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Endophytic Fungi Producing Indole Acetic Acid from Melastoma malabathricum L. and Rhodomyrtus tomentosa (Aiton) Hassk. In Indonesia

Fungi Endofit Penghasil Asam Indol Asetat Asal *Melastoma malabathricum L.* Dan *Rhodomyrtus tomentosa* (Aiton) Hassk. Di Indonesia

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ABSTRACT

Melastoma and Rhodomyrtus are known to have widespread distribution and considered as medicinal plants in Indonesia. However, information about the diversity of endophytic fungi in Melastoma malabathricum (MM) and Rhodomyrtus tomentosa (RT) is limited, and their potential as an alternative source of indole acetic acid (IAA) has not previously available. The purpose of this study was to determine the diversity of endophytic fungi from MM and RT which have the potential as an alternative source of IAA and potentially induce the germination of Capsicum annuum L. seeds. The research consisted of isolation of endophytic fungi, IAA production analysis, and capsicum seed germination test. Samples of MM and RT were obtained from Kelubi, East Belitung, Indonesia on August 7th, 2017. Endophytic fungi were isolated from various organs including : leaves, roots, stems, flowers, and fruits using surface sterilization method on the Malt Extract Agar (MEA). After purification, a total of 33 fungi were obtained (15 isolates from MM and 18 isolates from RT). Each endophytic colony shows unique and varied colony on Potato Dextrose Agar (PDA) . 17 endophytic fungal isolates in this study tested positive for IAA production. All isolates produced different levels of IAA, where MIVA2, MIVD1, and MIVA3 had the highest levels of IAA concentrations (89 ppm, 82 ppm, and 70 ppm respectively). In the term of seed germination, there were 5 isolates which have high potential in inducing C. annuum L. seeds, namely; MIVA3, MIVF3, RIVD2, RIVD5, RIVD6, RIVD9 (90%, 95%, 100%, 90%, 100%, 90%, respectively).

Keywords: *Melastoma malabathricum L., Rhodomyrtus tomentosa (Aiton) Hassk.,Endophytic Fungi, Divesity, IAA, Seeds Germination.*

ABSTRAK

Melastoma dan *Rhodomyrtus* merupakan tanaman yang terdistribusi luas dan digunakan sebagai bahan pengobatan tradisional di Indonesia. Namun hingga saat ini, informasi mengenai keragaman fungi endofit dari *Melastoma malabathricum* (MM) dan *Rhodomyrtus tomentosa* (RT) masih sangat terbatas dan potensinya sebagai sumber alternatif asam indol asetat (IAA) belum pernah dilaporkan sebelumnya. Tujuan dari penelitian ini adalah untuk mengetahui keragaman fungi endofit asal MM dan RT yang memiliki potensi sebagai sumber IAA guna meningkatkan perkecambahan biji *C. annuum* L. Penelitian dibagi menjadi 3 tahapan: isolasi fungi endofit, analisis produksi IAA, dan uji perkecambahan biji cabai. Sampel MM dan RT dikoleksi dari Desa Kelubi, Belitung Timur, Indonesia pada tanggal 7 Agustus 2017. Fungi endofit diisolasi dari beberapa organ tumbuhan diantaranya daun, akar, batang, bunga, dan buah dengan menggunakan teknik sterilisasi permukaan dan diisolasi pada *Malt Extract Agar* (MEA). Setelah pemurnian, sebanyak 33 fungi endofit (15 isolat dari MM dan 18 isolat dari RT) berhasil

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dikoleksi. Setiap koloni endofit menunjukkan karakter yang unik dan bervariasi pada *Potato Dextrose Agar* (PDA). Sebanyak 17 fungi endofit pada penelitian ini mampu memproduksi IAA. Semua isolat memiliki kadar produksi IAA yang berbeda, dengan MIVA2, MIVD1, dan MIVA3 memiliki konsentrasi paling tinggi (89 ppm, 82,ppm, dan 70 ppm secara berurutan). Sementara itu, 5 isolat fungi endofit menunjukkan aktivitas pemicu perkecambahan biji cabai tertinggi oleh MIVA3, MIVF3, RIVD2 RIVD5, RIVD6, RIVD9 (90%, 95%, 100%, 90%, 100%, 90% secara berurutan).

