Plant Pathology & Quarantine 11(1): 96–107 (2021) ISSN 2229-2217
www.ppqjournal.org **Article**

Doi 10.5943/ppq/11/1/11

Evaluation of *Trichoderma* isolates for biocontrol efficacy against plant fungal pathogens

Fan J[1], Zeng ZQ[1, 2*], Zhuang WY[2], Yu ZH[1*] and Hsiang T[3]

[1]College of Life Sciences, Yangtze University, Jingzhou, Hubei 434025, China
[2]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
[3]School of Environmental Sciences, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

Fan J, Zeng ZQ, Zhuang WY, Yu ZH, Hsiang T 2021 – Evaluation of *Trichoderma* isolates for biocontrol efficacy against plant fungal pathogens. Plant Pathology & Quarantine 11(1), 96–107, Doi 10.5943/ppq/11/1/11

Abstract

To reveal efficacious *Trichoderma* strains against four plant fungal pathogens (*Fusarium graminearum*, *F. oxysporum*, *Neocosmospora rubicola*, and *Rhizoctonia solani*), the antagonistic abilities of 48 isolates belonging to 14 *Trichoderma* species were investigated by dual culture. All plates were examined, and two types of antagonism, mycoparasitism and production of clear antagonistic zones, were observed. Mycoparasitism was evaluated by frequency of *Trichoderma* hyphal coilings around the pathogen hyphae and sporulation by the *Trichoderma* isolate on pathogenic fungal colonies**.** Among the mycoparasitic isolates, eight varying in antagonistic ability were further examined for their production of chitinase and β-1,3-glucanase. For all isolates producing antagonistic zones in dual cultures, their antifungal abilities were measured by inhibition of the phytopathogens growing on PDA plates mixed with their spent fermentation broth. Among the investigated species, isolates of *T. atrobrunneum*, *T. guizhouense*, *T. hamatum* and *T. koningiopsis* showed moderate to strong mycoparasitic abilities against at least three phytopathogens**.** A significant correlation between mycoparasitic coiling frequency and hydrolytic enzyme production was not detected. The fermentation broth of *T. brevicompactum* isolates showed high inhibition to *N. rubicola*, and they may have application potential in the field.

Keywords – antagonistic ability – antibiotic metabolite – enzyme activity – mycoparasitism

Introduction

Many plant diseases induced by fungal pathogens, such as species of *Botrytis*, *Fusarium*, *Neocosmospora* and *Rhizoctonia*, have broad distribution and severe effects on different crops of economic importance (Benítez et al. 2004, Sandoval-Denis et al. 2018, Maryani et al. 2019). Although chemical fungicides can be effective for control of crop diseases, there may be risks posed by residues of some pesticides which can cause problems in the environment and even for human health (Tjamos et al. 1992, Kumar et al. 2017, Launio et al. 2020). Consequently, discovery of eco-friendly, safe, long-lasting and effective methods to protect crops from phytopathogens is urgent, and efficacious biological control is needed.

Since Weindling (1932) found *Trichoderma lignorum* can attack *Rhizoctonia solani* causing citrus seedling disease, several species of *Trichoderma* have been used as biocontrol agents, especially those in the Clades of Viride and Harzianum in *Trichoderma* (Monte 2001, Howell 2003,

Submitted 2 June 2021, Accepted 17 August 2021, Published 7 October 2021
Corresponding Author: Zhaoqing Zeng – e-mail – zengzq@im.ac.cn, Zhihe Yu – e-mail – zhiheyu@hotmail.com

Fravel 2005, Kumar et al. 2017, He et al. 2019, Prisa 2019). The antagonistic mechanisms of these species include mycoparasitism, production of antibiotics, and competitions for nutrients and space (Hjeljord & Tronsmo 2003, Benítez et al. 2004, Monfil & Casas-Flores 2014). Mycoparasitism is one of the most important mechanisms, which consists of recognition, attachment, hyphal coiling, and enzymatic degradation of target cell walls (Dennis & Webster 1971, Viterbo et al. 2002, Kowsari et al. 2014). As the skeleton of filamentous fungal cell walls contains chitin and glucan, it is expected that both chitinases and β-glucanases may play significant roles in cell lysis during the antifungal activity by antagonistic agents (De Marco et al. 2000). Wang & Zhuang (2019) suggested that activities of chitinase and β-1,3-glucanase of *Trichoderma* related to mycoparasitism, and mycoparasitic abilities varied among species with some consistency among isolates within a species. Chitinase activity was treated as an indicator of antagonistic ability of *Trichoderma* strains (Zhang & Zhuang 2020). Although chitinase and β-1,3-glucanase can be expressed by *Trichoderma*, little is known regarding factors affecting production of these enzymes during mycoparasitism, and even whether the synthesized hydrolytic enzymes can act synergistically. Thus, a better understanding of the induction process of these enzymes is necessary to select the most efficient *Trichoderma* isolates for biocontrol.

The purpose of this study was to screen *Trichoderma* isolates for biocontrol ability, and investigate the relationships between their antagonistic abilities and specific enzyme activities. The antagonism of 48 isolates belonging to 14 *Trichoderma* species was evaluated against *Fusarium graminearum*, *F. oxysporum*, *Neocosmospora rubicola* and *Rhizoctonia solani*. Eight isolates with different antagonistic abilities were further examined for chitinase and β-1,3-glucanase production. Moreover, fermentation broths of nine isolates producing antagonistic zones on agar were tested for their inhibition efficacy on *N. rubicola*.

Materials & Methods

Fungal cultures

Forty-eight isolates of *Trichoderma*, and four isolates of the fungal plant pathogens, *Fusarium graminearum* PP18, *F. oxysporum* PP4, *Neocosmospora rubicola* PP23 and *Rhizoctonia solani* PP21 were obtained from the Beijing Academy of Agriculture and Forestry Sciences and the Technology Development and Transfer Center, Institute of Microbiology, Chinese Academy of Sciences (CAS). All the *Trichoderma* isolates were identified based on morphology and culture characteristics and molecular data (Jaklitsch & Voglmayr 2015, Zhuang 2020). The taxonomic positions of phytopathogens were confirmed by morphological features and phylogenetic analyses based on sequences of the fungal universal DNA barcode–ITS (nuclear ribosomal internal transcribed spacer) and TEF (translation elongation factor 1-α) regions. They have been maintained on potato dextrose agar (PDA) slants in the State Key Laboratory of Mycology, Institute of Microbiology, CAS. All isolates were grown on PDA at 25ºC for one week before use.

