 10 d, the transcripts abundance decreased sharply reaching almost the corresponding control level (Table 9). Results of the two barley cultivars had displayed almost the same manner.

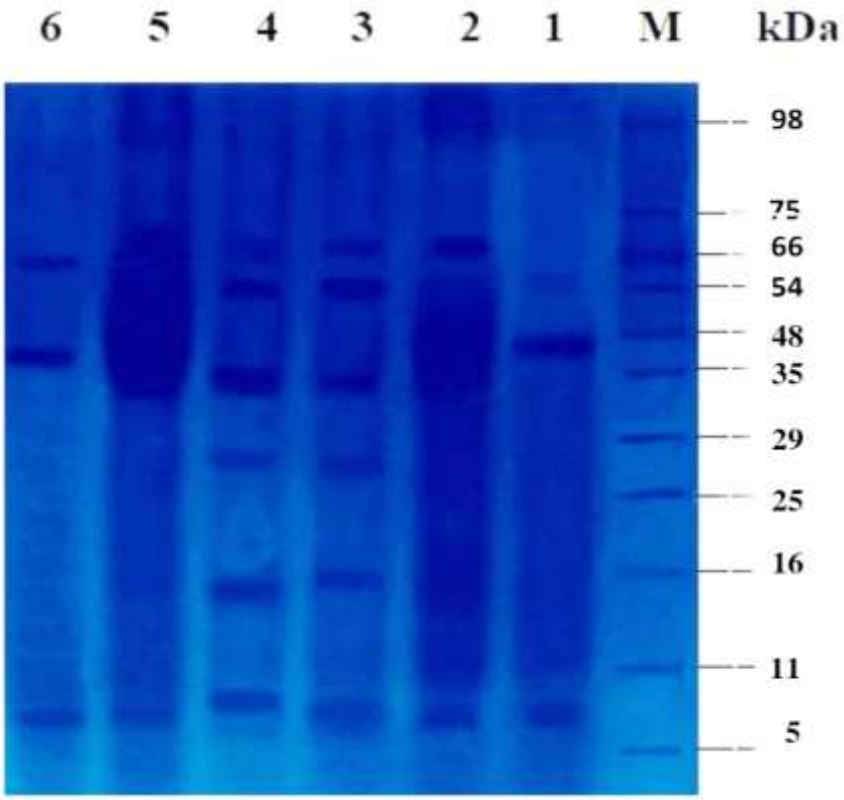

Fig. 4. SDS-PAGE protein expression pattern of two barley cultivars, mainly *Giza 126* and *Giza 2000* under the fungal infection stress. A standard protein molecular weight marker can be seen in Lane M. Lanes from 1 to 3 displayed the protein pattern of *cv*. *Giza 126*, while lanes from 4 to 6 represented *cv*. *Giza 2000*. Lanes 1 and 6 served as negative controls, lanes 3 and 4 acted as positive controls, and Lanes 2 and 5 were related to the application of TCF treatment