Kata kunci: *Melastoma malabathricum* L., *Rhodomyrtus tomentosa* (Aiton) Hassk. Fungi Endofit, Keragaman, IAA, Perkecambahan Biji

INTRODUCTION

Plants are facing many fluctuate circumstances in nature such as biotic and abiotic stress conditions. Symbioses with beneficial microbes can enhnace plant growth and its physiological status (Gamalero *et al*. 2009). Plants and fungi mutualistic association create a very positive impact for both partners. All plants associated with many kind of endophytic fungal communities (Arnold and Lutzoni 2007). Fungal endophytes are symbiotic organisms live in tissues of healthy plants to establish a harmonious relationship with their host without causing any visible disease symptoms **(**Schulz and Boyle 2005). Moreover, endophytic fungal can have many beneficial effects on plants, such as promoting growth, reducing disease severity, producing anti-herbivory products and inducing plant defense mechanisms, (Strobel and Daisy 2003; Melnick *et al*. 2011). This capability has been considered due to their potential to produce active metabolites and enzymes (Yuan *et al*. 2010). Fungi enhance plant growth by many mechanisms including production of phytohormones (Radhakrishnan *et al*. 2015). Among metabolites, plant hormones like Gas and auxin production is a new phenomenon in the endophytic fungi.

Many of the fungal endophytes isolated from diverse plants were reported for their ability to produce plant growth promoting hormones like indole acetic acid (IAA) (Hassan 2011; Waqas *et al*. 2012). Interestingly, most of endophytic fungi isolated from a particular host was shown to produce same chemical compound as produced by their respective host (Aly *et al*. 2010), which undoubtedly showed their importance for host-plants to enhance plant growth against biotic and abiotic stresses (Schulz and Boyle 2005). In addition, researcher reported that IAA secreted by fungal endophytes can improve plant growth and crop productivity (Hassan 2011) and also improve root proliferation and plant biomass (Hoffman *et al*. 2013). Recently, endophytic fungi such as *Aspergillus, Chaetomium, Exophiala, Fusarium, Paecilomyces, Penicillium* and *Phoma* colonizing the tissues of aerial plant parts have been shown to produce IAA (Mishra *et al*. 2014; Khan *et al*. 2015).

Sengon which is plant of *Melastoma* and *Rhodomyrtus* have traditionally been known for their medicinal uses in Indonesia. *Melastoma malabathricum* L. belongs to family Melastomaceae, and it is widely distributed in south-East Asian region and northern Australia (Meyer 2001). This plant is a small shrub, which being an important source in Chinese, Indian, Malay and Indonesian ethno-medicine. *Rhodomyrtus tomentosa* (Aiton) Hassk the member of the *Myrtaceae* family, is an abundant evergreen shrub native to Southeast Asia, with rose-pink flowers and dark purple edible bellshaped fruits (Winotai *et al*. 2005). In the previous study, 213 culturable endophytic fungi isolated from leaves and branches of RT from Thailand (Jeenkeawpieam *et al*. 2012), 93 endophytes fungi isolated from MM in India (Sarbadhikary *et al*. 2016), 20 isolates from MM in Malaysia (Ngieng 2013), and 28 isolates from MM in Indonesia (Octavianti *et al*. 2017). RT is commonly known as *keremunting* by local people in Belitung Island-Indonesia, and usually growth together with MM. Soil condition in Belitung Island generally has the properties of a low range of pH (below 5), which poses stress environmental condition for plant to grow (Panggabean *et al*. 2016).

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This condition makes the interaction of plants and their endophytic symbionts very interesting to be studied. Furthermore, limited information was found on the diversity of endophytic fungi from MM and RT in Indonesia as well as their potency as altenative source of IAA.

Present day agriculture is based on high inputs of agrochemicals. In order to optimize the conventional techniques of agriculture, innovative methods based on microbial inoculation are recently gaining more interest. As endophytes are generally harmless to the plant and have better plant growth promoting activities, enhancing plant growth promoting endophytes may open a new area in the field of organic agriculture. Considering the beneficial characteristics of endophytic microorganisms, the objective of this study was to observe the diversity and select endophytic fungi from MM and RT with potential for promoting the germination of *C. annuum* L. seedling, one of the important agriculture comodities in Indonesia.

MATERIAL AND METHOD

Sample Collection

Melastoma malabathricum L. (MM) and *Rhodomyrthus tomentosa* (Aiton) Hassk. (RT) were collected from Kelubi, East Belitung, Indonesia, on August 7th, 2017. Samples were taken from 2 plants at one location. Leaves, roots, stem, fruits, and flowers were cut directly from one tree. Samples were labelled and put in an ice box and brought to the laboratory. The isolation was performed on the day when the samples arrived at the laboratory. Environmental conditions of MM and RT were measured (Table 1) in situ.

