POTENTIAL OF ENDOPHYTIC FUNGI ISOLATED FROM MAIZE AS BIOLOGICAL AGENTS FOR SHEATH BLIGHT DISEASE ON MAIZE

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Abstract. Sheath blight caused by *Rhizoctonia solani* is an important constraint to sustainable maize production, and environmentally friendly control strategies are increasingly needed. This study aimed to isolate and characterize endophytic fungi from maize tissues and evaluate their potential as biological control agents against *R. solani*. Endophytic fungi were isolated from healthy maize plants collected from sheath blight–affected fields in Pudak Village, Jambi, Indonesia. Morphological characterization, pathogenicity, dual culture antagonism, volatile compound, chitinolytic, phosphate-solubilizing, and plant growth–promoting assays were conducted to evaluate their biocontrol potential. A total of 46 fungal isolates were successfully obtained from roots, stems, leaves, and fruits, with a high diversity index (H' = 3.66). Pathogenicity screening selected 18 non-pathogenic endophytic isolates for further evaluation. In dual culture assays, 17 of 18 isolates inhibited the growth of *R. solani*, with the highest inhibition recorded in sterile hyphae 3 (93.33%). Volatile assays showed that 11 isolates suppressed pathogen growth, with sterile hyphae 2 exhibiting the highest inhibition (68.78%). Chitinolytic activity was detected only in unidentified fungi 4, while none of the isolates showed phosphate-solubilizing activity. Several isolates also promoted maize seedling growth. These findings demonstrate that maize-associated endophytic fungi possess strong antagonistic activity against *R. solani* and have potential for development as environmentally friendly biological control agents in sustainable maize production systems.

Keywords: corn; fungi; induce resistance; parasitism; *Rhizoctonia solani*; *Zea mays*.

INTRODUCTION

Indonesia seeks maize self-sufficiency through increased sustainable production, but faces a major hurdle in the form of sheath blight disease, caused by *Rhizoctonia solani* (*R. solani*). This fungus can lead to yield reductions of up to 100%, jeopardizing the country's efforts for food security [38, 43, 49].

R. solani, a sterile hyphal fungus, survives in the soil through the formation of sclerotia-dense structures containing mycelia. These serve as defensive structures in soil and infected plant debris, germinating and developing under favorable conditions. Spread occurs through water, irrigation, contaminated soil, and plant residues, with optimal growth conditions requiring a relative humidity above 80% and a temperature range of 15°C to 35°C. This polyphagous fungus has a broad host range, infecting plants from the graminae, leguminoceae, Solanaceae, and Cucurbitaceae families [27, 30].

This fungus causes damping off in shoots, stem base rot, and sheath blight, with attacks occurring in nursery and field phases. Damping off disease can manifest as pre-emergence (rotting seeds) or post-emergence (wilting due to stem base rot). It thrives in humid conditions, reaching 80% intensity in the rainy season and 20–30% in the dry season. Additionally, *R. solani* is responsible for sheath blight, a prevalent disease in corn and sorghum plants [5, 6, 12, 13].

Symptoms of *R. solani* infection in maize include lesions and blight on leaf sheaths, followed by grayish discoloration of infected leaves. The spots will expand followed by the formation of white sclerotium, and become brown or blackish brown. Furthermore, the plant wilts and rot occur because the transportation of nutrients and water in the plant tissue is hampered. *R. solani* attacks can damage corn and sorghum plantings resulting in large yield losses. The spread of this disease is very widespread in almost all of Asia and several countries in the world. In the United States, it was reported that sheath blight disease caused a reduction in production of up to 50%. The introduced maize variety from CYMMYT is a variety that is susceptible to sheath blight disease, this disease attack causes disease intensity of up to 100%, even the ears of corn that are attacked rot [2, 27, 39].

Disease management is generally carried out using conventional methods, namely the use of synthetic fungicides. This technique is very effective to apply, easy and efficient. However, along with increasing public knowledge and awareness of maintaining environmental balance, there is a lot of demand for organic agricultural products that are free from synthetic chemicals. The application of disease control using synthetic pesticides is starting to be reduced, some of the side effects are not on target in the sense of killing microorganisms that do not cause disease, the emergence of new pathogens, phytotoxicity and disrupting the water system [10, 20, 41].

The use of non-pathogenic microorganisms known as biological agents is currently being developed as an environmentally friendly and sustainable control alternative. Several biological agents that have been widely used to control *R. solani* in corn plants include the application of the bacteria *Bacillus* sp., *Pseudomonas fluorescens* bacteria, *Trichoderma* sp. fungi, endophytic bacteria, endophytic fungi [9, 12, 32, 49].