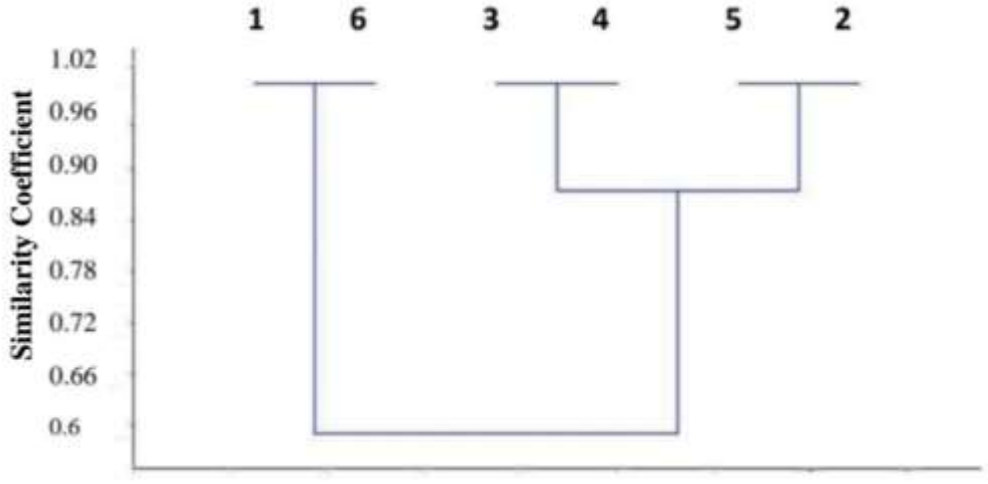

Fig. 5. SDS-PAGE-based dendrogram using the UPGMA cluster analysis for evaluating the electrophoretic protein patterns under the infection stress. Where; 1 and 6 represent the negative controls, 3 and 4 acted as positive controls, and 2 and 5 represent TCF treatment

Table 9: Regulation of *PAL* gene relative expression ratio the in two barley cultivars *cv. Giza 126* and *cv. Giza 2000*

Cultivation period (d)	Relative *PAL* gene expression ratio					
	cv. Giza 126			*cv. Giza 2000*		
	Negative control	Treatment with TCF	Positive control	Negative control	Treatment with TCF	Positive control
7	1.00± 0.05^{c}	2.62± 0.24^{a}	1.56± 0.18^{b}	1.00± 0.06^{c}	2.33± 0.2^{a}	1.63± 0.15^{b}
10	0.98± 0.09^{c}	0.95± 0.08^{c}	0.85± 0.07^{c}	0.93± 0.08^{c}	0.92± 0.07^{c}	0.86± 0.07^{c}

Where; Results are represented by means± SD for five replicates (n=5). The means of the same parameter with different superscript letters are significantly different (Tukey test at $p \leq 0.05$)

4. Discussion

The bioactive endophytic fungi as being eco-friendly alternatives to the synthetic fungicides, provide a promising potential against various phytopathogenic fungi. *Trichoderma* spp. were pronounced in their ability to grow in soil and compete with the other microflora for space and nutrients. Moreover, they are known for their antimicrobial potential against pathogenic fungi through the production of active antifungal compounds that alter the microbial growth (Pokhrel *et al*., 2022).

Our results displayed an obvious *in vitro* antagonism in dual culture assay that assumed *T. harzianum* reducing *D. graminea* growth. The methanol extract of the biocontrol strain that we obtained from Plant Pathology Research Institute and used in our work was analyzed in a previous study reported by Saleh *et al*., (2022) through HPLC chromatography, and displayed the presence of a main phenolic compound, known as kaempferol (233.4 mg/ g), which exhibited an antifungal activity

The *in vivo* evaluation of the inhibitory effect of *T. harzianum* bioagent on disease progress under greenhouse conditions showed a significant reduction in the disease severity, where *Trichoderma* strain suppressed the barley leaf-stripe fungal growth and elicited the plant induced resistance, in consistence with Saldajeno *et al*., (2014). Moreover, the application of *T. harzianum* culture filtrate in the open field against the leaf-stripe natural infection through two successive winter seasons displayed a high reduction in the final disease severity (FDS); AUDPC, and ACI values; where *T. harzianum* was able to produce a diverse range of antifungal compounds that suppress pathogen growth and activity that agreed with Esawy *et al*., (2021). Furthermore, field experiment results revealed that the application of *T. harzianum* culture filtrate improved the barley yield parameters with a significant increment in the vegetative growth parameters compared to the untreated barley plants, which is in agreement with the previous study conducted by Tyskiewicz *et al*., (2022). Explaining the plant signaling events generated by *Trichoderma* is of high importance in order to

recognize the molecular basis involving plant protection against the biotic stresses. It is well known that the infected plant tissue produces new proteins in response to infection called pathogenesis-related proteins. Protein pattern in our results showed that the negative control group produced bands of altered proteins with the creation of newly expressed proteins in positive control and after treatment with TCF. The current protein profile analysis expressed high similarity between the two barley cultivars in terms of their response to treatment with *T. harzianum* culture filtrate, as they were susceptible to infection, which is in agreement with the previous study reported by Ali *et al*., (2018). In accordance with the current work, Mayo *et al*., (2015) elucidated the confident properties of *T. harzianum* T019 on enhancing the bean plant response to *Rhizoctonia solani*, through its capability to stimulate the expression of seven plant defense-related genes including the *PAL* gene.

