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Isolation and identification of *rhizospheric* fungus under Mahoni (*Swietenia mahagoni*) stands and its ability to produce IAA (Indole Acetic Acid) hormones

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Abstract. Rhizosphere is a part of the soil that is in the roots of plants in which there are many soil microorganisms. One of the microorganisms found in the rhizosphere is fungi. Rhizosphere fungus plays an important role in increasing plant growth by various mechanisms that are carried out such as increasing nutrient absorption, as a biological control of pathogenic attacks and can produce growth hormones for plants. This study aims to identify and get the information of the diversity of rhizosphere fungi from mahogany stands in two provenances, and get information on IAA level production. The research methods include isolation of fungi and identification of rhizosphere fungi, as well as test of IAA production capability qualitatively and quantitatively. The results showed 17 rhizosphere fungus isolates were found under the mahogany stand in Takalar District those were included in the genus *Rhizopus*, *Fusarium*, *Aspergillus*, *Penicillium* and *Gliocladium*, whereas 11 Mahogany stands in the Maros Regency were included in the genus *Trichoderma*, *Gliocladium*, *Rhizopus* and *Aspergillus*. The whole genus is capable of producing IAA hormone, but the genus capable of producing the highest IAA is the *Trichoderma* genus.

1. Introduction

Rhizosphere is the soil zone around the roots of plants that are directly affected by soil microorganisms and plant roots exudation. Provision of nutrients to plants is strongly influenced by the composition of microorganisms in the rhizosphere [1]. The rhizospheric microorganisms physiological activities had an important role for influence soil properties, nutrient uptake, plant growth and development [2]. The plant roots produce some organic compounds including organic acids, vitamins and sugars [3]. The organic compound then used as nutrients or signals for fungal population. In contrary, the fungi release iron carriers, volatile compounds, and plant hormones that may enhance plant growth, either directly or indirectly, by increasing the nutrient availability of their host [4].



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Rhizosphere fungi are one of the microbial groups that have been reported to induce plant resistance to various diseases, both land-borne diseases and airborne diseases [5]. Rhizospheric fungi also known as source of some bioactive metabolites that will support plant growth. One of important bioactive metabolites produced by rhizosphere fungi is Indole Acetic Acid (IAA). IAA is an auxin phytohormone which is widely found in nature, including exogenous and endogenous IAA [6]. Endogenous IAA is a growth hormone produced by plants, while exogenous IAA is a hormone produced by fungi that can accelerate plant growth by spurring the process of differentiation in the roots in forming root hair. Some fungi that are known to produce auxin those were *Phanerochaete chrysosporium*, *Collectrichum gloeosporioides*, and *Aeschynomene*. IAA are produced by fungi as secondary metabolites that play a role in growth regulators [7]. One type of fungus that can produce IAA is *Penicillium sp* [8]. The objectives of this study was to isolate beneficial fungus producing IAA using direct and enrichment method on specific media and screen the isolated fungus for PGPR traits from two mahogany provenance in South Sulawesi.

2. Research methods

2.1. Tools and materials

The tools used were GPS, crowbar, plastic clips, plastic bags, cameras, ruler, labels and writing instruments, measuring cups, test tubes, ovens, analytical scales, drygalski, hot palte, magnetic stirrers, centrifuge tubes, Erlenmeyer, luminary air flow, autoclave, incubator, petri dish, glass bottle, preparatory needle, bunsen, glass object, glass deck, cork borer, filter paper, microscope, micropipette, vortex, centrifuge, freezer, spectrophotometer, and camera. The materials used are soil samples, jelly, distilled, PDA media (*Potato Dextrose Agar*), PDB media (*Potato Dextrose Broth*), glucose, synthetic IAA, methanol, L-Tryptophan, alcohol, lighters, plastic wrap, aluminum foil, tissue, tips, labels, H₂SO₄ and FeCl₃.6H₂O.

