Egyptian Journal of Biological Pest Control

RESEARCH

Open Access

First report of using *Trichoderma longibrachiatum* as a biocontrol agent against *Macrophomina pseudophaseolina* causing charcoal rot disease of lentil in Algeria

Mohamed El Amine Kouadri*, Ahmed Amine Bekkar and Souad Zaim

Abstract

Background Lentil (*Lens culinaris* Medik.) is one of the major pulse crops in the world. The present study aimed to evaluate the effect of *Trichoderma* isolates to control the charcoal rot disease caused by *Macrophomina pseudophaseolina* recently reported on lentil plants.

Results In this study, antagonistic effects of seven isolates of *Trichoderma* spp. isolated from the rhizosphere of lentil were evaluated in vitro against *Macrophomina pseudophaseolina* M1. All isolates of *Trichoderma* significantly reduced the mycelial growth of M1 in vitro. Maximum reduction of the pathogen growth was observed at T10 (72.53%), followed by T5 (63.7%) and T6 (62.88%), while T2 caused the minimum reduction (37.33%). Likewise, *Trichoderma* isolates significantly reduced the size and number microsclerotia. M1 produced the minimum number of microsclerotia in the presence of T10, followed by T1 causing reduction of 68.56 and 53.37% over control, respectively. The maximum reduction of microsclerotia's size was observed in the presence of T10 (54.92%), followed by T1 (49.84) and T8 (48.68%). Results also revealed that production of volatile metabolite, components and inhibition of the test pathogen by volatile metabolites varied among different antagonistic *Trichoderma*. T1 produced the most volatile compounds, followed by T2 and T3 and T10 causing mycelial growth inhibition of 41.66, 33.88, 30.55 and 30.37%, respectively. The efficacy of the antagonistic biocontrol agents on charcoal rot was evaluated in vivo. Seed treatment with T10 significantly protected lentil seedlings from *M. pseudophaseolina* as compared to untreated plants and also improved their growth. T10 isolate was identified based on molecular tools, using internal transcribed spaces and Translation elongation factor of ribosomal DNA. The results identified T10 as *Trichoderma longibrachiatum* with accession number MW818102 and OK500004 deposited in NCBI GenBank database.

Conclusion The study revealed the first report of using *Trichoderma longibrachiatum* as a promising biological agent against *M. pseudophaseolina* causing charcoal rot disease of lentil.

Keywords *Lens culinaris*, *Trichoderma longibrachiatum*, Charcoal rot, *Macrophomina pseudophaseolina*, Biocontrol

*Correspondence:
Mohamed El Amine Kouadri
melamine.kouadri@univ-mascara.dz
Laboratory of Research on Biological Systems and Geomatics (L.R.S.B.G), Department of Agronomy, Faculty of Life and Natural Sciences, University Mustapha Stambouli of Mascara, Mascara, Algeria

Background

Lentil (*Lens culinaris* Medik.) is one of the world's most important and the oldest legume, having been cultivated for over 7000 years (McVicar et al. 2010). Its seeds are rich of protein and micronutrients for human and animal diets (Laskar et al. 2019). This plant has been cultivated for its capacity to fix nitrogen naturally where included

in crop rotations with cereals for effective cultural control of pests, diseases and weeds, by breaking up their life cycles (Kumar 2013). In Algeria, lentil is one of the most cultivated legumes after faba bean and chickpea (FAOstat 2019). However, it is often affected by several constraints, including biotic and abiotic factors. Fungal pathogens are major factors that limit its overall yield and seeds quality such as *Fusarium oxysporum* f. sp. *lentis* causing Fusarium wilt disease, *Ascochyta lentis* responsible for ascochyta blight disease and *Uromyces viciae-fabae,* the causal agent of rust disease (Kouadri et al. 2021a).

In recent years, several new diseases were identified on lentil plants including; spot blotch and charcoal rot caused by *Bipolaris sorokiniana* and *Macrophomina phaseolina*. *Macrophomina phaseolina* is a fungus that incites charcoal rot disease. It has the widest host range as phytopathogenic fungus with more than 882 cultivated and wild plants including legumes and cereals (Farr and Rossman 2022). *M. phaseolina* is a soil and seed-borne pathogen that affects plants at all stages of development, from seedling to maturity (Purkayastha et al. 2006). The characteristic symptoms of charcoal rot are dryness of stem and roots, yellowing of leaves, and a black mass appearance of microsclerotia on stems and roots. *M. phaseolina* affects plants by secreting a number of cell wall degrading enzymes, including pectinases, xylanases, cellulases, and proteases (Javaid and Saddique 2012). In severe infection, plants eventually die from exposure to phaseoline toxin, along with vascular obstruction by fungal mycelium (Bhattacharya et al. 1994).

Recently, four new species of the genus *Macrophomina* were identified such as *M. pseudophaseolina*, *M. euphorbicola*, *M. vaccini* and *M. tecta* (Poudel et al. 2021). In 2020, *M. pseudophaseolina* was reported for the first time in Algeria as a new pathogen of lentil plants causing charcoal rot disease (Kouadri et al. 2021b). Control of charcoal rot disease has become difficult due to the long-term persistence of microsclerotia in soil and plant residues, pathogen's large host range, and lack of resistant cultivars; it is mainly managed by chemical fungicides (Iqbal and Mukhtar 2020a). Although chemical fungicides provide rapid and efficient protection against plant pathogens, their intensive application poses a serious threat to human health, environment and soil beneficial organisms (Aktar et al. 2009). However, the use of the same fungicides for the same pathogen leads to the development of resistant strains of the pathogens. Therefore, the researcher's efforts were focused on developing alternative ecofriendly disease control strategies, including the use of biological control agents such as *Trichoderma* bioagent. *Trichoderma* (teleomorph Hypocrea) fungi has long been used as a biocontrol agent against fungal plant pathogens (Atalla et al. 2020). *Trichoderma* spp. inhibit the pathogen's activity through various mechanisms such as nutrient competition, hydrolytic enzymes, antibiotic production, plant growth promotion and defense response activation (Pal and Gardener 2006).

This study aimed to assess the efficacy of different isolates of *Trichoderma* as a biocontrol agent against *M. pseudophaseolina* in vitro and under greenhouse conditions for the control of charcoal rot disease of lentil.

Methods

Fungal material

Pathogen

In the present study, one isolate of *M. pseudophaseolina* M1 (MW422257, OK500004) causing charcoal rot was obtained from lentil (*Lens culinaris* Medik) from Relizane (Algeria) and was deposited at the phytopathological herbarium of the Ecole Nationale Supérieure Agronomique (ENSA, ex. INA, Algiers, Algeria) (Kouadri et al. 2021b). The isolate was stored on potato dextrose agar (PDA, Difco) at 4 °C. Prior to use, it was maintained and grown on potato dextrose agar (PDA) at 25 °C in darkness for 7 days.

