Jurnal Perlindungan Tanaman Indonesia, Vol. 25, No. 1, 2021: 64–73
DOI: 10.22146/jpti.65290
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

In Vitro Evaluation of *Trichoderma asperellum* Isolate UGM-LHAF against *Rhizoctonia solani* Causing Sheath Blight Disease of Rice

Yeyet Nurhayati[1)]*, Suryanti[1)], & Arif Wibowo[1)]

[1)]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora No. 1, Bulaksumur, Sleman, Yogyakarta, 55281, Indonesia

**Corresponding author. E-mail: yeyet.nurhayati@mail.ugm.ac.id*

Received April 15, 2021; revised June 14, 2021; accepted July 24, 2021

ABSTRACT

Trichoderma spp. is a fungus widely used to control soil-borne pathogens, such as *Rhizoctonia solani* which is plant pathogenic fungi in widely host range, especially on rice. This research aimed to evaluate the ability of *Trichoderma asperellum* isolate UGM-LHAF against *R. solani* causing sheath blight disease of rice *in vitro* condition. *Trichoderma* sp. used in this research was obtained from The Biological Laboratory of Pakem, Yogyakarta, Indonesia, and *Rhizoctonia* sp. was obtained through isolation of diseased rice obtained from rice fields in Yogyakarta. The two isolates were characterized base on morphology and molecular identification based on ITS rDNA. The pathogenicity test of *Rhizoctonia* sp. was evaluated by adding four sclerotia of *Rhizoctonia* sp. near rice roots at 6 days after sowing. The *in vitro* test used dual culture and antifungal activity (0%, 10%, 25%, 50% culture filtrate of *Trichoderma* sp.) with three replicates of each treatment. Two isolates were identified as *T. asperellum* and *R. solani*. Sheath blight symptoms appeared after 12 days inoculation. In the *in vitro* test, *T. asperellum* isolate UGM-LHAF was able to inhibit the mycelial growth of *R. solani* (64.23% on dual culture and 68.5% on antifungal activity). This study suggests that *T. asperellum* isolate UGM-LHAF able to inhibit the growth of *R. solani* and can be a further potential candidate as a biocontrol agent against *R. solani* causing sheath blight disease of rice.

Keywords: *Rhizoctonia solani*; rice; sheath blight disease; *Trichoderma asperellum*

INTRODUCTION

Rice is an important staple food in Indonesia with the provinces of East, West, and Central Java producing the highest rates. From 2018 to 2020, the national rice production experienced fluctuations of 59.2, 54.6, and 55.2 million tonnes, respectively (*Badan Pusat Statistik*, 2020). According to Rabindran and Vidhyasekaran (1996), and Zhang *et al.* (2009), one of the factors that influence the fluctuation of rice production is plant disease, such as sheath blight disease caused by *Rhizoctonia* spp., with a yield loss of 2.5–50%. *R. solani* based on hyphal anastomosis was divided into 14 anastomosis groups (AG) namely AG1 to AG13, and bridging isolate (BI), while AG1 was divided into subgroups namely AG1-IA, AG1-IB, AG1-IC, and AG1-ID (Carling, 1996; Priyatmojo *et al.*, 2001; Carling *et al.*, 2002, Garcia *et al.*, 2006). P. Singh *et al.* (2019) stated that this disease is difficult to control due to the pathogen's high diversity, wide host, and ability to survive in the soil and plant residues by forming sclerotia.

Biological agents that have been reported to be effective against soil-borne pathogens were genus *Trichoderma* (Mukhopadhyay & Kumar, 2020). *Trichoderma* spp. such as *T. virens*, *T. harzianum*, and *T. asperellum* were known to be able to suppress sheath blight disease (Khan & Sinha, 2007; Naeimi *et al.*, 2010; de França *et al.*, 2015). *T. asperellum* was able to inhibit the growth of phytopathogenic fungi such as *Fusarium camptocerus*, *F. oxysporum*, *F. solani*, *F. camptocerus, Alternaria alternata*, *Colletotrichum gloeosporioides*, *Ganoderma applanatum*, *Botrytis cinerea*, and *Cytospora chrysosperma* (Yu *et al.*, 2021). *Trichoderma* spp. have often been multiplicated

in laboratories of the Indonesia Department of Agriculture to be used as biological agents in managing plant diseases (*Dinas Perkebunan Provinsi Kalimatan Timur*, 2017; Rismanto, 2020). One of them is *Trichoderma* sp. from The Biological Laboratory of Pakem which has not been identified by molecular identification. The study not only identifying *Trichoderma* sp. isolate from The Biological Laboratory of Pakem based on ITS ribosomal DNA, but it also evaluating its ability against *R. solani* causing sheath blight disease of rice under *in vitro* condition.

MATERIALS AND METHODS

The study was conducted at the Plant Disease Laboratory, Faculty of Agriculture, Universitas Gadjah Mada, Special Region of Yogyakarta, Indonesia from November 2019 until May 2020.

Isolation, Morphological Identification, and Pathogenicity Test

Trichoderma sp. used in this research was obtained from The Biological Laboratory of Pakem, Yogyakarta, Indonesia, then cultured on PDA and incubated at 28°C. *Rhizoctonia* sp. was isolated from diseased rice obtained of rice fields in Yogyakarta, Indonesia (7.8305 S, 110.3458 E). The leaf sheath sample was cut (2–3 mm), disinfected with 1.5% NaOCl for 1 minute, rinsed 3 times with sterile distilled water, dried on sterile filter paper, grown on PDA, and incubated for 2–3 days at a temperature of 28°C. Furthermore, fungal hyphae with the characteristics of *Rhizoctonia* sp. was cultured on a new PDA for further analysis (Wang *et al.*, 2015). The morphological features of *Trichoderma* sp. were observed, such as colony (color, size, shape), phialides (size), conidia (size, color, shape), and the growth rate (Bissett, 1991). Meanwhile, the morphological features of *Rhizoctonia* sp. were observed, i.e. colony, sclerotium characteristics (color, shape, number, and size), hyphae color and structure, as well as growth rate (Guleria *et al.*, 2007; Susheela & Reddy, 2013; Mishra *et al.*, 2014).

