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ORIGINAL ARTICLE

Biological Efficacy of *Trichoderma asperellum* Against Root Rot of Strawberry

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ABSTRACT

Strawberry crown and root rot represent major constraints to sustainable production worldwide, primarily due to infection by a complex of soilborne fungal pathogens. This study assessed the susceptibility of three commercial strawberry cultivars namely: 'Festival', 'Fortuna', and 'Sensation' to crown and root rot in Ismailia Governorate, Egypt. Isolation and morphological identification revealed the presence of three fungi: *Macrophomina phaseolina* (40%), *Fusarium solani* (35%), and *Rhizoctonia solani* (20%), with *M. phaseolina* being the most virulent and frequently isolated. Disease incidence peaked during warmer periods of September–October and April–May, closely associated with elevated soil temperatures. Among cultivars, 'Festival' showed the highest susceptibility, while 'Sensation' exhibited comparatively the lowest disease incidence. *In vitro* dual culture assays demonstrated that *Trichoderma asperellum* significantly inhibited mycelial growth of all tested pathogens, especially *M. phaseolina*. Gas chromatography–mass spectrometry (GC–MS) analysis of *T. asperellum* extract revealed the presence of diverse secondary metabolites, including fatty acid derivatives, and sugar alcohol, contributing to its antagonistic activities. Field application of *T. asperellum* reduced crown and root rot incidence by roughly 81% and significantly enhanced the plant growth and yield, particularly in the highly susceptible 'Festival' cultivar. The observed increase in yield was associated with remarkable biochemical changes in plant tissues, including elevated levels of chlorophylls, proteins, total phenols, and total sugars. These improvements are believed to contribute to enhanced plant resistance and productivity. These findings underscore the potential of *T. asperellum* as an effective biocontrol agent, offering a sustainable alternative to chemical fungicides.

Keywords: Strawberry, crown- root rot disease, *Trichoderma asperellum*, biological control

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INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.), a member of the Rosaceae family, is the most economically significant small berry crop cultivated worldwide (Whitaker *et al*., 2020). Valued for its unique flavor and rich nutritional profile, strawberry fruit has been globally consumed for centuries (Bianco *et al*., 2009). Regular consumption is linked to a range of health benefits, including blood pressure regulation, and anti-inflammatory and anticancer effects (Joseph *et al*., 2014). These beneficial properties are primarily attributed to the fruit's high content of vitamin C, anthocyanins, and phenolic compounds (Park *et al*., 2017). In recent years, global cultivation of strawberries has expanded considerably, with annual production surpassing 8.1 million tons over the past five years (FAOSTAT, 2021). In Egypt, it holds particular importance as an export crop due to its favorable agronomic traits, early market availability, and high economic return (Moussa *et al.*, 2019).

Despite its economic and nutritional value, strawberry production suffers significant losses due to various diseases, with pre- and postharvest losses estimated at 40 – 45% of the total yield (Hutton *et al*., 2013; Merve *et al*., 2016). Soilborne plant pathogens are widespread in strawberry cultivated soils, with root rot recognized as one of the most

significant and destructive diseases, leading to substantial economic losses in the strawberry industry (Lazcano *et al*., 2021). Root rot is a complex disease caused by a consortium of fungal pathogens, primarily *Macrophomina phaseolina* (Hutton *et al*., 2013), *Fusarium solani* (Koike and Gordon, 2015), and *Rhizoctonia solani* (Fang *et al*., 2013). The disease progresses rapidly, with few visible symptoms in its early stages. In the middle to late stages, infected plants typically exhibit sudden wilting and withering, particularly following rainfall (Dinler *et al*., 2016). Characteristic symptoms include progressive blackening and decay of the root system, leading inevitably to reduced plant vigor and yield (Ceja-Torres *et al*., 2014). Despite the implementation of various integrated strategies to manage this disease, *i. e.* selection and breeding of resistant cultivars, optimized cultivation practices, and improved soil drainage, chemical fungicides remain the predominant method of control (Abbas *et al*., 2022). This reliance is largely due to the high diversity of pathogenic species and the limited availability of resistant strawberry cultivars (Hong *et al*., 2022).

Recently, biological control has gained prominence as a sustainable and environmentally friendly alternative to chemical methods (Kumar *et al*., 2021). Several microbial genera, including *Trichoderma*, *Bacillus*, and *Streptomyces*, have been extensively studied and developed as biocontrol agents against strawberry root rot (Abd-El-Kareem *et al*., 2019; 2022). Among these, *Trichoderma* species belong to the Hypocreaceae family have received considerable attention due to their high reproductive capacity, ease of isolation and cultivation, and broad-spectrum mechanisms of action (Poveda *et al*., 2019). Several *Trichoderma* species have been extensively studied and tested in field assays as effective biocontrol agents, including *T. harzianum*, *T. hamatum*, *T. viride*, *T. atroviride*, and *T. asperellum*, among others (Olowe *et al*., 2022). These species exhibited strong antagonistic activity against a variety of fungal phytopathogens such as *Pythium*, *Phytophthora*, *Botrytis*, *Rhizoctonia*, and *Fusarium* (Ghasemi *et al*., 2020; Cai and Druzhinina, 2021). For instance, *T. harzianum* has been reported to produce chitinases and glucanases that degrade pathogen cell walls (Harman *et al*., 2004); *T. hamatum* exhibits strong antibiosis through secondary metabolite production (Lodi *et al*., 2023); *T. viride* and *T. atroviride* are known for competitive colonization and mycoparasitic activity (Vinale *et al*., 2008); while *T. asperellum* enhances plant systemic resistance via jasmonic acid and ethylene pathways (Shoresh *et al*., 2010).

The role of beneficial soil microbes in enhancing plant resistance against root rot disease remains largely unexplored. Investigating their potential in suppressing soilborne pathogens could offer valuable insights into multitrophic interactions and support the development of sustainable disease management strategies. Hence, the present study aimed to: (a) estimate the frequency of root rot disease occurring naturally in strawberry fields in Ismailia Governorate, Egypt; (b) isolate and identify the causal agents of strawberry root rot and assess their pathogenicity; (c) evaluate the antagonistic effects of various *Trichoderma* species on the mycelial growth of the isolated pathogens; and (d) assess the efficacy of *T. asperellum* in inhibiting the root rot infections in strawberry plants under field conditions.