Antagonists

Seven isolates of *Trichoderma* spp. were isolated from rhizosphere soils of healthy lentil plants collected from the northwest region of Algeria (Tiaret) during the period from 2019 to 2020, in order to be used as natural biocontrol agents. The samples were placed in polyethylene bags, closed tightly, and stored in a refrigerator at 4 °C until needed. Isolation of fungal antagonists was performed using a soil dilution plate method as described by Aneja (2003). One gram of dried soil samples was suspended in 9 ml sterile distilled water, agitated for 1 min. The suspension was subsequently diluted from 10^{-1} to 10^{-7}. The 0.1 ml soil dilutions of 10^{-3} to 10^{-7} were separately plated on potato dextrose agar (PDA) medium, in triplicate. The plates were then incubated at 25 °C for 3 days. After incubation, representative colonies with typical characteristics and morphological appearances of *Trichoderma* were selected and re-streaked on a new plate containing the same medium, to obtain pure colonies. Subsequently, the isolates were stored on PDA at 4 °C for until use.

Plant material

Lentil cultivar (Syrie 229), used in this experiment (in vivo), is a widely grown cultivar in Algeria and provided by the Algerian Office of Cereals.

Evaluation of antagonistic activity of *Trichoderma* isolates on mycelial growth of *M. pseudophaseolina* in vitro

Dual culture

In vitro inhibition of mycelial growth of *M. pseudophaseolina* (M1) by the *Trichoderma* isolates was tested using the dual culture technique described by Sivan and Chet (1989). A mycelial disc (5 mm) of 7-day-old culture from *M. pseudophaseolina* (M1) was placed in a Petri plate (90 mm diameter). At the opposite side of the Petri plate, a disc of *Trichoderma* (5 mm) of each isolate was placed separately with 3 cm of distance on a PDA medium for 5 days at 25 ± 1 °C. In control plates, the mycelial disc of test pathogen was put in the middle of the Petri plates. The experiment was repeated three times. After five days, the percentages of pathogen growth inhibition were calculated according to the following formula:

$$GI\ (\%) = (C - T)/C \times 100$$

Where: GI = Growth inhibition (%); C: radial growth of the pathogen in the control; T: radial growth of the pathogen in dual culture (with the antagonist).

After 2–6 days of incubation, the interface region was observed under light microscope. The effect of *Trichoderma* spp. on microsclerotia production was evaluated by counting the number of microsclerotia. The size of microsclerotia was measured using an ocular micrometer after calibration. The averages of 40 microsclerotia from each treatment were taken to calculate their size.

Fungal growth inhibition by Trichoderma volatile metabolites

Radial growth inhibition of the pathogen through the release of volatile substances was measured, following the method of Dennis and Webster (1971). Briefly, mycelial plug of 5 mm ø of each *Trichoderma* isolate was placed at the center of plate containing PDA. Then, the lid was replaced by another PDA plate with an *M. pseudophaseolina* mycelial plug of 5 mm ø at the center. The two plates were sealed together with parafilm and incubated at 25 °C for 5 days, registering mycelium growth every 24 h. As control, a non-inoculated PDA plate was used as a cover. Each test was replicated three times. Radial growth inhibition mycelial was calculated as describe previously in dual culture.

Efficacy of *Trichoderma* isolates on lentil charcoal rot in vivo

Fungal pathogen inoculum preparation

The pathogenic fungal of *M. pseudophaseolina* (M1) was used as a target pathogen in this experiment. The microsclerotia suspension was prepared by incubating the pathogenic fungal in potato dextrose agar (PDA) at 28 ± 2 °C for 7 days. The spore suspension was prepared by removing the microsclerotia from the culture edges and adding 5 ml of sterile distilled water for better separation. The suspension was filtered through two layers of sterile cheesecloth, subsequently, was determined to 10^4 microsclerotia/ml (Khanzada et al. 2012).

Trichoderma inoculum preparation

Trichoderma isolate (T10) was selected as the best and efficient antagonist in this experiment. T10 was grown on PDA plates at 25 °C till the colonies became green. Then, conidial concentration was adjusted to 10^8 conidia/ml with sterile distilled water (Zaim et al. 2018).

Pot experiment and seed treatment procedure

A pot experiment was conducted in 2019 and 2020 to evaluate the performance of *Trichoderma* as a biocontrol agent against *M. pseudophaseolina*. Four treatments and three replicates were done. Lentil seeds (cultivar Syrie 229) were surface sterilized with 1% sodium hypochlorite for 3 min and rinsed three times with sterile distilled water than dried. Each three lentil seeds were sowed in a 7 cm diameter sterilized plastic pot, filled 2/3 full with a sterilized soil mixture and peat (V/V). Sterilization was done at 120 °C for 1 h three times in three days.

The experiment included the following preparations:

Preparation 1 including the non-inoculated control, the seeds were immersed in sterile distilled water without fungal inoculum.

Preparation 2 seeds were immersed only in *M. microsclerotia* suspension (with pathogen and no *Trichoderma*).

Preparation 3 including seeds treated with *Trichoderma* and inoculated with *M. microsclerotia* suspension (with *Trichoderma* and pathogen).

Preparation 4 containing separately immersed seeds in conidial suspension of the *Trichoderma* isolate but without *M. microsclerotia* of the pathogen (*Trichoderma* + no pathogen).

Pots were distributed in a completely randomized block design with three replicates per treatment and kept under observation of Charcoal-rot symptoms for 5 weeks compared with the control pots. After 40 days of culture, 5 plants per treatment and control were analyzed. Dry and fresh weight of the aerial part and roots were determined.

Disease assessment

The charcoal rot disease severity (DS) of infection and colonization was assessed as described by Lamini et al. (2020). After 40 days of inoculation, charcoal rot disease symptoms on lentil plants were assessed using disease rating scale from 0 to 5 based on the level of infection and *M. microsclerotia* presence and

abundance observed in stem, root tissues, where 0: no symptoms on plants, 1: minor root infection with few *microsclerotia*, 2: 30% of root tissue showing lesions, 3: extensive lesions on roots and lower area of stem 4: most of the roots are affected, with high number of *microsclerotia* on stem and roots, 5: severe infection with plant mortality. Stem colonization results in a considerable amount of *microsclerotia* produced in the stem. It was measured according to the distance between the collar and the last *microsclerotia* appearing on the stem.

Disease incidence (DI) was assessed, using the following formula (Lamini et al. 2020):

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

The experiments were conducted using completely randomized block designs.