The pathogenicity test of *Rhizoctonia* sp. isolate UGM-RBAF was carried out by adding sclerotia on the rice roots. Rice seeds of IR64 that have been soaked and incubated for 24 hours were planted in the planting medium in the form of a mixture of soil and sterile manure 4:1 (v/v) with a total of 5 kg. Each pot consisted of five rice seeds. Inoculation of *Rhizoctonia* sp. isolate UGM-RBAF was carried out by placing four sclerotia around the rice roots at 6 days after sowing. Rice plants covered with transparent plastic to keep moisture then observed until symptom appearance.

DNA Extraction, Amplification, and Sequencing

Trichoderma sp. isolate UGM-LHAF and *Rhizoctonia* sp. isolate UGM-RBAF (5 mm diameter, 5 days old) were grown separately in 50 ml PDB medium and shaken in a shaker (130 rpm) at 28°C for 5 days. The DNA was extracted from growing mycelium, according to the 2% CTAB method (Doyle & Doyle, 1990). ITS1 (5'-TCCGTAGGTGAAC CTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGA TGC-3') primer pairs were used to amplify the DNA with T100 Thermal Cycler (White *et al.*, 1990).

PCR was performed in a total volume of 25 µl, which contained 12.5 µl PCR mix (Bioline®), 9.5 µl ddH_2O, 1 µl forward primer, 1 µl reverse primer, and 1 µl genomic DNA. PCR amplification for *Trichoderma* sp. isolate UGM-LHAF was programmed with an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 90 s, annealing at 55°C for 2 min, extension at 72°C for 3 min, and final extension at 72°C for 5 min (Jaisani & Pandey, 2017). PCR amplification for *Rhizoctonia* sp. isolate UGM-RBAF was programmed with an initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 15 s, annealing at 58°C for 15 s, extension at 72°C for 15 s, and final extension at 72°C for 7 min (Sandoval & Cumangun, 2019).

The PCR amplified products were resolved on 1% agarose gels and run at 100 volt for 25 min. The DNA bands were visualized with ethidium bromide under UV-illumination. Then the PCR products were sent to FirstBASE, Malaysia, for sequencing, with the sample gene sequence adjusted to the *Trichoderma* spp. and *Rhizoctonia* spp. gene sequences contained in the GenBank database via BLAST search at https://blast.ncbi.nlm.nih.gov/. ITS DNA sequences were aligned using Clustal W. Phylogenetic tree was constructed with Maximum Likelihood method by Mega-X programs (Tamura *et al.*, 2011). Each isolate sequence was deposited in GenBank.

Dual Culture

Dual culture test was carried out by culturing *Trichoderma* sp. isolate UGM-LHAF and *Rhizoctonia* sp. isolate UGM-RBAF (5 mm diameter, 5 days old) on PDA with the opposite side to each other (Xian *et al*., 2019). Two isolates were placed 2 cm from the edge of the Petri dish. PDA plate with only *Rhizoctonia* sp. isolate UGM-RBAF was included as a control. Three replicates were maintained for each treatment and incubated at 28°C for 3 days. The encounter of hyphae between the two isolates was observed under a light microscope (Olympus CX21, Tokyo, Japan). Furthermore, the percentage of pathogen growth inhibition in each test was calculated based on Saravanakumar *et al*. (2016) with the formula:

$$I = \frac{(C - T)}{C} \times 100$$

where *I* denotes growth inhibition of the pathogen (%), C and T indicate radial growth of the pathogen in control and treatment.

Antifungal Activity Test

Antifungal activity test was carried out based on Meena *et al*. (2017), where a hyphal disc of *Trichoderma* sp. isolate UGM-LHAF (6 mm diameter, 5 days old) was cultured in 50 ml of PDB medium, shaken using a shaker (130 rpm), and incubated at 28°C for 15 days. The culture was filtered with Whatman paper No.1 and centrifuged (10,000 rpm) for 15 minutes. Furthermore, the supernatant obtained was filtered with a 0.22 µm millipore to obtain the culture filtrate. *Rhizoctonia* sp. isolate UGM-RBAF (5 mm diameter, 5 days old) were grown on PDA medium containing 0%, 10%, 25%, and 50% culture filtrate of *Trichoderma* sp. isolate UGM-LHAF. The control plate, PDA medium contained an equal volume of sterile water which was inoculated with *Rhizoctonia* sp. isolate UGM-RBAF. Each treatment included three replicates and incubated at 28°C for 2–3 days. The mycelial growth was measured and the percentage of pathogen growth inhibition in each treatment was calculated according to the dual culture test formula.

Scanning Electron Microscopy (SEM)

Hyphae of *Rhizoctonia* sp. isolate UGM-RBAF growing on the culture filtrate of *Trichoderma* sp. isolate UGM-LHAF were observed under a scanning electron microscope (SEM). Observation using SEM was carried out by preparing the sample for one night in 0.05 M phosphate buffer (pH 7.3) containing 4% glutaraldehyde. The samples were washed 3 times (for 15 minutes each) with phosphate buffer the next day. Sample dehydration was carried out with stratified ethanol series (30, 50, 70, 80, 90, and 100%) for 15 minutes then dried with CO_2 for 5 minutes and immediately placed on aluminum pieces. Samples were placed on a 400 mesh grid. The sample was painted by negative painting used ammonium molybdate to bind the transmitted electron emission so that there was a color contrast. Then the samples were observed under SEM (Pandian *et al*., 2016).

Statistical Analysis

The data were analyzed with one-way ANOVA and followed by Duncan's Multiple Range Test at a significant level of 0.05 using DSASTAAT 1.101 program.

RESULTS AND DISCUSSION

Trichoderma sp. isolate UGM-LHAF had greenish-white colony color that turns dark green when approaching old age, hyaline hyphae with a septum, and pyramidal conidiophore with the phialide branches, the phialide size (diameter 10.31 × 3.29 µm), conidia (green and globose-shaped, diameter 3.74 × 3.25 µm), and growth rate of 34.43 mm/day (Figure 1a-c). The characteristics of *T. asperellum* isolate UGM-LHAF were similar to *T. asperellum* characterized by Shang *et al*. (2020). Oszako *et al*. (2020) stated that *T. asperellum* had conidiophores to which branched phialide, 2–3 in number, measuring 6.4 × 1.4 µm. Conidia were colored greenish, ellipsoid, 3.1 × 2.6 µm, and chlamydospores unicellular, terminal, and roughly spherical measuring 20.5 × 21.2 µm.