MATERIALS AND METHODS

Frequency and isolation of the associated root rot pathogenic fungi

The occurrence and incidence of root rot disease were screened monthly during the growing season 2020/2021 in three strawberry cultivars (Festival, Fortuna, and Sensation) grown commercially in Ismailia Governorate, Egypt. Representative diseased samples exhibiting typical root rot symptoms were collected from each cultivar (20 samples/month each cultivar) across five different locations in Ismailia Governorate. Infected roots were thoroughly washed, dried, surface-sterilized in 1% NaOCl solution for 3 min, rinsed several times with

sterile distilled water, and finally dried between two layers of sterile filter paper. Fungal pathogens associated with root rot were isolated on potato dextrose agar (PDA) medium. The resulting fungal colonies were purified using the hyphal tip technique as described by (Dhingra and Sinclair, 1973). Pure fungal cultures were examined microscopically and identified based on the cultural and morphological characteristics at the Central Lab of Organic Agriculture, Agriculture Research Center (ARC), Giza, Egypt. Identification was conducted using standard taxonomic keys and references, including those by Sneh *et al*. (1992) for *R. solani*, Booth (1971) for *F. solani* and Dhingra and Sinclair (1973) for *M. phaseolina*.

Pathogenicity test

Pathogenicity test was conducted following the method described by Haggag and El-Gamal (2012) to evaluate the virulence of isolated fungi and the susceptibility of three strawberry cultivars (Festival, Fortuna, and Sensation). The experiment was carried out under greenhouse conditions at the Central Laboratory of Organic Agriculture, Agricultural Research Center (ARC), Giza, Egypt. Sterilized plastic pots (20 cm diameter) were filled with disinfected clay loam soil previously treated with 5% formalin and left to aerate before use. Fungal inocula were prepared by culturing each pathogen on sterilized corn sand meal (CSM) medium, as described by (Ahmed and El-Fiki, 2017). The inoculum of each fungus was thoroughly mixed with the soil at 10 g kg^{-1} soil, ten days before transplanting. Control pots containing non-infested soil received an equivalent amount of autoclaved CSM medium. Seedlings of each strawberry cultivars (Festival, Fortuna, and Sensation) were transplanted in both infested and non-infested (control) soils (2 plants/pot). Each treatment consisted of ten pots (twenty plants per treatment). The plants were maintained in the greenhouse under ambient temperatures of 25/23 ˚C day/night, photoperiod of 16 h, light intensity of 600 – 800 lux, and relative humidity of 75±5% throughout the entire experiment. The plants were monitored weekly for a total period of seven weeks. The plants were arranged in split- split design and irrigated as needed and fertilized as usual.

Evaluation of antagonistic effect of different *Trichoderma* spp. against soil-borne pathogens

An *in vitro* dual culture assay was conducted to evaluate the antagonistic potential of three previously isolated *Trichoderma* species: *T. harzianum* (accession no. MT110634), *T. hamatum* (accession no. MT111894), and *T. asperellum* (accession no. MT110638) against three isolated soil-borne pathogenic fungi (*R. solani*, *F. solani*, and *M. phaseolina*). *Trichoderma* isolates were provided from the Central Lab of Organic Agriculture, Agricultural Research Center (ARC), Giza, Egypt. The assay was performed on gliotoxin fermentation agar (GFA) medium following the method described by Ahmed and El-Fiki (2017). Actively growing cultures of the *Trichoderma* spp. and the tested pathogens were used to obtain 5 mm diameter mycelial discs using a sterile corkborer. In each GFA plate, one disc of a *Trichoderma* isolates and one disc of a target pathogen were placed on opposite sides of the plate, equidistant from the center. Each treatment was replicated three times. Plates inoculated with the pathogens alone served as controls. All plates were incubated at 25-28 °C on laboratory benches. The antagonistic activity of each *Trichoderma* isolate was evaluated by measuring the radial growth of the pathogens five days after incubation. The percentage inhibition of pathogen growth was calculated using the formula described by Pandey *et al*. (2000) as follows:

$$\text{Mycelia growth reduction (MGR)\%} = \frac{DC - DT}{DC} \times 100$$

Where: DC = radial growth of the pathogen in control plates (cm)

DT = radial growth of the pathogen in dual culture plates (cm)

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Identification of potentially bioactive compounds of *T. asperellum* by gas chromatography mass spectrometry analysis

Trichoderma asperellum (exhibited the highest inhibitory effect on mycelial growth *in vitro*) was selected for the characterization of its bioactive compounds using GC– TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a TG–5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The GC oven temperature was initially held at 50 °C for 1 min, then increased at a rate of 5 °C min^{-1} to 250 °C, where it was held for 2 min. The total run time was 43 min. It was further increased to 300 °C at a rate of 30 °C/min and maintained for an additional 2 min. The injector and MS transfer line temperatures were set at 260 °C and 270 °C, respectively. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. A solvent delay of 4 min was applied. 1 µL aliquot of each diluted sample was injected in split mode using an AS1300 autosampler. Electron ionization (EI) was conducted at 70 eV, with mass spectra recorded in full scan mode over an m/z range of 50–650. The ion source temperature was maintained at 200 °C. Compound identification was achieved by comparing the mass spectra of the detected compounds with those in the WILEY 09 and NIST 14 mass spectral libraries, as described by Abd El-Kareem *et al*. (2016).