Molecular identification and phylogenetic analysis of efficient antagonistic *Trichoderma* T10

For DNA extraction, fungal isolate T10 was cultivated on PDA (slants) at 25 °C for 7 days. The total genomic DNA was extracted from T10 isolate, using the following protocol of the NucleoSpin® Food commercial kit from Macherey Nagel (Germany). An ITS and TEF regions of the 5.8S rDNA gene were amplified by PCR with universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′), ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and EF1-728F (5′-CATCGAGAAGTTCGAGAAGG-3′) (White et al. 1990; Carbone and Kohn 1999; Elsehemy et al. 2020), in a IcyclerdeBiorad type thermocycler (Thermal Cycler) (Biorad, USA).

A total volume of 25 µl containing 2 µl of T10 genomic DNA, 10 µM of each of the primers indicated above and five units of Taq polymerase (Solis Biodyn) was used to perform the PCR. Conditions for PCR amplification were initial denaturation of 1 cycle at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s; final extension step at 72 °C for 7 min. The amplified products were revealed after electrophoresis on 1.5% agarose gel and purified by Wizard® SV Gel kit and Promega's PCR Clean-Up System. The amplifiers b sequenced by Applied Biosystems Big Dye v3.1 kit and PCR primers used for amplification. The sequences obtained were analyzed and cleaned using the CHROMAS PRO software, and then identified by BLAST Program (https://blast.ncbi.nlm.nih.gov/Blast.cgi Blast). Also, a phylogenetic tree was constructed using MEGA X software with Neighbor-joining method based on 1000 bootstrap replicates (Kumar et al. 2018).

Statistical analysis

Collected data from in vitro and in vivo assays were analyzed by statistical variance (ANOVA), using SPSS software (Statistical Package for Social Science) and the treatments were compared by Tukey's test ($p < 0.05$). Each treatment consisted of three replicates. The experiment was conducted in a completely randomized design.

Results

Morphological and molecular identification of *Trichoderma longibrachiatum*

A total of seven *Trichoderma* isolates were obtained, by dilution isolation technique, from rhizosphere samples of lentil. Colony morphology and morphological features of the isolates typically resembled those of the genus *Trichoderma*. The T10 was the isolate that had the most important activity antagonist against *M. pseudophaseolina*, was selected for morphological observations and characterization by ITS and TEF sequences analysis.

T. longibrachiatum produces yellowish green pigmentation at the bottom of the culture plate. The phialids are solitary. The conidia are smooth and ellipsoid (Fig. 1). Chlamydospores are common, terminal or intercalated. The amplification of DNA region with ITS1-ITS4 and TEF1-α primers was successfully used to identify T10. A BLAST search of the obtained sequence revealed 99.83% and 100% similarity with several *T. longibrachiatum* isolates (MF102214, KY764833, MW193401) and (MF782844, MT881880, MN195113) for ITS and TEF sequences, respectively. A phylogenetic tree built for TEF region sequences with other *Trichoderma* spp. showed that the T10 isolate was clustered with *T. longibrachiatum* with a high bootstrap value (Fig. 2). T10 was identified as *T. longibrachiatum* and the sequence was deposited in NCBI GenBank under accession number MW818102 and OK500004 for ITS and TEF, respectively.

Antagonistic activity of *Trichoderma* spp. against *M. pseudophaseolina*

The results of the in vitro antagonistic activity determined by dual culture revealed that from 2 to 6 days after incubation, all isolates of *Trichoderma* were increasingly effective in inhibiting the mycelial growth of *M. pseudophaseolina* M1. Table 1 shows the percentage of radial growth inhibition by the different *Trichoderma* isolates against *M. pseudophaseolina* M1. All isolates of *Trichoderma* reduced the mycelial growth of M1 from 37.33 to 72.53%. T10, T5, T6, and T1 isolates were the most effective in vitro, inhibiting M1 growth by more than 50%. The highest inhibitory effect was noted in the isolate T10, in

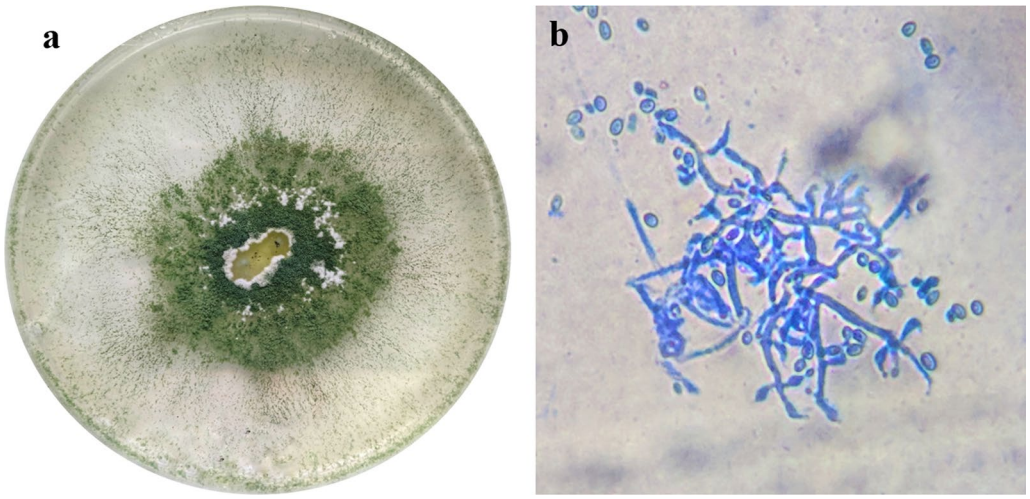

Fig. 1 Morphology of *Trichoderma longibrachiatum* (T10) **a** colony on PDA after 4 days, **b** microscopic observation of the fungus showing phialides and conidia

contrast to the isolate T2 showed the lowest inhibitory effect. After 10 days of incubation, the colony of *M. pseudophaseolina* was totally invaded by *T. longibrachiatum* (T10) with intense sporulation (Fig. 3c). In addition to mycelial growth, the number and size of microsclerotia were also affected by *Trichoderma* isolates. The control treatment showed the highest mycelium density and most branched hyphae (Fig. 3a). M1 produced a minimal number of *microsclerotia* in the presence of T10, followed by T1, causing 68.56 and 53.37% reduction, respectively over the control. On the other hand, T2 caused the minimum reductions of *microsclerotia* production, followed by T6, resulting in 28.59 and 32.85% reductions, respectively, over the control (Fig. 4a). Moreover, the isolates of *Trichoderma* spp. can significantly reduce the *microsclerotia* size of *M. pseudophaseolina*. The highest reduction in size of *microsclerotia* was caused by T10, while T6 caused the lowest reduction in size (Fig. 4b).