Rhizoctonia sp. isolate UGM-RBAF had perpendicular hyphae with a septum, a white colony that change to brownish-white when old, light brown to dark brown sclerotium with a round shape (diameter of 1.33–1.58 mm, the weight of 0.17 g/Petri dish, and amount of 19.5/petri), and a growth rate of 40 mm/day (Figure 1d-f). The characteristics of *R. solani* isolate UGM-RBAF were similar to *R. solani* characterized by R. Singh *et al*. (2015). Budiarti *et al*. (2020) reported that 41 *R. solani* isolates isolated

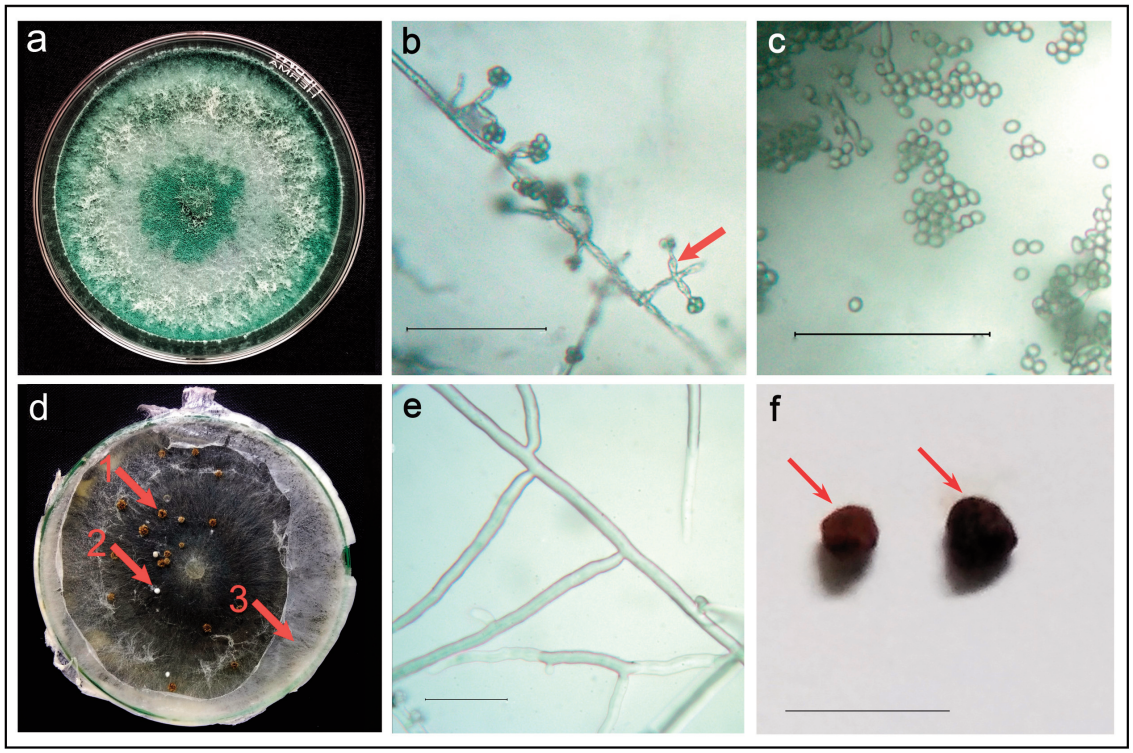

Figure 1. Morphology of fungal isolates. *Trichoderma* sp. isolate UGM-LHAF: (a) 6 days old culture plate on PDA, (b) phialide, and (c) conidia (bar = 50 µm). *Rhizoctonia* sp. isolate UGM-RBAF: (d) 15 days old culture plate on PDA (1 = mature sclerotia, 2 = young sclerotia, 3 = abundant mycelial growth), (e) hyphae (bar = 50 µm), and (f) sclerotia (bar = 5 mm).

from Indonesia had cultural and morphological characteristics, i.e. hyphae branching of *R. solani* 90 angles, had hyphal width (5.66–7.58 µm), a sparse fluffy of colony texture, sclerotia dark brown color, the irregular distribution pattern of sclerotia, micro, and macro sclerotial size (3.33 to 183.67 mm), and sclerotia on the surface of PDA. The pathogenicity test of *Rhizoctonia* sp. isolate UGM-RBAF explained that the first sheath blight symptom was observed at 12 days after inoculation. The mycelium was observed after 18 days inoculation around sheath blight symptoms (Figure 2a-b), while sclerotia were observed after 30 days inoculation (Figure 2c). This study indicated that *Rhizoctonia* sp. isolate UGM-RBAF was a virulent pathogen on rice.

The morphological identification of *Trichoderma* sp. isolate UGM-LHAF and *Rhizoctonia* sp. isolate UGM-RBAF were confirmed with molecular identification using ITS1-ITS4 Primers. The results showed that the possibility of amplifying PCR products with sizes around 600 bp for *Trichoderma* sp. isolate UGM-LHAF (Figure 3a), and around 700 bp for *Rhizoctonia* sp. isolate UGM-RBAF (Figure 3b). The result for the analysis of the two sequences using BLAST showed that *Trichoderma* sp. isolate UGM-LHAF was identified as *Trichoderma asperellum* (MT 102403) with 100% identity, while *Rhizoctonia* sp. isolate UGM-RBAF was identified as *Rhizoctonia solani* (MN365714) with 96.5% identity. It was strongly confirmed their morphological characteristic. The two sequences were then deposited at NCBI with the accession number MT367901 (*Trichoderma asperellum* isolate UGM-LHAF) and MT367900 (*Rhizoctonia solani* isolate UGM-RBAF).

