Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Evaluation of the antagonistic potential of *Trichoderma* spp. against *Fusarium oxysporum* F.28.1A

Nguyen Quoc Khuong[1], Cao Thi Thuy Trang[1], Do Thi Xuan[2], Le Thanh Quang[2], Tran Ngoc Huu[1], Ly Ngoc Thanh Xuan[3], Jun-Ichi Sakagami[4], Le Vinh Thuc[1]*

[1] Faculty of Crop Science, College of Agriculture, Can Tho University, Viet Nam

[2] Biotechnology Research and Development Institute, Can Tho University, Viet Nam

[3] Experimental and Practical Area, An Giang University – Vietnam National University, Ho Chi Minh City, Viet Nam

[4] Tropical Crop Science Laboratory, Faculty of Agriculture, Kagoshima University, Japan

Vol. 63, No. 1: 13–26, 2023

DOI: 10.24425/jppr.2023.144502

Received: May 27, 2022
Accepted: October 10, 2022
Online publication: February 17, 2023

*Corresponding address:
lvthuc@ctu.edu.vn

Responsible Editor:
Lidia Irzykowska

Abstract

The objective of this study was to evaluate the antagonistic activity of *Trichoderma* spp. against wild pathogen *Fusarium oxysporum* F.28.1A, which causes wilt disease on sesame. Twenty-six isolates of *Trichoderma* spp. isolated from soil samples were tested to control *F. oxysporum* F.28.1A. Prescreening showed that five isolates were T-02B1, T-18B2, T-20B1, T-28B1, and T-29A1, based on the lowest values of colony radius of *F. oxysporum* F.28.1A. The selected isolates were identified by their ITS region as *T. yunnanense* T-02B1, *T. lentiforme* T-18B2, *T. asperellum* T-20B1, *T. hamatum* T-28B1, and *T. hamatum* T-29A1, with similarities around 96–100%. The isolates selected were able to produce enzymes including chitinase, exo-β-1,3-glucanase, and endo-β-1,3-glucanase at levels of 0.34–0.44, 0.017–0.034, and 0.032–0.121 UI · ml^{-1}, respectively, which were considered to be a mechanism to prevent the growth of *F. oxysporum* F.28.1A. The isolates tested were applied in soil pots to prevent damage from *F. oxysporum* F.28.1A as a following experiment. The greenhouse experiment was arranged in a completely randomized design with 10 treatments, including a negative control, application of only *F. oxysporum* F.28.1A, application of both *F. oxysporum* F.28.1A and fungicide chemicals, application of both *F. oxysporum* F.28.1A and *Trichoderma* spp. DHCT, application of *T. yunnanense* T-02B1, application of *T. lentiforme* T-18B2, application of *T. asperellum* T-20B1, application of *T. hamatum* T-28B1, application of *T. hamatum* T-29A1 and a mixture of the five selected isolates of *Trichoderma* spp. with their total population equal to that in individual strain application. The results showed that the five mixed isolates of *Trichoderma* had a synergistic effect on the reduction of the disease's prevalence by 35% compared to the negative control treatment.

Keywords: *Fusarium* sp., sesame, *Trichoderma* spp., wilt disease

Introduction

Sesame (*Sesamum indicum* L.) seeds are widely used in Asian countries because of their high oil content, attractive taste, and health benefits (Zenawi and Mizan 2019). Globally, almost 7.4 million hectares of soil are devoted to cultivating sesame, generating roughly 5.53 million tons (Rahman *et al.* 2020). Sesame is considered to be an important oilseed crop, ranking as the 3rd highest in oilseed production worldwide (Mahmoud and Abdalla 2018). It is mainly produced in Asia and Africa, and together these two continents account for 97% of worldwide production (Myint *et al.* 2020). In Vietnam, sesame is grown under many different ecological conditions as a result of its broad adaptability, short growing period, high yield (820.1 kg · ha^{-1}), and high quantity (23,586 tons) (FAOSTAT 2021). However, sesame grain yield has recently decreased

due to various diseases, which are some of the most important factors affecting sesame yield (Egonyu *et al.* 2005; Ara *et al.* 2017). For instance, *Fusarium oxysporum* f. sp. *sesami* caused serious damage to sesame (Jyothi *et al.* 2011), resulting in a more than 50% reduction in grain yield (Khamari *et al.* 2018). It is one of the most prevalent soil-borne fungal diseases infecting roots, stems, and foliar components (Belay 2018). Sesame plants affected by *F. oxysporum* f. sp. *sesami* are recognized by wet rot at the neck, plant wilt, browning internal tissues, and sudden death (Dong-Hua *et al.* 2012). Unfortunately, the use of preventive pesticides can lead to an imbalance in the soil microflora (Egonyu *et al.* 2005; Komarek *et al.* 2010) and has low effectiveness because *F. oxysporum* f. sp. *sesami* forms spores which are resistant to adverse conditions and persist in plant and soil residues for a long period (Nehra *et al.* 2021). The management of arable land using biological methods such as antagonistic fungi instead of chemical methods could play an important role in maintaining microbial diversity in the soil community. Several studies have reported successful methods of using biological agents to control diseases in plants (Vinale *et al.* 2008; Parikh and Jha 2012). *Trichoderma* possesses a high ability to control *Fusarium* wilt disease, and its use has been shown for several crops, such as chili (Bhat *et al.* 2016), tomato (Sallam *et al.* 2019) and sesame (Ahmed and Abdel-Gayed 2017; Mahmoud and Abdalla 2018). The antagonism mechanisms of *Trichoderma* spp. toward pathogens of plant fungi have been found in parasitism, antibiotics, fungal-cell-wall-degrading enzymes (Monteiro *et al.* 2010; Ramada *et al.* 2016) and the ability to surround and curl the pathogenic mycelium and then secrete chemicals that degrade its cell walls (Ramada *et al.* 2016). According to Rai and Tewari (2016), *Trichoderma* has the potential to produce enzymes that can degrade the cell walls of parasitic fungi that cause plant diseases; and it has diverse filaments that result from different mechanisms. *Trichoderma* species have the ability to produce enzymes such as glucanase, chitinase, and protease, which are considered key factors for cell wall degradation (Silva *et al.* 2019). Evaluation of chitinase activity of fungal strains is an important means of biological control against fungal diseases (Kotasthane *et al.* 2015). *In vitro*, the activity of *T. viride* was effective against *F. oxysporum* f. sp. *ciceri* (62.9%) and *F. oxysporum* f. sp. *udum* (59.4%). On the other hand, *Trichoderma* spp. participates in direct antagonistic activity and reduces the effects of fungal diseases on plants (Saba *et al.* 2012). Wild *Trichoderma* are under consideration for potential use in fungal control because of its high adaptability to the environment, whereas exotic *Trichoderma* is limited by a new ecosystem and a poorer ability to adapt to climate and resistance (Su *et al.* 2018). Moreover, *Trichoderma* species are able to produce enzymes, which are considered as potential tools to prevent pathogen damage. Thus, *Trichoderma* strains were isolated from sesame cultivating soil. In this context, the study proposed detecting the species or mixed species of wild *Trichoderma* isolates to control *F. oxysporum* F.28.1A

Materials and Methods

Sources of sesame variety and fungi

Sesame variety: The ADB1 black sesame variety is commonly planted in the Mekong Delta. It was collected and kept by the Crop Science Department, College of Agriculture, Can Tho University, Vietnam.

Fungal source: *F. oxysporum* F.28.1A (Accession number: MZ781489) has the strongest ability to cause wilt disease on sesame and was stored at an experimental and practical area, in An Giang University, Vietnam (Trang *et al.* 2021).

The control fungi: *Trichoderma* spp. was used as a positive control. It was a powdered product which was sold in the market (Tricô-ĐHCT). *Trichoderma*-selective medium (TSM) was made of $MgSO_4 \cdot 7H_2O$ 0.2 g, KH_2PO_4 1.18 g, KC1 0.15 g, NH_4NO_3 1.0 g, glucose 0.5 g, and agar 20 g in 1 l of distilled water (DW) and adjusted to pH 6.5–6.8.

Production of pathogen: PDA medium consisted of potato 200 g, dextrose sugar 20 g and agar 20 g in 1 l of DW and pH was adjusted to 6.5–6.8. This PDA medium was used to grow *F. oxysporum* F.28.1A.

From December 2019 to October 2020, the experiments were carried out in the laboratory in An Giang University, Vietnam, while the greenhouse experiment was carried out at the Agricultural Research and Experimental Farm, College of Agriculture, Can Tho University. In this greenhouse, all factors of climate, water and soil were uniform.

Isolation and selection of *Trichoderma* spp. possessing ability to antagonize the fungus *Fusarium oxysporum* F.28.1A causing wilt disease on sesame under *in vitro* conditions

Sample collection

Twenty soil samples of healthy sesame root zones from 20 fields were collected in Binh Phu, Binh My and Binh Long communes in Cai Dau town and Vinh Thanh Trung commune in Chau Phu district, An Giang province. The samples were collected following the cross-diagonal method, with five samples per field, and put in plastic bags, labeled for location, stored under cold conditions, and taken to the laboratory for fungal isolation.

Isolation and collection of fungal sources were carried out by using the method of Kumar *et al.* (2018) in

specialized *Trichoderma* selective medium (TSM) medium. The soil sample was mixed with sterile DW at a ratio of 1.0 g of soil to 99 ml of sterile DW, shaken for 30 min and left to settle for 1 day at 28°C. After that, the soil sample was spread on TSM medium (Levy *et al.* 2015) that had been sterilized at 121°C for 20 min. After 5 days, the colonies were observed and compared with the morphological characteristics of *Trichoderma* spp. Then, the potential *Trichoderma* isolates were separated and purified in TSM medium for 5 days at 35°C. The morphological characteristics of mycelium (dispersion color and mycelium) and spores were observed under a 40× optical microscope to identify *Trichoderma*. After being determined as a pure isolate, the source was stored in a refrigerator at 4°C (in Eppendorf tubes and Petri dishes containing PDA medium). Finally, the isolates were named according to the genus *Trichoderma* (T) and the ordinal number of the sampling site. They were named A and B, with different morphologies based on the type of mycelium, growth process and colony color at 5 days of cultivation (Kumar *et al.* 2018).