The effect of volatile compounds on *M. pseudophaseolina* was evaluated to determine the mechanisms used by *Trichoderma* isolates. As presented in Table 1, all *Trichoderma* isolates have the capacity to produce volatile substances that inhibit the mycelial growth of *M. pseudophaseolina*. In comparison to the controls, T1 is the most isolate inhibitor of M1 mycelial growth by volatile compounds, followed by T2, T3 and T10. The inhibition rate varied from 11.11 to 41.66%. *T. longibrachiatum* (T10) isolate had the greatest antifungal activity against *M. pseudophaseolina* than the other *Trichoderma* spp. (Table 1). That is why this isolate was selected for further experiments in vivo.

Efficacy of *T. longibrachiatum* against *M. pseudophaseolina* under greenhouse experiment

Biocontrol efficacy of *T. longibrachiatum* T10 was evaluated in the greenhouse whose seeds were inoculated with *M. pseudophaseolina* M1 and treated with T10 (Table 2).

Typical symptoms of charcoal rot were noticed in treatments with the pathogen (Fig. 5b). Seedlings of Syria 229 cultivar showing chlorotic foliage, dry stem, and roots. On the other hand, no symptoms were observed on roots and stems when treated with *Trichoderma* and inoculated with the pathogen (Fig. 5a). The stem colonizing of M1 isolates was recorded at 24%. Figure 5b shows the colonization of lentil stem inoculated with M1 after 21 days. The *microsclerotia* clearly developed externally to the stem. No disease was noticed in 40 day-old control plants; whereas 91.7% reduction in disease incidence was recorded in the plants inoculated with M1 *M. pseudophaseolina* and treated *T. longibrachiatum* T10 as compared to the plants inoculated only with M1.

As shown in Table 2, a significant effect of the treatment on the charcoal rot disease incidence (DI) and severity (DS) at 40 days after sowing was recorded. In all seedlings, DI and DS were significantly reduced through the action of seed treated with T10. In fact, the treatment induced a significant decrease in the disease incidence DI values. Meanwhile, the DS values were significantly reduced to 82%. Disease severity score of lentil plants recorded 40 days after the sown has been compiled in (Table 2). Seeds inoculated with *M. pseudophaseolina* M1 exhibited the highest severity scores (5). Minimum disease severity with 0.9 scores was observed when T10 was applied (significant at $p \leq 0.05$).

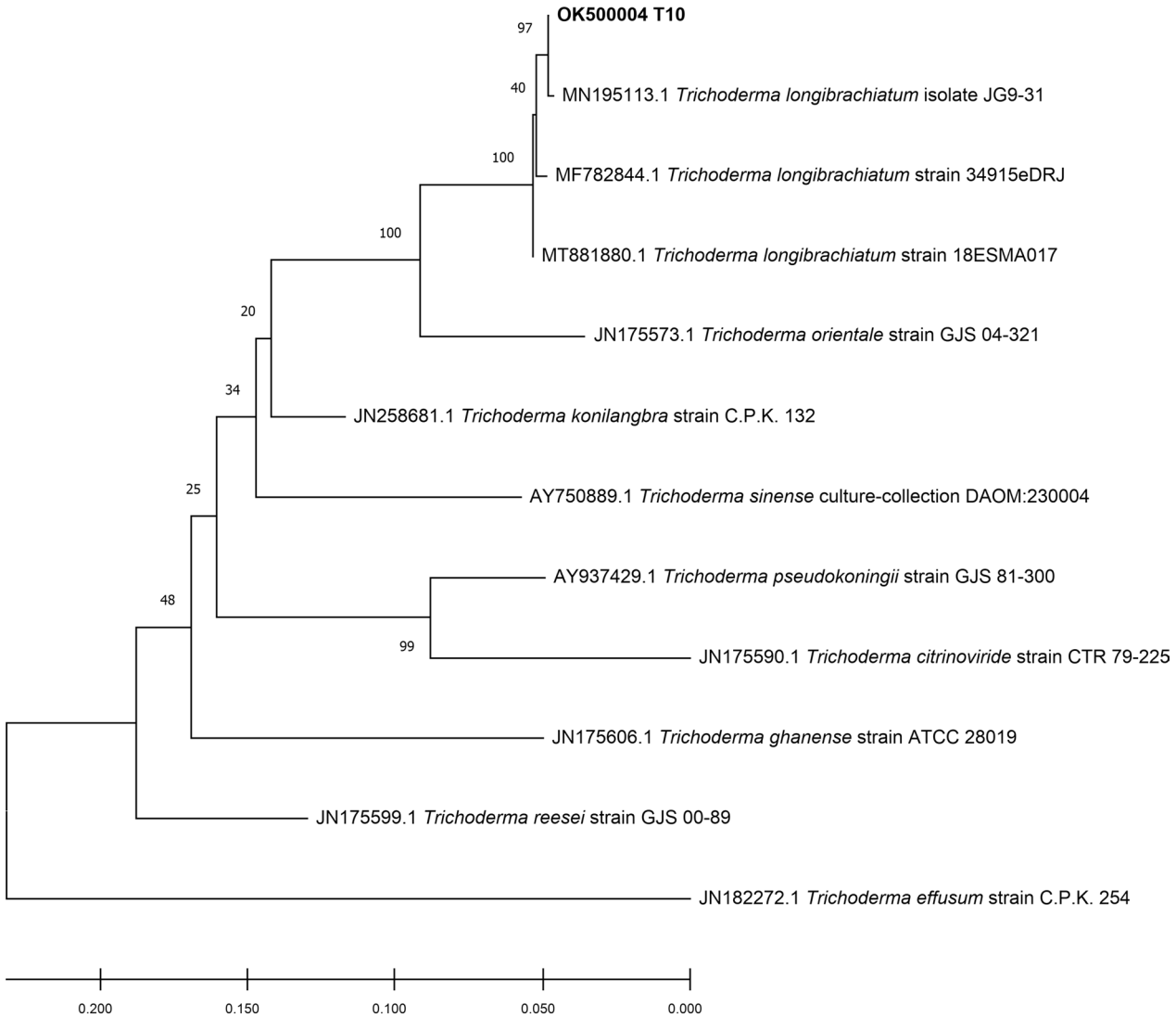

Fig. 2 Phylogenetic tree of the *Trichoderma* spp. inferred by Neighbor Joining method of TEF1-α sequence. The bootstrap values from 1000 replication are indicated on the branches

Table 1 In vitro effect of *Trichoderma* isolates on *Macrophomina pseudophaseolina* growth

