Imran *et al.*
Egyptian Journal of Biological Pest Control (2022) 32:40
https://doi.org/10.1186/s41938-022-00544-4

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Screening and biocontrol evaluation of indigenous native *Trichoderma* spp. against early blight disease and their field assessment to alleviate natural infection

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Abstract

Background: Early blight disease of tomato caused by pathogenic fungi *Alternaria solani* is the most significant and common disease throughout the world as well as in Kingdom of Saudi Arabia. The aim of this study was to isolate and identify native *Trichoderma* species from the Jeddah region in Saudi Arabia; evaluate their antagonistic potential against *A. solani*; and study their influence early blight disease severity in greenhouse and in open field.

Results: The present study focused to explore the biocontrolling potential of native *Trichoderma* spp. against *A. solani* strain to compare with a conventional fungicide. Out of 21, 3 *Trichoderma* isolates showed an antifungal activity and significantly inhibited the mycelial growth of pathogen that were identified as *Trichoderma atroviride, T. harzianum* and *T. longibrachiatum* by their ITS region sequence analysis. Strong in vitro mycelial growth suppression (70.66%) was also recorded at 400 ppm Mancozeb (90%WP®) fungicide. Further, these *Trichoderma* bioagents and fungicide were further evaluated in greenhouse (artificially inoculated) and in field on naturally infected tomato plants. In greenhouse, (13.74%) disease severity after *T. harzianum* treatment was recorded, followed by *T. longibrachiatum* (25.83%) and *T. atroviride* (21.67%). The disease severity after fungicide (50 mg/L; 10 ml per plant) application was (7.91%). Further, positive impact on the plant biomarkers was demonstrated by all selected *Trichoderma* isolates in greenhouse. Under natural infection in season I, the disease severity (%) after *T. longibrachiatum*, *T. atroviride* and *T. harzianum* treatments was 11.5, 13.26 and 16.81%, respectively, followed by control (32.12%), whereas 7.18% disease severity was recorded after fungicide application.

Conclusions: The results revealed that native *Trichoderma* of this region had potential to mitigate the early blight disease intensity in field.

Keywords: Early blight, *Alternaria solani*, Bio-control, *Trichoderma*, Plant biomarkers, Yield

Background

Tomato (*Solanum lycopersicum* L.) is susceptible to different pests present in the environment such as nematodes, viruses, bacteria and fungus. Early blight disease of tomato caused by pathogenic fungi *Alternaria solani* is the most significant and common disease throughout the world as well as in Kingdom of Saudi Arabia (Imran et al. 2021). This pathogen infects various solanaceous crops including eggplant, tomato, potato and pepper (Sallam and Abo-Elyousr, 2012). Numerous fungicides such as carbendazim, captan, mancozeb, propiconazole, copper oxychloride, propineb and tebuconazole are wildly used to fight against this pathogen (Deshmukh et al. 2020). However, among all fungicides Mancozeb fungicide with different formulations has widely been used to control

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the pathogen of early blight disease in tomato (Sowmya and Ram, 2021). Various studies have reported the significant growth suppression of fungal pathogens in field conditions (Yang et al. 2019). The successful control of this pathogen by fungicides clearly demonstrated the sensitivity of this pathogen toward this fungicide. Although the development of resistance risk of Mancozeb in *A. solani* is low (Yang et al. 2019), the frequent use of fungicide can lead to the development of resistance in pathogen. Agricultural practices and agro-chemical applications can control early blight disease and are generally considered effective (Adhikari et al. 2017). Inadequate utilization of the fungicides, however, implies serious environmental and human health hazards (Nishant et al. 2021). Furthermore, toxicity of fungicides confines applicability during fruiting period and multiple fungicide application may lead to resistance development in pathogens (Zhang et al. 2021). Therefore, biological control of plant diseases is considered a safe and eco-friendly alternative approach (Imran et al. 2022). In nature, some bacterial and fungal species are found harmless, protecting the roots of plants from rhizospheric pathogens. *Trichoderma* spp. are preeminent antagonists against various plants pathogens including nematodes, bacteria, and particularly fungi. They inhibit the growth of pathogens either directly as competitors for space and nutrients or by hyper-parasitism or indirectly by improving plant vigor, escalating stress lenience, promoting active uptake of nutrients and/or producing numerous secondary metabolites and pathogenesis-related (PR) proteins to plants (Zhang et al. 2017). Many soil borne pathogens are controlled by *T. harzianum* triggering-induced systemic resistance by rapid root colonization (Kamala and Devi, 2012). Secondary metabolites of *T. harzianum* such as pyrone, harzianopyridone, furanone, palmitic acid, stigmasterol and anthraquinone have antimicrobial effects (Ahluwalia et al. 2015). *T. viride* is also a significant bioagent and, due to its antagonistic activity, it has been widely used against many fungal plant pathogens (Mohamed and Gomaa, 2019). *T. longibrachiatum* as a promising biocontrol agent has been widely used because it secrets huge amount of peptaibols, small peptides approx. 5–20 amino acid residues in length, with a wide spectra of biological activities (Elegbede and Lateef, 2019).

