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# Dark septate endophytes symbiosis in Falcataria moluccana and its potency for forest and landscape restoration

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**ABSTRACT**

**BACKGROUND AND OBJECTIVES:** *Falcataria moluccana* is a potential forest tree species with high economic value in indonesia, and it is widely planted in various forests and landscape restorations. The planting of *F. moluccana* has experienced a decline in productivity attributed to a reduction in site quality and heightened susceptibility to pest and disease infestations. Dark septate endophytes are a group of root endophytic fungi that have potential as biological control and promoter agents. The objective of this study is to evaluate the symbiotic capabilities of various isolates of dark septate endophytes, assess the impact of dark septate endophytes on the sprouting of *F. moluccana* seeds, and identify the secondary metabolites generated as a result of this symbiotic relationship.

**METHODS:** The study encompassed the employment of three isolates of dark septate endophytes, specifically *Rhizopycnis vagum*, *Curvularia sp.*, and *Dendrothyrium sp*. Those isolates were inoculated on the seeds of *F. moluccana* and observed the endophytism and metabolite compounds produced after 23 hours and 14 days of inoculation time.

**FINDINGS:** Three new findings of this study were: 1) the dark septate endophytes isolates, i.e., *Rhizopycnis vagum*, *Curvularia sp.*, and *Dendrothyrium sp*. could colonize *F. moluccana* seeds on the surface with a colonization rate of 96 percent, 100 percent, and 32.5 percent, and entered the seed tissue and the sprout roots with a colonization rate of 93.3 percent, 93.3 percent, and 70 percent. The dark septate endophytes colonization could be seen at the beginning of germination, inoculation of 0-23 hours, and after germination until observation at the age of 14 days; 2) The dark septate endophytes has the potential to improve seed germination and vigor of *F. moluccana*, by speeding up the germination process and increasing the vigor index, this condition is expected to produce seeds that can grow and survive environmental stress; 3) The dark septate endophytes has the potential to trigger an increase secondary metabolite compounds in *F. moluccana* seeds, which can be as antibacterial, antifungal, and antioxidants such as the Hexamethyl Cyclotrisilaxane compound, 1-Isopropenyl-4-methyl-1,3-cyclohexadiene, and Acetamide, 2, 2,2-trifluoro-N-methyl-N- (trimethylsilyl) as a form of plant protection when the *F. moluccana* seeds grew and developed to be a normal seedling. These conditions are believed to produce high-quality seedlings that can grow and adapt well to their new environment after being outplanted.

**CONCLUSION:** Colonization of *F. moluccana* seeds by dark septate endophytes does not have a negative impact on seed viability. Therefore, inoculating seeds with dark septate endophytes can serve as an effective seed treatment method, resulting in the production of robust and healthy *F. moluccana* seedlings. This approach can contribute to enhancing the success of planting efforts aimed at restoring degraded forest and landscape areas.

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## INTRODUCTION

Endophytic microorganisms are defined as those microorganisms that inhabit plant tissues for a portion of their life cycle and are capable of establishing themselves within the host without inducing any disease symptoms (Kumar *et al.*, 2020). Endophytic symbiosis has the potential to produce secondary metabolites that function as antibacterial (Bhardwaj and Agrawal, 2014), antifungal (Chaudhary *et al*., 2022), fungicide, and antioxidants (Wen *et al.,* 2022), which can suppress disease in plants and increase the tolerance of host plants to biotic and abiotic environmental stress conditions (Sukapiring, 2016). Endophytes have been reported to provide numerous advantages to their host plants, such as promoting plant growth and defending plants against attacks by plant pathogens (Sari, 2020). Endophytes from the fungal group acting as biological control agents include *Acremonium zeae* (Auer and Ludwig-Müller, 2015), *Phomopis cassia* (Tewari *et al.*, 2019), *Periconia* sp., *Ampelomyces* sp., and *Neotyphodium lolii* (Segaran and Sathiavelu, 2019). Dark septate endophytes (DSE) belonging to the group of beneficial endophytic fungi have been identified as potential biological agents. However, their presence has not been extensively documented in comparison to other endophytic fungi, particularly in tropical Indonesia. DSE fungi are root endophytic fungi capable of colonizing plant tissues both intracellularly and intercellularly without causing disease symptoms while stimulating the growth of their host plants, which include agricultural (Surono and Narisawa, 2017) and forestry plants (Marfuah *et al.,* 2023). The precise nature of their symbiotic function remains unclear; however, numerous investigations have demonstrated that DSE plays a significant part in enhancing the ability of host plants to adapt to and withstand biotic (Dalimunthe *et al.,* 2023) and abiotic (Marfuah *et al.,* 2023) stresses. This enables the plants to maintain their health and vitality in comparison to non-symbiotic plants. Within this mutually beneficial partnership, diverse DSE strains have the capability to generate metabolite compounds that serve to shield plants from a range of stress factors (Dalimunthe *et al*., 2023). One of DSE's unique or distinctive characteristics is that DSE has melanin hyphae and forms microsclerotia in the host plant's root tissues, this is very useful because it makes the host plant tolerant to various stress conditions (Surono and Narisawa, 2018). DSE can suppress the growth of pathogens *Fusarium oxysporum* (Zaffan *et al.*, 2019), and *Ridigoporus microporus* (Dalimunte *et al*., 2023) with various mechanisms such as parasitism and the production of pathogenic antifungal compounds. DSE can suppress pathogens that cause fusarium wilt in *Lycium barbarum* plants (Chen *et al*., 2022) and *Asparagus officinalis* (Surono and Narisawa, 2018). The efficacy of DSE in managing pathogen attacks is intricately linked to its symbiotic relationship with the host plant and the precise method of inoculation. Studies on the association of DSE fungi with tropical forest tree species have not been widely reported. A Few forest trees are associated with DSE, including *Jatropha curcas* (Marfuah *et al.,* 2023), *Acacia leucopoea, Albizia* sp., *Lagerstroemia parviflora Roxb, Cassia fistula Linn* (Tripathi, 2014) and *Pinus merkusii* (Akhir *et al.*, 2024) and various pine plants (Chu *et al*., 2019) in subtropical regions. The potential of endophytes warrants further investigation for their application in the forestry industry, particularly in enhancing the growth of forest trees while addressing the numerous challenges posed by pests and diseases affecting these species. According to Malicka *et al.* (2022), the application of DSE to plants experiencing drought stress can improve the condition of the root system architecture, and physiology, and increase hydraulic properties. The facilitation of plant growth is underpinned by the ease of cultivating DSE, thereby enhancing the potential for DSE to be harnessed in phytoremediation, revegetation, and the restoration of degraded land projects. According to Widyani *et al*., (2024), DSE has the potential to be a green economic alternative that supports environmental sustainability efforts through the application of seed briquettes. Kumar *et al.* (2018) highlighted that DSE is frequently linked with plant species thriving in challenging environmental settings. The application of DSE fungal inoculation technology holds significant potential for the restoration and reforestation of delicate and degraded forest ecosystems (Pandey *et al*., 2016). DSE also has the potential to be used in future conservation programs (Pandey *et al*., 2016). Akhtar *et al.* (2021) suggest that utilizing DSE presents a significant potential for phytoremediation and land reclamation in areas affected by pollution. This will of course also support sustainable agriculture. The use of DSE is essential to be studied, such as in the *Falcataria moluccana* (Miq.) Plant. Barneby and J.W.

