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Exploration and characterization of indigenous *Trichoderma* spp. as antagonist of *Rhizoctonia solani* and plant growth promoter of maize

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[2]Department of Agrotechology, Faculty of Agriculture, Universitas Negeri Gorontalo. Jl. Prof. Dr. Eng. B. J. Habibie, Gorontalo 96554, Gorontalo, Indonesia. Tel.: +62-341-565845, ♥email: rida_iswati@ung.ac.id
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[4]Department of Soil Sains, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia

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Abstract. *Iswati R, Aini LQ, Soemarno, Abadi AL. 2024. Exploration and characterization of indigenous* Trichoderma *spp. as antagonist of* Rhizoctonia solani *and plant growth promoter of maize. Biodiversitas 25: 1375-1385.* Maize sheath blight disease, caused by *Rhizoctonia solani*, is the main maize disease in Gorontalo and is widespread from the lowlands to the highlands. The indigenous *Trichoderma* spp. is known to function as a biocontrol agent while producing secondary metabolites that promote plant growth. The objective of this study was to isolate, select, and identify indigenous *Trichoderma* strains that act as antagonistic agents and promote maize plants growth. The bulk of the soil samples were collected from the rhizosphere of maize plantations in the lowland (0-300 masl), medium (300-500 masl), and highland (500-1000 masl) regions at several sites in Gorontalo. *Trichoderma* spp. isolation was carried out using the serial dilution method on Rose Bengal chloramphenicol medium, and the strains were further purified in PDA medium, followed by morphological and molecular identification. The antagonistic effect was assessed using dual culture method. In addition, the plant growth-promoting traits examined were qualitative IAA production, potassium solubilization, phosphate solubilization, and in vivo for maize plant growth promotion. Molecular identification revealed that the indigenous fungal strains were dominated by three species, namely, *Trichoderma asperellum, T. virens,* and *T. brevicompactum.* Other strains identified were *T. ghanense, T. reesei,* and *T. dorothopsis.* Of the 30 *Trichoderma* spp. strains, 25 inhibited *R. solani* more than 50%. All *Trichoderma* spp. strains can produce IAA qualitatively and dissolve phosphate at different intensities; only one fungal strain namely *T. dorothopsis*-TZ31LU1, can solubilize potassium. In addition, several indigenous *Trichoderma* spp. strains were able to increase the growth of maize plants in vivo. Thus, 25 *Trichoderma* spp. strains had the potential to be developed as biological control agents for maize sheath blight disease as well as plant growth promoters in maize.

Keywords: Antagonist, biological agents, Gorontalo, growth promotion, maize, *Rhizoctonia solani*, sheath blight disease, *Trichoderma*

INTRODUCTION

Maize (*Zea mays*) is a main agricultural commodity that supports the economic growth of Gorontalo's province in Indonesia. Maize contributes 38.66% to gross regional domestic income (GRDP), 3.42% to economic growth for Gorontalo, and 4% to total national maize production (BPS 2022). However, maize productivity, specifically for Gorontalo, is only 4.7 tons/ha on average, lower than the average national productivity of 7.1 tons/ha. One of the reasons for the lower maize productivity in Gorontalo is thought to be caused by plant disease.

According to Haque et al. (2022) and Lim et al. (2023), plant diseases can be important obstacles to increasing maize productivity. Globally, there are more than 112 species of plant disease in maize (Rai and Singh 2018; Haque et al. 2022), 100 of which are prevalent in Indonesia (Rais 2016). The main maize diseases in Indonesia, as well as in Gorontalo, are downy mildew, leaf rust, leaf blight, leaf spot, and sheath blight, with disease intensities ranging from 5.5 to 50% (Tenteyali 2016; Talib 2017). Leaf-sheath blight disease, caused by *Rhizoctonia solani*, rank as the second major maize disease in Indonesia following downy mildew and presents a widespread issue across the country (Soenartiningsih et al. 2014). This pathogen infects the lower maize leaf sheath and can also spread to cobs, causing yield losses of up to 100% in susceptible varieties (Soenartiningsih et al. 2014). The disease symptoms include severe leaf rot accompanied by the death of the apex, which can infect all parts of the maize plant, such as leaf midribs, fruit rind cobs, and stems (Rai and Sigh 2018).

The development of maize sheath blight can be widespread throughout the season. The ability of the fungus to produce *sclerotia* in the soil and plant parts makes it difficult to control (Muimba-Kankolongo 2018; Mirsam et al. 2023a). The control measures for maize diseases in Gorontalo Province generally rely more on chemical control techniques using fungicides. In addition to being inefficient, the use of pesticides also affects environmental safety issues (Damalas and Eleftherohorinos 2011).

Along with the trend of limiting the use of chemical pesticides in crop cultivation, local biological agents have become an important alternative for controlling plant diseases. *Trichoderma* spp. are known as biocontrol agents,

can control fungal-caused plant diseases such as *R. solani*, *Sclerotinia sclerotia*, *Sclerotium rolfsii*, *and Fusarium oxysporum* (Saxena et al. 2015; Yusnawan et al. 2019; Herrera et al. 2020; Yadav et al. 2020). *Trichoderma* species can inhibit the growth of plant pathogens through several means, such as parasitism, secretion of fungal cell wall-degrading enzymes such as chitinase, and secretion of antibiotics or organic volatile compounds that are toxic to pathogens (Tzelepis et al. 2015; Li et al. 2019; Kamaruzzaman et al. 2021; Mukherjee et al. 2022). In addition, the ability of some *Trichoderma* species to produce growth hormones and provide nutrients for host plants gives this fungus the potential to promote plant growth. *Trichoderma* spp. are widely known to act as a producer of phytohormones and increase the supply of macronutrients to support plant growth (Khan et al. 2016; Kamal 2018; Ji et al. 2020; Yu et al. 2021).

Therefore, efforts are needed to isolate indigenous *Trichoderma* strains from Gorontalo, which function as biocontrol agents against maize plant diseases and promote maize plants growth. Exploration of *Trichoderma* requires recovery from the rhizospheres of maize plants grown at various altitudes from 0 to 1,000 meters above sea level (masl) to obtain variable isolates suitable for application in the field in Gorontalo. As a result, *Trichoderma*, which has traits that promote plant growth, is expected to potentially provide potential as a biocontrol agent for controlling the maize plant pathogen *R. solani*, which infects maize in Indonesia, especially Gorontalo. Thus, the objective of this study was to isolate, select, and identify indigenous *Trichoderma* strains that act as antagonistic agents and promote the growth of maize plants.