Considering the importance of bio-control agents and rising fungicides resistance problems, the present study aimed to: obtain and identify native *Trichoderma* species from the Jeddah region in Saudi Arabia; evaluate their antagonistic potential against *A. solani*; study their influence early blight disease severity in greenhouse and in open field; and compare bio-agents with a conventional fungicide with respect to fruit yield and disease control in open field under natural infection conditions. This is the first study on the isolation and evaluation of *Trichoderma* and their utilization against early blight disease in fields of the Kingdom of Saudi Arabia (KSA).

Methods

Fungal strain and growth conditions

Highly virulent strain of *A. solani* "9013" was previously isolated by Imran et al. (2022). This fungal strain was tested for pathogenicity and found highly virulent on tomato plants causing early blight disease. The strain was collected from the fungal stock culture of the laboratory. Strain was sub-cultured on PDA medium plates incubated at 27 °C for 7 days. Culture was maintained at 4 °C for further use.

Isolation and field-testing of Trichoderma as potential bioagents

For the isolation of naturally existing potential bioagents against fungal pathogens, soil samples were collected from the rhizosphere of healthy tomato plants. Then, 5 ml of sterilized double distilled water was added to each 2 g of soil sample, followed by mixing by vortexing. Dilutions 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} were prepared from supernatant from each sample (Bin et al. 2020) and inoculated on rose Bengal (RB) medium plates and incubated at 27 °C for 48 h. Germinating fungal colonies were further purified by single spore transfer (Dou et al. 2019). Colonies derived from single spores were further transferred to new PDA plates, and 3-mm-diameter mycelial disks from 3-day-old culture were transferred to PDA glass tubes. Fungal cultures were maintained in glycerol tubes and stored at 4 °C for further use.

In vitro *screening and selection of antagonists*

All isolates were tested for their antagonistic potential against the highly virulent *A. solani* strain. Antagonistic activity was determined on PDA plates. Bioagent isolates grown on PDA for 3 days were used to determine the antagonistic effect by dual culture assay against *A. solani* strain. Briefly, 5-mm-diameter mycelial disk from pathogen and identical diameter disks from each bioagent (priorly grown on PDA) were placed face to face on new PDA plates at equal distance from edges PDA plates containing pathogen disks were used as controls. Three replicates (each comprising 5 plates) were prepared for each potential bioagent isolate, and plates were incubated at 27 °C for 7 days. At this stage, in control plates the selected highly virulent *A. solani* strain would typically cover the whole plate. Diameters of pathogen isolate colonies on test plates were measured, and mean values were calculated to quantify the inhibitory effect of each potential bioagent isolate. The experiment was repeated twice. Based on pathogen colony mean diameters, bioagents

showing best antagonistic activity were selected for further experiments.

Morphological and molecular identification of bioagents

All potential bioagent isolates were classified morphologically based on colony color, length and width of conidia, hyphae length, and shape and diameter of conidia (Kumar et al. 2012). Selected bioagent isolates were classified by amplification and Sanger sequencing of internal transcribed spacer (ITS) regions using generic primer pair ITS5 (5′GGAAGTAAAAGTCGTAACAAGG3′) and ITS4 (5′TCCTCCGCTTATTGATATGC3′) (White et al. 1990). DNA of the selected isolates was extracted by CTAB as of pathogen. PCR was performed in a thermal cycler with the final volume 50 µl of reaction mixture with this primer pair as described above for pathogen isolates. PCR was performed as following: initial denaturation at 94 °C for 3 min followed by 30 cycles, denaturation at 94 °C for 1 min, 56 °C for 30 s, 72 °C for 30 s, and final incubation at 72 °C for 10 min. Reaction products were cooled to 4 °C for 10 min. PCR products were then separated on 2% agarose gel in 1× Tris–acetate (TAE) buffer and stained with 0.5 µg ethidium bromide solution for 10 min, and an Alpha Imager™ gel imager system was used to record fluorescence images. PCR products were submitted to Sanger sequencing by Macrogen Company, Seoul, South Korea. Obtained sequences of PCR products were compared ITS sequences available in public domain of National Center for Biotechnology Information (NCBI) library using Basic Local Alignment Search Tool (BLAST). Respective potential bioagent isolates were identified on the basis of their similarity to published reference sequences, and obtained new ITS sequences were submitted to NCBI under specific accession numbers. Phylogenetic trees were constructed from ITS1 sequence data with the help of the neighbor joining algorithm in MEGA 6X software package (Tamura et al. 2013).

Establishment of conventional control of A. solani with chemical fungicides

For in vitro mycelial inhibition virulent *A. solani* strain, different concentrations of a commercial fungicide Mancozeb (90% WP) [ethylenebisdicarbamates] were used that is recommended to control early blight disease in local commercial farming. Stock solution (0.5 g/L) of the fungicide was prepared in sterile double distilled water that was further used to prepare the tested concentrations, viz. 50, 100, 200 and 400 ppm. These concentrations were dissolved in PDA, and 5-mm mycelial disks from 7-day-old *A. solani* culture were placed face-down in the middle of fungicide-amended plates. PDA plate lacking fungicide but the pathogen was subjected as control. Plates were incubated at 27 °C for 7 days, and colony diameter was measured. Percent mycelial growth inhibition was calculated according to Bekker et al. (2006) as: Percent inhibition $= [(C - T)/C]^*100$, where, C representing colony diameter (mm) observed in controls, and T colony diameter (mm) observed in treatments. Experiments were performed in triplicates, with each replicate consisting of five plates. The experiment was repeated twice for the consistency of results.