Grimes are commonly known as falcata (Mazo *et al.*, 2020). *F. moluccana*, locally known as sengon is one of Indonesia's tropical forest plants with high economic value, especially in Java Island (Rahayu *et al.*, 2021). The growth of the *F. moluccana* tree is presently hindered by a range of pests and diseases. (Nuraeni *et al.*, 2016). Powdery mildew induced by the fungus *Oidium* sp., along with damping-off, caused by various pathogenic fungi such as *Pythium* spp., *Fusarium* spp., *Botryodiplodia* spp., and *Rhizoctonia* spp., as well as tumor rust disease caused by the fungus Uromycladium tepperianum, pose a notable challenge in falcata nurseries (Ryley, 2024). The absence of success in controlling pathogenic fungi in Indonesian *F. moluccana* seedlings, especially using various non-endophytic biological agents, encourages the efforts to use endophytic biological agents. Compared to other biological agents, the advantage of endophytes is their symbiotic potential with host plants so that they do not have to compete in new ecosystems or with microorganisms that have been in the ecosystem for a long time (Perez *et al.*, 2020). The DSE fungus has the potential to be used as a plant endophyte, so it is necessary to study the ability or potential of DSE to colonize *F. moluccana* roots and increase the adaptation and growth of *F. moluccana* which is widely planted in various forest and landscape restoration programs in Indonesia. The present study postulates that DSE fungi, which are acknowledged as endophytic fungi capable of promoting plant growth, may function as endophytes for trees in the forestry industry. DSE would help stimulate growth and protect forestry plants, such as *F. moluccana*, which are extensively cultivated in Indonesia for forest and landscape restoration programs, against both biotic and abiotic stresses. Consequently, additional research is required to confirm the ability and potential of DSE fungi to inhabit the roots of *F. moluccana* and enhance its growth. The aims of the current study were 1) to determine the symbiotic potential of DSE isolates, i.e., *Rhizopycnis vagum* MM12, *Curvularia* sp. PP 2.3, and *Dendrothyrium* sp. CPP 1.1.4 in *F. moluccana* and 2) to determine the effects of DSE on *F. moluccana* seed germination and secondary metabolites produced in those symbiotic interactions. This study was conducted at the Seed Testing Laboratory, Center for Standard and Instrument Implementation of Forestry and Environment, Bogor, and at the Soil Mycology Laboratory, Indonesian Soil Research Institute in Bogor, Indonesia, in 2020.

## MATERIALS AND METHODS

### *Materials*

The DSE fungi, i.e., *Rhizopycnis vagum* MM12, *Curvularia* sp. PP 2.3, and *Dendrothyrium* sp. CPP 1.1.4, was used in this study. The DSE fungal isolates were liquid inocula, namely pieces of mycelium in sterile water with a $10^5$ hyphae milliliter (mL)/ density. The *F. moluccana* seeds that were used have been stored for five years in an airtight container (in the refrigerator) with a temperature of 2-4 degrees Celsius (°C) and humidity of 40-50 percent (%). The seeds were from community forests in Kediri Regency, East Java, Indonesia (7°55'45.5" S, 112°11'38.3 E).

### *Inoculation of DSE isolates in F. moluccana seeds*

This study has been carried out at the Seed Testing Laboratory, Center for Standard and Instrument Implementation of Forestry and Environment, Bogor, and at the Soil Mycology Laboratory, Indonesian Soil Research Institute in Bogor, Indonesia. The seeds underwent sterilization through immersion in hot distilled water at a temperature ranging from 50 to 90 degrees Celsius, followed by a one-hour cooling period in a glass beaker. The seeds were soaked for 23 hours (h) in each DSE solution as treatment, namely *R. vagum, Curvularia* sp., and *Dendrothyrium* sp. Separately. *F. moluccana* seeds soaked in sterile distilled water were used as a control (ISTA, 2016). This activity was carried out at the Laboratory of the Center for Standard and Instrument Implementation of Forestry and Environment, Bogor, Indonesia.

### *Observation of DSE colonization on the surface of seeds, seed viability, and seedling growth*

The seeds that underwent treatment were germinated on sterile straw paper media and subsequently positioned within a Petri dish measuring 12 cm in diameter. The straw paper sterilization was done in an oven at 103°C for two hours. Germination was carried out in a germinator growth room type IPB 73-2A at a temperature of 24–30°C and a humidity of 90–95%. The experiment involved conducting four repetitions of each treatment, with each repetition utilizing 50 *F. moluccana* seeds. The assessment of DSE colonization in the seeds was determined by calculating the percentage of colonized seeds




Fig. 1: Germination of *Falcataria moluccana* in Petri dishes

in relation to the total number of seeds cultivated on the straw paper medium. This study used four treatments (three DSE and one control), with four replicates, each replicate consisted of 50 seeds. Dark hyphae growth was observed on the surface of the shoots, indicating the colonization of DSE fungi in *F. moluccana* seeds (Fig. 1).

The germination response was used to assess the seed viability, indicating the seeds' capacity to sprout under typical environmental conditions. Seed germination is the percentage of the number of seeds that grow into normal sprouts divided by the number of seeds planted (ISTA, 2016). Observations regarding germination criteria revealed that the sprouts measured twice the length of the seeds (ISTA, 2016). Germination observations were carried out every day until the 14th day after incubation or until no seeds germinated. The calculation of seed germination, using Eq. 1 (ISTA, 2016):

Germination percentage (%) =

$$\frac{\sum germinated\ seeds}{\sum sowed\ seeds} x100\ \% \quad (1)$$

The germination speed was observed every day so that the last day of observation for seeds was normal, using Eq. 2 (Lo Porto *et al*., 2019):

Germination speed (% /day) =

$$\frac{nihi+n2h2+n3h3+\ldots\ldots\ldots nihi}{n1+n2+n3+\ldots\ldots\ldots\ldots ni}=\frac{\sum nihi}{\sum ni} \quad (2)$$

ni = Number of seeds germinated on day i (item);

hi = The number of days it took to reach the tinier number of sprouts.

The vigor index was obtained by calculating the days it takes to germinate with the number of germinating seeds. The vigor index is calculated using Eq. 3 (Damalas *et al*., 2019):

$$\text{Vigor index} = \frac{G1}{D1} + \frac{G2}{D2} + \frac{G3}{D3} + \ldots\ldots\ldots \frac{Gn}{Dn} \quad (3)$$

G = the number of seeds that germinate on a certain day; D = time corresponding to that amount;

n = The number of days at the last calculation.