MATERIALS AND METHODS

Soil sampling

Sampling sites were established based on the ecological altitude range (Welman 1972): zone I 0-300 masl, zone II 300-500 masl, zone III 500-1,000 masl, zone IV 1,000-2,000 masl, and zone V 2,000 masl. In Gorontalo, maize plants generally cultivate from low to medium altitude up to Zone III, so the samples were collected from Zone I to Zone III. Subsequently, each zone was divided into two categories according to diseases incidence level. In each zone, samples were collected from two different sites, i.e., a disease-free site and a disease-endemic site; with data sourced government appointed observers of crop pest diseases. The sampling locations are listed in Figure 1.

Five rhizosphere soil samples at a depth of 20 cm were collected from each site and then composited. The samples were placed in a plastic bag and brought to the laboratory for further analysis.

Isolation of *Trichoderma* spp. and *Rhizoctonia* solani

Isolation of *Trichoderma* species was carried out using the serial dilution technique. A total of 10 g of soil was put into an Erlenmeyer flask containing 100 mL of sterile distilled water, stirred with a shaker for 30 minutes at a speed of 150 rpm, and then diluted serially until the dilution was 10^{-8}. A total of 0.1 mL of suspension was incubated in Rose Bengal Chloramphenicol (RBC) medium (Nikmah 2017). Incubation was carried out at room temperature for 3-5 days.

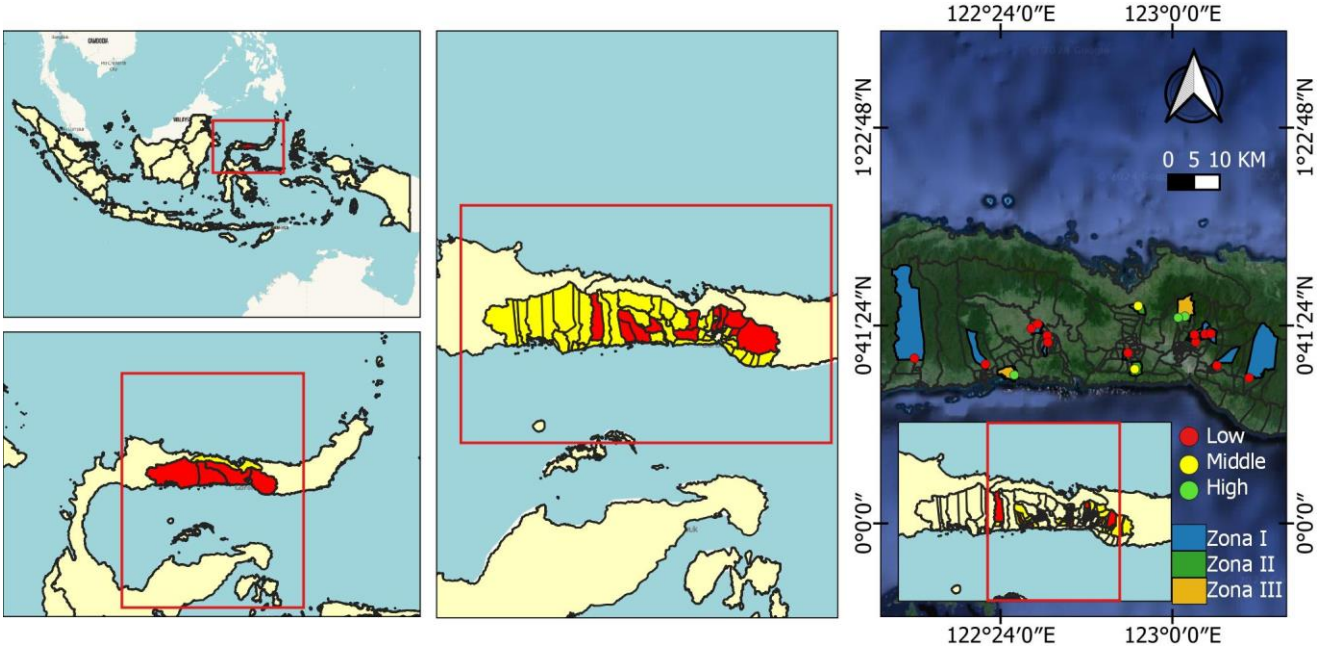


Figure 1. Site for maize rhizosphere sampling in Gorontalo, Indonesia

Observation and identification of *Trichoderma* spp. were conducted based on macroscopic and microscopic morphology (Zheng et al. 2021; Kubicek and Harman 2002), and the several strains were subsequently transferred to Malt Extract Agar (MEA) medium and the other strains transferred to Potato Dextrosa Agar (PDA) using the hyphal tip isolation technique to obtain pure isolates. Species of *Rhizoctonia* were isolated from diseased plant parts that were collected from the same location as the soil sampling location. Diseased specimens, such as stems, sheath, and leaves were cleaned with running water, air-dried, and cut into 0.5 cm pieces on the affected area adjacent to the healthy part. The samples were then dipped in 1% sodium hypochlorite for 1 min, followed by 70% alcohol for 1 min, washed with sterile distilled water, then air dried, and subsequently grown on PDA medium supplemented with chloramphenicol (12.5 mg/L) (Nurhayati et al. 2021). The growing *Rhizoctonia* sp. were identified macroscopically and microscopically and then purified by transferring the tips of hyphae to new PDA medium as a pure isolate (Barnett and Hunter 1988).

Molecular identification

Fungi that was propagated using single spores were rejuvenated on PDA medium and incubated for 4-6 days at 28°C. The fungal mycelium was collected from the colonies grown on PDA medium culture using inoculating loop and subsequently transferred into Eppendorf tube containing PBS buffer and centrifuged. This step was repeated until perfect cell pellets are obtained according to the required quantity.

Fungal DNA was extracted using the GES method, according to Pitcher et al. (1989), with minor modifications. The DNA was extracted using the BYF i-genomic DNA Extraction Mini Kit (iNtRON Biotechnology).

PCR amplification of the ITS DNA gene was carried out using Go-Taq Master Mix (Promega) and a universal primer pair (forward/reverse), namely, ITS 4 (5`-- TCC GCT TAT TGA TAT GC -- 3') and ITS 5 (5`-- GGA AGT AAA AGT CGT AAC AAG G -- 3'). The resulting PCR products (positive DNA) were subsequently subjected to Sanger sequencing using an ABI 3130 XL Genetic Analyser with the same primer pair. The sequencing data were subsequently analyzed using the BioEdit program software (http://www.mbio.nesu.edu) and ClustalW (http://www.clustal.org). The sequence data revealed from the analysis were subsequently compared with sequences in the NCBI GenBank database via the Basic Local Alignment Search Tool (BLAST-N) to determine similarities or homologies of the DNA samples with the genomic database. DNA sequence alignment and phylogenetic analysis were conducted using MEGA version 10 with the neighbor-joining method and 1000 replication bootstrapping.