Treatments	Radial growth inhibition percentage (%)		Number of *microsclerotia*	Size of *microsclerotia* (µm)
	Dual culture	Volatile compounds		
M_1+T_1	61.07^{ab}	41.66^{a}	87.5 ± 0.7^{ab}	39.49 ± 8.52^{a}
M_1+T_2	37.33^{c}	33.88^{b}	134 ± 2.82^{b}	46.66 ± 4.9^{bcd}
M_1+T_3	48.62^{bc}	30.55^{b}	98 ± 24.04^{ab}	41.24 ± 4.95^{abc}
M_1+T_5	63.7^{ab}	27.77^{bc}	105 ± 14.84^{ab}	47.91 ± 5.18^{cd}
M_1+T_6	62.88^{ab}	11.11^{d}	126 ± 30.40^{ab}	49.5 ± 4.05^{d}
M_1+T_8	57.14^{abc}	22.59^{c}	111.5 ± 21.92^{ab}	40.41 ± 8.8^{ab}
M_1+T_{10}	72.53^{a}	30.37^{b}	59 ± 11.31^{a}	35.49 ± 5.27^{a}
Control	/	/	187.66 ± 7.5^{c}	64.41 ± 10.82^{e}

Data shown (mean ± SD) with different letters denote the statistical significance determined by ANOVA followed by Tukey test ($p<0.05$)

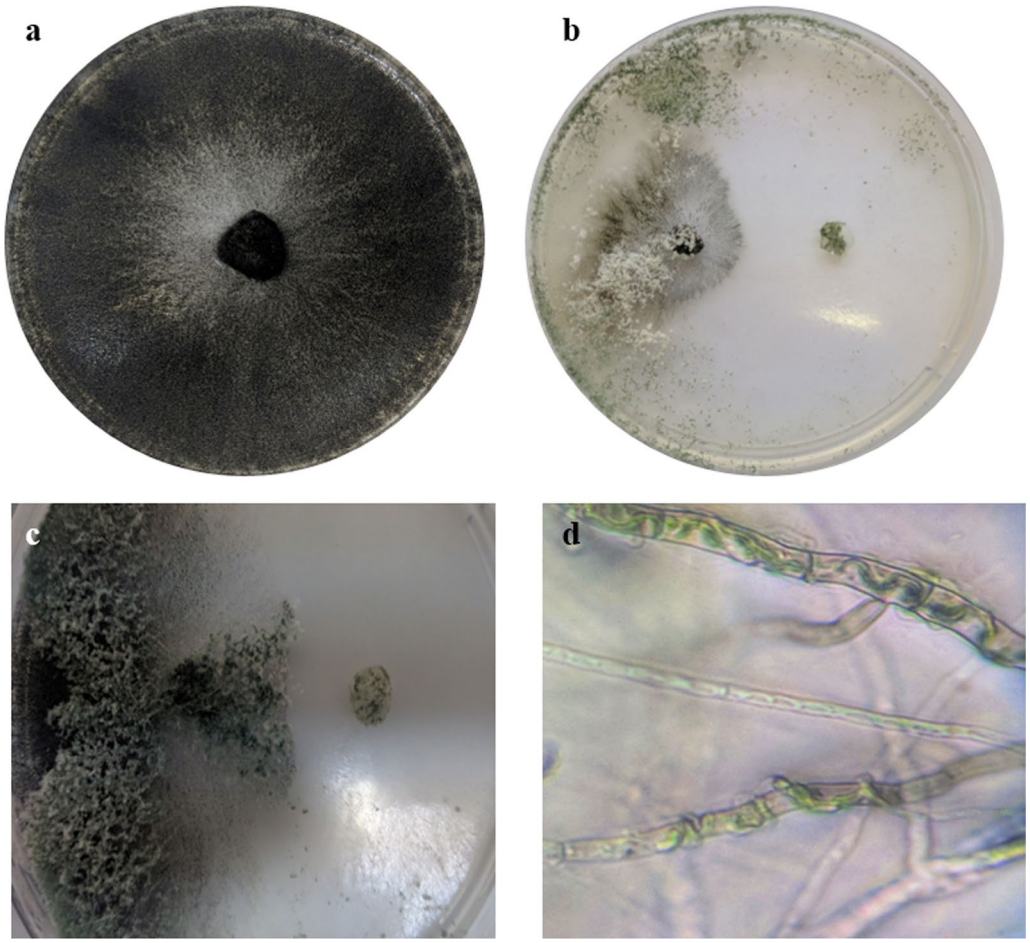

Fig. 3 Direct in vitro confrontation between *Trichoderma longibrachiatum* (T10) and *Macrophomina pseudophaseolina* (M1). **a** Colony of isolate M1 on PDA after 7 days of incubation. **b** Direct confrontation between T10 and M1. **c** Invasion of *M. pseudophaseolina* colony by T. *longibrachiatum* after 10 days. **d** Microscopic observation showing coiling hypha of *T. longibrachiatum* on *M. pseudophaseolina*

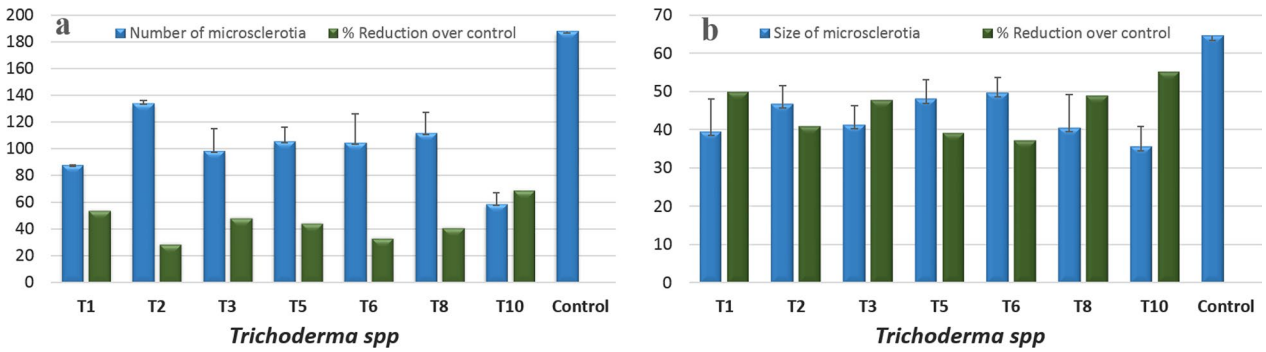

Fig. 4 Effect of Trichoderma isolate on number and size of microsclerotia of *Macrophomina pseudophaseolina*

Growth response measurement of lentil plants

Root alteration of plants lentil as a result of charcoal rot infection affect the root's ability to function properly, resulting in stunted growth and death of seedlings in the severe infection. In light of these changes, it is important to measure the stem and root length, fresh and dry weight of the infected and treated lentil plants in order to determine the degree of damage caused by this disease

Table 2 Effect of *Trichoderma longibrachiatum* on charcoal rot disease of lentil, and plant growth parameters under greenhouse conditions