Effect of potential Trchicoderma bioagents early blight disease severity

Under greenhouse condition

To test the efficacy of selected potential *Trichoderma* against early blight disease under greenhouse conditions relevant to horticultural praxis, experiments were conducted (in 2020) in greenhouses of the Department of Arid Land Agriculture, King Abdulaziz University Jeddah, Saudi Arabia, with tomato variety "Doucen." Briefly, tomato seeds were germinated and seedlings were grown in 18 cm plastic pots containing peat moss (1:3). At 3–4 leaf stage, seedlings were singled out to new pots. Selected potential *Trichoderma* isolates to be tested were grown on PDA for 5 days at 27 °C. Growing mycelia were scraped with a sterilized scraper and crushed in 20 ml of sterilized double distilled water, and resulting debris was filtered through 3 layers of cheese cloth to remove fragmented mycelium from spores. Twenty-days-old plants were sprayed with the resulting spore suspension (10 mL/plant) with a compression sprayer (Blue Stallion Co., Ltd, India) (Singh et al. 2019). Spore suspension of highly virulent *A. solani* strain priorly grown on PDA was prepared identical methods used for the preparation of *Trichoderma* suspension. Two days of spraying *Trichoderma*, pathogen spore suspension (10^4 spores mL^{-1} adjusted by hemo-cytometer) 5 mL/plant was sprayed with a compression sprayer (Blue Stallion Co., Ltd, India). Plants sprayed first with potential bio-agent, but then only with sterilized distilled water at the time of pathogen spraying served as "healthy" control. In contrast, "diseased" control plants were sprayed only with pathogen. Mancozeb fungicide as "conventional treatment" was sprayed at a concentration of 50 mg L^{-1} (25 mL per plant) was sprayed at the same time of pathogen inoculation (Gondal et al. 2012). After pathogen inoculation, plants were covered with sterile polythene bags for 3 days. Standard agronomic practices were carried out in greenhouse, and experiment was performed with 4 replicates for each treatment, with 3 plants for each replicate. The experiment was performed twice and disease severity was estimated with a grade 0–5 disease rating scale as: 0 = leaves free from leaf spots;

1 = 0–5% of leaf area infected; 2 = 6–20% of leaf area infected; 3 = 21–40% leaf area infected; 4 = 41–70% leaf area infected; 5 = more than 70% of leaf area infected (Gondal et al. 2012).

Prior to disease severity, plant height was calculated, and after this, fresh and dry weight of roots and shoots were measured to determine the dry weight; plants were placed in moisture dryer chamber at 60 °C for 3 days. Means of parameters were calculated and compared for different treatments.

Open field trials

The study was conducted for 2 consecutive seasons early (season I: Jan-Mar) and late (season II: Sep-Dec) 2020 to monitor the efficacy of potential *Trichoderma* under open field conditions. In season I, seedlings of the variety "Doucen" were grown in plastic seedling trays (50 holes) containing peat moss (1:3). At 3–4 leaf stage, tomato seedlings were transplanted to the field, maintaining 60 cm distance between rows and 45 cm within plants in a row. Suspensions of fungal *Trichoderma* were prepared as described above for greenhouse inoculation. Two weeks after transplanting, *Trichoderma* suspensions were applied as foliar spray (100 mL per plant) on tomato plants with a garden sprayer (Skybird Agro Ind. Amritsar, Punjab, India), while Mancozeb fungicide as 50 mg L^{-1} was sprayed (50 mL/plant) (Majumder et al. 2020) with hand sprayer (Taizhou Jiolong Machinery Co., Ltd, Zhejiang, China). Control plants were sprayed with sterile distilled water only. All treatments were applied in the evening hours, and plants were left for natural pathogen infection under open field conditions. After applying treatments, weather conditions were monitored to exclude washout of pesticides as well as *Trichoderma* suspensions due to rain. Standard agronomic practices were carried out in field. Disease severity was recorded based on a grade 0–9 disease rating scale as: 0 = no infection, 1 = 0–10%, 2 = 10–20%, 3 = 20–30%, 4 = 30–40%, 5 = 40–50%, 6 = 50–60%, 7 = 60–70%, 8 = 70–80%, 9 = 80–90% or more leaf area infected (Singh et al. 2014) and percent disease severity was calculated by above mentioned formula. Ripened tomato fruits were harvested regularly from all replicates in all treatments and fruit yield per treatment was calculated.

The experiments were conducted under complete randomized block design with 4 replicates, each carrying 4 plants. All recommended agronomic practices were adapted in experimental zone. Plants were randomly sprayed for each treatment. Experiment with same parameters was repeated in season II. Percent disease severity and fruit yield were calculated and compared among treatments.

