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In vitro inhibition mechanism of *Trichoderma asperellum* isolates from corn against *Rhizoctonia solani* causing banded leaf and sheath blight disease and its role in improving the growth of corn seedlings

Hishar Mirsam[1*], Suriani[1], Sri Kurniawati[1], Oky Dwi Purwanto[1], Amran Muis[1], Syahrir Pakki[1], A. Tenrirawe[1], Nurnina Nonci[1], Herawati[1], Muslimin[2] and Muhammad Azrai[3]

Abstract

Background One of the primary corn diseases, banded leaf and sheath blight, is carried on by the pathogenic fungus *Rhizoctonia solani*. Efforts to control *R. solani* are more directed at environmentally friendly control using a biological control agent, such as *Trichoderma* spp. as antagonistic agents and plant growth promoter. This study aimed to identify *T. asperellum* isolates based on molecular characteristics and to determine the in vitro inhibition mechanism against *R. solani* and its role in enhancing the growth of corn seedlings.

Results The HMRP7, HMRF7A, HMEDF1B, HMEDF6A, and CHM01 isolates were identified as *T. asperellum* with a genetic distance coefficient value of 0.000 and a very high similarity of 100%. Meanwhile, the RsHM isolate was identified as *R. solani* with a homology level of > 90% and genetic distance coefficient values ranging from 0.000 to 0.032. The antagonistic ability showed that the five *T. asperellum* isolates were able to inhibit the growth of *R. solani* in vitro on PDA medium with an inhibition percentage of ≥ 50%, so they were categorized as antagonist agents. *T. asperellum* showed the antagonistic mechanism in inhibiting the *R. solani* growth through the action of parasitism. The five *T. asperellum* isolates tested on corn seedlings showed a significantly high difference from the control treatment on the observational variables of maximum growth potential (MGP), growth rate (GtR), growth simultaneity (GS), vigor index (VI), germination rate (GR), and median germination time (T50).

Conclusion CHM01 isolate showed better potential than other isolates in inhibiting the growth of *R. solani* in vitro on PDA medium with a parasitism mechanism and enhancing the growth of corn seedlings.

Keywords Corn, Parasitism, Plant-growth inducer, *Trichoderma asperellum*

*Correspondence:
Hishar Mirsam
hish001@brin.go.id
Full list of author information is available at the end of the article

Background

In the past few years, efforts to increase corn production as food, feed, and industry have received serious attention from both farmers and product users. In actuality, numerous plant diseases are extensively present during corn cultivation in the field, which can decrease corn yields' quantity and quality. One of the major corn diseases, banded leaf and sheath blight, is brought on by the pathogenic fungus *Rhizoctonia solani*. This disease is commonly found attacking corn during the rainy season and reduces the production of corn in the field (Rotasouw et al. 2020). High-value economic crops, particularly corn, are susceptible to this disease causing high-yield losses (Dai et al. 2017). The epidemics of this disease have been reported in recent years in many countries, *i.e.* in Southeast Asia (Malaysia, Myanmar, Philippines, Thailand, Vietnam, Indonesia); South Asia (India, Sri Lanka, Nepal, Pakistan, Bhutan); East Asia (Japan, Korea, South China); West Africa (Sierra Leone, Ivory Coast, Nigeria); Europe (United Kingdom), South America (Venezuela); and United States (Arkansas) (Zhou et al. 2016).

Having a diverse host range, *R. solani* is a necrotrophic fungal disease (Adams 1988). This fungus is soil borne, causes different symptoms at each plant growth phase, including root, stem, and crown rots, damping-off, as well as foliar and sheath rot (Baker 1970). This pathogen can endure in soil under suitable environmental conditions for many years by forming sclerotia and mycelia on residual plant debries. In addition, *R. solani* is a complex fungus with various genetic groups (Matthew and Brooker 1991). These groups are known as anastomotic groups (AGs). Hyphae of the same AG expand their genetic diversity by fusing or anastomosing with each other (Webster and Weber 2007). Because the AG-BI group has been included in into AG-2, *R. solani* has been assigned to 13 AG, which consists of AG-1 to AG-13 (Carling et al. 2002). According to pathogenicity, morphology, host specificity, nutritional requirements, optimal temperature, and frequency of hyphal anastomosis, single anastomotic groups can be classified into subgroups (Sneh et al. 1991).

Several methods have been taken to overcome plant disease problems, such as the use of fungicides and breeding to produce disease-resistant plants. The use of synthetic pesticides can cause pollution to the environment, and kill non-target microorganisms, besides that being harmful to humans. However, the necessity to use biological control agents as an alternative to control plant pathogens has arisen due to rising organic food demand and public knowledge of the risks of synthetic fungicides (Suryadi et al. 2015).

One biological control agent, *Trichoderma* sp., is well known for antagonizing soil-borne pathogens both in vitro and in vivo (Mirsam et al. 2022). One of the *Trichoderma* species that has been widely reported to be able to control soil-borne pathogens is *T. asperellum*. This fungus was reported to be capable of inhibiting the growth of phytopathogenic fungi, including *R. solani*, *Fusarium verticillioides, F. oxysporum, F. solani, F. camptocerus, F. camptocerus, Alternaria alternata, Botrytis cinerea, Colletotrichum gloeosporioides, Cytospora chrysosperma,* and *Ganoderma applanatum* (Mirsam et al. 2022). Aside from being a biological control agent, *Trichoderma* sp. has also been widely reported to induce plant growth and development. *Trichoderma* sp. can indirectly and directly stimulate plant growth by enhancing germination rate and vigor, plant dry weight, promoting flowering, and enhancing plant tolerance to biotic and abiotic stresses, *Trichoderma* sp. can indirectly and directly stimulate plant growth (Stewart and Hill 2014). *Trichoderma* sp. stimulates plant growth by increasing the synthesis of phytohormones from both *Trichoderma* sp. and plants, promoting root development, generating vitamins, speeding up the metabolism of carbohydrates and photosynthesis, and enhancing nutrient absorption and translocation (Harman 2011). Auxin is a phytohormone produced by certain *Trichoderma* (Contreras-Cornejo et al. 2009), gibberellic acid (GA3), ethylene (Stewart & Hill 2014), and the ability to change the balance of auxins and cytokinins (Martínez-Medina et al. 2014). Therefore, efforts to control *R. solani* are more directed at environmentally friendly control by using biological control agents, namely *Trichoderma* sp., which is antagonistic and plant growth promoter. This study aimed to identify *T. asperellum* isolates based on molecular characteristics and to determine the in vitro inhibition mechanism against *R. solani* and its role in enhancing the growth of corn seedlings.

Methods

Isolation of *Rhizoctonia* sp. from corn

Rhizoctonia sp. was isolated from corn plants with banded leaf and sheath blight symptoms obtained from farmers' corn plantations in Maros Regency, South Sulawesi, Indonesia (4°13′–5°60′S and 119°42′–120°30′E). Symptomatic samples were cut to a size of 2–3 mm, surface sterilized for 1 min with 1.5% NaClO, and then rinsed three times with sterile distilled water. Next, the parts of the sample that exhibited symptoms were air-dried using sterile filter paper. These dried pieces were then placed on potato dextrose agar (PDA) medium and left to incubate for a period of 2–3 days at room temperature between 20 and 25 °C. Furthermore, the fungal hyphae identified as *Rhizoctonia* sp. were designated as RsHM isolates and propagated on new PDA medium for further examination (Wang et al. 2015).