Effect of *T. asperellum* on root rot incidence, severity, and fruit yield under field conditions

Field trails were conducted during two successive growing seasons 2021/2022 and 2022/2023 from October to May in Al-Deir village, Qalyubia Governorate, Egypt. The soil at the experimental site was light loamy in texture and had a history of root rot, as the crop was intensively cultivated in this area. Certified seedlings of strawberry cv. 'Festival' were transplanted into experimental plots, each measuring 4 × 8 m. Each plot consisted of eight rows, spaced 50 cm apart, with a planting distance of 25 cm between plants within rows. Each row contained 32 planting holes, with one seedling per hole. Prior to transplanting, the seedlings were inoculated with *T. asperellum* by dipping them in a spore suspension (1.0×10^7 CFU/ml). *Trichoderma asperellum* used in this study was mixed with 5% CMC to improve adhesion and ensure uniform distribution on the plant roots. Dipping was performed for 30 min**,** immediately before transplanting. The roots of the control plants were soaked in water without bioagent addition. Hence there were altogether two different treatments, *i.e.*, Control (Ctr.) and *T. asperellum* (Ta.). The experiments were laid out in a completely randomized design (CRD), with three replicates (plots) each treatment. Irrigation and agricultural practices were applied following standard horticultural practices. During the growing period, Trichoderma treatment was repeated 6 times by adding subsequent applications of 50 ml of spore suspension to each plant at 15-days intervals for a total period of 3 months. The development of disease symptoms on the plants was monitored regularly, and the percentages of disease incidence were calculated 100 days post-transplanting using the following formula:

$$\text{Disease incidence of root rot (\%)} = \frac{\text{Number of root rotted plants}}{\text{Total number of plants}} \times 100$$

The disease severity was assessed using a scoring system of 0–5, modified by Morocko (2006), where level 0 signifies an entire plant in a healthy status; level 1 indicates a root disease incidence of ≤ 30%, with normal leaves; level 2 characterizes a root disease incidence greater than 30% and ≤ 60%, with normal leaves; level 3 represents a root disease incidence greater than 60% and ≤ 80%, accompanied by yellowing leaves; level 4 indicates a root disease incidence exceeding 80%, leading to leaf wilting; and level 5 signifies complete plant mortality. The disease index and control efficiency were calculated as follows:

$$\text{Disease index} = \frac{\Sigma\,(\text{disease level} \times \text{number of plants at that level})}{(\text{total number of plants} \times \text{highest disease level}} \times 100$$

$$\text{Control efficiency} = \frac{(\text{control disease index} - \text{treatment disease index})}{\text{control disease index}} \times 100$$

The accumulated strawberry fruit yield (ton/feddan) was recorded during the period from 15 December to 15 January for each treatment. Surviving plants were counted, carefully uprooted, and subsequently used for further biochemical analysis.

Biochemical analysis

Representative samples of juvenile fully expanded leaves (0.1g) of infected control and treated plants were collected from the plants of each treatment and homogenized with 80% acetone at the end of the experiment. Then, the homogenates were filtered, and the contents of total chlorophyll pigments were measured spectrophotometrically in the filtrates according to Nornai (1982). Leaf total protein was determined according to Bradford (1976). Egg albumin was prepared in a diluted series and used as a standard to illustrate the calibration curve. The contents of protein were expressed as mg/g dw. The content of reduced, non-reduced and total sugars in the leaves was spectrophotometrically measured according to Nelson (1944), and were expressed as milligram glucose per gram dry weight (mg/g dw). Free and conjugated phenols were determined according to Simons and Ross (1971), and calculated as milligrams of gallic acid per one-gram dry weight (mg GA/g dw) according to standard curve of gallic acid.

Statistical analysis:

All data sets were analyzed by variance analysis (ANOVA) using SAS software. The differences between mean values were assessed using the least significant difference (LSD) test at $P \leq 0.05$.

RESULTS

Isolation and frequency of the associated root rot pathogenic fungi

The surveyed infected plants exhibited distinctly reduced growth, vigor and eventual collapse (Fig. 1 A and B). Their affected roots showed extensive black lesions and varying degrees of discoloration. In severe cases, the entire root system was significantly reduced, lacking cortical tissue, or completely absent, resulting in a distinctive "rat-tail" appearance (Fig. 1B). Additionally, typical symptoms such as vascular discoloration of the crown, tissue browning, and darkening were obvious in plants exhibiting advanced dieback (Fig. 1 C and D). Pathogenic soilborne fungi associated with root rot diseases were isolated from the symptomatic parts. The isolated and identified fungi were *F. solani*, *R. solani*, and *M. phaseolina*. As a general trend, the occurrence of these fungal pathogens peaked during warmer months of the growing season (September - April) in all surveyed cultivars and fields (Fig. 2). Data in Figure (2) showed clearly that Festival cultivar was the most sensitive, with isolation frequencies reached 20% for *F. solani*, 35% for *R. solani*, and 40% for *M. phaseolina*. In cultivar Fortuna, the corresponding frequencies were 20, 25, and 35%, respectively. The lowest disease incidence was observed in Sensation cultivar, with isolation frequency rate of 10% for *F. solani*, 15% for *R. solani*, and 20% for *M. phaseolina*. Overall, *M. phaseolina* was the most prevalent pathogens associated with root rot in all screened strawberry cultivars, followed by *R. solani* and *F. solani* (Fig. 2).

Pathogenicity tests

The pathogenicity of each purified fungi was evaluated on strawberry cultivars Festival, Fortuna, and Sensation under controlled greenhouse conditions. All isolated fungi were pathogenic, leading to characteristic root rot symptoms, reduced plant survival rates, and fulfilling Koch's postulates. The most severe disease symptoms appeared seven to eight weeks after transplanting. As shown in Table (1), *M. phaseolina* caused the highest root rot disease incidence, being 30, 28, and 22% in Festival, Fortuna, and Sensation, respectively. This was followed by *R. solani*, which caused disease incidences of 25, 27, and 23% in Festival, Fortuna, and Sensation, respectively. *F. solani* exhibited, however,

the lowest pathogenicity, with incidence rates averaged 20, 23, and 15% in Festival, Fortuna, and Sensation, respectively. In general, the cultivar Festival exhibited consistently the highest susceptibility to all fungal isolates.