In our results, it is difficult to determine a definite time point for the end of early defense responses, but the emerged seedling leaves appeared about 7-10 d of cultivation. So, on the 7th day after cultivation, *PAL* gene expression was estimated in the positive control leaves, which generated up-regulation in the two barely cultivars as a temporary response to the fungal infection, and its mRNA-transcript had been accumulated in 1.5 fold compared to the corresponding non-infected control. However, application of *T. harzianum* culture filtrate increased the defense-acquired response of the plant, which in turn increased the overexpression of *PAL* gene, consequently increased the accumulation of *PAL* mRNA transcripts about 2.5-fold compared to the corresponding non-infected control, in agreement with Pacheco-Trejo *et al*., (2022). But after 10 d of cultivation, PAL gene expression was down-regulated with a sharp decrease in the accumulation of its mRNA transcripts almost reaching the corresponding non-infected control with an infection progression. In this respect, Arabi *et al*., (2020) studied *PAL* gene expression in two barley cultivars, Banteng (*cv*. resistant) and Furat 1 (*cv*. susceptible) under the infection stress of *P. graminea*. Depending on qPCR analysis, they revealed that *PAL* gene expression in the resistant cultivar was higher and faster as compared with the susceptible one after infection with *P. graminea* at early points of infection (6 d post inoculation). In our results, *T. harzianum* as increased induced resistance of barley susceptible *cv*. (*Giza 126* and *Giza 2000*) that in turn leads to increase in *PAL* gene expression after seven days of cultivation.

Conclusion

Our work confirmed the antagonistic potential of *T. harzianum* particularly against *D. graminea*, and improved the barely plant-induced resistance under greenhouse conditions as well as in open fields. This was obvious through the significant variation in the protein profile and the expression patterns of *PA*L gene in the susceptible barley cultivars, *cv*. *Giza 126* and *Giza 2000*. In the future, this may represent an effective corresponding opportunity for an ecofriendly management of barely leaf-stripe disease.

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Conflict of interests

There are no conflicts of interest to declare.

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Ethical approval

None-applicable.

Author's Contributions

Conceptualization: M.F; Methodology: M.F and Sh.Ph.; Reviewing: M.F and Sh.Ph; Visualization:

M.F.; Statistical analysis: M.F; Editing and Writing - original draft: M.F.

5. References

Abadi, S.A.R.; Abdosalehi, A.S.; Pouyamehr, F. and Koohi, S. (2022). An accurate alignment-free protein sequence comparator based on physicochemical properties of amino acids. Scientific Report. 12(1): 11158. https://doi.org/10.1038/s41598-022-15266-8.

Abdulkerim, R.; Xiang, S.; Shi, Y.; Xie, X.; Li, L.; Chai, A. et al. (2023). Seed Pelleting with Gum Arabic-Encapsulated Biocontrol Bacteria for Effective Control of Club root Disease in Pak Choi. Plants. 12(21): 3702. https://doi.org/10.3390/plants12213702.

Ali, S.; Ganai, B.A.; Kamili, A.N.; Bhat, A.A.; Mir, Z.A.; Bhatd, J.A. et al. (2018). Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. Microbiological Research. 212: 29-37. https://doi.org/10.1016/j.micres.2018.04.008.

Arabi, M.I.E.; Alek, H.; Jawhar, M. and Al-Shehadah, E. (2020). Expression of *PAL* and *PR2* pathogenesis-related genes in barley plants challenged with closely related *Pyrenophora* species. Cereal Research Communications. 48: 211-216. https://doi.org/10.1007/s42976-020-00033-0.