*Observation of DSE colonization in Falcataria moluccana's radicles (roots)*

Root colonization was assessed through the purification and staining of the roots, following a modified procedure Kormanik *et al*. (1980). The number of roots observed for each treatment was 10 roots. The root purification process is soaking the *F. moluccana* root measuring ± 1 cm, in a 10% potassium hydroxide (KOH, Sigma-Aldrich). The submerged roots underwent heating in a water bath set at a temperature of 70°C for a duration of 15 minutes. The roots are rinsed with running water until slimy. The roots were immersed in a 1% hydrochloric acid (HCL, Sigma-Aldrich) solution and heated in a water bath at 70°C for five minutes. The HCl solution was disposed of, and the roots were thoroughly rinsed under running water until they were clean and prepared for the subsequent coloring process. The *F. moluccana* root was stained using 0.01% Fuchsin acid dye, namely 0.1 g of Fuchsin (Sigma Aldrich) dissolved in 1 liter of mixed lactic acid solution (a mixture of 875 ml of lactic acid, 63 ml of glycerol (Merck), and 62 ml

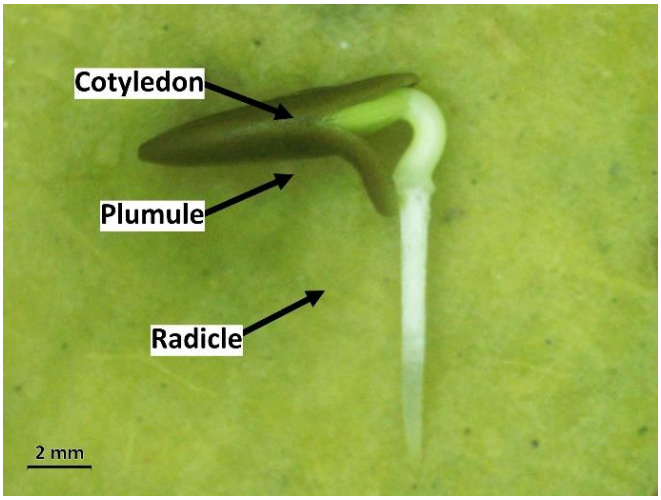



Fig. 2: Sprout structure of *Falcataria moluccana* seed

of ion-free water (Sigma-Aldrich). The *F. moluccana* root pieces were immersed in the lactic acid solution for 24 hours. Observations of DSE colonization were conducted on root segments by positioning the root samples on a glass slide. These preparations were examined microscopically at a magnification of 200 times. The roots-clearing process and the observation of the percentage of colonization in the roots were carried out at the Soil Mycology Laboratory at the Indonesian Soil Research Institute in Bogor, Indonesia. The evaluation of DSE colonization in sprouted roots involved calculating the percentage of colonization within the root shoots (radicles). This calculation was based on the ratio of infected roots exhibiting DSE fungi to the total number of roots observed, 14 days following the incubation of the root sprouts. Another observation response was the observation of the growth of the shoot structure, namely the length of the radicle and plumule (Fig. 2). Each treatment was replicated four times with a total of 10 sprouts in each replication.

*Analysis of secondary metabolite compounds*

The secondary metabolite compounds generated during the initial germination phase and after 14 days of aging of *F. moluccana* sprouts following inoculation with individual DSE isolates were identified. The secondary metabolite compounds were analyzed using gas chromatography-mass spectrometry (GC-MS). The approach for obtaining bioactive compounds from the seed-inoculated DSE fungal isolate was conducted based on the method described by Waruwu *et al*. (2016). The seed inoculated DSE isolate suspension was filtered gradually using Whatman filter paper No. 1. The suspension was centrifuged at 5,000 rpm for 20 minutes at 4°C. The obtained supernatant was filtered again. The Health Laboratory of the Local Government in Jakarta, Indonesia, utilized GC-MS for qualitative analysis of bioactive compounds. Analysis of bioactive compounds by GC–MS used Agilent Technologies 6890 Gas Chromatograph with AutoSampler and 5975N Mass Selective Detector and Chemstation data system at 70 eV with an HP 5 MS column (30 (m) x 0.25 (mm) I.D x 0.25 micrometer (µm) film thickness). The temperature of the injection port was set at 250oC, while the ion source temperature was maintained at 230oC. Additionally, the interface temperature was set to 280oC, and the quadrupole temperature was controlled at 140°C. The initial temperature at 70°C holds for 2 minutes, rising at 5°C per minute to 150°C hold for 10 minutes, rising at 10°C per minute to 250°C hold for 15 minutes. Helium was the carrier gas at a 0.9 mL per minute flow rate. The interpretation of the GC-MS spectrum involved a comparison with information found in the GC-MS library associated with the National Institute of Standards and Technology (NIST). The sample's peak and its corresponding ion fragment were compared against a database of known compounds, leading to

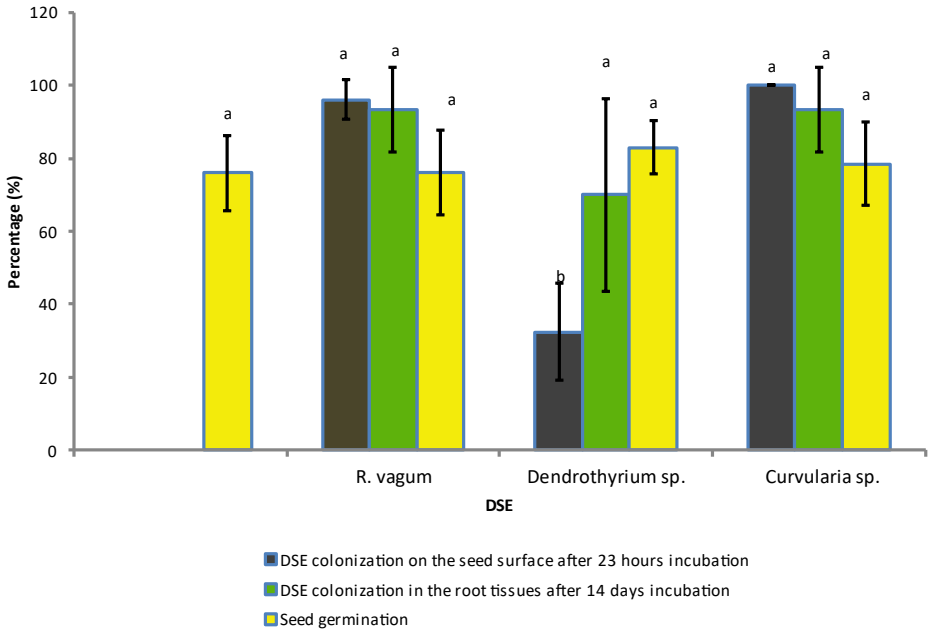



Fig. 3. The percentage of colonization of DSE fungal isolates on the surface of the seeds (after 23 hours incubation) and roots of *F. moluccana* sprouts (after 14 days incubation) and the percentage of germination. Values are means ± standard deviation (SD), total sample size (n) = 4. Means with the same letter were not significantly different ($P < 0.05$) after Tukey's Honestly Significant Difference test

the determination of the percentage similarity.

*Data analysis*

Analysis of variance was performed to determine the treatment effects on the percent colonization, germination percentage, germination speed, vigor index, radicle length, plumule, and secondary metabolite analysis. Prior to analysis, the data, which was presented in percentage form, underwent arcsine and square root transformations in order to achieve normalization. Tukey's honestly significant difference test (Tukey's HSD) was conducted to compare the cultivars if they were different for treatments. The Tukey's HSD test was conducted to compare the means across different sources of inoculum, with a significance level set at ($p < 0.05$). Variance analysis used SPSS 16 Software. The neural network analysis result is the data clustering into groups with similar petrophysical properties (classification). The classification was based on the values of the time-averaged shear-wave velocity to a depth of 30 meters (Vs30), and the horizontal-to-vertical spectral ratio (H/V value).

## RESULTS AND DISCUSSION

The colonization of DSE isolates on the surface of *F. moluccana* sprouts after 23 hours showed a significant correlation with the use of DSE inoculants ($p < 0.05$). Inoculants *R. vagum* and *Curvularia* sp. (96%; 100%) colonized were higher than *Dendrothyrium* sp. (32.5%) (Fig. 3). The colonization rate of the three DSE fungal isolates in the roots of *F. moluccana* sprouts was relatively consistent, with values ranging from 70.00% to 93.33% (Fig. 3). DSE colonized the seeds were still in good health conditions, and did not appear to show any damage or rot.