Endophytic fungi are able to penetrate the endoderm through the root cortex into the vascular tissue and then develop as endophytic fungi in stems, leaves, tubers, roots, fruit and other plant organs. The application of endophytic fungi as biological agents has many advantages compared to other fungi, for example rhizosphere fungi. This fungus lives and survives in plant tissue so that it can better withstand extreme

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conditions, and is able to increase plant defence through induce resistance. *Trichoderma* sp., *Fusarium* sp., and *Penicillium* sp. are examples of endophytic fungi which are reported to be capable of acting as biological control agents for soil-borne and air-borne diseases. Endophytic fungi can control plant diseases through several mechanisms, including competition, hyper parasitism, producing microbial inhibitor compounds (antibiotics, lysis enzymes, other physical or chemical disturbances), increasing plant resistance, and encouraging plant growth [11, 17, 19, 36, 37, 45, 46].

Based on the description above, it can be concluded that the use of endophytic fungi isolated from corn, both highland and lowland, has the potential as an alternative control of sheath blight caused by *R. solani*. This study aimed to evaluate the potential of maize endophytic fungi as biological control agents against sheath blight disease caused by *Rhizoctonia solani* through antagonistic, volatile-mediated, chitinolytic, phosphate-solubilizing, and plant growth-promoting activities.

MATERIALS AND METHOD

Isolation of Endophytic Fungi

Isolation of endophytic fungi using healthy corn plants, which were collected from corn fields in Pudak Village, Kumpeh Ulu District, Muaro Jambi Regency, Jambi Province, Indonesia. Samples of healthy plants were in corn planting areas that were attacked by sheath blight. The corn plant materials used were the roots, stems, leaves and fruit. Corn plants were cleaned first with running water before use. Plant parts were cut to a size of ± 1 x 1 cm, then separated and grouped according to the roots, stems, fruit and leaves [7].

Endophytic fungi were isolated by cutting the roots and leaves of the plant and then surface sterilizing using 70% alcohol for 30 seconds, then 1% NaClO for 1 minute. Cut stems and fruit using 70% alcohol for 1 minute, 1% NaClO for 2 minutes, then rinsed with sterile distilled water 3 times and then dried on sterile tissue. Samples were placed on PDA media and incubated for 7 days [45, 46, 48].

The final sterile distilled water used for rinsing plant parts was isolated into PDA media, then incubated for 3-5 days and the growth of other fungi was observed as a control. If fungi grow on the control medium, then the plant parts isolated in Petri dishes cannot be used, because the surface of the plant sample contains endophytic fungi [16, 45, 52, 54].

After passing the sterility test, the mycelium growing from plant tissue segments was observed daily. Emerging fungal colonies were purified to obtain pure cultures of candidate endophytic fungi. Identification of fungal isolates was conducted based on macroscopic and microscopic morphological characteristics using the identification keys of Barnett and Hunter [4] and Watanabe [57]. Molecular identification was not performed in the present study due to limited sequencing facilities. Therefore, several isolates were retained as sterile hyphae or unidentified fungi. Further molecular characterization using ITS rDNA sequencing is recommended, particularly for isolates showing strong antagonistic activity against *Rhizoctonia solani*.

Isolation of the fungus *Rhizoctonia solani*

The fungus *R. solani* was isolated from leaves and leaf sheaths of maize plants showing symptoms of sheath blight disease. Corn leaves were cleaned before use. Leaves were cut to size 1 x 1 cm, then sterilized with 2% NaClO solution for 10 seconds. Then, the leaves were rinsed with sterile distilled water three times and isolated on PDA media. The growing mycelium was purified again on new PDA media [6, 27, 33]. Identification of pathogenic fungi using a microscope and adjusted according to Barnett and Hunter [4]. The fungi that have been identified were multiplied and an inoculum stock was made for use in research.

Diversity index

The diversity index is analyzing the number of individuals in a community. The diversity index value of growing fungi was calculated using the Shannon-Wiener diversity index [16, 28]. The index formula is as follows: $\sum_{i=1}^{n} Pi\ ln\ P\ i$

where: Pi is the proportion of the abundance of the i-th species in the total abundance, and N is the total abundance. The criteria for the diversity index value of a fungus are as follows: H' ≤ 1: low diversity, 1 < H'< 3: medium diversity, dan H'≥ 3: high diversity.

Endophytic fungi pathogenicity test

Tests were carried out using corn seeds grown in Petri containing endophytic fungal cultures in PDA media. Prior to use, the seeds are sterilized using 70% alcohol for 30 seconds and 1% NaClO for 1 minute, then rinsed using sterile distilled water three times and dried on sterile tissue. Petri dishes containing pure culture colonies of endophytic fungi were planted with 10 corn seeds and repeated 3 times then incubated for 5-7 days at room temperature [48].