Benkorteby-Lyazidi, H.; Zeghar, I.; Hanifi-Mekliche, L. and Bouznad, Z. (2018). Barley leaf stripe disease in Algeria: Evaluation of virulent *Pyrenophora graminea* isolates and identification of resistant Algerian barley genotypes. Journal of Agricultural Science. 25: 367-372. https://doi.org/10.15832/ankutbd.421747

Biselli, C.; Urso, S.; Bernardo, L.; Tondelli, A.; Tacconi, G.; Martino, V. et al. (2010). Identification and mapping of the leaf stripe resistance gene *Rdg1a* in *Hordeum spontaneum*. Theoretical Applied Genetics. 120: 1207-1218. https://doi:10.1007/s00122-009-1248-2

Brunelle, J.L. and Green, R. (2014). Coomassie Blue Staining. Methods in Enzymology. 541: 161-167. https://doi.org/10.1016/B978-0-12-420119-4.00013-6

Cynthia, L.C.; Peraldi, A.; Dowd, P.F.; Mottiar, Y.; Santoro, N.; Karlen, S.D. et al. (2015). Effects of *PAL* knockdown on cell wall composition, biomass digestibility, and biotic and abiotic stress responses in *Brachypodium*. Journal of Experimental Botany. 66: 4317-4335. https://doi.org/10.1093/jxb/erv269.

Dinglasan, E.; Hickey, L.; Ziems, L.; Fowler, R.; Anisimova, N.; Baranova, O. et al. (2019). Genetic characterization of resistance to *Pyrenophora teres* f. sp. *teres* in the international barley differential Canadian lake shore. Frontier in Plant Science.10: 326-334. https://doi.org/10.3389/fpls.2019.00326.

Draz, I.S.; Abou-Elseoud, M.S.; Kamara, A.M.; Alaa-Eldein, O.A. and El-Bebany, A.F. (2015). Screening of wheat genotypes for leaf rust resistance along with grain yield. Annals of Agricultural Science. 60: 29-39. https://doi.org/10.1016/j.aoas.2015.01.001

Egyptian Ministry of Agriculture and Land Reclamation. (2020/21c). Agricultural Statistics Bulletin. Retrieved from: https://www.moa.gov.eg.

Esawy, A.A.; Elsharkawy, M.M.; Omara, R.I.; Khalifa, M.A.; Fadel, F.M. and El-Naggar, M.M. (2021). Biological control of *Golovinomyces cichoracearum*, the causal pathogen of sunflower powdery mildew. Egypt Journal of Biological Pest Control. 31: 133. https://doi.org/10.1186/s41938-021-00479-2

Ghorbanpour, M.; Omidvari, M.; Abbaszadeh-Dahaji, P.; Omidvar, R. and Kariman, K. (2018). Mechanisms underlying the protective effects of beneficial fungi against plant diseases. Biological

Control. 117: 147-157. https://doi.org/10.1016/j.biocontrol.2017.11.006.

Giraldo, P.; Benavente, E.; Manzano-Agugliaro, F. and Estela Gimenez, E. (2019). Worldwide Research Trends on Wheat and Barley: A Bibliometric Comparative Analysis. Agronomy. 9(7): 352. https://doi.org/10.3390/agronomy9070352

Guzmán-Guzmán, P.; Kumar, A.; de los Santos-Villalobos, S.; Parra-Cota, F.I.; Orozco-Mosqueda, M.d.C.; Fadiji, A.E. et al. (2023). *Trichoderma* species: Our best fungal allies in the biocontrol of plant diseases-A review. Plants. 12: 432-466. https://doi.org/10.3390/plants12030432.

Hafez, E.M. and Abou El Hassan, W.H. (2015). Nitrogen and water utilization efficiency of barley subjected to desiccated conditions in moderately salt-affected soil. Egyptian Journal of Agronomy. 37(2): 231-249. https://doi.org/10.21608/AGRO.2015.203.

Hussien, M.M.; Mostafa, N.A. and EL-Shamy, M.M. (2009). Control of barley leaf stripe (*Drechslera graminea*) by fungicidal seed treatments. Journal of Agricultural Science. Mansoura University. 34(6): 6907-6913. https://doi.org/10.21608/JPPP.2009.217169.

Khan, R.A.; Najeeb, S.; Hussain, S.; Xie, B. and Li, Y. (2020). Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. Microorganisms. 8(6): 817-831. https://doi.org/10.3390/microorganisms8060817

Kohler, A.; Schwindling, S. and Conrath, U. (2002). Benzothiadiazole-induced priming for potentiated responses to pathogen infection, wounding, and infiltration of water into leaves requires the *NPR1/ NIM1* gene in *Arabidopsis*. Plant Physiology. 128: 1046-1056. https://doi.org/10.1104/pp.010744.

