

RESEARCH PAPER

Biological control of maize sheath blight caused by *Rhizoctonia solani* using rhizosphere fungi isolated from wild *Solanum lasiocarpum* Dunal: an in vitro study

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ABSTRACT

Maize sheath blight caused by *Rhizoctonia solani* is a major constraint to maize production, particularly in tropical peatland ecosystems where environmental conditions favor pathogen development. This study evaluated the antagonistic potential of rhizosphere fungi isolated from the indigenous plant *Solanum lasiocarpum* Dunal (*Terung Asam*) against *R. solani* under in vitro conditions. Rhizosphere fungi were isolated and identified using morphological characteristics and ITS rDNA sequencing, while their antagonistic activity and volatile organic compound (VOC)-mediated inhibition were assessed using dual culture and sealed plate assays. Six fungal isolates were successfully recovered from the rhizosphere of *S. lasiocarpum* and identified as belonging to the genera *Trichoderma* (TA-1–TA-3) and *Aspergillus* (TA-4–TA-6). Among them, *Trichoderma* isolate TA-1 (82.8%) and *Aspergillus* isolate TA-4 (81.4%) exhibited the highest inhibition of *R. solani*, indicating strong antagonistic activity through competition, mycoparasitism, and antibiosis. In the VOC assay, *Aspergillus* isolates TA-4 and TA-5 showed the strongest inhibition of pathogen growth, suggesting the production of antifungal volatile metabolites. Molecular identification based on ITS sequencing and BLAST analysis confirmed that isolate TA-1 was *Trichoderma asperellum* (576 bp) and isolate TA-4 was *Aspergillus welwitschiae* (574 bp), both sharing 100% sequence similarity with reference sequences in GenBank. To our knowledge, this is the first report describing antagonistic fungi isolated from the rhizosphere of wild *S. lasiocarpum* as potential biological control agents against maize sheath blight caused by *R. solani*. These indigenous fungi represent promising candidates for the development of environmentally sustainable disease management strategies in tropical peatland agriculture.

Keywords: Antagonistic fungi, biological control, ITS sequencing, maize sheath blight, *Rhizoctonia solani*, *Solanum lasiocarpum*

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important food crops in Indonesia after rice and soybean. Besides serving as a staple food, maize is a major raw material for the livestock feed industry and therefore plays a strategic role in national food security and agricultural development (Fahrul, 2024). However, maize productivity is constrained by several diseases, among which sheath blight caused by *Rhizoctonia solani* Kühn is one of the most destructive soil-borne diseases.

Rhizoctonia solani infects maize under warm and humid conditions and can attack leaf sheaths, stems, husks, and ears, resulting in severe blight symptoms and plant death. Yield losses caused by this pathogen may reach 40% in several maize-growing regions

and can become much higher under highly conducive conditions (Soenartiningih et al., 2014; Li et al., 2023; Juliana et al., 2024). The pathogen survives for long periods in soil through mycelia and sclerotia, making the disease difficult to manage once established (Mirsam et al., 2023). Disease development is favored by relative humidity above 80% and temperatures between 15 and 35 °C, conditions commonly occurring during the rainy season in tropical regions (Rotasouw et al., 2020).

In Indonesia, *R. solani* is widely distributed, including in the peatland ecosystems of Central Kalimantan. Peat soils are characterized by high organic matter content, acidic pH, and prolonged moisture retention, creating favorable conditions for the survival and spread of soil-borne pathogens (Sumartini, 2012; Soenartiningih et al., 2015). Recent field observations in maize-growing areas around Palangka Raya indicated that sheath blight incidence reached approximately 60%, highlighting the increasing importance of this disease in peatland-based maize. The persistence of *R. solani* in peat soils

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further complicates disease management and poses a significant challenge to sustainable maize cultivation in the region.

Management of sheath blight still relies largely on synthetic fungicides. Although fungicides can reduce disease severity, their continuous application often results in environmental contamination, disruption of beneficial soil microorganisms, and the development of fungicide-resistant pathogen populations (Suciati et al., 2016; Di et al., 2023). Moreover, chemical control is frequently less effective in peatland ecosystems because high organic matter can reduce fungicide persistence and activity. Consequently, environmentally friendly and locally adapted disease management strategies are urgently needed.

Biological control using indigenous antagonistic microorganisms represents a promising alternative. Rhizosphere fungi are particularly attractive because they naturally colonize plant root zones, compete effectively for nutrients and space, and may produce antifungal metabolites or induce plant resistance (Soplanit et al., 2021). Species of *Trichoderma* have been widely recognized as effective antagonists of *R. solani*. Local isolates of *Trichoderma asperellum* and *T. harzianum*, for example, were reported to suppress the growth of *R. solani* by more than 65% in vitro (Ariyanti et al., 2021). Other studies in Central Kalimantan showed that rhizosphere-derived *Trichoderma* and *Gliocladium* isolates could inhibit several soil-borne pathogens and improve seedling performance (Supriati et al., 2023; Aizah et al., 2024).

One indigenous plant that has received increasing attention is *Solanum lasiocarpum* Dunal (syn. *S. ferox*), locally known as *terung asam* or *terong Dayak*. This perennial Solanaceae species grows naturally in peatland areas of Kalimantan and is commonly used as a vegetable and flavoring ingredient by local communities. In addition to its nutritional value, the plant contains phenolics and flavonoids with strong antioxidant activity and has traditionally been used for medicinal purposes (Nion & Kamillah, 2023; Myat & Myint, 2024; Rahman et al., 2019). Extracts of *S. lasiocarpum* have also shown antimicrobial activity against several plant pathogens (Ibrahim et al., 2022).

Recent studies suggest that the rhizosphere of *S. lasiocarpum* harbors diverse beneficial fungi. Isolates belonging to *Penicillium*, *Trichoderma asperellum*, *T. hamatum*, and *T. harzianum* obtained from its rhizosphere were reported to inhibit *Colletotrichum gloeosporioides* by up to 68.53% in vitro (Mulyani et al., 2024). The rich root exudates and organic matter surrounding Solanaceae roots are known to support

the proliferation of antagonistic microorganisms such as *Trichoderma*, *Gliocladium*, and *Aspergillus* (Mukaromah et al., 2025). Despite these findings, information regarding rhizosphere fungi associated with *S. lasiocarpum* and their ability to suppress *R. solani*, the causal agent of maize sheath blight, remains very limited.

Although *S. lasiocarpum* has been reported to contain bioactive phytochemicals and its rhizosphere harbors beneficial microorganisms, studies investigating the antagonistic fungi associated with its rhizosphere remain limited, particularly regarding their potential for controlling *R. solani* (Mulyani et al., 2024; Rushdy et al., 2025). This lack of information represents an important research gap, especially for peatland agriculture, where indigenous microbial resources may provide more adaptive and sustainable disease management solutions than introduced biocontrol agents. Therefore, the present study aimed to isolate and evaluate rhizosphere fungi from wild *S. lasiocarpum* growing in Central Kalimantan for their antagonistic activity against *R. solani* under in vitro conditions. In addition, the most effective fungal isolates were characterized using molecular identification to determine their taxonomic identity and assess their potential as biocontrol agents for maize sheath blight management.