The parameters observed were the ability of the seeds to germinate and the necrotic symptoms seen in the germinated seeds. If the seeds are unable to germinate, the fungus isolate is pathogenic and cannot be used as an antagonistic agent. Seeds that can germinate were transferred into polybags containing sterile soil, then observed for symptoms of disease that arise due to inoculation of endophytic fungi.

Testing the inhibitory power of endophytic fungi against *Rhizoctonia solani in vitro*

Each endophytic fungus isolate obtained was tested for its inhibitory ability against *R. solani* using the multiple test method [23], *R. solani* fungi on 7 day old PDA media were cut with a cork borer (diameter 5 mm) and taken using an ose needle, then placed on the test PDA media at a distance of 3 cm from the edge of the Petri dish. The pieces of endophytic fungus (diameter 5 mm) were placed in the same petri dish, at a distance of 3 cm from the pathogenic fungus (Fig. 1). Each

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treatment consists of 3 (three) replications. As a control, *R. solani* was grown on PDA media without endophytic fungi. Each Petri was labeled and incubated at room temperature for 7 days. Qualitative observations were conducted on the 7th day after isolation.

Observations were made on day 7 by measuring the mycelium radius of pathogenic fungi compared to controls. The percentage of inhibitory power can be calculated using the following formula [20]:

$$P(\%) = \frac{R - r}{R} \times 100$$

where, P is percentage of inhibition, r is the radius of of the fungus colony *R. solani* which was inoculated with antagonistic fungi and R is the maximum radius of of the fungus colony *R. solani* (control).

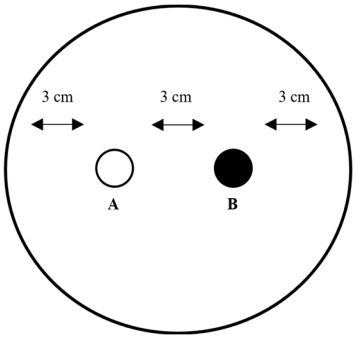

Figure 1. Scheme of inhibitory test of endophytic fungus isolates against *R. Solani*: (A) *R. solani* isolate; (B) Antagonist fungus isolate.

Phosphate Solvent Test

Phosphate dissolution testing was carried out using the fungus culture method grown on PDA medium for 7-10 days at a temperature of 25°C. The test was carried out to see the phosphate dissolving activity on Pikovskaya agar (PKV) media (5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 \times 7H_2O$, 0.2 g NaCl, 0.2 g KCl, 0.003 g $FeSO_4 \times 7H_2O$, 0.003 g $MnSO_4.H_2O$, 10.0 g glucose, 0.5 g yeast extract, 15.0 g agar in 1 L distilled water. After inoculation, the fungus culture was allowed to grow for 10 days at temperature room with three replications, observations were made in the clear zone around the fungus culture colony. Moreover, a phosphate-solubilizing fungal isolate was included as a positive control to verify the suitability of the assay conditions, and then observation data was presented descriptively.

Chitinolytic test

Chitinolytic test is a test to see the chitinolytic activity of endophytic fungi where it was carried out on colloidal chitin media. The composition of colloidal chitin media was colloidal chitin 5% (40 mL), $MgSO_4$ 0.15 g; $(NH_4)SO_4$ 1.5 g; KH_2PO_4 1 g; citric acid 0.5 g; NA 1.6 g Bromo cresol purple 0.15 g; distilled water 80 ml agar 7 g.

Endophytic fungus cultures that grow on PDA media aged 10 days were collected using a cork borer (diameter 3 mm) and then grown on colloidal chitin media at room temperature for 10 days. If there is a clear zone around the endophytic fungus colony, this indicates that the fungus has chitinolytic activity [1].

Volatile test

The volatile test was carried out using the steam method, each pathogenic fungus and endophytic fungus were cultured on PDA media, the culture was taken using a cork borer (d=3 mm) and isolated in the middle of a Petri dish (Fig. 2). Then the two petris are glued together. The growth of pathogenic fungi will be hampered due to the volatile compounds released by endophytic fungi. Observations were made on the 10th day by measuring the diameter of the fungus and comparing it with the control [24, 54].