2.2. Research implementation methods

2.2.1. Land sampling. The soil was taken around the rhizosphere in the mahogany stand in Takalar District, Patalassang Subdistrict, Pappa Village and Mahogany stand in Maros Regency, Cenrana Sub-District, Poccoe Limam Village. Taking soil samples from under the mahogany stand on two provenances by determining ten trees that would be used as sampling points for local superior plants, then taking soil samples on four sides in the roots with a soil depth of 0-25 cm. Then mixed and put into a plastic bag and labeled with plants, location of collection, date of collection and name of the sample taker.

2.2.2. Making media of fungus. 19.5 grams Potato Dextrosa Agar (PDA), put in Erlenmeyer with 10.5 grams of gelatin or jelly, 5 grams of glucose and 500 ml of distilled water. The solution was heated on a hot plate and stirred using a magnetic stirrer until it was homogeneous. After homogeneous the solution was sterilized using autoclave for ± two hours at 121°C. The sterile solution was transferred to the Laminary Air Flow then 0.5 grams of antibiotic was added and then homogenized. The solution was poured into 25 sterilized petri dishes of 20 ml for each then allowed to solidify.

2.2.3. Isolation of rhizosphere fungi. The method of fungus growth used in this study was the dilution method. The procedure was to fill 9 ml of distilled for each in four test tubes. Weighing 1 gram of soil sample was then put into a test tube containing sterilized distilled water and homogenized for a few minutes using vortex. 1 ml of homogeneous soil sample was inserted into the test tube 1 using a micropipette to obtain a 10⁻¹ dilution, then took 1 ml of the first test tube inserted into the second test tube to obtain a 10⁻² dilution, until 10⁻³ dilution. The growth of the fungus was carried out by taking a sample of 0.5 ml using a micropipette from a dilution of 10⁻² and 10⁻³ then spraying it on the PDA

media and leveling it using a spatula then covered with plastic wrap. The fungi colonies grew \pm four days after isolation

2.2.4. Fungus Purification. Fungus purification was carried out by removing one fungus colony on the new PDA medium using a preparatory needle into the Laminary air flow cabinet. The purified fungus was then stored in the incubator room for \pm three days.

2.2.5. Fungus Identification. Identification of fungi was carried out after pure fungus grew in PDA media for \pm one week. Identification of fungi refers to the mushroom identification book, which is the key to determining The Pictorial Atlas of Soil and Seed Fungi [9]. Identification of fungi was carried out descriptively, determining genus level by looking at the macroscopic characteristics including color, diameter and texture, microscopic features including hyphae and spores. Microscopic identification of fungus was carried out by taking the fungus growing on PDA media using preparatory needles and placed on glass objects that had been dripped with distilled water and then closed using a glass deck, then observing the fungus used a microscope with 400x magnification. The images obtained were adjusted to the literature image.

2.2.6. Measurement of the content of indole acetic acid (IAA). IAA content measurement was done by the modified [10] method. The method was to make the GDP (Potato Dextrosa Broth) media liquid first by homogenizing 24 grams of GDP, 20 grams of glucose, L-tryptophan 0.1 gram with 1 liter of distilled water into the hot plate stirrer. The media was then poured on media bottles according to the number of isolates to be grown and then sterilized in an autoclave for \pm 2 hours with a temperature of 121°C. After sterile liquid media for 5 pieces of fungus were put into a bottle of liquid media using cork borer. The bottle was then shaken using a shaker with 1000 rpm for 5 days with room temperature until the fungus isolates grew on the media. 5 ml of liquid GDP media that had been overgrown with fungus isolates was put into a centrifugation tube, then centrifuged using a centrifugator for 30 minutes at a speed of 8000 rpm. 1 ml of the obtained supernatant was added with 4 ml of Kalcowski reagent (75 ml of concentrated H₂SO₄, 125 ml of sterile distilled 3.75 ml of FeCl₃, 6H₂O 0.5 M) were put into each test tube and incubated in the dark room for 30 minutes. Changing the color of the sample to pink after incubation identified that the isolate produced IAA. IAA concentration was measured using a spectrophotometer at a wavelength of 520 nm. The IAA concentration was calculated after being compared with the absorbance of the IAA standard solution. With the equation: $Y = 0.018X - 0.001$, where Y = Absorbance Value and X = Concentration (ppm).