MATERIALS AND METHODS

Research Site. This study was conducted from August to December 2024 at the Agricultural Cultivation Laboratory, Faculty of Agriculture, University of Palangka Raya, Indonesia. Molecular identification through DNA sequencing was performed at PT. Genetic Sciences Indonesia, Tangerang, Indonesia.

Experimental Design. The experiment was conducted in vitro using a completely randomized design (CRD) consisting of seven treatments: one control (*R. solani* alone) and six rhizosphere fungal isolates (TA1–TA6) obtained from the rhizosphere of wild *S. lasiocarpum* growing on peatland. Each treatment was replicated three times, and each petri dish represented one experimental unit.

Isolation of Rhizosphere Fungi. Rhizospheric soil samples were collected from healthy wild *S. lasiocarpum* plants growing on peatland in Central Kalimantan. Ten grams of soil were suspended in 100 mL sterile distilled water and shaken for 20 min. Serial dilutions were prepared up to 10⁻⁵ one milliliter

of the final dilution was transferred into sterile Petri dishes containing potato dextrose agar (PDA; Merck, Germany) supplemented with chloramphenicol to suppress bacterial growth. Plates were incubated at 25 ± 2 °C for 5–7 days.

Emerging fungal colonies were selected based on differences in colony morphology, color, and shape, then purified by single-colony transfer using a sterile inoculation needle onto fresh PDA plates following the method of Anggraeni & Usman (2015). Six purified isolates were obtained and designated TA1–TA6.

Isolation *Rhizoctonia solani*. The pathogen *R. solani* was isolated from naturally infected maize sheath tissues showing typical sheath blight symptoms following the method described by Soplanit et al. (2021). Small tissue pieces from the lesion margins were surface sterilized, rinsed with sterile distilled water, dried on sterile filter paper, and cultured on PDA. Pure cultures were maintained on PDA for further experiments.

Morphological Identification. Rhizosphere fungi and *R. solani* were identified based on colony morphology and microscopic characteristics. Colony color, texture, growth pattern, and margin characteristics were observed on PDA after seven days of incubation. Microscopic observations were performed using methyl blue-stained preparations under a compound microscope at 400× magnification. Identification was based on the taxonomic keys of Barnett & Hunter (1998) and Watanabe (2002).

Molecular Identification. The most promising antagonistic isolates were identified molecularly using DNA barcoding. Genomic DNA was extracted using the Quick-DNA Fungal MagBead Plus Kit (Zymo Research, USA). DNA quality and concentration were determined using a NanoDrop spectrophotometer (Table 1).

The internal transcribed spacer (ITS) region of rDNA was amplified using universal primers ITS1 and ITS4. PCR amplification was performed using MyTaq HS Red Mix (Bioline, UK). PCR products were separated on 1% agarose gel stained with GelRed and visualized under UV illumination using a 100 bp DNA ladder.

Purified PCR products were sequenced bidirectionally using the Sanger sequencing method. Consensus sequences were compared with those available in the NCBI GenBank database using BLASTn for species identification.

Dual Culture Assay. The antagonistic activity of rhizosphere fungi against *R. solani* was evaluated using the dual culture technique. A 5-mm-diameter mycelial plug of *R. solani* and a 5-mm plug of each rhizosphere fungal isolate were placed on opposite sides of a PDA plate, approximately 3 cm apart (or the actual distance used), whereas the control consisted of *R. solani* cultured alone (Armila et al., 2019).

Cultures were incubated at 25 ± 2 °C for seven days. Colony diameters were measured daily, and the percentage inhibition of radial growth (PIRG) was calculated using the formula.

$$\text{PIRG} = \frac{R_1 - R_2}{R_1} \times 100\%$$

PIRG = Percentage inhibition of radial growth (%);
 R_1 = The radial growth of *R. solani* in the control;
 R_2 = The radial growth of *R. solani* toward the antagonist.

The interaction between both fungi was also examined microscopically to determine the antagonistic mechanism, including competition, antibiosis, and mycoparasitism, based on the criteria described by Agustina et al. (2019).

Volatile Compound Assay. The inhibitory effect of volatile organic compounds (VOCs) produced by rhizosphere fungi was evaluated using the sealed plate method. A 5-mm mycelial plug of each antagonist and a 5-mm plug of *R. solani* were inoculated separately onto PDA plates. The bottoms of the two Petri dishes were placed face-to-face and sealed with parafilm to prevent volatile loss while avoiding direct contact between the fungi.

Control plates consisted of *R. solani* paired with uninoculated PDA plates. Colony diameter of *R. solani* was measured daily for seven days, and PIRG values were calculated as described above. Inhibitory activity was classified as low (<50%), moderate (50–60%),

Table 1. DNA quantification results using NanoDrop

No.	Isolate	Concentration (ng/μL)	A260/280	A260/230	Volume (μL)
1	TA-1	82.0	1.94	2.47	35
2	TA-4	46.6	1.91	2.22	35

high (60–75%), or very high (>75%) according to Picardal et al. (2019).

Data Analysis. Data were subjected to analysis of variance (ANOVA) using a completely randomized design. When significant treatment effects were detected ($P < 0.05$), means were separated using the Least Significant Difference (LSD) test at the 5% significance level. Percentage data were examined for normality and homogeneity of variance prior to analysis and transformed using the arcsine square-root transformation when necessary. Statistical analyses were performed using Smartstat XL.

RESULTS AND DISCUSSION

Rhizoctonia solani Causing Maize Sheath Blight.

Maize plants grown on peatland and naturally infected with sheath blight initially exhibited reddish-brown lesions on the leaf sheath, which gradually enlarged and turned gray as the disease progressed. Under severe infection, plants became wilted and rotted due to disruption of water and nutrient transport.

Morphologically, the isolated pathogen produced rapidly growing white colonies with hyaline hyphae on potato dextrose agar (PDA), which gradually turned yellowish-brown to dark brown with age (Figure 1A). Microscopically, the fungus produced septate hyphae with characteristic right-angle branching and constrictions near the branch point (Figures 1B–C).

The morphological characteristics observed in this study agree with previous descriptions of *R. solani* isolated from maize and other crops (Kumalasari et

al., 2021). The formation of abundant mycelia and sclerotia explains the persistence of the pathogen in peat soils, where high moisture and acidic conditions favor disease development.

Characteristics of Rhizospheric Fungi from *S. lasiocarpum*. Isolation of fungi from the rhizosphere of wild *S. lasiocarpum* growing on peatland yielded six morphologically distinct isolates (Table 2). Based on macroscopic and microscopic observations, the isolates were classified into two genera, *Trichoderma* and *Aspergillus*, following the identification keys of Barnett & Hunter (1998) and Watanabe (2002).