Data analysis

All in vitro experiments were conducted in triplicates, while field experiment was conducted with 4 replicates. Field experiments were performed in a complete randomized design, and all collected data were analyzed by using statistix 8.1 (Analytical software, statistix; Tallahassee, FL, USA, 1985–2003) software. The data for disease severity were transformed into arcsine values, and a one-way analysis of variance (ANOVA) was performed. Means of replicates in all treatments were compared using Fisher's least significant difference test at $p = 0.05$ (Steel et al. 1996).

Results

Isolation and identification of potential Trichoderma

A total of 21 fungal isolates were collected from rhizosphere of healthy tomato fields as starting material to identify potentially beneficial bioagents. Morphological characterization like mycelial growth rate, colony facade, conidial shape, structure of conidiophores and branching pattern of phialides was consistent with species of genus *Trichoderma*. Colony color was green/yellow to dark green/green; conidia were green to dark green. Most of the isolates showed green conidia color with 2.5–5.3 µm size having globose to subglobose/obovoid conidial shape, while the phialide were slender, hooked, and flask shaped, as well as elongated with size 3.0–11.5 µm × 2–6.2 µm (data not shown). Therefore, all isolates were identified as members of genus *Trichoderma*. Based on morphological identification, isolates were used to study their antagonistic behavior against highly virulent *A. solani* strain.

In vitro *application of Mancozeb fungicide*

All the tested concentrations of Mancozeb fungicide effectively inhibited the mycelial growth of *A. solani*. However, increase in the concentration of fungicide suppressed the mycelial growth of *A. solani*, and at 400 ppm concentration, the mycelial growth was significantly inhibited (70.66%) than the control. In these results, all the used concentrations remained effective against the mycelial growth suppression of *A. solani*. Results showed that an increase in fungicide concentration reduced the growth of *A. solani* (Fig. 1).

Screening of antagonists and molecular characterization

All 21 potential bioagent isolates showed antagonistic activity against *A. solani* strain 9013 in vitro. Pathogen mycelial growth inhibited by the isolates 1006, 1007 and 1013 was significant (30.6, 27.3nd 23.3 mm), respectively, as compared to all other fungal bioagents. The colony diameter of pathogen growth in the dual culture plates

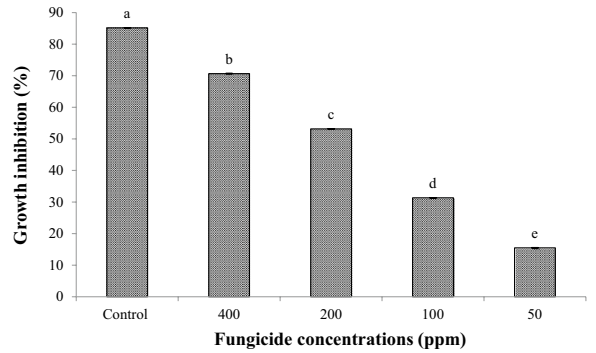

Fig. 1 In vitro application of fungicides against highly virulent isolate of *Alternaria solani*. Values followed by different letters indicate that means are significantly different from each according to fisher's least significant difference at $p = 0.05$. Error bars on graphs represent the Mean ± SE

by the isolates 1018, 1021, 1003, 1011 and 1015 was 73.5, 46.5, 45.6 44.6 and 46.3 mm, respectively, over control (82.66 mm) and other isolates (Fig. 2).

Potential bioagent isolates 1006, 1007 and 1013 identified by PCR amplification of internal transcribed spacer (ITS) regions showed approximately 560–680 bp length. Sequences were submitted to NCBI database under accession numbers MW590689, MW590687 and MW590688, respectively. Comparison of obtained ITS sequences to NCBI database entries by basic local alignment search tool (BLAST) indicated the isolate 1007 into the species *Trichoderma harzianum* (KR868283.1), 1013 into *T. longibrachiatum* (MT409889.1), and the isolate 1006 into *T. atroviride* (MT604177.1).

Phylogenetic analysis

The phylogenetic tree of 3 selected *Trichoderma* bioagents (1006, 1007 and 1013) is represented in Fig. 3a–c constructed with 80–90% nucleotide sequences similarity from NCBI. In Fig. 3a, red dot demonstrated the identified isolate *T. atroviride* supported with a bootstrap value of 100% (Fig. 3b) *T. harzianum* was supported by a bootstrap value of 97% and Fig. 3c T. *longibrachiatum* is supported by a bootstrap value of 99%.