There is limited research on the colonization of endophytic fungi in forestry tree seeds or during the germination process. The information about the colonization process of seeds from the beginning of germination until the age of 14 days, especially for *F. moluccana* seeds has not been reported as well as seeds from other plants. The DSE fungus exhibits the capability to inhabit host plant seeds following inoculation and during the germination phase. DSE could colonize both on the surface of the seeds and penetrate the *F. moluccana* seed tissues. *F. moluccana*

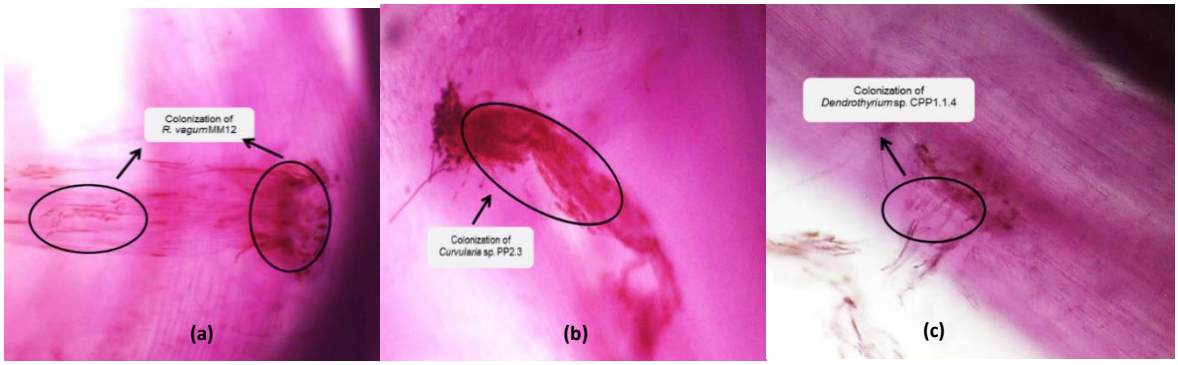



Fig. 4: DSE fungal isolates microscopic colonization of (a) *R. vagum* MM 12; (b) *Curvularia* sp. PP 2.3; and (c) *Dendrothyrium* sp. CPP1.1.4 on the roots of *F. moluccana* seedlings after 14 days of inoculation

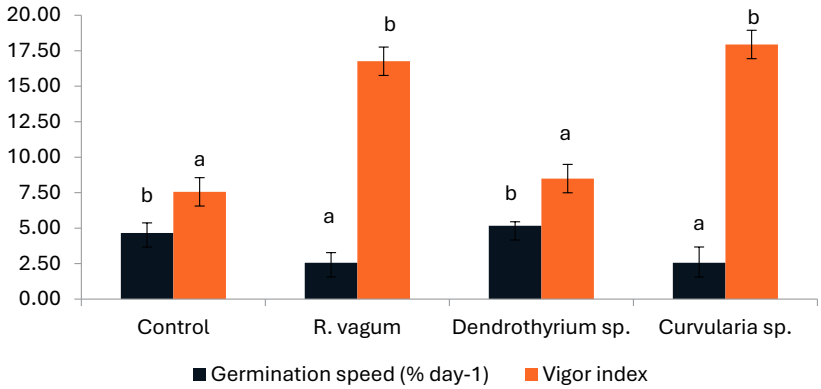



Fig. 5: Germination speed and vigor index of *F. moluccana* seeds on the control and DSE inoculation treatment. Values are means ± SD, n = 4. Means with the same letter were not significantly different ($P < 0.05$) after Tukey's Honestly Significant Difference test.

sprouts inoculated with the DSE fungal species, *R. vagum,* and *Dendrothyrium* sp., showed colonization on the seeds' surface (96% -100%) and in the root sprout's tissue (70.00%-93.33%). The microscopic analysis revealed the colonization of F. moluccana sprout roots by the DSE function, as depicted in Fig. 4. This indicates the ability of the DSE function to form a symbiotic relationship with the roots of *F. moluccana* sprouts. This colonization showed that two DSE fungi have the potency to symbiosis with the *F. moluccana* plant since the beginning of germination. Boddy and Hiscox (2016) stated that if a plant were a suitable host for a fungus, it would colonize more plant tissues. This study presents the initial documentation of the DSE colonization process on *F. moluccana* seeds following a 23-hour seed immersion treatment, as well as the subsequent colonization of seedlings up to 14 days post-inoculation.

The initial mechanism of DSE colonization in *F. moluccana* sprouts was identified by the presence of dark mycelium, specifically melanin, on the plumule. Hyphae may have infected the entire sprout tissue, including the radicle (root candidate), macroscopically the mycelium is not visible on the surface of the radicle. The *F. moluccana* sprouts inoculated by the *Dendrothyrium* sp. exhibited a low colonization rate (32.5%) on the surface of the seeds, while showing a high colonization rate (70%) at the roots. Each of the DSE species tested showed differences in their colonization ability, although the three DSE species colonized the *F. moluccana* seeds at the beginning of the treatment. The efficacy of DSE inoculation in seeds from the point of inoculation to germination is of significant importance as it serves as a protective measure against pathogenic fungi that tend to target seeds during the critical stage of germination. Post hoc test analysis results of the germination speed and vigor index of *F. moluccana* seeds can be seen in Fig. 5.

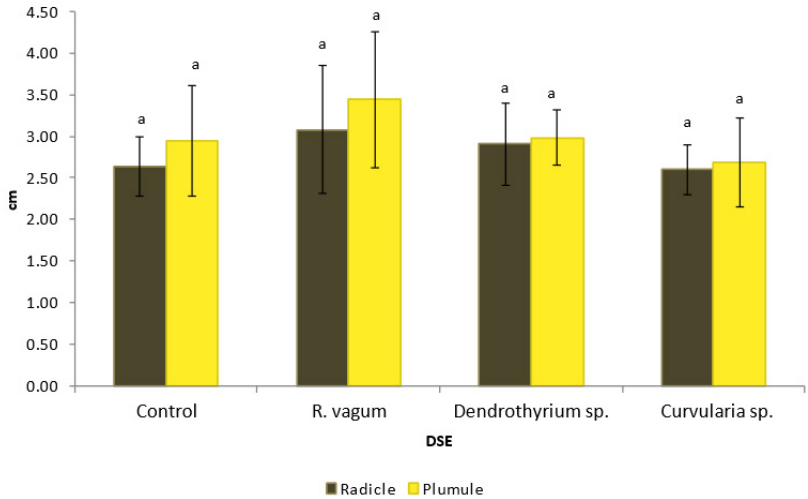



Fig. 6: The radicle and plumule length of *F. moluccana* sprouts aged 14 days after inoculation with DSE and control. Values are means ± SD, n = 4. Means with the same letter were not significantly different (P < 0.05) after Tukey's Honestly Significant Difference test.