A stock preparation of *Trichoderma* sp. isolates

The isolates used in this study were five *Trichoderma* spp. isolates originating from various ecological niches of corn, namely HMRP7 isolate from rhizoplane, HMRF7A and CHM01 isolates from rhizosphere, HMEDF6A isolate from root endophytic, and HMEDF1B isolate from stem endophytic. The five isolates were obtained from the Research Center for Food Crops, National Research and Innovation Agency, Indonesia. These isolates were then cultured on PDA medium and subjected to incubation at a temperature of 28 °C. Furthermore, stock isolates for testing were prepared by re-cultured on slanting PDA medium, then incubated at 28 °C for 7 days in an incubator. Once the fungi had colonized the slanted medium, they were transferred to a refrigerator set at 4 °C and kept as a stock culture for future use.

Identification of *Trichoderma* sp. and *Rhizoctonia* sp. isolates based on morphological characters

The morphological characters were identified on pure *Trichoderma* sp. and *Rhizoctonia* sp. isolates. The identification of *Trichoderma* sp. isolates was based on their morphological characteristics, such as colony color, size, and shape, as well as the size and shape of their phialides and conidia, pigmentation, and growth rate (Matas-Baca et al. 2022). Meanwhile, *Rhizoctonia* sp. isolate was examined based on its colony characteristics (color, size, shape), the color and shape of its sclerotia, number and size of sclerotia, the color and structure of its hyphae, and its growth rate (Mishra et al. 2014).

Identification of *Trichoderma* sp. and *Rhizoctonia* sp. isolates based on molecular characters

Preparation of Trichoderma sp. and Rhizoctonia sp. pellets

Trichoderma sp. and *Rhizoctonia* sp. isolates from the stock were re-cultured on PDA medium. The 7-day-old *Rhizoctonia* sp. and *Trichoderma* sp. isolates were suspended using sterile distilled water. To each Petri dish containing *Trichoderma* sp. and *Rhizoctonia* sp. isolates, 100 ml of sterile distilled water was added. Next, a spatula was used to collect the suspensions of conidia and mycelium. The conidia and mycelium suspensions were placed in collection bottles. *Trichoderma* sp. and *Rhizoctonia* sp. suspensions were each placed into a 1.5 ml microtube and centrifuged for 5 min at 10,000 rpm. After centrifugation, the supernatant was discarded and the resulting pellet was utilized for DNA extraction.

Extraction of Trichoderma sp. and Rhizoctonia sp. DNA

DNA extraction followed the method Mirsam et al. (2022) carried out by using the Zymo Research Quick-DNA Fungal/Bacterial Miniprep Kit. The working principle of this Extraction Kit was that the sample was lysed using bead beating without proteinase. The result of extraction using this kit was Ultra-pure DNA.

Amplification of Trichoderma sp. and Rhizoctonia sp. DNA

The PCR method was used to amplify the extracted DNA. The PCR reaction involved combining 12.5 µl of KAPA Taq ReadyMix PCR, 8.5 µl of ddH2O, 1 µl of forward primer, 1 µl of reverse primer, and 2 µl of DNA template. The primer used was a universal fungus primer, ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4(5'TCCTCCGCTTA TTGATATGC-3'). The PCR cycle consisted of a pre-denaturation step at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 5 °C for 30 s, extension at 72 °C for 1 min, and final extension at 72 °C for 3 min. A total of 4 µl of indigenous microbial DNA applicon was loaded into the well of a 0.8% agarose gel and electrophoresed at 110 V for 50 min. The electrophoresis results were visualized using a UV transilluminator, and photos were taken with a camera.

Nucleotide sequence analysis

The amplicons obtained were sent to FirstBase services in Malaysia for sequencing. The nucleotide sequences obtained were then analyzed using Bioedit Sequence Alignment Editor 7.2 software with the ClustalW multiple alignment tool for contig analysis of both forward and reverse DNA sequences. The consensus sequences obtained from the contig analysis were subjected to basic local alignment search tool (BLASTN 2.13.0+) analysis to identify the DNA sequence information available in the National Center for Biotechnology Information (NCBI) database. A phylogenetic analysis between the isolates was carried out using the Molecular Evolutionary Genetic Analysis Software version 11 (MEGA11), with 1000 times replications using the bootstrap method.

Antagonistic ability test of *Trichoderma* sp. isolate based on the dual-culture method

The antagonistic ability of *Trichoderma* sp. against *Rhizoctonia* sp. was carried out using the dual-culture method. The 7-day-old *Trichoderma* sp. and *Rhizoctonia* sp. isolates were taken using a cork borer with a diameter of 5 mm and placed in the same Petri dish. The control (−) was carried out by preparing PDA medium mixed with a synthetic fungicide with active chemical ingredients of dimethomorph and pyraclostrobin with a concentration of 10%. The PDA medium containing synthetic fungicide was then taken with a diameter of 5 mm using a cork borer and placed in the same Petri dish as *Rhizoctonia* sp. Meanwhile, for the control (+), only using *Rhizoctonia* sp. Each fungus was arranged facing

each other at a distance of 3 cm. The fungus was then incubated for 7 days at room temperature. Observations of the radial growth of fungal colonies were carried out every 24 h. until the growth of fungi in control (+) filled the Petri dish. The test was arranged using a completely randomized design consisting of 7 treatments, namely 5 *Trichoderma* sp. isolates, 1 synthetic fungicide as control (-), and 1 distilled water as control (+). The test was repeated 3 times. Percentage growth inhibition (PGI) was calculated using the equation below (Eq. 1).

$$PGI = \frac{R1 - R2}{R1} \times 100\% \quad (1)$$

where R1 is the *R. solani* radius growing in the opposite direction to the antagonistic microorganism, and R2 is the *R. solani* radius growing towards the antagonistic microorganism (Seema 2012).

In-planta potential test of *Trichoderma* sp. isolate as plant growth promoter on corn seedling in greenhouse

The *in-planta* potential test of *Trichoderma* sp. isolates as an inducer of corn growth was carried out in a greenhouse using seedling trays and followed the method Mirsam et al. (2021). *Trichoderma* sp. isolates were first re-cultured on PDA medium and incubated at room temperature for 7 days. The seeds of the Anoman variety were first sorted based on their physical characteristics (shape, size, color, and no mechanical damage). The seeds were sterilized by hot water treatment at 60 °C for 2 min, then dried on sterile filter paper in laminar airflow for 20 min. *Trichoderma* sp. isolates were suspended using sterile distilled water at a conidia concentration of 10^8 cfu/ml. The corn seeds were soaked with *Trichoderma* sp. suspension in Erlenmeyer and covered with sterile cotton and aluminum foil, and then incubated using a rotary shaker at 180 rpm for 24 h. After soaking, the seeds were air-dried on sterile filter paper in laminar airflow. Furthermore, the seeds were planted in a seedling tray with sterile soil and combined medium (1:1), and then the seeds that have been planted in the tray were arranged in a greenhouse. This test was arranged using a completely randomized design consisting of 6 treatments, namely 5 *Trichoderma* sp. isolates and 1 distilled water as a control. The test was repeated 3 times. Observation was made 7 days after planting (DAP). The observed variables are seedling height, root length, root wet weight, root dry weight, shoot wet weight, and shoot dry weight. The dry weight was calculated by weighing the plant samples which had been dried in an oven at 60 °C for 48 h. Other observational parameters were maximum growth potential (MGP), growth rate (GtR), growth simultaneity (GS), vigor index (VI), germination rate (GR), and median germination time (T50) based on International Seed Testing Association (ISTA 2023).