Efficacy of Trichoderma spp. on disease severity under greenhouse conditions

Application of three *Trichoderma* isolates effectively reduced in early blight disease severity in greenhouse. Symptoms appeared after 15 days of inoculation as browning of tissues followed by necrosis. Earlier, oval shape spots were observed which extended and converted to concentric rings and changed to dark brown lesions. Disease severity of infected control was significantly

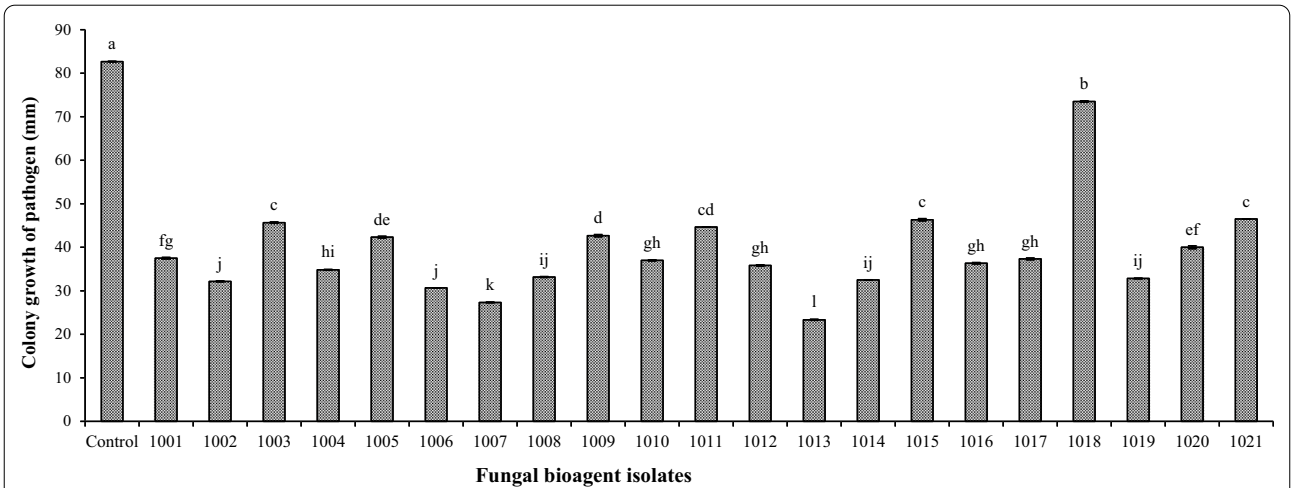

Fig. 2 In vitro screening of bio-agents against highly virulent isolate of *Alternaria solani*. Values followed by different letters indicate that means are significantly different from each according to fisher's least significant difference at $p = 0.05$. Error bars on graphs represent the Mean ± SE

(See figure on next page.)

Fig. 3 Phylogenetic trees of identified isolates of *Trichoderma*, constructed by the analysis of ITS1 sequences with neighbor joining algorithm in MEGA 6X package. The numbers over branches represent the coefficient of bootstrap, tested by 100 replications. **a** Isolate 1006, identified as *Trichoderma atroviride* (accession no. MW590689); **b** Isolate 1007, identified as *Trichoderma harzianum* (accession no. MW590687); **c** Isolate 1013, identified as *Trichoderma longibrachiatum* (accession no. MW590688). A red dot in each figure represents the highest similarity of identified isolates based on the sequences available in NCBI

