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Research Article

Endophytes and Rhizosphere Fungi from Galam (*Melaleuca cajuputi* Powell.) which has the Potential to Produce Indole Acetic Acid (IAA)

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ABSTRACT

Some types of fungi are known to have the ability to produce Indole Acetic Acid (IAA). Fungi can be isolated from the rhizosphere and tissues of various plants, including from the rhizosphere and the root "Galam" (*Melaleuca cajuputi* Powell.), which grow predominantly in peatlands. Therefore, the purposes of this study were: (a) to isolate and measure the potential of fungi from endophytic and rhizospheric of "Galam" (*M. cajuputi*) as a producer of IAA hormone, (b) determine the types of fungal interaction that occur and their potential to increase the total IAA hormone produced. This research begins with isolation, purification, isolate screening, analysis of IAA hormone production, data analysis, seed germination test and isolates identification. The result showed that the concentration of IAA produced by *Penicillium* sp. IRZ15 was $5.86 \pm 0.47 \mu\text{g.mL}^{-1}$ to $8.46 \pm 0.26 \mu\text{g.mL}^{-1}$ and *Syncephalastrum* sp. AG15 is $4.77 \pm 0.44 \mu\text{g.mL}^{-1}$ to $8.77 \pm 0.25 \mu\text{g.mL}^{-1}$. Meanwhile, the combination of rhizospheric fungi *Penicillium* sp. IRZ15 and endophytic fungi *Syncephalastrum* sp. AG15 does not produce significantly different IAA concentrations ($6.42 \pm 0.34 \mu\text{g.mL}^{-1}$ to $9.19 \pm 0.50 \mu\text{g.mL}^{-1}$) compared to fungi used alone without combinations.

Keywords: endophytic fungi, Galam, *Melaleuca cajuputi* Powell., Indole Acetic Acid, peatland, rhizospheric fungi

INTRODUCTION

Indole acetic acid (IAA) is a hormone included in the auxin group (Wojciech Szajdak & Maryganova 2007). The IAA hormone plays an important role in triggering the extension and division of plant cells. Auxin is a hormone composed of indole rings and can trigger the growth and development of plants (Setyowati et al. 2017). The IAA hormone can support root extension and increase the absorption of nutrients from the soil (Suebrasri et al. 2020). The presence of endogenous IAA hormones (produced by plants) and the addition of exogenous IAAs can affect plant growth rates.

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There have been many studies that show the presence of soil fungi and endophytic fungi capable of producing the IAA hormone (Larosa et al. 2013). Endophytic fungi can be isolated from various types of plants, one of which is “Galam” plants. “Galam” (*M. cajuputi*) is a plant that grows predominantly on peatlands. These plants are distributed in the peatlands of Central Kalimantan, South Kalimantan, and the coastal part of South Sumatra. “Galam” wood is classified as a pioneer plant that can grow in burnt areas and is tolerant of flooded areas (Supriyati et al. 2014), and generally can survive in acid sulfate swamp conditions with humus and peat soils. Therefore, it is assumed that “Galam” plants might be colonized by endophytic fungi that have the potential to help the host plants to survive under conditions of environmental stress by secreting secondary metabolites that are profitable, especially IAA. 7

Waqas et al. (2012), reported that endophytic fungi isolated from the roots of plants can secrete gibberellin, auxin, and cytokinin. Endophytic fungi such as *Penicillium* and *Phoma* which are isolated from cucumber plant tissue are known to have the ability to produce IAA hormones (Subowo 2016; Waqas et al. 2012). In addition to endophytic fungi, several types of fungi originated from the soil rhizosphere are known to have the ability to produce hormones (Unyayar 2000; Maor et al. 2004; Astriani et al. 2014). *Phanerochaete chrysosporium* (Unyayar 2000), *Aeschynomene*, and *Colletotrichum gloeosporioides* isolated from the rhizosphere are known to have the ability to produce hormones, such as IAA (Robinson et al. 1998; Maor et al. 2004; Astriani et al. 2014). 12

The interaction between fungi in the plant tissue (endophytes) and fungi around the rooting area (rhizosphere) can certainly give a diverse effect on plant growth, either positive or negative. One form of positive interaction is known as synergism. This synergism can be utilized both to support the growth of host plants and between species of fungus that interact with each other (Hestrin et al. 2019). Research by (Suebrasri et al. 2020) showed that endophytic fungi *Diaporthe phaseolorum* and *Macrophomina phaseolina* isolated from siam weed and ginger can produce the IAA hormone and increase the growth of sunchoke plant.