In vitro antagonistic assay of *Trichoderma* spp. against *Rhizoctonia solani*

The antagonistic effects of *Trichoderma* spp. against *R. solani* (that has been tested for its virulence with an average of 69.75%) were tested using the dual culture method to determine the mode of action, i.e., parasitism, competition, or antibiosis. A five-millimeter-diameter culture disc of *Trichoderma* spp. was transferred to a new PDA medium surface in a 9 cm Petri dish, and then *R. solani* agar disc was also placed on the same PDA medium surface at a distance of 3 cm. The culture was then incubated until 7 days at room temperature. The growth of *R. solani* was then assessed daily by measuring the radius of *R. solani* colony growing toward *Trichoderma* spp. and the opposite radius toward the edge of Petri dish (Figure 2). The measurements were then calculated using the following formula (Skidmore and Dickinson 1976):

$$I = \frac{R1 - R2}{R1} \; x \; 100\%$$

Where:

I : Percentage of inhibition

R1 : The radius of *R. solani* toward the edge of the Petridish

R2 : The radius of *R. solani* toward the *Trichoderma* spp. antagonist fungus

In vitro growth-promoting assay of *Trichoderma* spp.

IAA production assay

The ability of *Trichoderma* spp. to generate IAA was assayed qualitatively using a method described by Lesmana et al. (2019). Potato dextrose broth (PDB) containing L-tryptophan (1 g/L) was used to cultivate each *Trichoderma* spp. isolate. Two 0.5-cm-diameter mycelial agar discs of *Trichoderma* sp. were added to 25 mL of sterile PDB, and the mixture was then incubated for 72 hours. Next, 5 mL of the culture was spun for five minutes at 3000 rpm. One milliliter of the supernatant was transferred to a test tube, and 3 mL of Salkowski reagent was added. A change in color from yellow to brick red was observed. The more intense the red color formed, the greater the amount of IAA dissolved in the filtrate.

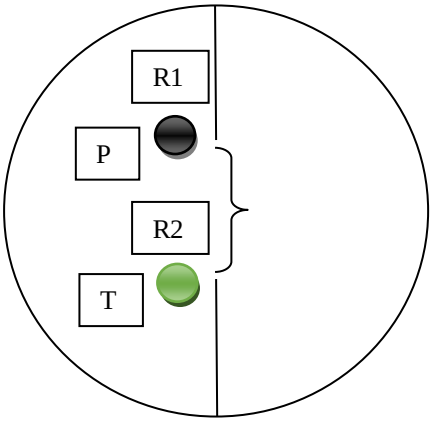


Figure 2. Scheme of antagonistic test on PDA media between *R. solani* (P) and *Trichoderma* spp. (T), the radius of *R. solani* toward the edge of the petri dish (R1), and the radius of *R. solani* toward *Trichoderma* spp. (R2)

Phosphate solubilization assay

A phosphate solubilization assay was carried out on Pikovskaya medium (HiMedia) by dissolving 31.3 g of the media in one liter of distilled water. 0.5 cm agar disc of 7-day-old *Trichoderma* sp. mycelia was grown on the Pikovskaya media in a Petri dish and incubated at room temperature, after which the diameter of the clear zone around the colony of *Trichoderma* sp. was measured after 3 days. The phosphate dissolution index (PDI) was calculated using the following formula of Sharon et al. (2016):

Phosphate Dissolution Index = (colony diameter + clear zone diameter)/colony diameter

Potassium solubilization assay

A potassium solubilization assay of *Trichoderma* spp. indigenous isolates was carried out using modified Alexanderove media (Hu et al. 2006). 0.5-cm agar disc of 7-day-old *Trichoderma* sp. mycelia was grown in Petri dishes containing Alexanderove medium and incubated at room temperature. The clear zone and colony diameter were measured after 3 days. The potassium dissolution index was calculated using the formula (Setiawati and Mutmainnah 2016):

Potassium dissolution index = clear zone diameter/ colony diameter

Plant growth promotion assay of *Trichoderma* spp. on maize plants in the pot experiment

The ability of *Trichoderma* spp. indigenous isolates to promote growth was tested in experimental pots in the greenhouse. 7 days old of *Trichoderma* sp. isolate pieces were inoculated in sterile rice medium and incubated for 7 days at room temperature. Pots containing 1 kg sterile soil were inoculated with 10 g of 7-day-old *Trichoderma* sp. inoculum in rice medium by spreading it evenly on the surface of the ground then covered it again with soil. After 7 days, 2 maize seeds of the Anom cultivar were planted on soil medium per pot. Each treatment involving the *Trichoderma* spp. isolate was repeated three times and the pots were arranged in a completely randomized design. Observations of plant height, number of leaves, length of roots, and number of roots were performed 21 days after planting.

Data analysis

The observation data were statistical analysis of variance (ANOVA) and compared the treatment means with least significance tests at $P<0.05$ using Star software (Akbar 2021).