The phylogenetic analysis based on the ITS sequence observed that *T. asperellum* isolate UGM-LHAF was located in the clade *T. asperellum* strain MF22552, PANCOM8 isolate, and Tasum66 isolate with 100% bootstrap value (Figure 3a). *T. asperellum* strain MF22552 was isolated from lakes in India, *T. asperellum* isolate PANCOM8 isolated from soil in Indian rice fields, and *T. asperellum* isolate Tasum66 isolated from soil in China. The phylogenetic tree based on the ITS sequence (Figure 3b) showed that *R. solani* isolate UGM-RBAF was grouped with *Thanatephorus cucumeris* isolate JZ2, *R. solani* strain APHyd,

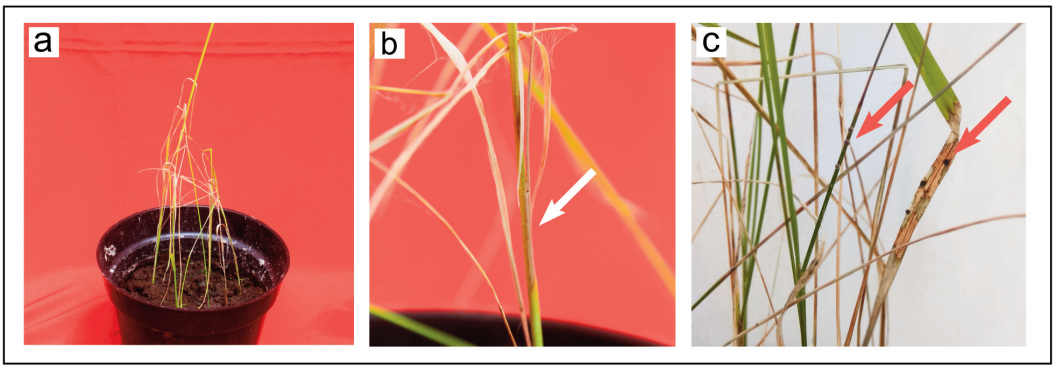

Figure 2. Pathogenicity test of *Rhizoctonia* sp. isolate UGM-RBAF. (a-b) symptoms of sheath blight disease (white arrow) 18 days after inoculation on IR64 rice. (c) sclerotia (red arrow) was observed on 30 days after inoculation.

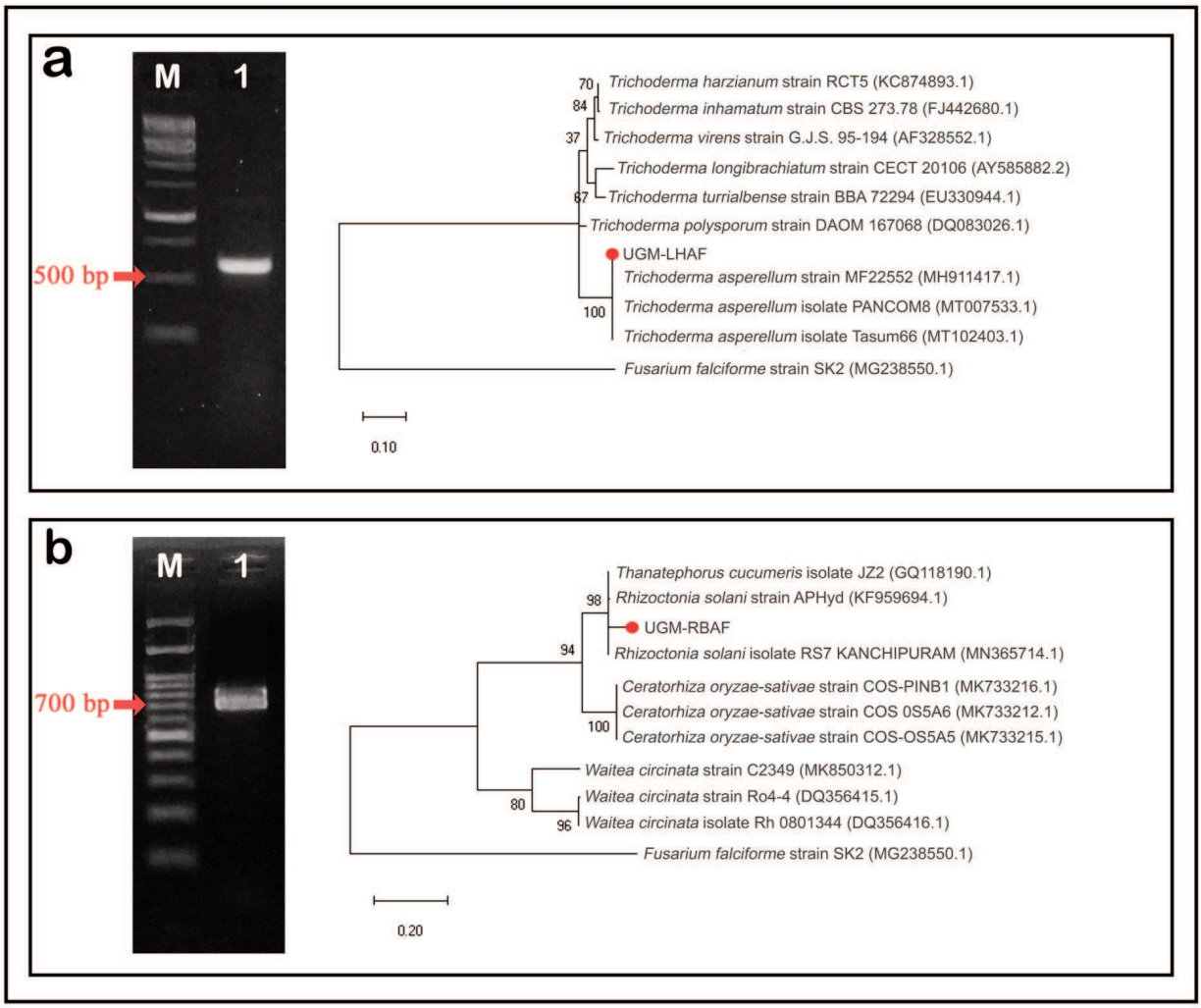

Figure 3. Molecular identification and phylogenetic tree analysis. (a) M = 1 Kb ladder, lane 1 = *Trichoderma asperellum* isolate UGM-LHAF; (b) M = 100 bp ladder, lane 1 = *Rhizoctonia solani* isolate UGM-RBAF. Bootstrap values with 1000 replicates are used in phylogenetic analysis. Isolates from this study are in red node. *Fusarium falciforme* is an outgroup.