Investigation of the antagonistic ability of *Trichoderma* spp. in Petri dishes against the fungus *F. oxysporum* F.28.1A causing wilt disease on sesame: The experiment was arranged in a completely randomized design with each treatment being a fungal strain with four replications. The radius of pathogenic fungal colonies was observed and recorded at 24, 48, 72, 84 and 96 h. The fungal strains *Trichoderma* spp. and *F. oxysporum* F.28.1A were cultured in PDA medium for 7 days in the same Petri dish (ϕ = 9.5 mm), with a 3-cm focal distance between the two colonies (ϕ = 5.0 mm). *Fusarium oxysporum* F.28.1A was inoculated within 12 h. The Petri dishes were placed in an incubator (25°C) with a uniform light source. Antagonism efficacy (AE) of *Trichoderma* spp. was monitored through the colony radius of *F. oxysporum* and the antagonistic efficiency was determined, as high resistance ≥ 60%, average resistance 40% ≤ AE ≤ 59%, weak resistance 20% ≤ AE ≤ 39%, not antagonistic ≤ 19% at 24, 48, 72, 84 and 96 h after culture according to the formula below (Kakraliya *et al.* 2017).

$$\mathrm{AE}(\%) = \frac{(C-T)}{C} \times 100,$$

where: C – the colony radius of the pathogenic fungus *F. oxysporum* F.28.1A, which is considered as the control treatment, and T – the colony radius of the pathogenic fungal strain corresponding to the treatment containing one of the strains of *Trichoderma* spp. The fungal strains of *Trichoderma* spp. having a good growth and antagonistic ability under selected laboratory conditions were chosen to evaluate chitinase production.

Investigation of the ability to secrete chitinase, exo-β-1,3-glucanase, endo-β-1,3-glucanase of *Trichoderma* spp. having effective antagonism against *Fusarium* sp.

Chitinase-secreting ability of Trichoderma spp.

All 26 isolates of *Trichoderma* spp. from rhizospheric soil for sesame cultivation and the control were cultured and analyzed for their chitinase secretion. The experiment was arranged in a completely randomized design, with four replications. *Trichoderma* isolates after 7 days of culture on PDA medium were used to proliferate in 50 ml test tubes containing 10 ml of liquid TSM medium, supplemented with 0.5% chitin as precursor, with a density of 10^7 spores · ml^{-1}, under room conditions, and shaken at 120 rpm for 5 days. Then, 2 ml of the post-enrichment solution was aspirated and centrifuged at 5,000 rpm for 20 min; after that, the biomass was removed and the supernatant was to be used as the crude extracellular enzyme source of the fungal strains. The chitinase enzyme activity was investigated following the method used by Bruce *et al.* (1995). This method for determining chitinase enzyme activity is based on the amount of reducing sugars produced through the intermediate reaction with Nelson-Somogyi reagent, the amount of reducing sugars reacting with a copper reagent when heated to brick red and when reacting with Aseno-moblybdate reagent turns blue, absorbs at 520 nm. The chitinase resolution was checked by spectrophotometric densitometry at a wavelength of 490 nm. The chitinase was measured by reacting 1 ml of enzyme with 1 ml of chitinase solution (1% chitin in acetate buffer pH = 6.0) at 40°C for 60 min, and the amount of reduced sugars produced by the method of chitinase was recorded. For Nelson's method of enzyme activity, a unit of enzyme activity (Ul · ml^{-1}) is the amount of reducing sugar produced in 1 min per 1 ml of enzyme at 40°C and pH = 6.0.

Method of endo-β-1,3-glucanase and exo-β-1,3-glucanase detection of Trichoderma spp.

After 7 days of culture on PDA medium, isolated strains of *Trichoderma* were subjected to growth in 50 ml tubes containing 10 ml of liquid TSM medium added with 0.5% chitin precursor at room temperature and shaken at 120 rpm for 7 days. The fungal density was 10^6 spores · ml^{-1}. Then, 2 ml of culture broth was centrifuged at 5,000 rpm for 20 min. Biomass was removed and the obtained liquid was used as a source of extracellular enzyme of fungal strains. The method was described by Nelson (1944). The enzyme activity of endo-β-1,3-glucanase was measured by adding 1 ml of enzyme to react with 1 ml of carboxymethyl cellulose (CMC, 1% in acetate buffer, pH = 5.0) at 40°C for 60 min. Then, the reduced glucose content was determined.

The enzyme activity of exo-β-1,3-glucanase was measured as follows

One ml of enzyme reacted with 1 ml of cellulose powder (1% cellulose powder in acetate buffer, pH = 5.0) at 40°C for 60 min. The measurement was based on the amount of glucose reduced. The unit of enzyme activity was (UI · ml^{-1}) which corresponded to the amount of glucose produced by 1 ml of enzyme for 1 min at 40°C and pH = 5.0.

Investigation of the cellulase-secreting ability of Trichoderma spp.

The fungal strains *Trichoderma* spp. were isolated after 7 days of culture on PDA medium and proliferated in 50 ml test tubes containing 10 ml of liquid TSM medium, supplemented with precursor 0.5% carboxymethyl cellulose (CMC) with a density of 10^6 spores · ml^{-1}, placed at room temperature and shaken at 120 rpm for 7 days. After that, 2 ml of the post-enrichment solution was withdrawn and centrifuged at 5,000 rpm for 20 min, the biomass was removed, and the supernatant was used as a crude extracellular enzyme source of the fungal strains. Twenty µl of this extracellular enzyme solution was withdrawn and pumped into the agar well of the TSM medium supplemented with 0.5% CMC. The experiment was conducted in three wells (0.5 cm), corresponding to three replicates, for the fungal strains and one control well, which was pumped with sterile DW. They were incubated at 37°C for 5 days. After incubation, Lugol staining was carried out. The Lugol solution was poured onto the agar surface, and after 5 min, the iodine reacted with the cellulose, forming a black-green complex and a colorless halo ring appeared around the agar well containing the enzyme solution of the investigated fungal strains, indicating the cellulose-degrading capacity of these strains (Orpin 1977).

Identification of the tested fungus Trichoderma spp. against F. oxysporum F.28.1A

Strains *Trichoderma* spp. T-02B1, T-18B2, T-20B1, T-28B1 and T-29A1 were extracted their DNA from filamentous fungal colonies. Specifically, the fungal colony spores were shaken well in 2.2 ml Eppendorf after 7 days of PDA medium cultivation and incubated at room temperature for 10 min. They were then centrifuged at 13,000 rpm for 5 min, and the extract was transferred to fresh Eppendorf and the precipitate was washed with 500 µl of 70% ethanol, centrifuged at 13,000 rpm for 5 min, and then vacuumed dried. The DNA was dissolved in 100 µl TE 0.1 X. Then, PCR reaction was conducted with primer pair ITS 1: 5'-TCCGTAGGTGAACCTGCGG-3'; ITS 4: 5'-TCCTCCGCTTATTGATATGC-3' (White *et al.* 1990). PCR reaction was performed with a total volume of 50 µl through reaction stages: Denaturation at 95°C for 5 min and (95°C for 90 s) × 30 cycles, annealing (52°C for 60 s) × 30 cycles, extension (72°C for 90 s and 72°C for 5 s) × 30 cycles and the reaction was stopped at room temperature. PCR products were purified and sequenced using an automated sequencing system. The result was compared to the GenBank database on NCBI using the BLASTN tool.

Evaluation of the efficacy of *Trichoderma* spp. in prevention of wilt disease on sesame caused by *Fusarium oxysporum* under greenhouse conditions

Soil, fungi, chemical fungicide and seed preparation

Alluvial soil cultivated with sesame in the field was collected at a depth of 0–30 cm and dried. The organic residues were then removed from the soil, mixed well and crushed. This experimental soil was collected from the same area of soil for isolation. Finally, the homogenous sample was autoclaved twice at 121°C for 30 min. After being sterilized, 3.0 kg per pot of soil was used. *F. oxysporum* F.28.1A was cultured on PDA medium over 7 days. Then the spores were collected and the density of the fungal spores was determined by calculating density through a spectrophotometer at a wavelength of 550 nm, using the dilution method to adjust the spore density at 5 × 10^6 spores · g^{-1} soil. *Trichoderma* spp. was also added at the same concentration. Ridomil gold 68WG was prepared according to the manufacturer's guide, 6.25 g · l^{-1}. Sesame seeds were soaked in 50°C warm water for 15 min, then taken out, incubated, and sown in a plastic tray in rows. When the seedlings reached two true leaves, five healthy plants were cultivated in each pot.

Experimental design

The pot experiment was arranged in a completely randomized block design, with 10 treatments and four replications, and one plant per each pot, giving a total of four plants. The treatments included (i) no addition of *Trichoderma* spp. and *Fusarium* sp., (ii) addition of only *F. oxysporum* F.28.1A, (iii) addition of *F. oxysporum* F.28.1A and chemical fungicides (Ridomil gold 68WG), (iv) addition of *F. oxysporum* F.28.1A and commercial production, *Trichoderma* sp., (v) addition of *F. oxysporum* F.28.1A and *T. yunnanense* T-02B1, (vi) addition of *F. oxysporum* F.28.1A and *T. lentiforme* T-18B2, (vii) addition of *F. oxysporum* F.28.1A and *T. asperellum* T-20B1, (viii) addition of *F. oxysporum* F.28.1A and *T. hamatum* T-28B1, (ix) addition of *F. oxysporum* F.28.1A and *T. hamatum* T-29A1 and (x) addition of *F. oxysporum* F.28.1A and a mixture of five fungal isolates (T-02B1, T-18B2, T-20B1, T-28B1 and T-29A1).

Experimental performance

10 ml of *F. oxysporum* F.28.1A spores were mixed into the sterilized soil at a density of 5×10^6 CUF · g^{-1} soil. After treatment, the soil pot was placed in a net house and irrigated with tap water once a day with a sprayer. Then, the soil was inoculated with 10 ml of *Trichoderma* spp. equivalent to the treatment 15, 30 and 45 days after planting (DAP). To the control treatment 10 ml of sterile DW was added. In the Mekong Delta, the recommended fertilizer formula for sesame is 90N-60P_2O_5-30K_2O. Phosphorus fertilizer was applied 1 day before sowing. The first application was 33.3% of nitrogen fertilizer plus 50% of potassium fertilizer at 10 DAP; the second application was 33.3% of nitrogen fertilizer at 30 DAP; and the third application was 33.3% of nitrogen fertilizer plus 50% of potassium fertilizer at 45 DAP.