RESULTS AND DISCUSSION

Isolation and identification

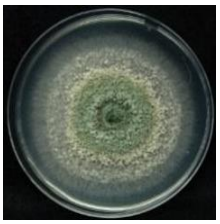
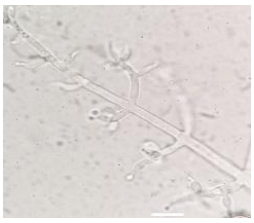
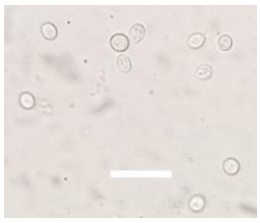
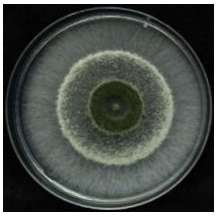
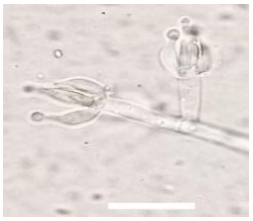
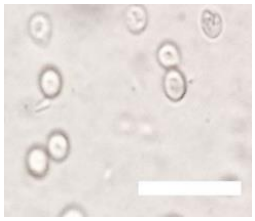
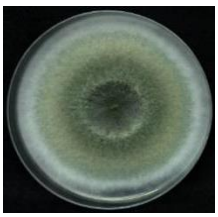
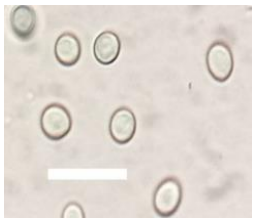
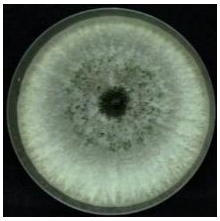
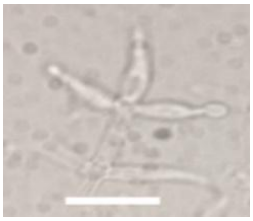
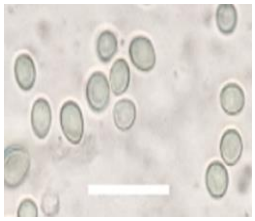
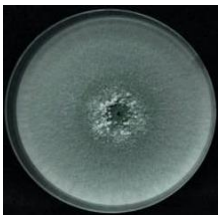
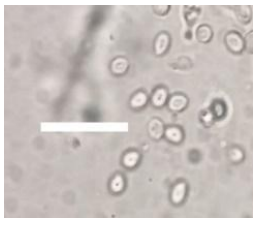
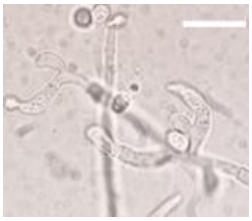
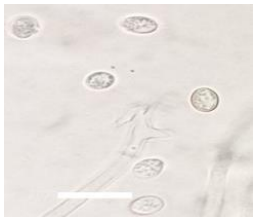
A total of 30 isolates of *Trichoderma* spp. were obtained from the soil rhizosphere samples. A total of 25 *Trichoderma* spp. isolates were successfully recovered from lowland areas (0-300 masl), two isolates from medium plains (300-500 masl), and three isolates from highland (500-1,000 masl) sites around Gorontalo Province. A significant differences was observed in *Trichoderma* population at various altitudes. The distribution and composition of *Trichoderma* species is influenced by several factors such as ecosystem, region, altitude, latitude and longitude (Ma et al. 2020). An important finding of present study was that *Trichoderma* was more dominant at lowest altitude around 83.33%. This may be as lowest altitude environmental conditions are more suitable for the growth and development of *Trichoderma*. The wide distribution of *Trichoderma* is very profitable for agriculture in Gorontalo considering that maize farming businesses in Gorontalo are spread from lowland to highland and dominant in the lowlands. It was also recorded that 63.33% of *Trichoderma* spp. spread in areas with high disease incidence. The morphological characteristics of 30 isolates of *Trichoderma* spp. based on macroscopic and microscopic identification are listed in Table 1.

Molecular identification

The results of molecular identification showed that all the indigenous fungal isolates belonged to the genus *Trichoderma* (Table 2). The fungal strains were dominated by three species, namely, 12 strains (40%) of *T. asperellum*, 10 strains (33.33%) of *T. brevicompactum*, and 5 strains (16.67%) of *T. virens* (Table 2). Moreover, the results of molecular identification included *T. dorothopsis* (TZ31LU1), *T. ghanense* (TZ12PO1), and *T. reesei* (TZ31DU1) species. Based on the site of sample, in lowland had 25 isolates (83.83%) consists of 4 strains, namely *T. asperellum* strains (11 isolates), *T. brevicompactum* strain (10 isolates) *T. virens* strains (3 isolates), and *T. ghanense* strain (1 isolate); 1 strain that is *T. virens* (2 isolates) in medium plains; and 3 strains consists of *T. asperellum* (1 isolate), *T. dorothopsis* strain (1 isolate), and *T. reesei* (1 isolate) in highland (500-1,000 masl). Several studies have revealed that *T. asperellum*, *T. brevicompactum*, and *T. virens* play a role in plant pathogens biocontrol and plant growth promotion. Several species of *T. asperellum*, *T. brevicompactum*, and *T. virens* are also known to be able to act as plant growth promoters (Shentu et al. 2013; Jogaiah et al. 2018; Yu et al. 2021).

Phylogenetic tree (Figure 3) analysis revealed that group of species was divided into 2 main clusters. The first cluster with a bootstrap value of 74% was similar to *Trichoderma asperellum* which consists of isolate codes TZ32TP1, TZ12BU1, TZ11BO1, TZ12BU5, TZ11BO2, TZ12BN1, TZ12BU4, TZ11MO1, TZ12BU3, TZ11DI1, TZ11WO1 and TZ12BU2. Apart from that, in this cluster there was a sub-cluster which with a bootstrap value of 99% is close to *Trichoderma dorothopsis*, namely isolate code TZ31LU1. Meanwhile, the second cluster with a bootstrap value of 73% had 2 sub-clusters. The first sub-cluster had a bootstrap value of 95% which showed closeness to *Trichoderma brevicompactum* with isolate codes consisting of TZ12BN2, TZ12HU3, TZ12HU1, TZ12KB2, TZ12KB3, TZ12OW1, TZ12KB1, TZ12HU4, TZ12HU2, and TZ12MN1.

Table 1. Morphological characteristics of *Trichoderma* spp. isolates from the rhizosphere of maize plants

Morphological characteristics			Morphological description	Isolates code
Colony	Microscopic view			
			The colony was fast-growing (radius 6.0 cm) on MEA at 27±2°C temperature; brass-greenish white aerial mycelium on MEA medium. Conidia production was visible after 3 days. Phialide were ampulliform, lageniform hyaline, and 4-5.5 (long)$\bar{x}$: 5.1) µm. Conidia were greenish-hyaline, globose to ovoid, (2.2-)3.5 x 2.0(2.5) µm, and smooth surface	TZ11BO1 TZ11BO2 TZ11WO1 TZ11MO1 TZ12BN1 TZ12BU1 TZ12BU2 TZ12BU3 TZ12BU4 TZ12BU5 TZ32TP1 TZ11DI1
			The colony was fast-growing (radius 4.7 cm) on MEA at 27±2°C temperature; white-green aerial mycelium on MEA medium. Conidia production was visible after 3 days. Phialide were ampulliform, lageniform hyaline, and length 4.1-7.8 ($\bar{x}$: 5.6) µm. Conidia were greenish, globose to ovoid, (1.8-)2.4 x 1.4(2.0) µm, and smooth surface.	TZ12BN2 TZ12HU1 TZ12HU2 TZ12HU3 TZ12HU4 TZ12KB1 TZ12KB2 TZ12KB3 TZ12OW1 TZ12MN1
			The colony was fast-growing (radius 7.5 cm) on MEA at 27±2°C temperature, white-green aerial mycelium on MEA medium Conidia production was visible after 3 days. Phialide were ampulliform, hyaline, and 4-6 ($\bar{x}$: 5.1) µm. Conidia were greenish, globose to ovoid, (3.1-)4.0 x 2.5(3.1) µm, and smooth surface.	TZ11BO3 TZ21BT1 TZ11BT1 TZ22MM1 TZ11PI1
			The colony was fast-growing (radius 8-8.4 cm) on MEA at 27±2°C temperature. Colonies were grayish green with yellow pigment diffused on the medium. Conidia production was visible after 3 days, starting from the initial point of inoculum growth. Phialide were ampulliform, *lageniform* hyaline, and 5-8 ($\bar{x}$: 6.63) µm. Conidia were greenish-hyaline, globose to ovoid, (2.6-)3.4 x 2.2(2.7) µm, and smooth surface.	TZ31DU1
			The colony was fast-growing (radius 6.9 cm) on MEA at 27±2°C temperature, white-green aerial mycelium on MEA medium. Conidia production was visible after 3 days. Phialide were ampulliform, lageniform hyaline, and 4-6 ($\bar{x}$: 5.0) µm. Conidia were greenish-hyaline, globose to ovoid, (2.5-)3.6 x 2.0(2.5) µm, and smooth surface.	TZ31LU1
			The colony was slow-growing (radius 4-4.1 cm) on MEA at 27±2°C temperature. Submerged colonies and yellow-green aerial mycelium on PDA medium. Conidia production was visible after 7 days. Phialide were *lageniform* curved at the tip of the conidiogen, hyaline, and 4-8 ($\bar{x}$: 6.0) µm. Conidia were greenish-hyaline, globose to ovoid, (3.1-)4.1 x 2.2(3.0) µm, and rough surface.	TZ12PO1