The production of IAA hormone by fungi can be optimized to perform combinations between fungi from various origins. However, there is less data on the research of rhizosphere fungi and endophytic fungi growing in peatlands in South Kalimantan and the forms of their interactions that have the potential to produce the IAA hormone. Therefore, it is necessary to perform research related to the exploration of rhizosphere and endophytic fungi from the “Galam” (*M. cajuputi*) and its interactions that have the potential to produce the IAA hormone. So that the information can be used for further studies on the utilization of fungi from peatland trees to support peatland farming.

MATERIALS AND METHODS

Materials

Materials used are plant roots of “Galam” (*M. cajuputi*), peat soil (10 gr), potato dextrose agar (PDA), and broth (PDB) media added with 1% chloramphenicol, NaOCl 5%, ethanol 70%, tryptophan, methanol, synthetic IAA solution, and Salkowski reagent (50 mL, 35% sulfuric acid, 1 mL of 0.5 mol FeCl₃ solution). 11

Methods

“Galam” (*M. cajuputi*) Rhizosphere Fungi Isolation

Soil samples (10 gr) were collected from the area around the plant roots

(rhizosphere) *M. cajuputi* with a depth of 10-20 cm. The isolation fungi according to Larekeng et al. (2019) method with modification, that was the pour plate method with multilevel dilution from 10^{-1} until 10^{-5} . Cultures were incubated at 22°C - 25°C for 5-7 days.

“Galam” (*M. cajuputi*) Roots Endophytic Fungi Isolation

Isolation of endophyte fungi was carried out by direct method. Endophyte fungi were isolated from “Galam” plants with a height of 3 meters and a diameter of 10 cm. The root used was the lateral root part close to the root hair. The roots were rinsed with water. Furthermore, sterilized the surface by soaking for 1 minute in ethanol 70%, 3 minutes in a solution of NaOCl 5.3%, and another 30 seconds in ethanol 70%. Then dried and cut into 1 cm sizes. Each root piece (3 pieces) was placed on PDA media with chloramphenicol on Petri dish, incubated at 22°C - 25°C for 5-7 days (Yunaedi et al. 2016).

In Vitro Screening of Rhizospheric and Endophytic Fungi

Screening rhizosphere and endophytic fungi isolate was performed by several methods which were: observation and measurement of the growth rate of fungi, pathogenicity test, and the test of antagonism.

a. Fungi Growth Rate

The growth rate of a single isolate of rhizospheric and endophytic fungi that have been isolated and purified was measured. The growth of fungi isolates was measured by the colony surface area every day after inoculation for 4 days. Measurement of growth rate was done according to (Miyashira et al. 2010) with modification by calculating the difference of the fungi's surface area divided by the time difference in the measurement of fungi.

b. Pathogenicity Test

The pathogenicity test of fungi was carried out using the healthy orchid (*Phalaenopsis* sp.) leaves. First, orchid leaves were washed using water and cut into 3 x 3 Cm². Furthermore, surface-sterilized orchid leaf surface with 10% Clorox, 10% alcohol, and distilled water. Leaf samples were then placed in a Petri dish with an inverted surface. Orchid leaf pierced by 3 punctures per leaf using a sterile needle. Next, the fungi colonies to be tested were affixed to the puncture section that has been made. The presence or absence of disease progression was calculated based on the score disease rating (Chung et al. 2011).

c. In Vitro Antagonistic Test

Each non-pathogenic rhizospheric fungi isolate was interacted with non-pathogenic endophytic fungi isolates using multiple culture test techniques. Rhizospheric fungi isolates were grown in pairs with endophytic fungi. The pieces isolate were placed in pairs at a distance of 3 cm from the edge of the media PDA on the Petri dish and were incubated at 26°C - 28°C. The diameter colony of both fungi isolates was measured every day for one week. The inhibition percentage was calculated using the formula according to (Skidmore & Dickinson 1976). The interaction between the two fungi isolates was also observed microscopically by the slide culture method (Rabha et al. 2014). A pair of isolates that showed the lowest percentage of inhibition would be analyzed for the production of the IAA hormone.