Treatments	Disease incidence (%)	Stem colonization (%)	Severity	Growth parameters			
				Fresh weight (g)	Dry weight (g)	Stem length (cm)	Root length (cm)
M1	100	24	5 ± 0.00	0.1 ± 0.00^{c}	0.04 ± 0.002^{c}	16 ± 0.4^{c}	4 ± 0.00^{d}
M1 + T10	8.3	0	0.9 ± 0.264	1.5 ± 0.07^{b}	0.2 ± 0.03^{b}	17 ± 0.9^{bc}	19.8 ± 0.197^{c}
T10	/	/	/	1.8 ± 0.048^{a}	0.34 ± 0.003^{a}	20 ± 1.0^{a}	24.6 ± 0.01^{a}
Control	/	/	/	1.7 ± 0.012^{a}	0.22 ± 0.005^{b}	18 ± 0.5^{b}	21.2 ± 0.4^{b}

Data shown (mean ± Std. deviation) with different letters denote the statistical significance determined by ANOVA followed by Tukey test ($p < 0.05$)

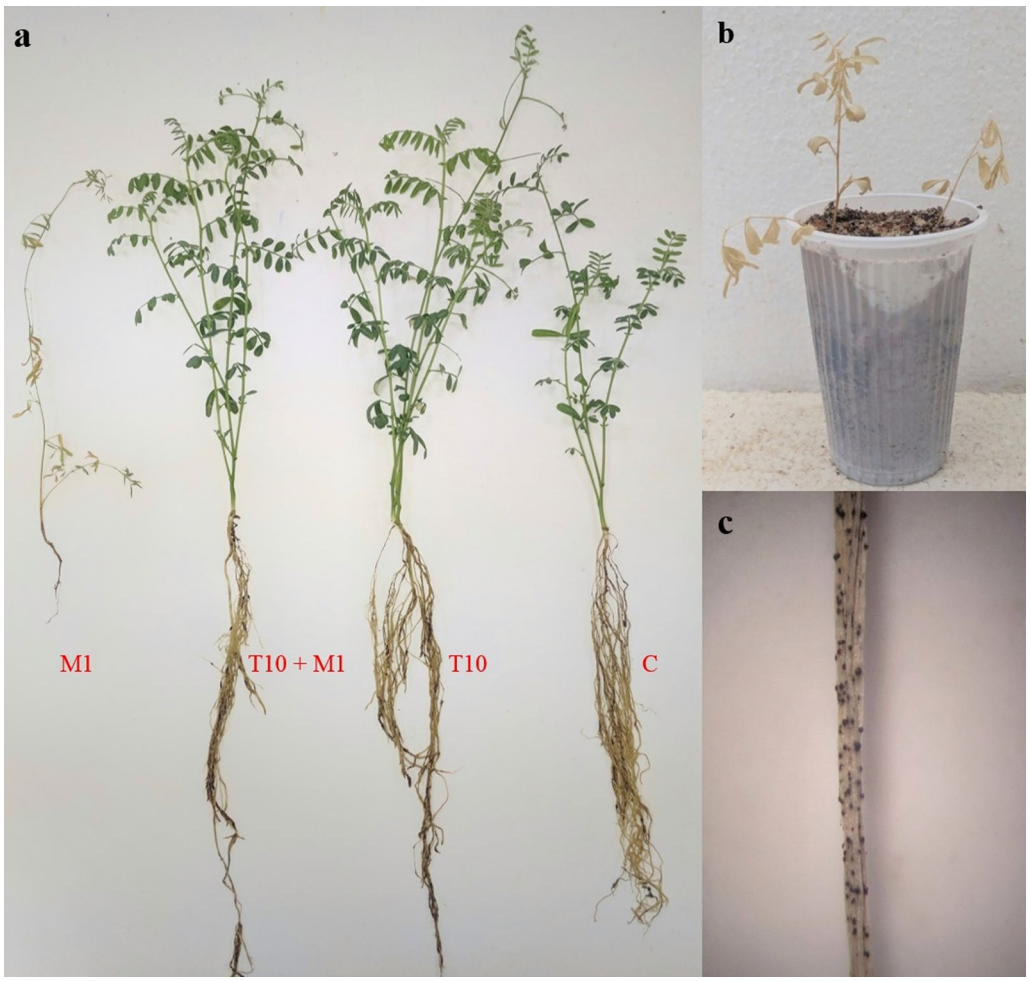

Fig. 5 Biocontrol efficacy of *Trichoderma longibrachiatum* species against *Macrophomina. pseudophaseolina* under greenhouse conditions. **a** In vivo test, **b** symptoms of charcoal rot (chlorotic foliage) on lentil plant, **c** colonization of lentil stem by the pathogen's microsclerotia

and the impact of the biocontrol agent on growth parameters. Lentil plant growth was measured 40 days after *T. longibrachiatum* T10 inoculation. The results pertaining to the efficacy of T10 in disease suppression and plant growth enhancement against the charcoal rot disease of lentil have been compiled in (Table 2). In contrast to untreated plants, the use of T10 with or without M1 significantly ($p \leq 0.05$) enhanced the plant growth parameters. Compared to the un-inoculated control, infection by M1 caused significant decreases in plant height (16 cm), root length (14.76 cm), fresh and dry weights (0.1 and 0.04 g/plant, respectively) (Table 2). The seedling

growth parameters were significantly increased ($p < 0.05$) in plants colonized by *T. longibrachiatum* T10 as compared to untreated seedlings (Table 2). Accordingly, T10 increased the stem and root length by 11.11 and 16.03%, respectively, and fresh and dry weights by 5.88 and 54.54%, respectively.

Discussions

M. pseudophaseolina was first detected on lentil in Algeria in 2021 (Kouadri et al. 2021b). In order to fight against this pathogen, several control methods have been used (Marquez et al. 2021). Therefore, its biocontrol by antagonistic agents such as *Trichoderma* allows less reliance on chemical pesticides, are commonly employed to control plant pathogens (Topolovec-Pintarić 2019). The use of native isolates in biological control is a crucial factor because they contribute to the balance of agro-systems, while expressing the desired agronomic characteristics such as phytoprotection and phytostimulation of cultivated plants (Zin and Badaluddin 2020). *Trichoderma* isolates used in the present study are indigenous isolated from the rhizosphere of healthy lentils.

Vinale et al. (2008) have demonstrated that hyperparasitism is connected with the direct contact of an antagonist with a pathogen and is composed of such stages as: pathogen recognition, attack, gradual penetration of the pathogen cells and death.

The present results demonstrated that *T. longibrachiatum* was the most effective and antagonistic against *M. pseudophaseolina*. The ability of *Trichoderma* spp. to overgrow and degrade pathogen mycelia with an inhibition in *microsclerotia* production was positively correlated with *M. pseudophaseolina* growth inhibitions during antagonism. The significant antagonistic property of *Trichoderma* against *Macrophomina* sp. has been reported by many workers (Bastakoti et al. 2017). Sridharan et al. (2020) reported that the antagonistic activity of *T. longibrachiatum* caused an inhibition in *microsclerotia* production of *Macrophomina* sp.