Note: Scale bar =10 µm

The second sub-cluster with a bootstrap value of 43% consists of 2 sub-sub-clusters, namely the first with a bootstrap value of 98% which was similar to *Trichoderma virens* (TZ11BT1, TZ21BT1, TZ11BO3, TZ11PI1, and TZ22MM1) and the second with a bootstrap value of 96% which shows similarity to *Trichoderma ghanense* (bootstrap value 86% with isolate code TZ12PO1) and *Trichoderma reesei* (bootstrap value 84% with isolate code TZ31DU1). The outgroup in the phylogenetic tree was used to show differences in genetic closeness between isolates and the comparison gene, i.e. *Gliocladium viride*. The results of molecular identification using Blast-N search are presented in Table 2.

In vitro antagonistic activity against *Rhizoctonia solani*

Antagonistic assays showed that several *Trichoderma* species strongly inhibited the growth of *R. solani* in vitro. Among the 30 fungal strains, 25 inhibited *R. solani* by more than 50% (Table 3). The five strains had less than 50% lower inhibitory effect on *R. solani*, i.e., *T. asperellum* TZ12BU3 (42.2%), *T. asperellum* TZ12BU4 (22.2%), *T. asperellum* TZ12BU5 (35.2%), *T. virens* TZ21BT1 (46.7%), and *T. virens* TZ22MM1 (46.6%). Six *Trichoderma* spp. strains were able to inhibit more than 70%, i.e., *T. asperellum*-TZ11DI1 (77.4%), *T. asperellum* TZ12BU1 (75%), *T. asperellum* TZ12BU2 (76.2%), *T. brevicompactum* TZ12OW1 (77.4%), *T. reesei* TZ31DU1 (75.1%), and *T. asperellum* TZ32TP1 (75.1%). These results suggest that most *Trichoderma* spp. strains were able to inhibit the growth of the plant pathogen *R. solani*. *Trichoderma* sp. is a biological agent that acts as a biocontrol agent to suppress plant diseases in vitro (Manandhar et al. 2019; Ngo et al. 2021). Other reports have shown that five strains of *T. asperellum* have antagonistic effects on more than 50% of maize plants through parasitism and increased growth (Mirsam et al. 2023).

Fourteen *Trichoderma* spp. strains, including *T. brevicompactum*, are known to be capable of inhibiting the growth of the plant pathogen *Fusarium solani* on potato plants (Ommati and Zaker 2012). *T. virens* has also been reported to inhibit the growth of *R. solani* by secreting cell wall-degrading enzymes (CWDEs) such as chitinase and cellulase (Ghasemi et al. 2020). *T. ghanense* also efficiently inhibited the growth of *F. solani*, and a moderate antagonism against *S. sclerotia* or *R. solani* (Qualhato et al. 2013). *T. reesei* is also known to inhibit the growth of *Alternaria alternata* by 75%. The results of the present study revealed consistent traits of *Trichoderma* spp. as a biocontrol agent that can antagonistically inhibit the growth of plant pathogens. *Trichoderma* spp. are widely known as biocontrol agents for suppressing plant pathogens through antibiosis, competition, and parasitism mechanisms (Stracquadanio et al. 2020; Mukherjee et al. 2022).

Table 2. Results of BLAST-N searches of *Trichoderma* spp. and *Rhizoctonia solani* isolates

Strains	Nearest species/ Accession no.	Similarity (%)
TZ11BO1	*Trichoderma asperellum* (KU059966.1)	100%
TZ11BO2	*Trichoderma asperellum* (MH215555.1)	100%
TZ11BO3	*Trichoderma virens* (MG707198.1)	100%
TZ11BT1	*Trichoderma virens* (KY767626.1)	100%
TZ11WO1	*Trichoderma asperellum* (MH215555.1)	100%
TZ11MO1	*Trichoderma asperellum* (MH215554.1)	100%
TZ11DI1	*Trichoderma asperellum* (MH215555.1)	100%
TZ11PI1	*Trichoderma virens* (MG707198.1)	100%
TZ12BN1	*Trichoderma asperellum* (KU059966.1)	100%
TZ12BN2	*Trichoderma brevicompactum* (MH624142.1)	100%
TZ12BU2	*Trichoderma asperellum* (MF871562.1)	100%
TZ12BU3	*Trichoderma asperellum* (KU059966.1)	100%
TZ12BU4	*Trichoderma asperellum* (KU059966.1)	100%
TZ12HU1	*Trichoderma brevicompactum* (MH624142.1)	100%
TZ12HU2	*Trichoderma brevicompactum* (MH624142.1)	100%
TZ12HU3	*Trichoderma brevicompactum* (MH624142.1)	100%
TZ12HU4	*Trichoderma brevicompactum* (MH624147.1)	100%
TZ12KB1	*Trichoderma brevicompactum* (MH624147.1)	100%
TZ12KB2	*Trichoderma brevicompactum* (MH624147.1)	100%
TZ12KB3	*Trichoderma brevicompactum* (MH624147.1)	100%
TZ12MN1	*Trichoderma brevicompactum* (MH624147.1)	100%
TZ21BT1	*Trichoderma virens* (MN427919.1)	100%
TZ22MM1	*Trichoderma virens* (MH624149.1)	100%
TZ31LU1	*Trichoderma dorothopsis* (MH624143.1)	100%
TZ32TP1	*Trichoderma asperellum* (MH215554.1)	100%
TZ12BU1	*Trichoderma asperellum* (MH215555.1)	100%
TZ12PO1	*Trichoderma ghanense* (KT792972.1)	100%
TZ12OW1	*Trichoderma brevicompactum* (MH624147.1)	100%
TZ31DU1	*Trichoderma reesei* (LC535971.1)	100%
TZ12BU5	*Trichoderma asperellum* (KU059966.1)	100%
R2S	*Rhizoctonia solani* (MT620736.1)	100%