Maximum growth potential (MGP)

MGP is the percentage of seeds grown, calculated on the 7th day of observation using Eq. (2):

$$MGP = \frac{\sum \text{germinated seeds}}{\sum \text{seeds grown}} \text{x } 100\% \quad (2)$$

Growth rate (GtR)

The GtR was observed daily on normal seedlings and calculated using Eq. (3).

$$GtR(\%\ /\text{etmal}) = \frac{n1}{D1} + \frac{n2}{D2} + \ldots + \frac{n7}{D7} \quad (3)$$

where n = percentage of seedlings that are considered normal for each observation period (%); D = duration of each observation period per 24 h (etmal).

Growth simultaneity (GS)

Observation of GS was carried out on the third and sixth days after the seeds were initially planted. The formula used is (Eq. 4):

$$GS = \frac{\sum NS}{\sum SG} \text{x } 100\% \quad (4)$$

where NS is normal seedling during 1st and 2nd observation, SG is seeds grown.

Vigor index (VI)

To determine VI, the number of seeds that exhibited normal germination on the first day of observation (day 3) was calculated using the following equation (Eq. 5):

$$VI = \frac{\sum NS}{\sum SG} \text{x } 100\% \quad (5)$$

where NS is normal seedlings; SG is seeds grown.

Germination rate (GR)

To measure GR, the number of days that each seed required to germinate was calculated using the following formula (Eq. 6):

$$GR = \frac{N1T1 + N2T2 + \ldots + NxTx}{\sum \text{germinated seeds}} \quad (6)$$

where N = number of seeds germinating within each daily observation interval, T = length of time between the start of seeding and the end of the predetermined observation period.

Median germination time (T50)

T50 is the time required for the seeds to reach 50% germination of the total seeds sown by calculating the number of seeds grown every day. T50 describes seed vigor, calculated by the formula (Eq. 7):

$$T50 = ti + \left(\frac{n50\% - ni}{nj - ni}\right)(tj - ti) \quad (7)$$

where ti = intermediate time at or before seed germination 50%; tj = intermediate time after the seeds germinate 50%; n50% = number of seeds that germinated (50% of the total germinated seed); nj = number of seeds germinated at time tj; ti = number of seeds germinated at time ti.

Data analysis

This experiment was set up in a completely randomized design. The antagonistic ability test consisted of 5 treatments of *Trichoderma* sp. isolates, 1 synthetic fungicide treatment as a control (-) and 1 aquadest treatment as a control (+). Meanwhile, the in-planta test also consisted of the same treatments as the antagonistic ability test, but only used a (+) control. The experiment was repeated three times. Observational data were statistically tested using analysis of variance, and then the Least Significant Difference (LSD) test was performed at the 5% significance level ($\alpha = 0.05$).

Results

Morphological characters of *Trichoderma* sp. and *Rhizoctonia* sp.

The macroscopic observation of *Trichoderma* sp. colonies showed a flat surface, rough-like fibrous, and smooth edges. The colony was initially white and the middle part was light green; then, the colony became dark green in the shape of a circle with clear boundaries, while the edges were white like cotton. The color of the colony turned into a dark green on the entire upper surface at 7 days old after being cultured (Fig. 1a). Meanwhile, the microscopic characters of *Trichoderma* sp. were greenish-white hyphae, short phialides stalk, greenish globose-shaped conidia that grew at the ends and some conidia formed clusters of light green color on the surface of the conidiophore cells. The conidiophore branches and phialides were ± 10 µm and ± 14 µm in length, respectively. Many conidiophore branches were like pyramids, namely the longer branches below, the phialides were arranged in

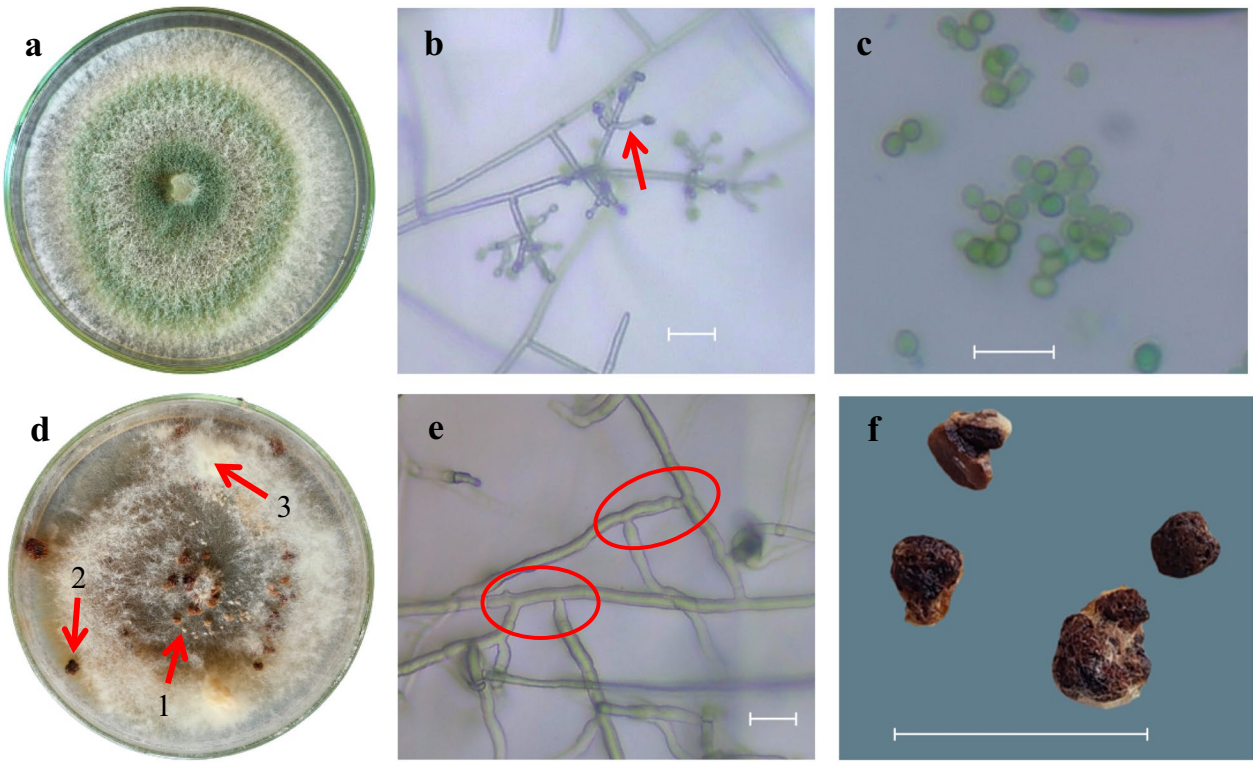

Fig. 1 Morphological characters of the fungal isolates. *Trichoderma* sp. isolate: **a** 5 days old culture plate on PDA; **b** phialide (bar scale = 50 µm); and **c** conidia (bar scale = 5 µm). *Rhizoctonia* sp. isolates: **d** 13 days old culture plate on PDA (1 = young sclerotia, 2 = mature sclerotia, 3 = abundant mycelial growth); **e** hyphae (hyphae branching of 45–90 angles, bar scale = 50 µm); and **f** sclerotia (bar scale = 5 mm)

different groups, and there were 2–3 phialides per group (Fig. 1b and c).