Laemmili, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London). 227: 680-685. https://doi.org/10.1038/227680a0.

Li, N.; Alfiky, A.; Wang, W.; Islam, M.; Nourollahi, K.; Liu, X. et al. (2018). Volatile compound-mediated recognition and inhibition between *Trichoderma* biocontrol agents and *Fusarium oxysporum*. Frontiers in Microbiology. 31(9): 2614-2629. https://doi.org/10.3389/fmicb.2018.02614.

Mahmoud, A.M.A.; Eisa, N.A.; El-Habbaa, G.M.; El-Nashar, F.K. and El-Fiki, I. A.I. (2020). Efficacy of Some Fungicides, Commercial Plant Oils and Bio-Agents against *Drechslera Graminea* Inciting Barley Leaf Stripe Disease. Annals of Agricultural Science, Moshtohor. 58(4): 1063-1078. https://doi.org/10.21608/assjm.2020.155392.

Mayo, S.; Gutiérrez, S.; Malmierca, M.G.; Lorenzana, A.; Campelo, M.P.; Hermosa, R. et al. (2015). Influence of *Rhizoctonia solani* and *Trichoderma* spp. in growth of bean (*Phaseolus vulgaris* L.) and in the induction of plant defense-related genes. Frontier in Plant Science. 6: 685-695. https://doi.org/10.3389/fpls.2015.00685.

Mehiar, F.F.; Wasfy, E.H. and El-Samra, I.A. (1976). New leaf diseases of barley in Egypt. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Zweite Naturwissenschaftliche Abteilung: Allgemeine, Landwirtschaftliche und Technische Mikrobiologie. 131: 757-759. https://doi.org/10.1016/s0044-4057(76)80066-4

Miyashira, C.H.; Tanigushi, D.G.; Gugliotta, A.M. and Santos, D.Y. (2010). Comparison of radial growth rate of the mutualistic fungus of *Atta sexdens rubropilosa forel* in two culture media. Brazilian Journal of Microbiology 41(2): 506-511. https://doi.org/10.1590/S1517-838220100002000035.

Narwal, S.; Kumar, D.; Sheoran, S.; Verma, R.P.S and Gupta, R.K. (2017). Hulless barley as a promising source to improve the nutritional quality of wheat products. Journal of Food and Science Technology. 54(9): 2638-2644. https://doi.org/10.1007/s13197-017-2669-6.

Oszako, T.; Voitka, D.; Stocki, M.; Stocka, N.; Nowakowska, J.A.; Linkiewicz, A. et al. (2021). *Trichoderma asperellum* efficiently protects *Quercusrobur* leaves against *Erysiphea lphitoides*. European Journal of Plant Pathology. (159): 295-308. https://doi.org/10.1007/s10658-020-02162-y.

Pacheco-Trejo, J.; Aquino-Torres, E.; Reyes-Santamaría, M.I.; Islas-Pelcastre, M.; Pérez-Ríos, S.R.; Madariaga-Navarrete, A. et al. (2022). Plant Defensive Responses Triggered by *Trichoderma* spp. as Tools to Face Stressful Conditions. Horticulturae. 8: 1181-1201. https://doi.org/10.3390/horticulturae8121181.

Pathan, A.K. and Park, R.F. (2006). Evaluation of seedling and adult plant resistance to leaf rust in European wheat cultivars. Euphytica. 149: 327-342. https://doi.org/10.1007/s10681-006-9308-z.

Pokhrel, A.; Adhikari, A.; Oli, D.; Paudel, B.; Pandit, S.; Bigyan, G.C. et al. (2022). Biocontrol Potential and Mode of Action of *Trichoderma* Against Fungal Plant Diseases. Acta Scientific Agriculture. 6(10): 10-21. https://doi.org/10.31080/ASAG.2022.06.1184.

Raj, R.; Shams, R.; Pandey, V.K.; Dash, K.K.; Singh, P. and Bashir, O. (2023). Barley phytochemicals and health promoting benefits: A comprehensive review. Journal of Agriculture and Food Research. 14: 100677. https://doi.org/10.1016/j.jafr.2023.100677.