The predominance of *Trichoderma* and *Aspergillus* may be associated with the unique rhizosphere environment of *S. lasiocarpum*. Root exudates, including phenolics, flavonoids, organic acids, and other secondary metabolites, provide nutrients and signaling molecules that regulate microbial diversity and stimulate colonization by beneficial microorganisms while limiting pathogen establishment (Adhi & Suganda, 2020; Bernadip et al., 2014; Soon & Ding, 2021; Ibrahim et al., 2022; Myat & Myint, 2024).

The recovery of *Trichoderma* and *Aspergillus* as the dominant fungal genera is consistent with their ecological characteristics. Both genera are fast-growing saprophytic fungi that compete efficiently for nutrients and space and produce a wide range of extracellular enzymes, antibiotics, and volatile metabolites involved in pathogen suppression. In particular, *Trichoderma* spp. have been widely recognized as effective biological control agents against *R. solani* through

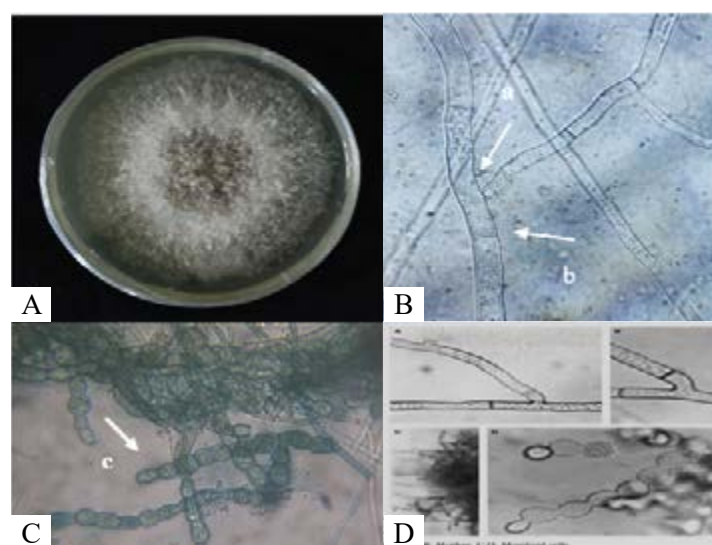


Figure 1. Morphological characteristics of *Rhizoctonia solani* causing maize sheath blight. A. Colony morphology on PDA; B–C. Microscopic characteristics of the isolate; D. diagnostic morphological features of *R. solani* adapted from Watanabe (2002). showing (a). Hyphae branch; (b). Septum; (c). Monilioid cell.

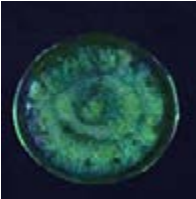
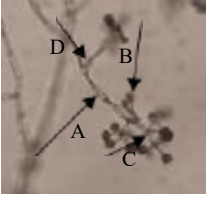
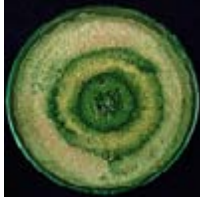
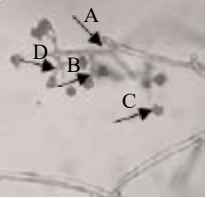
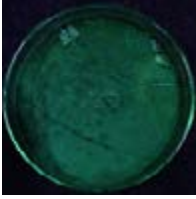
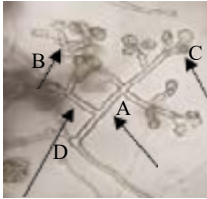
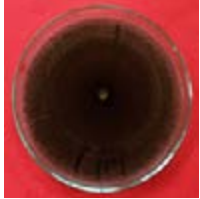
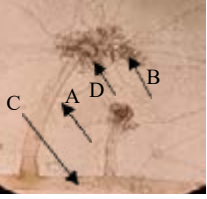
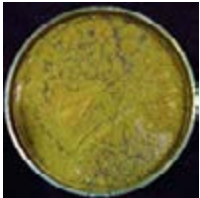
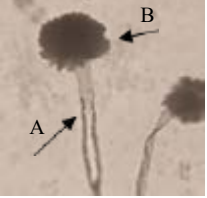
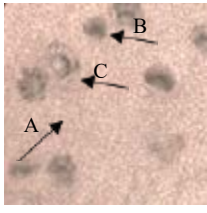
competition, mycoparasitism, and the production of antifungal secondary metabolites (Di et al., 2023).

Previous studies have demonstrated that rhizosphere-derived antagonistic fungi possess considerable potential for controlling plant diseases. For example, *Trichoderma*, *Penicillium*, and *Chaetomium* isolated from the rhizosphere of rubber plants inhibited

Fusarium spp. by up to 51.08%, whereas isolates from onion rhizospheres suppressed *Alternaria porri* by 4.49–59.90% (Adhi & Suganda, 2020).

Antagonistic Activity of Rhizospheric Fungi Against *R. solani*. All six rhizospheric fungal isolates inhibited the radial growth of *R. solani*, with

Table 2. Morphological characteristics of rhizospheric fungal isolates obtained from *Solanum lasiocarpum*

Isolate	Morphological characteristics		Image	
	Macroscopic	Microscopic	Macroscopic	Microscopic
TA-1 (<i>Trichoderma</i>)	Dark green colony; smooth and thick; Rapid and radial growth	A. Branched conidiophores; B. 2–4 phialides per branch; C. Conidia ovoid to cylindrical; D. Conidiophores with complex branching		
TA-2 (<i>Trichoderma</i>)	Circular colony, whitish-green; velvety surface; rapid growth forming concentric rings	A. Straight, verticillately branched conidiophores; B. Short, thick phialides; C. Ovoid conidia; D. Branched conidiophor		
TA-3 (<i>Trichoderma</i>)	Green colony covering the PDA surface; rough, powdery texture	A. Branched conidiophores; B. Short phialides; C. Globose conidia; D. Elongated conidiophores		
TA-4 (<i>Aspergillus</i>)	Circular dark brown to black colony with white marginal mycelia; slightly rough surface with smooth margins	A. Branched conidiophores; B. Globose conidia; C. Septate hyphae; D. Spherical vesicles		
TA-5 (<i>Aspergillus</i>)	Yellowish-green colony; powdery surface; irregular colony expansion	A. Erect conidiophores; B. Globose conidia produced in chains		
TA-6 (<i>Aspergillus</i>)	Brown colony surrounded by white mycelia; cottony surface; irregular radial growth	A. Erect conidiophores; B. Globose conidia produced in chains; C. Hemispherical vesicles		

inhibition percentages exceeding 60% (Figure 2). Although ANOVA indicated no significant differences among isolates ($P > 0.05$), all isolates demonstrated consistently high antagonistic activity. Among them, *Trichoderma* isolate TA-1 (82.8%) and *Aspergillus* isolate TA-4 (81.4%) showed the highest inhibition percentages and were classified as having very high antagonistic activity according to the criteria of Picardal et al. (2019). Similar inhibition levels have also been categorized as highly effective by Syarifah et al. (2024). The inhibitory interaction between *R. solani* and the rhizospheric fungal isolates is presented in the dual culture assay (Figure 3).