Data collection

Classification of disease levels on sesame stems included disease level 1 – healthy plant; disease level 2 – plant wilt, which appears as discolored chlorophyll in the lower leaves; disease level 3 – necrosis on the lower leaves, as seen by yellowing leaves; and disease level 4 – the plant wilts and dies. Rate of diseased leaves: Method of calculating disease rate (RDL) (Ziedan *et al.* 2011) is:

$$\text{RDL}\ (\%) = A/B \times 100,$$

where: A – the number of infected sesame leaves, B – the total number of sesame plants in each treatment.

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using SPSS software package version 13.0, and comparison for significant differences between treatments was done using Duncan's test at $p < 0.05$.

Results

Isolation and selection of antagonistic *Trichoderma* spp. against *Fusarium oxysporum* F.28.1A causing wilt disease in sesame *in vitro*

Isolating Trichoderma spp. from soil samples

There were 26 isolates of *Trichoderma* spp. obtained from 20 soil samples of alluvial soil such as that found in dikes. Colonies were initially white (white-bright red, white-bright purple, white-bright yellow, and purely white), then turned green 48 h after incubation. The mycelia were slightly spongy, spread fast, and formed a concentric circle on the surface of Petri dishes. The fungal isolates developed long, thick conidiophores with branches. The peak of each branch had the shape of a short bottle. There were small quantities of egg-shaped (oval) conidia. Spores formed into many chlamydospores, gathering into small round and oval clusters at 72 h of incubation on PDA medium (Fig. 1). Most of the isolates were able to form many chlamydospores after 3 to 4 days of incubation (Fig. 1S, in supplementary material).

Growth rate of Trichoderma spp. on PDA medium

The mean growth rate of *Trichoderma* spp. isolates varied significantly at the 1% level after 24, 48 and 72 h of incubation. After 48 h of inoculation, isolates with broad colony diameters included T-29A1, T-25A2, T-25B2, T-18A1, T-18B1, T-02B1, T-29B1 and T-18B2. Among them, the isolates with the highest growth

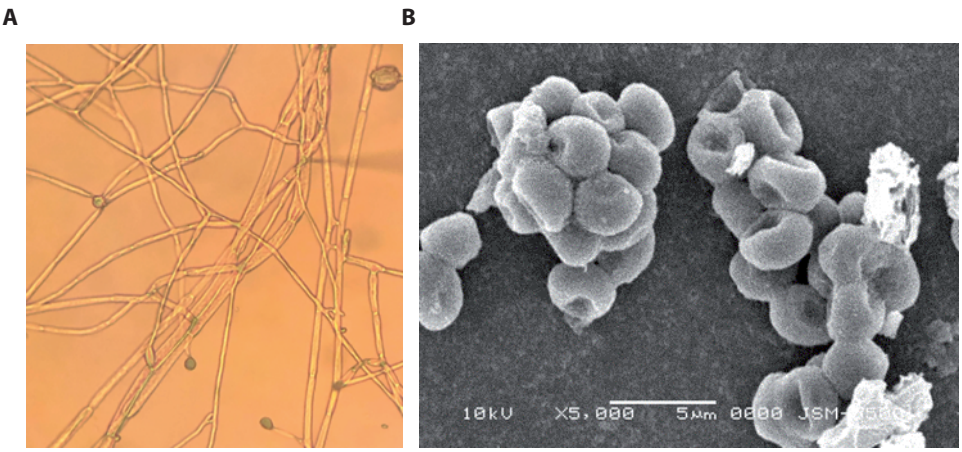

Fig. 1. Morphological characteristics of mycelium 72 h after incubation under a 40× optical microscope – A and chlamydospores forms of *Trichoderma asperellum* T-20B1 96 h after incubation under a Scanning Electron Microscopy – B

rate were T-18B1 (24.00 mm · day^{-1}) and T-28B1 (26.00 mm · day^{-1}) (Table 1).

Ability of *Trichoderma* spp. to antagonize *Fusarium oxysporum* F.28.1A *in vitro*

At 48 h after inoculation, the antagonistic efficacies of *Trichoderma* spp. isolates against *F. oxysporum* F.28.1A were insignificantly different. Nevertheless, at 72 h of inoculation, the efficacy values among isolates of *Trichoderma* spp. were statistically different at 5%, ranging from 42.3 to 65.5% (Table 2). In detail, an ability to repress the growth of *F. oxysporum* F.28.1A was found for *T. yunnanense* T-02B1, *T. lentiforme* T-18B2, *T. asperellum* T-20B1, and *T. hamatum* T-28B1 on PDA medium (Fig. 2S A–D, in supplementary material). At 168 h after inoculation on PDA medium, the pathogenic hyphae completely died out, *Trichoderma* spp. spread all over the Petri dishes, and *F. oxysporum* F.28.1A hyphae were deformed and covered the whole dish (Fig. 2). Based on the antagonistic efficiencies, the proportion of highly antagonistic isolates was 15.4%, including T-18B2 with the highest efficacy (65.5%), T-28B1 (62.7%), T-02B1 (61.9%), and T-20B1 (61.3%) after 72 h of inoculation. The isolates with higher than 50% antagonism were T-02A1, T-07B1, T-14A1, T-20A1, T-27A1, T-27B1, T-27B2, T-28A1, and T-29A2. However, the mean value came to only 34.6% (Table 2).

Table 1. Growth rate of each isolate of *Trichoderma* spp. on PDA medium

Strains	Growth rate of *Trichoderma* spp. [mm]			
	24 h	48 h	72 h	96 h
T–02A1	23.3 bcd	46.7 abc	63.3 c–f	88.3
T–02B1	23.7 a–d	44.3 a–d	67.3 b–e	88.0
T–07A1	21.3 cde	40.0 bcd	67.3 b–e	90.0
T–07B1	17.67 ef	40.0 bcd	63.3 c–f	90.0
T–14A1	23.0 bcd	42.0 a–d	66.7 b–e	87.7
T–14B1	22.3 bcd	45.7 a–d	66.3 cde	90.0
T–16A2	23.0 bcd	44.3 a–d	60.0 ef	90.0
T–16B2	23.3 bcd	45.3 a–d	65.7 cde	90.0
T–18A1	25.0 a–d	46.0 a–d	68.3 b–e	90.0
T–18B1	24.0 a–d	48.0 ab	67.7 b–e	90.0
T–18B2	24.0 a–d	49.3 ab	71.3 abc	90.0
T–19A2	20.3 de	42.3 a–d	67.3 b–e	90.0
T–19B2	23.3 bcd	47.0 abc	67.0 b–e	90.0
T–20A1	23.0 bcd	46.7 abc	66.0 cde	89.0
T–20B1	23.3 bcd	47.7 abc	69.3 a–d	88.0
T–25A2	27.0 ab	50.3 a	71.3 abc	88.0
T–25B2	25.7 abc	49.0 ab	77.6 a	90.0
T–27A1	21.0 cde	43.7 a–d	60.3 ef	90.0
T–27B1	23.3 bcd	44.7 a–d	62.0 def	84.3
T–27A2	23.7 a–d	45.7 a–d	66.3 cde	90.0
T–27B2	24.3 a–d	36.0 d	70.6 a–d	90.0
T–28A1	14.7 f	37.6 cd	55.7 f	90.0
T–28B1	22.3 bcd	48.3 ab	71.0 abc	90.0
T–29A1	28.3 a	48.3 ab	75.3 ab	90.0
T–29B1	25.3 a–d	48.7 ab	69.6 a–d	90.0
T–29A2	23.0 bcd	43.0 a–d	72.0 abc	90.0
Significant difference	*	*	*	ns
CV (%)	14.4	12.3	8.84	4.78

Values are means of four replications. Different lowercase letters in the same column indicate significant differences at $p < 0.05$ (*); and ns is no significant difference at $p > 0.05$

Table 2. Antagonistic efficacy of isolates *Trichoderma* spp. against *Fusarium oxysporum* F.28.1A causing wilt disease on sesame *in vitro*

Strains	Antagonistic efficacy [%]		Inhibition diameter of fungus *F. oxysporum* F.28.1A [cm]	
	48 h	72 h	48 h	72 h
Control	–	–	1.73 fg ± 0.06	4.73 a ± 0.20
T-02A1	5.7	58.5 bc	1.96 ef ± 0.06	1.97 gh ± 0.11
T-02B1	5.8	61.9 ab	1.80 fg ± 0.10	1.80 hi ± 0.17
T-07A1	17.3	45.8 fg	2.57 ab ± 0.03	2.56 bc ± 0.05
T-07B1	9.6	50.0 cde	2.37 bcd ± 0.08	2.37 cde ± 0.15
T-14A1	7.7	57.7 bc	2.00 ef ± 0.05	2.00 gh ± 0.10
T-14B1	17.3	47.9 efg	2.47 ab ± 0.12	2.46 bcd ± 0.20
T-16A2	15.4	47.9 efg	2.46 ab ± 0.15	2.46 bcd ± 0.25
T-16B2	11.5	47.9 efg	2.46 ab ± 0.09	2.47 bcd ± 0.15
T-18A1	15.4	47.9 efg	2.46 ab ± 0.03	2.47 bcd ± 0.05
T-18B1	11.5	46.5 fg	2.53 ab ± 0.03	2.53 bc ± 0.05
T-18B2	13.5	65.5 a	1.63 g ± 0.12	1.63 i ± 0.20
T-19A2	11.5	48.6 efg	2.43 abc ± 0.03	2.43 bcd ± 0.05
T-19B2	13.5	47.9 efg	2.47 ab ± 0.03	2.47 bcd ± 0.06
T-20A1	5.7	59.2 bc	1.93 ef ± 0.08	1.93 gh ± 0.15
T-20B1	9.6	61.3 ab	1.83 fg ± 0.09	1.93 gh ± 0.15
T-25A2	15.4	42.3 f	2.73 a ± 0.18	2.73 b ± 0.32
T-25B2	13.5	46.5 fg	2.53 ab ± 0.08	2.53 bc ± 0.15
T-27A1	9.6	59.9 abc	1.90 efg ± 0.06	1.90 ghi ± 0.10
T-27B1	11.5	57.7 bc	2.00 ef ± 0.06	2.00 gh ± 0.10
T-27A2	9.6	46.5 fg	2.53 ab ± 0.03	2.53 bc ± 0.06
T-27B2	9.6	50.0 cde	2.36 bcd ± 0.13	2.37 cde ± 0.23
T-28A1	9.6	54.9 cd	2.13 de ± 0.18	2.13 fg ± 0.30
T-28B1	9.6	62.7 ab	1.76 fg ± 0.03	1.76 hi ± 0.06
T-29A1	9.6	46.5 fg	2.53 ab ± 0.03	2.53 bc ± 0.06
T-29B1	9.6	49.3 cde	2.4 bcd ± 0.06	2.40 cde ± 0.10
T-29A2	13.5	54.2 cde	2.17 cde ± 0.03	2.17 efg ± 0.06
Significant difference	ns	*	*	*
CV (%)	51.8	13.8	15.3	12.5