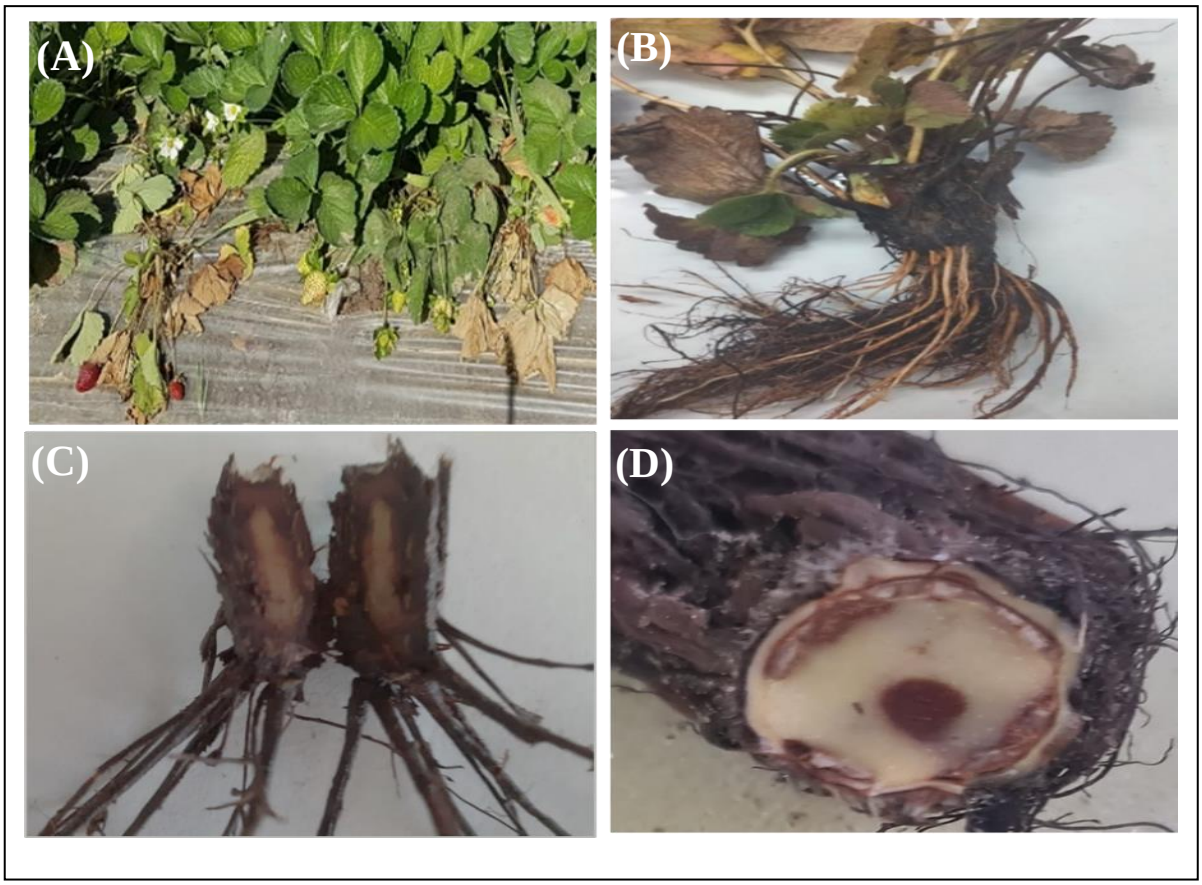

Fig. 1. Visual symptoms of natural infections with root rot disease in a commercial strawberry field survey in Ismailia Governorate, Egypt. (A): Typical wilt/death symptoms of strawberry plant, (B): root discoloration and "rat tail" appearance on strawberry plants, (C): Internal rotting in infected crown tissue, (D): Advanced stages of the disease show soft rot or decay with in the crown.

Table 1. Pathogenicity test of the isolated root rot pathogenic fungi on three different strawberry cultivars under greenhouse conditions.

Pathogens	Root rot incidence (%)		
	Festival	Fortuna	Sensation
Control	0.00 ^{c} ±0.00	00.0 ^{c} ±0.00	00.0 ^{c} ±0.00
M. phaseolina	30.00 ^{a} ±5.00	28.33 ^{a} ±2.89	21.67 ^{a} ±2.89
R. solani	25.00 ^{a} ±5.00	26.67 ^{a} ±2.89	23.33 ^{a} ±5.77
F. solani	20.0 ^{b} ±5.00	23.33 ^{b} ±2.89	15.00 ^{b} ±5.00

Each value represents the mean of twenty replicates ± standard errors. Significant differences ($P \leq 0.05$) between treatments are indicated by different letters, LSD test.