Isolation of Endophytic Fungi

Endophytic fungi were isolated according to Okane *et al*. (2008). Dried samples were cut into 4 segments (0.5 x 0.5 cm^2) and put on half-strength MEA (SIGMA) and incubated at 25˚C for 1-2 weeks. Mycelium which growing from each plant segments was isolated and purified by hyphal tip method using fine tungsten needle in the PDA(1L : 200 gr of potato, 20 gr of dextrose, 15 gr of agar).

Morphology Observation of Fungal Endophyte

The fungi were firstly grouped on the bases of their colony appearance on PDA such as colony shape, color, elevation, texture, mycelia type, edges, density, and diameter (Putra *et al.* 2015). Fungal colonies with similar characteristics were grouped into the same morphotypes. Microscopic observation was also carried out to verify the morphotyping by using OLYMPUS BX53 (Olympus, Japan).

Colonization rates

The colonization formula was defined as follows:

$$\text{Colonization rates} = \frac{\text{Total number of segments colonized by endophytic fungi}}{\text{Total number of segments observed}} \times 100\%$$

IAA Production Analysis

IAA was detected calorimetrically in the supernatants of the fungal cultures using Gordon and Weber's reagent (1 ml 8.12% $FeCl_3.6H_2O$, 50 ml $HClO_4$35% in dark bottle). The isolates were grown overnight in modified nutrient broth M26 (0.5% NaCl, 1% pepton, 1% *beef extract*). About 100 µl overnight culture were added to 10 ml of minimal salt medium (0.136% KH_2PO_4, 0.213% Na_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$, and 10 ml trace element. Trace element was consisted of 700 mg $CaCl_2 \cdot 2H_2O$, 300 mg $FeSO_4 \cdot 7H_20$, 20 mg $MnSO_4 \cdot H_20$, 40 mg $CuSO_4 \cdot 5H_2O$, 20 mg $ZnSO_4 \cdot 7H_2O$, 3 mg H_3BO_3, 7 mg $CoCl_2 \cdot 6H_2O$, 4 mg $Na_2MoO_4 \cdot H_2O$, and 1 ml H_2SO_4 per 1 liter) with supplemented by 1 ml L-tryptophan (10% glucose, 1% L-Triptofan and 0.1% yeast extract, filtered by using *milipore* 0.2 µm). After further incubation for 48 h, IAA test was performed as described by Gordon and Weber (1951). Fungal cells were removed from the culture medium by centrifugation (8000 rpm, 4ºC, 10 min), 2 ml of the above reagent mixed with 1 ml of culture

supernatant, followed by incubation at room temperature for 25 min. The absorbance at 530 nm was measured by spectrophotometer using minimal salt medium with tryptophan (3 replicates for each strain.). The concentration of IAA in each culture medium was determined by comparison to a standard curve of IAA (Gordon and Weber 1951).

Seed Germination Test

Seed germination was done (3 replications) reffering to Willia *et al*. (2013) with modification. The surface of *C. annuum* L. seeds (Tanjung variety from Vegetable Research Center, Lembang) were sterilized in 50°C hot water for 15 minutes, then followed by 70% of ethanol for 60 seconds. *C. annuum L.* seeds were then inoculated to pure culture of endophytic fungi isolates in the Petri dish and incubated at 25˚C for 7 days.

RESULTS AND DISCUSSION

Samples of MM and RT were collected from natural forest ecosystem (Table 1). The plant samples grew vigorously (not producing any disease symptoms) and healthy in ideal enviromental condition, so it is assumed that the collected fungi was not pathogen. The total number of endophytic fungi appeared from parts of plant varied. Most of organs harbored one colony of fungal endophytes, but some were either free or contained more than one fungal endophyte (Table 2). The first fungal endophyte colony emerged after 3 days incubation, and most of the colonies showed up between 3-7 days after incubation. After purification, a total of 33 fungal were obtained **(**15 isolates from MM and 18 isolates from RT).

MM has contained fewer number of endophytic fungi compared to RT. The population of fungi per plant organ also varied. All host organs of MM have endophytic partner except in the flower as well as stem, flower and fruit of RT. Putra *et al*. (2015) reported that the distribution of some fungal species within the host also varied depending on their microhabitat. Moreover, this is also probably caused by the isolation method used in this research is limited to non-biotrophic symbionts. Leaves are the organ with the highest number of fungi isolated from RT in this study. The number of endophytes isolated from each plant may be affected by plant species and organ types. Arnold and Lutzoni (2007) reported that single plant may poses hundreds of different endophytic fungi.