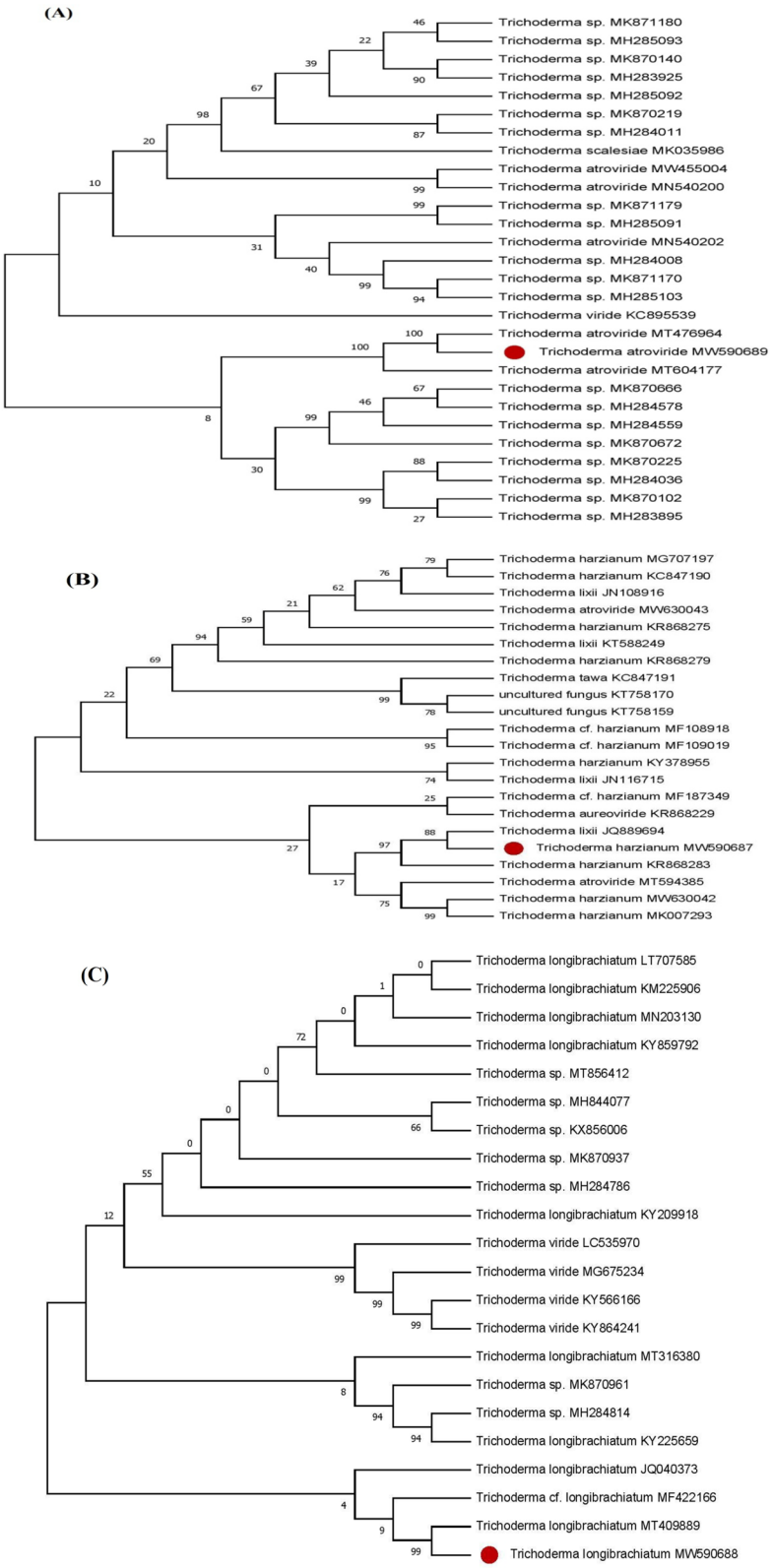

Fig. 3 (See legend on previous page.)

higher (77.91%) than other treatments, which conferred that the *A. solani* isolate "9013" was highly virulent (Fig. 4). Plants treated with Mancozeb fungicide showed lowest disease severity (7.91%). In comparison, the disease severity in plants treated with *T. atroviride* isolate 1006 was 21.67%, while disease severity of plants treated with *T. harzianum* isolate 1007 and *T. longibrachiatum* isolate 1013 was 13.71and 25.83%, respectively.

Plant biomarkers

Results demonstrated that *T. harzianum* isolate 1007 and *T. longibrachiatum* isolate 1013 considerably increased the fresh and dry weight over infected plants that where otherwise untreated, indicating the efficacy of them as bio-control agents. Plants treated with Mancozeb fungicide and bioagents also sustained the height of plants over infected plants that where otherwise untreated. The results (Table 1) showed that the inoculation of tomato with *Trichoderma* bioagents has an invigorating effect on plants. All treatments also improved the root weight as fresh and dry weight was higher than infected control.

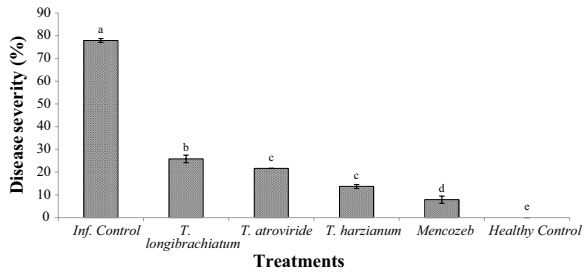

Fig. 4 Efficacy of bioagents on disease severity (%) of artificially inoculated virulent isolate of *Alternaria solani* on tomato plants in greenhouse. Values followed by different letters indicate that means are significantly different from one another according to Fisher's least significant difference at $p = 0.05$. Error bars on graphs represents the Mean ± SE

Open field trials

In the field, percent disease severity was almost constant in differing between seasons (Fig. 5) among all treatments after 15 days of transplanting. Application of Mancozeb in field showed lower severity disease (%) in both seasons (7.18 and 6.31%). Treatments with *Trichoderma* spp. also showed promising and comparable effects for the reduction in disease severity over naturally infected control. However, in season I, treatment with *T. atroviride* isolate 1006, *T. harzianum* isolate 1007 and *T. longibrachiatum* isolate 1013 showed promising potential in the reduction in disease severity as 13.26, 16.81% and 11.5%, respectively, recorded. While in season II, disease severity after applying *T. harzianum* slightly reduced to 16.5%, disease severity percent after the application of *T. atroviride* and *T. longibrachiatum* was 16.12 and 16.5% that was lower than control (31.68%) but higher than fungicide treatment (6.31%) (Fig. 5). Alternatively, under field experiments, plant height was higher after treatment with *Trichoderma* bioagents compared to fungicide and water

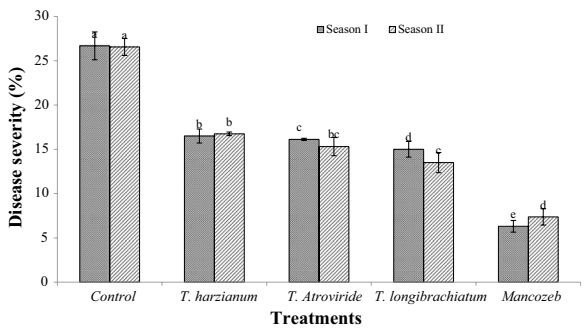

Fig. 5 Open field efficacy of *Trichoderma* bioagents against natural infection of *Alternaria solani* on tomato plants in two different seasons. Values followed by different letters indicates that means are significantly different from one another, while values followed by same letter indicate no significant difference in means according to Fisher's least significant difference at $p = 0.05$. Error bars on graphs represent the Mean ± SE