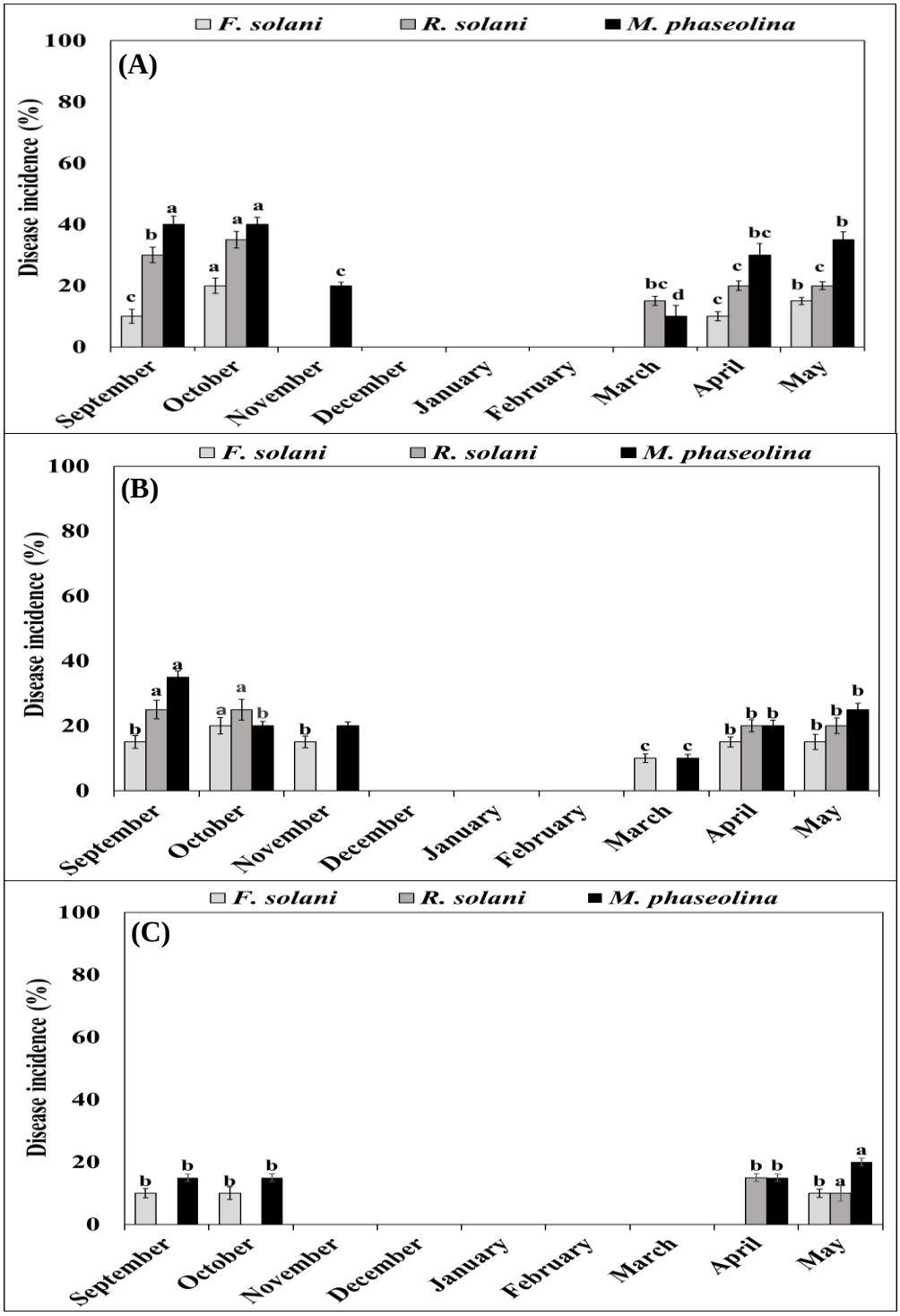

Fig. 2. Occurrence of the causal agents of root rot diseases *F. solani*, *R. solani*, and *M. phaseolina* in different strawberry cultivars: *Fragaria* × *ananassa* cv. Festival (A), Fortuna (B), and Sensation (C) during the growing season 2020/2021 in Ismailia Governorate. Each bar represe nts the mean value of 100 samples, with error bars indicating standard errors. Columns sharing the same letter are not significantly different at $P \leq 0.05$**,** LSD test.

Evaluation of antagonistic effects of different *Trichoderma* spp. on root rot pathogenic fungi

In vitro antagonistic activities of three *Trichoderma* species (*T. harzianum*, *T. hamatum*, and *T. asperellum*) were examined against the three pathogenic fungi: *M. phaseolina*, *F. solani*, and *R. solani*, which were proved to cause root rot in strawberry plants. Data presented in Table (2) and Figure (3) reveal that *Trichoderma* species remarkably reduced the mycelial growth of the pathogenic fungi compared to the control treatment. Additionally, significant variations in the antagonistic activities among the tested *Trichoderma* species against the causal pathogens were observed. Among the tested isolates, *T. asperellum* exhibited the highest antagonistic potential, achieving inhibition percentages of 54%, 52%, and 50% against *M. phaseolina*, *R. solani*, and *F. solani*, respectively (Table 2, Fig. 3). Both *T. harzianum* and *T. hamatum* showed comparable lower inhibitory effects on the linear growth of *M. phaseolina*, *R. solani*, and *F. solani* (Table 2, Fig. 3).

Table 2. Antagonistic effect of different *Trichoderma* spp. on growth of root rot fungal pathogens*: F. solani; R. solani;* and *M. phaseolina* evaluated using a dual culture assay, 7 days of *in vitro* incubation

Pathogenic fungi	Reduction in the linear mycelial growth (%)		
	T. harzianum	***T. hamatum***	***T. asperellum***
Control	0.00 [c] ±0.00	00.0 [c] ±0.00	0.00 [d] ±0.00
M. phaseolina	50.8 [a] ±0.69	50.6 [a] ±0.00	54.3 [a] ±1.19
R. solani	51.2 [a] ±1.04	50.6 [a] ±0.60	52.4 [b] ±0.60
F. solani	46.77 [b] ±0.59	47.13 [b] ±0.06	50.4 [c] ±0.53

Each value represents the mean of twenty replicates ± standard errors. Significant differences ($P \leq 0.05$) between treatments are indicated by different letters, LSD test.

Identification of potentially bioactive compounds of *T. asperellum* extract

GC-MS analysis of the ethyl acetate extracts of *T. asperellum* revealed the presence of eleven distinct bioactive compounds as summarized in Table (3). These compounds were identified based on their retention time and mass spectrum. The identified metabolites include various classes of chemicals such as fatty acid esters, phenols, alkenes, and aromatic compounds. Among the detected compounds, six have antifungal, and antibacterial literature background. These include: 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl); 1-Hexadecanol; 1-Nonadecene; 1-Docosene; and 1-Hexadecanol, 2-methyl. Additionally, four compounds demonstrated antioxidant activity: 2,2-Dideutero Octadecanal; 9,12-Octadecadienoic acid (Z, Z); 9-Octadecenoic acid (Z)-methyl ester; 1-Docosene**,**1-Hexadecanol, 2-methyl**.**

Effect of *T. asperellum* on strawberry root rot incidence, severity, fruit yield and some biochemical parameters under field conditions

Under control conditions, root rot incidence averaged 38%, with disease severity reached 75% in non-treated strawberry plants (Table 4). *Trichoderma asperellum* treatment substantially lowered the incidence and severity of root rot by 84 and 72%, respectively, compared to the controls (Table 4). As shown in Table (4),

Table 3. Screening of potentially bioactive compounds of *T. asperellum* extract using GC-MS

Sr No.	Name of compound	Molecular formula	Molecular weight	RT (min.)	Peak area %	Specific role
1	1-Methoxy-2-propyl acetate	$C_6H_{12}O_3$	132	3.42	67.01	Solvent
2	2,5-Cyclohexadiene-1,4-dione, 2,6-bis (1,1-dimethyl ethyl)	$C_{14}H_{20}O_2$	220	16.10	0.8	Antifungal
3	1-Hexadecanol	$C_{16}H_{34}O$	242	18.60	4.33	Antifungal
4	1-Nonadecene	$C_{19}H_{38}$	266	22.32	7.12	Antibacterial, Antifungal
5	Tert- Hexadecanethiol	$C_{16}H_{34}S$	258	22.43	0.47	Antioxidant, Antibacterial
6	Phthalic acid, butyl tetra decyl ester	$C_{26}H_{42}O_4$	418	23.72	1.27	Antioxidant
7	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	$C_{22}H_{34}O_4$	362	25.32	1.91	Antimicrobial
8	1-Docosene	$C_{22}H_{44}$	308	25.70	5.80	Antifungal
9	2,2-Dideutero octadecanal	$C_{18}H_{34}D_2O$	270	27.22	0.41	Antioxidant
10	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	294	27.33	8.68	Antioxidant
11	9-octadecenoic acid (z)-, methyl ester	$C_{19}H_{36}O_2$	296	27.42	4.74	Antioxidant
12	1-Docosene, 1-hexadecanol, 2- methyl	$C_{17}H_{36}O$	256	31.63	2.31	Antimicrobial

