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Trichoderma afroharzianum B3R12: a potent biocontrol agent against *Stromatinia cepivora*, the causal agent of onion white rot

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Abstract

Stromatinia cepivora (Berk.) Whetzel, the causal agent of onion white rot, is a devastating disease globally affecting onion crop production, leading to considerable losses. Control of *S. cepivora* is difficult due to its sclerotial high survival rate that can extend for decades in the soil. In this study, forty-nine *Trichoderma* species were isolated from the rhizosphere and tissues of 25 plant species collected from different sites across four Egyptian governorates. The antagonistic activity of all isolated *Trichoderma* strains was screened against *S. cepivora* BYAN1 *in vitro*. Microscopic examination showed that isolate B3R12 (identified as *T. afroharzianum* B3R12) has the greatest mycoparasitic level. Moreover, this isolate showed high *in vitro* inhibitory effect on *S. cepivora* BYAN1 growth by the production of both volatile and nonvolatile antifungal metabolites, recording inhibition of 74.32 % and 71.68 %, respectively. In the greenhouse experiment, *T. afroharzianum* B3R12 culture filtrate led to complete reduction in disease severity in the pretreated onion plants. In addition, pretreating onion plants with *T. afroharzianum* B3R12 enhanced several plant growth parameters and photosynthesis pigments, and increased total protein, carbohydrates, phenols, and flavonoid contents. Our results suggested that *T. afroharzianum* B3R12 represents a promising bioagent for biocontrol of onion white rot and promoting the plant growth; however, field evaluation in the future studies is necessary before the final recommendation.

Keywords: Antifungal, Bioactive compounds, Onion, *Sclerotium cepivorum*, *Stromatinia cepivora*, White rot, *Trichoderma* spp.

1. Introduction

Stromatinia cepivora (Berk.) Whetzel (Anamorph *Sclerotium cepivorum* Berk.) is an economically important soil borne ascomycetes pathogen, which causes white rot of onion (*Allium cepa*) and several other *Allium* species such as garlic and leeks. Its destructive threat limits the onion production worldwide and leads to significant yield losses up to 50 % (Thineshkumar *et al*., 2022). This fungus grows as a white sterile mycelium, but in the absence of its host, it remains in the soil as compact masses of small (200-500 µm) spherical, black-hardened mycelia; named as sclerotia. These sclerotia may survive in the soil for up to 20 years and are the primary source of infection. Volatile organic compounds emitted from onion root are essential for sclerotial growth activation. After germination, the hyphae spread through the soil until they reach the host plant, where they invade the roots and destroy the host radicular system (Lourenço *et al*., 2018). This fungus can infect the plant at any growth stage causing some distinct symptoms such as leaf yellowing, wilting, root white molding, and death. By the time these symptoms appear, the pathogen has already spread into the roots, bulb, and leaves. White fungal growth, which indicates root deterioration, is frequently seen at the soil line. Once this pathogen is established, it is almost impossible to eradicate it (Thineshkumar *et al*., 2022). Resistant plant varieties are the best strategy used for managing this disease. Several chemical fungicides have been used to control this disease; however, their effectiveness become low, due to fungal resistance that has developed as a result of improper application of these fungicides, in addition to their un-safety, as they cause certain health deleterious effects on both humans and animals.

Biological fungicides represent good alternatives to control many plant diseases, and many biological control agents have been studied to manage the current pathogen due to their safety, sustainability, and being eco-friendly (Rashad *et al*., 2020b; 2022a, b). In a recent study, Amin and Ahmed, (2023) reported a white rot severity significant reduction (78.6 %) in onion plants treated with *Bacillus subtilis* isolate 2 under field conditions. In addition, this biocontrol agent showed strong inhibitory activity (75.8 %) against growth of *S. cepivora in vitro*. *Trichoderma* sp. represents one of the most widespread fungi used in biocontrol of various plant diseases, where more than 50 % of the bio-control products worldwide are based on *Trichoderma* spp. (Rashad and Moussa, 2019). Moreover, Madbouly *et al*., (2023) reported a high potential of *T. harzianum* AYM3 on biodegradation of Aflatoxin B1 produced by the toxigenic fungus *Aspergillus flavus* infecting maize grains. *In vitro*, *T. harzianum* AYM3 showed an inhibition activity against *A. flavus* growth and its related Aflatoxin B1 biosynthesis genes. *Trichoderma* spp. antagonistic mechanisms have been extensively studied for decades. They include space and nutrients competition, antibiosis through the production of antifungal volatile and non-volatile metabolites (El-Sharkawy *et al*., 2018), lytic enzymes, and mycoparasitism (Asad, 2022). Moreover, these beneficial fungi are known to promote the plant growth and trigger its defense responses *via* jasmonates (JA) and ethylene (ET)-dependent pathways (Ram *et al*., 2020; Rodríguez-Hernández *et al*., 2023). Rivera-Méndez *et al*., (2020) reported an inhibition by 90 % in growth of *S. cepivora* using *T. asperellum in vitro*. In addition, this strain led to a significant reduction in onion white rot under greenhouse and field conditions. The objective of this study was to *in vitro* investigate the antagonistic mechanisms of *Trichoderma* sp. against *S. cepivora*, it's *in vivo* biocontrol potential against onion white rot under greenhouse conditions, plant growth promotion, and resistance responses.

Material and methods

2.1. Samples collection

Rhizosphere soil samples and wild plants were collected from various sites across four Egyptian governorates; namely Port Said, Damietta, Al-Dakahlia, and Alexandria (Table 1). For soil sampling, around 250 g of rhizosphere soil were collected from each location at a depth of 20 cm, placed in clean paper bags, and tagged in the field. For plant samples, whole plants were uprooted and placed in clean plastic bags, labeled in the field, and then all the collected samples were brought to the lab. and were preserved at 4 °C until needed.

2.2. Isolation and identification of the fungal isolates

Trichoderma spp. were isolated from the collected plants and their related rhizosphere soil. For isolation from wild plants, each plant was washed with tap water and its parts (*i.e*., roots, stems, and leaves) were cut into small pieces of 0.5 cm using a sterile scalpel, surface sterilized with 5 % sodium hypochlorite solution for 3 min., dried on sterile filter papers, and aseptically placed individually on plates of *Trichoderma* selective medium (TSM) (composed of: 0.2 g $MgSO_4$, 0.9 g K_2HPO_4, 0.15 g KC1, 1.0 g NH_4NO_3, 3.0 g glucose, 0.25 g chloramphenicol, 0.3 g di-methyl-amino-benzene-diazo sodium sulfonate, 0.2 g penta-chloro-nitro-benzene, and 20 g agar, dissolved in 1l of distilled water) (Askew and Laing, 1993). For isolation from soil, 1 g from each soil sample was utilized to make serial dilutions (10^{-1} to 10^{-6}). For each dilution, 1 ml was aseptically spread using a sterile glass spreader on TSM plate amended with Rose Bengal (0.15 g/ l). For each sample three replicate plates were applied. The plates were then incubated at 28 °C for 72 h. After incubation, the grown *Trichoderma* hyphal tips were picked, purified on potato dextrose agar (PDA) plates using a single spore technique, and incubated at 28 °C for 72 h. All *Trichoderma* isolates were kept on PDA slants at 4 °C until further use. Identification of the selected isolate was performed based on its phenotypical characterization such as its cultural and microscopic characteristics according to Gams and Bissett, (2002); Jaklitsch and Voglmayr, (2015).