R. solani isolate RS7 KANCHIPURAM by 96% bootstrap value. *R. solani* isolate RS7 KANCHIPURAM was the result of isolation from symptoms of sheath blight on rice leaves in India, and *Thanatephorus cucumeris* isolate JZ2 was also the result of isolation from rice plants in China.V. Singh *et al.* (2018) reported that *R. solani* APHyd strains were obtained from the isolation of symptoms of sheath blight on maize in India which were grouped into AG1 -IA based on ITS sequences. This showed that *R. solani* isolate UGM-RBAF belongs to the AG1-1A subgroup.

The dual culture test showed that hyphal of *T. asperellum* isolate UGM-LHAF after one day incubation was contacted with hyphal *R. solani* isolate UGM-RBAF (Figure 4a-b). The colony diameter of *R. solani* isolate UGM-RBAF after 3 days incubation had 23.5 mm (in dual culture Petri dish) and 65.7 mm (control Petri dish). *T. asperellum* isolate UGM-LHAF was able to suppress the radial growth of *R. solani* isolate UGM-RBAF. The inhibition of *T. asperellum* isolate UGM-LHAF against *R. solani* isolate UGM-RBAF in mycelial growth was found as 64.23%. The effectiveness of *T. asperellum* isolate UGM-LHAF in controlling *R. solani in vitro* by a dual culture test in this study was in line with Asad *et al.* (2014) and Chinnaswami *et al.* (2021) studies, which stated that *T. asperellum* was able to suppress the radial growth of *R. solani* at a percentage range of 67.74–74.40%. The inhibition zone of the two isolates illustrated the coiling of *T. asperellum* isolate UGM-LHAF hyphae against *R. solani* hyphae (Figure 4c). In line with Jiang *et al.* (2016) reported that *T. asperellum* coiled and penetrated the hyphae pathogen then breaks it down into smaller fragments so that causes the death of pathogenic hyphae.

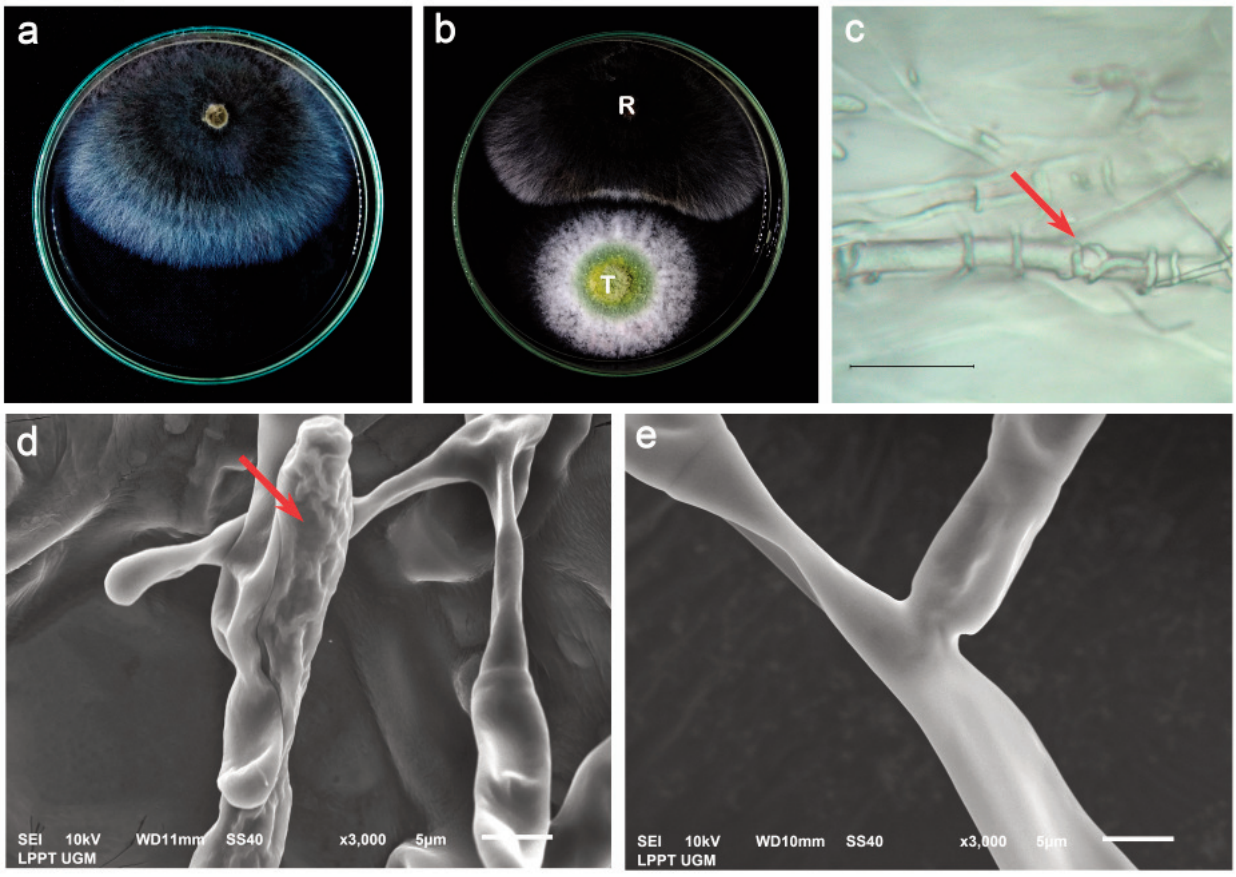

Figure 4. The inhibition effect of *Trichoderma asperellum* isolate UGM-LHAF toward mycelial growth of *Rhizoctonia solani* isolate UGM-RBAF. Dual culture test: (a) *R. solani* isolate UGM-RBAF as a control plate, (b) interaction between *T. asperellum* isolate UGM-LHAF and *R. solani* isolate UGM-RBAF at 28ºC for 1 day after incubation, and (c) hyphae coiling after 2 days confrontation (bar = 100 µm). SEM micrographs of *R. solani* isolate UGM-RBAF hyphae after 3 days exposure by (d) 50% and (e) control: 0% culture filtrate of *T. asperellum* isolate UGM-LHAF (bar = 5 µm).