The high inhibitory activity observed in the present study agrees with previous reports demonstrating the effectiveness of *Trichoderma* spp. against *R. solani*. Iswati et al. (2024) reported that several *Trichoderma*

species, including *T. asperellum*, *T. brevicompactum*, *T. virens*, *T. dorthopsis*, *T. ghanense*, and *T. reesei*, exhibited strong antagonistic activity toward *R. solani*. Likewise, *T. atroviride* suppressed *Fusarium oxysporum* f. sp. *lycopersici* by 92.11% (Nofal et al., 2021), while *T. harzianum* reduced disease severity caused by *Plasmopara viticola* by 82.9% (Kamble et al., 2021). These findings support the high antagonistic performance of isolate TA-1 observed in this study.

The antagonistic activity of *Trichoderma* is primarily attributed to multiple complementary mechanisms, including rapid competition for nutrients and space, mycoparasitism, and antibiosis (Mukherjee et al., 2022). During mycoparasitism, *Trichoderma* recognizes and coils around the hyphae of *R. solani*, forms appressoria, and secretes extracellular hydrolytic enzymes such as chitinase,

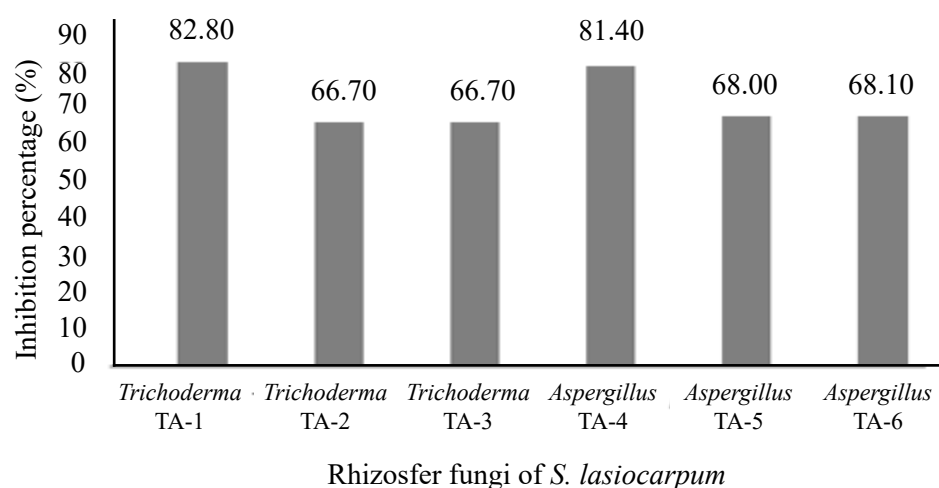


Figure 2. Inhibition (%) of *R. solani* by rhizospheric fungal isolates obtained from wild *S. lasiocarpum* in the dual culture assay.

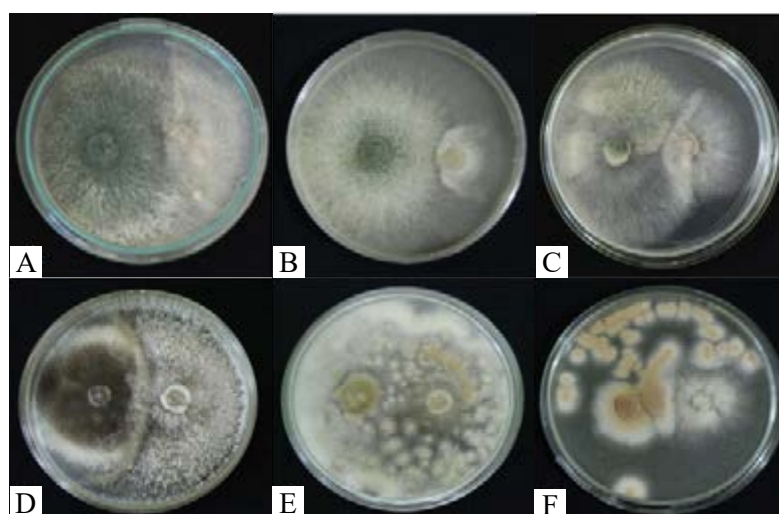


Figure 3. Dual culture assay showing the inhibition of *R. solani* by rhizospheric fungi isolates from wild *S. lasiocarpum*. A. *Trichoderma* isolate TA-1; B. *Trichoderma* isolate TA-2; C. *Trichoderma* isolate TA-3; D. *Aspergillus* isolate TA-4; E. *Aspergillus* isolate TA-5; F. *Aspergillus* isolate TA-6.

β -1,3-glucanase, cellulase, protease, and acid phosphatase that degrade the pathogen cell wall (Muslim, 2019). In addition, *Trichoderma* produces antifungal secondary metabolites, including harzianic acid, peptaibols, 6-pentyl- α -pyrone, and gliovirin, which further inhibit pathogen development (Khairul et al., 2017; Di et al., 2023). Similar antagonistic activity has also been reported for rhizosphere fungi isolated from *S. lasiocarpum*, where *Penicillium* sp., *T. asperellum*, *T. hamatum*, and *T. harzianum* inhibited *C. gloeosporioides* by 66.27–68.53% through competition, antibiosis, and mycoparasitism (Mulyani et al., 2024).

In addition to *Trichoderma*, isolates belonging to the genus *Aspergillus* also showed strong antagonistic activity against *R. solani*. Previous studies reported inhibition values ranging from 61.11% to 90% for different *Aspergillus* species (Matondang & Aini, 2022). The high inhibition recorded for isolate TA-4 (81.4%) is therefore consistent with earlier findings. Several *Aspergillus* species, including *A. versicolor* and *A. aculeatus*, produce extracellular enzymes and antifungal metabolites such as versicolorin B and sterigmatocystin that suppress pathogens including *Colletotrichum*, *Fusarium*, and *Penicillium*. Likewise, *Aspergillus* sp. R3 produces cyclopiazonic acid (CPA), which has been identified as the principal metabolite responsible for inhibiting *R. solani* (Ngo et al., 2021), suggesting that secondary metabolite production may also contribute to the antagonistic activity observed in isolate TA-4.