Evaluation of antagonistic abilities

Direct confrontation assay of *Trichoderma* isolates against four phytopathogenic species were tested following Zhang & Zhuang (2017), in which mycelial plugs of two species were placed 5 cm apart in each 9-cm-diameter petri dish containing PDA with three replications for individual pairings. Plates inoculated with only a pathogen were used as controls. The dual culture plates were incubated at 25°C for 30 days and observed every 7 days. Colonies of *Trichoderma* and pathogens in dual culture were photographed on days 3, 10 and 30 after inoculation using a Canon G15 digital camera (Tokyo, Japan). Small blocks (1 cm × 1 cm) were cut from the intersection zone between each pair of colonies within 24–48 hours after they made contact. Lactophenol cotton blue was used as mounting medium for observation of hyphal interactions. Photographs were taken with a Zeiss AxioCamMRc 5 digital camera (Jena, Germany) attached to an Imager A2 microscope (Göttingen, Germany). Growth rates of pathogens in each treatment were measured. The antagonistic abilities were evaluated by frequency of *Trichoderma* hyphal coilings and sporulation

on target fungal colonies. Different degrees of antagonism were recognized, i.e. strong, moderate, weak, and none as defined by Zhang & Zhuang (2017).

Determination of chitinase and β-1,3-glucanase activities

Mycoparasitic isolates of *Trichoderma* with different antagonistic abilities were further tested for their chitinase and β-1,3-glucanase production**.** PDA plates of the *Trichoderma* isolates were incubated at 25°C for 7 days, and then washed with 5 mL sterile water to make spore suspensions. Spore suspension concentrations were adjusted to $5.0 \pm 0.05 \times 10^6$ spores mL^{-1}. Aliquots of 0.5 mL spore suspension were added to each 250 mL flask with 50 mL TLE [0.1% (w/v) peptone, 0.03% (w/v) urea, 0.2% (w/v) KH_2PO_4, 0.14% (w/v) $(NH_4)_2SO_4$, 0.03% (w/v) $MgSO_4 \cdot 7H_2O$, 0.01% (w/v) $CaCl_2 \cdot 6H_2O$, 0.03% (w/v) glucose, 0.1% trace elements (Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+})] supplemented with 0.5% (w/v) pathogen cell walls as carbon source (Lopes et al. 2012). The cultures were incubated with shaking at 200 rpm at 25°C for 7 days, and then centrifuged at 12 000g to collect the supernatant for enzyme assay. Chitinase and β-1,3-glucanase activity were measured following Wang & Zhuang (2019).

The pathogen cell walls were obtained following Huang et al. (2011). Each flask containing 250 mL potato dextrose broth (PDB, potato 200 $g \cdot L^{-1}$, glucose 20 $g \cdot L^{-1}$) (BD Dicfo, Sparks, Maryland) was incubated with six 0.5 cm × 0.5 cm mycelial blocks of each pathogen isolate separately at 25°C for 7 days. Mycelia were collected by filtration through 2 layers of sterile gauze and washed with distilled water five times, and then oven-dried and stored at –20°C before use.

Antifungal assay by fermentation broth test

For testing antifungal activities of the *Trichoderma* isolates which produced antagonistic zones, two mycelial blocks taken from the margin of each actively growing colony were inoculated into each 250 mL flask containing 50 mL PDB, and incubated for 7 days at 180 rpm at 25°C (You et al. 2016). Antifungal activity was measured following Wang & Zhuang (2019). A plug of *N. rubicola* was inoculated onto a plate containing 20 ml PDA amended with spent fermentation broth of each *Trichoderma* isolate and incubated for 8 days at 25°C. The spent broth had been mixed into molten PDA after it cooled to 55°C. The inhibition percentage (I %) of each *Trichoderma* isolate against *N. rubicola* was calculated using the formula: I % = (C–T)/C × 100 (where C and T refer to growth radius of *N. rubicola* in control and in different treatments, respectively).

Statistical analysis of data

All experiments were repeated three times using a completely randomized design. The SPSS 20 software (Chicago, Illinois) was used for analyses. Means and standard deviations of the data were calculated, and all data were subjected to analyses of variance (ANOVA) and Duncan's Multiple Range tests ($P<0.05$).

Results

Mycoparasitic abilities of *Trichoderma* isolates

Two types of antagonism, mycoparasitism and production of antagonistic zones, were observed. Among the 48 isolates of *Trichoderma*, eight were of moderate antagonism against *F. graminearum* (Fig. 1), 12 showed moderate antagonism against *F. oxysporum* (Fig. 2), seven and six showed moderate (Fig. 3) and strong (Fig. 4) antagonism against *N. rubicola*, respectively, and 24 were with moderate antagonism against *R. solani* (Table 1).

The isolates having moderate to strong antagonistic abilities against at least three pathogens were from *T. atrobrunneum*, *T. guizhouense*, *T. hamatum* and *T. koningiopsis*. Meanwhile, most isolates of *T. polysporum* and *T. viridulum* exhibited weak or no antagonism.

Activities of chitinase and β-1,3-glucanase tested for mycoparasitism

Eight isolates of *Trichoderma* belonging to six species with different antagonistic abilities

were further tested for their chitinase and β-1,3-glucanase activities. The results indicated that all of them produced chitinases. In particular, *T. koningiopsis* strain 9100 possessed the highest activity (82.4 U mL^{-1}) when cell walls of *F. oxysporum* PP4 was used as the carbon source (Fig. 5), which was positively correlated with the abundant hyphal coilings surrounding hyphae of the phytopathogen (Table 2).