Table 1. The inhibition effect of culture filtrate of *Trichoderma asperellum* isolate UGM-LHAF toward mycelial growth of *Rhizoctonia solani* isolate UGM-RBAF at 2 days

Concentration	Radial growth[1] (mm)	Inhibitory effect[2] (%)
0% (control)	90.00	0 d
10%	79.22	11.98 c
25%	69.35	22.94 b
50%	28.35	68.50 a

[1]The radial growth of *Rhizoctonia solani* isolate UGM-RBAF was measured on four sides in each treatment plate.
[2]The numbers followed by the same letter in the same column show no significant difference in the 5% DMRT test.

According to Wu *et al.* (2017), *T. asperellum* had a mycoparasitic mechanism in the form of hyphae coiling involving cell wall-degrading enzymes (CWDEs) comprising of chitinase, glucanase, and protease which degraded pathogenic cell walls. *Trichoderma* hyphae coiled pathogenic hyphae and caused hyphal abnormalities and pathogenic cell lysis (Romero-Cortes *et al.*, 2019; Zhang & Zhuang, 2020).

The culture filtrate inhibitor of *T. asperellum* isolate UGM-LHAF also had significant differences with control in reducing the mycelium growth of *R. solani*. Table 1 showed that the radial growth of *R. solani* mycelium at 10%, 25%, and 50% concentration of culture filtrate of *T. asperellum* isolate UGM-LHAF was 79.22 mm, 69.35 mm, 28.35 mm, respectively. The percentage of growth inhibition of *R. solani* after 2 days incubation in each treatment were 11.98%, 22.94%, and 68.5%, respectively. Mayo-Prieto *et al.* (2020) reported that *Trichoderma* isolate was able to inhibit *R. solani* growth more than 80% by an antifungal activity test. Among antifungal treatment of *T. asperellum* isolate UGM-LHAF, 50% culture filtrate of *T. asperellum* isolate UGM-LHAF showed the strongest radial growth inhibition, while 10% and 25% culture filtrate of *T. asperellum* isolate UGM-LHAF showed the lowest. Furthermore, SEM observation indicated that the culture filtrate of *T. asperellum* isolate UGM-LHAF caused abnormalities of *R. solani* isolate UGM-RBAF hyphae compared with the control (Figure 4d-e). The culture filtrate of *Trichoderma* species decreased germ tube length of pathogens (El-Rafai *et al.*, 2003). In the antifungal activity test, the abnormality of *R. solani* hyphae was probably caused by the secondary metabolite contents in the culture filtrate of *T. asperellum* isolate UGM-LHAF. The culture filtrate of *T. asperellum* was also known to contain secondary metabolites in the form of trichodermaerin, asperilin, methylcordysinin A, steroid, ergosta, beta-sitosterol, adenine nucleoside, sesquiterpene, pyrone, viridin, viridiol, butenolide, harzianolide, ferulic acid, viridiofungin A, cyclonerodiol, massoilactone, and gliovirin which were antifungal (Vinale *et al.*, 2009; Stracquadanio *et al.*, 2020). Harzianic acid produced by *Trichoderma* species exhibited antibiotic activity against *R. solani* under *in vitro* conditions (Manganiello *et al.*, 2018).

CONCLUSION

Trichoderma sp. and *Rhizoctonia* sp. were identified as *T. asperellum* and *R. solani* based on ITS ribosomal DNA. *In vitro* test showed that *T. asperellum* isolate UGM-LHAF was able to inhibit the growth of *R. solani* on dual culture and antifungal test.

ACKNOWLEDGEMENT

This research article is part of the first author's thesis. The authors would like to thank The Indonesian Endowment Fund for Education (LPDP)–Ministry of Finance, Indonesia, for giving a National-International Olympic Affirmation Scholarship and funding this research.

LITERATURE CITED

Asad, S.A., Ali, N., Hameed, A., Khan, S.A., Ahmad, R., Bilal, M., … Tabassum, A. (2014). Biocontrol Efficacy of Different Isolates of *Trichoderma* against Soil Borne Pathogen *Rhizoctonia solani*. *Polish Journal of Microbiology, 63*(1), 95–103. https://doi.org/10.33073/pjm-2014-014

Badan Pusat Statistik. (2020). *Harvested Area, Productivity, and Production of Paddy by Province, BPS Statistics Indonesia*. Retrieved from https://www.bps.go.id/indicator/53/1498/1/harvested-area-productivity-and-production-of-paddy-by-province.html

Bissett, J. (1991). A Revision of the Genus *Trichoderma*. II. Infrageneric Classification. *Canadian Journal of Botany, 69*(11), 2357–2372. https://doi.org/10.1139/b91-297

Budiarti, S.W., Lukman, R., Wibowo, A., Sumardiyono, C., & Priyatmojo, A. (2020). The Cultural and Morphological Variability among *Rhizoctonia solani* Isolates Causing Banded Leaf and Sheath Blight of Maize in Indonesia. *Archives of Phytopathology and Plant Protection, 53*(1–2),17–36. https://doi.org/10.1080/03235408.2020.1715765

Carling, D.E. (1996). Grouping in *Rhizoctonia solani* by Hyphal Anastomosis Reaction. *In* B. Sneh, S. Jabaji-Hare, S. Neate, G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 35–47), Dordrecht, Netherlands: Springer. https://doi.org/10.1007/978-94-017-2901-7_3

Carling, D.E., Baird, R.E., Gitaitis, R.D., Brainard, K.A., & Kuninaga, S. (2002). Characterization of AG-13, a Newly Reported Anastomosis Group of *Rhizoctonia solani*. *Phytopathology*, *92*(8), 893–899. https://doi.org/10.1094/PHYTO.2002.92.8.893

Chinnaswami, K., Mishra, D., Miriyala, A., Vellaichamy, P., Kurubar, B., Gompa, … Raman, M.S. (2021). Native Isolates of *Trichoderma* as Biosuppressants against Sheath Blight and Stem Rot Pathogens of Rice. *Egyptian Journal of Biological Pest Control, 31*, 12. https://doi.org/10.1186/s41938-020-00356-4

de França, S.K.S., Cardoso, A.F., Lustosa, D.C., Ramos, E.M.L.S., de Filippi, M.C.C., & da Silva, G.B. (2015). Biocontrol of Sheath Blight by *Trichoderma asperellum* in Tropical Lowland Rice. *Agronomy for Sustainable Development, 35*(1), 317–324. https://doi.org/10.1007/s13593-014-02443

Dinas Perkebunan Provinsi Kalimatan Timur. (2017). *Manfaat Trichoderma* sp. *dan Cara Perkembangbiakannya.* Retrieved from https://disbun.kaltimprov.go.id/artikel/manfaat-trichoderma-sp-cara-pembiakkannya

Doyle, J.J. & Doyle, J.L. (1990). Isolation of Plant DNA from Fresh Tissue. *Focus, 12*(1), 13–15.

