Journal of Natural Resources and Environmental Management
https://dx.doi.org/10.29244/jpsl.16.4.469

RESEARCH ARTICLE

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Growth Performance, Volatile Compound Production, and Pathogenicity Assessment of Jakaba BHP01 under Varying Culture Conditions

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Article History
Received 30 October 2025
Revised 26 May 2026
Accepted 03 January 2026

Keywords
biocontrol, culture media, Falcataria moluccana, Jakaba BHP01, volatile organic compounds

ABSTRACT

Jakaba or *Jamur Keberuntungan Abadi*, classified in the Ascomycota phylum, is a fungus that is thought to have benefits in the form of nutrients for plants, and thus has the potential for further cultivation. The success of a pure culture depends on proper media preparation and the suitable environmental conditions for mycelial growth. Accordingly, this study investigated the growth characteristics of Jakaba BHP01 under different culture media, pH, and temperature conditions. Three factors were tested: media type (potato dextrose agar/PDA, malt extract agar/MEA, and malt peptone agar/MPA), media pH (pH 4, pH 6, and pH 8), and incubation temperature (10 ± 0.1 °C, 28 ± 0.1 °C, and 35 ± 0.1 °C). Thus, there were 27 treatment combinations with three replicates. Macroscopic and microscopic observations revealed differences in colony texture, color, and hyphal characteristics under different treatments. The highest average diameter of Jakaba isolates was found in the M2P1S2 treatment combination, MEA medium, pH 4, and incubation temperature 28 ± 0.1 °C. The results of three-way ANOVA showed that the type of medium and pH did not have a significant effect on the growth of Jakaba isolate diameter, while temperature had a significant effect ($p < 0.0001$). Pathogenicity tests on sengon seeds indicated that Jakaba BHP01 was non-pathogenic and significantly enhanced seedling length on MEA medium. Moreover, Jakaba BHP01 produced volatile compounds that inhibited *Colletotrichum sp.* pathogen growth by up to 38% on MEA. These findings provide preliminary baseline information for future studies on the cultivation and potential application of Jakaba BHP01 under controlled laboratory conditions in the future.

Introduction

The intensive use of inorganic fertilizers in nurseries has become common practice because of their ability to quickly increase yields. Indonesia's chemical fertilizer usage reaches 6.5 million tons, ranking it among the world's top consumers of chemical fertilizers [1]. The heavy reliance on chemical fertilizers poses a risk of harmful residue accumulation, potentially contributing to climate change. Soil contamination by chemical fertilizers can disrupt the soil microbiome and decrease its natural fertility. Excessive use of chemical fertilizers disrupts soil microorganisms [2]. Further impacts include increased seedling susceptibility to abiotic stress and pathogens, which can reduce plant quality after transplantation. Therefore, alternatives, such as microbial-based liquid organic fertilizers, are urgently needed to support the sustainability of organic-based seedling production.

Jakaba is a locally derived microbial bioresource that has gained increasing attention in Indonesian organic cultivation practices. Jakaba, also known as "*Jamur Keberuntungan Abadi*" is a fungus produced through the fermentation of rice washing water with the addition of other organic materials [3]. The high carbohydrate content can aid in the production of hormones, such as auxin and gibberellin, as well as amino acids, such as

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alanine. These hormones play a role in stimulate leaf bud growth and transport nutrients to essential cells in the leaves and stems [4]. Previous reports have suggested that Jakaba may have potential applications related to plant growth promotion and suppression of *Fusarium* sp. wilt disease, although these effects remain insufficiently validated under controlled experimental conditions [5]. Biological control is an eco-friendly approach to plant disease management, as it is harmless to living beings and the environment [6]. From a microbial ecology perspective, understanding the environmental response and volatile organic compounds (VOC) mediated interactions of Jakaba is important for evaluating its ecological adaptability and biocontrol potential.

Based on these potential benefits, Jakaba may serve as a complementary microbial resource for sustainable plant cultivation and biological control. However, information regarding the optimal growth conditions for Jakaba mycelium, such as medium type, pH, and incubation temperature, is still lacking. Research on these aspects is important to support future studies on the cultivation and biological characterization of Jakaba under controlled conditions. In general, mushroom cultivation involves several stages, including the production of pure cultures, mother cultures, parent spawn, and production spawn [7]. The success of pure culture development depends on proper media preparation and the suitability of the medium for fungal mycelial growth. Factors such as medium type, pH, and incubation temperature can influence the physicochemical properties of fungi [8]. Optimal growth conditions are crucial for determining the production of bioactive metabolites and the efficiency of large-scale cultivation.