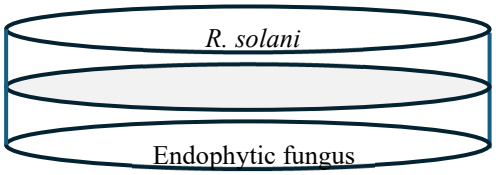

Figure 2. The volatile test of endophytic fungus and *R.solani*

The percentage of inhibition was calculated using the formula:

$$Pi = \frac{dk - dp}{dk}$$

where: Pi is Percentage of inhibition (%), dk is diameter of *R. solani* at control treatment, and dp is diameter *R. solani* at antagonistic fungi treatment.

Ability test of endophytic fungi as plant growth promoters

Testing the ability of endophytic fungi to stimulate plant growth was determined from the response of seeds after being treated with endophytic fungi. Growth-promoting endophytic fungi are fungi that have the ability to increase plant height and root length and result in plants being able to absorb nutrients well and have greater vigor. Isolates that have the potential to stimulate the growth of chili seedlings are then identified with the help of the Watanabe identification key [50].

Experimental design and statistical analysis

All experiments were arranged in a completely randomized design (CRD). Each treatment consisted of three replications. Data obtained from volatile assays and plant growth–promoting assays were subjected to analysis of variance (ANOVA). When significant differences were detected, mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$. Statistical analyses were conducted using SPSS software.

RESULTS

Isolation of endophytic fungi in maize plants

Exploration results showed that as many as 46 isolates of candidate endophytic fungi were successfully isolated from the roots, stems, leaves and fruit of corn

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plants. Candidate endophytic fungi were then selected until 18 endophytic fungi were obtained which were used for further tests.

The abundance of fungus isolates that were successfully isolated based on the Shannon-Wieber index was 3.66, which means that the diversity of isolates that were isolated was relatively high, namely H'>3, with a relative density for each isolated tissue, namely roots 2.78, stems 2.45 leaves 1.75 and fruit 1.19 (Table 1).

Table 1. Exploration results and diversity index value of candidate isolate of endophytic fungi from maize plant tissue

Plant tissue	Total	Diversity index value
Root	19	2.78
Stem	11	2.45
Leaf	7	1.75
Fruit	9	1.19

Table 1 indicated that all plant tissues were infected with endophytic fungi. Endophyte diversity was influenced by corn genotype, agroecosystem conditions, and cultivation practices, including pesticide use and soil type [39, 43]. Potshangbam *et al*. [39] reported that the Shannon-Wiener Diversity Index in corn showed the highest diversity in leaves, although its distribution was even throughout the plant. Endophytic fungi isolated from rice and corn plants were generally not tissue- or host-specific, thus these isolates have the potential to be widely applied to various host plants.

Other studies have shown the dominance of the Ascomycota phylum in corn seeds, including *Fusarium*, *Cladosporium*, *Penicillium*, and *Alternaria* [51]. Gao *et al*. [12] reported that endophyte diversity increased during germination, with young roots showing a higher number of isolates than seeds, with Fusarium and *Penicillium* predominating. These changes indicate that endophyte colonization is dynamic during the early stages of plant growth.

Fan & Shi [10] stated that corn has a moderate level of endophyte diversity, dominated by *Aspergillus*, *Cladosporium*, and *Penicillium*. However, corn endophytic isolates tend to produce unique secondary metabolites with antimicrobial potential. This finding aligns with a report by Manganyi [31] which demonstrated high diversity in corn roots and stems from various ecosystems in South Africa, as well as a 20–35% increase in plant growth after inoculation with certain isolates.

Candidate isolates of endophytic fungi were then tested for pathogenicity on corn seeds, seeds that germinated normally were considered to be endophytic fungi, because they did not cause disease in plants (Fig. 3A).

A total of 46 candidate isolates of endophytic fungi were selected using a pathogenicity test on corn with 3 replications. The results indicated that 34 isolates were endophytic fungi and 12 isolates were pathogens. Observations were made on seeds that were able to germinate normally and did not show symptoms of necrosis, which were endophytic fungi. The test was continued by planting seeds in the planting medium until 18 endophytic fungi were obtained which showed normal plant growth and showed no symptoms of disease. Then identification was carried out using a light microscope at a magnification of 10x40 (Table 2).

Inhibitory power and volatile test of candidate endophytic fungi against *Rhizoctonia solani in vitro*

The inhibitory activity of endophytic fungi against *R. solani* varied among isolates. The results revealed that 17 candidate isolates of endophytic fungi were able to inhibit the growth of the fungus *R. solani* out of a total of 18 fungi tested in the dual culture assay (Fig. 4). Sterile hyphae 3 showed the highest inhibitory capacity (93.33%) in the antagonist test.