Colony characters of *Rhizoctonia* sp. on PDA medium generally had white mycelium, then changed to yellowish white to dark brown (Fig. 4d). After several days of incubation on a PDA medium, the colonies produced sclerotia of various sizes and shapes (Fig. 4f). While the microscopic character of *Rhizoctonia* sp., *i.e.,* young hyphae had branches with an angle of 45 °C, the more mature the branches were perpendicular, stiff, and uniform. The color of the young hyphae was hyaline and the old hyphae were brown; the average diameter of the hyphae was around 6–10 µm (Fig. 4e).

Molecular characters of *Trichoderma* sp. and *Rhizoctonia* sp. based on the rDNA-ITS region

Morphological characters of *Trichoderma* sp. isolate and *Rhizoctonia* sp. isolates were verified by analyzing their molecular features through the use of universal primers ITS1/ITS4. The results of the molecular analysis indicated that PCR using ITS1/ITS4 primers successfully amplified DNA bands with a size of ± 500 bp for *Trichoderma* isolates (Fig. 2A) and ± 700 bp for *Rhizoctonia* sp. isolate RsHM (Fig. 2B). The ITS-1/ITS-4 primers were generally used to amplify fungal DNA in the internal transcribed spacer (ITS) region. This ITS region was a conserved, non-encoded functional protein located in the ribosomal DNA (rDNA) region.

According to the BLASTN 2.13.0 program, the sequencing analysis results indicated that all *Trichoderma* sp. isolates (HMRP7, HMRF7A, HMEDF1B, HMEDF6A, CHM01) had similarities to *T. asperellum* isolates from Nigeria, South Korea, Yogyakarta (Indonesia), China, and India with sequence identity matrix values of 1.000 (100% homology) (Table 1). Meanwhile, the RsHM isolate had similarities to *R. solani* isolates from Vietnam, Malaysia, China, Brazil, and India, with sequence identity matrix values ranging from 0.917 to 0.929 (homology 91.7–92.9%) (Table 2). The data of Blast result from NCBI showed the highest similarity (> 90%) with an e value of 0.0.

Phylogenetic analysis showed that *Trichoderma* sp. isolates (HMRP7, HMRF7A, HMEDF1B, HMEDF6A, CHM01) were closely related and grouped with *T. asperellum* isolate RCZID-3 from Nigeria, strain YS-27 from South Korea, strain PK1J2 from Yogyakarta (Indonesia), isolate Tasum66 from China, and isolate PANCOM8 from India with a genetic distance coefficient of 0.000. Meanwhile, RsHM isolates were closely related and grouped with *R. solani* isolate RORC9 from India with genetic distance coefficient values ranging from 0.000 to 0.032. In addition, RsHM isolate was also quite closely related to *R. solani* strain 2773 from Brazil, JK-2016-14 isolates from China, CSU4 isolates from Malaysia, and CTCR02-2 isolates from Vietnam, with a genetic distance coefficient ranging from 0.002 to 0.007. *Trichoderma* sp. isolates were very distantly related to RsHM isolate, exhibiting a genetic distance coefficient of 0.448 (Fig. 3).

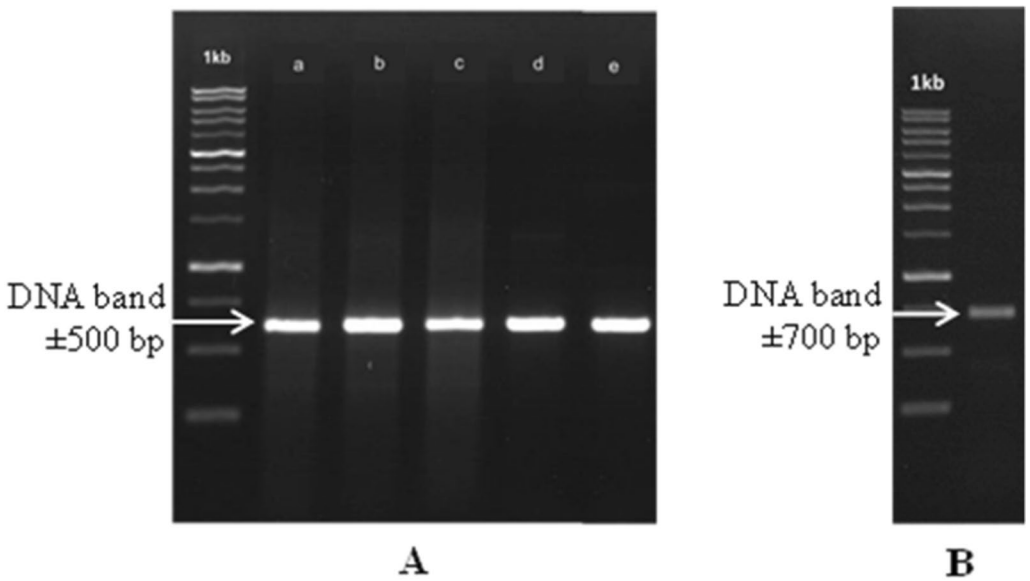

Fig. 2 Molecular identification based on polymerase chain reaction at 1% agarose gel with 4 µl of DNA ladder loaded per lane and 4 µl of sample loaded per lane. 1kb, Marker/DNA ladder; A, *Trichoderma* sp. isolates: **a** HMRP7, **b** HMRF7A, **c** HMEDF6A, **d** HMEDF1B, **e** CHM01; B, *Rhizoctonia* sp. isolate

Table 1 DNA sequence identity matrix of *Trichoderma* sp., which is juxtaposed with isolates in GenBank

Isolate Code	HMRP7	HMRF7A	HMEDF1B	HMEDF6A	CHM01	Ts1	Ts2	Ts3	Ts4	Ts5
HMRP7	ID									
HMRF7A	1.000	ID								
HMEDF1B	1.000	1.000	ID							
HMEDF6A	1.000	1.000	1.000	ID						
CHM01	1.000	1.000	1.000	1.000	ID					
Ts1	1.000	1.000	1.000	1.000	1.000	ID				
Ts2	1.000	1.000	1.000	1.000	1.000	1.000	ID			
Ts3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	ID		
Ts4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	ID	
Ts5	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	ID

Ts1, isolate RCZID-3 from Nigeria; Ts2, strain YS-27 from South Korea; Ts3, strain PK1J2 from Yogyakarta (Indonesia); Ts4, isolate Tasum66 from China; Ts5, isolate PANCOM8 from India