Analysis of Indole Acetic Acid Production

Analysis of IAA hormone production was carried out by the method of Gordon and Weber (1951). Rhizospheric fungi isolates, endophytic fungi, and a combination of both fungi were inoculated into a 5 mL potato dextrose broth (PDB) medium which was enriched with 100 µg.mL⁻¹

tryptophan and potato dextrose broth (PDB) medium without tryptophan. Next, both media were incubated for 17 days at a speed of 150 rpm in a non-light condition and centrifuged for 15 minutes at a speed of 6000 rpm. 1 mL of the supernatant was transferred to a sterile test tube and 4 mL of Salkowski reagent was added. The suspension was then incubated in a non-light condition for 60 minutes at room temperature. If the color of the solution turned pink, it means that the solutions contained IAA hormone. Next, absorbance measurements were carried out using a spectrophotometer with a wavelength of 530 nm. The determination of IAA concentration was done by comparing the absorbance value obtained with the IAA standard curve through regression analysis (Astriani et al. 2014).

Germination Test Using Chilli Seeds

The fungi which have the potential of IAA production were tested directly on the chili seeds. The first step was to surface sterilized the seed with 1% NaOCl. The chili seeds were then soaked for 1 day in sterile distilled water as a control and in the media which contain fungi isolates. The chili seeds were then placed on a Petri dish which contained 2 sheets of moist filter paper. The number of germination seeds, hypocotyl length, and epicotyl length measured every day in one week (Syamsia et al. 2015).

Data Analysis

The data of IAA hormone production that was obtained were presented in figures, tables, and descriptive analysis. The data also would be analyzed to the middle-value test (Astriani et al. 2014) and Duncan's Multiple Range Test (DMRT).

Identification of Rhizospheric and Endophytic Fungi

Rhizospheric and endophytic fungi that have the smallest inhibitory and proven potential to produce hormones IAA were identified. Identification was carried out by observing its macroscopic and microscopic morphological character based on the identification book *Illustrated Genera of Imperfect Fungi* (Barnett & Hunter 1987).

RESULTS AND DISCUSSION

Results

The Growth Rate and Patogenicity of “Galam” Root Rhizospheric and Endophytic Fungi

In total, there were 16 isolates of rhizospheric (IRZ) and 22 endophytic (AG) fungi successfully isolated during this study. The average rhizospheric and endophytic fungi growth rate is presented in Table 1 and Table 2.

The high value of the growth rate average is shown on IRZ7, IRZ8, IRZ10, IRZ13, IRZ15, AG10, AG11, AG15, AG18, and AG20 isolate.

The pathogenicity test showed no symptoms of the disease in control, IRZ7, IRZ8, IRZ10, IRZ15, and AG15 (Table 3). Meanwhile, the necrosis on AG18 isolate appeared from the first day with a score of disease rating of 2. The necrosis on the IRZ13, AG10, and AG11 appeared on the second day with a score of disease rating of 2. However, on isolates AG10 and AG11 consecutively on the fifth and fourth day, the symptoms of the disease were accompanied by the appearance of soft rot on the leaves so that the score of the disease rises to 3. Therefore, only isolate rhizospheric fungi IRZ7, IRZ8, IRZ10, IRZ15, and AG15 endophytic fungi were used in antagonism tests because they were classified as non-pathogenic based on disease scores arising from leaves.

Table 1. The rhizospheric fungal growth rate.

| Isolate | Colony Growth Rate (Cm ² /day) | | | |
|--------------------|---|----------------------|----------------------|----------------------|
| | 1 th -DAI | 2 th -DAI | 3 th -DAI | 4 th -DAI |
| IRZ1 | 2 | 2 | 2 | 4 |
| IRZ2 | 3 | 2 | 1 | 3 |
| IRZ3 | 1 | 2 | 4 | 4 |
| IRZ4 | 3 | 1 | 0 | 7 |
| IRZ5 | 2 | 0 | 0 | 0 |
| IRZ6 | 1 | 2 | 0 | 1 |
| IRZ7 ^T | 2 | 4 | 4 | 5 |
| IRZ8 ^T | 2 | 2 | 4 | 4 |
| IRZ9 | 1 | 3 | 3 | 3 |
| IRZ10 ^T | 2 | 3 | 3 | 3 |
| IRZ11 | 1 | 2 | 2 | 4 |
| IRZ12 | 1 | 0 | 0 | 0 |
| IRZ13 ^T | 10 | 12 | 15 | 4 |
| IRZ14 | 3 | 0 | 2 | 3 |
| IRZ15 ^T | 2 | 5 | 46 | 0 |
| IRZ16 | 1 | 0 | 0 | 0 |

Note: ^T selected isolate (the five isolates with the high colony growth rate), DAI (Day After Incubation).