In this study, the inhibitory mechanism of *Trichoderma* spp. against *R. solani* involved two mechanisms, namely, antibiosis, in which a clear zone formed around the colony on PDA media, and competition (Figure 4). This study revealed that each *Trichoderma* spp. strain had a different antagonistic mechanism against *R. solani*. A clear zone was produced by *Trichoderma* spp. due to the release of antibiosis compounds that are toxic to the mycelia of *R. solani*. *Trichoderma* sp. can inhibit pathogen growth by producing VOCs, causing antibiosis, parasitism, activating defense enzymes, and inducing plant resistance (Sood et al. 2020). *Trichoderma* sp. as a biocontrol agent, releases antibiotic compounds and lytic enzymes that are toxic to pathogens (Asad et al. 2015). Most volatile organic compounds (VOCs) produced by *Trichoderma* spp. are monoterpene and sesquiterpene compounds, play a role in protecting plants from pathogen infection (Tabarestani et al. 2016). *T. asperellum* produces as many as 32 volatile organic compounds (VOCs) with antifungal activity that effectively influence the growth of the pathogens *B. dinner* and *S. clerotiorum* (Kamaruzzaman et al. 2021). *Trichoderma* sp. produce several enzyme pathogenic cell wall-degrading enzymes (CWDEs), such as β-1,6-glucanase and chitinase, which play important roles in parasitism (Jayalakshmi et al. 2009). In the present study, *Trichoderma* spp. strains were able to inhibit the growth of *R. solani* by releasing antibiosis compounds (Figure 4.A) and competing (Figure 4.B).

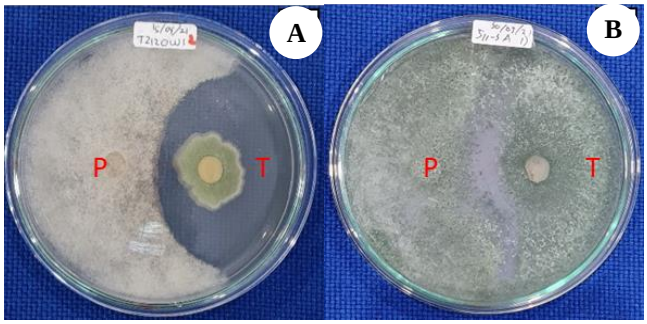


Figure 4. Inhibition of *R. solani by Trichoderma* sp.: A. Antibiosis, B. Competition. T: *Trichoderma* sp., P: *Rhizoctonia solani*

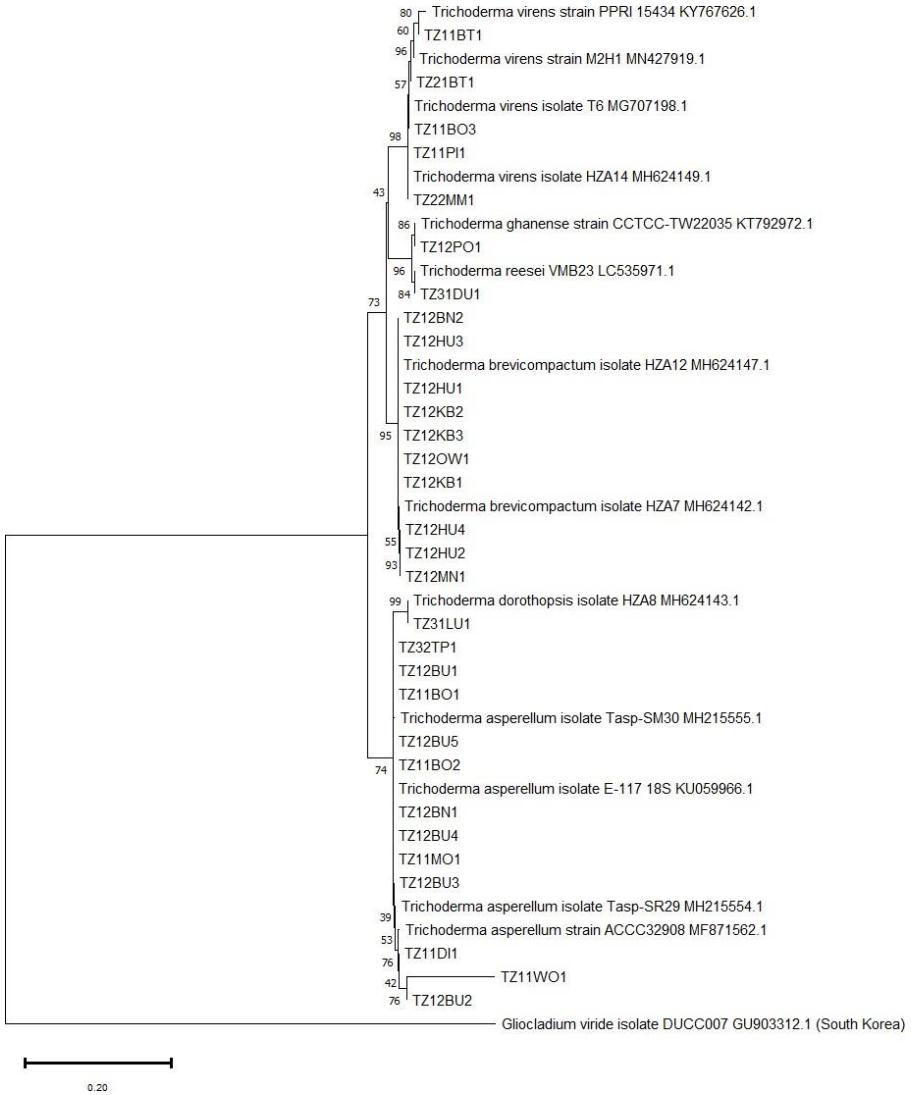


Figure 3. Phylogenetic tree of indigenous *Trichoderma* spp.

Table 3. Inhibition of *R. solani* by *Trichoderma* spp.