Table 2 DNA sequence identity matrix of isolates of *Rhizoctonia* sp. which is juxtaposed with isolates in GenBank

Isolate Code	RsHM	Rs1	Rs2	Rs3	Rs4	Rs5
RsHM	ID					
Rs1	0.917	ID				
Rs2	0.917	1	ID			
Rs3	0.929	0.984	0.984	ID		
Rs4	0.917	1.000	1.000	0.984	ID	
Rs5	0.917	1.000	1.000	0.984	1.000	ID

Rs1, isolate CTCR02-2 from Vietnam; Rs2, isolate CSU4 from Malaysia; Rs3, isolate RORC9 from India; Rs4, strain 2773 from Brazil; Rs5, isolate JK-2016–14 from China

In vitro antagonistic ability of *T. asperellum* against *R. solani*

According to the dual culture method used for the antagonism test of *T. asperellum* isolates, all isolates were able to inhibit the growth of *R. solani* on PDA medium, with a percentage of inhibition ranging from 59.57 to 75.25%. However, the inhibition percentage with synthetic fungicide treatment/control (−) was only 56.54% (Fig. 4). The inhibition values of HMEDF6A and CHM01 isolates significantly differed from the control (−) in the LSD test level of 5% with 75.09 and 75.25% inhibition percentages, respectively. The LSD test at the 5% level revealed that the inhibitory abilities of the HMRP7, HMRF7A, and HMEDF1B isolates were non-significantly different from the control (−), with inhibition percentages of 72.17, 59.57, and 68.06%, respectively.

The antagonistic ability showed that the five *T. asperelluum* isolates could inhibit the growth of *R. solani* in vitro on PDA medium with an inhibition percentage of > 50% so that these isolates could be categorized as antagonistic agents. *T. asperellum* showed the antagonistic mechanis*m* in inhibiting the growth and reproduction of *R. solani* through the parasitism mechanism. In this study, *T. asperellum* and *R. solani* formed antagonistic zone indicating competition and then hyperparasites. As the antagonist time progresses, *R. solani* mycelium gradually thins, stops growing, and does not form sclerotia (Fig. 5).

Inhibition effect of *T. asperellum* to mycelial growth of *R. solani*

The microscopic observations showed that there was a mechanism of parasitism/ hyperparasitism of *T. asperellum* against *R. solani* in PDA medium (Fig. 6). The *T. asperellum* mycelium spirally coiled around and penetrated the *R. solani* mycelium (Fig. 7b and c). This inhibitory mechanism led to cytoplasmic shrinkage and coagulation (Fig. 6d) and swollen malformation of *R. solani* mycelium (Fig. 6a).

In-planta ability of *T. asperellum* isolate to induce the growth of corn seedlings in green house

The ability of *T. asperellum* isolates to induce the growth of corn seedlings is shown in Table 3. The CHM01 isolate could generally induced corn seedlings' growth better than other isolates. CHM01 isolate had a better ability to increase GtR and VI compared to other isolates, where the GtR and VI values were 29.09and 47.22%, respectively, and significantly high the control treatment LSD test at a significance level of 5%. However, the CHM01 isolate had a lower GR value compared to other isolates.

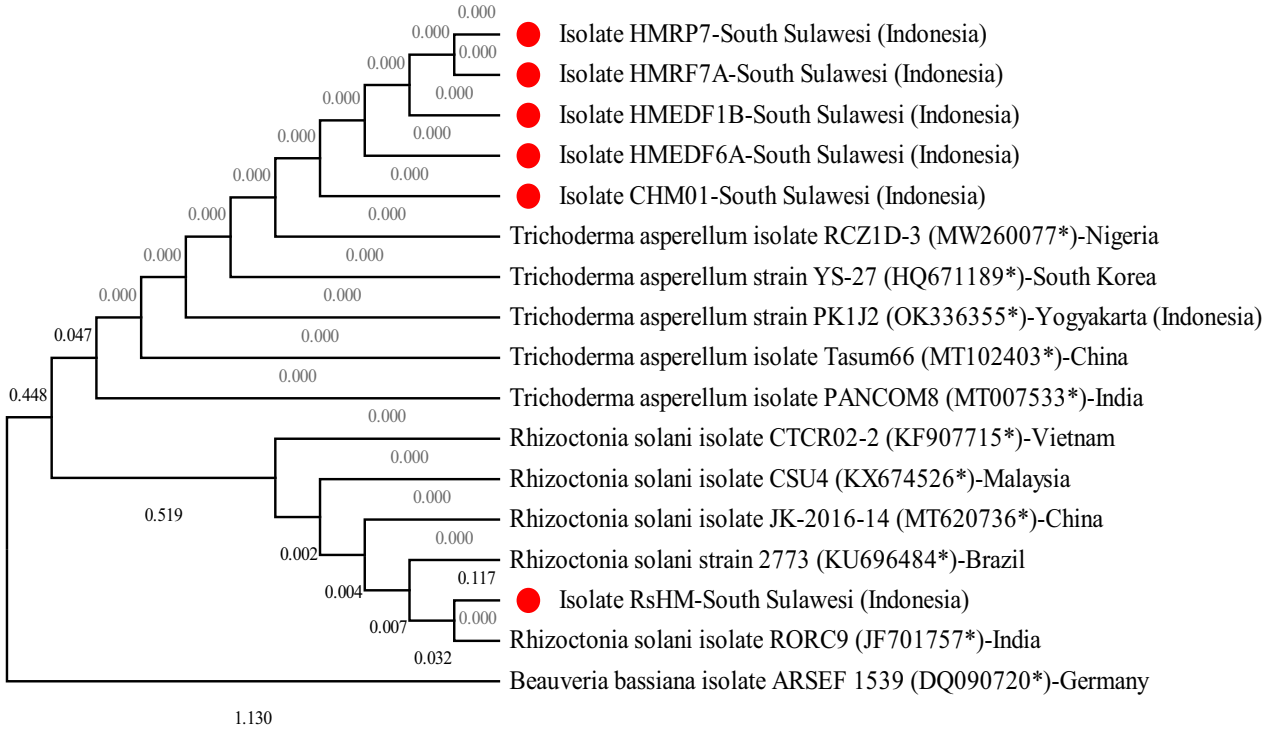

Fig. 3 The phylogenetic tree of *Trichoderma* sp. and *Rhizoctonia* sp. isolates based on Neighbor-Joining analysis with Maximum Composite Likelihood model and the bootstrap genetic distance matrix calculation for 1000 replicates was analyzed using Bioedit 7.2 and MEGA11 software. The scale under the branch is a scale of the coefficient of genetic distance, which describes the average number of nucleotide changes between isolates. *NCBI accession number.; (red circled) research sample