Table 2. The endophytic fungal growth rate.

| Isolate | Colony Growth Rate (Cm ² /day) | | | |
|-------------------|---|----------------------|----------------------|----------------------|
| | 1 th -DAI | 2 th -DAI | 3 th -DAI | 4 th -DAI |
| AG1 | 2 | 4 | 1 | 0 |
| AG2 | 1 | 0 | 0 | 0 |
| AG3 | 1 | 5 | 1 | 0 |
| AG4 | 1 | 4 | 2 | 5 |
| AG5 | 1 | 4 | 2 | 4 |
| AG6 | 3 | 13 | 0 | 0 |
| AG7 | 1 | 3 | 1 | 1 |
| AG8 | 43 | 24 | 1 | 0 |
| AG9 | 2 | 3 | 0 | 0 |
| AG10 ^T | 5 | 18 | 26 | 0 |
| AG11 ^T | 4 | 18 | 20 | 15 |
| AG12 | 4 | 12 | 17 | 0 |
| AG13 | 4 | 0 | 1 | 0 |
| AG14 | 1 | 0 | 0 | 0 |
| AG15 ^T | 5 | 9 | 11 | 9 |
| AG16 | 5 | 8 | 10 | 0 |
| AG17 | 2 | 0 | 0 | 0 |
| AG18 ^T | 13 | 39 | 1 | 5 |
| AG19 | 2 | 5 | 4 | 3 |
| AG20 ^T | 4 | 14 | 17 | 1 |
| AG21 | 1 | 0 | 0 | 0 |
| AG22 | 4 | 0 | 0 | 0 |

Note: ^T selected isolate (the five isolates with the high colony growth rate), DAI (Day After Incubation).

Table 3. Pathogenicity test in vitro on the leaves of *P. amabilis* for 5 DAI.

| No | Isolat | Score Disease Rating | | | | |
|----|-------------------------------|----------------------|---------|---------|---------|---------|
| | | 1th-DAI | 2th-DAI | 3th-DAI | 4th-DAI | 5th-DAI |
| 1 | Leaf without fungal infection | 0 | 0 | 0 | 0 | 0 |
| 2 | IRZ7 | 0 | 0 | 0 | 0 | 0 |
| 3 | IRZ8 | 0 | 0 | 0 | 0 | 0 |
| 4 | IRZ10 | 0 | 0 | 0 | 0 | 0 |
| 5 | IRZ13 | 0 | 2 | 2 | 2 | 2 |
| 6 | IRZ15 | 0 | 0 | 0 | 0 | 0 |
| 7 | AG10 | 0 | 2 | 2 | 2 | 3 |
| 8 | AG11 | 0 | 2 | 3 | 3 | 3 |
| 9 | AG15 | 0 | 0 | 0 | 0 | 0 |
| 10 | AG18 | 2 | 2 | 2 | 2 | 2 |
| 11 | AG20 | 0 | 0 | 0 | 2 | 2 |

Note: 0 = no symptoms of leaf disease, 1 = necrosis diameter ≤ 2 mm, 2 = necrosis diameter > 2 mm, 3 = necrosis diameter > 2 mm, and soft rot on the leaves. DAI (Day After Incubation).

In Vitro Antagonistic Test

Figure 1 showed that there was inhibition between the interaction of each isolate. The percentage of inhibition continued to increase until the seventh day. The interaction of AG15 and IRZ15 has a relatively slow increase in inhibition percentage and has a smaller value compared to other isolate combinations, so this combination was chosen for the analysis of IAA hormone production.

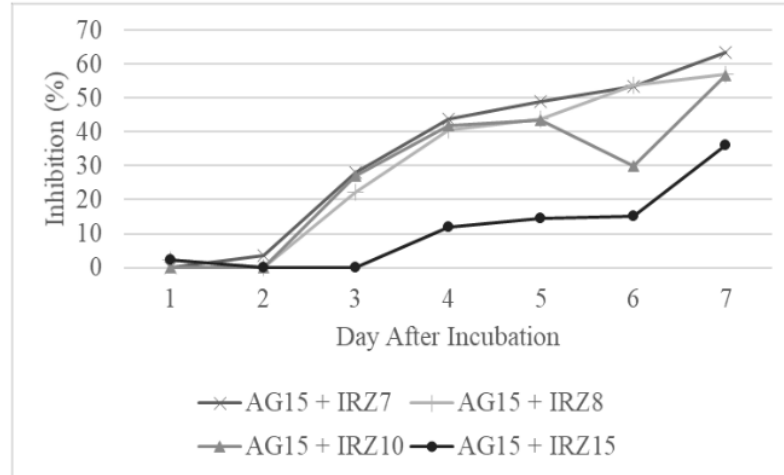


Figure 1. Inhibition rate between endophytic fungi and rhizospheric fungi tested by dual culture method, AG15 (the endophytic fungi selected isolate), IRZ7,8,10,15 (the rhizospheric fungi selected isolates).