Macroscopic observations of the dual culture assay together with microscopic examination of the interaction zone (Figure 4) revealed several antagonistic mechanisms. Antibiosis was indicated by the formation of inhibition zones and deformation of pathogen hyphae, whereas competition and mycoparasitism were characterized by hyphal coiling, swelling, bending, lysis, entanglement, and restricted hyphal growth. Similar morphological changes have been widely recognized as indicators of successful fungal antagonism against *R. solani* (Mukherjee et al., 2022; Di et al., 2023). Interestingly, abundant sclerotia were also observed in the interaction zone. Sclerotium formation is considered a survival response of *R. solani* under physiological stress, allowing the pathogen to withstand unfavorable environmental conditions. The occurrence of numerous sclerotia in the presence of antagonistic fungi suggests that the pathogen experienced substantial stress during fungal interaction (Ningsih et al., 2023).

Volatile Organic Compounds (VOCs) Produced by Rhizospheric Fungi. The inhibitory effect of volatile organic compounds (VOCs) produced by rhizospheric fungi against *R. solani* was evaluated using the sealed plate method (Figure 5). Analysis using the LSD test revealed significant differences ($P < 0.05$) among the fungal isolates in their ability to suppress the growth of *R. solani* through volatile-mediated interactions.

The VOC assay demonstrated considerable variation among the isolates (Figure 6). The highest inhibition was recorded for *Aspergillus* isolate TA-5 (83.1%), followed by *Aspergillus* isolate TA-4 (81.9%) and *Trichoderma* isolate TA-1 (70.5%). These results indicate that isolates belonging to the genera *Aspergillus* and *Trichoderma* produce volatile metabolites capable of suppressing pathogen growth even in the absence of direct physical contact. Such findings suggest that antibiosis mediated by VOCs represents an important antagonistic mechanism in addition to competition and mycoparasitism.

The strong VOC-mediated inhibition observed in this study is consistent with previous reports demonstrating that several *Trichoderma* species, including *T. virens*, *T. hamatum*, *T. amazonicum*, and *T. atroviride*, produce volatile metabolites that effectively suppress the growth of *Rigidoporus microporus* (Amaria et al., 2015). Fungal VOCs are generally low-molecular-weight compounds that readily diffuse through air-filled pores in soil and can inhibit pathogen growth without direct hyphal contact (El Jaddaoui et al., 2023). Besides their direct antifungal activity, VOCs may also influence plant physiology by inducing defense responses and altering plant metabolic pathways (Sarkar & Sadhukhan, 2023).

The ability to produce VOCs has been reported in many groups of fungi, particularly soil-borne and mycoparasitic fungi such as *T. asperellum* and *T. harzianum*. These fungi release diverse volatile metabolites, including alcohols, ketones, terpenes, pyrones, and other bioactive compounds that inhibit pathogen development and may induce plant defense-related genes (Razo-Belmán et al., 2023). The high inhibition exhibited by isolates TA-4 and TA-5 suggests that similar volatile metabolites may contribute to the suppression of *R. solani*, although the specific chemical composition of these VOCs remains to be determined.

Volatile metabolites are known to inhibit fungal pathogens through multiple mechanisms, including disruption of cell membrane integrity, interference with energy metabolism, inhibition of hyphal elongation, and suppression of sclerotial development. Some VOCs also induce oxidative stress by increasing

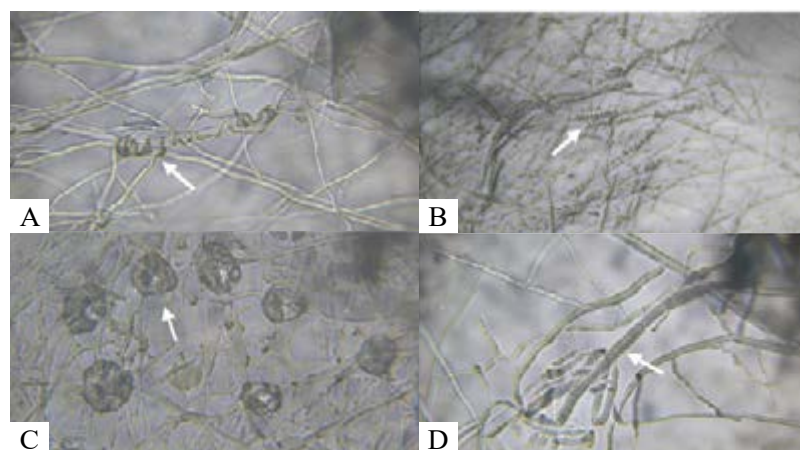


Figure 4. Microscopic observations of antagonistic interactions between rhizospheric fungi and *R. solani*. A. Hyphal coiling; B. Hyphal thinning and lysis; C. Sclerotial formation by *R. solani*; D. Hyphal swelling (400× magnification).

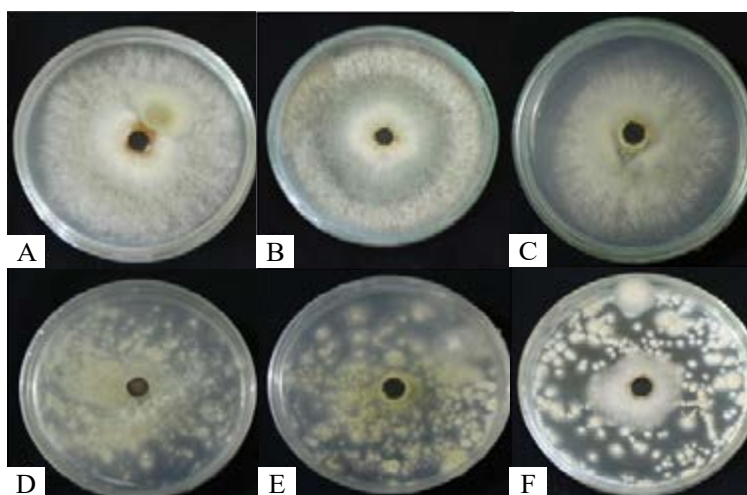


Figure 5. Sealed plate assay used to evaluate the inhibitory effect of volatile organic compounds (VOCs) produced by rhizospheric fungal isolates from wild *S. lasiocarpum* against *R. solani* (top view)

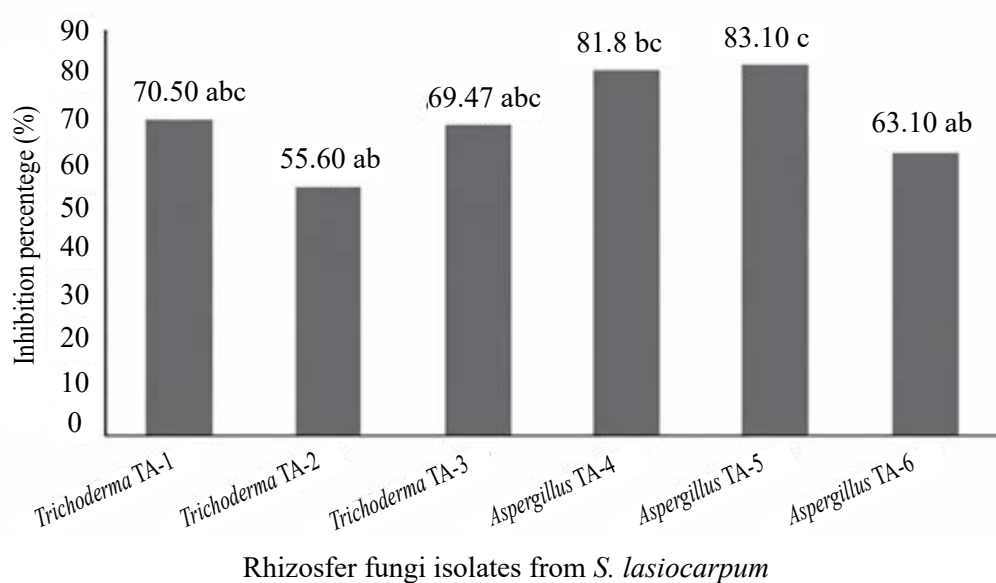


Figure 6. Percentage inhibition of *R. solani* by volatile organic compounds (VOCs) produced by rhizospheric fungal isolates from wild *S. lasiocarpum*.