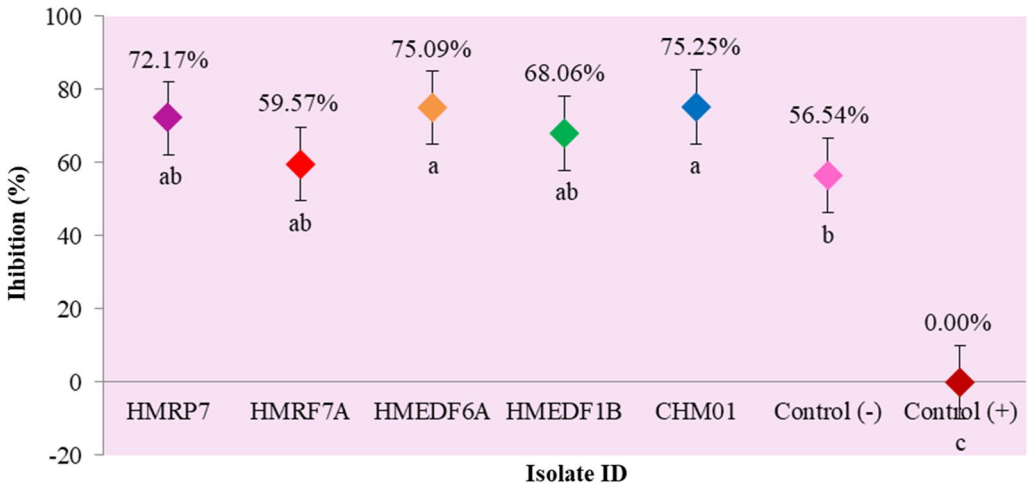

Fig. 4 Percentage of in vitro inhibition of *Trichoderma asperellum* isolates against *Rhizoctonia solani* on PDA. The same letters on the graph showed that there was non-significant difference between treatments according to the LSD test at the 5% level

In the GS variable, all isolates had lower GS values than the control, but the CHM01 isolate did not differ significantly from the control. This indicated that the simultaneous growth of corn seedlings was relatively unaffected by the application of CHM01. Furthermore, the isolates HMRP7 (84.72%), HMRF7A (83.33%), and CHM01 (86.11%) had MGP values that were higher than the control treatment of 79.17%. However, the LSD test at the

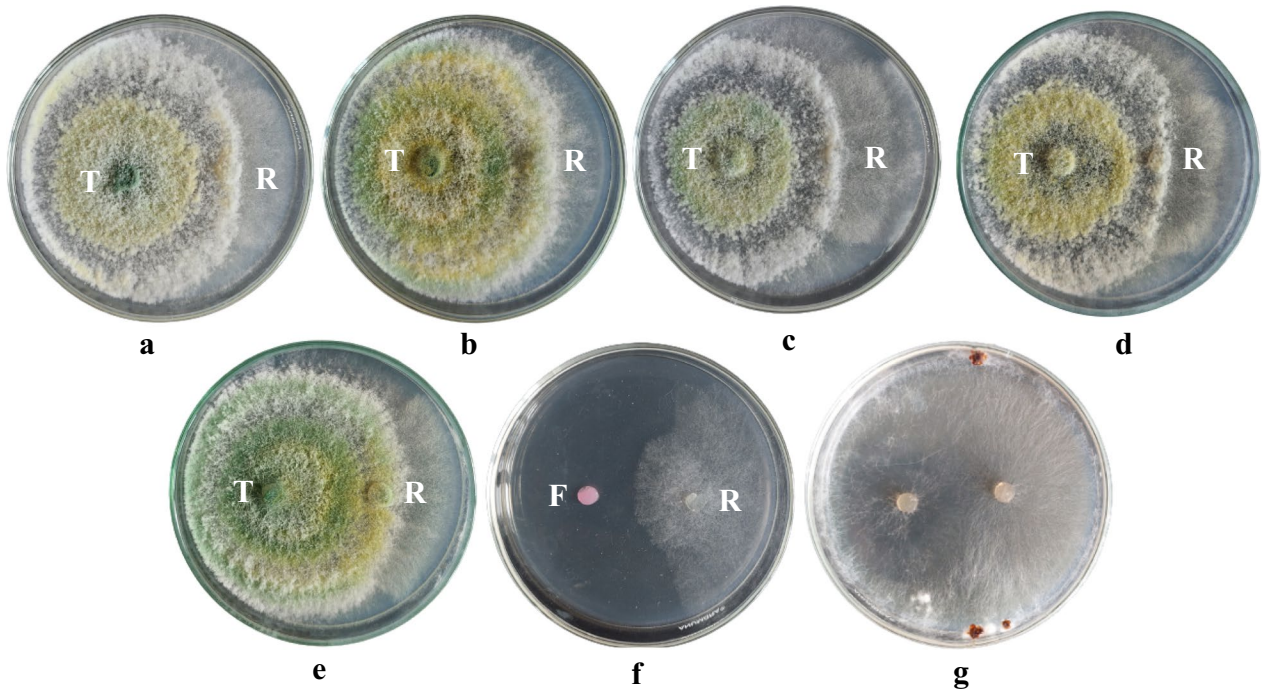

Fig. 5 Interaction between *Trichoderma asperellum* isolate and *Rhizoctonia solani* isolate on PDA medium at room temperature for 7 days after incubation. **a** HMRP7; **b** HMRF7A; **c** HMEDF6A; **d** HMEDF1B; **e** CHM01; **f** Control (); **g** Control (+). T, *T. asperellum*; R, *R. solani*; F, synthetic fungicide

5% level found non- statistically significant difference between the isolates' MGP values. In the T50 variable, there was non-significant difference between all isolates and the control, but the T50 value of the CHM01 isolate was 4.65 days higher than the control which was only 4.47 days.

The LSD test at 5% level showed a significant difference between *T. asperellum* isolates for plant height and root length, but not for root number. HMEDF6A and CHM01 isolates increased plant height and root length by 60.97 and 118.59%, respectively, than the control, whereas in the variable number of roots, all isolates showed higher number of roots than the control, but not significantly different in the LSD test at the 5% level (Table 4).

Discussion

The microscopic characters of the *Trichoderma* sp. isolates obtained in this study were similar to the characters of *T. asperellum* reported by Oszako et al. (2020), namely *T. asperellum* had 2—3 phialide-branched conidiophores and measuring 6.4×1.4 µm. The conidia were greenish, globose to sub-globose, ellipsoid, measuring 3.1×2.6 µm. Meanwhile, the chlamydospores were unicellular, terminal and spherical, measuring 20.5×21.2 µm with inconspicuous ornamentation.

The tested pathogen isolates, *Rhizoctonia* sp., showed traits with previously reported *Rhizoctonia* sp., such as the tendency of young colonies to be white and the ability of older isolates to change brown (Misawa and Kurose 2018). The difference in colony color is caused by pigments produced by pathogens in the media (Taheri et al. 2007). Sclerotia are categorized into macrosclerotia and microsclerotia based on size. Macrosclerotia typically measures 1 mm in diameter, are tiny, spherical, and blackish-brown in color (Lal and Kandhari 2009). Meanwhile, on PDA medium, microsclerotia takes on a form resembling thin crusts, which are small and white (Kumar et al. 2008). The size of the sclerotia was inconsistent, arranged in the center, the edges, scattered or even absent (Abdel-Sattar et al. 2017).