For isolation of the fungal pathogen, onion plant samples exhibiting typical white rot symptoms were collected from several Egyptian onion fields (Al-Dakahlia, Tanta, and Assiut), and then properly washed with tap water. The separated roots of each sample were cut into 0.5 cm small pieces, surface sterilized using 5 % sodium hypochlorite solution, dried on filter papers, placed on the surface of PDA media plates, and incubated at 18 ± 2 °C. After 7 d of incubation, the grown fungi were purified on new PDA plates using the hyphal tip technique and incubated at 18 ± 2 °C for 7 d. All fungal isolates were kept on PDA slants at 4 °C until needed. The isolated fungi were phenotypically identified based on their cultural and microscopic characteristics according to Mordue, (1976).

2.3. Screening the isolated *Trichoderma* spp. for their *in vitro* antagonistic activity against *S. cepivora* BYAN1

2.3.1. Dual culture test

To assess the antagonistic potential of *Trichoderma* spp. against *S. cepivora*, a 6-mm diameter disc cut using a sterile cork borer from actively growing culture of the tested *Trichoderma* spp. and from *S. cepivora* BYAN1were oppositely placed at 2 cm apart from the edge of a PDA plate. PDA plates inoculated with the pathogen disc only were used as control treatment and three replicate plates were used for each treatment (Oldenburg *et al*., 1996). All plates were incubated at 18 ± 2 °C for 10 d and then inhibition diameter (mm) in radial growth of the pathogen was measured using a calibrated ruler after 3, 7, and 10 d of incubation. The experiment ended when the control plate of *Trichoderma* reached its full growth. The growth inhibition (GI %) was calculated using the following equation (Phu *et al*., 2023):

$$\text{GI}\ (\%) = \frac{R1 - R2}{R1} \times 100$$

Where R1 is the radial growth of *S. cepivora* BYAN1 in the control plate and R2 is the radial growth of *S. cepivora* BYAN1 in the dual culture plate

Table 1: Location of plant and rhizosphere samples collected for isolation of *Trichoderma* spp. in this study

Isolate no.	Location	Isolation part	Plant name
B2R28, B2R13	Borg Al Arab Al Gadida City,	Rhizosphere	*Thymelaea hirsute* (L.) Endl.
B2R29, B2R30	Alexandria	Rhizosphere	*Onopordum alexandrinum* (L.)
B2R11		Rhizosphere	*Echinops spinosissimus* Turra 1765 not Freyn 1895
B2R10		Rhizosphere	*Fagonia arabica* (L.)
B2R33		Rhizosphere	*Anabasis articulata* (Forssk.) Moq.
B2R16		Rhizosphere	*Atractylis cancellata* L.
B2R17	Bahig, Borg El Arab,	Rhizosphere	*Thymus serpyllum* L.
B2Pr26 B2R27	Alexandria	Rhizosphere	*Arthrocnemum macrostachyum* (Moric.) Piirainen & G. Kadereit
B2R32, B2Pr31		Rhizosphere, Plant root	*Suaeda pruinosa Lange*
B2R14, B2R15		Rhizosphere	*Limoniastrum monoppetalum* (L.) Boiss.
B2R18, B2R19		Rhizosphere	*Salicornia europaea* (L.)
B1R3	New Damietta city, Damietta	Rhizosphere	*Tetraena alba (L.f.) Beier & Thulin*
B1R1, B1R2		Rhizosphere	*Salicornia europaea* (L.)
B1R5, B1Pr9		Rhizosphere, Plant root	*Mesembryanthemum crystallinum* L.
B1R6		Rhizosphere	*Juncus rigidus* Desf.
B1R7, B1R8		Rhizosphere	*Mesembryanthemum nodiflorum* L.
B1R4		Rhizosphere	*Diplotaxis harra* (Forssk.) Boiss.
B1R21		Rhizosphere	*Tetraena alba* (L.f.) Beier & Thulin
B1R22	Belqas, Dakahlia	Rhizosphere	*Salicornia europaea* L.
B3R20	Al Daqahleya Desert, Al-Dakahlia	Rhizosphere	*Mesembryanthemum crystallinum* L.
B3R34		Rhizosphere	*Zygophyllum decumbens* Delile
B3R35		Rhizosphere	*Diplotaxis acris* (Forssk.) Boiss.
B3R23		Rhizosphere	*Suaeda pruinosa Lange*
B3R36, B3Pr37, B3Pl38, B3Ps39	El Manasra, Port Said	Rhizosphere Plant root Plant leaf Plant stem	*Tetraena alba* (L.f.) Beier & Thulin
B3R40	Al Daqahleya Desert, Al-Dakahlia	Rhizosphere	*Sarcocornia fruticose* (L.) L.
B3R41	Ashtoum Elgameel, El Manasra, Port Said	Rhizosphere	*Suaeda pruinosa Lange*
B3R24, B3R42		Rhizosphere	*Atriplex portulacoides* (L.) Aellen
B3R12		Rhizosphere	*Mesembryanthemum forsskalii* L.
B3R25		Rhizosphere	*Silybum marianum* (L.) Gaertn.
B3R43, B3R44		Rhizosphere	*Mesembryanthemum nodiflorum* L.
B3R45		Rhizosphere	Polypogon monspeliensis (L.) Desf.
B3R46, B3Pr48		Rhizosphere, Plant root	*Cyperus conglomeratus* Rottb.
B3R47, B3Pr49		Rhizosphere, Plant root	*Suaeda maritima* (L.) Dumort.

2.3.2. Microscopic observation for mycoparasitism

Using slide culture technique, the isolated *Trichoderma* spp. were tested for mycoparasitism against *S. cepivora*. A 6-mm- diameter disc of each *Trichoderma* isolate was placed aseptically on a sterile glass slide coated with a thin layer of molten water gar and a 6-mm- diameter disc of *S. cepivora* BYAN1 was placed 1 cm apart from the disc of *Trichoderma* isolate (Naeimi *et al*., 2010). The dual inoculated slides were transferred individually to sterilized Petri plates containing two layers of water saturated filter papers to maintain humidity. All plates were incubated at 25 °C for 4 d. The hyphal interaction between both *Trichoderma* and *S. cepivora* BYAN1 was examined and photographed using an Optical light microscope equipped with Canon kiss4 digital camera.

2.3.3. Antifungal assay of the diffusible metabolites

Trichoderma afroharzianum B3R12 was selected based on the results of the dual culture test and microscopic observation for mycoparasitism. This isolate was cultured by placing a 6-mm-diameter disc taken from an actively growing culture on a PDA plate; where a sterile cellophane membrane was laid on the PDA medium. The plates were incubated at 25 ±1 °C for 4 d. After that, the cellophane membrane including the grown *Trichoderma* colony was aseptically removed. The plate was then inoculated using a 6 mm diameter disc taken from a newly grown culture of *S. cepivora* BYAN1. PDA plate inoculated only with *S. cepivora* BYAN1 served as control treatment. The plates were incubated at 18± 2 °C for 7 d. After incubation, d the radial growth of the pathogen colony was recorded (mm) and percent inhibition was calculated according to Küçük and Kivanç, (2003).

2.3.4. Antifungal assay of the volatile metabolites

Antifungal activity of *T. afroharzianum* B3R12 volatile metabolites was assessed using the inverted plate technique reported by Dennis and Webster, (1971). PDA plates were individually inoculated with 6 mm diameter discs taken from newly grown cultures of *T. afroharzianum* B3R12 and *S. cepivora* BYAN1. The top lid of one inoculated plate was replaced by the bottom part of the other fungus and then both plates' bottoms were sealed to each other using parafilm. PDA plates singly inoculated with each fungus were used as controls. Three plates were used for each test. All plates were incubated at 25± 1 °C until the control plate of the pathogen got full growth. Antifungal impact of the volatile metabolites was estimated by calculating the inhibition (%) in radial growth of pathogen colony compared to the control plate.