Values are means of four replications. Different lowercase letters in the same column indicate significant differences at $p < 0.05$ (*); and ns is no significant difference at $p > 0.05$

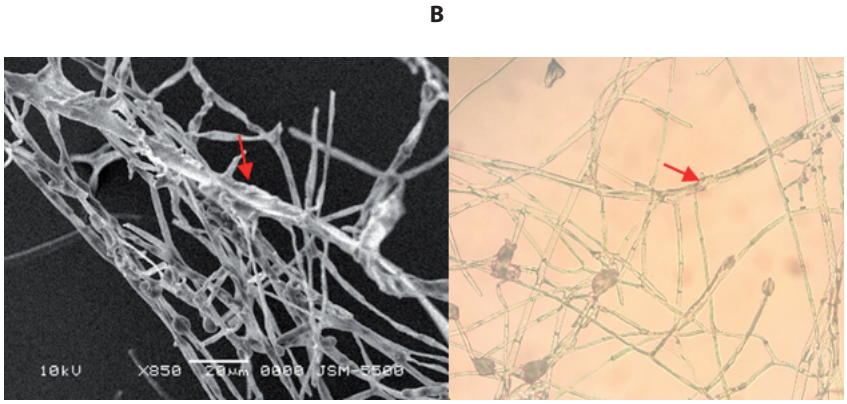

Fig. 2. *Trichoderma asperellum* T-20B1 wreathed and killed *Fusarium oxysporum* F.28.1A under observation by 40× optical microscope – A and Scanning Electron Microscopy at the end point of arrow – B

Ability of *Trichoderma* spp. isolates to secrete enzymes chitinase, endo-β-1,3-glucanase, exo-β-1,3-glucanase and cellulose *in vitro*

Ability to produce chitinase

The chitinase production of *Trichoderma* spp. isolates was statistically different at the 1% level. Isolates of *Trichoderma* spp. with chitinase amounts lower than 0.3 UI · ml^{-1} consisted of T-14A1, T-25A2, T-27A1, and T-28A1, making up 15.4% of the total. Meanwhile, isolates with high chitinase content accounted for 84.6%. Among them, five isolates of *Trichoderma* spp. had the highest chitinase enzyme production and were ranked in the following order: T-29A1 > T-28B1 ~ T-18B2 ~ T-20B1 ~ T27A2, with a corresponding chitinase concentration of 0.44 > 0.39 ~ 0.38 ~ 0.36 ~ 0.34 UI · ml^{-1}, respectively. Most importantly, strain T-29A1 produced the highest quantity of chitinase (Table 3).

Ability to produce endo-β-1,3-glucanase

Isolates of *Trichoderma* spp. with the ability to secrete a large amount of endo-β-1,3-glucanase included T-18B1, T-07A1, T-14B1, and T-18B2, whose enzyme content was 0.163, 0.135, 0.132, and 0.121 UI · ml^{-1}, respectively, and the differences were insignificant. However, low endo-β-1,3-glucanase production was found in isolates T-20B1 (0.032 UI · ml^{-1}), T-25B2 (0.038 UI · ml^{-1}), and T-29A1 (0.046 UI · ml^{-1}) (Table 3).

Ability to produce exo-β-1,3-glucanase

The quantity of exo-β-1,3-glucanase enzyme in isolate T-07B1 (0.060 UI · ml^{-1}) was the highest among all isolates. Five selected isolates (T-02B1, T-18B2, T-20B1,

Table 3. Ability of isolates *Trichoderma* spp. to produce chitinase, endo-β-1,3-glucanase, exo-β-1,3-glucanase and cellulose *in vitro*

Strains	Chitinase [UI · ml^{-1}]	Endo-β-1,3-glucanase [UI · ml^{-1}]	Exo-β-1,3 glucanase [UI · ml^{-1}]	Cellulase [cm]
T-02A1	0.31 efg	0.088 b–f	0.024 de	1.13 ikl
T-02B1	0.34 cde	0.068 d–g	0.026 cde	2.03 bcd
T-07A1	0.31 efg	0.135 ab	0.025 cde	1.07 kl
T-07B1	0.32 d–g	0.039 efg	0.060 a	1.17 h–l
T-14A1	0.29 gh	0.068 d–g	0.027 b–e	1.60 e–k
T-14B1	0.31 efg	0.132 ab	0.016 de	1.23 e–l
T-16A2	0.33 c–f	0.077 c–g	0.020 de	2.20 bcd
T-16B2	0.31 efg	0.056 d–g	0.022 de	1.70 d–i
T-18A1	0.31 efg	0.064 d–g	0.036 bcd	1.83 bcd
T-18B1	0.32 d–g	0.163 a	0.030 b–e	1.23 e–l
T-18B2	0.38 b	0.121 abc	0.030 b–e	1.67 d–i
T-19A2	0.33 c–f	0.071 c–g	0.022 de	1.80 c–g
T-19B2	0.31 efg	0.067 d–g	0.027 b–e	0.90 k
T-20A1	0.34 cde	0.079 c–g	0.024 de	1.37 f–l
T-20B1	0.36 bc	0.032 g	0.008 e	1.60 e–k
T-25A2	0.271 hi	0.060 d–g	0.030 b–e	1.73 c–h
T-25B2	0.31 efg	0.038 fg	0.050 ab	3.23 a
T-27A1	0.25 i	0.0911 be	0.031 bcd	1.00 l
T-27B1	0.32 d–g	0.063 d–g	0.023 de	1.57 e–k
T-27A2	0.35 bcd	0.063 d–g	0.023 de	1.33 f–l
T-27B2	0.32 efg	0.081 c–g	0.027 b–e	1.33 f–l
T-28A1	0.29 fgh	0.069 d–g	0.019 de	1.67 d–i
T-28B1	0.39 b	0.106 bcd	0.034 bcd	1.17 h–l
T-29A1	0.44 a	0.046 efg	0.017 de	1.73 c–h
T-29B1	0.33 c–f	0.062 d–g	0.047 abc	2.63 b
T-29A2	0.31 efg	0.082 c–g	0.026 cde	2.27 bc
Significant difference	*	*	*	*
CV (%)	12.2	48.9	51.4	35.4

Values are means of four replications. Different lowercase letters in the same column indicate significant differences at $p < 0.05$ (*)

T-28B1, and T-29A1) possessed an exo-β-1,3-glucanase content of 0.008–0.034 UI · ml^{-1} (Table 3).

Ability to produce cellulase

Isolate T-25B2, with a halo diameter of 3.23 cm, secreted the largest amount of cellulose. The following ones had the largest amounts: T-02B1, T-16A2, T-18A1, T-29B1, and T-29A2, whose halo diameter values were 2.03, 2.20, 1.83, 2.63, and 2.27 cm, respectively (Table 3). The halo diameter of selected isolates was illustrated in Fig. 3S (in supplementary material).

Identification of Trichoderma spp.

The neighbor-joining phylogenetic tree was reconstructed based on ITS gene sequences of the selected strains, including the closely related strains from the GenBank database with a similarity of 96–100%. Specifically, the percentage of similarity of *T. yunnanense* T-02B1 and *T. hamatum* T-29A1 was 96%; and that of *T. hamatum* T-28B1, *T. lentiforme* T-18B2, and *T. asperellum* T-20B1 was 100%, with accession numbers MZ812829, MZ812833, MZ812832, MZ812830, and MZ812831, respectively. *Aspergillus flavus* strain 14-1 (AF033802.1) was considered as the outgroup strain (Fig. 3).

Efficacy of *Trichoderma* spp. supplementation in preventing sesame wilt disease caused by *Fusarium oxysporum* F.28.1A under greenhouse conditions

Influence of the selected Trichoderma spp. supplement on wilt disease levels induced by Fusarium oxysporum F.28.1A on sesame

Symptoms of the wilt disease did not appear until the 7th day after infection. However, on day 11, disease levels varied significantly at 5% between the treatments. The negative control treatment infected with *F. oxysporum* F.28.1A showed the highest level of wilt disease (1.1), with symptomatic withered leaves. Treatments inoculated with *T. asperellum* T-02B1, *T. lentiforme* T-18B2, or *T. hamatum* T-28B1 individually had lower disease levels (0.2). Nevertheless, during this period, disease symptoms were absent from plants treated with *T. hamatum* T-29A1. On day 14 after infection, differences in disease levels were insignificant between treatments, and the levels fluctuated from 1.0 to 1.5. Between days 21 and 28 after infection, there were remarkable differences at the 5% level in disease among treatments, with 50% of the leaves being yellow for the sesame plants. On day 35 after infection, in plants treated with T-02B1 and the positive control treatment,