3. Results and discussion

3.1. Isolation of rhizosphere fungi

The diversity of fungi originating from rhizosphere soil under mahogany stands in two provenances can be known through the isolation stage, with a dilution rate of 10^{-2} and 10^{-3} as the figures shown and growing times ranging from three to four day. The next step is purification of the fungus which aims to separate each fungus colony according to the morphological differences macroscopically, so as to produce isolates that are truly pure and uncontaminated.

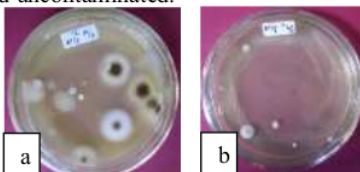


Figure 1. Results of Isolation of the Rhizosphere of the Mahogany Stand with Dilution Rate 10^{-2} (a) and Dilution 10^{-3} (b)

The results of isolation and fungus purification came from ten trees in the mahogany stand in Takalar District, Patalassang Subdistrict, Pappa Village, 17 isolates were obtained. In the mahogany stand in Maros Regency, Cenrana Subdistrict, Limam Poccoe Village, obtained 11 Isolates. The most fungal isolates were found in trees five and six in the Mahogany stand in Takalar District, each of which was three isolates while the other trees were only found in one to two isolates. The number of isolates in each tree varies in the number and type of fungi obtained.

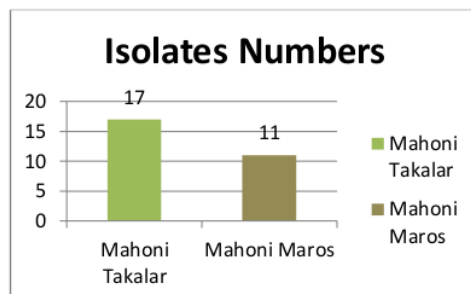


Figure 2. Results of Fungi Isolation, Takalar and Maros Mahogany.

If viewed from the results of research conducted by [11] that explored rhizosphere fungi on the roots of stands of mahogany community forest, *elmerilla ovalis* (uru) and ebony were found the isolates results on five different trees from each stand that was observed obtained from fungi growing from two *elmerilla ovalis* isolates, from the mahogany stand, six isolates and one ebony isolate from the ebony stand. According to [12] rhizosphere fungus depends on root exudates so that it will affect the diversity and number of fungus populations in the soil.

3.2. Morphological characteristic of rhizosphere fungi

Morphological characteristics of 17 rhizosphere fungi isolates in mahogany stands in Takalar District and 11 rhizosphere fungi isolates in Maros Regency were very diverse, it can be seen in the color of the fungus colonies on the upper and lower parts having varied colors, and the texture of the fungus varied with the growth rate different hyphae for each isolate. The development of fungi on PDA media was observed on the seventh day to see the difference between isolates one and the other isolates.

Table 1. Growth Diameter and Texture of Rhizospheric Isolates of Mahogany Stands in Two Provenance, on PDA Media for Seven Days

Origin of Isolate	Isolate Code	Diameter after 7 days of growth	Texture
Takalar Regency Patalassang District Pappa Village	MT 1.1	6.4 cm	Velvet
	MT 2.1	7.7 cm	Smooth Cotton
	MT 2.3	Spread	Rude Cotton
	MT 3.1	Spread	Smooth Cotton
	MT 3.2	Spread	Velvet
	MT 4.2	Spread	Velvet
	MT 4.3	4,9 cm	Rude Cotton
	MT 5.3	Spread	Velvet
	MT 5.4	7 cm	Rude Cotton
	MT 5.5	9 cm	Smooth Cotton
	MT 6.1	Spread	Velvet