2.4. The greenhouse experiment

Plastic pots (35 cm in diameter) were utilized, each containing with 2 kg of sterilized soil (sand/clay ratio 3:1). Each pot was seeded with five onion bulbs (*cv*. Giza 20, 2 months old) that were carefully selected to be of the same size, length, and appeared to be healthy. Three *T. afroharzianum* B3R12 formulations (*i.e*., culture filtrate, cell-free filtrate, and Talc-based powder) were evaluated in this experiment against onion white rot pathogen under greenhouse conditions. For inoculum preparation, *T. afroharzianum* B3R12 was inoculated on sterilized PDB medium and incubated for 10 d at 28 ± 1°C. The spore concentration was adjusted to 3×10^9 spore/ ml using a hemocytometer. For cell-free filtrate, the culture filtrate was centrifuged at 500 rpm for 30 min. The supernatant was collected and used as a cell-free filtrate. Talc-based powder formulation was prepared as described by Vidhyasekaran and Muthamilan, (1995). Ten grams of carboxymethyl cellulose were mixed with 1 kg of talc powder (pH = 7), the mixture was autoclaved for 30 min. on two consecutive days, then one kilogram of the talc mixture was mixed with 400 ml of *T. afroharzianum* B3R12 inoculum (3×10^9 spore/ ml) under sterile conditions, and dried under shade to get the moisture content ≤ 20 %. The three formulations were applied as a bulb coating by

immersing the bulbs in each treatment for 1 h. For pathogen inoculum preparation, 250 g of autoclaved sorghum grains were inoculated with 6 mm diameter discs taken from a newly grown culture of *S. cepivora* BYAN1 and incubated at 18 ± 1 °C for two weeks. For soil infestation, the pathogen inoculum was mixed with the soil at 2.5 %. The five used treatments were as follows: untreated and non-infected control (C), untreated and infected (P), *T. afroharzianum* B3R12 culture filtrate and infected (TF + P), *T. afroharzianum* B3R12 cell-free filtrate and infected (TC + P), and *T. afroharzianum* B3R12 Talc-based powder and infected (TL + P). For each treatment, three replicates were used. All pots were kept under the greenhouse conditions at 22/ 18 °C Day/night temperature, 80 % relative humidity, and 12/12 h light/dark photoperiod for 2 months. The pots were arranged in a completely randomized design. For the first irrigation, the pots were allowed to drain until field capacity, and were regularly irrigated as needed to keep the soil moisture ≈ 40 %. No fertilization practices were applied. Six weeks after inoculation, three plants from each treatment were carefully uprooted, washed under running water to remove soil particles, and evaluated for disease severity and different growth parameters as follows:

2.4.1. Disease severity and incidence

Disease severity was rated on a 6 degrees scale according to bulbs' health and rotting percentages (Tian and Bertolini, 1995). 0 = healthy bulbs; 1 = mycelia appeared but no rotting; 2 = 1–25 % rotting; 3 = 25–50 % rotting; 4 = 50–75 % rotting, and 5 = 75–100 % rotting. The disease incidence (DI %) was calculated using the following equation (Morón-Ríos *et al*., 2017):

$$\text{DI\%} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

2.4.2. Growth parameters

Three plants were selected from each treatment for measuring their shoot and root lengths, and for determining the plant fresh and dry weights after being oven-dried at 80 °C for 72 h.

2.4.3. Estimation of the photosynthetic pigments

The photosynthetic pigments were extracted from 1 g of onion tubular leaves crushed in methanol (96 %, 20 ml/ g) using a mortar and pestle, homogenized using a homogenizer at 1000 rpm for about 5 min. and filtered using a cheesecloth. The extract was centrifuged at 5000 rpm for 10 min. and the supernatant was collected for measuring its absorbance at 470, 653, and 666 nm using UV-visible spectrophotometer (Janeway 7315, UK). Photosynthetic pigments were calculated and expressed as mg/ g fresh weight according to the following equations reported by Lichtenthaler and Wellburn, (1983).

$$\textit{Chlorophyll } a \text{ (Chl. } a) = (15.65 \times A666) - (7.34 \times A653)$$

$$\textit{Chlorophyll } b \text{ (Chl. } b) = (27.05 \times A653) - (11.21 \times A666)$$

$$\text{Carotenoids} = \frac{(1000 \times A470) - (2.860 \times \text{Chl. } a) - (129.2 \times \text{Chl. } b)}{245}$$

Where A470 is the measured absorbance at 470 nm, A653 is the measured absorbance at 653 nm, and A666 is the measured absorbance at 666 nm

2.4.4. Estimation of carbohydrates

Fresh leaves (100 mg) were grounded in 5 ml of 2.5 N HCl, heated in a water bath for 3 h, neutralized using sodium carbonate, and filtered through Whatman filter paper no. 1. The filtrate was used for carbohydrate determination according to the anthrone-sulphuric acid assay adopted by Leyva *et al*., (2008) using glucose solution as a standard and the carbohydrates content was expressed as glucose equivalent (mg/ g fresh weight).

2.4.5. Estimation of total protein content

One-hundred mg fresh leaves were crushed in 10 ml of sodium phosphate buffer (pH 7.5), homogenized, and then centrifuged for 10 min. at 10000 rpm. 0.1 ml of the supernatant was diluted up to 1 ml and used for protein content estimation using

Lowry *et al*., (1951) method. The absorbance was measured at 650 nm using UV-visible spectrophotometer (Janeway 7315, UK) and bovine serum albumin (BSA) was used as a standard. The amount of total protein was expressed as BSA equivalent (mg/ g fresh weight).

2.4.6. Estimation of total phenolic content

Onion leaves (0.1 g) were homogenized in 10 ml of 70 % acetone, and centrifuged at 5000 rpm for 10 min. 1 ml of the resulting supernatant was mixed with 2.5 ml Folin-Ciocalteu reagent to estimate the total phenolic content (Kupina *et al*., 2019), where the absorbance was measured at 750 nm using UV-visible spectrophotometer (Janeway 7315, UK). The phenolic compounds content was expressed as gallic acid equivalent (GAE mg/ g).

2.4.7. Determination of total flavonoid content

Using 0.1 g of onion leaves was homogenized in 10 ml of 80 % ethanol, centrifuged at 5000 rpm for 10 min., and the obtained supernatant was used to estimate the total flavonoid content (Crozier *et al*., 1997). The absorbance was measured at 430 nm and the flavonoid content was expressed as quercetin equivalent (QE mg/ g).

2.5. Statistical analysis

All experiments were conducted in triplicate and the results are presented as the mean ± standard deviation (SD). The Tukey's HSD test ($p \leq 0.05$) was utilized to compare the mean values among the control and treatments using CoStat software (v. 6.4) (CoStat. 2005).

3. Results

3.1. Screening *Trichoderma* spp. for their antagonistic activity against *S. cepivora* BYAN1

In the present study, forty-nine *Trichoderma* spp. were isolated from the rhizosphere and tissues of 25 plant species collected from different locations across four Egyptian governorates; mainly Port Said, Damietta, Al-Dakahlia, and Alexandria. Sixteen *Trichoderma* isolates were obtained from Port Said, 10 from Damietta, 6 from Al-Dakahlia, and 17 Alexandria (Table 1). All obtained *Trichoderma* isolates were screened for their antagonistic potential against *S. cepivora* BYAN1 *in vitro*. Most isolates showed varying levels of antifungal activity ranging from 5 % to 85 %. Both B3R41 and B3R20 isolates had the highest inhibitory efficacy of *S. cepivora* BYAN1 growth by 85.18 % and 80.61 %, respectively. Meanwhile, the growth of two *Trichoderma* isolates (B1R5 and B1R6) was suppressed by *S. cepivora* BYAN1 by -24.4 % and -52.3 %, respectively (Table 2). Also, the tested *Trichoderma* isolates showed varied over-growth rates and lytic activity against *S. cepivora* BYAN1, while B3Pr37 and B3R12 isolates had the highest over-growth and lytic activity, the B3R43, B3R44, B3R45, B3R46, and B3R47 isolates showed neither over-growth nor lytic activity (Table 2).