Fungal metabolites play an important role in plant–pathogen interactions and have the potential to stimulate plant growth and defense mechanisms. In addition to optimizing culture conditions, the safety of using Jakaba fungi in liquid organic products must be validated through pathogenicity tests. Previous studies have shown that Jakaba fungi are not pathogenic to corn plants [9]. Based on these findings, further research is needed to determine whether Jakaba is pathogenic to other plant species, particularly to forest tree species. Given its potential biological activity, Jakaba BHP01 may provide useful preliminary information for future nursery-related studies. Preliminary observations suggest that Jakaba BHP01 may have potential biological activity, warranting further investigation under broader experimental conditions. Therefore, identifying optimal culture conditions is an important preliminary step for future studies on fungal formulations and biological applications. Although Jakaba has been locally recognized in Indonesia as a beneficial fungus derived from traditional organic fermentation, previous studies have been limited to regional observations and have not systematically characterized purified isolates under controlled laboratory conditions. These findings provide preliminary scientific information regarding the biological characteristics of Jakaba BHP01 under laboratory conditions and may provide a basis for future studies related to sustainable nursery management. This study is the first to comprehensively evaluate Jakaba BHP01 by integrating growth condition optimization, VOC antagonism, and pathogenicity assessment, thereby advancing beyond earlier regional reports. This study integrated growth condition optimization, VOC-mediated biocontrol evaluation, and pathogenicity safety assessment to provide a more comprehensive laboratory-scale characterization of Jakaba BHP01.

Materials and Methods

Sample Collection

This research was conducted from October 2023 to February 2024 at the Silviculture Department, Faculty of Forestry and Environment, IPB University. The materials used in this study were pure culture isolates of Jakaba with isolate code BHP01, which was collected from liquid organic fertilizer produced by the Forest Pathology Laboratory. The isolate was maintained on potato dextrose agar (PDA) medium prior to experimentation to preserve the culture viability and purity. Subculturing was performed under aseptic conditions before each assay.

Preparation of Culture Media for Mycelial Growth Testing

The media used to test the growth diameter of the Jakaba mycelial colonies were PDA, MEA, and MPA. These three types of media were used based on their nutritional content and growth considerations in fungal cultures [10]. The potato dextrose agar (PDA) medium was prepared using 30 g of PDA dissolved in 1 L of distilled water. The malt extract agar (MEA) medium consisted of 15 g of malt extract, 16 g of pure agar, and 1 L of distilled water. The malt peptone agar (MPA) medium consisted of 15 g of malt extract, 20 g of D-(+)-Glucose (*monohydrate*), 5 g of bacteriological peptone, 16 g of pure agar, and 1 Lof distilled water. Each medium was prepared by dissolving the respective ingredients in 1 Lof distilled water, followed by the addition of antibiotics. All culture media (PDA, MEA, and MPA) were supplemented with chloramphenicol at

100 mg L^{-1} to suppress bacterial contamination. This concentration aligns with the typical levels used in yeast and mold enumeration media compliant with ISO 6611, as well as in antibiotic-supplemented PDA, which is commonly applied for fungal isolation. The solution was boiled and stirred until homogeneous. Before sterilization, the pH of each medium was adjusted to 4, 6, and 8 by adding 0.1 M HCl and 0.1 M NaOH. The solution was then sterilized in an autoclave at 121 °C and 1 atm for 15 min. After sterilization, the medium was poured into sterile 90 mm petri dishes, each containing approximately 10 mL. The plates were allowed to solidify for 30 min and then stored at room temperature for 24 h to equilibrate moisture before inoculation.

Testing the Growth of Jakaba BHP01 Mycelial Colony Diameter in Response to Medium Type, pH, and Temperature