	17		
	MT 6.2	1,6 cm	Smooth Cotton
	MT 6.3	Spread	Smooth Cotton
	MT 7.2	Spread	Velvet
	MT 8.5	Spread	Smooth Cotton
	MT 9.2	8.7 cm	Mid-Velvet, Side
	MT 10.4	Spread	Smooth Cotton Velvet
Maros Regency Cenrana District Liman Paccoc Village	MB 1	9 cm	Rude Cotton
	MB 2.1	9 cm	Rude Cotton
	MB 3	Spread	Velvet
	MB 4.2	9 cm	Rude Cotton
	MB 6.1	2.8 cm	Smooth Cotton
	MB 6.2	9 cm	Rude Cotton
	MB 7.1	9 cm	Rude Cotton
	MB 7.2	5.2 cm	Rude Cotton
	MB 9.2	Spread	Rude Cotton
	MB 9.3	3.8 cm	Rude Cotton
	MB 10.2	7.3 cm	Rude Cotton

Note : MT (Takalar Mahogany), MB (Bengo Mahogany)

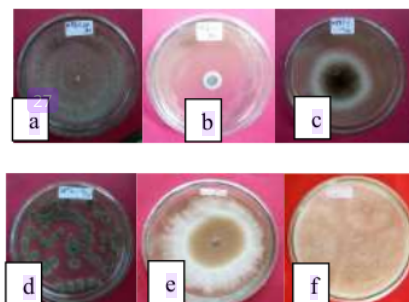


Figure 3. Color of Rhizosphere Fungus Colony; White (a), Middle Gray, White Edge (b), Middle Black, White Edge (c), Grayish Green (d) Middle Brown, White Edge (e) Brownish White (f).

Morphological observations of fungi were carried out on isolates that had been purified for seven days of growth. The isolate diameter growth found was different until the seventh days (Table 1). Some isolates were able to grow to fill the cup (nine cm) and there were several isolates that grew spread so that the diameter of the isolate was difficult to measure. The texture of all fungus isolates was Smooth Cotton, Rude Cotton and Velvet. The dominant texture was found in the texture of rude cotton. Morphological observations on the color of the Rhizosphere colonies (Figure 3) were found in white, Middle Gray, White Edge, Middle Black, White Edge, Gray Green, Middle Brown, White Edge, Brownish White.

3.3. Identification of fungus isolates

At the identification stage we used a microscope to identify rhizosphere fungi isolates that had grown for seven days on PDA media. At the identification stage, we observed the vegetative structure (hyphae) and subsequent generative structures (spores) then for book references, it was used the key book of the fungus determination of *Pictorial Atlas of Soil and Seed Fungi* [9] wherein we could

match the microscopic characteristics of fungi observed with using a microscope so that we could know the genus of fungi that we observed. The observations results can be seen in Table 2.

Table 2. Microscopic Characteristics of Growth of Rhizosphere Fungus Isolates in Mahogany Stands in two provenances.

Sample Location	Isolate Code	Isolate Numbers	Genus
Takalar Regency, Patallasang District, Pappa Village	MT 5.5	1	<i>Rhizopus</i>
	MT 2.1	1	<i>Fusarium</i>
	MT 1.1, MT 2.3, MT 4.2, MT 4.3, MT 5.4, MT 6.2, MT 6.3, MT 8.5.	8	<i>Aspergillus</i>
	MT 3.1, MT 3.2, MT 5.3,	5	<i>Penicillium</i>
	MT 6.1, MT 7.2, MT 9.2, MT 10.4	2	<i>Gliocladium</i>
	MB 1, MB 6.2, MB 7.1, MB 7.2	4	<i>Trichoderma</i>
	MB 3, MB 9.2.	2	<i>Gliocladium</i>
	MB 2.1, MB 4.2, MB 6.1, MB 10.2.	4	<i>Rhizopus</i>
	MB 9.3	1	<i>Aspergillus</i>

Note : MT (Mahogany of Takalar), MB (Mahogany of Bengo)

The results of microscopic observations made can be seen that in the mahogany stand in Takalar District which was successfully identified to the genus level including *Rhizopus*, *Fusarium*, *Aspergillus*, *Penicillium*, *Gliocladium*. Whereas the mahogany stand in Maros regency which was identified successfully to the genus level such as, *Aspergillus*, *Rhizopus*, *Trichoderma*, and *Gliocladium* can be seen in Figure 3 below.