3.2. Microscopic observations of mycoparasitism

Based on the obtained results, the top 14 isolates of *Trichoderma* spp. that showed overgrowth and lytic activity towards *S. cepivora* BYAN1 where microscopically examined for their ability to parasitize *S. cepivora* BYAN1 using the slide culture technique (Table 3). The highest mycoparasitic level was observed for the isolate B3R12 recording a high coiling and lysis effect on *S. cepivora* BYAN1 (Fig. 1). Based on this result, this isolate was selected and identified as *T. afroharzianum* B3R12.

3.3. Production of non-volatile metabolites

To investigate the antagonistic mechanisms of *T. afroharzianum* B3R12, the antifungal activity of its non-volatile metabolites against *S. cepivora* BYAN1 was *in vitro* determined using the cellophane assay (Fig. 2). The obtained results revealed a significant inhibition by 74.32 % in the mycelial growth *S. cepivora* BYAN1 compared to the untreated control, which indicated the production of non-volatile metabolites by *T. afroharzianum* B3R12 (Table 4).

Table 2: *In vitro* antagonistic activity of the isolated *Trichoderma* spp. against *Stromatinia cepivora* BYAN1

Isolate no.	Inhibition (%) after		Overgrowth	Lysis
	4 d	7 d		
B1R1	14.29 ± 0.51^{tu}	35.09 ± 0.33^{q}	-	-
B1R2	25.58 ± 0.50^{opq}	10.40 ± 0.95^{s}	-	-
B1R3	RI	5.31 ± 0.10^{t}	-	-
B1R4	18.24 ± 0.31^{rs}	40.17 ± 0.29^{op}	-	-
B1R5	RI	RI	-	-
B1R6	3.50 ± 0.07^{w}	RI	-	-
B1R7	RI	11.45 ± 0.93^{s}	-	-
B1R8	40.00 ± 0.01^{h}	58.12 ± 0.23^{hij}	++	++
B1Pr9	45.00 ± 0.04^{ef}	50.00 ± 1.14^{kl}	++	+++
B2R10	27.50 ± 0.07^{nop}	44.29 ± 1.65^{mno}	-	-
B2R11	12.83 ± 0.05^{uv}	42.30 ± 1.14^{no}	+	++
B3R12	36.00 ± 0.10^{ij}	74.71 ± 0.98^{cd}	+++	+++
B2R13	40.00 ± 0.13^{h}	75.00 ± 0.99^{cd}	+	+
B2R14	20.00 ± 0.21^{r}	62.49 ± 1.05^{fgh}	-	-
B2R15	16.00 ± 0.30^{st}	65.33 ± 1.30^{ef}	-	-
B2R16	40.00 ± 0.24^{h}	73.15 ± 0.97^{cd}	-	+
B2R17	RI	55.00 ± 0.56^{ijk}	-	-
B2R18	RI	57.04 ± 0.81^{ij}	+	-
B2R19	28.00 ± 0.41^{mno}	70.00 ± 1.31^{de}	+	-
B3R20	70.70 ± 0.39^{a}	80.61 ± 1.25^{ab}	++	+++
B3R21	41.00 ± 0.20^{gh}	53.37 ± 0.92^{jk}	-	-
B3R22	17.94 ± 0.16^{rs}	36.86 ± 0.35^{pq}	-	-
B3R23	2.51 ± 0.03^{wx}	29.41 ± 0.24^{r}	-	-
B3R24	38.21 ± 0.19^{hi}	55.46 ± 0.09^{ij}	+	-
B3R25	35.52 ± 0.40^{ijk}	50.00 ± 1.03^{kl}	-	-
B2R26	28.10 ± 0.10^{mno}	43.13 ± 0.14^{mno}	-	-
B2R27	43.47 ± 0.47^{fg}	57.17 ± 1.44^{ij}	-	-
B2R28	28.47 ± 0.20^{mn}	46.30 ± 1.95^{lmn}	-	-
B2R29	15.00 ± 0.18^{tu}	46.20 ± 1.52^{lmn}	-	-
B2R30	20.36 ± 0.31^{r}	41.88 ± 1.63^{no}	-	-
B2Pr31	52.95 ± 0.74^{c}	67.09 ± 1.11^{ef}	-	-
B2R32	53.73 ± 0.16^{c}	70.00 ± 1.87^{de}	+	++
B2R33	48.50 ± 0.34^{d}	65.30 ± 1.54^{ef}	-	-
B3R34	62.53 ± 1.24^{b}	77.24 ± 1.04^{bc}	-	+
B3R35	46.15 ± 0.51^{def}	74.03 ± 0.87^{cd}	+	++
B3R36	33.30 ± 0.52^{jkl}	55.15 ± 0.69^{ij}	++	+++
B3Pr37	47.01 ± 1.17^{de}	70.00 ± 1.08^{de}	+++	+++
B3Pl38	53.80 ± 0.45^{c}	78.13 ± 1.14^{bc}	+	+
B3Ps39	33.04 ± 0.51^{kl}	64.73 ± 0.45^{fg}	+	++
B3R40	25.12 ± 0.71^{pq}	55.00 ± 0.55^{ijk}	-	+
B3R41	64.10 ± 0.15^{b}	85.18 ± 1.86^{a}	+	+
B3R42	10.09 ± 0.17^{v}	50.00 ± 0.67^{kl}	+	+
B3R43	23.75 ± 1.18^{q}	53.33 ± 0.68^{jk}	NO	NO
B3R44	32.96 ± 0.64^{kl}	60.00 ± 1.36^{ghi}	NO	NO
B3R45	18.76 ± 0.55^{rs}	47.37 ± 1.02^{lm}	NO	NO
B3R46	30.70 ± 0.17^{lm}	55.00 ± 1.16^{ijk}	NO	NO
B3R47	44.79 ± 2.23^{ef}	65.00 ± 0.88^{efg}	NO	NO
B3Pr48	12.81 ± 0.54^{uv}	43.30 ± 0.42^{mno}	+	+
B3Pr49	53.41 ± 0.65^{c}	70.00 ± 1.07^{de}	++	++

*Values in each column followed by different letter are significantly different according to Tukey's HSD test ($p \leq 0.05$), each value is the mean of three replicates ± SE. Where; (NO): no overgrowth or lysis, (-): very low level, (+): low level, (++): moderate level, (+++): high level, and RI: Reverse inhibition

Table 3: Microscopic assessment of 14 selected isolates of *Trichoderma* spp. for mycoparasitism against *Stromatinia cepivora* BYAN1