In dual culture plate tests or through the production of volatile compounds, *Trichoderma* isolates showed a significant inhibition of *Macrophomina* sp. growth. Sridharan et al. (2020) demonstrated that antibiosis via microbial volatile organic compounds (mVOCs) was observed in addition to inhibiting the growth of *M. phaseolina* during a direct interaction with *T. longibrachiatum*. These mVOCs reduce *M. phaseolina* mycelial growth by altering the mycelial structure.

Sharma and Sankaran (1988) reported that the fast growth rate of *T. longibrachiatum* is characteristic of a promising antagonist. According to Sobowale et al. (2010), *T. longibrachiatum* has a fast and high sporulation rate, which allows it to cover more space per time on a Petri plate; it was also suggested that the competition for nutrients and space is part of inhibition mode of the pathogen.

The activity of *Trichoderma* isolates in inhibiting *M. pseudophaseolina* growth is attributed to mycoparasitic activity and production of active compounds, including antibiotics and/or lytic enzymes (Zaim et al. 2018). Many studies have demonstrated that *Trichoderma* spp. produces a variety of secondary metabolites, some of which are toxic to the pathogens and enzyme production, including chitinases, proteases and glucanases (Gajera and Vakharia 2012). *Trichoderma* species are also capable of producing cell wall degrading enzymes, such as cellulase, xylanase, pectinase, glucanase, lipase, amylase, arabinase, and protease (Strakowska et al. 2014), as well as many volatile metabolites, such as 6-n-pentyl-2H--pyran-2-one (6-PAP).

Trichoderma species are also capable of producing a wide range of volatile secondary metabolites such as alcohols, ketones, alkanes, furanes and pyrone 6-PP (6-n-pentyl-2H- -pyran-2-one) which can interfere with various biological processes such as biocontrol and plant growth promotion (Garnica-Vergara et al. 2016). Vinale et al. (2008) reported that 6-PP is one of the major volatile organic compounds VOCs, which has antimicrobial activity at high concentrations and can be involved in plant–fungus signaling leading to the activation of plant defense mechanisms and the regulation of plant growth in tomato, pea, and canola.

Following interaction with *Trichoderma* isolates, damage on the *Macrophomina* (M1) *microsclerotia* was observed. *Trichoderma* spp. had the ability to reduce the number and size of *microsclerotia*. Similar results were reported for *Trichoderma* species having a significant effect on reducing *Macrophomina phaseolina* microsclerotia size and number (Iqbal and Mukhtar 2020b). Previous published studies gave an evidence that the rhizospheric *Trichoderma* have been isolated and tested for their antagonistic effects against *Macrophomina* spp. Several isolates were quite effective in reducing disease incidence and promoting host plant growth traits (Martínez-Salgado et al. 2021).

In most of the earlier studies, *Trichoderma* mediated plant growth promotion has been attributed to indirect mechanisms viz. control of plant pathogens and induced resistance. The results presented here also demonstrated a significant increase in growth of lentil plants for each of the parameters; plant height, fresh and dry weight, under greenhouse condition.

T. longibrachiatum T10 isolate was selected to be applied in the greenhouse conditions as it recorded the highest inhibition rate accompanied with reduction in size and number of *microsclerotia*. The obtained results

clearly showed that seed treatment with *T. longibrachiatum* reduced the incidence of charcoal rot disease and improved lentil plant growth parameters. In addition, plants treated with T10 had higher length and weight of stem and root than other treatments. Several reports indicated that *Trichoderma* species can effectively suppress charcoal rot caused by *M. phaseolina* on different plants such as soybean and Mung bean (Iqbal and Mukhtar 2020b). Other results obtained by Khaledi and Taheri (2016) revealed that inoculating soybean seeds with *Trichoderma* suspension not only suppressed the development of charcoal rot disease, but also increased the activity of peroxidase enzyme and phenolic contents of the soybean roots, resulting in defense activation and plant growth promotion.

Beside the suppression of the pathogen's growth, the results showed that plant height and weight were increased in plants treated with *T. longibrachiatum*, and this may be due to the production of secondary metabolites hormones enhancing root and stem growth as well as making some nutrients more available to plants. This is in line with the findings showing that *Trichoderma* can promote plant growth by producing IAA and ACC deaminase (Ali et al. 2014).

Conclusion

The present study is the first report to use *T. longibrachiatum* as a biocontrol agent against *M. pseudophaseolina*, a new pathogen of lentil plants causing charcoal rot disease in Algeria. The results demonstrated that *T. longibrachiatum* had the ability to control the pathogenic fungus, reduce the infection of charcoal rot on lentil, and promote plant growth. Therefore, these findings strongly support the use of *T. longibrachiatum* as an effective biological control agent in the management of charcoal rot disease. Future studies will aim to determine the efficacy of the selected isolates under field conditions.

Abbreviations

ANOVA	Analysis of variance
ITS1	Internal transcribed spacers
TEF1	Translation elongation factor1-alpha
GI (%)	Growth inhibition
DI	Disease incidence
DS	Disease severity

Acknowledgements

Not applicable.

Author contributions

KM initiated the research, conceived and performed the experiments, data analysis and curation, wrote the first draft manuscript. BA contributed to conceptualization, data analysis and validation, writing—original draft, review and editing. ZS contributed in Methodology, data analysis, revision of the manuscript, approving the final draft. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

All data generated and analyzed during this study are indicated in the manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 26 December 2022 Accepted: 21 March 2023
Published online: 10 April 2023

References

Aktar W, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: Their benefits and hazards. Interdiscip Toxicol 2:1–12

Ali S, Charles TC, Glick BR (2014) Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol Biochem 80:160–167

Aneja KR (2003) Experiments in microbiology and plant pathology. New Age International Pvt. Ltd., New Delhi

Atalla SM, Abdel-Kader MM, El-Gamal NG, El-Mougy NS (2020) Using maize wastes, fermented by co-cultures of *Trichoderma harzianum* and *Pseudomonas fluorescens*, as grain dressing against m maize diseases under field conditions. Egypt J Biol Pest Control 30(1):1–8

Bastakoti S, Belbase S, Manandhar S, Arjyal C (2017) Trichoderma species as biocontrol agent against soil borne fungal pathogens. Nepal J Biotechnol 5(1):39–45

Bhattacharya D, Dhar TK, Siddiqui KA, Ali E (1994) Inhibition of seed germination by *Macrophomina phaseolinais* related to phaseolinone production. J Appl Bact 77:129–133

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3):553–556

Dennis C, Webster J (1971) Antagonistic properties of specific group of Trichoderma. II. Production of volatile antibiotics. Trans Br Mycol Soc 57:41–48