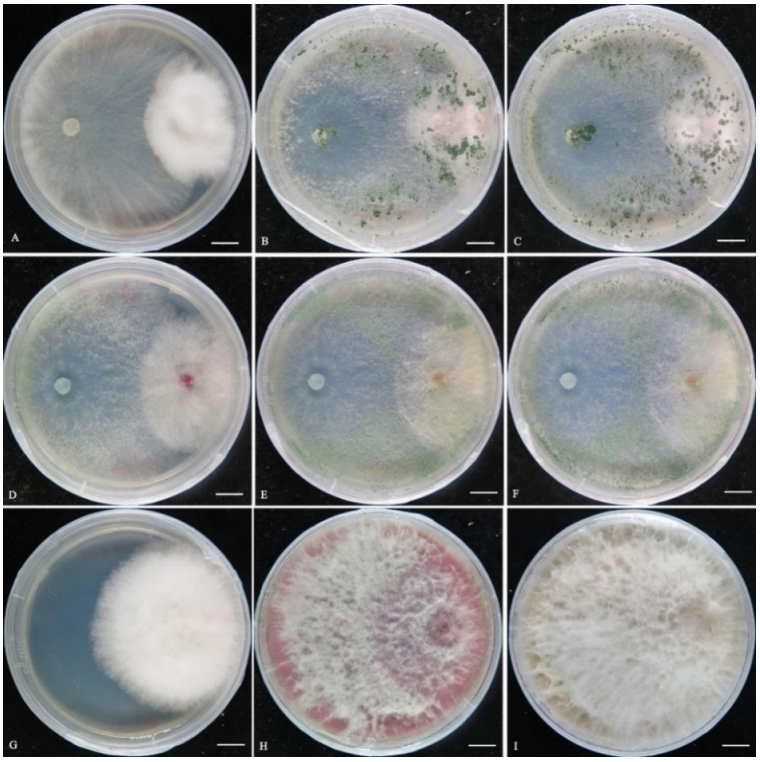

Fig. 1 – Colonies of *Trichoderma* (left) and phytopathogen (right) in dual culture. A–C *T. brevicrassum* TC967 against *F. graminearum* PP18. D–F *T. koningiopsis* 7819 against *F. graminearum* PP18. G–I *F. graminearum* PP18. A, D and G Colonies after 3 days. G as the control, plate inoculated with only pathogen plug. B, E and H Colonies after 10 days. C, F and I Colonies after 30 days. Scale bars: A–I = 10 mm.

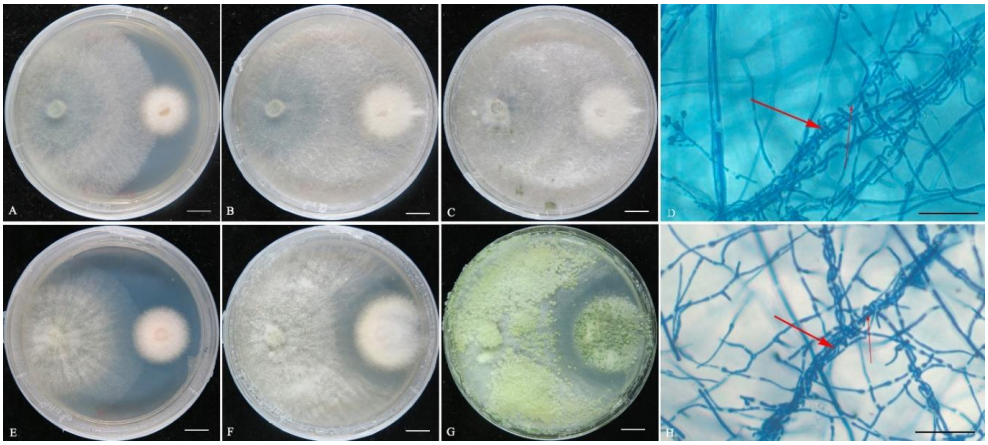

Fig. 2 – Colonies of *Trichoderma* (left) and pathogen (right) in dual culture. A–D *T. koningiopsis* 9100 against *F. oxysporum* PP4. E–H *T. hamatum* TC636 against *F. oxysporum* PP4. A, E Colonies after 3 days. B, F Colonies after 10 days. C, G Colonies after 30 days. D, H Hyphal coilings of *Trichoderma* (thick hyphae) surrounding the pathogen (thin hyphae). Scale bars: A–C, E–G = 10 mm, D, H = 50 μm.

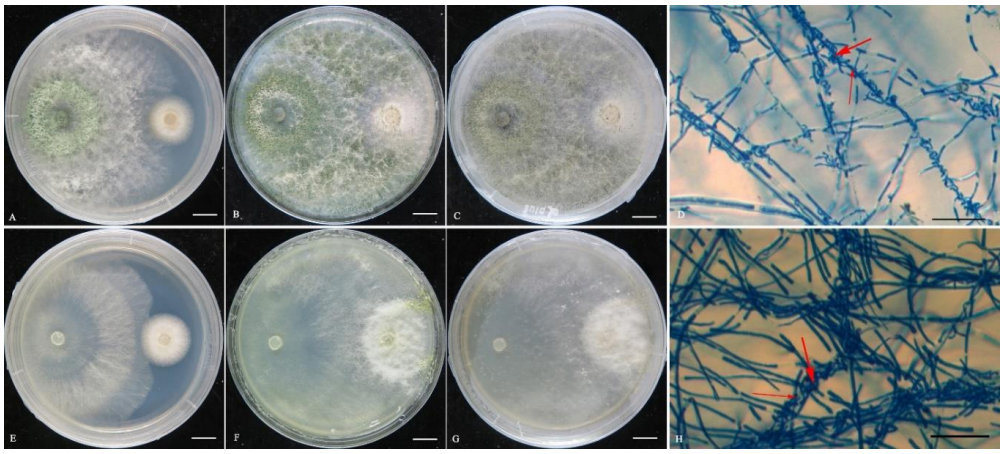

Fig. 3 – Colonies of *Trichoderma* (left) and pathogen (right) in dual culture. A–D *T. simmonsii* 9036 against *N. rubicola* PP23. E–H *T. hamatum* TC66 against *N. rubicola* PP23. A, E Colonies after 3 days. B, F Colonies after 10 days. C, G Colonies after 30 days. D, H Hyphal coilings of *Trichoderma* (thick hyphae) surrounding the pathogen (thin hyphae). Scale bars: A–C, E–G = 10 mm, D, H = 50 μm.