intracellular reactive oxygen species (ROS), leading to cellular damage and impaired fungal growth (Rajani et al., 2021). Therefore, characterization of the VOC profiles produced by the most effective isolates would be an important next step toward understanding their mode of action and evaluating their potential as biological control agents for maize sheath blight.

Molecular Identification of Selected Isolates. To confirm the taxonomic identity of the most effective antagonistic isolates, molecular identification was performed using ITS rDNA sequencing. Amplification of the ITS region with the universal primers ITS1 and ITS4 produced single DNA fragments of approximately 550–600 bp for isolates TA-1 and TA-4, indicating successful amplification and suitable DNA quality for sequencing (Figure 7). The amplified PCR products were subsequently subjected to bidirectional Sanger sequencing.

BLAST analysis of the ITS sequences revealed that isolate TA-1 shared 100% sequence similarity with *T. asperellum*, whereas isolate TA-4 showed 100% sequence similarity with *Aspergillus welwitschiae* (Table 3). Phylogenetic analyses based on ITS sequences (approximately 576 bp for *T. asperellum* and 574 bp for *A. welwitschiae*) further confirmed the taxonomic placement of both isolates by clustering them with reference sequences retrieved from the ten closest BLAST matches in the NCBI GenBank database, excluding uncultured fungi (Figures 8 and 9). According to Simanjuntak & Safni (2025), ITS sequence similarities of $\geq 97\%$ are generally considered sufficient for reliable species-level identification of fungi. These molecular data were consistent with the morphological identification, confirming that the two most effective antagonistic isolates belonged to the

genera *Trichoderma* and *Aspergillus*.

The molecular identification of isolate TA-1 as *T. asperellum* is consistent with its strong antagonistic activity observed in the dual culture assay. *Trichoderma asperellum* is one of the most extensively studied fungal biological control agents and has demonstrated high efficacy against numerous soil-borne pathogens. Previous studies reported that *T. asperellum* effectively suppressed Fusarium wilt and anthracnose in chili pepper, reduced disease severity caused by *R. solani*, and controlled cocoa pod rot caused by *Phytophthora palmivora* (Herrera et al., 2020; Mirsam et al., 2023). Its antagonistic activity is associated with rapid rhizosphere colonization, competition for nutrients and space, secretion of cell wall-degrading enzymes such as chitinase and β -1,3-glucanase, and production of antifungal secondary metabolites (Jati et al., 2023). Similar biological control potential has also been reported for other *Trichoderma* species, including *T. atroviride*, *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. virens*, and *T. viride* (Kubiak et al., 2023). More recently, three strains of *T. asperellum* exhibited antagonistic efficiencies of 86.9–87.6% against *Neurospora* spp., further demonstrating the broad-spectrum antagonistic capability of this species (Khuong et al., 2025). These findings support the strong inhibitory activity of isolate TA-1 against *R. solani* observed in the present study.

Interestingly, isolate TA-4 was identified as *A. welwitschiae*, a species known to exhibit diverse ecological roles. Although *A. welwitschiae* has been reported as an opportunistic pathogen associated with bole rot of sisal and postharvest diseases in several crops (Duarte et al., 2018; Ying et al., 2019), recent studies have demonstrated that certain isolates produce biologically active secondary metabolites

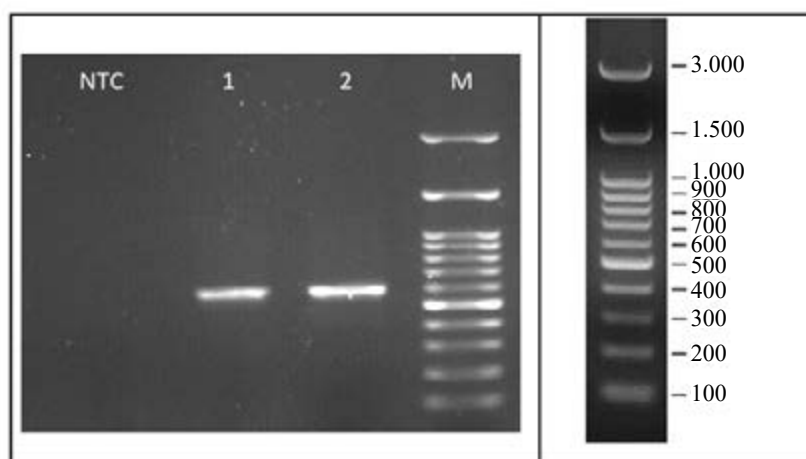


Figure 7. Agarose gel electrophoresis of ITS PCR amplification products from *Trichoderma* isolate TA-1 (lane 1) and *Aspergillus* isolate TA-4 (lane 2). NTC = No-template control; M = 100 bp DNA ladder.

Table 3. Molecular identification of selected rhizospheric fungal isolates based on NCBI BLAST analysis