The endophytic fungi AG15 was tested for interaction with the four selected rhizospheric fungi isolates through the dual culture method. The microscopic data (Figure 3) showed that the hyphae of AG15 attached to hyphae of IRZ7, IRZ8, IRZ10, and IRZ15. This interaction is suspected as the mycoparasitic interaction scheme. The AG15 breaks out the walls and

structure of the IRZ7, IRZ8, and IRZ10. Furthermore, the macroscopic data showed that AG15 isolate competes with IRZ7, IRZ8, and IRZ10. AG15 isolate grows faster than rhizospheric fungi isolates that cause the inhibition of rhizospheric fungi growth. Meanwhile, interactions between AG15 and IRZ15 showed an inhibitory zone (Figure 2, d). This indicates that there was an antibiotic mechanism that occurred between the fields.

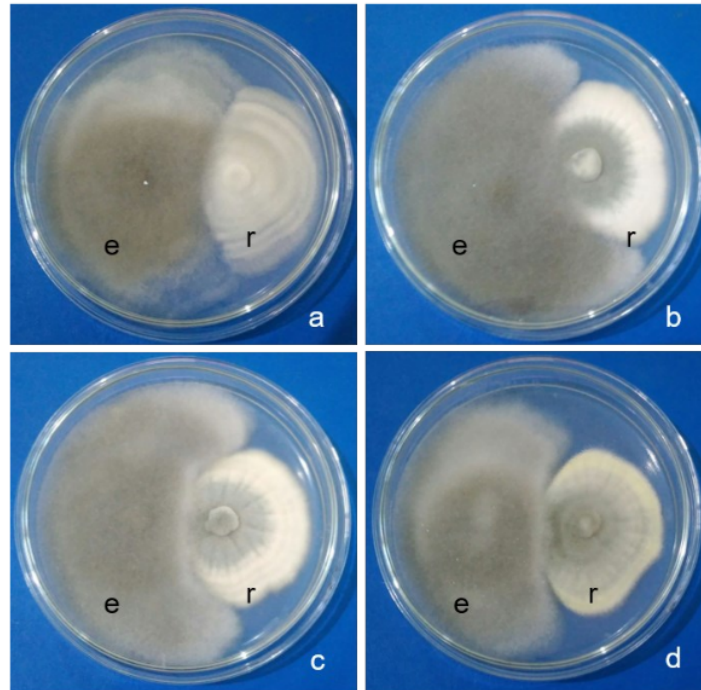


Figure 2. The interaction between (a) AG15 and IRZ7, (b) AG15 and IRZ8, (c) AG15 and IRZ10, (d) AG15 and IRZ15; e = endophytic fungi; r = rhizospheric fungi.

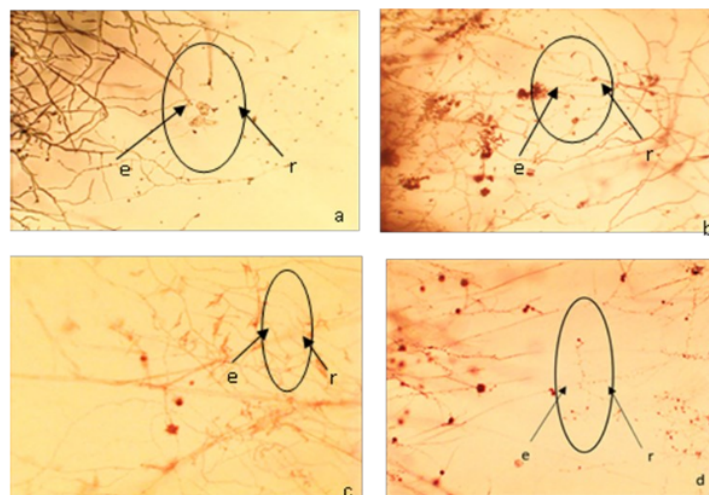


Figure 3. The mycoparasitic interaction between endophytic and rhizosphere fungi at 40X magnification (a) AG15 and IRZ7, (b) AG15 and IRZ8, (c) AG15 and IRZ10, and antibiosis (d) AG15 and IRZ15; e = endophytic fungi; r = rhizospheric fungi.