The outcomes of the analysis of variance indicated that the introduction of the DSE fungi had no impact on the percentage of *F. moluccana* seed germination (Fig. 3) and the length growth of *F. moluccana* sprouts radicles and plumules (Fig. 6). However, there was a notable difference in germination speed and vigor index (Fig. 5), and no disease symptoms were observed in *F. moluccana* seedlings .*F. moluccana* seeds inoculated with the fungus *Dendrothyrium* sp. and *Curvularia* sp. showed a higher germination percentage than the control (without DSE inoculation). The seed germination rate of *F. moluccana* varies between 76.0% and 83.0%, as illustrated in Fig. 3. Similarly, the development of *F. moluccana* sprouts was influenced by the inoculation of *R. vagum* fungi, which resulted in an increase in the length of both the radicle and plumule compared to the control group; however, this difference was not statistically significant (Fig. 6). The existence of DSE fungi in the *F. moluccana* rooting sprouts does not cause a negative influence based on the performance of germinated seeds. The three DSE isolates successfully colonized the seeds and sprouts of *F. moluccana*, exhibiting no signs of disease. Furthermore, the seeds of *F. moluccana* were able to grow robustly without experiencing any damage rotting. Until the 14th day, the *F. moluccana* sprouts still look fresh and healthy. The germination ability of *F. moluccana* seeds infected with the DSE fungi was relatively high (76% –83%) and did not differ from control (76%). The germination ability is not different from the results of other studies, which are the germination percentage of fresh and healthy seeds of *F. moluccana* ranging from 71% (Mazo *et al*., 2020) to 88% (Istikorini *et al*., 2019). The *R. vagum* and *Curvularia* sp. can accelerate germination, or the time needed for germination is shorter with high vigor than the control (without DSE inoculation). The non-pathogenic (endophytic) fungi *R. vagum, Curvularia* sp*., and Dendrothyrium* sp. have been found to not only be harmless to *F. moluccana* seeds, but also to actively support their growth and development. The germination speed of *F. moluccana* seeds inoculated by *Dendrothyrium* sp. was not significantly different from the control but significantly different from the germination speed of the seeds inoculated by *R. vagum* and *Curvularia* sp. (Fig. 5). The control's vigor index value did not show a significant difference compared to the seeds inoculated by *Dendrothyrium sp*, however, it did exhibit a significant variance when compared to the vigor index value of the seeds inoculated by *R. vagum* and *Curvularia* sp. (Fig. 5). The *F. moluccana* seeds inoculated by *R. vagum* and *Curvularia* sp. germinate faster (2.56 days) than the control (4.6 days), and the *F. moluccana* seeds inoculated by *Dendrothyrium* sp. (5.6 days). Figs. 4 and 5 show the vigor index value of the seeds inoculated by *R. vagum* and *Curvularia* sp. (16.7 and 17.9) were higher than the vigor index

value of the control (7.5) and the seeds inoculated by *Dendrothyrium* sp. (8.5). Seeds exhibiting high vigor demonstrate an enhanced capacity for rapid germination. These results indicated that the *R. vagum* and *Curvularia* sp. application could increase the vigor the seeds germinate faster. It has been observed that DSE fungi, such as *R. vagum* and *Dendrothyrium* sp., possess the capacity to promote the growth of *F. moluccana*, as demonstrated by the growth rate of *F. moluccana* seedlings. There was an increase in radicle and plumule length in seedlings colonized by *R. vagum* and *Dendrothyrium* sp. compared to controls which is 17.11% and 10.27% radicles and 17.0% and 1.36% for plumules. According to Surono and Narisawa (2017), the DSE *Phialocephala fortinii* was found to have a positive impact on the growth of *Asparagus officinalis*. Nevertheless, it is crucial to acknowledge that some isolates of this species may actually hinder growth. DSE fungi isolated from the roots of *Astragalus adsurgens* can increase *Pinus tabulaeformis* seedlings and have the potential to prevent wilting caused by the nematode *Bursaphelenchus xylophilus* (Chu *et al.*, 2019). The outcomes of this study suggest that the application of DSE fungi on *F. moluccana* seeds can be considered as a valuable seed treatment approach to boost germination, thereby serving as a fundamental stage in the cultivation of superior quality *F. moluccana* seeds. Endophytic microbe›s mechanism in inducing resistance is by colonizing plant tissue to stimulate plants to increase the production of metabolite compounds in the form of peroxidase enzymes that play a role in plant resistance (Mengistu, 2020). The mechanism of the DSE function in protecting plants against pathogenic attack includes indirect inhibition through the formation of secondary metabolites such as salicylic acid, jasmonic acid, and ethylene. These metabolites function to bolster plant resistance to pathogens or act as antimicrobials, including phytoalexin (Kaur *et al.*, 2022). The identification of secondary metabolite composition plays a vital role in identifying bioactive compounds that act as plant

Table 1: Identification results and levels (%) of *Falcataria moluccana* seed active compounds, without DSE inoculation (control) and with DSE inoculation treatments after 23 hours of inoculation using the GC-MS method

| No. | Compound | DSE | | | |
|---|---|---|---|---|---|
| | | Control | *R. vagum* | *Curvularia* sp. | *Dendrothyrium* sp. |
| 1 | Acetamide, 2,2,2-trifluoro-N-methyl-N-(trimethylsilyl) | - | 25.20 | 26.17 | 23,33 |
| 2 | Hexamethyl Cyclotrisilaxane | 29.35 | - | - | - |
| 3 | 2,2,4,4,6,6-Hexamethyl-1,3,5,2,4,6-trioxatrisilinane | 13.38 | - | - | - |
| 4 | 1-Isopropenyl-4-methyl-1,3-cyclohexadiene | - | 43.88 | 43.06 | 44.66 |
| 5 | Acetamide,2,2,2-trifluoro-N-N-bis (trimethylsilyl) | - | 2.40 | 2.32 | 2.37 |
| 6 | N-Tetramethyl-N-(trimethylsilyl) silanamine | - | 2.56 | 2.59 | 2.58 |
| 7 | 2,2,7,7-tetramethyl-3,6-dioxa-2,7-Disilaoctane | - | 2.82 | 2.85 | - |
| 8 | Ethylene glycol, DI-TMS | - | - | - | 2.98 |
| 9 | Diethyl disulfide | - | 1.51 | 1.60 | 1.55 |
| 10 | 2.2.4.4.6.6.8.8-Octamethyl-1.3.5.7.2.4.6.8-tetraoxatetrasilocane | 24.48 | - | - | - |
| 11 | 1-Butanol.2-iodo- | 4.20 | - | - | - |
| 12 | Tris (trimethylsilyl) amine | - | 9.33 | 9.17 | 9.68 |
| 13 | Decamethylcyclopentasiloxane | 8.12 | - | - | - |
| 14 | Silane. trimethyl [ (1-propylnonyl)oxy]- atau 4-Trimethylsilyloxydodecane | - | 7.54 | 7.24 | - |
| 15 | 1-Ethyl-1-pentanonyloxyl-1-silacyclopentane | - | - | - | 7.66 |
| 16 | Dodecamethylcyclohexasiloxane | 1.70 | - | - | - |
| 17 | 4-hydroxy-2.9-dimethoxy-1.10-oxy-1.10B-[ (N-Methyl)Iminoethano] | 4.08 | - | - | - |
| 18 | Hexadecanoic Acid | 1.95 | - | - | - |
| 19 | 3-Alpha-Chloro-4-4-dimethyl-3-beta-nitro5.alpha-Cholestane | 4.08 | - | - | - |
| 20 | Acetamide.2-(adamantan-1-yl)-N-[2-(adamantan-1-yloxy)propoyl]- | 1.95 | - | - | - |