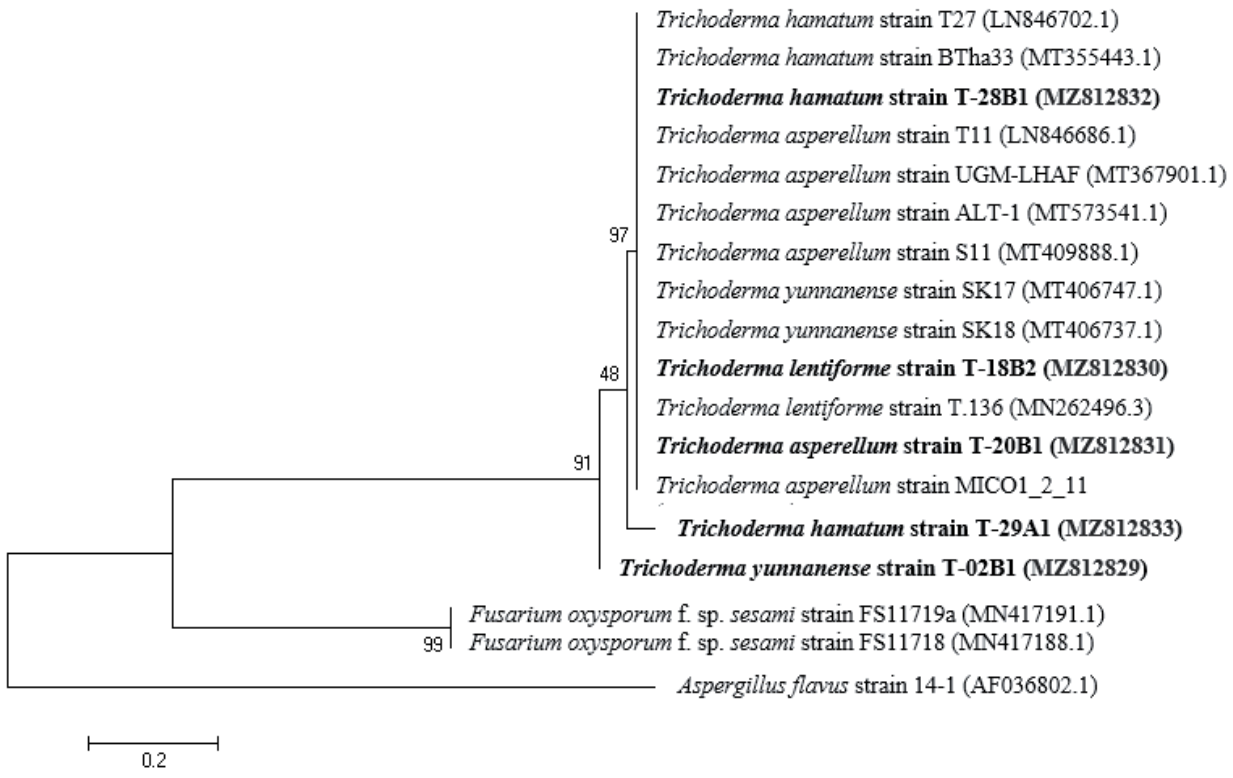

Fig. 3. Neighbor-joining phylogenetic trees based on ITS sequences of five selected strains *Trichoderma* spp. compared to the closely related strains in the GenBank database. The percentage levels of bootstrap analysis of 1,000 replicates are indicated at each node. Bar, 0.2 substitutions per nucleotide position. *Aspergillus flavus* strain 14–1 was used as the outgroup strain. Access numbers of GenBank sequences are implied in brackets

Trichoderma sp. had significantly lower disease levels, ranging from 2.7 to 2.8, than the negative one, which received inoculation only from *F. oxysporum* F.28.1A (3.7) (Table 4). Levels of the wilt disease are also illustrated in Fig. 4S (in supplementary material).

Influence of the selected Trichoderma spp. supplement on the rate of wilt disease caused by Fusarium oxysporum on sesame

On day 11 after infection, sesame plants in every treatment had wilting symptoms from the stem to the peak, and the rates ranged from 5 to 20%. However, on the 14th and 35th days after infection, disease rates differed significantly at the 5% level in treatments. On day 35, the disease rate for treatment with a mixture of *Trichoderma* spp. (65%) was statistically the same as the positive control treatment using commercial fungi *Trichoderma* sp. (60%). Both had noticeably lower disease rates than the negative control treatment infected only by *F. oxysporum* F.28.1A (100%) and were equivalent to that of the chemical fungicide method (80%) (Table 5).

Table 4. Effects of the selected *Trichoderma* spp. supplementation on wilt disease levels induced by *Fusarium oxysporum* F.28.1A on sesame under greenhouse conditions

Treatment	Days after *F. oxysporum* F.28.1A infection				
	11	14	21	28	35
No *Trichoderma* spp. + No *F. oxysporum* F.28.1A	0.6 abc	1.3	1.8 ab	2.6 a–d	3.6 ab
Negative control, *F. oxysporum* F.28.1A	1.1 a	1.5	2.3 a	2.7 abc	3.7 ab
Chemical fungicide	0.9 ab	1.2	1.7 ab	2.1 cde	3.2 abc
Positive control, *Trichoderma* sp. DHCT	0.9 ab	1.0	1.3 b	2.0 de	2.8 c
T. yunnanense T-02B1	0.2 c	1.5	1.7 ab	1.9 e	2.7 c
T. lentiforme T-18B2	0.2 c	1.2	1.7 ab	2.0 cd	3.2 abc
T. asperellum T-20B1	1.1 a	1.2	1.4 b	2.4 a–d	3.1 bc
T. hamatum T-28B1	0.1 c	1.0	1.7 ab	3.0 a	3.8 a
T. hamatum T-29A1	0.0 c	1.0	1.7 ab	2.8 ab	3.5 ab
A mixture of 5 strains	0.5 bc	1.0	1.6 b	3.1 a	3.4 ab
Significant difference	*	ns	*	*	*
CV (%)	11.5	32.1	24.7	23.2	14.7

Values are means of four replications. Different lowercase letters in the same column indicate significant differences at $p < 0.05$ (*); and ns is no significant difference at $p > 0.05$

Table 5. Effects of the selected *Trichoderma* spp. supplementation on the rate of wilt disease caused by *Fusarium oxysporum* F.28.1A on sesame under greenhouse conditions

Treatment	Days after *F. oxysporum* F.28.1A infection				
	11	14	21	28	35
No *Trichoderma* spp. + No *F. oxysporum* F.28.1A	10	15 bc	30 ab	70 a	95 a
Negative control, *F. oxysporum* F.28.1A	15	30 abc	40 ab	70 a	100 a
Chemical fungicide	20	25 abc	25 ab	65 ab	80 abc
Positive control, *Trichoderma* sp. DHCT	15	20 abc	30 ab	35 ab	60 c
T. yunnanense T-02B1	25	40 ab	55 a	75 a	95 a
T. lentiforme T-18B2	25	45 a	60 a	70 a	90 ab
T. asperellum T-20B1	20	25 abc	35 ab	70 a	90 ab
T. hamatum T-28B1	5	5 c	40 ab	60 ab	85 abc
T. hamatum T-29A1	5	5 c	10 b	60 ab	85 abc
A mixture of 5 strains	10	10 c	10 b	25 b	65 b
Significant difference	ns	*	*	*	*
CV (%)	91.6	49.2	47.2	22.9	10.1

Values are means of four replications. Different lowercase letters in the same column indicate significant differences at $p < 0.05$ (*); and ns is no significant difference at $p > 0.05$.
Chemical fungicide was used Ridomil gold 68WG

Discussion

Twenty-six isolates of *Trichoderma* spp. were derived from 20 samples of rhizospheric soil from cultivated sesame in four communes (Binh Phu, Binh My, Binh Long, and Vinh Thanh Trung) and Cai Dau town, Chau Phu district, An Giang Province. Out of all the isolates, the highest antagonistic fungi came from Binh My (15.4%), Binh Phu (57.7%), and Cai Dau town (26.9%). The average growth rate of the isolates was 21.95 mm · day^{-1}, and the rate peaked at 48–72 h after inoculation (22.24 mm · day^{-1}). The isolates with the highest growth rate were T-27B2 (34.67 mm · day^{-1}), T-25B2 (28.67 mm · day^{-1}), T-29A2 (29.00 mm · day^{-1}), and T-29A1 (27.00 mm · day^{-1}) (Table 2). These results were in accordance with *T. harzianum's* growth rate on PDA medium (22.86 mm · day^{-1}) (Kim *et al.* 2019). Similarly, according to Matrood *et al.* (2020), *T. harzianum* has a mean diameter of 42.5–56.5 mm at 25–30°C, 72 h after inoculation. After 96 h of inoculation, mycelia from the isolates covered the surface of the Petri dishes and differed insignificantly from each other, with a growth rate of 20.44 mm · day^{-1}. The mycelia grew and over-covered the dishes' surfaces after 4 to 5 days of inoculation, and the hyphae turned bright or dark green in color. The morphological features described in this study were identical for *Trichoderma* spp. In the same way, hyphae of *Trichoderma* spp. had soft fibers, lean growth, and a green color *in vitro* (Sundaramoorthy and Balabaskar 2013; Kotasthane *et al.* 2015).

All 26 isolates of *Trichoderma* spp. were able to antagonize *F. oxysporum* F.28.1A (Table 3; Fig. 4S – in supplementary material). According to Puyam *et al.* (2013), after 7 days of inoculation, the isolates have an antagonistic rate of 100%. Hyphae of *Fusarium* spp. began dying after 8 to 10 days of observation (Fig. 2). *Trichoderma* spp. are capable of breaking lipid membranes and participating in antagonistic activity against pathogenic fungi (Saba *et al.* 2015).

Antagonistic efficacy and chitinase producing capacity in the selected isolates ranged from medium to high. This result was consistent with Akrami *et al.* (2011), who found that the antagonistic efficacy of *T. asperellum* and *T. harzianum* against *F. oxysporum* causing wilt disease on lentils was 68–71%. The antagonistic mechanism may be due to the fact that chitinase is able to cut oligomer-chitin into monomer chitin, resulting in *Trichoderma* spp. controlling pathogenic fungi (Silva *et al.* 2019). In this study, the fast growth rate of *Trichoderma* spp. hyphae eliminated pathogenic hyphae on day 4 after inoculation. Thus, the selected isolates had the potential to control *F. oxysporum* F.28.1A causing wilt disease on sesame. Furthermore, *Trichoderma* destroys the cell walls of pathogenic fungi via the combination of chitinase, endo-β-1,3-glucanase, and exo-β-1,3-glucanase and increases regressive efficiency by degrading cell walls and deforming pathogenic hyphae and cells (Kaur *et al.* 2021). As reported by Edison *et al.* (2018), *Trichoderma* secretes enzymes in two forms: endo--β-1,3-glucanase and exo-β-1,3-glucanase, which cut glucose-containing segments from the unreduced ends of polymers and oligomers, leading to the formation of glucose that breaks cells. In this study, exo--β-1,3-glucanase (UI · ml^{-1}) content secreted from the isolates ranged from 0.016 to 0.060 UI · ml^{-1}. Among the isolates, T-02B1 had an exo-β-1,3-glucanase content of 0.026 UI · ml^{-1}, T-18B2 had 0.030 UI · ml^{-1}, T-20B1 had 0.008 UI · ml^{-1}, T-28B1 had 0.034 UI · ml^{-1}, and T-29A1 had 0.017 UI · ml^{-1}. Additionally, the isolates with a moderately high amount of exo-β-1,3-glucanase were T-07B1 (0.060 UI · ml^{-1}), T-25B2 (0.050 UI · ml^{-1}) and T-29B1 (0.047 UI · ml^{-1}). The exo-β-1,3-glucanase production is proportional to the amount of glucan appearing in degradation reactions (Kumar *et al.* 2018). Furthermore, cellulose production was revealed by the mean halo diameter, which ranged from 1.83 to 3.23 cm (Table 3). The more cellulase produced, the better cellulose degradation the *Trichoderma* spp. isolate had (Li *et al.* 2019).