Isolate No.	Mycelial overgrowth (mm) after*			Coiling	Lysis
	24 h	48 h	72 h		
B1R8	5.33 ± 0.33^{fg}	10.00 ± 0.10^{g}	12.67 ± 0.67^{h}	-	-
B1Pr9	2.00 ± 0.33^{g}	8.67 ± 0.33^{g}	21.00 ± 0.58^{fg}	-	+
B2R11	10.00 ± 1.15^{def}	15.67 ± 0.33^{ef}	24.00 ± 0.89^{efg}	-	+
B3R12	46.00 ± 1.52^{a}	66.67 ± 0.33^{a}	75.67 ± 1.45^{a}	++	+++
B2R13	18.67 ± 1.67^{bc}	27.33 ± 1.33^{c}	33.67 ± 1.72^{cd}	+	+
B3R20	5.67 ± 0.67^{fg}	12.33 ± 0.41^{fg}	21.00 ± 1.33^{fg}	-	-
B3R24	23.67 ± 2.33^{b}	33.33 ± 0.37^{b}	45.67 ± 1.20^{b}	++	++
B2R32	15.33 ± 1.76^{cd}	30.33 ± 1.33^{bc}	34.67 ± 0.67^{c}	-	+
B3R35	4.00 ± 0.01^{fg}	21.33 ± 0.67^{d}	28.67 ± 0.33^{de}	-	+
B3R36	16.67 ± 0.33^{cd}	20.00 ± 0.94^{de}	28.00 ± 0.77^{de}	+	++
B3Pr37	8.00 ± 0.01^{efg}	17.33 ± 0.33^{def}	20.33 ± 0.33^{fg}	+	+
B3Pl38	13.00 ± 0.87^{cde}	20.67 ± 0.47^{de}	25.00 ± 0.12^{ef}	-	-
B3Ps39	8.33 ± 2.02^{efg}	13.00 ± 0.57^{fg}	18.33 ± 0.37^{gh}	-	+
B3Pr49	10.33 ± 1.45^{def}	17.00 ± 1.52^{def}	21.00 ± 0.11^{fg}	-	+

*Values in each column followed by the same letter(s) are not significantly different according to Tukey's HSD test ($p \leq 0.05$), each value is the mean of three replicates ± SE. Where; (-) = very weak effect, (+) = weak effect, (+) = good effect, and (+++) = very good effect

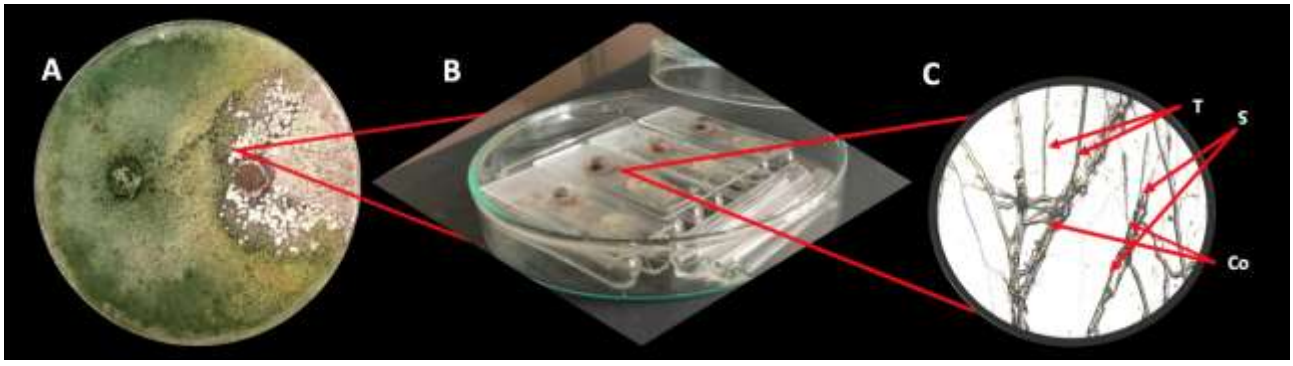

Fig. 1. Microscopic examination of *Trichoderma afroharzianum* B3R12 for mycoparasitism against *Stromatinia cepivora* BYAN1, where; A: dual culture assay, B: slide culture technique, C: mycoparasitism under light microscope. T: *Trichoderma* mycelium S: *S. cepivora* BYAN1, and Co: Coiling of *T. afroharzianum* B3R12 around *S. cepivora* BYAN1

Fig. 2. Antifungal activity of the non-volatile metabolites of *Trichoderma afroharzianum* B3R12 against *Stromatinia cepivora* BYAN1 *in vitro*

Table 4: Antifungal activity of the non-volatile metabolites of *Trichoderma afroharzianum* B3R12 against *Stromatinia cepivora* BYAN1 *in vitro* after 7 d of incubation[*]

Treatment	Mycelial growth (cm)	Inhibition (%)
Control (*S. cepivora* BYAN1)	6.83 ± 0.14^{a}	0.00^{b}
Non-volatile metabolites + *S. cepivora* BYAN1	1.75 ± 0.08^{b}	74.32 ± 0.93^{a}

3.4. Assay for production of antifungal volatile metabolites

Trichoderma afroharzianum B3R12 was examined also to produce antifungal volatile metabolites against *S. cepivora* BYAN1 *in vitro* (Fig. 3). The obtained results demonstrated a significant inhibition (71.68 %) in mycelial growth of *S. cepivora* BYAN1 compared to the untreated control plate (Table 5).

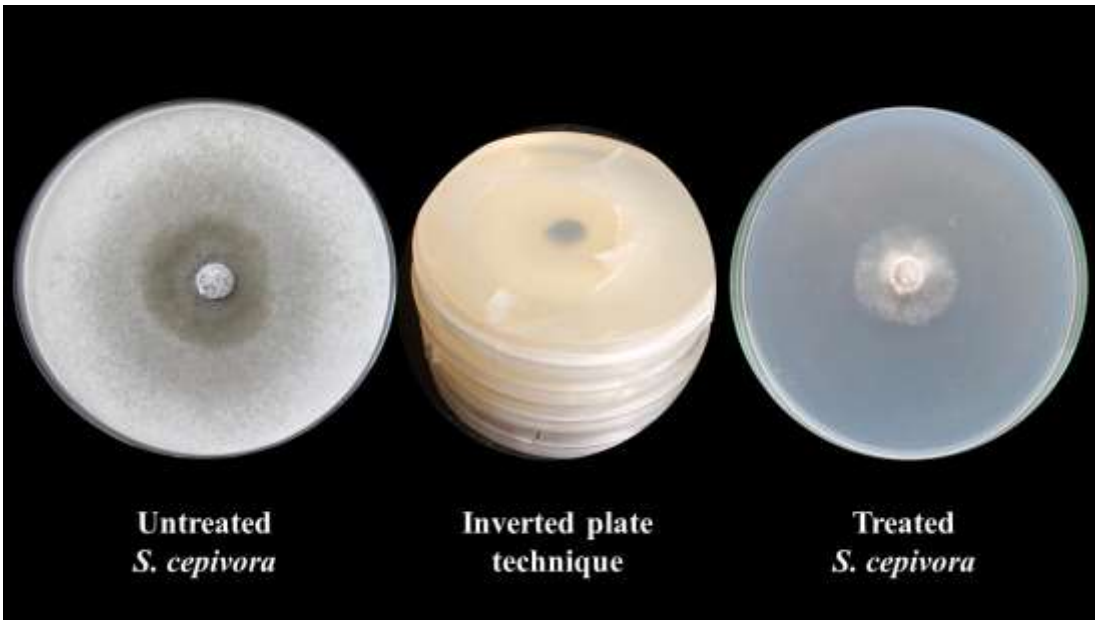

Fig. 3: Antifungal assay of the volatile metabolites produced by *Trichoderma afroharzianum* B3R12 against *Stromatinia cepivora* BYAN1 *in vitro* using inverted plate technique

Table 5: Antifungal activity of the volatile metabolites produced by *Trichoderma afroharzianum* B3R12 against *Stromatinia cepivora* BYAN1 *in vitro* after 7 d of incubation*

Treatment	Mycelial growth (cm)	Inhibition (%)
Control (*S. cepivora* BYAN1)	8.16 ± 0.12^{a}	0.00^{b}
Volatile metabolites + *S. cepivora* BYAN1	2.31 ± 0.05^{b}	71.68 ± 1.13^{a}

*Values in each column followed by different letter are significantly different according to Tukey's HSD test ($p \leq 0.05$), each value is the mean of three replicates ± SE.