Table 4. Effect of *T. asperellum* treatment on root rot incidence, severity and total yield in strawberry plant under field conditions

Treatment	Disease incidence (%)	Disease severity (%)	Yield (Ton/ feddan)
Ctr.	38.0 [a] ± 5.2	75.0 [a] ± 3.0	2.5 [b] ± 2.6
T. asperellum	6.0 [b] ± 0.4	21.0 [b] ± 2.5	3.9 [a] ± 1.2

Means ± standard errors within a column followed by the same letter are not significantly ($P \leq 0.05$) different according to LSD test.

T. asperellum treatment positively affected the yield of strawberry plants, resulting in 56% increase in yield compared to the control. *Trichoderma asperellum* treatment enhanced the leaf total chlorophyll and protein contents by 93 and 89%, respectively, compared to the untreated controls (Fig. 4 A and B). Although *T. asperellum* significantly decreased leaf content of reduced sugars by 13%, it led to increase the non-reducing sugars by about 400% and total sugars by 42% (Fig. 4 C). Furthermore, *T. asperellum* significantly altered the leaf phenolic compounds, leading to 156% and 57% increases in the free and total phenolic compounds, respectively, compared to the untreated controls (Fig. 4 D).

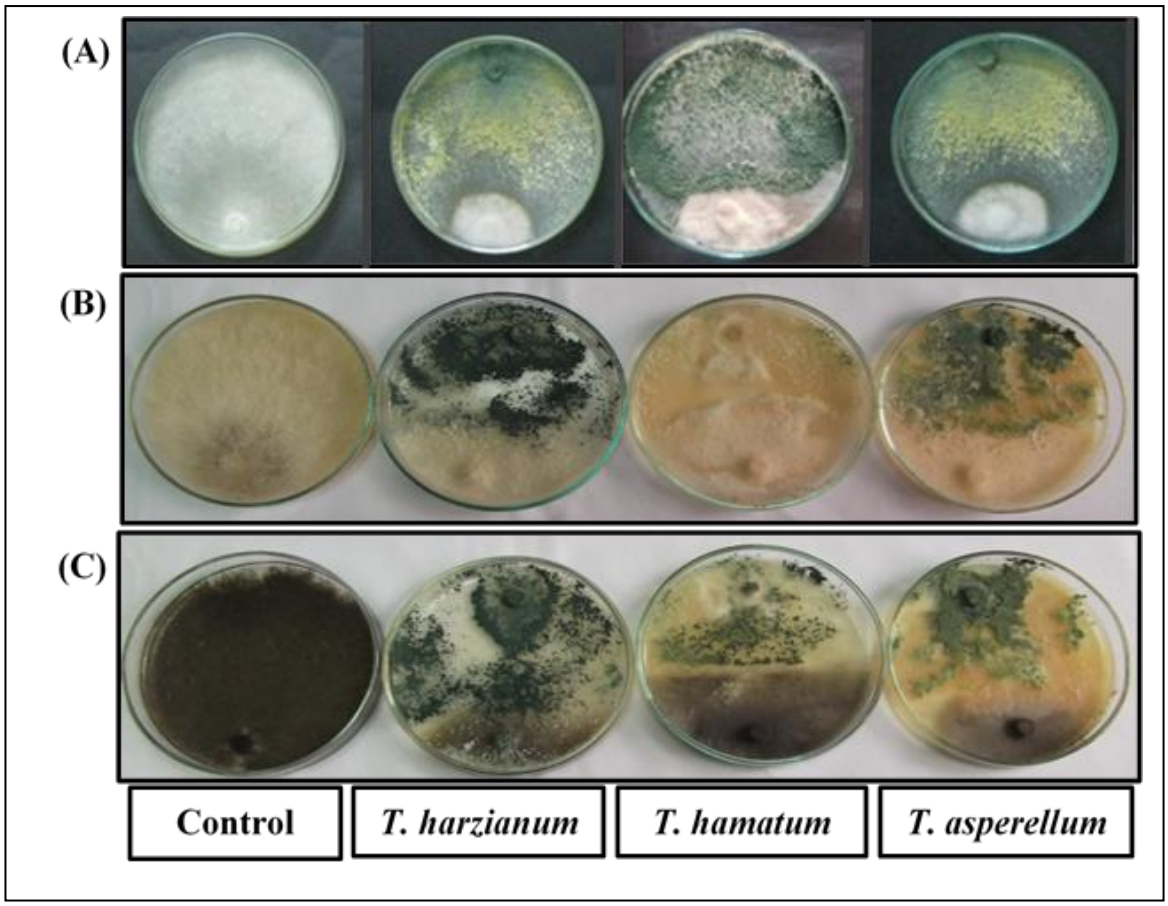

Fig. 3. Antagonistic activity of three different *Trichoderma* sp. against soil-borne pathogen causing strawberry root rot. (A) *F. solani*; (B) *R. solan*i and (C) *M. phaseolina* evaluated using a dual culture assay, 7 days of *in vitro* incubation.