Table 2: The active compound identification results of *Falcataria moluccana* seedlings without DSE inoculation (control) and DSE inoculation treatments after 14 days of inoculation using the GC-MS method

| No. | Compound | DSE | | | |
|---|---|---|---|---|---|
| | | Control | *R. vagum* | *Curvularia* sp. | *Dendrothyrium* sp. |
| 1 | Acetamide. 2.2.2-trifluoro-N-methyl-N-(trimethylsilyl) | 8.43 | 9.67 | 14.18 | 26.15 |
| 2 | 1-piperidicbecarboxylic acid. 4-(1-methyl propyl)-methyl ester | 17.55 | - | 13.52 | - |
| 3 | 1-Isopropenyl-4-methyl-1.3-cyclohexadiene | 44.16 | 43.53 | 42.68 | 43.34 |
| 4 | Acetamide.2.2.2-trifluoro-N-n-bis (trimethylsilyl) | 2.38 | - | 2.27 | 2.39 |
| 5 | Bis (trimethylsilyl) trifluoroacetamide | - | 2.28 | - | - |
| 6 | N-Tetramethyl-N-(trimethylsilyl) silanamine | 2.53 | 2.51 | 2.45 | 2.46 |
| 7 | Bis(trimethylsilyl) carbonate | 2.71 | 2.62 | 2.62 | 2.59 |
| 8 | Diethyl disulfide | 1.51 | 1.53 | 1.48 | 1.51 |
| 9 | Tris (trimethylsilyl) amine | 9.13 | 8.83 | 8.86 | 9.26 |
| 10 | 2-Quinazolinamine | - | 7.20 | - | 6.97 |
| 11 | Isopropyl triethylsilyl ether/ dodecanol | 6.43 | - | 6.92 | - |

defenses. The identification secondary metabolites present in *F. moluccana* seeds, both in the absence of DSE inoculation (control) and following DSE inoculation (DSE treatments), was performed after a duration of 23 hours. This was achieved by comparing the ion fragments of each sample against the NIST database on GC MS library (Table 1). The major compound in control was hexamethyl cyclo trisiloxane which was identified by appearing at three higher peaks of ion precursor at mass-to-charge ratio (m/z) 207, 208, and 96. Meanwhile, in three different isolates, the major compound was 1-isopropenyl-4-methyl-1,3-cyclohexadiene which also was verified by appearing higher peak at m/z 119, 91, and 134. A diverse array of secondary metabolite compounds was observed in both control and treatment groups following 23 hours of inoculation, indicating a difference in the metabolite compounds produced. Metabolite compounds in control group (seeds that did not receive DSE inoculation) exhibited significant differences compared to those in the DSE treatment group. The compounds in the DSE treatment were almost similar between each isolate; only a few different compounds were produced, such as seeds that were inoculated with *R. vagum* MM12 and *Curvularia* sp. PP2.3 to produce 2,2,7,7-tetramethyl-3,6-dioxa- compounds. 2,7-Disilaoctane and Silane, trimethyl [(1-propylnonyl) oxy] - or 4-Trimethylsilyloxydodecane, while the seeds inoculated with isolate *Dendrothyrium* sp. CPP1.1.4 did not produce either of these compounds. Furthermore, the metabolite compound 1-Ethyl-1-pentanonyloxyl-1-silacyclopentane was exclusively generated by seeds that were inoculated with *Dendrothyrium* sp. CPP 1.1.4.

The metabolite content analysis of *F. moluccana* seedlings after 14 days of inoculation (DSE treatments and control) can be seen in Table 2. As described, the identification of compounds in both the control and DSE treatment was conducted through the utilization of the NIST database. The highest peak from the GC-MS spectrum of control and DSE treatment was the same at retention time 3.507-3.521 with higher ion fragments found at m/z 119, 91, and 134. Referring to the NIST library, this compound was identified as 1-Isopropenyl-4-methyl-1,3-cyclohexadiene. Additionally, this method was employed to identify the potential compound associated with each peak. There are 11 secondary metabolites in the root section of *F. moluccana* sprouts, namely seeds without DSE inoculation (control) and seeds with DSE after 14 days of inoculation. Variations in secondary metabolites were noted in the control treatment post 23 hours of incubation and 14 days after inoculation (Tables 1 and 2). After 14 days of inoculation, seeds treated with control and DSE treatments were both able to produce the same metabolite compounds. Metabolite compounds such as Acetamide, 2,2,2-trifluoro-N-methyl-N- (trimethylsilyl), 1-isopropenyl-4-methyl-1,3-cyclohexadiene, N-Tetramethyl-N-(trimethylsilyl) silanamine, Diethyl disulfide, and Tris (trimethylsilyl) amine (Table 2). Metabolites of 1-piperidicbecarboxylic acid, 4- (1-methyl propyl) -methyl ester, and Isopropyl triethylsilyl ether/

dodecanol were only produced by the seeds that were not inoculated and inoculated with *Curvularia* sp. PP 2.3 (Table 2). The Acetamide, 2,2,2-trifluoro-N-n-bis (trimethylsilyl) metabolite compound was produced by seeds that were not inoculated with DSE, inoculated with *Curvularia* sp. PP2.3 and inoculated with *Dendrothyrium* sp. CPP 1.1.4 (Table 2). Whereas metabolite compounds, such as 2-Quinazolinamine, were exclusively synthesized by seeds inoculated with *R. vagum* MM12 and *Dendrothyrium sp* CPP1.1.4, as these compounds were not generated by seeds that lacked DSE inoculation. The Bis (trimethylsilyl) trifluoroacetamide metabolite compound was only produced by seeds inoculated with *R. vagum* MM12 (Table 2).