Furthermore, descriptive tests that were carried out on endophytic fungi showed that only one fungus had chitinolytic capabilities, namely unidentified fungi 4. Meanwhile, testing on phosphate solvent for all fungi did not show a clear zone around the fungus colony which mean no fungi were found that had phosphatase capabilities (Table 2, Fig. 3B). The formation of a clear halo zone by the positive control confirmed that the assay conditions were suitable for detecting phosphate-solubilizing activity.

Volatile compounds produced by 11 of 18 endophytic fungal isolates significantly inhibited the growth of *Rhizoctonia solani*, with inhibition values ranging from 30.56% to 68.78%. The highest inhibition percentage was recorded in sterile hyphae 2 (68.78%) based on DMRT analysis at $p \leq 0.05$ (Table 3).

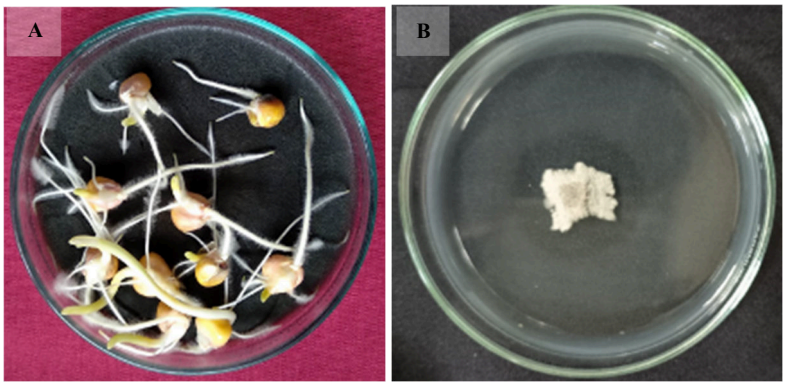

Figure 3. Pathogenicity testing of candidate endophytic fungi on corn seeds (**A**) and chitin solvent testing on colloidal chitin agar media shows a clear zone around the endophytic fungi (**B**).

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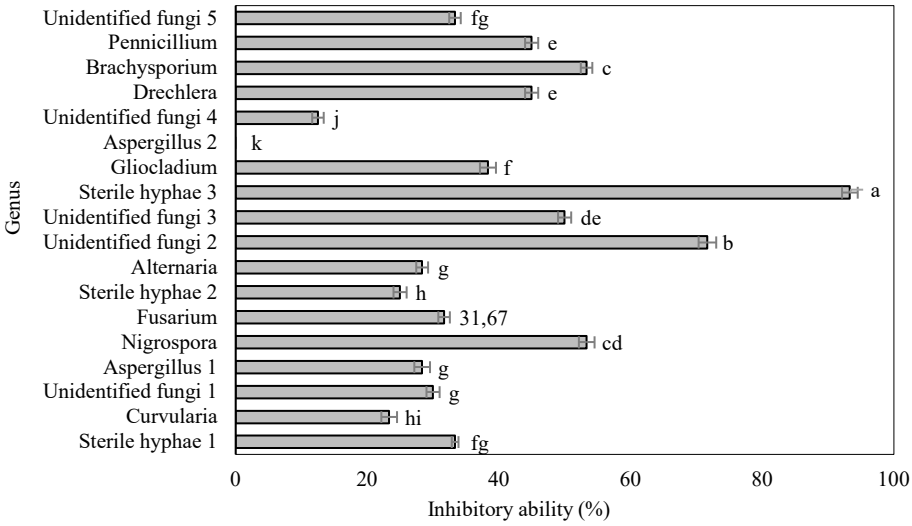

Figure 4. Direct inhibitory activity of endophytic fungal isolates against *Rhizoctonia solani* in dual culture assay. Error bars represent standard error (SE) of three replications. Different letters above bars indicate significant differences according to Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

Table 2. Endophytic fungi that were successfully identified after pathogenesis selection and results of descriptive tests for phosphate solvents and chitinolytic capability.

No	Treatment	Chitinolytic	Phosphate
1	control	+	+
2	Sterile hyphae 1	-	-
3	*Curvularia*	-	-
4	Unidentified fungi 1	-	-
5	*Aspergillus* 1	-	-
6	*Nigrospora*	-	-
7	*Fusarium*	-	-
8	Sterile hyphae 2	-	-
9	*Alternaria*	-	-
10	Unidentified fungi 2	-	-
11	Unidentified fungi 3	-	-
12	Sterile hyphae 3	-	-
13	*Gliocladium*	-	-
14	*Aspergillus* 2	-	-
15	Unidentified fungi 4	+	-
16	*Drechslera*	-	-
17	*Brachysporium*	-	-
18	*Penicillium*	-	-
19	Unidentified fungi 5	-	-

Table 3. Diameter of *R. solani* colonies and percentage of growth inhibition in volatile tests with endophytic fungi.