Strains	Percentage (%)	Inhibition types
*T. asperellum*TZ11BO1*	51.52	Competition
T. asperellum -TZ11BO2*	60.00	Competition
*T. green*TZ11BO3*	59.52	Competition
T. green -TZ11BT1*	55.15	Competition
*T. asperellum*TZ11WO1*	58.59	Competition
T. asperellum-TZ11MO1*	60.71	Competition
T. asperellum-TZ11DI1*	77.40	Competition
*T. green*TZ11PI1*	55.15	Competition
T. asperellum -TZ12BN1*	61.11	Competition
T. brevicompactum-TZ12BN2*	67.44	Antibiosis
*T. asperellum*TZ12BU1*	75.00	Antibiosis
T. asperellum-TZ12BU2*	76.27	Competition
T. asperellum-TZ12BU3	42.22	Competition
T. asperellum-TZ12BU4	22.22	Competition
*T. asperellum*TZ12BU5	35.52	Antibiosis
T. ghanense-TZ12PO1*	57.06	Antibiosis
T. brevicompactum-TZ12HU1*	65.56	Competition
T. brevicompactum-TZ12HU2*	63.10	Competition
T. brevicompactum-TZ12HU3*	51.19	Antibiosis
T. brevicompactum-TZ12HU4*	66.67	Competition
T. brevicompactum-TZ12KB1*	60.00	Competition
T. brevicompactum-TZ12KB2*	68.89	Antibiosis
T. brevicompactum-TZ12KB3*	67.44	Competition
T. brevicompactum-TZ12OW1*	77.40	Antibiosis
T. brevicompactum-TZ12MN1*	64.55	Competition
*T. green*TZ21BT1	46.70	Competition
*T. green*TZ22MM1	46.67	Competition
T. reesei-TZ31DU1*	75.14	Competition
T. dorothopsis-TZ31LU1*	69.49	Competition
*T. asperellum*TZ32TP1*	75.14	Competition

Note: *: Inhibition index ≥ 50%.

Plant Growth Promotion (PGP) traits

The results also revealed the activities of *Trichoderma* spp. plant growth-promoting agents in terms of IAA hormone production, phosphate solubilization, and potassium solubilization. The results of the qualitative assay of IAA production showed that after 12 hours of incubation, *Trichoderma* spp. strains produced a color change from yellow to brick red with different intensities for each isolate. These results suggest that all the strains of indigenous *Trichoderma* spp. can produce IAA qualitatively at different concentrations. Moreover, 13 strains produce a strong dark brick red colour, indicated high concentration of IAA: *T. asperellum* TZ11DI1, *T. brevicompactum* TZ12BN2, *T. asperellum* TZ12BU1, *T. asperellum* TZ12BU2, *T. asperellum* TZ12BU3, *T. asperellum* TZ12BU5, *T. ghanense* TZ12PO1, *T. brevicompactum* TZ12HU1, *T. brevicompactum* TZ12HU4, *T. brevicompactum* TZ12KB1, *T. virens* TZ21BT1, *T. reesei* TZ31DU1, and *T. dorothopsis* TZ31LU1 (Table 4). Some *Trichoderma* spp. can produce hormone IAA in vitro (Saxena et al. 2015; Bader et al. 2020). The results of also confirmed that *Trichoderma* sp. strain was capable of producing IAA, and the application of *Trichoderma* sp. can increase plant growth. IAA is an important hormone in plants that develops lateral roots and promotes root hair development (Zamioudis et al. 2013). Applying *T. atroviride* and *T. virens* increased plant growth by regulating IAA production in *Arabidopsis thaliana* (Contreras-Cornejo et al. 2009).

The ability of indigenous *Trichoderma* spp. as a PGP, especially as a biofertilizer were assayed for phosphate and potassium solubilization activity. The ability of *Trichoderma* spp. to dissolve phosphate was determined by the clear zone around the colonies that formed in the Pikovskaya medium. All the *Trichoderma* spp. strains were able to dissolve phosphate (Table 4). Two strains have the highest ability to solubilize phosphate, namely, *T. asperellum* TZ12BN1 (1.8) and *T. brevicompactum* TZ12HU1 (1.4). The results showed that *Trichoderma* sp. can act as a phosphate solubilizer, a macronutrient for plant growth. Additionally, phosphate is a macronutrient that plays an important role in plant growth and is involved in the physiological response of plants to abiotic stress (Khan et al. 2023). Several reports have shown that many *Trichoderma* species, such as *T. brevicompactum, T. gamsii*, and *T. harzianum*, can solubilize phosphate (Bader et al. 2020). The application of *T. asperellum* Q1 was shown to increase plant growth under salt stress, and this fungus can dissolve organic and inorganic phosphate as well as phosphatase and phytase activities (Zhao and Zhang 2015). Therefore, the results of present study exhibited that several *Trichoderma* spp. strains were able to solubilize phosphate, which plays a role in increasing plant growth. The ability of *Trichoderma* sp. to solubilize potassium was determined by the clear zone surrounding the fungal colony grown on the Alexandrov medium. The comparison showed that, with an index of 0.57, only one strain of *Trichoderma* sp. could solubilize potassium, namely, *T. dorothopsis* TZ31LU1. Potassium is known to play a role in plant growth and metabolism. Inoculation with Trichoderma can increase the amount of NPK nutrients used for plant growth (Silva et al. 2023). Therefore, applying *Trichoderma* sp. to plants can be used as biological fertilizers to increase plant growth.

The effect of *Trichoderma* spp. application on maize plants in pots

The study results showed that inoculation with *Trichoderma* spp. increased the growth of maize plants (Table 5). The height of the plants inoculated with *Trichoderma* spp. was higher than that of control. Similarly, the number of leaves on plants inoculated with *Trichoderma* spp. was higher than control plants, except for those in the two treatments, namely, *T. brevicompactum* TZ12BN2 and *T. asperellum* TZ32TPI1. *Trichoderma* spp. inoculation also increased the root length compared to that of the control, except for the *T. asperellum* TZ11BO1, *T. asperellum* TZ11 WO1, *T. brevicompactum* TZ12BN2, *T. asperellum* TZ12BU5, and *T. brevicompactum* TZ12MN1 strains. However, these strains showed slightly higher values than the control but not statistically significant. Similarly, the number of roots increased in the plants treated with *Trichoderma* spp. compared to the control, except for the *T. asperellum* TZ11BO2, *T. virens* TZ11BO3, *T. virens* TZ11BT1, *T. asperellum* TZ11WO1, and *T. asperellum* TZ11DI1 strains. These results follow various studies that applying *Trichoderma* sp. to plants can

increase plant growth (Susiana et al. 2018; Bayoumi et al. 2019; He et al. 2019). *Trichoderma* sp. is a biological fertilizer that can increase plant growth by producing phytohormones and increasing plant nutrient uptake (Zhang et al. 2013). Moreover, our study confirmed that *Trichoderma* spp. increases the growth of maize plants.

Table 4. Plant growth-promoting activities of *Trichoderma* spp.