The identification results of five isolates at 96–100% similarity included *T. yunnanense* T-02B1, *T. lentiforme* T-18B2, *T. asperellum* T-20B1, *T. hamatum* T-28B1, and *T. hamatum* T-29A1 (Fig. 3). Previous studies have shown that *T. hamatum* is popularly applied in treating diseases on sesame. In detail, *in vitro*, *T. hamatum* has an antagonistic activity against *F. oxysporum* f. sp. *sesame* causing wilt disease on sesame at a rate of 76.6% (Selima 2018). According to El-Fiki *et al.* (2004). *T. hamatum* is used to prevent and cure sesame charcoal rot disease caused by *Mycorrhizae phaseolina* at the rate of 80% in Egypt. Moreover, *T. hamatum*is is applied to treat charcoal rot disease caused by *M. phaseolina* and wilt disease caused by *F. oxysporum* on sesame under greenhouse conditions (Mahdy *et al.* 2007).

In the present study, despite the application of five *Trichoderma* spp. isolates for treating *F. oxysporum* F.28.1A in sesame, the plants caught the disease on the 23rd day after infection. Therefore, treatments including *Trichoderma* spp. isolates had good antagonistic activity against pathogen performance 7–21 days after infection. Strains T-18B2 and T-02B1 dominantly expressed disease antagonism, compared with other isolates.

Trichoderma asperellum is applied to treat *Alternaria* leaf spot disease on sesame, reducing disease rate 15 days after inoculation (Meena and Ezhilarasi 2019). Furthermore, *T. asperellum* reaches an antagonistic efficacy of 78.6% against *F. oxysporum* causing wilt

disease on cucumber (Mei *et al.* 2019). *T. asperellum* is successfully applied to reduce *Fusarium* spp. disease rate, at a level above 85%, by increasing the activities of phenol, peroxidase, and polyphenoloxidase to protect and stimulate tomato growth (Patel *et al.* 2017). It is interesting that in this study, *T. lentiforme* was found in alluvial soil from dikes for sesame-rice rotation, which was novel in research about pathogens in sesame in Vietnam. However, the antagonism of *T. lentiforme* against *F. oxysporum* f. sp. *niveum* causing wilt disease in watermelon is about 67.0% (Nuangmek *et al.* 2021).

According to Cruz-Quiroz *et al.* (2018), *T. yunnanense* produces exo-glucanase (1.7 Ul · g^{-1}), endo-glucanase (32.8 Ul · g^{-1}), and chitinase (18.3 Ul · g^{-1}) and can control *P. capsici* and *C. gloeosprioides in vitro*. Nevertheless, Yu *et al.* (2007) claims that the genus of *T. yunnanense* still belongs to *T. hamatum*. As reported by Oljira *et al.* (2020), isolation and identification of *T. yunnanense* Th4 on rice-cultivating soil improves indole-3-acetic acid production, which enhanced the growth and tolerance of rice on acidic soil. However, an isolation of *T. yunnanense* for antagonism against wilt disease on sesame has not been found. This was the first report to reveal that *T. yunnanense* can control *F. oxysporum* causing wilt disease in sesame. Moreover, *Trichoderma producing* cell-wall-degrading enzymes (chitnase, protease, and β-glucanases) contributed to antagonist activity against *F. graminearum* (Li *et al.* 2016). Thus, biological control agents exhibit potential against pathogens and remarkably enhance photosynthesis, plant growth, and nutrient use efficiency to significantly improve crop yields (Asad 2022).

On the other hand, the disease levels in the mixed *Trichoderma* treatment and treatments with neither T-28.1B nor T-29A1 increased dramatically (3.1 ~ 3.0 ~ 2.8, respectively) 28 days after infection. This was equal to the level in the negative control treatment (2.7) (Table 4). This indicated that the antagonistic mechanism was better in the early stages. On the 35th day after infection, plants with an extremely high disease level were inoculated with T-28.1B, T-29A1, and a mixture of the five selected isolates; the values were 3.8 ~ 3.5 ~ 3.4, respectively, and were equivalent to the negative treatment with only *F. oxysporum* F.28.A1 (3.7). However, during the development of the disease inside the stem, the progress from the advent of the disease to leaf loss and death happened rapidly 7–10 days after disease incubation.

Under greenhouse conditions, 20–25 days after infection, the pathogen attacked bark cells, invaded xylem tissue, deformed parenchymal cells of plant bark, browned stems, and withered leaves. According to Ahmed *et al.* (2017), *Trichoderma* spp. can trigger a rhizosphere defense mechanism via direct interaction between hyphae and epidermal root cells to protect cultivars. Furthermore, internal observation of infected cells showed that pathogenic hyphae blocked the vascular system that carried water and nutrients to over-ground parts of plants, leading to plants withering quickly (Ara *et al.* 2017). Based on the number of dead plants 21–28 days after infection, supplementation with *Trichoderma* spp. was able to ease the toxicity of the pathogenic fungus. Similarly, application of *T. Harzianum* and *T. viride* reduces the damage to sesame by 74–77% under greenhouse conditions (Mahmoud and Abdalla 2018).

Conclusions

Five potential strains of *Trichoderma* spp. with antagonism against *F. oxysporum* F.28.1A, including *T. yunnanense* T-02B1, *T. lentiforme* T-18B2, *T. asperellum* T-20B1, *T. hamatum* T-28B1, and *T. hamatum* T-29A1, were highly efficient at antagonizing F.28.1A, with rates of 61.3–65.5% on sesame. The production of chitinase in the selected isolates ranged from 0.34 to 0.44 UI · ml^{-1}. Additionally, exo-β-1,3-glucanase content was about 0.017–0.034 UI · ml^{-1} and endo-β--1,3-glucanase was quantified at 0.032–0.121 UI · ml^{-1}. The selected isolates were capable of secreting enzymes that degraded cell walls, which is considered an effective mechanism to control pathogens *in vitro*. The application of either *T. hamatum* T-28B1 and T-29A1 or the mixture of the five selected isolates of *Trichoderma* spp. (T-02B1, T-18B2, T-20B1, T28B1 and T-29A1) was effective in reducing prevalence and disease levels under unsterilized soil conditions. The efficacy of the five selected *Trichoderma* spp. isolates in controlling *F. oxysporum* F.28.1A should be evaluated in sesame-cultivating fields.

Acknowledgements

This project was funded by the author's affiliated institution.

References

Ahmed H.A., Abdel-Gayed M.A. 2017. Safe approach to control *Fusarium oxysporum* in sesame crop. Zagazig Journal of Agricultural Research 44: 2529–2540. DOI: 10.21608/ZJAR.2017.51339

Akrami M., Golzary H., Ahmadzadeh M. 2011. Evaluation of different combinations of *Trichoderma* species for controlling *Fusarium* rot of lentil. African Journal of Biotechnology 10 (14): 2653–2658. DOI: 10.5897/AJB10.1274

Ara A., Akram A., Ajmal M., Akhund S., Nayyar B.G., Seerat W., Chaudhry S.M. 2017. Histopathological studies of sesame (*Sesamum indicum*) seedlings infected with *Fusarium oxy-*

sporum. Plant Pathology & Quarantine 7 (1): 82–90. DOI 10.5943/ppq/7/1/10

Asad S.A. 2022. Mechanisms of action and biocontrol potential of *Trichoderma* against fungal plant diseases – A review. Ecological Complexity 49: 100978. DOI: 10.1016/j.ecocom.2021.100978

Belay Y. 2018. Screening of *Fusarium* wilt, bacterial blight and phyllody diseases resistant sesame genotypes in sesame growing areas of northern Ethiopia. Journal of Agriculture and Ecology Research International 15 (2): 1–12. DOI: 10.9734/JAERI/2018/41414

Bhat M.N., Mesta R., Yenjerappa S.T., Tatagar M.H., Sardana H.R., Singh D., Vennila S., Sabir N., Ahmad M. 2016. Biological control of *Fusarium* wilt of chillies using *Trichoderma* spp. Indian Journal of Horticulture 73 (1): 74–77. DOI: 10.5958/0974-0112.2016.00021.9

Bruce A., Srinivasan U., Staines H.J., Highley T.L. 1995. Chitinase and laminarinase production in liquid culture by *Trichoderma* spp. and their role in biocontrol of wood decay fungi. International Biodeterioration & Biodegradation 35 (4): 337–353. DOI: 10.1016/0964-8305(95)00047-3

Dong-Hua L., Lin-Hai W., Yan-Xin Z., Hai-Xia L., Xiao-Qiong Q., Wen-Liang W., Xiu-Rong Z. 2012. Pathogenic variation and molecular characterization of *Fusarium* species isolated from wilted sesame in China. African Journal of Microbiology Research 6: 149–154. DOI: 10.5897/AJMR11.1081

Edison L.K., Shiburaj S., Pradeep N.S. 2018. Microbial beta glucanase in agriculture. Advances in microbial biotechnology: Current trends and future prospects. Apple Academic Press. 590 pp.

Egonyu J.P., Kyamanywa S., Anyanga W., Ssekabembe C.K. 2005. Review of pests and diseases of sesame in Uganda. African Crop Science Conference Proceedings 7 (3): 1411–1416.

El-Fiki A.I.I., Mohamed F.G., El-Deeb A.A., Khalifa M.M.A. 2004. Some applicable methods for controlling sesame charcoal rot disease (*Macrophomina phaseolina*) under greenhouse conditions. Egyptian Journal of Phytopathology 32: 87–101.