During the initial stage of inoculation (23 hours), the predominant metabolite found in the seeds of *F. moluccana* control was Hexamethylcyclotrisilaxane, constituting 29.35% of the total composition. This compound is also reported as the main component of the stem in *Syzygium alternifolim* (Yugandhar and Savithramma, 2017) and is also found in medicinal plants such as *Olea europaea* (Zohdi *et al.*, 2023) and *Bauhinia acuminate* (Krishna *et al*., 2015). Hexamethylcyclotrisiloxane is a secondary metabolite that can withstand the entry of bacteria (antibacterial) (Nurain *et al*., 2017). Other compounds that also tend to be the main compounds are 2, 2, 4, 4, 6, 6, 8, 8-Octamethyl-1,3,5,7,2,4,6,8- tetraoxatetra silocane compounds (24,48%) which are antimicrobial (Krishna *et al.,* 2015). After 14 days of germination, the two secondary metabolites were not detected. Secondary metabolite compounds that are relatively low in *F. moluccana* seeds without DSE inoculation (control) are Hexadecanoic Acid (Palmitic Acid) (1.95%) which acts as an antifungal (El-Sheekh *et al*., 2022), and antimicrobial (Krishnaveni *et al*., 2014). The findings suggest that the seeds of *F. moluccana* possess inherent antifungal and antimicrobial compounds. Several research results stated that the content of secondary metabolite compounds from the *F. moluccana* plant came from the flavonoid, saponin, tannin, terpenes, and alkaloid groups (Rumidatul *et al.*, 2021). Differences in secondary metabolite synthesis are evident among distinct parts of the same plant species. The leaves of *F. moluccana* contain alkaloids, potential to produce natural antioxidants (Rumidatul *et al*., 2021), while the stem contains flavonoids, saponins, phenolic hydroquinones, tannins, triterpenoids, and steroids but does not contain alkaloids. The analysis conducted by Rahmawati *et al*. (2019) demonstrated the existence of alkaloids, tannins, flavonoids, saponins, steroids, anthraquinone, and triterpenoids in the extract obtained from *F. moluccana* sawdust. Alkaloid secondary metabolite compounds can be found in both seeds and fruit (Abegaz *et al.,* 2019). The alkaloids can protect the plant from pests, act as plant boosters, and act as hormone regulators. Rahmawati *et al*. (2019) reported Triterpenoids and steroids in the stems of *F. moluccana* make the plants that were resistant to tumor rust. Even these results were reported by Rahmawati *et al*., (2019), who reported that *F. moluccana* trees resistant to tumor rust contained higher levels of flavonoids, saponins, triterpenoids, and steroids than susceptible trees. The flavonoid group is involved in various processes including safeguarding against ultraviolet radiation, contributing to pigmentation, and enhancing resistance to diseases. The GC-MS analysis showed that the inoculation process and the interaction between the *F. moluccana* seeds and the DSE fungi tested produced secondary metabolites that were very different from those of the control, especially at the beginning or during the 23 hours of the inoculation process. After the introduction of *R. vagum*, *Curvularia* sp., and *Dendrothyrium* sp., a number of secondary metabolites were exclusively generated in *F. moluccana* seeds. Those secondary metabolites are Acetamide compound, 2,2,2-trifluoro-N-methyl-N-(trimethylsilyl); 1-Isopropenyl-4-methyl-1,3-cyclohexadiene; Acetamide, 2,2,2-trifluoro-N-N-bis (trimethylsilyl); N-Tetramethyl-N- (trimethylsilyl) silanamine; Diethyl disulfide; and Tris (trimethylsilyl) amine. The majority of these substances are TMS derivatives that serve as antifungal (Nurain *et al*., 2017) and antibacterial functions (Abdel-Aziz *et al*., 2019). Meanwhile, The secondary metabolites produced by the seeds that were not inoculated by DSE (control) were Hexamethyl Cyclotrisilaxane; 2,2,4,4,6,6,8,8-Octamethyl-1,3,5,7,2,4,6,8-tetraoxa tetrasilocanel; 1-Butanol, 2-iodo; Decamethylcyclopenta siloxane; Dodecamethylcyclo hexasiloxane; 4-hydroxy-2,9-dimethoxy-1,10-oxy-1,10B- [(N-Methyl) Iminoethano]; Hexadecanoic Acid; 3-Alpha-Chloro-4-4-dimethyl-3-beta-nitro5. alpha-Cholestane; Acetamide, 2- (adamantan-1-yl) -N- [2- (adamantan-1-yloxy) propoyl]. Hexamethyl

Cyclotrisilaxane is an antibacterial compound, and the Hexadecanoic Acid compound is antifungal; this means that the seeds of *F. moluccana* already contain secondary metabolites that can withstand the entry of microbes (bacteria or fungi). Following 14 days of germination (Table 2), variations in the secondary metabolites generated in the control seeds were observed, which were absent at the initial stage (23 hours inoculation). The metabolic compounds are Acetamide, 2,2,2-trifluoro-N-methyl-N-(trimethylsilyl); N-Tetramethyl-N- (trimethylsilyl) silanamine; Tris (trimethylsilyl) amine; Bis (trimethylsilyl) carbonate; Diethyl disulfide, and 1-Isopropenyl-4-methyl-1,3-cyclohexadiene. The production of six secondary metabolites was observed in seeds inoculated with DSE as early as 23 hours post-inoculation. This indicates the ability of the DSE species (*R. vagum, Curvularia* sp., and *Dendrothyrium* sp.) to spur the formation of metabolite compounds more quickly for defense in the seeds or sprouts of *F. moluccana* as a form of adaptation to environmental conditions, all of which function as antibacterial and antifungal. *Dendrothyrium* sp., at the beginning of inoculation (23 hours), produced ethylene glycol, Trimethylsilyl (DI-TMS), and 1-Ethyl-1-pentanonyloxyl-1-silacyclopentane, which were not produced by seeds inoculated by *R. vagum*, *Curvularia* sp., and control). DI-TMS, an ethylene glycol compound, exhibits antibacterial properties according to Moghayedi *et al*. (2017). Similarly, Cadophora sp. also demonstrates antibacterial activity (Tan *et al*., 2018). The 1-Ethyl-1-pentanonyloxyl-1-silacyclopentane compound is responsible for the pharmacological activity of antioxidants, antimicrobials, and antifungals (Arumugam *et al*., 2018). Secondary metabolite compounds with a high percentage on the 14th day after DSE inoculated seeds are 1-Isopropenyl-4-methyl-1,3-cyclohexadiene (42.68%–43.53%), this compound can function as an antifungal (Wibawa *et al.*, 2019), and Acetamide, 2,2,2-trifluoro-N-methyl-N- (trimethylsilyl) (9.67%–26.15%), which is a flavonoid group and can be an antimicrobial compound (Nurain *et al*., 2017). On the 14th day of observation, 1-Isopropenyl-4-methyl-1,3-cyclohexadiene and Acetamide, 2,2,2-trifluoro-N-methyl-N- (trimethylsilyl) were also found in lower percentages in control *F. moluccana* seeds. The potential of DSE leads to an enhancement in the production of secondary metabolite compounds within *F. moluccana* seeds, which may exhibit antimicrobial and antifungal characteristics, serving as a mechanism for plant defense. DSE's ability to trigger an increase in secondary metabolites related to disease resistance in plants certainly influences the percentage increase in plant life. This results have a substantial influence on the effectiveness of forest reforestation and rehabilitation programs, ultimately contributing to environmental sustainability. The research conducted by Spagnoletti *et al*. (2020) supports the notion that DSE can play a crucial role in combating plant infections caused by pathogens, enhancing nutrient uptake in the soil, and minimizing the negative consequences of challenging environmental conditions, ultimately fostering the growth and well-being of plants in adverse environments. This is also supported by Spagnoletti *et al.* (2020) research, which summarized the specific response of DSE which is one of the efforts to increase plant tolerance to climate change. Adinugroho *et al*. (2024) demonstrated that the utilization of indigenous tree species in rehabilitation endeavors leads to promising results in carbon sequestration, emphasizing the essential requirement for well-growth plant species. In this study, the inoculation of DSE on *F. moluccana* has a beneficial impact on promoting plant growth. The findings of this study suggest that the observed effect is linked to the synthesis of metabolite compounds by DSE, which not only promote growth but also offer protection against various biotic and abiotic stressors. This study is the first report of symbiotic interaction between DSE and *F. moluccana*, specifically focusing on the seed-to-seedling stage. The results of this study contribute additional insigjt regarding the significant impact of DSE in supporting growth from seed development seedling stage. According to Lin *et al.* (2023), DSE can also be used in *Cinnamomum kanehirae* conservation because it can increase seedling height, fresh weight, and chlorophyll concentrations. The findings suggest that DSE has the potential to promote the development of *F. moluccana* seeds, despite the fact that the DSE strains utilized originated from rice and chili plant tissues rather than the intended plant species. This finding supports the claims made by other studies on DSE, which indicate that numerous selected DSE strains can establish a symbiotic relationship not only with

their original host plant but also with a diverse range of other plants. According to Yinli *et al*. (2023), DSE can help *Morus alba* to increase soil carbon and nitrogen absorption. It also can increase plant height, crown size, and root and leaf biomass of *M. alba*. Furthermore, DSE has the potential to enhance carbon sequestration. Utilizing DSE in leaf applications may prove beneficial in the ecological rehabilitation of mining sites, serving as a means to introduce novel microbial fertilizers that aid in restoration efforts and the recovery of former mining lands (Yinli *et al*., 2023). According to Yinli *et al*. (2023), DSE and the metabolites produced can increase the growth of *Medicago sativa* L. plants, affecting photosynthesis. In the future, this can be used in green microbial fertilizer development as a means of ecological restoration in mining areas.