Elsehemy IA, El Deen AMN, Awad HM, Kalaba MH, Moghannem SA, Tolba IH, Farid MA (2020) Structural, physical characteristics and biological activities assessment of scleroglucan from a local strain *Athelia rolfsii* TEMG. Int J Biol Macromol 163:1196–1207

FAOstat, Food and Agriculture Organization of the United Nations—agricultural data (2019) https://www.fao.org/faostat/en/#home

Farr DF, Rossman AY (2022) Fungal databases, U.S. National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/. Accessed 17 Dec 2022

Gajera HP, Vakharia DN (2012) Production of lytic enzymes by *Trichoderma* isolates during in vitro antagonism with *Aspergillus niger*, the causal agent of collar rot of peanut. Braz J Microbiol 43:43–52

Garnica-Vergara A, Barrera-Ortiz S, Muñoz-Parra E, Raya-González J, Méndez-Bravo A, Macías-Rodríguez L, López-Bucio J (2016) The volatile 6-pentyl-2H-pyran-2-one from *Trichoderma atroviride* regulates *Arabidopsis thaliana* root morphogenesis via auxin signaling and ETHYLENE INSENSITIVE 2 functioning. New Phytol 209(4):1496–1512

Iqbal U, Mukhtar T (2020a) Inhibitory effects of some fungicides against *Macrophomina phaseolina* causing charcoal rot. Pak J Zool 52(2):709–715

Iqbal U, Mukhtar T (2020b) Evaluation of biocontrol potential of seven indigenous Trichoderma species against charcoal rot causing fungus, *Macrophomina Phaseolina*. Gesunde Pflanz 72(2):195–202

Javaid A, Saddique A (2012) Control of charcoal rot fungus *Macrophomina phaseolina* by extracts of Datura metel. Nat Prod Res 26:1715–1720. https://doi.org/10.1080/14786419.2011.605363
Khaledi N, Taheri P (2016) Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. J Plant Prot Res 56(1):21–31. https://doi.org/10.1515/jppr-2016-0004
Khanzada AM, Lodhi AM, Shah N, Maitlo SA (2012) Effect of different inoculation methods and inoculum levels of *Macrophomina phaseolina* on Okra. Int J Phytopathol 1:1–5. https://doi.org/10.33687/phytopath.001.01.0009
Kouadri MEA, Zaim S, Bekkar AA (2021a) Lentil rust in north-west of Algeria caused by*Uromyces viciae-fabae*. Mycopath 18(2)
Kouadri MEA, Zaim S, Bekkar AA (2021b) First report of *Macrophomina pseudophaseolina* infecting *Lens culinaris*. Austral Plant Dis Not 16(1):1–3. https://doi.org/10.1007/s13314-021-00440-0
Kumar S (2013) Trichoderma: a biological weapon for managing plant diseases and promoting sustainability. Int J Agric Sci Vet Med 1(3):106–121
Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549
Lamini S, Cornelius EW, Kusi F, Danquah A, Attamah P, Mukhtaru Z, Mensah G (2020) Prevalence, incidence and severity of a new root rot disease of cowpea caused by *Macrophomina phaseolina* (Tassi) Goid in Northern Ghana. West Afr J Appl Ecol 28(2):140–154
Laskar RA, Khan S, Deb CR, Tomlekova N, Rafiq MW, Raina A, Amin R (2019) Lentil (*Lens culinaris* Medik.) diversity, cytogenetics and breeding. In: Advances in plant breeding strategies: legumes, pp 319–369. https://doi.org/10.1007/978-3-030-23400-3_9
Marquez N, Giachero ML, Declerck S, Ducasse DA (2021) *Macrophomina phaseolina*: general characteristics of pathogenicity and methods of control. Front in Plant Sci 12:634397
Martínez-Salgado SJ, Andrade-Hoyos P, Parraguirre Lezama C, Rivera-Tapia A, Luna-Cruz A, Romero-Arenas O (2021) Biological control of charcoal rot in peanut crop through strains of *Trichoderma* spp., in Puebla, Mexico. Plants 10(12):2630
McVicar R, McCall P, Brenzil C, Hartley S, Panchuk K, Mooleki P (2010) Lentils in Saskatchewan. Saskatchewan Ministry of Agriculture
Pal KK, Gardener BM (2006) Biological control of plant pathogens. Plant Health Inst. https://doi.org/10.1094/PHI-A-2006-1117-02
Poudel B, Shivas RG, Adorada DL, Barbetti MJ, Bithell SL, Kelly LA, Vaghefi N (2021) Hidden diversity of *Macrophomina* associated with broadacre and horticultural crops in Australia. Eur J Plant Pathol 161:1–23
Purkayastha S, Kaur B, Dilbaghi N, Chaudhury A (2006) Characterization of *Macrophomina phaseolina*, the charcoal rot pathogen of cluster bean, using conventional techniques and PCR-based molecular markers. Plant Pathol 55:106–116. https://doi.org/10.1111/j.1365-3059.2005.01317.x
Sharma JK, Sankaran KV (1988) Biocontrol of rust and leaf spot diseases. In: Biocontrol of plant diseases, pp 1–23
Sivan A, Chet I (1989) Degradation of fungal cell walls by lytic enzymes of *Trichoderma harzianum*. Microbiology 135:675–682. https://doi.org/10.1099/00221287-135-3-675
Sobowale AA, Jonathan SG, Odu BO, Ayansina ADV, Ojikutu TK (2010) *Trichoderma longibrachiatum* as an antagonist of *Botrydiplodia theobromae*. Arch Phytopathol Plant Prot 43(5):479–484
Sridharan AP, Thankappan S, Karthikeyan G, Uthandi S (2020) Comprehensive profiling of the VOCs of *Trichoderma longibrachiatum* EF5 while interacting with *Sclerotium rolfsii* and *Macrophomina phaseolina*. Microbiol Res 236:126436
Strakowska J, Błaszczyk L, Chełkowski J (2014) The significance of cellulolytic enzymes produced by *Trichoderma* in opportunistic lifestyle of this fungus. J Basic Microbiol 54(S1):S2–S13
Topolovec-Pintarić S (2019) Trichoderma: invisible partner for visible impact on agriculture. In: Trichoderma-the most widely used fungicide, p 15
Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, Woo SL, Lorito M (2008) A novel role for *Trichoderma* secondary metabolites in the interactions with plants. Physiol Mol Plant Pathol 72:80–86
White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1
Zaim S, Bekkar AA, Belabid L (2018) Efficacy of *Bacillus subtilis* and *Trichoderma harzianum* combination on chickpea Fusarium wilt caused by *F. oxysporum* f. sp. *ciceris*. Arch Phytopathol Plant Prot 51(3–4):217–226
Zin NA, Badaluddin NA (2020) Biological functions of *Trichoderma* spp. for agriculture applications. Ann Agric Sci 65(2):168–178

Publisher's Note