El-Rafai, I.M., Asswah, S.M.W., & Awdalla, O.A. (2003). Biocontrol of Some Tomato Disease Using Some Antagonistic Microorganisms. *Pakistan Journal of Biological Sciences, 6*(4), 399–406. https://doi.org/10.3923/pjbs.2003.399.406

Garcia, V.G., Onco, M.A.P., & Susan, V.R. (2006). Review. Biology and Systematic of the Form Genus *Rhizoctonia*. *Spanish Journal of Agricultural Research, 4* (1), 55–79. https://doi.org/10.5424/sjar/2006041-178

Guleria, S., Aggarwal, R., Thind, T.S., & Sharma, T.R. (2007). Morphological and Pathological Variability in Rice Isolates of *Rhizoctonia solani* and Molecular Analysis of their Genetic Variability. *Journal of Phytopathology, 155*(11–12), 654–661. https:// doi.org/10.1111/j.1439-0434.2007.01291.x

Jaisani, P. & Pandey, R.N. (2017). Morphological and Molecular Characterization for Identification of Isolates of *Trichoderma* spp. from Rhizospheric Soils of Crops in Middle Gujarat. *Indian Phytopathology, 70*(2), 238–245. http://doi.org/10.24838/ip.2017v70.i2.71652

Jiang, H., Zhang, L., Zhang, L-Z., Ojaghian, M.R., & Hyde, K.D. (2016). Antagonistic Interaction between *Trichoderma asperellum* and *Phytophthora capsici* in Vitro. *Journal of Zhejiang University Science B, 17*(4), 271–281. https://doi.org/10.1631/jzus.B1500243

Khan, A.A. & Sinha, A.P. (2007). Screening of *Trichoderma* spp. against *Rhizoctonia solani* the Causal Agent of Rice Sheath Blight. *Indian Phytopathology, 60*(4), 450–456. Retrieved from https://epubs.icar.org.in/ejournal/index.php/IPPJ/article/view/13922/6910

Manganiello, G., Sacco, A., Ercolano, M.R., Vinale, F., Lanzuise, S., Pascale, A., … Woo, S.L. (2018). Modulation of Tomato Response to *Rhizoctonia solani* by *Trichoderma harzianum* and Its Secondary Metabolite Harzianic Acid. *Frontiers in Microbiology, 9,* 1996. https://doi.org/10.3389/fmicb.2018. 01966

Mayo-Prieto, S., Campelo, M.P., Lorenzana, A., Rodríguez-González, A., Reinoso, B., Gutiérrez, S., & Casquero, P.A. (2020). Antifungal Activity and Bean Growth Promotion of *Trichoderma* Strains Isolated from Seed vs Soil. *European Journal of Plant Pathology*, *158*(4), 817–828. https://doi.org/10.1007/s10658-020-02069-8

Meena, M., Swapnil, P., Zehra, A., Dubey, M.K., & Upadhyay, R.S. (2017). Antagonistic Assessment of *Trichoderma* spp. by Producing Volatile and Non-volatile Compounds against Different Fungal Pathogens. *Archives of Phytopathology and Plant Protection*, *50*(13–14), 629–648. https://doi.org/10.1080/03235408.2017.1357360

Mishra, P.K., Gogoi, R., Singh, P.K., Rai, S.N., Singode, A., Kumar, A., & Manjunatha, C. (2014). Morpho-cultural and Pathogenic Variability in *Rhizoctonia solani* Isolates from Rice, Maize and Green Gram. *Indian Phytopathology, 67*(2), 147–154. Retrieved from https://epubs.icar.org.in/ejournal/index.php/IPPJ/article/view/40913/18622

Mukhopadhyay, R. & Kumar, D. (2020). Trichoderma: A Beneficial Antifungal Agent and Insights into its Mechanism of Biocontrol Potential. *Egyptian Journal of Biological Pest Control, 30*(1), 1–8. https://doi.org/10.1186/s41938-020-00333-x

Naeimi, S., Okhovvat, S.M., Javan-Nikkhah, M., Vágvölgyi, C., Khosravi, V., & Kredics, L. (2010). Biological Control of *Rhizoctonia solani* AG1-1A, the Causal Agent of Rice Sheath Blight with *Trichoderma* Strains. *Phytopathologia Mediterranea, 49*(3), 287–300. Retrieved from https://www.jstor.org/stable/26458654

Oszako, T., Voitka, D., Stocki, M., Stocka, N., Nowakowska, J.A., Linkiewicz, A., ... Malewski, T. (2020). *Trichoderma asperellum* Efficiently Protects *Quercus robur* Leaves against *Erysiphe alphitoides*. *European Journal of Plant Pathology*, *159*(2), 295–308. https://doi.org/10.1007/s10658-020-02162-y

Pandian, R.T.P., Raja, M., Kumar, A., & Sharma, P. (2016). Morphological and Molecular Characterization of *Trichoderma asperellum* strain *Ta*13. *Indian Phytopathology, 69*(3), 298–303. Retrieved from https://epubs.icar.org.in/ejournal/index.php/IPPJ/article/view/60166/24995

Priyatmojo, A., Escopalao, V.E., Tangonan, N.G., Pascual, C.B., Suga, H., Kageyama, K., & Hyakumachi, M. (2001). Characterization of a New Subgroup of *Rhizoctonia solani* Anastomosis Group 1 (AG-1-ID), Causal Agent of a Necrotic Leaf Spot on Coffee. *Phytopathology*®, *91*(11), 1054–1061. https://doi.org/10.1094/phyto.2001.91.11.1054

Rabindran, R., & Vidhyasekaran, P. (1996). Development of a Formulation of *Pseudomonas fluorescens* PfALR2 for Management of Rice Sheath Blight. *Crop Protection*, *15*(8), 715–721. https://doi.org/10.1016/s0261-2194(96)00045-2