FAOSTAT. Food and Agriculture Organization Statistical Databases. 2021. [Available on: http://faostat.fao.org/] [Accessed on: 19 June 2022].

Jyothi B., Ansari N.A., Vijay Y., Anuradha G., Sarkar A., Sudhakar R., Siddiq E.A. 2011. Assessment of resistance to *Fusarium* wilt disease in sesame (*Sesamum indicum* L.) germplasm. Australasian Plant Pathology 40 (5): 471–475. DOI: 10.1007/s13313-011-0070-x

Kakraliya S., Choskit D., Pandit D., Abrol S., 2017. Effect of bioagents, neem leaf extract and fungicides against *Alternaria* leaf blight of wheat (*Triticum aestivum* L.). Natural Products Chemistry & Research 6: 23–34. DOI: 10.4172/2329-6836.1000295

Kaur R., Kalia A., Lore J.S., Kaur A., Yadav I., Sharma P., Sandhu J.S. 2021. *Trichoderma* sp. endochitinase and β-1, 3-glucanase impede *Rhizoctonia solani* growth independently, and their combined use does not enhance impediment. Plant Pathology 70 (6): 1388–1396. DOI: 10.1111/ppa.13381

Khamari B., Beura S.K., Ranasingh N. 2018. Status of sesame diseases grown in different agroclimatic zones of Odisha. International Journal of Current Microbiology and Applied Sciences 7 (11): 945–948. DOI: 10.20546/ijcmas.2018.711.110

Kim J.Y., Kwon H.W., Lee D.H., Ko H.K., Kim S.H. 2019. Isolation and characterization of airborne mushroom damaging *Trichoderma* spp. from indoor air of cultivation houses used for oak wood mushroom production using sawdust media. The Plant Pathology Journal 35 (6): 674–683. DOI: 10.5423/PPJ.FT.10.2019.0261

Komarek M., Cadkova E., Chrasny V., Boras F., Bollinger J. 2010. Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. Environment International 36 (1): 138–151. DOI: 10.1016/j.envint.2009.10.005

Kotasthane A., Agrawal T., Kushwah R., Rahatkar O.V. 2015. *In-vitro* antagonism of *Trichoderma* spp. against *Sclerotium rolfsii* and *Rhizoctonia solani* and their response towards growth of cucumber, bottle gourd and bitter gourd. European Journal of Plant Pathology 141 (3): 523–543. DOI: 10.1007/s10658-014-0560-0

Kumar M., Brar A., Yadav M., Chawade A., Vivekanand V., Pareek N. 2018. Chitinases potential candidates for enhanced plant resistance towards fungal pathogens. Agriculture 8: 88–100. DOI: 10.3390/agriculture8070088

Levy O.N., MellerHarel Y., Haile Z.M., Elad Y., Rav-David E., Jurkevitch E., Katan J. 2015. Induced resistance to foliar diseases by soil solarization and *Trichoderma harzianum.* Plant Pathology 64 (2): 365–374. DOI: 10.1111/ppa.12255

Li C., Pang A.P., Yang H., Roụing Lv., Zhou Z., Wu F.G., Lin F. 2019. Tracking localization and secretion of cellulase spatiotemporally and directly in living *Trichoderma reesei.* Biotechnol Biofuels 12: 1–12. DOI: 10.1186/s13068-019-1538-0

Li Y., Sun R., Yu J., Saravanakumar K., Chen J. 2016. Antagonistic and biocontrol potential of *Trichoderma asperellum* ZJSX5003 against the maize stalk rot pathogen *Fusarium graminearum*. Indian Journal Microbiology 56 (3): 318–327. DOI: 10.1007/s12088-016-0581-9

Mahdy A.M.M., Nawal A.E., Faten M., El-Wakil A.A. El-Wakil D.A. 2007. Effect of antagonistic fungi on the growth and control of *Macrophomina phaseolina* and *Fusarium oxysporum* the causal agents of charcoal rot and wilt of sesame. Annals of Agriculture Science, Moshtohor 45: 577–585.

Mahmoud A.F., Abdalla O.A. 2018. Biocontrol efficacy of *Trichoderma* spp. against sesame wilt caused by *Fusarium oxysporum* f. sp. *sesami*. Archives of Phytopathology and Plant Protection 51: 277–287. DOI: 10.1080/03235408.2018.1471837.

Matrood A.A., Khrieba M.I., Okon O.G. 2020. Synergistic interaction of *Glomus mosseae* T. and *Trichoderma harzianum* R. in the induction of systemic resistance of *Cucumis sativus* L. to *Alternaria alternata.* Plant Science Today 7 (1): 101–108. DOI: 10.14719/pst.2020.7.1.629

Mei L.I., Hua L.I.A.N., Su X.L., Ying T.I.A.N., Huang W.K., Jie M.E.I., Jiang X.L. 2019. The effects of *Trichoderma* on preventing cucumber *fusarium* wilt and regulating cucumber physiology. Journal of Integrative Agriculture 18 (3): 607–617. DOI: 10.1016/S2095-3119(18)62057-X

Monteiro V.N., do Nascimento Silva R., Steindorff A.S., Costa F.T., Noronha E.F., Ricart C.A.O., de Sousa M.V., Vainstein M.H., Ulhoa C.J. 2010. New insights in *Trichoderma harzianum* antagonism of fungal plant pathogens by secreted protein analysis. Current Microbiology 61: 298–305. DOI: 10.1007/s00284-010-9611-8

Myint D., Gilani S.A., Kawase M., Watanabe K.N. 2020. Sustainable sesame (*Sesamum indicum* L.) production through improved technology: An overview of production, challenges, and opportunities in Myanmar. Sustainability 12 (9): 3515–3527. DOI: 10.3390/su12093515

Nehra S., Gothwal R.K., Varshney A.K., Solanki P.S., Chandra S., Meena P., Trivedi P.C., Ghosh P. 2021. Biomanagement of *Fusarium* spp. associated with oil crops. p. 453–474. In: "Microbiome Stimulants for Crops" (J. White, A. Kumar, S. Droby, eds.). Woodhead Publishing. DOI: 10.1016/B978-0-12-822122-8.00026-1

Nelson N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. Journal of Biological Chemistry 153 (2): 375–380. DOI: 10.1016/S0021-9258(18)71980-7

Nuangmek W., Aiduang W., Kumla J., Lumyong S., Suwannarach N. 2021. Evaluation of a newly identified endophytic fungus, *Trichoderma phayaoense* for plant growth promo-

tion and biological control of gummy stem blight and wilt of Muskmelon. Frontiers Microbiology 12: 410-424. DOI: 10.3389/fmicb.2021.634772.

Oljira A.M., Hussain T., Waghmode T.R., Zhao H., Sun H., Liu X., Wang X., Liu B. 2020. *Trichoderma* enhances net photosynthesis, water use efficiency, and growth of wheat (*Triticum aestivum* L.) under salt stress. Microorganisms 8: 1565–1583. DOI: 10.3390/microorganisms8101565

Orpin C.G. 1977. The occurrence of chitin in the cell walls of the rumen organisms *Neocallimastix frontalis*, *Piromonas communis and Sphaeromonas communis*. Journal of General Microbiology 99: 215–218. DOI: 10.1099/00221287-99-1-215

Parikh K., Jha A. 2012. Biocontrol features in an indigenous bacterial strain isolated from agricultural soil of Gujarat, India. Journal of Soil Science and Plant Nutrition 12 (2): 245–252. DOI: 10.4067/S0718-95162012000200004.

Patel S., Saraf M. 2017. Biocontrol efficacy of *Trichoderma asperellum* MSST against tomato wilting by *Fusarium oxysporum* f. sp. *lycopersici*. Archives of Phytopathology and Plant Protection 50(5-6): 228-238. DOI: 10.1080/03235408.2017.1287236.

Puyam A., Srivastava M., Singh A. 2013. Effect of different physiological parameters on growth and sporulation of *Trichoder maviride*. Plant Disease Research 28 (2): 146–151.

Rahman A., Bhattarai S., Akbar D., Thomson M., Trotter T., Timilsina S. 2020. Market analysis of sesame seed. CQUniversity. Report. DOI: https://doi.org/10.25946/15079245.v1

Rai D., Tewari A.K. 2016. Evaluation of different carbon and nitrogen sources for better growth and sporulation of T. *harzianum* (Th14). Journal of Agricultural Biotechnology and Sustainable Development 8 (8): 67–70. DOI: 10.5897/JABSD2016.0262

Ramada M.H.S., Steindorff A.S., Bloch Jr C., Ulhoa C.J. 2016. Secretome analysis of the mycoparasitic fungus *Trichoderma harzianum* ALL 42 cultivated in different media supplemented with *Fusarium solani* cell wall or glucose. Proteomics 16: 477–490. DOI: 10.1002/pmic.201400546.

Saba H., Vibhash D., Manisha M., Prashant K.S., Farhan H., Tauseef A. 2012. *Trichoderma* a promising plant growth stimulator and biocontrol agent. Mycosphere 3 (4): 524–531. DOI 10.5943/mycosphere/3/4/14

Sallam N.M.A., Eraky A.M.I., Sallam A. 2019. Effect of *Trichoderma* spp. on *Fusarium* wilt disease of tomato. Molecular Biology Report 46: 4463–4470. DOI: 10.1007/s11033-019-04901-9.

Selima K. 2018. Integrated disease management strategy of *Fusarium oxysporum* f. sp. *sesami* causing wilt of sesame. Journal Mycopathology Research 56: 89–99.

Silva R.N., Monteiro V.N., Steindorff A.S., Gomes E.V., Noronha E.F., Ulhoa C.J. 2019. *Trichoderma*/pathogen/plant interaction in pre-harvest food security. Fungal Biology 123: 565–583. DOI: 10.1016/j.funbio.2019.06.010

Su D., Ding L., He S. 2018. Marine-derived *Trichoderma* species as a promising source of bioactive secondary metabolites. Mini Reviews in Medicinal Chemistry 18: 1702–1713. DOI: 10.2174/1389557518666180727130826.