The colonization degree of organ-based endophytic fungi suggests that there were endophytic fungal preference to the host's organ (Figure 1). The colonization rates on the leaves were higher than roots in RT, while MM fruit were more colonized by endophytes compared to RT. Similar to other organs, level of colonization in both hosts is not particularly patterned. Glienke *et al*. (2002) reported that 81% of the total 433 endophytes successfully isolated came from leaf organs. This suggests that the colonization of endophytes concentrated in the leaves may influenced by the ability of the organs to provide a preferred growth environment by endophytes. Moreover, leaves are the organ which known actively performs photosynthesis so that nutrition is not a limiting growth factor for endophytic fungi. Previous studies confirmed that leaves were hot spot for the diversity of endophytic fungi in the tropics (Arnold and Lutzoni 2007)**.** However, endophytic fungi obtain from MM fruit were higher than leaf. It is likely because fruit also contain high nutrition concentration for fungi endophytes in MM. Thirty-three fungal isolates from MM and RT were recovered and the morphologichal properties were characteristized. Each endophytic colony shows unique and varied appearance of colonies on PDA (Figure 2; Table 3). Based on colony diameter, some of them were identified as slow growing endophyte while the others colonize PDA with larger diameter in 11 days.

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Table 1. Environmental Parameters of MM and RT Ecosystem

Parameters	Details
Latitude	-2.8954823
Longitude	108.1107134
Height (m ASL)	500
RH (%)	76
T(°C)	27
Wind (MPH)	14.3

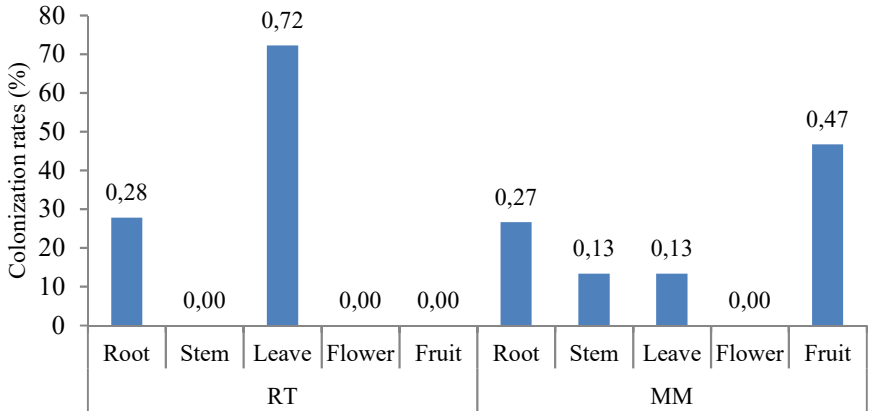

Figure 1. Colonization rates of endophytic fungi

Table 2. The number of endophytic fungi isolated from each plant's organ

No	Host	Total Isolates	No	Host Organ	Isolate Number	Isolate code
1	RT	18	1	Root	5	RIVA1, RIVA2, RIVA3, RIVA4, RIVA5
			2	Stem	-	-
			3	Leave	13	RIVD1, RIVD2, RIVD3, RIVD4, RIVD5, RIVD6, RIVD7, RIVD8, RIVD9, RIVD10, RIVD11, RIVD12, RIVD13
			4	Flower	-	-
			5	Fruit	-	-
	Total					18
2	MM	15	1	Root	4	MIVA1,MIVA2,MIVA3,MIVA4
			2	Stem	2	MIVB1,MIVB2
			3	Leave	2	MIVD1,MIVD2
			4	Flower	-	-
			5	Fruit	7	MIVF1,MIVF2,MIVF3,MIVF3,MIVF4,MIVF5,MIVF6,MIVF7
	Total					15