Isolate	Results																																																																													
<i>Trichoderma</i> isolate TA-1	<table><tr><th>Description</th><th>Max Score</th><th>Total Score</th><th>Query Cover</th><th>E value</th><th>Per Ident</th><th>Accession</th></tr><tr><td><i>Trichoderma asperellum</i> strain IMCC2012 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1064</td><td>1064</td><td>100%</td><td>0.0</td><td>100.00%</td><td>PP476263.1</td></tr><tr><td><i>Trichoderma asperellum</i> isolate TR21 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1064</td><td>1064</td><td>100%</td><td>0.0</td><td>100.00%</td><td>MH620815.1</td></tr><tr><td><i>Trichoderma asperellum</i> isolate TR10 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1064</td><td>1064</td><td>100%</td><td>0.0</td><td>100.00%</td><td>MH620809.1</td></tr><tr><td><i>Trichoderma yunnanense</i> isolate SST3 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1064</td><td>1064</td><td>100%</td><td>0.0</td><td>100.00%</td><td>Q0264430.1</td></tr><tr><td><i>Trichoderma asperellum</i> unilaPO genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence</td><td>1064</td><td>1064</td><td>100%</td><td>0.0</td><td>100.00%</td><td>LC655157.1</td></tr><tr><td><i>Trichoderma yunnanense</i> isolate SST34 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1064</td><td>1064</td><td>100%</td><td>0.0</td><td>100.00%</td><td>Q0264440.1</td></tr><tr><td><i>Trichoderma asperellum</i> isolate Ting Ho 1 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1064</td><td>1064</td><td>100%</td><td>0.0</td><td>100.00%</td><td>Q0091256.1</td></tr><tr><td><i>Trichoderma asperellum</i> strain DF256 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1064</td><td>1064</td><td>100%</td><td>0.0</td><td>100.00%</td><td>Q0617926.1</td></tr><tr><td><i>Trichoderma</i> sp. strain MWU14-9201 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1064</td><td>1064</td><td>100%</td><td>0.0</td><td>100.00%</td><td>MT150399.1</td></tr><tr><td><i>Trichoderma asperellum</i> isolate WT10 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1064</td><td>1064</td><td>100%</td><td>0.0</td><td>100.00%</td><td>OL597343.1</td></tr></table>	Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession	<i>Trichoderma asperellum</i> strain IMCC2012 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1064	1064	100%	0.0	100.00%	PP476263.1	<i>Trichoderma asperellum</i> isolate TR21 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1064	1064	100%	0.0	100.00%	MH620815.1	<i>Trichoderma asperellum</i> isolate TR10 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1064	1064	100%	0.0	100.00%	MH620809.1	<i>Trichoderma yunnanense</i> isolate SST3 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1064	1064	100%	0.0	100.00%	Q0264430.1	<i>Trichoderma asperellum</i> unilaPO genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	1064	1064	100%	0.0	100.00%	LC655157.1	<i>Trichoderma yunnanense</i> isolate SST34 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1064	1064	100%	0.0	100.00%	Q0264440.1	<i>Trichoderma asperellum</i> isolate Ting Ho 1 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1064	1064	100%	0.0	100.00%	Q0091256.1	<i>Trichoderma asperellum</i> strain DF256 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1064	1064	100%	0.0	100.00%	Q0617926.1	<i>Trichoderma</i> sp. strain MWU14-9201 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1064	1064	100%	0.0	100.00%	MT150399.1	<i>Trichoderma asperellum</i> isolate WT10 small subunit ribosomal RNA gene, partial sequence, internal transcribed 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<i>Trichoderma asperellum</i>																																																																														

<i>Aspergillus</i> isolate TA-4	<table><tr><th>Description</th><th>Max Score</th><th>Total Score</th><th>Query Cover</th><th>E value</th><th>Per Ident</th><th>Accession</th></tr><tr><td><i>Aspergillus welwitschiae</i> strain TM3 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1061</td><td>1061</td><td>100%</td><td>0.0</td><td>100.00%</td><td>PP465553.1</td></tr><tr><td><i>Aspergillus niger</i> strain D95_83 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1055</td><td>1055</td><td>100%</td><td>0.0</td><td>99.83%</td><td>Q0509877.1</td></tr><tr><td><i>Aspergillus</i> sp. isolate A11 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1056</td><td>1056</td><td>100%</td><td>0.0</td><td>99.83%</td><td>MN266668.1</td></tr><tr><td><i>Aspergillus welwitschiae</i> strain CMV5845, 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1055</td><td>1055</td><td>100%</td><td>0.0</td><td>99.83%</td><td>MK450651.1</td></tr><tr><td><i>Aspergillus foetidus</i> isolate DM2 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1055</td><td>1055</td><td>100%</td><td>0.0</td><td>99.83%</td><td>MK311057.1</td></tr><tr><td><i>Aspergillus niger</i> isolate M2C-10 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1055</td><td>1055</td><td>100%</td><td>0.0</td><td>99.83%</td><td>MN050571.1</td></tr><tr><td><i>Aspergillus niger</i> isolate N-501 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1055</td><td>1055</td><td>100%</td><td>0.0</td><td>99.83%</td><td>MK311057.1</td></tr><tr><td><i>Aspergillus niger</i> isolate M234 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1055</td><td>1055</td><td>100%</td><td>0.0</td><td>99.83%</td><td>MT132219.1</td></tr><tr><td><i>Aspergillus niger</i> isolate IMCC NMTP01 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1055</td><td>1055</td><td>100%</td><td>0.0</td><td>99.83%</td><td>MN050457.1</td></tr><tr><td><i>Aspergillus niger</i> strain BOPCST9 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2</td><td>1055</td><td>1055</td><td>100%</td><td>0.0</td><td>99.83%</td><td>MT132219.1</td></tr></table>	Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession	<i>Aspergillus welwitschiae</i> strain TM3 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1061	1061	100%	0.0	100.00%	PP465553.1	<i>Aspergillus niger</i> strain D95_83 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1055	1055	100%	0.0	99.83%	Q0509877.1	<i>Aspergillus</i> sp. isolate A11 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1056	1056	100%	0.0	99.83%	MN266668.1	<i>Aspergillus welwitschiae</i> strain CMV5845, 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1055	1055	100%	0.0	99.83%	MK450651.1	<i>Aspergillus foetidus</i> isolate DM2 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1055	1055	100%	0.0	99.83%	MK311057.1	<i>Aspergillus niger</i> isolate M2C-10 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1055	1055	100%	0.0	99.83%	MN050571.1	<i>Aspergillus niger</i> isolate N-501 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1055	1055	100%	0.0	99.83%	MK311057.1	<i>Aspergillus niger</i> isolate M234 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2	1055	1055	100%	0.0	99.83%	MT132219.1	<i>Aspergillus niger</i> isolate IMCC NMTP01 small subunit ribosomal RNA gene, 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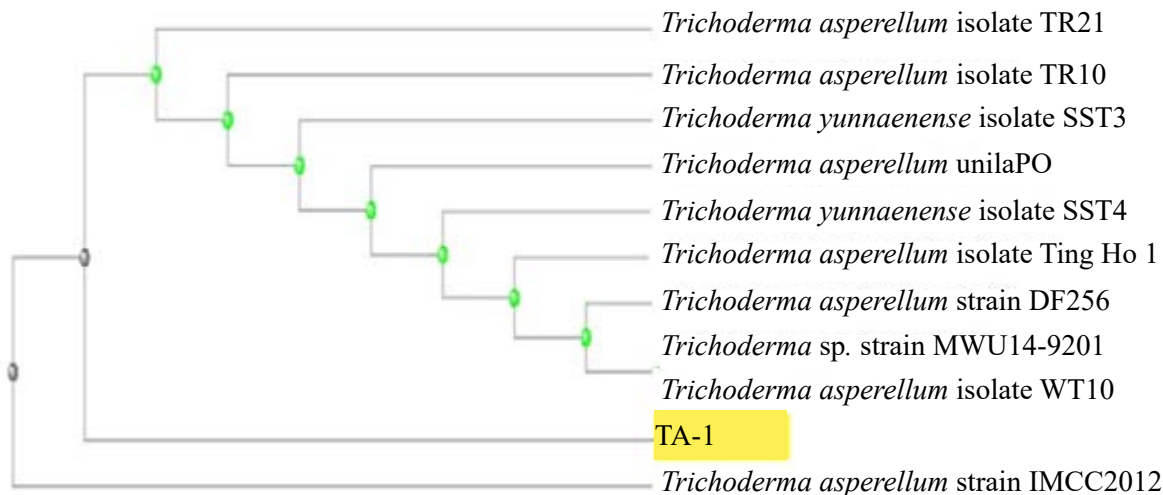