No	Treatment	Mean colony diameter (cm)	Inhibition percentage (%)
1	Control	9.00 a	0.00
2	Sterile hyphae 1	3.63 d	59.67
3	*Curvularia*	9.00 a	0.00
4	Unidentified fungi 1	3.11 d	65.44
5	*Aspergillus*1	4.11 d	54.33
6	*Nigrospora*	4.38 d	51.33
7	*Fusarium*	3.63 d	59.67
8	Sterile hyphae 2	2.81 d	68.78
9	*Alternaria*	9.00 a	0.00
10	Unidentified fungi 2	5.48 dc	39.11
11	Unidentified fungi 3	9.00 a	0.00
12	Sterile hyphae 3	5.19 d	42.33
13	*Gliocladium*	6.25 bc	30.56
14	*Aspergillus* 2	3.78 d	58.00
15	Unidentified fungi 4	6.19 c	31.22
16	*Drechslera*	9.00 a	0.00
17	*Brachysporium*	9.00 a	0.00
18	*Penicillium*	9.00 a	0.00
19	Unidentified fungi 5	9.00 a	0.00

Note: Means followed by different letters within the same column are significantly different according to Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$

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Table 4. Effect of endophytic fungi on plant height and root length of maize (mean ± SD).

No	Treatment	Root length (cm)	Height (cm)
1	Control	109.33 ± 10.07 a	26.67 ± 2.89 bcd
2	Sterile hyphae 1	100.33 ± 1.53 a	32.67 ± 11.85 ab
3	*Curvularia*	70.33 ± 2.52 cd	27.67 ± 7.23 abcd
4	Unidentified fungi 1	71.00 ± 1.00 cd	22.00 ± 4.36 cde
5	*Aspergillus* 1	61.33 ± 4.16 de	27.00 ± 1.73 bcd
6	*Nigrospora*	66.00 ± 7.00 cde	19.00 ± 3.00 de
7	*Fusarium*	74.00 ± 2.65 cd	28.00 ± 6.00 abcd
8	Sterile hyphae 2	66.00 ± 5.57 cde	24.00 ± 14.00 bcde
9	*Alternaria*	56.33 ± 1.53 e	17.67 ± 4.93 e
10	Unidentified fungi 2	73.33 ± 1.53 cd	19.00 ± 3.00 de
11	Unidentified fungi 3	75.33 ± 3.51 cd	14.67 ± 7.37 e
12	Sterile hyphae 3	77.67 ± 9.45 c	22.00 ± 5.57 cde
13	*Gliocladium*	68.00 ± 14.11 cd	20.00 ± 3.46 de
14	*Aspergillus* 2	72.33 ± 17.62 cd	23.33 ± 2.08 bcde
15	Unidentified fungi 4	72.00 ± 2.65 cd	30.33 ± 9.29 abc
16	*Drechslera*	61.33 ± 9.07 de	31.33 ± 2.31 ab
17	*Brachysporium*	53.00 ± 11.79 e	34.00 ± 4.58 a
18	*Penicillium*	69.33 ± 3.21 cd	34.33 ± 1.15 a
19	Unidentified fungi 5	53.00 ± 10.15 e	17.33 ± 5.03 e

Note: Values represent mean ± standard deviation of three replications. Means followed by different letters in the same column are significantly different according to Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

The experimental results showed that inoculation of several endophytic fungi on corn plants had an effect on plant growth (Table 4). Some isolates suppressed plant growth compared to the control such as Unidentified fungi 1, *Nigrospora,* Sterile hyphae 2, *Alternaria,* Unidentified fungi 2, Unidentified fungi 3, Sterile hyphae 3, *Gliocladium, Aspergillus* 2, and Unidentified fungi 5. However, endophytic fungi will show a real influence in increasing plant growth. Due to observations were only made in the vegetative phase, 1 month after planting, so the effect as a growth inducer was not yet visible (Table 4).

DISCUSSION

The most commonly found genus were *Aspergillus*, *Curvularia* and sterile hyphae, but there were differences in each isolated tissue, such as *Gliocladium* which is only found in plant root tissue. Similar to Terna *et al*. [52] that has successfully isolated endophytic fungi from sweet corn, namely *Fusarium verticillioides*, *Fusarium sacchari*, and *Penicillium citrinum*. Potshangbam *et al*. [39] successfully isolated 123 isolates of endophytic fungi from corn and rice and the most dominant genera in corn were *Fusarium, Sarocladium, Aspergillus, Penicillium,* and *Acremonium*. These genera were not tissue specific and were found in roots, stems, and leaves. *Trichoderma koningiopsis* was successfully isolated only in corn plant stems. *Talaromyces pinophilus* and *epicoccum sorghi* were only found in corn plant roots, while *Rhizomucor* sp. and *Eutypella scoparia* were only found in corn plant leaves.