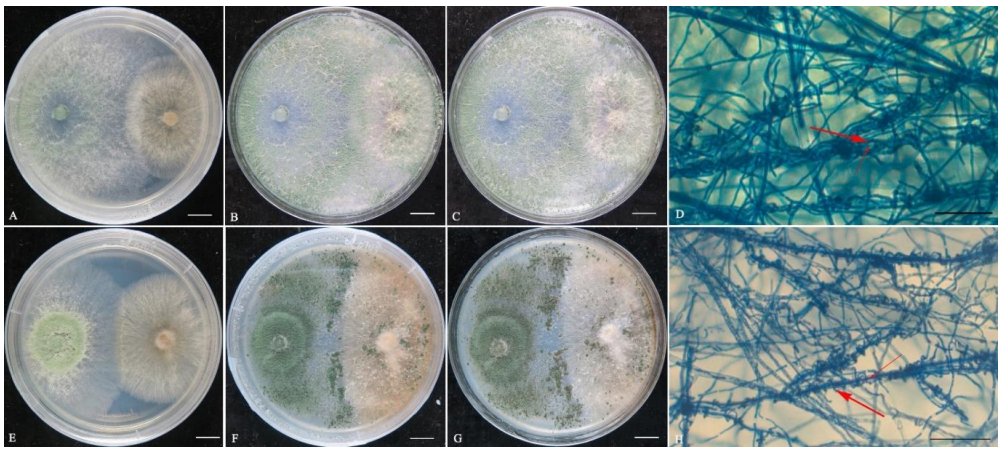

Fig. 4 – Colonies of *Trichoderma* (left) and pathogen (right) in dual culture. A–D *T. koningiopsis* 7819 against *R. solani* PP21. E–H *T. ingratum* TC34 against *R. solani* PP21. A, E Colonies after 3 days. B, F Colonies after 10 days. C, G Colonies after 30 days. D, H Hyphal coilings of *Trichoderma* (thick hyphae) surrounding the pathogen (thin hyphae). Scale bars: A–C, E–G = 10 mm; D, H = 50 μm.

All isolates showed relatively high β-1,3-glucanase activity (Fig. 6). The highest activity was found with *T. ingratum* TC34 (493.9 U mL^{-1}) when cells walls of *N. rubicola* used as the carbon source (Fig. 6). *Trichoderma koningiopsis* 8717 displayed the lowest activity (100 U mL^{-1} or less) (Table 1). There was no strong relationship between *Trichoderma* β-1,3-glucanase activity and its antagonism against the four pathogens.

Antifungal metabolite produced by *Trichoderma* isolates

Nine *Trichoderma* isolates with weak antagonism during the dual culture testing were further examined for their antifungal abilities (Fig. 7). The fermentation broths of two stains of *T. brevicompactum*, TC708 and TC711, showed relatively high inhibition rates (> 40%) after 8 days, while other isolates displayed low inhibition (Table 3). Further investigations might be required to uncover the specific bioactive metabolites and evaluate whether they might be applicable for practical plant disease control.

Table 1 The types and abilities of antagonism of *Trichoderma* isolates

Trichoderma		**Pathogens**							
		Fusarium graminearum		***Fusarium oxysporum***		***Neocosmospora rubicola***		***Rhizoctonia solani***	
Species	**Isolate**	**Types of antagonism**	**Ability**	**Type of antagonism**	**Ability**	**Type of antagonism**	**Ability**	**Type of antagonism**	**Ability**
T. atrobrunneum	TC436	mycoparasitism	moderate	mycoparasitism	moderate	antagonistic zones	-	mycoparasitism	moderate
	TC474	mycoparasitism	weak	mycoparasitism	weak	antagonistic zones	-	mycoparasitism	moderate
	11027	mycoparasitism	weak	mycoparasitism	weak	antagonistic zones	-	mycoparasitism	moderate
T. brevicompactum	TC708	mycoparasitism	none	mycoparasitism	weak	antagonistic zones	-	mycoparasitism	moderate
	TC711	mycoparasitism	none	mycoparasitism	weak	antagonistic zones	-	mycoparasitism	moderate
T. brevicrassum	TC967	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	strong
	TC968	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	moderate
T. guizhouense	TC428	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	moderate
	TC521	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	moderate
	TC609	mycoparasitism	none	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	moderate
	TC730	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	moderate
	10027	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	strong
T. hamatum	TC66	mycoparasitism	none	mycoparasitism	none	mycoparasitism	moderate	mycoparasitism	none
	TC570	mycoparasitism	none	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	weak
	TC611	mycoparasitism	none	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	weak
	TC636	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	moderate	mycoparasitism	strong
T. harzianum	TC785	mycoparasitism	none	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	moderate
T. ingratum	TC34	mycoparasitism	none	mycoparasitism	moderate	mycoparasitism	none	mycoparasitism	strong
	TC183	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	moderate
	11666	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	none	mycoparasitism	weak
T. koningii	TC106	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	none	mycoparasitism	moderate
	9032	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	moderate
	11818	mycoparasitism	weak	mycoparasitism	none	mycoparasitism	none	mycoparasitism	none
T. koningiopsis	7819	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	strong
	8717	mycoparasitism	none	mycoparasitism	none	antagonistic zones	-	mycoparasitism	weak
	8985	mycoparasitism	none	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	weak
	9100	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	none
T. polysporum	TC264	mycoparasitism	none	mycoparasitism	none	mycoparasitism	weak	mycoparasitism	none
	TC536	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	none	mycoparasitism	moderate
	TC622	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	none	mycoparasitism	none
	10684	mycoparasitism	none	mycoparasitism	none	mycoparasitism	weak	mycoparasitism	none
T. pyramidale	TC84	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	none	mycoparasitism	moderate
	TC617	mycoparasitism	none	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	none

Table 1 Continued.