Figure 8. Phylogenetic tree based on ITS rDNA sequences showing the relationship of rhizosphere isolate TA-1 from *S. lasiocarpum* with reference *Trichoderma* species retrieved from the NCBI GenBank database. The tree was constructed using the Neighbor-Joining method with 1000 bootstrap replicates.

with potential agricultural applications. For example, *A. welwitschiae* produces $\alpha\beta$ -dehydrocurvularin, a metabolite with nematicidal activity, suggesting its potential as a source of bioactive compounds for sustainable crop protection (Ngo et al., 2021; Li et al., 2023). In addition, endophytic isolates of *A. welwitschiae* have been reported to promote plant growth and enhance antioxidant defense responses under heavy-metal stress (Husna et al., 2021). These findings indicate that the ecological function of *A. welwitschiae* is isolate-dependent and influenced by host association and environmental conditions.

Beyond its role in plant-associated environments, *A. welwitschiae* has also been reported to produce industrially important enzymes, including inulinases involved in the bioconversion of inulin-rich substrates, highlighting its broad metabolic versatility (de Carvalho Cardoso et al., 2025).

The high antagonistic activity of isolate TA-4 against *R. solani* suggests that this isolate may produce antifungal metabolites that contribute to pathogen suppression. However, because *A. welwitschiae* has also been reported as an opportunistic plant pathogen and certain strains are capable of producing mycotoxins,

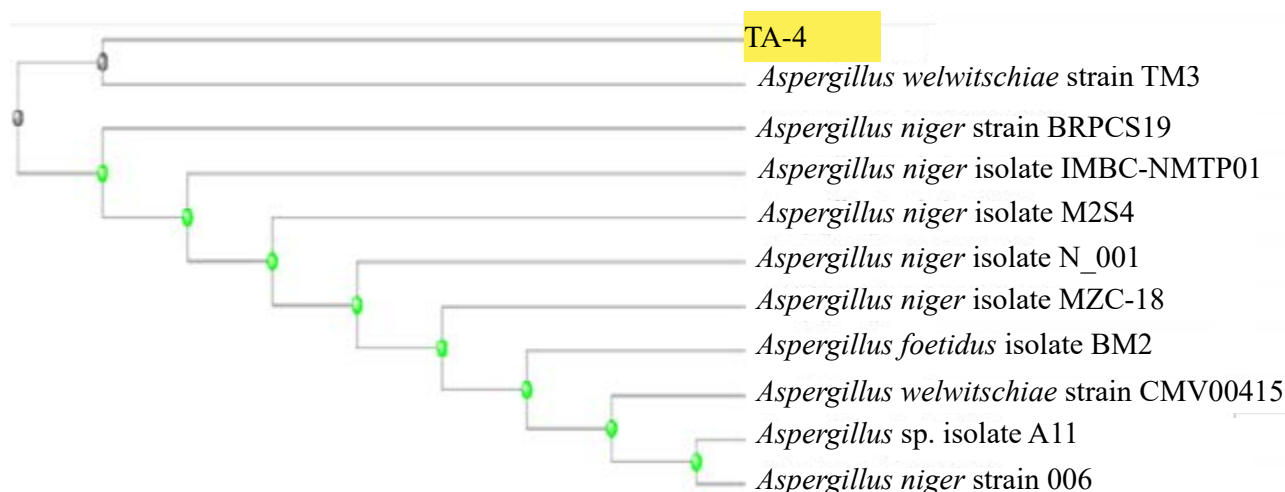


Figure 9. Phylogenetic tree based on ITS rDNA sequences showing the relationship of rhizosphere isolate TA-4 from *S. lasiocarpum* with reference *Aspergillus* species retrieved from the NCBI GenBank database. The tree was constructed using the Neighbor-Joining method with 1000 bootstrap replicates.

its application as a biological control agent requires careful evaluation. Further studies should investigate the pathogenicity, toxigenicity, and environmental safety of isolate TA-4 before its practical use in greenhouse or field conditions. Nevertheless, its strong in vitro antagonistic activity highlights the potential of peatland-derived *A. welwitschiae* as a valuable source of antifungal metabolites.

Taken together, the dual culture assay, VOC assay, and molecular identification demonstrated that indigenous fungi isolated from the rhizosphere of *S. lasiocarpum* possess considerable potential as biological control agents against *R. solani*. Among the tested isolates, *T. asperellum* TA-1 represents the most promising candidate because of its consistently high antagonistic activity and well-established safety profile, whereas *A. welwitschiae* TA-4 warrants further biosafety evaluation before field application.

CONCLUSION

The results of this study demonstrates that rhizosphere fungi isolated from *Solanum lasiocarpum* have considerable potential as indigenous biocontrol agents against *Rhizoctonia solani*, the causal agent of maize sheath blight. All six fungal isolates exhibited antagonistic activity in vitro, with *Trichoderma* isolate TA-1 and *Aspergillus* isolate TA-4 showing the highest inhibition of pathogen growth. Molecular identification based on ITS rDNA sequencing and BLAST analysis confirmed that isolate TA-1 was closely related to *Trichoderma asperellum* (576 bp), whereas isolate TA-4 was identified as *Aspergillus welwitschiae* (574 bp),

both sharing 100% sequence similarity with reference sequences in GenBank. These findings demonstrate that the rhizosphere of *S. lasiocarpum* represents a promising source of locally adapted fungal antagonists for sustainable management of maize sheath blight in peatland ecosystems. However, because this study was limited to in vitro evaluation, further research under greenhouse and field conditions is required to verify their efficacy, environmental safety, and consistency under natural conditions. Future studies should also investigate formulation development, mechanisms of antagonism, compatibility with integrated disease management practices, and long-term effects on soil health and maize productivity.

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AUTHORS' CONTRIBUTIONS

YAN conceived and supervised the study and designed the research methodology. RBM and YAN contributed to the study design, data interpretation, and manuscript preparation. R and PL collected field samples and performed laboratory work, including fungal isolation, purification, and dual culture assays. YAN conducted the molecular analyses. RBM, YAN, AAD, and KVA interpreted the results and drafted the manuscript. All authors reviewed, revised, and approved the final manuscript.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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