The *Fusarium* genus was successfully isolated from roots and stems, according to research conducted by Jin *et al*. [18] in cotton plants that succeeded in isolating endophytic fungi with a Shannon index of 3.848 with a diversity of endophytic fungi in each different tissue, the fungus genus that was successfully isolated was also different in each tissue. The abundance of endophytic fungi depends on environmental conditions, fungal spores that are free in the air will colonize the plant's phyllosphere, and direct transmission can also occur through the roots around the plant's rhizosphere [3, 34].

The endophytic fungi that have been isolated have differences in each part of the corn plant tissue, according to Khiralla *et al*. [22], endophytic microbes are microbes that have the characteristic that they are plant specific and even plant tissue specific. The species of endophytic fungi between one plant variety and another can be the same or different types. This difference may be influenced by the environmental conditions of origin of the plant samples used. According to Sieber and Grünig [46], there are several things that influence the diversity of endophytic fungi on a plant, including environmental factors, vegetation type, root patterns, and interactions with various microbes in the planted land.

Pathogenicity test results showed that 18 isolates of endophytic fungi did not inhibit corn seed germination (Fig. 3A). The seeds do not show symptoms of necrosis or rot at the base of the stem so they can grow well. This is because endophytic fungi are fungi that are not pathogenic so they do not cause disease in host plants. Endophytic fungi complete all or part of their life cycle in healthy plant tissue and do not cause disease in their hosts [35]. This is in accordance with the opinion of Carroll [8] who stated that endophytic fungi are fungi that live on the inside of healthy plant tissue and do not cause disease symptoms in the host plant.

The results of the dual culture test showed that most endophytic fungal isolates obtained from maize tissues had varying antagonistic abilities against *Rhizoctonia solani*, with inhibition percentages ranging from 12.50% to 93.33%. Sterile hyphae isolate 3 demonstrated the highest direct inhibitory activity (93.33%), followed by several other genera such as *Aspergillus*, *Fusarium*, and *Nigrospora* with moderate inhibition levels (Fig. 4). These dual culture assay results indicate that several endophytic fungi possess direct antagonistic and

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antibiosis activities against *Rhizoctonia solani*. The antibiosis mechanism is characterized by the formation of an inhibition zone between the endophytic fungi and the pathogenic fungi. The inhibitory activity observed in this study could involve antifungal metabolites previously reported in endophytic fungi. Several studies have reported that endophytic species from the genera *Aspergillus* and *Fusarium* can produce phenolic compounds, lactones, organic acids, and polyketides such as terrein, butyrolactone, and gliotoxin, which are toxic to pathogenic fungi [22, 44].

Microscopically, high inhibition is usually accompanied by morphological changes in pathogenic hyphae, such as vacuolization, swollen hyphal tips, or fragmentation of the mycelial structure. The chitinolytic activity observed may indicate the potential involvement of hydrolytic enzymes such as chitinase in fungal antagonism. Although in this study, only one isolate (Unidentified Fungi 4) demonstrated clear chitinolytic activity (Fig. 3B), it is likely that isolates with high inhibitory activity, such as sterile hyphae 3 and *Aspergillus* sp., also produce low levels of hydrolytic enzymes that contribute to their antibiotic effects. The detection of chitinolytic activity in only one isolate suggests that hydrolytic enzyme production was isolate-specific and not commonly expressed among the tested maize endophytic fungi. This finding aligns with the report by Huang *et al*. [15] which showed that endophytes produce a combination of secondary metabolites and hydrolytic enzymes as an antibiosis mechanism. The chitinolytic assay in this study was conducted qualitatively based on clear zone formation, intended as a qualitative screening based on clear zone formation on colloidal chitin medium; therefore, quantitative enzyme activity was not determined.

The enhancement of plant growth observed in this study could be linked to mechanisms previously reported in endophytic fungi, including phytohormone production. Research by Waqas *et al*. [58] stated that the endophytic fungi *Phomaglomerata* and *Penicillium* sp. are able to againstmetabolites, in the form of the hormone gibberellin (GA) and indole acetic acid (IAA), which function to reduce abiotic stresses in plants such as salinity and drought so that plants can grow well. The symbiosis of endophytic fungi with plants is able to assimilate important nutrients for plants such as potassium, calcium and magnesium.

Endophytic fungi are reported to have antimicrobial substances, plants associated with endophytes produce several metabolites that induce resistance. The symbiotic relationship between the two is able to activate the plant's defense system more quickly compared to non-symbiotic plants with endophytic fungi [14].