Trichoderma		**Pathogens**							
		Fusarium graminearum		***Fusarium oxysporum***		***Neocosmospora rubicola***		***Rhizoctonia solani***	
Species	**Isolate**	**Types of antagonism**	**Ability**	**Type of antagonism**	**Ability**	**Type of antagonism**	**Ability**	**Type of antagonism**	**Ability**
	TC900	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	strong
	8931	Mycoparasitism	weak	mycoparasitism	none	antagonistic zones	-	mycoparasitism	moderate
	8991	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	moderate
T. simmonsii	8798	mycoparasitism	none	mycoparasitism	none	mycoparasitism	none	mycoparasitism	weak
	9036	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	moderate
	9154	mycoparasitism	weak	mycoparasitism	weak	mycoparasitism	none	mycoparasitism	moderate
	10094	mycoparasitism	weak	mycoparasitism	moderate	mycoparasitism	weak	mycoparasitism	moderate
	10125	mycoparasitism	weak	mycoparasitism	none	mycoparasitism	weak	mycoparasitism	moderate
T. virens	TC36	mycoparasitism	none	mycoparasitism	moderate	mycoparasitism	none	mycoparasitism	moderate
	TC540	mycoparasitism	none	mycoparasitism	none	mycoparasitism	none	mycoparasitism	none
	TC792	mycoparasitism	none	mycoparasitism	moderate	antagonistic zones	-	mycoparasitism	moderate
	TC897	mycoparasitism	none	mycoparasitism	none	antagonistic zones	-	mycoparasitism	weak
	TC955	mycoparasitism	none	mycoparasitism	none	mycoparasitism	none	mycoparasitism	none
T. viridulum	8707b	mycoparasitism	none	mycoparasitism	none	mycoparasitism	none	mycoparasitism	weak
	9767	mycoparasitism	none	mycoparasitism	none	mycoparasitism	none	mycoparasitism	weak

Table 2 Hyphal coilings of Trichoderma isolates surrounding phytopathogen

***Trichoderma* species**	**Isolate**	**Hyphal coiling**			
		Fusarium graminearum	***Fusarium oxysporum***	***Neocosmospora rubicola***	***Rhizoctonia solani***
T. koningiopsis	7819	no coiling	no coiling	no coiling	high coiling
T. hamatum	TC636	no coiling	high coiling	no coiling	high coiling
T. ingratum	TC34	no coiling	high coiling	no coiling	high coiling
T. koningiopsis	9100	low coiling	high coiling	no coiling	no coiling
T. guizhouense	TC428	no coiling	no coiling	no coiling	no coiling
T. koningii	9032	no coiling	no coiling	no coiling	high coiling
T. koningiopsis	8717	no coiling	no coiling	no coiling	no coiling
T. polysporum	TC264	no coiling	no coiling	no coiling	no coiling

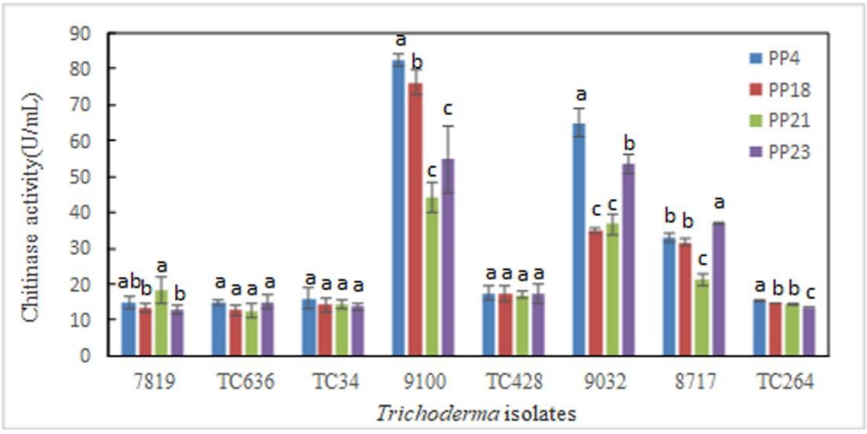

Fig. 5 – Chitinase activities of *Trichoderma* isolates growing on purified cell walls of pathogens after 7 days. Different letters above the bars indicate significant differences among isolates according to Duncan's test at $P < 0.05$. PP4 = *F. oxysporum*, PP18 = *F. graminearum*, PP21 = *Rhizoctonia solani*, PP23 = *Neocosmospora rubicola.*

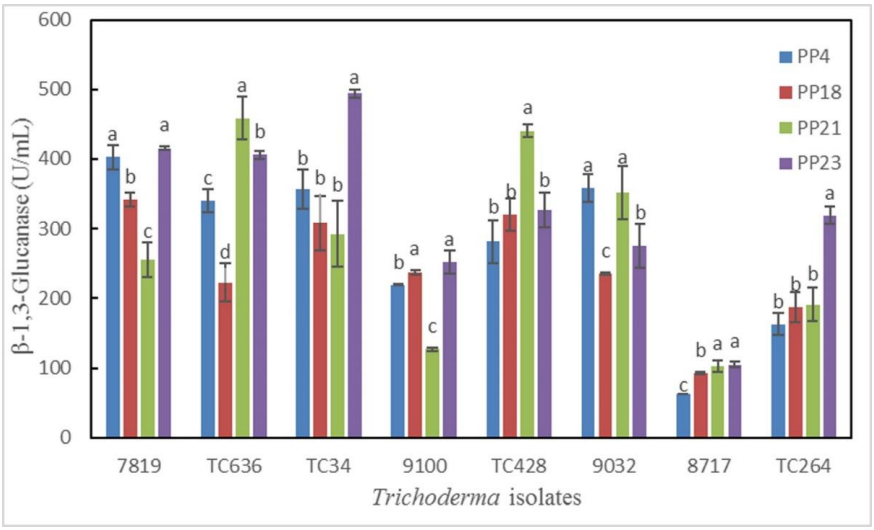

Fig. 6 – β-1,3-glucanase activities of *Trichoderma* isolates growing on purified cell wall of pathogens after 7 days. Different letters above the bars indicate significant differences among isolates according to Duncan test at $P < 0.05$. PP4 = *F. oxysporum*, PP18 = *F. graminearum*, PP21 = *Rhizoctonia solani*, PP23 = *Neocosmospora rubicola.*