Rismanto, T.A.L. (2020). *Eksplorasi dan Isolasi APH Trichoderma sp.* (*Exploration and Isolation of Biological Agents of Trichoderma sp.*). Retrieved from https://dpkp.jogjaprov.go.id/

Romero-Cortes, T., López-Pérez, P.A., Pérez España, V.H., Medina-Toledo, A.K., Aparicio-Burgos, J.E., & Cuervo-Parra, J.A. (2019). Confrontation of *Trichoderma asperellum* VSL80 against *Aspergillus niger* via The Effect of Enzymatic Production. *Chilean Journal of Agricultural & Animal Sciences*, *35*(1), 68–80. https://doi.org/10.4067/s0719-38902019005000202

Sandoval, R.F.C., & Cumagun, C.J.R. (2019). Phenotypic and Molecular Analyses of *Rhizoctonia* spp. Associated with Rice and Other Hosts. *Microorganisms*, *7*(3), 88. https://doi.org/10.3390/microorganisms7030088

Saravanakumar, K., Yu, C., Dou, K., Wang, M., Li, Y., & Chen, J. (2016). Synergistic Effect of *Trichoderma*-derived Antifungal Metabolites and Cell Wall Degrading Enzymes on Enhanced Biocontrol of *Fusarium oxysporum* f. sp. *cucumerinum*. *Biological Control, 94*, 37–46. https://doi.org/10.1016/j.biocontrol.2015. 12.001

Shang, J., Liu, B., & Xu, Z. (2020). Efficacy of *Trichoderma asperellum* TC01 against Anthracnose and Growth Promotion of *Camellia sinensis* Seedlings. *Biological Control*, *143*, 104205. https://doi.org/10.1016/j.biocontrol.2020.104205

Singh, P., Mazumdar, P., Harikrishna, J.A., & Babu, S. (2019). Sheath Blight of Rice: A Review and Identification of Priorities for Future Research. *Planta*, *250*(5), 1387–1407. https://doi.org/10.1007/s00425-019-03246-8

Singh, R., Murti, S., Mehilal, Tomer, A., & Prasad, D. (2015). Virulence Diversity in *Rhizoctonia Solani* Causing Sheath Blight in Rice Pathogenicity. *Journal of Plant Pathology & Microbiology, 6*(8), 296. https://doi.org/10.4172/2157-7471.1000296

Singh, V., Amaradasa, B. S., Karjagi, C. G., Lakshman, D. K., Hooda, K. S., & Kumar, A. (2018). Morphological and Molecular Variability among Indian Isolates of *Rhizoctonia solani* Causing Banded Leaf and Sheath Blight in Maize. *European Journal of Plant Pathology, 152*(1), 45–60. https://doi.org/10.1007/s10658-018-1447-2

Stracquadanio, C., Quiles, J.M., Meca, G., & Cacciola, S.O. (2020). Antifungal Activity of Bioactive Metabolites Produced by *Trichoderma asperellum* and *Trichoderma atroviride* in Liquid Medium. *Journal of Fungi*, *6*(4), 263. https://doi.org/10.3390/jof6040263

Susheela, K., & Reddy, C.S. (2013). Variability in *Rhizoctonia solani* (AG1IA) Isolates Causing Sheath Blight of Rice in India. *Indian Phytopathology, 66* (4), 341–350. Retrieved from https://epubs.icar.org.in/ejournal/index.php/IPPJ/article/view/36009/15962

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, *28* (10), 2731–2739. https://doi.org/10.1093/molbev/msr121

Vinale, F., Ghisalberti, E., Sivasithamparam, K., Marra, R., Ritieni, A., Ferracane, R., ... Lorito, M. (2009). Factors Affecting the Production of *Trichoderma harzianum* Secondary Metabolites during the Interaction with Different Plant Pathogens. *Letters in Applied Microbiology*, *48*(6), 705–711. https://doi.org/10.1111/j.1472-765x.2009.02599.x

Wang, L., Liu, L.M., Hou, Y.X., Li, L., & Huang, S.W. (2015). Pathotypic and Genetic Diversity in the Population of *Rhizoctonia solani* AG1-IA Causing Rice Sheath Blight in China. *Plant Phytopathology, 64*(3), 718–728. https://doi.org/10.1111/ppa.12299

White, T.J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In M.A. Innis, D.H. Gelfand, J.J. Sninsky, & J.J. White (Eds.), *PCR Protocols: A Guide to Methods and Applications* (pp. 315–322). San Diego, United States: Academic Press. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Wu, Q., Sun, R., Ni, M., Yu, J., Li, Y., Yu, C., ... Chen, J. (2017). Identification of a Novel Fungus, *Trichoderma asperellum* GDFS1009, and Comprehensive Evaluation of its Biocontrol Efficacy. *PLoS ONE, 12*(6), e0179957. https://doi.org/10.1371/journal .pone.0179957

Xian, H., Liu, L., Li, Y., Yang, Y., & Yang, S. (2019). Molecular Tagging of Biocontrol Fungus *Trichoderma asperellum* and its Colonization in Soil. *Journal of Applied Microbiology*, *128*(1), 255–264. https://doi.org/10.1111/jam.14457

Yu, Z., Wang, Z., Zhang, Y., Wang, Y., & Liu, Z. (2021). Biocontrol and Growth-Promoting Effect of *Trichoderma asperellum* TaspHu1 Isolate from *Juglans mandshurica* Rhizosphere Soil. *Microbiological Research*, *242*, 126596. https://doi.org/10.1016/j.micres.2020.126596

Zhang, C., Liu, Y., Ma, X., Feng, Z., & Ma, Z. (2009). Characterization of Sensitivity of *Rhizoctonia solani,* Causing Rice Sheath Blight, to Mepronil and Boscalid. *Crop Protection*, *28*(5), 381–386. https://doi.org/10.1016/j.cropro.2008.12.004

Zhang, Y., & Zhuang, W.-Y. (2020). *Trichoderma brevicrassum* strain TC967 with Capacities of Diminishing Cucumber Disease Caused by *Rhizoctonia solani* and Promoting Plant Growth. *Biological Control*, *142*, 104151. https://doi.org/10.1016/j.biocontrol.2019.104151