3.5. Evaluation of the *in vivo* biocontrol efficacy of *T. afroharzianum* B3R12 against onion white rot pathogen under the greenhouse conditions

3.5.1. Disease severity and incidence

Results of greenhouse experiment showed that onion plants inoculated with *S. cepivora* BYAN1 had the highest disease severity (3.7) and incidence (31.3%). All applied formulations of *T. afroharzianum* B3R12 were effective in reducing the disease severity and incidence of onion white rot. The highest biocontrol activity was recorded in onion plants pretreated with *T. afroharzianum* B3R12 culture filtrate and inoculated with *S. cepivora* BYAN1 (TF + P) and those pretreated with *T. afroharzianum* B3R12 Talc-based powder (TL + P). The recorded disease severity was of 0 and 0.7, while the incidence was of 2.9 % and 4.1 %; respectively, with no significant differences recorded between both treatments in this regard. No disease symptoms were observed for those onion plants that did not receive inoculum of *S. cepivora* BYAN1 and were not pre-treated (Fig. 4).

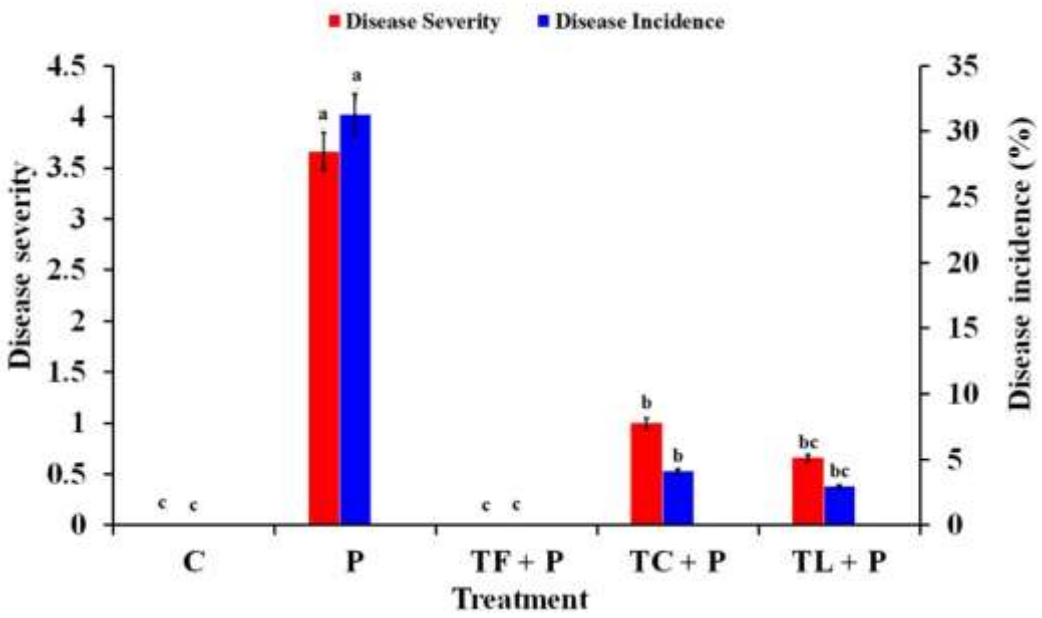

Fig. 4: Disease severity and incidence of onion white rot in response to *Trichoderma afroharzianum* B3R12 different formulations under the greenhouse conditions. Where, C: untreated and non-infected control, P: untreated and infected, TF + P: treated with culture filtrate of *T. afroharzianum* B3R12 and infected, TC + P: treated with cell-free filtrate of *T. afroharzianum* B3R12 and infected, and TL + P: treated with Talc-based powder of *T. afroharzianum* B3R12 and infected

3.5.2. Effect of *Trichoderma afroharzianum* B3R12 different formulations on onion growth in the greenhouse

The obtained results showed that onion plants white rot infection considerably reduced all evaluated growth parameters compared to the control plants (Table 6). Treatment of the infected plants with the three tested formulations of *T. afroharzianum* B3R12 enhanced the shoot length of plants compared to the untreated-infected plants. The highest shoot length was recorded in infected plants pretreated with *T. afroharzianum* B3R12 culture filtrate and those pre-treated with *T. afroharzianum* B3R12 Talc-based powder recording 64.33 ± 1.15 and 60.00 ± 2.16 cm, respectively. Regarding root length, results expressed that the infected plants pre-treated with *T. afroharzianum* B3R12 Talc-based powder recorded the highest value (17.66 ± 1.44 cm). The highest fresh weight was recorded for the infected plants that were pre-treated with cell-free filtrate of *T. afroharzianum* B3R12 and those that were pre-treated with the culture filtrate of *T. afroharzianum* B3R12 recording 19.00 ± 1.13 and 18.66 ± 1.15 g, respectively. While the infected plants that were pre-treated with the culture filtrate of *T. afroharzianum* B3R12 displayed the highest dry weight (3.31 ± 0.17 g).

Table 6: Mean growth parameters of onion plants inoculated with *Stromatinia cepivora* BYAN1 and pretreated with *Trichoderma afroharzianum* B3R12 different formulations after 60 d of inoculation under greenhouse conditions

Treatment	Shoot length (cm)	Root length (cm)	Plant fresh weight (g)	Plant dry weight (g)
C	71.66 ± 2.88^{a}	10.66 ± 1.15^{c}	16.33 ± 0.57^{b}	3.03 ± 0.08^{b}
P	34.33 ± 1.15^{d}	5.33 ± 0.57^{d}	11.00 ± 0.95^{c}	1.20 ± 0.16^{d}
TF + P	64.33 ± 1.15^{b}	15.33 ± 0.57^{b}	18.66 ± 1.15^{a}	3.31 ± 0.17^{a}
TC + P	58.00 ± 3.60^{c}	12.00 ± 1.03^{c}	19.00 ± 1.13^{a}	2.80 ± 0.11^{c}
TL + P	60.00 ± 2.16^{bc}	17.66 ± 1.44^{a}	15.00 ± 1.06^{b}	2.71 ± 0.15^{c}

3.5.3. Effect of *Trichoderma afroharzianum* B3R12 different formulations on photosynthetic pigment contents

The results of photosynthetic pigments mean contents in onion plant leaves inoculated with *S. cepivora* BYAN1and pre-treated with *T. afroharzianum* B3R12 different formulations (Table 7) showed significant reduction in photosynthetic pigments Chl. *a*, Chl. *b*, and carotenoids in infected plants compared to the non-infected ones. Application of any tested *T. afroharzianum* B3R12 formulation improved the photosynthetic pigments' content compared to the untreated-infected plants. The highest values were recorded for plants pre-treated with *T. afroharzianum* B3R12 culture filtrate and infected, recording total contents of 10.82 ± 0.25 mg/ g fresh weight, without significant differences compared to the untreated plants.