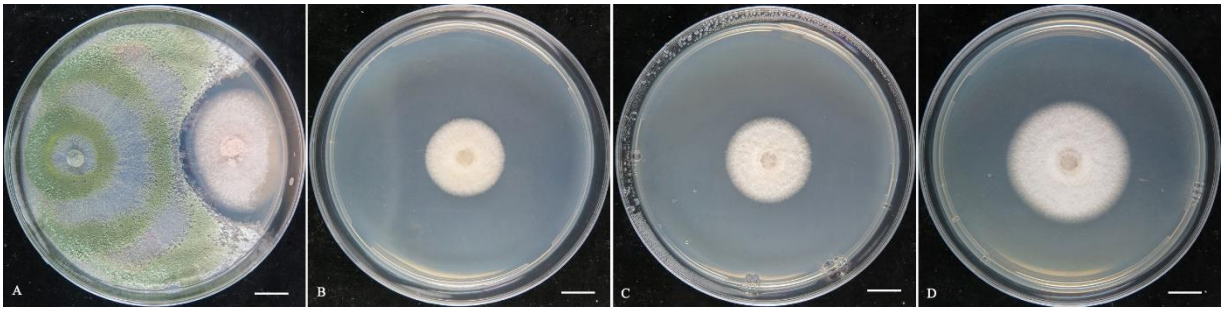

Fig. 7 – Antagonistic zone between isolates of *Trichoderma* and pathogen and colonies of *N. rubicola* on different media at 25°C after 4 days. A: Antagonistic band between *T. brevicompactum* TC708 (left) and *N. rubicola* (right) in dual culture; B: On PDA amended with fermentation broth of *T. brevicompactum* TC708; C: On PDA amended with fermentation broth of *T. brevicompactum* TC711; D: On PDA.

Table 3 Antifungal activities of nine Trichoderma isolates against N. Rubicola

Species	Isolates	Antifungal activity (% inhibition)	
		4 d	8 d
T. koningiopsis	8717	3.25±2.52 e*	2.22±1.92 ef
T. virens	TC792	2.44±2.44 e	1.69±2.89 ef
	TC897	3.66±1.34 e	1.11±1.11 f
T. pyramidale	8931	5.69±1.26 e	3.57±1.34 e
T. brevicompactum	TC711	34.15±0.00 b	40.00±0.00 b
	TC708	40.65±1.26 a	47.22±0.59 a
T. atrobrunneum	TC436	12.8±2.56 c	13.89±2.33 c
	11027	8.17±0.00 d	9.67±0.97 d
	TC474	9.55±1.00 c	10.93±0.68 cd

*Different letters indicate significant differences among isolates according to Duncan test at $P < 0.05$. Percent inhibition = (C–T)/C × 100 (where C and T refer to growth radius of *N. rubicola* in control and in different treatments, respectively

Discussion

In this study, the antagonistic activities of the 48 isolates from 14 *Trichoderma* species were tested against four important plant pathogens. The results showed that the antagonism of individual isolates of the same species varied against different pathogens, which are congruent with the previous studies (Saxena et al. 2015, You et al. 2016). For example, *T. koningiopsis* 7819 showed strong antagonism against *R. solani*, moderate antagonism against *F. graminearum* and *N. rubicola*, and a weak effect against *F. oxysporum*, while *T. koningiopsis* 8717 displayed weak antagonism against *R. solani*, and no antagonism against either *F. graminearum* or *F. oxysporum*.

The isolates of *T. atrobrunneum*, *T. guizhouense*, *T. hamatum* and *T. koningiopsis* had moderate to strong antagonism against at least three of the pathogens**.** They belong to the Viride and Harzianum species complexes, which have been used as biocontrol agents (Monte 2001, Howell 2003, Kumar et al. 2017). Furthermore, *T. ingratum* TC34 showed strong and moderate antagonism against *R. solani* and *F. oxysporum*. To our knowledge, this is the first report of *T. ingratum* possessing suppressive capacity against *F. oxysporum* and *R. solani*. The above tested *Trichoderma* species are valuable for further research in the lab or in field investigations.

The ability to produce hyphal coilings around plant pathogens is an important criterion for effective biocontrol agents (Almeida et al. 2007). In the present study, the coiling production capacities of the 48 *Trichoderma* isolates against four plant pathogens were investigated. The results showed that many of them produced dense hyphae coiled around those of the pathogens as reported in previous studies (De Marco et al. 2000, El-Katatny et al. 2001, Innocenti et al. 2003).

Hydrolytic enzymes, especially chitinase, play crucial roles in hyphal coiling capacity (Howell 2003, Kubicek et al. 2011). These enzymes were induced efficiently by pathogen cell walls (Almeida et al. 2007), and among 15 investigated strains of *T. harzianum*, only one showed positive correlation between the coiling capacity and activities of chitinase and β-1,3-glucanase when tested against *R. solani* in dual culture. Many studies have failed to find a correlation between enzyme production and coiling frequency of *Trichoderma* against pathogens (Roberts & Lumsden 1990, Ordentlich et al. 1991, Worasatit et al. 1994). Similarly, Innocenti et al. (2003) found a negative relationship by using r2 value between disease incidence and enzymatic activity of β-1,3-glucanase. Therefore, we propose speculate that chitinase and β-1,3-glucanase activities in vitro cannot be simply used to evaluate mycoparasitic abilities of *Trichoderma* isolates.

Among 256 *Trichoderma* species accepted by Bissett et al. (2015), the most extensively studied for their secondary metabolites are *T. brevicompactum*, *T. ghanense*, *T. harzianum*, *T. parceramosum*, *T. virens* and *T. viride* (Neumann et al. 2015, Stoppacher et al. 2013). We found that *T. brevicompactum* TC708 and TC711 suppressed mycelial growth of the plant pathogens, and likely by producing bioactive metabolites as indicated in the study of Nielsen et al. (2005).