Sundaramoorthy S., Balabaskar P. 2013. Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. Journal of Applied Biology and Biotechnology 1 (3): 36–40. DOI: 10.7324/JABB.2013.1306

Trang C.T.T, Thuc L.V., Xuan L.N.T., Huu T.N. Khuong N.Q. 2021. Isolation, characterization and evaluation of potential to cause the wild disease in sesame (*Sesamum indicum* L.) by *Fusarium* spp. in Chau Phu district, An Giang province. Science and Technology Journal of Agriculture & Rural Development 17: 23–30. (English abstract)

Vinale F., Sivasithamparam K., Ghisalberti E.L., Marra R., Woo S.L., Lorito M. 2008. *Trichoderma* – plant – pathogen interaction. Soil Biology Biochemestry 40: 1–10. DOI: 10.1016/j.soilbio.2007.07.002

White T.J., Bruns T., Lee S.J.W.T., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18 (1): 315–322.

Yu Z.F., Qiao M., Zhang Y., Zhang K.Q. 2007. Two new species of *Trichoderma* from Yunnan, China. Antonie van Leeuwenhoek 92: 101–108. DOI: 10.1007/s10482-006-9140-4

Zenawi G., Mizan A. 2019. Effect of nitrogen fertilization on the growth and seed yield of sesame (*Sesamum indicum* L.). International Journal of Agronomy 2019: Article ID 5027254. DOI: 10.1155/2019/5027254

Ziedan E.S.H., Sadek Elewa I., Mostafa H.M., Sahab A.F. 2011. Application of *Mycorrhizae* for controlling root diseases of sesame. Journal Plant Protection Research 51 (4): 355–361. DOI: 10.2478/v10045-011-0058-0

Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Evaluation of the antagonistic potential of *Trichoderma* spp. against *Fusarium oxysporum* F.28.1A

Nguyen Quoc Khuong[1], Cao Thi Thuy Trang[1], Do Thi Xuan[2], Le Thanh Quang[2], Tran Ngoc Huu[1], Ly Ngoc Thanh Xuan[3], Jun-Ichi Sakagami[4], Le Vinh Thuc[1]*

[1] Faculty of Crop Science, College of Agriculture, Can Tho University, Viet Nam
[2] Biotechnology Research and Development Institute, Can Tho University, Viet Nam
[3] Experimental and Practical Area, An Giang University – Vietnam National University, Ho Chi Minh City, Viet Nam
[4] Tropical Crop Science Laboratory, Faculty of Agriculture, Kagoshima University, Japan

Vol. 63, No. 1: 27–29, 2023

DOI: 10.24425/jppr.2023.144502

Received: May 27, 2022
Accepted: October 10, 2022
Online publication: February 17, 2023

*Corresponding address:
lvthuc@ctu.edu.vn

Responsible Editor:
Lidia Irzykowska

SUPPLEMENTARY MATERIAL

The authors are fully responsible for both the content and the formal aspects of the supplementary material. No editorial adjustments were made.

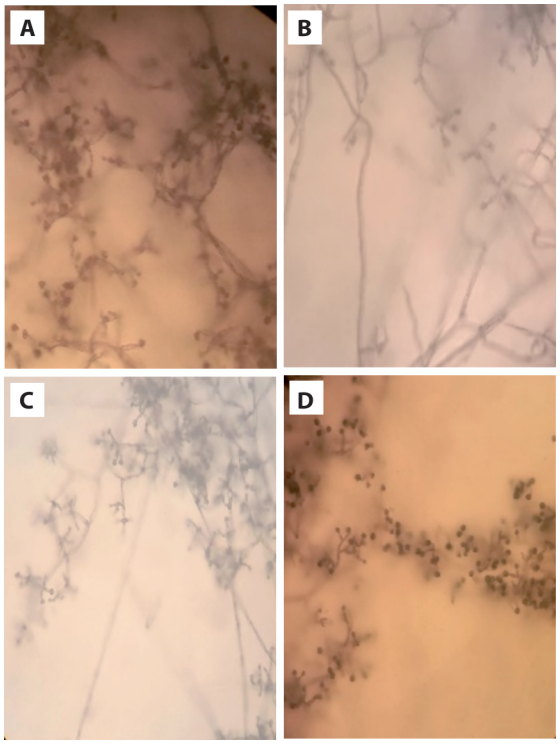

Fig. 1S. Morphological characteristics of mycelium of *Trichoderma* spp.: A – *T. yunnanense* T-02B1, B – *T. lentiforme* T-18B2, C – *T. asperellum* T-20B1, D – *T. hamatum* T-28B1 72 h after incubation under a 40× optical microscope

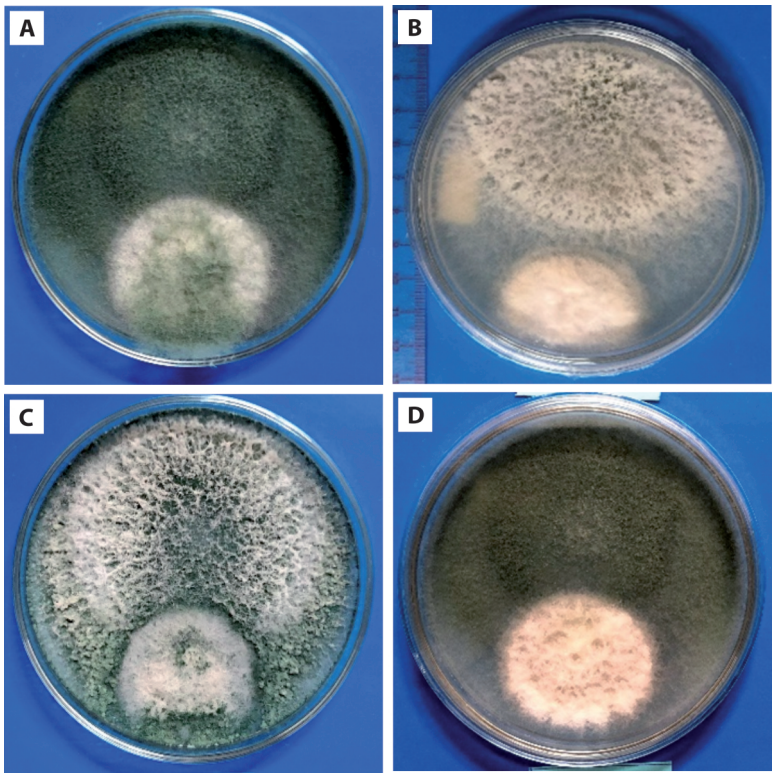

Fig. 2S. Ability of *Trichoderma* spp.: A – *T. yunnanense* T-02B1, B – *T. lentiforme* T-18B2, C – *T. asperellum* T-20B1, D – *T. hamatum* T-28B1 to inhibit *Fusarium oxysporum* F.28.1A *in vitro*

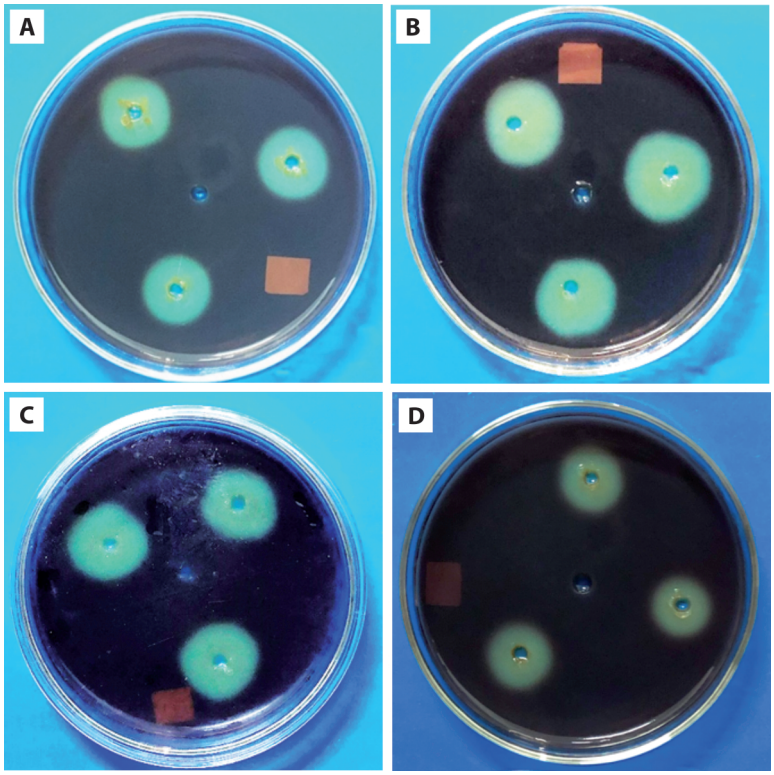

Fig. 3S. Ability of *Trichoderma* spp.: *T. yunnanense* T-02B1 – A, *T. lentiforme* T-18B2 – B, *T. asperellum* T-20B1 – C, *T. hamatum* T-28B1 – D to produce cellulose *in vitro*

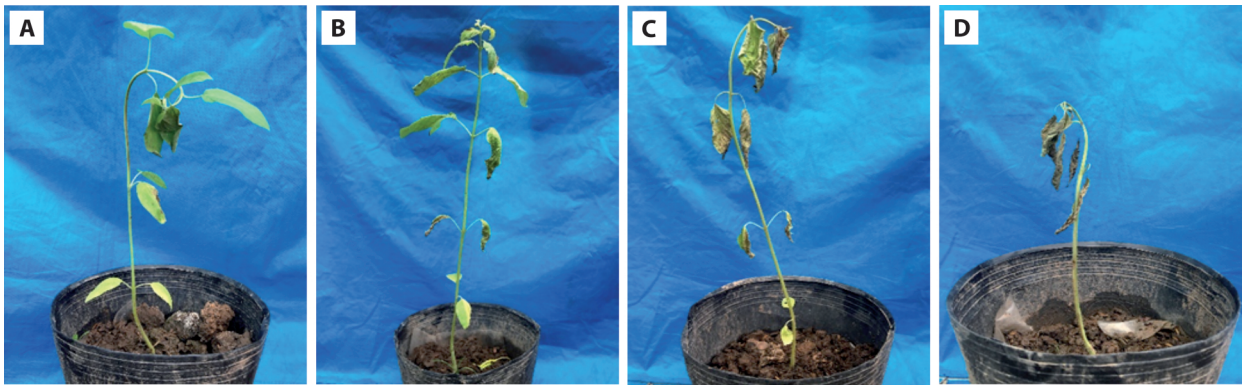

Fig. 4S. Levels of the wilt disease on sesame: level 1 – A, level 2 – B, level 3 – C, level 4 – D