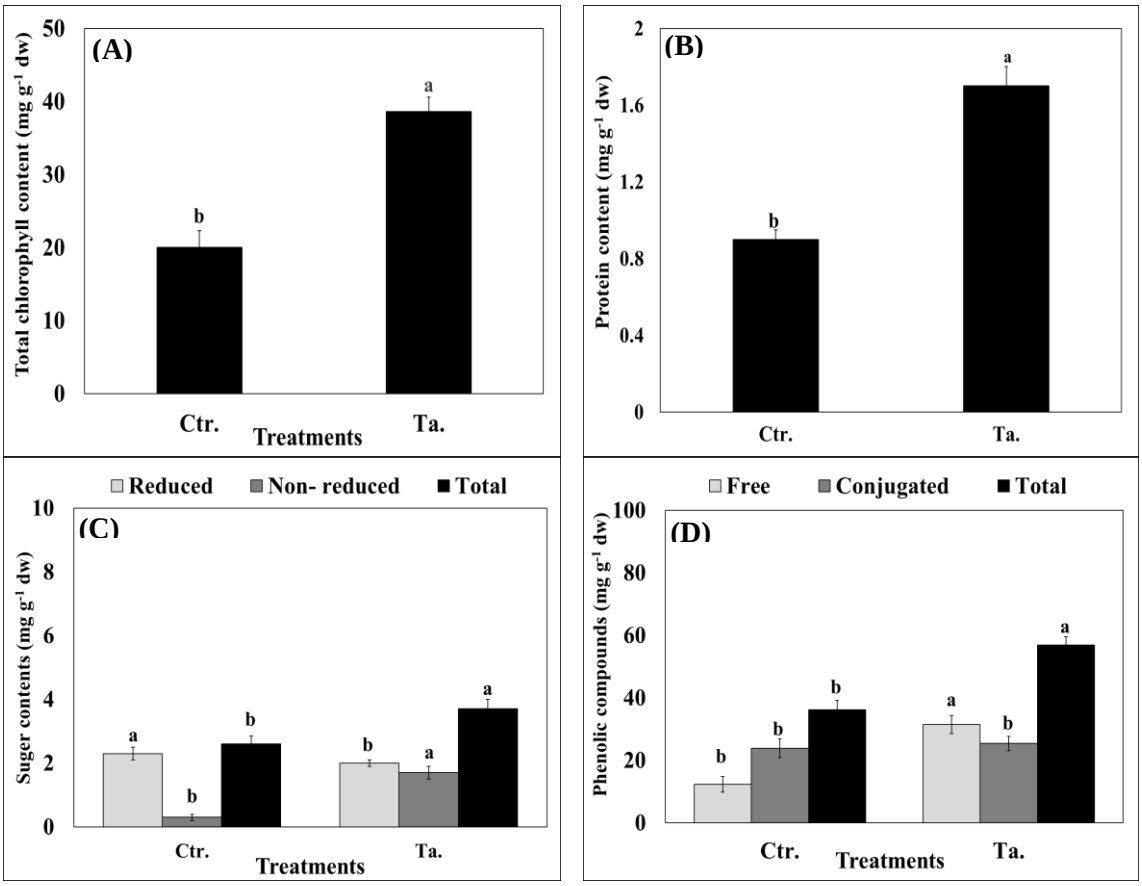

Fig. 4. Effect of *T. asperellum* on total chlorophyll contents **(**A); total protein contents (B); phenolic compounds (C) and total sugar contents (D). Each bar represents the mean value of ten collected samples, with error bars indicating standard errors. Columns sharing the same letter are not significantly different at $P \leq 0.05$**,** LSD test

DISCUSSION

Strawberry root rot is considered one of the most destructive diseases limiting its sustainable and profitable production worldwide (Ceja-Torres *et al*., 2014). Based on the morphological identification, *M. phaseolina*, *R. solani* and *F. solani* were isolated pathogens associated with crown and root rot of strawberry. Among these pathogens, *M. phaseolina* was the most frequently isolated. This finding is supported by recent research (Baggio *et al*., 2021), which identified *M. phaseolina* as a primary causal agent of crown and root rot of strawberries. The pathogens colonize the vascular tissues of the crown, leading to characteristic reddish-brown discoloration, wilting of older leaves and ultimately plant death (Mertely *et al*., 2005; Avilés *et al*., 2008). Abd-El-Kareem *et al*. (2019) documented the widespread presence of *Fusarium* and *Rhizoctonia* sp. in the Egyptian agricultural soils, particularly in the Nile Delta and reclaimed lands. Field survey results demonstrated that crown and root rot of strawberry exhibited clear seasonal trends, with significantly higher incidence observed in September, October, April, and May across all evaluated months. This fluctuation appeared closely tied to environmental factors, particularly high air and soil temperatures and relative humidity. Notably, soil temperature during the initial growth phase (September–October) was especially influential, suggesting that pathogen infection and establishment occur shortly after cultivation. *Macrophomina phaseolina* thrives in warm, dry, arid to semi-arid subtropical regions and has been

reported in key strawberry-growing areas, including Florida, Israel, and Spain (Mertely *et al*., 2005; Avilés *et al*., 2008). *Rhizoctonia solani* was frequently isolated from the roots of symptomatic strawberry plants and is known to became more aggressive in summer, as higher soil temperatures favor increased pathogen activity (Manici *et al*., 2005). Similarly, *Fusarium* species demonstrate enhanced mycelial growth under warm conditions. Their adaptability to both high temperature and rainfall likely contributes to their widespread distribution and infection potential (Hassan and Chang, 2022). It has been observed that disease severity consistently intensified later in the production season, particularly in April and May. High temperatures during this period may accelerate fruit maturation and exacerbate stress, thereby promoting disease progression (Koike *et al*., 2016).

Under field conditions, the incidence of pathogenic fungi varied among the tested cultivars. The cultivars 'Festival' and 'Fortuna' exhibited higher susceptibility. These findings corroborate previous studies: Sánchez *et al*. (2016) reported over 40% mortality in 'Festival' plants inoculated with *M. phaseolina*. Mertely *et al*. (2005) observed 100% mortality under similar conditions in Florida. Baggio *et al*. (2021) classified 'Festival' as a highly susceptible cultivar. The cultivar Fortuna was identified as moderately susceptible, which was in contrast to a previous study that characterized 'Fortuna' as highly susceptible to *M. phasolina*, with high mortality rates (100% in California and 60% in Chile) (Koike *et al*., 2016). In contrast, the cultivar 'Sensation' exhibited relatively low susceptibility, with disease incidence remained lower than 50% for all isolated causal agents. These findings underscore significant variability in cultivar responses to root rot pathogens, with 'Festival' exhibited consistently the highest levels of disease incidence in both roots and crowns.