Although some commercial *Trichoderma* products have been used commercially such as *T. afroharzianum*, *T. atroviride* and *T. asperellum* (Woo et al. 2014, Chaverri et al. 2015, Samuels & Hebbar 2015, Li et al. 2018), many species of the genus have not been investigated for their biological control potential (Wang & Zhuang 2019). More than 80 new species of *Trichoderma* have been reported in recent studies from our research group (Qin & Zhuang 2016a, b, 2017, Zhu & Zhuang 2015, Chen & Zhuang 2016, 2017a, b, c). Future evaluations of a broad spectrum of *Trichoderma* species for their biological activities may speed up utilization and exploitation of this group of fungi as important natural resources.

Conclusion

This study was performed to investigate the antagonistic and antifungal abilities of 48 *Trichoderma* isolates against *F. graminearum*, *F. oxysporum*, *N. rubicola* and *R. solani*. Our results suggest that *T. atrobrunneum* TC436, *T. brevicompactum* TC 708, *T. guizhouense* TC521, *T. hamatum* TC636 and *T. koningiopsis* 7819 have promising prospects as biocontrol agents, and should be further investigated in field studies.

Acknowledgements

This work was financed by the National Natural Science Foundation of China (31750001, 31870012, 31570022) and Frontier Key Program of Chinese Academy of Sciences (QYZDY-SSW-SMC029). We are grateful to Drs. Zhaoxiang Zhu, Wentao Qin, Kai Chen and Yubo Zhang (Institute of Microbiology, CAS) for isolating the *Trichoderma* strains for this study, and Prof. Jiyan Qiu (Beijing Academy of Agriculture and Forestry Sciences), Prof. Zhengqiang Ma (Nanjing Agricultural University) and Shenzhan Fu (Institute of Microbiology, CAS) for providing strains of the phytopathogens.

References

Almeida FBdR, Cerqueira FM, Silva RdN, Ulhoa CJ et al. 2007 – Mycoparasitism studies of *Trichoderma harzianum* strains against *Rhizoctonia solani*: evaluation of coiling and hydrolytic enzyme production. Biotechnology Letters 29, 1189–1193.

Benítez T, Rincón AM, Limón MC, Codón AC. 2004 – Biocontrol mechanisms of *Trichoderma* strains. International Microbiology 7, 249–260.

Bissett J, Gams W, Jaklitsch W, Samuels GJ. 2015 – Accepted *Trichoderma* names in the year 2015. IMA Fungus 6, 263–295.

Chaverri P, Branco-Rocha F, Jaklitsch W, Gazis R et al. 2015 – Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. Mycologia 107, 558–590.

Chen K, Zhuang WY. 2016 – *Trichoderma shennongjianum* and *Trichoderma tibetense*, two new soil-inhabiting species in the Strictipile clade. Mycoscience 57, 311–319.

Chen K, Zhuang WY. 2017a – Discovery from a large-scaled survey of *Trichoderma* in soil of China. Scientific Reports, 7: 9090.

Chen K, Zhuang WY. 2017b – Seven soil-inhabiting new species of the genus *Trichoderma* in the Viride clade. Phytotaxa 312, 28–46.

Chen K, Zhuang WY. 2017c – Three new soil-inhabiting species of *Trichoderma* in the Stromaticum clade with test of their antagonism to pathogens. Current Microbiology 74, 1049–1060.

De Marco JL, Lima LHC, Valle de Souza M, Felix CR. 2000 – A *Trichoderma harzianum* chitinase destroys the cell wall of the phytopathogen *Crinipellis perniciosa*, the causal agent of witches broom disease of cocoa. World Journal of Microbiology and Biotechnology 16, 383–386.

Dennis C, Webster J. 1971 – Antagonistic properties of species-groups of *Trichoderma*: III. Hyphae interaction. Transactions of British Mycological Society 57, 363–369.

El-Katatny M, Gudelj M, Robra KH, Elnaghy MA et al. 2001 – Characterization of a chitinase and an endo-β-1,3-glucanase from *Trichoderma harzianum* Rifai T24 involved in control of the phytopathogen *Sclerotium rolfsii*. Applied Microbiology and Biotechnology 56, 137–143.

Fravel DR. 2005 – Commercialization and implementation of biocontrol. Annual Review of Phytopathology 43, 337–359.

He A, Sun JA, Wang XH, Zou LW et al. 2019 – Reprogrammed endophytic microbial community in maize stalk induced by *Trichoderma asperellum* biocontrol agent against *Fusarium* diseases and mycotoxin accumulation. Fungal Biology 123, 448–455.

Hjeljord LG, Tronsmo A. 2003 – Effect of germination initiation on competitive capacity of *Trichoderma atroviride* P1 conidia. Phytopathology 93, 1593–1598.

Howell CR. 2003 – Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Disease 87, 4–10.

Huang XQ, Chen LH, Ran W, Shen QR et al. 2011 – *Trichoderma harzianum* strain SQR-T37 and its bio-organic fertilizer could control *Rhizoctonia solani* damping-off disease in cucumber seedlings mainly by the mycoparasitism. Applied Microbiology and Biotechnology 91, 741–755.

Innocenti G, Roberti R, Montanari M, Zakrisson E. 2003 – Efficacy of microorganisms antagonistic to *Rhizoctonia cerealis* and their cell degrading enzymatic activities. Mycological Research 107, 421–427.

Jaklitsch WM, Voglmayr H. 2015 – Biodiversity of *Trichoderma* (Hypocreaceae) in Southern Europe and Macaronesia. Studies in Mycology 80, 1–87.

Kowsari M, Zamani MR, Motallebi M. 2014 – Enhancement of *Trichoderma harzianum* activity against *Sclerotinia sclerotiorum* by over expression of Chit42. Iranian Journal of Biotechnology 12, 41–52.

Kubicek CP, Herrera-Estrella A, Seidl-Seiboth V, Martinez DA et al. 2011 – Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. Genome Biology 12, R40.