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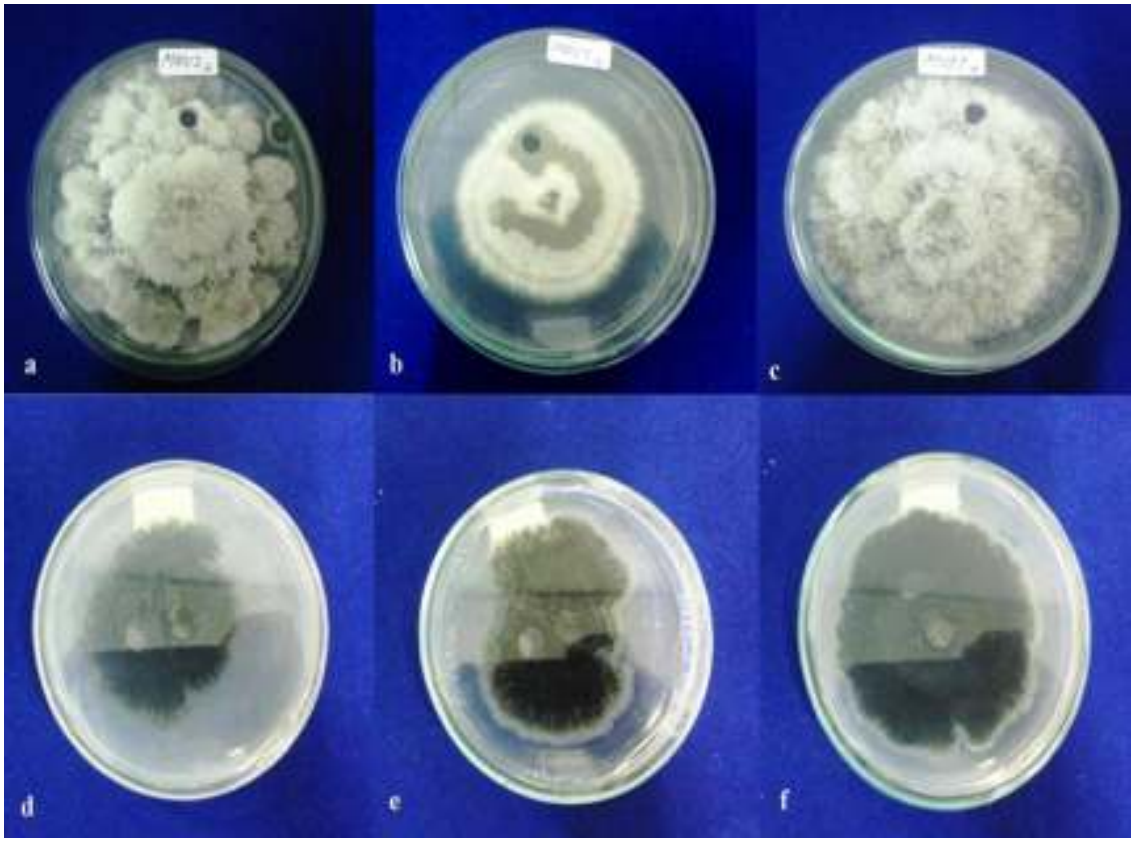

Figure 2. Some cultures of endophytic fungi from this study grown on PDA at room temperature after 7 days. a: MIVA2; b: MIVA3; c: MIVD1; d: RIVD5; e: RIVD10; f: RIVD11

Table 3. Colony characteristics of the Endophytic Fungi on PDA

No	Isolate code	Shape	Colour		Elevation	Texture	Mycelium	Edge	Size (cm) of colony after 11 days
			Above	Reverse					
1	RIVA1	Circullar	Brown	Cream To Yellow	Raised	Cottony	Aerial	Entire	7.2
2	RIVA2	Circullar	Dark Green	Cream To Yellow	Raised	Cottony	Aerial	Entire	7.2
3	RIVA3	Irregular	White	Cream	Raised	Fluffy	Aerial	Dentate	8
4	RIVA4	Circullar	White	Cream	Flat	Cottony	Aerial	Filamentous	8.5
5	RIVA5	Circullar	White	Cream	Flat	Velvety	Aerial	Filamentous	9.5
6	RIVD1	Irregular	White,Black	Cream To Yellow	Convex	Rocky	Immersed	Dentate	9
7	RIVD2	Circullar	White	Cream	Raised	Rocky	Aerial	Entire	3
8	RIVD3	Irregular	Black,White	Cream To Yellow	Convex	Rocky	Immersed	Dentate	7.4
9	RIVD5	Irregular	Black,White	Cream To	Convex	Rocky	Immersed	Dentate	2.5