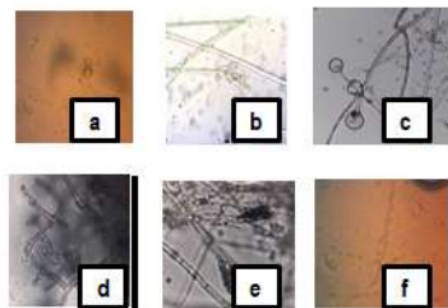


Figure 4. (a) *Aspergillus* Genus, (b) *Penicillium* Genus, (c) *Rhizopus* Genus (d) *Trichoderma* Genus

Aspergillus is a type of fungus commonly found in tropical regions with black, green, brown and yellow colonies [13]. *Aspergillus sp.* is a type of fungus that has an important role in the degradation of organic substrates, especially plant material. *Aspergillus sp.*, is found in all regions both low, medium and high [14]. *Aspergillus* comprises a large group of filamentous, anamorph fungi classified into 837 species [15], which are human and plant pathogens (e.g. *A. fumigatus*, and *A. avus*), as well as producers of specific industrially important and bio- products, e.g., *A. niger*, *A. terreus* and *A. oryzae* [16]. The results of the microscope observations showed that these isolates had conidia that were round and dark brown in color, had conidiophores that stand, long, and not branched. *Aspergillus* has a large and dense conidial carrier head, round and black.

Penicillium is known for its ability to produce a number of compounds that possess many biological activities. Cholesterol-lowering activity of secondary metabolites from *Penicillium* species has spurred efforts towards the isolation of these fungi from different natural sources. *Penicillium* has fast, flat, stringy, velvet and cotton-textured growth. When viewed from the microscopic form *Penicillium* has the characteristic of septic hyphae and forms a spore body called conidium. The conidium is different from sporangium, because it does not have a protective sheath like sporangium. Conidium stalks are called conidiophores, and the spores they produce are called conidia. This conidium has branches called phialides so that it appears to form a barrow [17]. The macroscopic observations made showed that these fungi had white and dark green colonies, whereas microscopic observations showed isolates had branched conidiophores.

When viewed macroscopically the *Rhizopus* fungus is textured like smooth cotton and white. *Rhizopus* is one of the fungi that in anaerobic conditions is able to produce extracellular amylase enzymes. *Rhizopus* can generally form colonies quickly forming stolons and rhizoid [18]. The observations carried out showed that the fungus colonies were initially gray in color which would eventually turn browned, the sporangiophore grows from stolon and leads to air either singly or in groups (up to five sporesiforas).

Gliocladium has a velvety texture and gray color, can be seen *Gliocladium* is a fungus that is widespread in the soil and weathering of plants. *Gliocladium* is saprophytic and mycoparasite life and has not been reported as a disease-causing agent in humans and plants. When viewed microscopically *Gliocladium* has septic hyphae, the upper branches of the conidiophores are shaped like lumps in the middle [17].

Trichoderma is smooth cotton textured and brownish white in color, *Trichoderma* fungus is also referred to as one type of fungus that is commonly found in almost all types of soil and in various habitats. *Trichoderma* can be used as a biological control agent because *Trichoderma* is capable of removing gliotoksin and viridin compounds so that it can inhibit plant pathogens [19].