Rajani, P.; Rajasekaran, C.; Vasanthakumari, M.M.; Olsson, S.B.; Ravikanth, G. and Shaanker, R.U. (2021). Inhibition of plant pathogenic fungi by endophytic *Trichoderma* spp. through mycoparasitism and volatile organic compounds. Microbiological Research. 1(242): 126595. https://doi.org/10.1016/j.micres.2020.126595.

Rocha, I.; Ma, Y.; Souza-Alonso, P.; Vosátka, M.; Freitas, H. and Oliveira, R.S. (2019). Seed Coating: A Tool for Delivering Beneficial Microbes to Agricultural Crops. Frontier in Plant Science. 10: 485509. https://doi.org/10.3389/fpls.2019.01357

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4: 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454.

Saldajeno, M.G.B.; Naznin, H.A.; Elsharkawy, M.M.; Shimizu, M. and Hyakumachi, M. (2014). Enhanced Resistance of Plants to Disease Using *Trichoderma* spp. Biotechnology and Biology of *Trichoderma*. 1: 477-493. https://doi.org/10.1016/B978-0-444-59576-8.00035-7

Saleh, R.A.; Ata, A.A.; Eid, H.E.; El-shazly, M.M. and Ahmed, A.Y. (2022). Efficacy of the active substances of some *Trichoderma* isolates to control early blight in potatoes plants. Middle East Journal of Agriculture Research. 11(4): 1291-1306. https://doi.org/10.36632/mejar/2022.11.4.87.

Stracquadanio, C.; Quiles, J.M.; Meca, G. and Cacciola, S.O. (2020). Antifungal activity of bioactive metabolites produced by *Trichoderma asperellum* and *Trichoderma atroviride* in liquid medium. Journal of Fungi. 6(4): 263. https://doi.org/10.3390/jof6040263.

Szczepanek, M.; Lemanczyk, G.; Lamparski, R.; Wilczewski, E.; Graczyk, R.; Nowak, R. et al. (2020). Ancient wheat species (*Triticum sphaerococcum* Perc. and *T. persicum* Vav.) in organic farming: influence of sowing density on agronomic traits, pests and diseases occurrence, and weed infestation. Agriculture. 10(11): 556. https://doi.org/10.3390/agriculture10110556

Tamura, K.; Stecher, G. and Kumar, S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution. 38: 3022-3027. https://doi.org/10.1093/molbev/msab120.

Tanwar, V.K.; Shekhawat, P.S.; Sain, S.K.; Kanwar, H.; Saini, K. and Paul, D. (2022). Development of prediction model for strip disease of barley caused by *Drechslera graminea* in Rajasthan. Scientist (Philadelphia, Pa.). 1(3): 2901-2913. https://doi.org/10.5281/zenodo.7336639.

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. 23(4): 2329. https://doi:10.3390/ijms23042329.

USDA. (2024). United States Department of Agriculture. Barley Explorer (usda.gov).

Wahiba, A. and Fadhila, S. (2021). Comparative study of *Pyrenophora graminea* incidence on *Hordeum vulgare* L. hybrid populations and their parents. Journal of Oasis Agriculture and Sustainable Development. 3(3): 64-71. https://doi.org/10.56027/JOASD.spiss102021.

White, T.J.; Bruns, T.; Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications (Innis, M.A.; Gelfand, D.H.; Sninsky, J.J. and White, T.J. Editors). pp. 315-322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.

Yuceler, H.S.; Karakaya, A. and Oguz, A.C. (2022). Barley stripe disease in Turkey – current situation. Bioengineering Studies Review Article. 3(1): 1-6. https://doi.org/10.37868/bes.v3i1.id205.

Zeng, Y.; Pu, X.; Du, J.; Yang, X.; Li, X.; Mandal, M.S.N. et al. (2020). Molecular Mechanism of Functional Ingredients in Barley to Combat Human Chronic Diseases. Oxidative Medicine and Cellular Longevity. 3836172. https://doi.org/10.1155/2020/3836172

Zhang, C.; Wang, X.; Zhang, F.; Dong, L.; Wu, J.; Cheng, Q. et al. (2017). Phenylalanine ammonia-lyase2.1 contributes to the soybean response towards *Phytophthora sojae* infection. Scientific Reports. 7: 7242. https://doi.org/10.1038/s41598-017-07832-2.