Table 1 Effect of bioagents on growth parameters of tomato plants after inoculation with *Alternaria solani* under greenhouse conditions

Treatments	Plant height (cm)	Shoot weight (g)		Root weight (g)	
		Fresh	Dry	Fresh	Dry
Mancozeb	29.91 ± 1.38 c	3.77 ± 0.71 c	0.76 ± 0.16 b	4.19 ± 0.75 b	0.97 ± 0.33 c
T. atroviride	29.25 ± 1.82 c	3.68 ± 0.79 c	0.69 ± 0.13 b	4.15 ± 0.56 b	1.26 ± 0.23 b
T. harzianum	32.66 ± 1.56 b	6.12 ± 1.31 b	1.5 ± 0.44 a	3.48 ± 0.49 c	0.64 ± 0.15 d
T. longibrachiatum	32.41 ± 2.57 b	4.82 ± 1.23 c	0.79 ± 0.18 b	3.72 ± 0.22 bc	0.72 ± 0.28 d
Infected Ctrl	18.25 ± 1.22 d	2.07 ± 0.63 d	0.28 ± 0.08 c	2.18 ± 0.12 d	0.21 ± 0.08 e
Healthy Ctrl	34.83 ± 1.59 a	10.29 ± 1.24 a	1.67 ± 0.33 a	5.19 ± 0.74 a	1.56 ± 0.16 a

In a column, means followed by a same letter(s) indicate no significant difference between the treatments according to least significant difference at $p = 0.05$

treatment which indicated that *Trichoderma* bioagents might have acted as plant growth promoters.

In field conditions, the efficacy of *Trichoderma* bioagents was assessed and compared with conventional fungicide treatment. Fruits were randomly harvested for at least 3–4 times in each season. Fruit yield after application of Mancozeb fungicide was 19.69 kg per plant in season I and 18.31 kg in season II, compared to 8.67 kg in season I and 8.08 kg in season II for untreated control plants. Thus, after fungicide application fruit yield was consistently higher in both seasons. Application of *T. harzianum* isolate 1007, *T. atroviride* isolate 1006 and *T. longibrachiatum* isolate 1013 also effectively increased the fruit yield in both seasons (Fig. 6). In season I, fruit yield after the application of *T. harzianum* isolate 1007, and *T. longibrachiatum* isolate 1013 was 13.92 kg and 12.1 kg, respectively, followed by *T. atroviride* isolate 1006 (10.91 kg). In season II, fruit yield after the application of *T. harzianum* was 12.78 kg compared to *T. atroviride* (9.85 kg), and *T. longibrachiatum* (9.43 kg). Results showed that *Trichoderma* bioagents indeed protected fruit yield under field conditions, but not too the same extent as conventional fungicide treatment.

Discussion

Early blight disease of tomato caused by a fungal pathogen *A. solani* is the most significant disease (Rahmatzai et al. 2017). Significant mycelial growth inhibition was recorded with mancozeb fungicide at 400 ppm over control. The fungicide concentrations used herein this study showed that the increase in the concentration of fungicide significantly suppressed the mycelial growth of *A. solani*, and most probably at 2000 ppm the mycelial growth might be completely inhibited because the previous studies reported 86.4–100% mycelial growth inhibition of *A. solani* with the 0.2% concentration of mancozeb (Adhikari et al. 2017). Further, the significant reduction in the mycelial growth of *Fusarium oxysporum* was obtained at 10, and 100 and 1000 ppm (Shah et al. 2006). More, the highest reduction in the growth (86.4%) of *A. solani* was achieved at 1500 ppm concentration of identical fungicide (Sadana and Didwania, 2015). A study by Vanitha et al. (2013) revealed that increase in the concentration significantly suppresses the A. solani growth these previously reported studies strongly support present results.

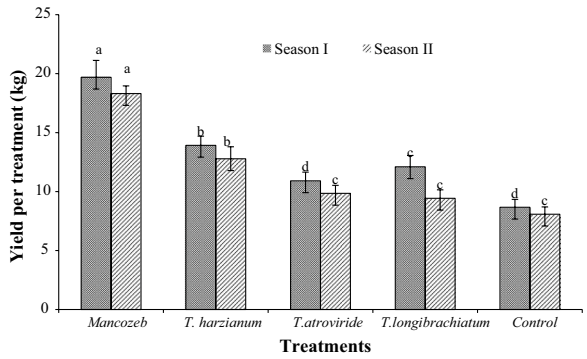

Fig. 6 Open field efficacy of *Trichoderma* bioagents on yield of tomato after natural infection of *Alternaria solani* in two different seasons. Values followed by different letters indicate that means are significantly different from one another, while values followed by same letter indicate no significant difference in means according to Fisher's least significant difference at $p = 0.05$. Error bars on graphs represent the Mean ± SE