Using the universal primer pair ITS1/ITS4 to amplify the ITS region of ribosomal DNA (rDNA), species of *Trichoderma* sp. and *Rhizoctonia* sp. isolates were successfully identified. The HMRP7, HMRF7A, HMEDF1B, HMEDF6A, and CHM01 isolates were identified as *T. asperellum* with a genetic distance coefficient value of 0.000 and a very high similarity of 100%. With a homology level of > 90% and genetic distance coefficient values ranging from 0.000 to 0.032, the RsHM isolate was identified as *R. solani*. Identification of fungi by PCR technique generally uses the universal primer pair ITS1/ITS4

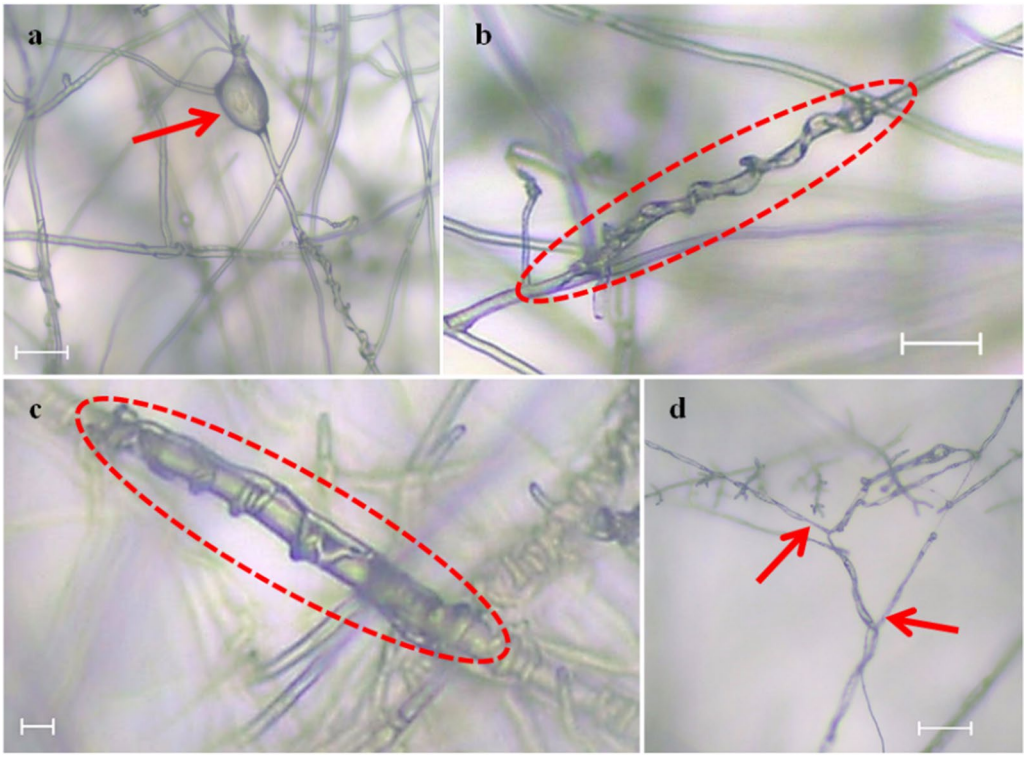

Fig. 6 Inhibition mechanism of *Trichoderma asperellum* on *Rhizoctonia solani* mycelium growth on PDA medium with dual culture method. **a** *R. solani* hyphae swollen malformation (bar scale = 100 µm); **b** *T. asperellum* hyphae coiling in *R. solani* hyphae after 1-day confrontation (bar scale = 100 µm) **c** *T. asperellum* hyphae coiling in *R. solani* hyphae after 3 days confrontation (bar scale = 100 µm); **d** shriveled hyphae

to amplify the ITS region of ribosomal DNA (rDNA) because of its universal presence, conservative, and abundant presence. The rDNA is the coding region of the genome for the ribosomal DNA component. In addition, the ITS region also has a clear barcode to distinguish species and the presence of inter- and intra-specific nucleotide base variations (Buehler et al. 2017).

In vitro, antagonistic activity against *R. solani* was further assessed for the isolates HMRP7, HMRF7A, HMEDF1B, HMEDF6A, and CHM01. According to the test results, the five *T. asperellum* isolates had a > 50% ability to inhibit the growth of *R. solani* on PDA medium. The *Trichoderma* sp. antagonist mechanism was also reported by Harwoko et al. (2019) that the main biological control mechanism for *Trichoderma* species was hyperparasitism. *Trichoderma* sp. recognizes pathogens by identifying lectins secreted by pathogens (Kredics et al. 2018). Furthermore, *Trichoderma* sp. induces a series of hyperparasite-associated signaling pathways in its body (Stappler et al. 2017).

The mechanism of parasitism shown by *T. asperellum* in inhibiting the growth of *R. solani*, namely swollen malformation and hyphae coiling. *R. solani*'s hyphae died due to the *T. asperellum* hyphae's coiling, penetration, and subsequent crushing of the hyphae into smaller fragments. The mechanism of *Trichoderma* sp. parasitism, in which the hyphae coiled on *R. solani* until haustoria formed inside the pathogenic hyphae, and then the haustoria took the cytoplasm until the hyphae shriveled (Jiang et al. 2016). According to Wu et al. (2017), *T. asperellum* utilizes a method of mycoparasitism whereby it wraps its hyphae around pathogens and secretes cell wall-degrading enzymes (CWDEs) containing chitinase, glucanase, and protease. The enzymes caused degradation of the cell walls in the pathogens, while the hyphae of *Trichoderma* sp. coiled around the hyphae of the pathogen, resulting in abnormalities in hyphal growth and lysis of pathogenic cells (Zhang and Zhuang 2020).

In addition to having antagonistic abilities, the five *T. asperellum* isolates also had the ability to enhance the growth of corn seed in planta. The results for the potential test of *T. asperellum* isolates in enhancing growth showed that the isolate was thought to produce bioactive compounds that could induce growth. A typical approach for promoting the growth of plants was through the capacity of *T. asperellum* isolates to colonize roots and supply them with nutrients and minerals. This study aimed to investigate the potential of