Table 7: Photosynthetic pigments mean contents (mg/ g fresh weight) in onion leaves inoculated with *Stromatinia cepivora* BYAN1 and pretreated with different *Trichoderma afroharzianum* B3R12 formulations after 60 d of inoculation under greenhouse conditions

Treatment	Chl. *a*	Chl. *b*	Carotenoids	Total photosynthetic pigments
C	5.25 ± 0.19^{a}	4.06 ± 0.23^{ab}	0.59 ± 0.04^{b}	9.90 ± 0.42^{ab}
P	2.45 ± 0.12^{c}	2.11 ± 0.11^{c}	0.36 ± 0.11^{c}	4.92 ± 0.30^{d}
TF + P	5.34 ± 0.23^{a}	4.52 ± 0.14^{a}	0.96 ± 0.02^{a}	10.82 ± 0.25^{a}
TC + P	3.58 ± 0.38^{b}	3.35 ± 0.43^{b}	0.68 ± 0.03^{b}	7.62 ± 0.51^{c}
TL + P	5.31 ± 0.14^{a}	3.53 ± 0.55^{b}	0.92 ± 0.03^{a}	9.76 ± 0.38^{b}

*Values in each column followed by different letter are significantly different according to Tukey's HSD test ($p \leq 0.05$), each value is the mean of three replicates ± SE. Where; C: untreated and non-infected control, P: untreated and infected, TF + P: treated with culture filtrate of *T. afroharzianum* B3R12 and infected, TC + P: treated with cell-free filtrate of *T. afroharzianum* B3R12 and infected, and TL + P: treated with Talc-based powder of *T. afroharzianum* B3R12 and infected

Effect of different *T. afroharzianum* B3R12 formulations on contents of several biochemical compounds

The results of total protein, carbohydrates, phenols, and flavonoids mean contents in onion plants inoculated with *S. cepivora* BYAN1 in response to pre-treatment with *T. afroharzianum* B3R12 different formulations (Table 8) revealed that the infected plants had considerable reduction in all the studied contents compared to the untreated and non-infected plants. However, all tested formulations of *T. afroharzianum* B3R12 significantly enhanced these biochemical parameters compared to the untreated-infected plants. In this regard, the highest total protein content was recorded in plants pre-treated with *T. afroharzianum* B3R12 cell-free filtrate and infected, recording 232.90 ± 4.55 mg/ g compared to the untreated-infected plants (144.03 ± 3.68 mg/ g). The highest total carbohydrates content was recorded in both *T. afroharzianum* B3R12 Talc-based powder and cell-free filtrate pre-treated plants and infected, recording 150.33 ± 10.90 and 143.10 ± 3.75 mg/ g, respectively. The highest total phenolic content was recorded in cell-free filtrate *T. afroharzianum* B3R12 pre-treated and infected plants, recording 223.00 ± 6.92 µg/ g compared to the untreated-infected plants (90.47 ± 7.67 µg/ g). While the highest total flavonoids content was recorded in plants pre-treated with both culture filtrate and Talc-based powder *T. afroharzianum* B3R12 and infected, recording 15.63 ± 0.07 and 15.05 ± 0.05 µg/ g, respectively.

Table 8: Mean total contents of protein, carbohydrates, phenols, and flavonoids in onion plants inoculated with *Stromatinia cepivora* BYAN1 and pretreated with different formulations of *Trichoderma afroharzianum* B3R12 at 60 d after inoculation under greenhouse conditions

Treatment	Total protein (mg/ g)	Total carbohydrate (mg/ g)	Total phenols (µg/ g)	Total flavonoids (µg/ g)
C	203.03 ± 3.18^{b}	126.01 ± 4.06^{b}	140.00 ± 20.62^{b}	14.70 ± 0.32^{b}
P	144.03 ± 3.68^{d}	56.53 ± 11.58^{d}	90.47 ± 7.67^{c}	11.13 ± 0.41^{c}
TF + P	205.27 ± 6.02^{b}	77.10 ± 3.70^{c}	111.80 ± 3.35^{b}	15.05 ± 0.05^{ab}
TC + P	232.90 ± 4.55^{a}	143.10 ± 3.75^{ab}	223.00 ± 6.92^{a}	15.63 ± 0.07^{a}
TL + P	179.97 ± 6.12^{c}	150.33 ± 10.90^{a}	139.91 ± 3.05^{b}	14.67 ± 0.34^{b}

*Values in each column followed by different letter are significantly different according to Tukey's HSD test ($p \leq 0.05$), each value is the mean of three replicates ± SE. Where; C: untreated and non-infected control, P: untreated and infected, TF + P: treated with culture filtrate of *T. afroharzianum* B3R12 and infected, TC + P: treated with cell-free filtrate of *T. afroharzianum* B3R12 and infected, and TL + P: treated with Talc-based powder of *T. afroharzianum* B3R12 and infected

4. Discussion

White rot represents a destructive disease that negatively affects onion plants and leads to high losses in crop production. In this study, forty-nine *Trichoderma* species were isolated from the rhizosphere and tissues of 25 wild plant species collected from different sites across four Egyptian governorates. All isolated *Trichoderma* spp. were *in vitro* screened for their antagonistic activity against *S. cepivora* BYAN1. Varied antifungal activity levels were recorded for most of the tested isolates, while two *Trichoderma* isolates were suppressed by *S. cepivora* BYAN1. This result indicated that the produced secondary metabolites of *Trichoderma* isolates may vary according to the isolate's origin and/or the surrounding environmental conditions. In line with this result, Mayo-Prieto *et al*., (2020) found that among 55 *Trichoderma* strains isolated from bean seeds and rhizosphere, the *Trichoderma* strains isolated from the soils exhibited a higher plant growth-promoting potential than those isolated from the seeds.

Trichoderma spp. are recognized to express antifungal activity against a wide range of pathogenic fungi by producing various antifungal secondary metabolites (Montes Vergara *et al*., 2022). These metabolites can be classified into two types: 1) low molecular weight and volatile metabolites, including simple aromatic compounds, and polyketides such as pyrones and butanolides, volatile terpenes, and isocyanate metabolites, and 2) high-molecular-weight metabolites such as peptaibols, diketopiperazine-like gliotoxin, and gliovirin compounds. These compounds can cause vacuolation, coagulation, disintegration, enzymatic inactivation, disintegration in the fungal cell wall permeability, and induce pathogens cell lysis (Rashad and Abdel-Azeem, 2020). Diversity in the antagonistic mechanisms of *Trichoderma* spp. against different pathogenic fungi makes it necessary to select the most efficient *Trichoderma* isolates for

each genus of plant pathogens. The current observed inhibition zones in the dual culture assay; even without direct hyphal contact between the *Trichoderma* isolates and *S. cepivora* BYAN1, indicated that they may release inhibitory substances that diffused into the growth media and hindered growth of the *S. cepivora* BYAN1, while the rapid growth rate of *Trichoderma* isolates revealed high levels of competition. Several studies reported that different antagonistic mechanisms have been used by *Trichoderma* strains, including competition for space and/or nutrients, antibiosis by production of antimicrobial molecules and/or lytic enzymes, and mycoparasitism on the pathogenic fungi. However, the antagonistic mechanisms differ based on the *Trichoderma* strain, the pathogenic fungus, and the surrounding conditions (Rashad and Moussa, 2019).