Kumar G, Maharshi A, Patel J, Sarma BK. 2017 – *Trichoderma*: A potential fungal antagonist to control plant diseases. SATSA Mukhapatra-Annual Technical Issue 21, 206–218.

Launio CC, Labon KO, Bañez AA, Batani RS. 2020 – Adoption and economic analysis of using biological control in Philippine highland farms: Case of *Trichoderma koningii* strain KA. Crop Protection 136, 105177.

Li YT, Hwang SG, Huang YM, Huang CH. 2018 – Effects of *Trichoderma asperellum* on nutrient uptake and *Fusarium* wilt of tomato. Crop Protection 110, 275–282.

Lopes FAC, Steindorff AS, Geraldine AM, Brandão RS et al. 2012 – Biochemical and metabolic profiles of *Trichoderma* strains isolated from common bean crops in the Brazilian Cerrado, and potential antagonism against *Sclerotinia sclerotiorum*. Fungal Biology 116, 815–824.

Maryani N, Lombard L, Poerba YS, Subandiyah S et al. 2019 – Phylogeny and genetic diversity of the banana *Fusarium* wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin. Studies in Mycology 92, 155–194.

Monfil VO, Casas-Flores S. 2014 – Molecular mechanisms of biocontrol in *Trichoderma* spp. and their applications in agriculture. *Edited by*: Gupta VG, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy M. Biotechnology and Biology of *Trichoderma*. Elsevier Press, Amsterdam. pp. 429–453.

Monte E. 2001 – Understanding *Trichoderma*: between biotechnology and microbial ecology. International Journal of Microbiology 4, 1–4.

Neumann NKN, Stoppacher N, Zeilinger S, Degenkolb T et al. 2015 – The peptaibiotics database – a comprehensive online resource. Chemistry & Biodiversity 12, 743–751.

Nielsen KF, Graefenhan T, Zafari D, Thrane U. 2005 – Trichothecene production by *Trichoderma brevicompactum*. Journal of Agricultural and Food Chemistry 53, 8190–8196.

Ordentlich A, Migheli Q, Chet I. 1991 – Biological control activity of three *Trichoderma* isolates against *Fusarium* wilt of cotton and muskmelon and lack of correlation with their lytic enzymes. Journal of Phytopathology 133, 177–186.

Prisa D. 2019 – *Trichoderma harzianum*: biocontrol to *Rhizoctonia solani* and biostimulation in *Pachyphytumoviferum* and *Crassula falcata*. World Journal of Advanced Research and Reviews, 3(3), 11–18.

Qin WT, Zhuang WY. 2016a – Four new species of *Trichoderma* with hyaline ascospores from central China. Mycological Progress 15, 811–825.

Qin WT, Zhuang WY. 2016b – Seven wood-inhabiting new species of the genus *Trichoderma* (Fungi, Ascomycota) in Viride clade. Scientific Reports 6, 27074.

Qin WT, Zhuang WY. 2017 – Seven new species of *Trichoderma* (Hypocreales) in the Harzianum and Strictipile clades. Phytotaxa 305, 121–139.

Roberts DP, Lumsden RD. 1990 – Effects of extracellular metabolites from *Gliocladium virens* on germination of sporangia and mycelial growth of *Pythium ultimum*. Phytopathology 80, 461–465.

Samuels G, Hebbar P. 2015 – *Trichoderma* Identification and Agricultural Applications. American Phytopathological Society Press, St. Paul.

Sandoval-Denis M, Guarnaccia V, Polizzi G, Crous PW. 2018 – Symptomatic *Citrus* trees reveal a new pathogenic lineage in *Fusarium* and two new *Neocosmospora* species. Persoonia 40, 1–25.

Saxena A, Raghuwanshi R, Singh HB. 2015 – *Trichoderma* species mediated differential tolerance against biotic stress of phytopathogens in *Cicerarietinum* L. Journal of Basic Microbiology 55, 195–206.

Stoppacher N, Neumann NK, Burgstaller L, Zeilinger S et al. 2013 – The comprehensive peptaibiotics database. Chemistry and Biodiversity 10, 734–743.

Tjamos EC, Papavizas GC, Cook RJ. 1992 – Biological control of plant diseases. Progress and challenges for the future. Plenum Press, New York.

Viterbo A, Montero M, Ramot O, Friesem D et al. 2002 – Expression regulation of the endochitinase chit36 from *Trichoderma asperellum* (*T. harzianum* T-203). Current Genetics 42, 114–122.

Wang C, Zhuang WY. 2019 – Evaluating effective *Trichoderma* isolates for biocontrol of *Rhizoctonia solani* causing root rot of *Vigna unguiculata*. Journal of Integrative Agriculture 18, 2–9.

Weindling R. 1932 – *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology 22, 837–845.

Woo SL, Ruocco M, Vinale F, Lorito M. 2014 – *Trichoderma*-based products and their widespread use in agriculture. The Open Mycology Journal 8, 71–126.

Worasatit N, Sivasithamparam K, Ghisalberti EL, Rowland C. 1994 – Variation in pyrone production, lytic enzymes and control of *Rhizoctonia* root rot of wheat among single-spore isolates of *Trichoderma koningii*. Mycological Research 12, 1357–1363.

You J, Zhang J, Wu M, Long Y et al. 2016 – Multiple criteria-based screening of *Trichoderma* isolates for biological control of *Botrytis cinerea* on tomato. Biological Control 101, 31–38.

Zhang YB, Zhuang WY. 2017 – First step evaluation of *Trichoderma* antagonism against plant pathogenic fungi in dual culture. Mycosystema 36, 1251–1259.

Zhang Y, Zhuang WY. 2020 – *Trichoderma brevicrassum* strain TC967 with capacities of diminishing cucumber disease caused by *Rhizoctonia solani* and promoting plant growth. Biological Control 142, 104151.

Zhu ZX, Zhuang WY. 2015 – *Trichoderma* (*Hypocrea*) species with green ascospores from China. Persoonia 34, 113–29.

Zhuang WY. 2020 – Flora Fungorum Sinicorum, Vol 60. Hypocreaceae. Science Press, Beijing, pp 1–225 [in Chinese].