In pathogenicity assays, all identified fungal pathogens were confirmed to be pathogenic, as inoculated plants developed typical crown and root rot symptoms. However, the pathogens exhibited varying degrees of aggressiveness, expressed as disease incidence. The highest virulence was recorded for *M. phaseolina*, followed by *R. solani* and *F. solani*. The observed variation in severity of infection among different fungal isolates might be influenced by the diversity of root exudates produced by different plant cultivars. These exudates can either stimulate or inhibit the activity of rhizospheric microorganisms, including saprophytes. As a result, they may encourage the proliferation of beneficial saprophytes that suppress pathogens or, conversely, reduce these protective populations, creating favorable conditions for pathogenic fungi to infect healthy roots and cause disease (Sun *et al*., 2023).

The dual culture test revealed the antagonistic potential of *T. asperellum*, which effectively inhibited the growth of *M. phaseolina*, *R. solani*, and *F. solani*. The strongest inhibition was observed against *M. phaseolina*, suggesting a particularly pronounced antagonistic effect toward this pathogen. These results align with previous studies showing that *T. asperellum* exhibits strong antifungal activity against diverse soil-borne pathogens through multiple mechanisms, including competition, antibiosis, and mycoparasitism. The fungus produces mycolytic enzymes, such as chitinase and β-1,3-glucanase, which degrade the cell walls of pathogenic fungi. Notably, these enzymes are overexpressed during pathogen interactions, emphasizing their crucial role in the biological control activity of *T. asperellum* (Benítez *et al.* 2004; Sharma *et al.* 2011; Win *et al.* 2021). Similarly, Liu *et al*. (2024) reported that *T.* asperellum strain CMT10 showed strong antagonistic activity against *Neopestalotiopsis clavispora* CMGF3 via competition, antimicrobial metabolite production, and hyperparasitism *in vitro*.

Several previous reports demonstrated that *Trichoderma* species are prolific producers of diverse secondary metabolites with antifungal, antibacterial, and cytotoxic properties and can boost plant development, making plants more resistant to a wide range

of phytopathogens (Khan *et al*., 2020; Zhang *et al*. 2021). Consistent with earlier findings (Sehim *et al*., 2023; Elshahawy and Marrez, 2023), GC–MS analysis of the *T. asperellum* crude extract in the present study identified 11 bioactive compounds reported to possess antimicrobial or toxic activities against various phytopathogens. Among these, several fatty acids and their derivatives such 1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester and 1-Hexadecanol, 9-Octadecenoic acid, methyl ester and 9,12,15-Octadecatrienoic acid methyl ester, (Z, Z) are well known for their antifungal activity against important plant pathogenic fungi (Walters *et al*., 2004; Liu *et al*., 2008). Their broad-spectrum efficacy, notably against *Fusarium* spp., *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*, has been well documented (Elad *et al*., 1983; Gajera *et al*., 2020). Additionally, sugar alcohol such as 2,5-Cyclohexadiene-1,4-dione, 2,6-bis (1,1-dimethyl ethyl) (myo-inositol) was also detected. In addition to its antifungal properties, myo-inositol is suggested to play crucial role in providing the energy required for the defenses mechanism and serves as signals for regulating defense genes in plant-microbe interactions (Bolton, 2009). It further plays essential roles in stress responses, development, and numerous physiological processes (Valluru and Van den Ende, 2011). These bioactive metabolites collectively may contribute to the suppression of soilborne pathogens, protecting strawberry plants from root rot disease.

In the present work, *Trichoderma asperellum* treatment significantly decreased the incidence and severity of root and crown rot of strawberry cultivars "Festival" under field conditions. The ability of *T. asperellum* to mitigate disease caused by *Pythium sp.* and *Fusarium* spp. has been consistently confirmed across several studies (Li *et al*., 2018; Win *et al*., 2021; Intana *et al*., 2022; Elshahawy and Marrez, 2023). This biocontrol efficacy is attributed to several synergistic mechanisms, including competition for nutrients and space, antibiosis, mycoparasitism, cell wall degradation, and effective rhizosphere colonization. Yukun *et al*. (2023) demonstrated that *T. asperellum* strain 576 effectively inhibits *Exserohilum turcicum* 101 via volatile and non-volatile metabolites and cell wall–degrading enzymes. Likewise, Vechithran *et al*. (2025) reported that *T. asperellum* (PP256386) suppresses *Pythium aphanidermatum* through extracellular enzymes as well as volatile and non-volatile metabolites.

The application of *T. asperellum* significantly improved total yield, consistent with the findings of Cherif *et al*. (2007) and Prlak and Kose (2009), who also reported yield improvements following *Trichoderma* treatment. In the present study, this yield increase was accompanied by notable biochemical changes in plant tissues, including elevated levels of chlorophyll, proteins, total phenols, and total sugars. Similarly, Segarra *et al*. (2007) demonstrated that *T. asperellum* T34 modulates protein expression in cucumber, particularly those involved in energy metabolism and stress defense. In addition, *T. afroharzianum* T22 has been shown to enhance chlorophyll biosynthesis and photosynthetic efficiency in soybean (Kabir *et al*., 2022). Moreover, *Trichoderma* enhances carbohydrate metabolism and photosynthesis in maize, thereby increasing the plant's energy and carbon resources (Harman *et al*., 2004; Shoresh & Harman, 2008). In tomato plants, the application of *T. asperelloides* Ta41 significantly increased chlorophyll and total phenol contents under *R. solani* infection (Heflish *et al*., 2021).

CONCLUSION

In conclusion, the present study confirmed that *T. asperellum* exhibits strong antifungal activity against *M. phaseolina*, *R. solani* and *F. solani*. It justified the potential of *T. asperellum* as a promising biocontrol agent for integrated management programs against strawberry root rot. However, it should be mentioned that *T. asperellum* is

not only a promising biocontrol agent for root rot disease management in strawberry, but also through deep understanding of its physiological and molecular interactions with phytopathogens, would provide a possible route to enhance disease resistance in other phyto-pathosystems.

COMPETING INTERESTS

The authors declare that they have no competing interests. The contents of the manuscript have neither been published nor under consideration for publication elsewhere.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author (s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

STATEMENT AND ETHICS DECLARATIONS

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article. Ethical approval was not required for this study.

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