				Yellow					
10	RIVD6	Irregular	Black,White	Cream To Yellow	Convex	Rocky	Immersed	Dentate	2.5
11	RIVD7	Irregular	Black,White	Cream	Convex	Rocky	Immersed	Dentate	2.8
12	RIVD8	Circullar	White	Cream	Raised	Cottony	Aerial	Entire	2.7
13	RIVD9	Irregular	Black,White	Cream To Yellow	Raised	Rocky	Immersed	Dentate	9
14	RIVD10	Irregular	Black,White	Cream To Yellow	Convex	Rocky	Immersed	Dentate	2.6
15	RIVD11	Irregular	Black,White	Cream To Yellow	Convex	Rocky	Immersed	Dentate	3.2
16	RIVD14	Circullar	White	Cream	Raised	Cottony	Aerial	Entire	1.5
17	RIVD15	Circullar	White	Cream	Raised	Cottony	Aerial	Entire	8.4
18	RIVD16	Irregular	Black,White	Cream To Yellow	Convex	Rocky	Immersed	Dentate	9
19	MIVF3	Irregular	Grey	Grey	Flat	Fluffy	Aerial	Undulate	2
20	MIVF4	Circullar	White	Cream	Raised	Cottony	Aerial	Undulate	8.3
21	MIVF5	Circullar	White	Cream	Convex	Fluffy	Aerial	Filamentous	8.3
22	MIVF6	Circullar	White	Cream	Convex	Cottony	Aerial	Dentate	8.2
23	MIVF7	Circullar	White	Cream	Raised	Cottony	Aerial	Dentate	8.5
24	MIVA1	Circullar	White	Cream	Raised	Rocky	Immersed	Entire	2.5
25	MIVA2	Irregular	White	Cream	Flat	Cottony	Aerial	Dentate	9
26	MIVA3	Circullar	White	Cream	Raised	Cottony	Aerial	Entire	9
27	MIVA4	Circullar	White,Cream	Cream To Yellow	Raised	Cottony	Aerial	Entire	9
28	MIVB1	Irregular	White	Cream	Raised	Cottony	Aerial	Dentate	6.5
29	MIVB2	Circullar	Grey	Grey	Flat	Fluffy	Aerial	Filamentous	7
30	MIVD1	Irregular	White	Cream	Flat	Fluffy	Aerial	Entire	7
31	MIVD2	Circullar	White	Cream	Flat	Cottony	Aerial	Entire	9
32	MIVF1	Circullar	White	Cream	Flat	Cottony	Aerial	Filamentous	9
33	MIVF2	Irregular	White	Cream	Flat	Cottony	Aerial	Entire	9

To the best of our knowledge, this is the first report of endophityc fungi producing IAA from MM and RT in Indonesia. Endophytic fungi has been reported as a vital necessity for the host plant to survive the unforeseen climatic and environmental changes in the rhizosphere and phyllosphere (Rodriguez *et al*. 2009; Rim *et al*. 2007). Reports by Khan *et al*. (2015) and Mishra *et al*. (2014), have shown that endophytic symbiosis can assist the host in any environmental conditions by the potential to produce various types of biologically active metabolites and enzymes such as gibberellins and IAA. 17 endophytic fungal isolates in this study tested positive for IAA production. The isolates possed different levels of IAA production with MIVA2, MIVD1,and MIVA3 were produced the highest IAA concentration levels (89 ppm, 82 ppm, and 70 ppm respectively) (Figure 3). Several studies have reported the potential of endophytic fungi to produce IAA (Khan *et al*. 2015; Waqas *et al*. 2012). IAA

production by endophytic fungi in this study varied in levels of concentration (Fig. 3). However, it seems that IAA production in culture medium was not always correlatively positive with seed germination which inoculated with endophytic fungi.

Turning to seed germination (Table 4), all of the isolates induced *C. annuum* L. Seed**.** The pods number of the malica soybean plant (133.80 pieces) was significantly higher than the pods number of 3 Prida (110.17). The different genetic characteristics between the two soybean varieties are thought to have an effect on their appearance. In the opinion of Gabesius et al. (2012) stated that the diversity of the number of pods produced between varieties is influenced by the genetics of the dominant variety, and is also supported by germination. Subash *et al*. (2014) reported that plant regulators including IAA have important roles on seed germination and seedling quality character. There were 6 isolates which have high potential of inducing *C. annuum* L. seed germination in this research, namely; MIVA3, MIVF3, RIVD2, RIVD5, RIVD6, RIVD9 (90%, 95%, 100%, 90%, 100%, 90%, respectively). RIVD2 and RIVD6 induced the highest seed vaiability in this research. Based on IAA concentration in culture medium, MIVA3 was the only isolate which consistently performed high result both in medium and seed germination. In this study, *C. annuum* L. germination test is carried out at 25ºC for 7 days. According to Maynard and Hochmuth (1997), *C. annuum* L. germination is optimal between 22 and 30ºC, and the optimal germination time is 8-10 days.