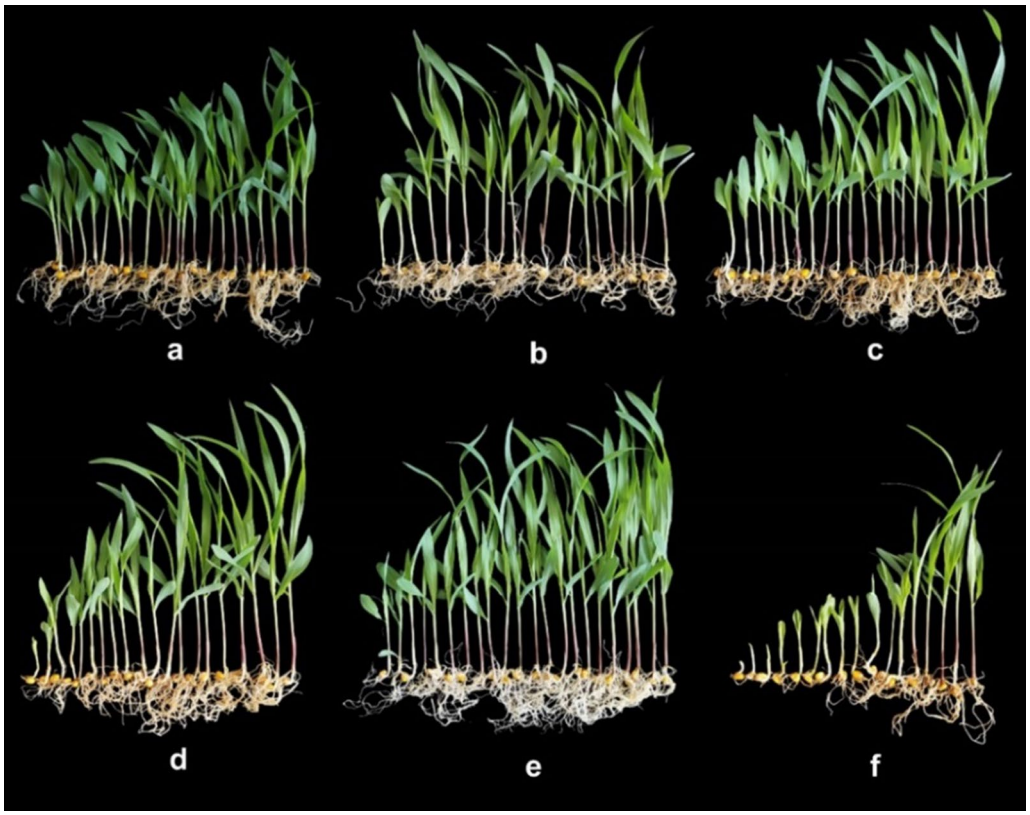

Fig. 7 Effect of *Trichoderma asperellum* suspension soaking treatment on the corn growth in seedling trays. **a** HMRP7; **b** HMRF7A; **c** HMEDF6A; **d** HMEDF1B; **e** CHM01; **f** Control

Table 3 Effect of antagonistic fungi isolates on the vigor and viability of corn seed

Isolate ID	MGP (%)	GtR (%)	GS (%)	VI (%)	GR (average days)	T50 (average days)
HMRP7	84.72	20.09b	16.67c	9.72c	4.57a	3.62
HMRF7A	83.33	18.71b	30.56b	15.28bc	4.93a	4.34
HMEDF6A	56.94	13.73b	23.61bc	12.50c	4.34a	5.13
HMEDF1B	68.06	17.63b	29.17bc	22.22bc	4.18a	4.65
CHM01	86.11	29.09a	50.00a	47.22a	3.29b	2.33
Control	79.17	19.10b	54.17a	29.17b	4.77a	4.47
LSD 5%	-	7.33	13.07	14.93	0.78	-
SE (%)	13.05	3.36	6.00	6.85	0.36	0.80
CV (%)	20.93	20.88	21.60	36.99	10.14	24.11

MGP maximum growth potential; *GtR* growth rate; *GS* growth simultaneity; *VI* vigor index; *GR* germination rate; *T50* median germination time; *LSD* least significant difference; *SE* standard error; *CV* coefficient of variation

Letters followed in the same column are not significantly different according to 5% LSD (α 0.05)

T. asperellum as a saprophytic antagonist capable of enhancing corn seedlings. The growth of plants can be stimulated by *T. asperellum*, which was accomplished through the production of growth regulators (Glick 2012). A study reported by Hoyos-Carvajal et al. (2009) indicated out of 106 *Trichoderma* sp. isolates, 60% demonstrated the capacity to generate IAA and analogs of auxin.

Trichoderma sp. is a filamentous saprophytic fungus that lives in the rhizosphere and plant tissue, which can stimulate plant growth indirectly (Doni et al. 2014). The mechanism of *Trichoderma* sp. in enhancing growth is

Table 4 The effect of antagonistic fungi isolates on the growth of corn seedling

Isolate ID	Seedling height (cm)	Root length (cm)	Number of roots
HMRP7	16.42bc	9.81bc	9.29a
HMRF7A	18.29bc	10.16bc	8.16a
HMEDF6A	19.82ab	12.00ab	8.75a
HMEDF1B	18.53bc	8.73 cd	8.38a
CHM01	24.00a	14.58a	9.81a
Control	14.91c	6.67d	7.57a
LSD 5%	4.71	2.67	1.55
SE (%)	2.16	0.22	0.71
CV (%)	14.19	14.52	10.06

Letters followed in the same column are not significantly different according to 5% LSD (α 0.05)

LSD least significant difference; *SE* standard error; *CV* coefficient of variation

carried out by colonizing the rhizosphere zone of plant roots. The rhizosphere is influenced by the secretion of bioactive compounds in the form of highly hydrated mono- and di-saccharides by plant roots, which stimulate the growth and survival of fungi in the rhizosphere zone. Plant-derived sucrose serves as a carbon source for *Trichoderma* sp., which enables the colonization of roots, coordinates defense mechanisms, and enhances photosynthetic rates (Vargas et al. 2009). Several phytostimulatory mechanisms of *Trichoderma* sp. had been reported previously such as increased root development and production of auxin (Contreras-Cornejo et al. 2009), produce siderophores (Chowdappa et al. 2013), improved tolerance for drought (Shukla et al. 2012), defense protein expression in plants (Thakur et al. 2013), phosphate solubility (Saravanakumar et al. 2013), elicitor release (Nawrocka et al. 2013), and improved plant tolerance under conditions of salinity (Contreras-Cornejo et al. 2014). In addition, there have been reports on various *Trichoderma* species that have the potential to influence plant growth and development directly. This includes promoting nutrient uptake, reducing reliance on synthetic chemical fertilizers, enhancing seed germination rates, and inducing plant resistance to biotic and abiotic stresses (Shoresh et al. 2010).

Conclusion

The five *Trichoderma* sp. isolates and *Rhizoctonia* sp. isolates were identified as *T. asperellum* and *R. solani* based on the characteristics of the ITS ribosomal DNA region by PCR technique. *T. asperellum* inhibited the growth of *R. solani* in vitro on PDA medium with a parasitism mechanism. CHM01 isolate showed better potential than other isolates in enhancing the growth of corn seedlings. Further research is needed to confirm the results of laboratory and greenhouse tests so that the effectiveness of *T. asperellum* in controlling *R. solani* is known on a large scale in the field.

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Author contributions
All authors listed have significantly contributed to the development and the writing of this article. All authors read and approved the final manuscript.

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Availability of data and materials
The data and materials of this study are presented in the manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that there are no conflicts of interest.

Author details
[1]Research Center for Food Crops, Research Organization for Agriculture and Food, National Research and Innovation Agency, Jalan Raya Bogor Km. 46, Cibinong 16911, Republic of Indonesia. [2]Research Center for Behavioral and Circular Economics, Research Organization for Economic Governance and Public Welfare, National Research and Innovation Agency, B.J. Habibie Building 15Th-24Th Floor, Jl. M.H. Thamrin No. 8, Jakarta Pusat 10340, Republic of Indonesia. [3]Departement of Agrotechnology, Faculty of Agriculture, Hasanuddin University, Jl. Perintis Kemerdkaan Km.10, Makassar 90245, South Sulawesi, Republic of Indonesia.

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