Indole Acetic Acid Production

Table 4 showed the concentration of IAA produced has varying values. However, the fungi isolates with tryptophan enriched media showed higher concentration values and significantly different than IAA concentrations produced by isolates on media that were not enriched with tryptophan, which was $8.77 \pm 0.25 \mu\text{g.mL}^{-1}$ (from endophytic fungi AG15), $8.46 \pm 0.26 \mu\text{g.mL}^{-1}$ (from rhizosphere fungi IRZ15), and $9.19 \pm 0.50 \mu\text{g.mL}^{-1}$ (from a combination of rhizosphere and endophytic fungi). Besides, the combination of rhizospheric fungi and endophytes can increase the total amount of IAA concentration produced by rhizospheric fungi and endophytic fungi. However, the result of the concentration measurement was not significantly different from the use of fungal isolate in a single.

Table 4. The concentration of IAA hormones produced by selected isolates.

| Code | Concentration ($\mu\text{g.mL}^{-1}$) |
|--------|---|
| AG15T | 8.77 ± 0.30^c |
| IRZ15T | 8.46 ± 0.32^c |
| KART | 9.19 ± 0.61^c |
| AG15 | 4.77 ± 0.53^a |
| IRZ15 | 5.86 ± 0.58^b |
| KAR | 6.42 ± 0.46^b |

Note: AG15T: endophytic fungi isolate with tryptophan enriched media; IRZ15T: rhizospheric fungi isolate with tryptophan enriched media; KART: Combination of endophytic and rhizospheric fungi isolate with tryptophan enriched media; AG15: endophytic fungi isolate without tryptophan enriched media; IRZ15: rhizospheric fungi isolate without tryptophan enriched media; KAR: Combination of endophytic and rhizospheric fungi isolate without tryptophan enriched media.

Seed Germination

The treatment with AG15 isolate had a significant effect on increasing the number of germination and epicotyl lengths compared to other treatments and controls. However, hypocotyl length measurements did not show significantly different results from control (Table 5).

Identification of genus

Penicillium sp. IRZ15 obtained in this study has a greyish white color with a smooth colony surface (such as velvet). The microscopic morphology fungi showed a conidium that is spherical in shape, metula, conidiophores, and

Table 5. The effect of fungi extract treatment on germination number, hypocotyl length, and epicotyl length on chili sprouts.

| Treatment | Number of germinated seed | Hypocotyl Length (mm) | Epicotyl Length (mm) |
|-----------|---------------------------|-----------------------|----------------------|
| Control | 3.9 ± 3.03^{ab} | 4.80 ± 0.00^b | 3.80 ± 0.00^b |
| AG15 | 5.05 ± 3.50^c | 5.73 ± 2.26^b | 7.77 ± 2.02^c |
| IRZ15 | 3.62 ± 3.33^{bc} | 0.00 ± 0.00^a | 0.00 ± 0.00^a |
| KAR | 2.71 ± 3.24^{ab} | 0.00 ± 0.00^a | 0.00 ± 0.00^a |
| AG15T | 1.90 ± 2.44^a | 0.23 ± 0.40^a | 0.37 ± 0.64^a |
| IRZ15T | 3.43 ± 3.12^{ab} | 0.00 ± 0.00^a | 0.00 ± 0.00^a |
| KART | 1.95 ± 2.25^a | 0.46 ± 1.30^a | 1.37 ± 2.88^a |

hyphae (Figure 4). Macroscopically, *Syncephalastrum* sp. AG15 has blackish-grey color morphology with a cotton-like surface. Microscopic observations showed that the *Syncephalastrum* sp. AG15 has wide hyphae with septa and a large sporangiophores (6). Macroscopic and microscopic morphological character based on the identification book *Illustrated Genera of Imperfect Fungi* (Barnett & Hunter 1987).