However, since seed germination is a complex adaptive trait which is influenced by a large number of genes and environmental factors (Koornneef *et al*. 2002) as well as plant growth hormone, water, light, oxygen, salinity, and temperature are also important factors in seed germination (Zia and Khan 2004), it is imply that there is a need to examine those factors in our future research.

Table 4. IAA production and Seed Germination Test Using Endophytic Isolates

No.	Isolate	IAA production (ppm)	Seed Viability (%)	No.	Isolate	IAA production (ppm)	Seed Viability (%)
1	MIVA1	14	85	10	RIVD 3	14	85
2	MIVA2	89	80	11	RIVD 5	18	90
3	MIVA3	70	90	12	RIVD 6	18	100
4	MIVA4	3	85	13	RIVD 7	10	50
5	MIVD1	82	55	14	RIVD 9	8	90
6	MIVF3	30	95	15	RIVD 10	14	85
7	RIVA4	20	60	16	RIVD 16	8	65
8	RIVD1	9	80	17	RIVD 11	3	85
9	RIVD2	9	100				

CONCLUSION

A total of 33 fungi were obtained (15 isolates from MM and 18 isolates from RT) from this study. Each endophytic colony shows unique morphological charasteristic on PDA. MIVA2, MIVD1, and MIVA3 were the isolates which produced the highest levels of IAA concentrations (89 ppm, 82 ppm and 70 ppm respectively). MIVA3, MIVF3, RIVD2, RIVD5, RIVD6, RIVD9 induced the highest germination of *C. annuum* L. seed (90%, 95%, 100%, 90%, 100%, 90%, respectively). However, MIVA3 was the only endophytic fungi poses both high production of IAA in culture and enhance high germination of *C. annuum* L. seed. MIVA3 is considered to be our potential isolate in the future works.

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REFERENCES

Aly, A. H., Debbab, A., Kjer, J., & Proksch, P. 2010. Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. *Fungal Diversity*, 41(1),1-16.

Arnold, A. E., & Lutzoni, F. 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots?. *Ecology*, *88*(3), 541-549.

Gamalero, E., Lingua, G., Berta, G., & Glick, B. R. 2009. Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. *Canadian Journal of Microbiology*, *55*(5), 501-514.

Glienke-Blanco, C., Aguilar-Vildoso, C. I., Vieira, M. L. C., Barroso, P. A. V., & Azevedo, J. L. 2002. Genetic variability in the endophytic fungus Guignardia citricarpa isolated from citrus plants. *Genetics and Molecular Biology*, *25*(2), 251-255. Gordon, S. A., & Weber, R. P. 1951. Colorimetric estimation of indoleacetic acid. *Plant physiology*, *26*(1), 192.

Hasan, H. A. H. 2011. Gibberellin and auxin production by plant root-fungi and their biosynthesis under salinity-calcium interaction.*Plant, Soil and Environment,* 48(3), 101-106.

Hoffman, M. T., Gunatilaka, M. K., Wijeratne, K., Gunatilaka, L., & Arnold, A. E. 2013.Endohyphal Bacterium Enhances Production of Indole-3-Acetic Acid by a Foliar Fungal Endophyte. *PLoS ONE*, 8(9), e73132.

Jeenkeawpieam, J., Phongpaichit, S., Rukachaisirikul, V., & Sakayaroj, J. 2012. Antifungal activity and molecular identification of endophytic fungi from the angiosperm *Rhodomyrtus tomentosa*. *African Journal of Biotechnology*, *11*(75), 14007-14016.

Khan, A. R., Ullah, I., Waqas, M., Shahzad, R., Hong, S. J., Park, G. S., & Shin, J. H. 2015. Plant growth-promoting potential of endophytic fungi isolated from *Solanum nigrum* leaves. *World Journal of Microbiology and Biotechnology*, *31*(9),1461-1466.

Koornneef, M., Bentsink, L., & Hilhorst, H.2002. Seed dormancy and germination. *Current opinion in plant biology*, *5*(1), 33-36.

Maynard, D. N., & Hochmuth, G. J. 1997. *Knott's handbook for vegetable growers* (No. Ed. 4). John Wiley and Sons, Inc.

Melnick, R. L., Suárez, C., Bailey, B. A., & Backman, P. A. 2011. Isolation of endophytic endospore-forming bacteria from *Theobroma cacao* as potential biological control

agents of cacao diseases. *Biological Control*, *57*(3), 236-245.

Meyer, K.2001. Revision of the Southeast Asian genus *Melastoma* (Melastomataceae).Blumea: *Journal of Plant Taxonomy and Plant Geography*. 46. 351-398.