In this study, the potent 14 *Trichoderma* isolates that showed overgrowth and significant *in vitro* inhibition of *S. cepivora* BYAN1 were selected for further microscopic examination for their mycoparasitic ability. Strain *T. afroharzianum* B3R12 displayed the highest levels of coiling and lysis against *S. cepivora* BYAN1. The concept that mycoparasitism is one of the primary mechanisms by which *Trichoderma* can antagonize pathogens is supported by a previous study (Mukherjee *et al*., 2022). The selected strain *T. afroharzianum* B3R12 was further tested for production of volatile and non-volatile metabolites. It showed strong suppressive activity against *S. cepivora* BYAN1 suggesting the contribution of some volatile and non-volatile metabolites in the aggressive nature of *T. afroharzianum* B3R12. This result is consistent with the findings of Gualtieri *et al*., (2022) who reported the over-emission of 2-pentyl-furan, 6PP, acetophenone, and p-cymene by *T. asperellum* B6, *T. atroviride* P1, *T. afroharzianum* T22, and *T. longibrachiatum* MK1. In a recent study, it has been found that the volatile organic compound 6-Pentyl-2H-pyran-2-one that emitted by *T. harzianum* CECT 2413 contributed to its antifungal activity against *Botrytis cinerea* 98 (Rubio *et al*., 2023). In addition, the volatile compounds 2-methyl-1-butanol and 2-pentyl furan emitted by *T. asperelloides* PSU-P1 have been reported to be responsible for the growth suppression of *Ganoderma* sp., *Penicillium oxalicum*, *S. rolfsii*, and *S. cucurbitacearum* (Phoka *et al*., 2020). Moreover, the volatile compounds emitted by *T. asperelloides* PSU-P1 have been found to be involved in growth promotion and resistance induction in *Arabidopsis thaliana*. In general, the volatile compounds emitted by *Trichoderma* spp. diffuse over a distance that affect growth of the pathogen, while the non-volatile compounds directly diffuse in the medium or soil to suppress their antagonists (Gonzalez *et al*., 2023). Yogalakshmi *et al*., (2021) reported 6-pentyl - 2H-pyran-2-one, quinoline, phenol, 2-(6-hydrazino-3-pyridazinyl), and heptadecane as the antifungal metabolites responsible for the antagonistic behavior of *T. atroviride* against *Fusarium oxysporum* f.sp. *lycopersici*. Meanwhile, Epipolythiodioxopiperazines are fungitoxic metabolites that have been reported to be produced by *Trichoderma* spp. and can inactivate the proteins by generation of reactive oxygen species (ROS). Trichorzianine A1 and B1 produced by *T. harzianum* can suppress spore germination and mycelial growth of several pathogenic fungi (Khan *et al*., 2020). Production of antifungal enzymes such as chitinase, 1,3-glucanase, and trichodemic acid by *T. spirale* has been also reported (Mukhopadhyay and Kumar, 2020). These antifungal enzymes may contribute to the microparasitic behavior of *Trichoderma* spp.

Control of *S. cepivora* is difficult due to the high survival rate of its sclerotia under various environmental conditions, which can extend to decades in the soil (Lourenço *et al*., 2018). Many researches have reported that *T. hamatum*, *T. harzianum*, *T. reesei*, *T. virens*, and *T. viride* demonstrated efficient antagonistic activity against *S. cepivora* under laboratory conditions, under pots, and field conditions (Lodi *et al*., 2023). In this study, the biocontrol efficacy of three formulations of *T. afroharzianum* B3R12 was evaluated against *S.*

cepivora BYAN1 under greenhouse conditions. The highest biocontrol activity was recorded for infected onion plants that were pre-treated with culture filtrate of *T. afroharzianum* B3R12 and those which were pretreated with Talc-based powder of *T. afroharzianum* B3R12. This result is in agreement with that of Bouanaka *et al*., (2021) who reported a reduction of 63 % in severity of *Fusarium* crown rot of wheat plants, caused by *F. culmorum*, when treated with *T. afroharzianum* T14. Developing different formulations of *Trichoderma* spp. that serve as carrier substances play great roles in enhancing the shelf life, viability, efficacy, and protection of the used bioagent (Martinez *et al*., 2023). In this concern, Mahendra *et al*., (2022) reported that talc-based formulation of *T. harzianum* recorded the highest inoculum density at room temperature and at 4 °C compared to the other tested formulations. Induction of plant defense responses by inoculation with *Trichoderma* spp. has been extensively reported (Yao *et al*., 2023). In this study, this result was supported by the recorded increment in the total phenolic and flavonoid contents in the infected plants pre-treated with *T. afroharzianum* B3R12. In accordance, the phenolic fungitoxic compounds produced by the plant against the attacking pathogens are regarded as indicators for the plant resistance level (Rashad *et al*., 2020a). Different antifungal mechanisms have been reported for the polyphenols, including cell wall distortion, disintegration of the cell membrane permeability, enzymes inactivation, oxidative elicitation, DNA damage, and repression of virulence genes (Kumar *et al*., 2020). In addition, various elicitors produced by *Trichoderma* spp. have been reported to induce plant resistance, including antitoxins, polypeptides, lipopeptides, cellulases, terpenoids, phenol derivatives, glycosidic ligands, and flavonoids (Pocurull *et al*., 2020).

Moreover, current application of T. *afroharzianum* B3R12 on the infected onion plants led to an enhancement of the plants growth and improved their protein and carbohydrates contents, and content of the photosynthetic pigments in the plant leaves. This result is in consistence with that obtained by Kakabouki *et al*., (2021) who found that inoculation of *T. harzianum* in hemp plants enhanced their inflorescences number, fresh weight moisture, and compactness. *Trichoderma* spp. are characterized by their ability to rapidly uptake elements found in the rhizosphere in trace amounts. For example, Fe is chelated by *Trichoderma* spp. due to the production of siderophores (Tyśkiewicz *et al*., 2022). Furthermore, *Trichoderma* spp. can enhance the chlorophyll and carotenoid contents and stimulate the uptake of micro- and macro elements when applied to the ornamental plants (Andrzejak and Janowska, 2022). Chlorophyll is a diverse photosynthetic pigment found in higher plants, crucial for converting light energy into chemical energy during photosynthesis. The concentration of chlorophyll in leaves directly affects the plant's photosynthetic machinery. *Trichoderma* spp. can enhance plant photosynthesis by increasing the photosynthetic pigments and/ or upregulating the genes related to chlorophyll biosynthesis and the Calvin cycle (Harman *et al*., 2021).

Moreover, *Trichoderma* spp. can produce a variety of phytohormones, including auxin (indole-3-acetic acid), abscisic acid, salicylic acid, cytokinin, and gibberellic acid, which contribute to balancing of the plant phytohormone network and promote the plant growth and development (Illescas *et al*., 2021).

Conclusion

This study demonstrated that *T. afroharzianum* B3R12 had strong antagonistic activity against *S. cepivora* BYAN1 *in vitro*. Multiple antifungal modes of action were confirmed to contribute to the antagonistic behavior of *T. afroharzianum* B3R12, including competition, antibiosis through the production of volatile and non-volatile antifungal metabolites, and mycoparasitism. In the greenhouse experiment, pre-treatment of onion plants with *T. afroharzianum* B3R12 led to a significant reduction in the disease's severity and incidence of white rot.

A considerable promotion in the plants growth and enhancement in their contents of the photosynthetic pigments, protein, and carbohydrates was achieved. In addition, induction in the onion resistance was also recorded *via* an increment in the total phenolic and flavonoid contents. Based on the obtained results, we can conclude that *T. afroharzianum* B3R12 may represent a probable promising bioagent for biocontrol of white rot of onion and promotion of the host growth. However, field evaluation in the future studies is necessary before the final recommendation.

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Conflict of interests

The authors declare that there are no conflicts of interest.

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Ethical approval

None applicable.

Authors' Contributions

Belal Natey: Investigation and Writing original draft; Rashad YM: Conceptualization, Supervision, Writing, Review & editing original draft and Formal analysis; Kasem AMMA: Conceptualization, Supervision, Review & editing final draft; Abo-Dahab NF: Supervision, and reviewing final draft.

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