Strains	PGP activities			No	Strains	PGP activities		
	Phosphate Solubilization index	Potassium Solubilization Index	IAA			Phosphate Solubilization index	Potassium Solubilization index	IAA
TZ11BO1	1.27	-	+	16	TZ12PO1	1.03	-	+++
TZ11BO2	1.24	-	++	17	TZ12HU1	1.42	-	+++
TZ11BO3	1.00	-	++	18	TZ12HU2	1.08	-	++
TZ11BT1	1.00	-	++	19	TZ12HU3	1.23	-	++
TZ11WO1	1.19	-	++	20	TZ12HU4	1,00	-	+++
TZ11MO1	1.20	-	++	21	TZ12KB1	1,00	-	+++
TZ11DI1	1.16	-	+++	22	TZ12KB2	1.03	-	++
TZ11PI1	1.00	-	++	23	TZ12KB3	1.28	-	++
TZ12BN1	1.80	-	++	24	TZ12OW1	1.12	-	++
TZ12BN2	1.06	-	+++	25	TZ12MN1	1.27	-	++
TZ12BU1	1.20	-	+++	26	TZ21BT1	1,00	-	+++
TZ12BU2	1.14	-	+++	27	TZ22MM1	1,14	-	++
TZ12BU3	1.20	-	+++	28	TZ31DU1	1,00	-	+++
TZ12BU4	1.23	-	+	29	TZ31LU1	1,00	0.57	+++
TZ12BU5	1.15	-	+++	30	TZ32TP1	1.15	-	+

Note: -: no activities, +: light brick red (low concentration), ++: medium brick red (medium concentration), +++: strong dark brick red (high concentration)

Table 5. The effect of *Trichoderma* spp. on maize plant growth

Isolates code	Plant growth parameters			
	Plant height (cm)	Number of leaves	Root length (cm)	Number of roots
Control	60.50a (0%)	4.50a (0%)	14.00a (0%)	6.00a (0%)
T. asperellum - TZ11BO1	73.50b (17.69%)	7.00b (35.71%)	14.60a (4.11%)	11.00b (45.45%)
T. asperellum - TZ11BO2	77.00b (21.43%)	7.50c (40.00%)	19.10b (26.70%)	8.00a (25.00%)
T. virens - TZ11BO3	75.00b (19.33%)	8.00c (43.75%)	20.21d (30.73%)	8.00a (25.00%)
T. virens - TZ11BT1	83.00c (27.11%)	7.50c (40.00%)	22.50c (37.78%)	8.00a (25.00%)
T. asperellum - TZ11WO1	77.00b (21.43%)	7.50c (40.00%)	17.00a (17.65%)	8.00a (25.00%)
T. asperellum - TZ11MO1	85.50c (29.24%)	7.50c (40.00%)	22.26c (37.11%)	9.00b (33.33%)
T. asperellum - TZ11DI1	74.50b (18.79%)	6.50b (30.77%)	19.60b (28.57%)	8.00a (25.00%)
T. virens - TZ11PI1	70.00b (13.57%)	8.00c (43.75%)	27.00d (48.15%)	9.00b (33.33%)
T. asperellum - TZ12BN1	70.00b (13.57%)	7.00b (35.71%)	22.94c (38.97%)	12.00c (50.00%)
T. brevicompactum - TZ12BN2	78.50b (22.93%)	5.50a (18.18%)	16.06a (12.83%)	10.00b (40.00%)
T. asperellum - TZ12BU1	75.50b (19.87%)	7.50c (40.00%)	18.44b (24.08%)	11.00b (45.45%)
T. asperellum - TZ12BU2	72.00b (15.97%)	7.00b (35.71%)	25.70c (45.53%)	9.00b (33.33%)
T. asperellum - TZ12BU3	73.00b (17.12%)	8.00c (43.75%)	19.70b (28.93%)	9.00b (33.33%)
T. asperellum - TZ12BU4	79.00b (23.42%)	7.00b (35.71%)	20.44b (31.51%)	9.00b (33.33%)
T. asperellum - TZ12BU5	81.50b (25.77%)	7.50c (40.00%)	17.08a (18.03%)	9.00b (33.33%)
T. ghanense - TZ12PO1	77.00b (21.43%)	7.00b (35.71%)	22.20c (36.94%)	14.00c (57.14%)
T. brevicompactum - TZ12HU1	76.00b (20.39%)	7.50c (40.00%)	30.50e (54.10%)	13.00c (53.85%)
T. brevicompactum - TZ12HU2	84.00c (27.98%)	8.00c (43.75%)	23.28c (39.86%)	12.00c (50.00%)
T. brevicompactum - TZ12HU3	75.50b (19.87%)	6.50b (30.77%)	22.25c (37.08%)	12.00c (50.00%)
T. brevicompactum - TZ12HU4	77.00b (21.43%)	6.50b (30.77%)	22.62c (38.11%)	15.00d (60.00%)
T. brevicompactum - TZ12KB1	74.00b (18.24%)	7.50c (40.00%)	21.52b (34.94%)	11.00b (45.45%)
T. brevicompactum - TZ12KB2	74.00b (18.24%)	6.50b (30.77%)	22.00c (36.36%)	10.00b (40.00%)
T. brevicompactum - TZ12KB3	73.50b (17.69%)	8.00c (43.75%)	19.04b (26.47%)	16.00d (62.50%)
T. brevicompactum - TZ12OW1	79.50b (23.90%)	7.50c (40.00%)	22.44c (37.61%)	14.00c (57.14%)
T. brevicompactum - TZ12MN1	73.50b (17.69%)	7.00b (35.71%)	17.50a (20.00%)	10.00b (40.00%)
T. virens - TZ21BT1	82.00b (26.22%)	7.50c (40.00%)	18.20b (23.08%)	12.00c (50.00%)
T. virens - TZ22MM1	69.50b (12.95%)	6.50b (30.77%)	20.19b (30.66%)	12.00c (50.00%)
T. reesei - TZ31DU1	71.50b (15.38%)	6.50b (30.77%)	23.24c (39.76%)	12.00c (50.00%)
T. dorothopsis - TZ31LU1	86.00c (29.65%)	8.00c (43.75%)	26.00d (46.15%)	13.00c (53.85%)
T. asperellum - TZ32TP1	87.50c (30.86%)	4.50a (0.00%)	19.10b (26.70%)	14.00c (57.14%)

Note: Means followed by the same letter are not significantly different.

In conclusion, the result revealed that 30 *Trichoderma* spp. isolates were found which included 6 strains, namely 12 stains *T. asperellum*, 10 strains *T. brevicompactum*, 5 strains *T. virens*, 1 strain *T. Dorothopsis*, 1 strain *T. Ganense*, and 1 strain *T. reseei*. All isolates were found antagonistic to *R. solani*. This indicates that isolates can function as a biocontrol against *R. solani*. All isolates produced IAA and dissolved phosphate, and only 1 isolate dissolved potassium in vitro. Almost all isolates had a significant effect on plant height, number of leaves, number and length of roots in greenhouse, that showed the isolate potential as a growth promoter and biofertilizer. A comprehensive study should be conducted to investigate the ability of indigenous *Trichoderma* spp. isolates to promote growth and induce resistance in maize plants.

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