Recent approaches have been made to use more eco-friendly bioagents to control early blight disease (Behiry et al. 2020) because biological control is considered an important approach over chemical control with fungal and bacterial antagonists to early blight pathogen. In the present study, 21 isolates of *Trichoderma* spp. were obtained from field sites with healthy tomato plants. Former studies have reported the antagonistic behavior of *Trichoderma* strains as effective bio-control agents against different disease such as *T. harzianum* against cucumber *Rhizoctonia solani* (Srivastava, 2021) and blight late disease in potato (Purwantisari et al. 2021). Many *Trichoderma*, such as *T. atroviride*, *T. virens* and *T. harzianum* as bio-control can inhibit the pathogen growth (Kumar et al. 2012). *Trichoderma* spp., showed significant mycelial growth suppression against many fungal pathogens by exhibiting the capability to contend for space and nutrients (Hirpara et al. 2017) and ability for rapid growth was significant advantage for nutrient and space competition (Amira et al. 2017). These findings evidently and sturdily support these results.

Considerable increase in plant height, fresh and dry weight of roots and shoots was recorded over infected control. *Trichoderma* spp. as potential bio-control agents was reported with beneficial effects on the repression of pathogen by producing antifungal metabolites and antibiotic promoted the plant growth and caused significant yield improvement under greenhouse and field conditions (Koley et al. 2015). This may be due to the synergetic mode of action of *Trichoderma* which hindered the phytopathogen development (Alabouvette et al. 2006). Further, similar findings were recorded when *T. viride* was used against *A. solani* of potato in greenhouse and significant reduction in disease severity was observed (Udhav, 2013). Similar results reported by various researchers strongly and clearly supported our findings and our finding corroborated that previous.

The present results showed remarkable difference (25.83, 21.67 and 13.74% disease severity after *T. longibrachiatum*, *T. atroviride* and *T. harzianum*,, respectively,

treatment, followed by infected control (77.91%). Between all treatments, disease severity (%) after the application of mancozeb 90% WP (7.91%) in greenhouse was significant in comparison with all selected microbial antagonists. Similar findings have been reported when Ridomil (Mancozeb 64% + Metalaxyl 4%) along with selected bioagents was used against *A. solani* on tomato plants in greenhouse over treated control and recorded significant reduction in the intensity of early blight (Ngoc, 2013). *Trichoderma* spp. have also been reported as plant growth promoters (Sánchez-Montesinos et al. 2020). Field application of selected *Trichoderma* bioagents showed significant reduction in disease severity in both seasons. The results here in showed that disease severity in both seasons was reduced after the application of *Trichoderma* bioagents than naturally infected control. Similar results were recorded by Kulimushi et al. (2021) upon field application of *Trichoderma* against early blight which caused significant reduction in disease severity (%), which strongly supported the present results. As in the results, fruit yield after treatments with *T. harzianum* was higher in both seasons than the control and followed by *T. atroviride* and *T. longibrachiatum*. However, studies reported the difference in yield with same antagonists (Ngoc 2013) and these variations might be due to the type of antagonists used, varieties and environmental conditions because antagonists could be influenced by diverse environmental factors like relative humidity temperature and pH (Benítez et al. 2004).

Conclusion

The results presented in this study showed that native *Trichoderma* spp. could reduce the severity of early blight of tomato under the conditions in the Kingdom of Saudi Arabia. Further, these isolates may promote the growth and development of plants and ultimately increase the fruit yield. These isolates might be integrated with other management strategies to reduce the disease losses and sustainable tomato production.

Abbreviations

PDA: Potato dextrose agar; PCR: Polymerase chain reaction; ITS: Internal transcribed spacer; ANOVA: Analysis of variance.

Acknowledgements

This project was funded by the Deanship of Scientific Research (DSR) at King AbdulAziz University, Jeddah, Saudi Arabia under grant no (DG: 085-155-1443). The authors, therefore, acknowledge with thanks DSR for technical and financial support.

Author contributions

MI involved in conceptualization, methodology, formal analysis, writing—original draft. KAMA-E took part in supervision, review and editing. MM involved in conceptualization, formal analysis review and editing. MMS took part in formal analysis, review and editing. All authors read and approved the final manuscript.

Funding

This project was funded by the Deanship of Scientific Research (DSR) at King AbdulAziz University, Jeddah, Saudi Arabia under grant no (DG: 085-155-1443)

Availability of data and materials

Data for sequence of Trichoderma spp)Trichoderma atroviride (accession no. MW590689); Trichoderma harzianum (accession no. MW590687) and Trichoderma longibrachiatum (accession no. MW590688) are available at website https://www.ncbi.nlm.nih.gov.

Declarations

Ethics approval and consent to participate

Not applicable. This manuscript is in accordance with the guide for authors available on the journal's website. Also, this work has not been published previously and is approved by all authors and host authorities.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 29 December 2021 Accepted: 12 April 2022
Published online: 18 April 2022

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