Chitinolytic microbes are demonstrated by the ability to produce clear zones around their colonies on media containing chitin. The clear zone is formed because chitin is hydrolyzed into soluble monomers or derivatives, especially GlcNAc, by extracellular chitinase produced by microbes. Then the degradation products are absorbed by microbial cells as a source of carbon and nitrogen [31, 47]. All endophytic fungi do not show a clear zone on colloidal chitin media, but the ability of endophytic fungi to solubilize phosphate is not the main characteristic for controlling pathogenic fungi [18].

Based on the volatile assay, inhibition levels ranged from 30.56% to 68.78%, with the highest inhibition shown by sterile hyphae 2 (68.78%). This volatile test examined the ability of endophytic fungi to suppress the growth of pathogenic fungi through non-contact mechanisms. Volatile compounds are organic molecules that can diffuse through the air and have fungistatic and fungicidal effects (Table 3). This study showed that *R. solani* tested with endophytic fungi experienced slowed colony growth and a brownish discoloration. Several studies have shown that VOCs cause damage to fungal hyphae, inhibit mycelial growth, and change the morphology and structure of mycelium and spores in fungi [29]. However, the volatile compounds responsible for pathogen inhibition were not chemically characterized in the present study; therefore, further analysis using analytical techniques such as GC-MS is required to identify the specific compounds involved.

Several major volatile compounds identified from the *Aspergillus* genus and sterile hyphae include benzaldehyde, 1-octen-3-ol, acetoin, 2,3-butanediol, and ethyl acetate [34]. These compounds are known to act through diverse biochemical mechanisms [11, 55]. Moreover, Fontana *et al*. [11] and Tao *et al*. [51] stated that in addition to directly inhibiting pathogens, volatiles also play a role in inducing plant resistance. Compounds such as 2,3-butanediol and acetoin have been reported to induce the expression of plant defense genes, including PR1, PAL (phenylalanine ammonia lyase), and CHI (chitinase), as well as increase peroxidase enzyme activity. Increased root length and plant height in endophyte treatments can also be attributed to the dual effects of volatiles: inhibiting pathogens and stimulating plant growth.

Volatile organic compounds (VOCs) are a large group of carbon-based compounds produced by some microbes with low molecular weights that evaporate easily and inhibit the growth of other microbes [53]. According to Siri-udom *et al.* [48], some endophytic fungi can produce volatile antibiotic compounds or alkaloids. VOCs released by endophytic fungi cause partial or total inhibition of the growth of pathogenic fungi. Kaddes *et al*. [19] stated that VOCs have the potential to be anti-microbial, the vapor released by endophytic fungi can be used as an alternative fungicide to control pathogenic fungi. However, VOC activity depends on the antagonist agent, dose and application technique. The abundance of endophytic fungi is abundant and has high genetic diversity, which is a potential that must be developed as a bio fungicide.

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Antibiotic compounds produced by antagonistic microbes can have direct and indirect effects on plants. First, these compounds can directly act as fungicides to control pathogens, but this mechanism can suppress plant growth. This mechanism functions as an agent that induces plant resistance to disease [25]. The second indirect effect has the potential to act as an inducer of plant resistance rather than as a fungicide. Antibiotic compounds have a role in increasing plant growth so that they can defend themselves from pathogen attacks. The positive effect of several isolates on maize seedling growth may be related to mechanisms previously reported in endophytic fungi, including nutrient mobilization and phytohormone-related activity.

Endophytic fungi are thought to have an indirect role, namely increasing plant growth so that they can trigger plant resistance to pathogen infection. This role requires direct physical contact with the pathogen, and the population of this fungus must be large in plant tissue. The production of antibiotics in large quantities will also have a negative impact on plants, while their presence in plant tissue is less than that of pathogenic fungi. According to research by Mourhofer *et al*. [34] on the endophytic bacteria *Pseudomodas* spp., the resulting antibiotics such as pyoluteorin and 2,4-diacetylphloroglucinol (DPAG) are phytotoxic at high concentrations, having a negative impact on plant growth.

Our research found that 46 isolates of endophytic fungi from maize plants were successfully isolated. There were 18 (eighteen) isolates of endophytic fungi which have potential as biological agents which do not cause disease symptoms in corn seeds or seedlings in pathogenicity tests and have the ability to inhibit directly in *in vitro* tests. This research contributes to the development of sustainable and environmentally friendly plant disease control strategies, with potential applications in the form of bio fungicides and biofertilizers based on endophytic fungi isolated from corn plants grown in Jambi City, Indonesia.

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