Fusarium fungus has a mycelium shape like smooth cotton which grows fast with white, while the microscopic results of this isolate show oval-shaped spores. Microscopically the genus of this isolate is easily recognized by its long spore shape. Based on observations using a microscope it can be seen

that the microscopic form of *Fusarium* is shaped like a caterpillar that is adjacent to each other and the number is very large [17].

3.4. Production capability test of fungi isolate IAA

Based on the results of capability test of IAA production on 28 fungus isolates isolated from Mahogany Stands on two provenances which were incubated for 30 minutes, they showed discoloration in each isolate. The color changes that occur can be seen in each isolate grown in liquid GDP media and added to the *calcowski reagent* solution. The fungus isolate mixed with the *calcowski reagent* solution would change color. Based on the results of observations the most discoloration is the color change from yellow (control) to solid yellow. The results of the observations obtained were different from those of [20] which showed a color change to pink in all isolates of rhizosphere fungi isolated from kaloko paddy aromatic after the gift of *salkowski reagent* compared to the control. The difference in the results obtained is thought to be due to the origin of the fungus isolates and the types of fungi that are not the same.

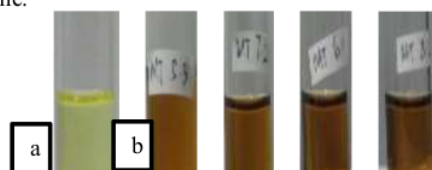


Figure 5. IAA Qualitative Test, (a) Salkowski (right), (b) Highest IAA concentration

The measurement results of IAA concentration levels with spectrophotometer showed that each fungus isolate had different concentrations. The fungus isolates in the mahogany stand in Takalar District, obtained 3 isolates that had high IAA concentrations including 18.08 ppm, 19.52 ppm and 19.69 ppm with the sample code (MT 7.2, MT 6.1 and MT 3.2, Genus *Penicillium*), while 3 isolates that had concentrations Low IAA includes 10.27 ppm (MT 1.1, Genus *Aspergillus*), 12.13 ppm (MT 8.5, Genus *Aspergillus*) and 12.47 ppm (MT 10.4, Genus *Gliocladium*). The concentration of IAA in the mahogany stand in Maros Regency was obtained by 3 isolates which had high concentrations including 20.36 ppm (MB 7.2, Genus *Trichoderma*), 19.66 ppm (MB 6.1, Genus *Rhizopus*) and 18.38 ppm (MB 3, Genus *Gliocladium*), while 3 isolates that have low concentrations include 11.41 ppm (MB 9.3, Genus *Aspergillus*), 14.02 ppm (MB 1, Genus *Trichoderma*), and 15.41 ppm (MB 7.1, Genus *Trichoderma*).

Difference in the results of IAA concentrations can be influenced by differences in the tryptophan concentration added to the media, the higher the tryptophan concentration, the higher the IAA concentration produced. The difference in IAA concentration was also due to the conditions of each sampling location, type of microbes, amount of nutrients, incubation time, growth rate and ability to convert L-tryptophan contained in the media.

Table 3. Measurement Results of IAA Concentration of Isolates of Rhizosphere fungus Mahogany stand in Takalar District, with a Wavelength of 520 nm

Takalar Isolate Code	Absorbance (nm)	Concentration of IAA (ppm)
MT 3.2	0.35	19.69
MT 6.1	0.35	19.52
MT 7.2	0.32	18.08
MT 5.3	0.32	17.94

MT 9.2	0.31	17.63
MT 5.4	0.30	16.94
MT 2.3	0.28	15.61
MT 3.1	0.26	14.83
MT 6.3	0.26	14.63
MT 4.2	0.25	14.33
MT 4.3	0.25	14.33
MT 5.5	0.24	13.47
MT 6.2	0.24	13.44
MT 2.1	0.22	12.80
MT 10.4	0.22	12.47
MT 8.5	0.21	12.13
MT 1.1	0.18	10.27

Table 4. Measurement Results of IAA Concentration of Isolates of Rhizosphere fungus Mahogany stand in Maros Regency, with a Wavelength of 520 nm