Mishra, Y., Singh, A., Batra, A., & Sharma, M. M. 2014. Understanding the biodiversity and biological applications of endophytic fungi: a review. *J. Microbiol. Biochem. Technol. S*, *8*.

Ngieng, N. S., Zulkharnain, A., Roslan, H. A., & Husaini, A. 2013. Decolourisation of synthetic dyes by endophytic fungal flora isolated from senduduk plant (*Melastoma malabathricum*). *ISRN biotechnology*, 1-7.

Octavianti, R., harahap, I., & Elsie, E. 2017. Isolation of Fungal Endophytes from Melastoma malabathricum L. and Their Potential as Antifungal. *BIO-SITE*, *Biologi Dan Sains Terapan*, 3(1), 19-24.

Okane, I., Toyama, K., Nakagiri, A., Suzuki, K. I., Srikitikulchai, P., Sivichai, S., ... & Læssøe, T. 2008. Study of endophytic Xylariaceae in Thailand: diversity and taxonomy inferred from rDNA sequence analyses with saprobes forming fruit bodies in the field. *Mycoscience*, *49*(6), 359-372.

Panggabean, M.T., Amanah, S. & Tjitropranoto, P. 2016. Persepsi Petani Lada terhadap Diseminasi Teknologi Usahatani Lada di Bangka Belitung. *Jurnal Penyuluhan*, 12 (1): 61-73.

Putra, I. P., Rahayu, G., & Hidayat, I. 2015. Impact of domestication on the endophytic fungal diversity associated with wild Zingiberaceae at Mount Halimun Salak National Park. *HAYATI Journal of Biosciences*, *22*(4), 157-162.

Radhakrishnan, R., Khan, A. L., Kang, S. M., & Lee, I. J. 2015. A comparative study of phosphate solubilization and the host plant growth promotion ability of *Fusarium verticillioides* RK01 and *Humicola* sp. KNU01 under salt stress. *Annals of microbiology*, *65*(1), 585-593.

Rim, S. O., Lee, J. H., Khan, S. A., Lee, I. J., Rhee, I. K., Lee, K. S., & Kim, J. G. 2007. Isolation and identification of fungal strains producing gibberellins from the root of plants. *Microbiology and Biotechnology Letters*, *35*(4), 357-363.

Rodriguez, R. J., White Jr, J. F., Arnold, A. E., & Redman, A. R. A. 2009. Fungal endophytes: diversity and functional roles. *New phytologist*, *182*(2), 314-330.

Sarbadhikary, S. B., Pal, B., & Mandal, N. C.2016. Antimicrobial and antioxidant activities of two endophytic fungi isolated from *Melastoma malabathricum* L. leaves. *Int J Curr Pharm Res*, *8*(3), 88-92. ISSN- 0975-7066

Schulz, B., & Boyle, C. 2005. The endophyte continuum. *Mycological Research*. 109(6): 661-686.

Strobel, G., & Daisy, B. 2003. Bioprospecting for microbial endophytes and their natural products. *Microbiol. Mol. Biol. Rev.*, *67*(4), 491-502.

Subash, M., Rafath, H., & Lalitha, J. 2014. Influence of GA3 and IAA and their Frequency of Application on Seed Germination and Seedling Quality Characters. *International Letters of Natural Sciences,* 30, 44–48.

Waqas, M., Khan, A. L., Kamran, M., Hamayun, M., Kang, S. M., Kim, Y. H., & Lee, I. J. 2012. Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. *Molecules*, 17(9), 10754-10773.

Wilia, W., Widodo, Wiyono, S. 2013. Exploration of Endophytic Fungi from Pepper as Biological Control Agents of Anthracnose

(*Colletotrichum acutatum L.*). *Bioplantae*, 2(1), 9-15.

Winotai, A., Wright, T., & Goolsby, J. A. 2005. Herbivores in Thailand on *Rhodomyrtus tomentosa* (Myrtaceae), an invasive weed in Florida. *Florida Entomologist*, *88*(1), 104-106.

Yuan, Z. L., Lin, F. C., Zhang, C. L., & Kubicek, C. P. 2010. A new species of Harpophora (Magnaporthaceae) recovered from healthy wild rice (*Oryza granulata*) roots, representing a novel member of a beneficial dark septate endophyte. *FEMS microbiology letters*, *307*(1), 94-101.

Zia, S., & Khan, M. A. 2004. Effect of light, salinity, and temperature on seed germination of *Limonium stocksii*. *Canadian Journal of Botany*, *82*(2), 151-157.