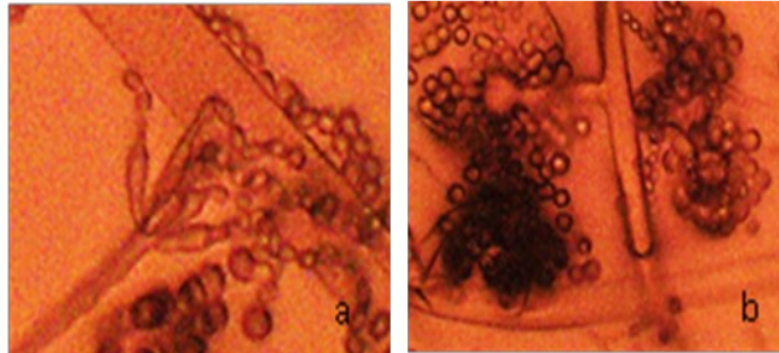


Figure 4. The microscopic morphology of *Penicillium* sp IRZ15 (a) and *Syncephalastrum* sp AG15 (b) at 100X magnification.

9 Discussion

A total of 16 isolates of fungi from the rhizosphere and 22 isolates of “Galam” plant were successfully isolated. Each isolate has a different growth rate character on the PDA medium (Tables 1 and 2). Growth area ranges from 0.25 to 13.25 Cm²/day for rhizospheric isolates and from 0.25 to 14.25 cm²/day for endophytic isolates, respectively. Five rhizospheric fungi isolates (IRZ7, IRZ8, IRZ10, IRZ13, IRZ15) and five endophytic fungi isolates (AG10, AG11, AG15, AG18, and AG20) have the best growth rate and selected for further testing. The disadvantage of this test is that it is done without repetition, so isolate is selected based on only one data.

Each type of fungi certainly has a different rate of growth. This is because each type of fungi has optimal conditions to grow. Several factors that can affect the isolation process were nutrient (8) humidity, oxygen availability, temperature, acidity, and the content of organic matter in the soil (Alexander 1978). Besides, the diversity of endophytic fungi obtained in the isolation process can be affected by the sensitivity of the host plant to environmental factors, such as light intensity, soil temperature, humidity, nutrients, and soil pH (Manaroinsong & Lolong 2016).

The ten isolates were then tested for pathogenicity, from ten isolates obtained four isolate rhizospheric fungi (IRZ7, IRZ8, IRZ10, IRZ15) and one isolate endophytic fungi AG15 that are not pathogens (Table 3). Based on in vitro antagonist test, rhizospheric fungi isolate and endophytic fungi isolate, IRZ15 and AG15 isolate were the lowest pair showing antagonism, so these two isolates are tested for the ability to produce IAA (figure 2).

Both isolates were based on identification known as *Syncephalastrum* sp. and *Penicillium* sp. *Syncephalastrum* is known as a non-pathogenic saprophyte fungi (Richardson & Rautemaa-Richardson 2020) and *Penicillium* is also reported as fungi supporting plant growth (Hossain et al. 2014) although also some species are reported as pathogens in garlic (Valdez et al. 2009). The results obtained are similar to Huda et al. (2019) that isolated *Syncephalastrum* sp. on “Galam” root. *Penicillium* was also found in peatland (Omar et al. 2012). This is because tropical peatlands generally consist of lignin consisting mostly of wood, so many species of fungi can be found.

In this study the IAA concentrations produced by *Penicillium* sp. IRZ15 ($5.86 \pm 0.47 \mu\text{g.mL}^{-1}$ to $8.46 \pm 0.26 \mu\text{g.mL}^{-1}$) after a three-day incubation period is higher than reported by Syamsia et al. (2015) $0.635 \pm 0.651 \mu\text{g.mL}^{-1}$ obtained from rice endophytic fungi. The concentration of the IAA produced by each isolate could be influenced by the enriched media of tryptophan as a precursor. The presence of tryptophan could increase the biosynthesis of IAA up to 2.7 times compared without tryptophan addition. Tryptophan increases enzymatic activity in fungi so that IAA could be produced (Tudzynski & Sharon 2002). The more concentrations of tryptophan produced are directly proportional to the increasing concentration of IAA produced (Suebrasri et al. 2020). However, the results obtained also showed that the isolate of *Syncephalastrum* sp. AG15 and *Penicillium* sp. IRZ15 were able to produce the IAA hormone in media conditions without tryptophan enriched. This was because fungi were able to maintain enzymes to synthesize IAA at low concentrations even though there is no addition of tryptophan (Astriani et al. 2014; Maor et al. 2004).