## CONCLUSION

This study reports for the first time that DSE could colonize the *F. moluccana* (economic value tree, widely planted for forest and landscape restoration in Indonesia) seed at the initial inoculation and germination period and produce compounds that function as antioxidants and antimicrobial pathogens. During the initial stages of germination, *F. moluccana* seeds may be colonized by the DSE species *Rhizopycnis vagum*, *Curvularia* sp., and *Dendrothyrium* sp. Inoculation can occur within the first 23 hours after germination, and colonization may continue up to 14 days post-inoculation. The DSE isolates (*R. vagum*, *Curvularia* sp., and *Dendrothyrium* sp.) could colonize seeds on the surface with a colonization rate of 96%, 100, and 32.5%, and entered the seed tissue and the sprout roots with a colonization rate of 93.3%, 93.3%, and 70%. DSE colonization on the *F. moluccana* seeds did not significantly affect the percentage of seed germination, although there was a tendency for the germination percentage of the seeds infected with the DSE fungus to be relatively high (76%–83%) compared to the control (76%). The seeds of *F. moluccana* that were inoculated with *R. vagum* and *Curvularia* sp. exhibited a quicker germination process, taking only 2.56 days compared to the control group which took 4.6 days. On the other hand, the seeds of *F. moluccana* that were inoculated with *Dendrothyrium* sp. took 5.6 days to germinate. The vigor index value of the seeds inoculated by *R. vagum* and *Curvularia* sp. (16.7 and 17.9) was higher than the vigor index value of the control (7.5) and the seeds inoculated by *Dendrothyrium* sp. (8.5). Seeds with high vigor will be able to germinate faster, indicated that the application of the *R. vagum* and *Curvularia* sp. could increase the vigor so that the seeds germinate faster. The presence of DSE fungi has been shown to enhance the production of secondary metabolites in *F. moluccana* seeds, which include various antibacterial, antifungal, and antioxidant compounds such as the Hexamethyl Cyclotrisilaxane compound, 1-Isopropenyl-4-methyl-1,3-cyclohexadiene, and Acetamide, 2, 2,2-trifluoro-N-methyl-N-(trimethylsilyl). DSE fungi, *R. vagum, Curvularia* sp., and *Dendrothyrium* sp., can stimulate the formation of metabolite compounds more quickly for defense in *F. moluccana* seeds or sprouts as a form of adaptation to environmental conditions, all of which function as antibacterial and antifungal. By adhering to this condition, *F. moluccana* seeds and seedlings are able to thrive and exhibit increased vitality in their growth process. DSE inoculation, that were inoculated by *R. vagum*, was able to increase growth which was characterized by faster growth of radicle and plumule length. The accelerated development of radicles and plumules in seedlings will result in the production of seeds that generate “target seedlings” more rapidly, which are defined as normal seedlings prepared for out-planting. The colonization of the seed by DSE enables the establishment of a seed defense system against both abiotic (environmental) and biotic (pest and disease) stress conditions. DSE inoculation on *F. moluccana* seeds can be used as a seed treatment and can produce healthy and vigor seedlings, thereby increasing the success of *F. moluccana* planting in the context of forest and landscape restoration.

## AUTHOR CONTRIBUTIONS

S. Surono contributed to this work by conceptualizing the study, applying for funding, designing the experimental work, analyzing the data, writing the initial manuscript, and finalizing the manuscript. Y. Yulianti contributed to this work by conceptualizing the study, applying for funding, designing the experimental work, analyzing the data, writing the initial manuscript, and reviewing and finalizing the manuscript. K.P. Putri contributed to this work by conceptualizing the study, designing the experimental work, analyzing the data, writing the initial manuscript, and finalizing the manuscript. D.J.

Sudrajat contributed to this work by conceptualizing the study, designing the experimental work, analyzing the data, and supporting and finalizing the manuscript. Y.M.M.A. Nugraheni contributed to performing experiments, analyzing the data, and supporting manuscript writing and corresponding author duties. C. Sriherwanto contributed to supporting the manuscript writing, reviewing, and revising. M.A. Syabana contributed to performing experiments, analyzing the data, and supporting manuscript writing. N. Widyani contributed to analyzing the data and supporting manuscript writing. N. Yuniarti contributed to analyzing the data and supporting manuscript writing. M. Zanzibar contributed to analyzing the data and supporting manuscript writing. E. Rustam contributed to analyzing the data and supporting the manuscript writing. R.U.D. Sianturi contributed to analyzing the data, supporting manuscript writing, and finalizing the manuscript.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript. The ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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## ABBREVIATIONS

| | |
|---|---|
| *%* | Percent |
| *µL* | Microliter |
| *µm* | Micrometer |
| *$^0$C* | Degree Celcius |
| *cm* | Centimeter |
| *D* | The time corresponding to that amount |
| *DSE* | Dark septate endophytes |
| *DSE CPP.1.1.4* | *Dendrothyrium* sp. CPP 1.1.4 |
| *DSE PP2.3* | *Curvularia* sp. PP 2.3 |
| *DSE MM12* | *Rhizopycnis vagum* MM12 |
| *DI-TMS* | Trimethylsilyl (or double TMS) |
| *Eq.* | Equation |
| *et al.* | And others |
| *H* | Hours |
| *hi* | The number of days it took to reach the smaller number of sprouts |
| *HCL* | Hydrochloric acid |
| *i.e.* | Id est (that is) |
| *IPB* | *Bogor Agricultural University* |
| *IST*A | International Seed Testing Association |
| *G* | The number of seeds that germinate on a certain day |
| *g* | Gram |

| | |
|---|---|
| *GC-MS* | Gas chromatography-mass spectrometry |
| *H/V* | The horizontal-to-vertical spectral ratio |
| *Kg* | Kilogram |
| *Km* | Kilometer |
| *KOH* | Potassium hydroxide |
| *m* | Meter |
| *mm* | Millimetre |
| *mL* | Milliliter |
| *mm* | Millimeter |
| *m/z* | mass-to-charge ratio |
| *N* | total sample size |
| *N* | Number of total samples or the number of days at the last calculation |
| *ni* | Number of seeds germinated on day i |
| *NIST* | National Institute Standard Technology |
| *p <0.05* | P value <0.05 |
| *Rpm* | Revolutions per minute |
| *SD* | Standard deviation |
| *sp.* | Species |
| *spp.* | Several species |
| *SPSS* | Statistical Package for the Social Sciences |
| *Tukey's HSD* | Tukey's honestly significant difference test |
| *Vs30* | The time-averaged shear-wave velocity to a depth of 30 meters |
| *TMS* | Trimethylsilyl |

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