Maros Isolate Code	Absorbance (nm)	Concentration of IAA (ppm)
MB 7.2	0.36	20.36
MB 6.1	0.35	19.66
MB 3	0.33	18.38
MB 9.2	0.31	17.58
MB 10.2	0.29	16.58
MB 2.1	0.28	15.88
MB 4.2	0.28	15.69
MB 6.2	0.28	15.63
MB 7.1	0.27	15.41
MB 1	0.25	14.02
MB 9.3	0.20	11.41

The results of measuring IAA concentration levels quantitatively were not significant with IAA measurements qualitatively, where in the qualitative test the color changes obtained were color changes from yellow (control) to solid yellow. Quantitative measurements of the IAA concentration levels in the mahogany stand in Takalar District and in Maros Regency, had not significantly different where the IAA levels were high in the mahogany stand in Takalar District, at 19.69 ppm, the sample code (MT 3.2, Genus *Penicillium*), while in the mahogany stand in Maros Regency, at 20.36 ppm (MB 7.2, Genus *Trichoderma*), from the two samples of different provenances, it can be seen that geographical factors and environmental factors did not show a significant difference, where samples in Takalar District had the pH content is 6.5 with an altitude of 22 mdpl, while the sample in Maros Regency has a pH content of 6.1 with an altitude of 418 mdpl.

The research results conducted by [21] said that the production of IAA tended to increase in the 2 days incubation period and decreased during the 6 days incubation period. The isolates of the *Aspergillus* genus had the lowest IAA concentration compared to all of the isolates which were 10.27 ppm. The results obtained in this study were higher when compared with the results obtained from [22] which were 1.88 ppm after 7 days of incubation. This isolate had a low ability to produce IAA, but it could add to the completeness of the fungus as a microbial constituent of biological fertilizers. In

fungal isolates with the *Fusarium* genus having IAA concentrations of 12.80 ppm, the results obtained in this study were lower when compared with the results obtained from [11] which obtained the highest number in *Fusarium* isolates of 15.44 ppm which were incubated for 7 days, whereas in isolates with the genus *Penicillium* with sample code MT 7.2 with 19.69 ppm IAA concentration. The results obtained from this research were higher if compared with the results of the research conducted by [23], namely 1.9 ppm for the *Penicillium sp.* genus which was incubated for 5 days.

Rhizopus isolate has a concentration of IAA between 13.47 ppm to 19.66 ppm. The results obtained in this research were higher than the results obtained by [24], which were 13.38 ppm incubated for 7 days. *Trichoderma* isolates had IAA concentrations between 14.02 ppm to 20.36 ppm. The results obtained in this research were higher when compared with the results obtained by [25] which was 9,656 ppm with an incubation period of 3 days, but at the seven-day incubation period IAA production decreased to 4,049 ppm. The IAA concentration produced by these isolates is indeed very small but these isolates could stimulate plant growth because these fungi produce plant growth fitohormones [25].

According to [26] obtained fungal isolates capable of producing IAA can provide new formulations in improving plant growth. Isolates capable of producing IAA can be used as biological controllers through competition, antibiotic production, plant resistance induction, fit-hormone production and increased nutrient availability through N fixation or increased organic and inorganic phosphate solubility. The fungus isolates found in Mahogany Stands at two different provenances were able to produce different IAA concentrations, Therefore, isolates that have been found in Mahogany Standing plants can be applied as biological fertilizers and can be applied directly to the soil around the plant, as plant growth booster to get optimal growth.

4. Conclusion

Based on the results of the research conducted, it can be concluded that the results of exploration of rhizosphere fungi in Mahogany stands in Takalar Regency were obtained 17 which belonged to 5 genus namely *Rhizopus*, *Fusarium*, *Aspergillus*, *Penicillium* and *Gliocladium*, while Mahogany stands in Maros Regency were obtained 11 which belonged to 4 genus namely *Trichoderma*, *Gliocladium*, *Rhizopus* and *Aspergillus*.

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