On the contrary, the combination of rhizospheric fungi and endophytic fungi was able to increase the total amount of IAA concentration. However, the result of concentration measurement was not significantly different from the use of fungi isolates singly. The interactions conducted by *Syncephalastrum* sp. AG15 and *Penicillium* sp. IRZ15 was suspected of antibiosis. The antibiosis mechanism that occurred in this study is in line with the mechanism reported in the study of Mendoza et al. (2015) which reports antibiosis among some species of *Trichoderma* with *Macrophomina* *phaseolina*. *Penicillium* sp. known to produce antifungal compounds to inhibit the growth of other fungi. It is also following the results of microscopic observations that show that the endophytic hyphae *Syncephalastrum* sp. AG15 grows away from the fungi *Penicillium* sp. IRZ15.

The treatment by *Penicillium* sp. IRZ15, a combination of *Syncephalastrum* sp. AG15. and *Penicillium* sp. IRZ15 without the addition of tryptophan and the treatment of *Syncephalastrum* sp. AG15, *Penicillium* sp. IRZ15 and the combination of both with the addition of tryptophan in the analysis of previous IAA levels showed higher results compared with IAA levels produced by *Syncephalastrum* sp. AG15 without tryptophan, which ranges from $5.86 \pm 0.47 \mu\text{g.mL}^{-1}$ (*Penicillium* sp. IRZ15 without tryptophan) to $9.19 \pm 0.50 \mu\text{g.mL}^{-1}$ (a combination of *Penicillium* sp. IRZ15, and *Syncephalastrum* sp. AG15 with tryptophan) so that it could inhibit the growth of hypocotyl and epicotyl lengths in seeds. According to Ludwig-Müller (2011), auxin concentrations that exceed the needs of each plant can be an inhibitor of plant root growth. Meanwhile, *Syncephalastrum* sp. AG15 produces IAA without tryptophan addition with the lowest concentration compared to other treatments, which was $4.77 \pm 0.44 \mu\text{g.mL}^{-1}$. This result is lower when compared to auxin produced by endophyte fungi from some medicinal plants which are 4 to $55 \mu\text{g.mL}^{-1}$ with the addition of 0.2% tryptophan (Suebrasri et al. 2020). This was because fungi are able to maintain enzymes to synthesize IAA at low concentrations even though there is no addition of tryptophan (Astriani et al. 2014). These results are in accordance with statements from Woodward and Bartel (2005) and Astriani et al. (2014), that there are two paths to the formation of IAA, namely tryptophan dependent and tryptophan independent.

Exogenous auxins such as IAA can influence the metabolism of endogenous auxins which will result in reverse inhibition of IAA biosynthesis (Ribnicky et al. 1996; Imaningsih et al. 2019). Based on the results, the direct treatment of a single isolate *Syncephalastrum* sp. AG15 gives the best results to accelerate germination, hypocotyl, and epicotyl length compared to the combination of *Syncephalastrum* sp. AG15 with *Penicillium* sp. IRZ15.

CONCLUSION

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The result showed that the concentration of IAA produced by *Penicillium* sp. IRZ15 was $5.86 \pm 0.47 \mu\text{g.mL}^{-1}$ to $8.46 \pm 0.26 \mu\text{g.mL}^{-1}$ and *Syncephalastrum* sp. AG15 was $4.77 \pm 0.44 \mu\text{g.mL}^{-1}$ to $8.77 \pm 0.25 \mu\text{g.mL}^{-1}$. Meanwhile, a combination of rhizospheric fungus *Penicillium* sp. IRZ15 and endophytic fungus *Syncephalastrum* sp. AG15 does not show significantly different concentration values with fungal isolates without combinations. Interaction between rhizospheric fungi *Penicillium* sp. IRZ15 and endophytic fungi *Syncephalastrum* sp. AG15 able to produced IAA hormones ranging from $6.42 \pm 0.34 \mu\text{g.mL}^{-1}$ to $9.19 \pm 0.50 \mu\text{g.mL}^{-1}$. Interactions that occur between the *Penicillium* sp. IRZ15 and *Syncephalastrum* sp. AG15 was suspected of antibiosis.

AUTHORS CONTRIBUTION

W.I designed the research and supervised all the process, analyzed the data and wrote manuscript, S.S.H designed the research and supervised all the process, N.D.R collected and analyzed the data and wrote the manuscript, S.S.H designed the research and supervised all the process.

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CONFLICT OF INTEREST

The author declare that there is no conflict of interest.

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