Pieces of the Jakaba isolate cut using a 0.8 cm diameter cork borer were aseptically inoculated onto various types of media with adjusted pH levels. The inoculum was taken from the edge of 7-day old active colonies to ensure the inclusion of active growth zones. All procedures were performed in a sterile workstation, specifically within a laminar airflow cabinet (LAF). The diameter of Jakaba mycelial colonies was measured every 24 h over a 17-day incubation period at predetermined temperatures of 10 °C, 28 ± 1 °C, and 35 °C. Treatments at 10 °C were stored in a walk-in environmental test chamber with a temperature stability of ±0.1 °C, whereas treatments at 28 ± 1 °C were placed in a controlled room equipped with a thermohygrometer, and treatments at 35 °C were incubated in an incubator with a temperature stability of ±0.2 °C. Temperature and relative humidity were recorded daily, and all plates were incubated in complete darkness to avoid photomorphogenic variation with no light/dark cycle applied. The experiment involved three factors: medium type, pH, and incubation temperature, each with three levels. The medium type of factor (M) consisted of three levels: M1 (PDA), M2 (MEA), and M3 (MPA). The pH factors included P1 (pH 4), P2 (pH 6), and P3 (pH 8). The incubation temperature factor (S) consisted of S1 (10 °C), S2 (28 $\pm$ 1 °C), and S3 (35 °C). This resulted in 27 treatment combinations, each replicated thrice, resulting in a total of 81 Petri dishes. In this study, one replicate was defined as an independent petri plate containing a single fungal colony. To avoid positional bias within the incubator, the placement of petri plates was randomized at the start of incubation and re-randomized daily during the measurement period. All treatments were performed concurrently, and the entire experiment was performed once (single experimental run). The mycelial colony diameter growth was calculated based on the formula proposed by Achmad and Mulyaningsih [11] as shown in Figure 1.

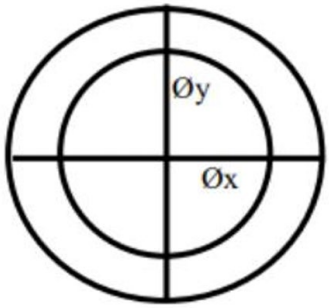

Figure 1. Design for measuring the diameter of mycelium colonies. The diagram shows the two perpendicular axes, d_1 and d_2, drawn across the petri dish to record colony diameter at each observation. This measurement scheme was applied daily to monitor the radial growth of the Jakaba BHP01 mycelium colony under the tested combinations of culture media, pH, and incubation temperature.

Two perpendicular lines were drawn on the bottom of the petri dish, intersecting at the inoculation point. The colony diameter was measured along both axes (d_1 and d_2), and the mean diameter was calculated as in Equation 1.

$$\text{Radial diameter} = \frac{d1+d2}{2} \quad (1)$$

Measurements were performed using a transparent acrylic ruler with 0.1-cm resolution.

Description:

Øx = diameter of axis x (cm);

Øy = diameter of axis y (cm)

Pathogenicity Test

This test was conducted to determine the pathogenic potential of Jakaba on plants. The method used followed that described by Surono and Narisawa [12], which involved the use of sengon seeds. The sengon seeds were surface-sterilized using 70% alcohol for 1.5 minutes, 1% NaOCl for 3 minutes, and rinsed three times with sterile water, and then subjected to dormancy breaking in warm water for 24 hours. Sengon seeds were cultured in jars containing PDA and MEA media. The inoculated jars were incubated under uniform room temperature conditions (25 ± 2 °C), and all treatments were exposed to the same environment throughout the assay. All sterilization, rinsing, drying, and sowing steps were performed aseptically inside a laminar airflow cabinet to maintain axenic conditions. These media were either inoculated with pure Jakaba isolates and incubated for 14 days or left uninoculated as control treatments. Each treatment consisted of five seeds and was replicated thrice, resulting in a total of 60 seeds. Pathogenicity was assessed 14 days after sowing using seedling damping-off (pre- or post-emergence collapse and tissue softening at the hypocotyl) and abnormal growth (severe stunting, chlorosis, or necrotic lesions) as indicators of pathogenic infection, whereas upright seedlings with normal coloration and morphology were classified as healthy.

Testing the Production Potential of Volatile Compounds

The effect of volatile organic compounds (VOCs) produced by Jakaba on pathogen growth was tested using two Petri dishes of uniform diameter [13]. Jakaba and the pathogens were cultured on two types of media, PDA and MEA. The use of two types of media was intended to determine the effect of media composition on the production of volatile compounds. The two Petri dishes were then stacked, with the dish containing Jakaba placed at the bottom and the one containing the pathogen at the top and sealed with plastic wrap (Figure 2). The VOC assay was conducted using uniform Petri plates measuring 90 mm in diameter and 15 mm in height. Each paired plate was tightly sealed with a double layer of plastic sealing film to ensure secure closure and minimize external gas exchange. Pathogen inhibition was quantified using colony radial diameters measured on day 14 of incubation, which was designated as the endpoint for calculating percent inhibition. The VOC experiment was performed once as a single independent assay, with replicate plate pairs included within the same experimental run [14]. All treatments were conducted concurrently under controlled laboratory conditions to minimize environmental variations among the experimental units during the observation period. This study represents a preliminary laboratory-scale evaluation of Jakaba BHP01 under standardized experimental conditions.

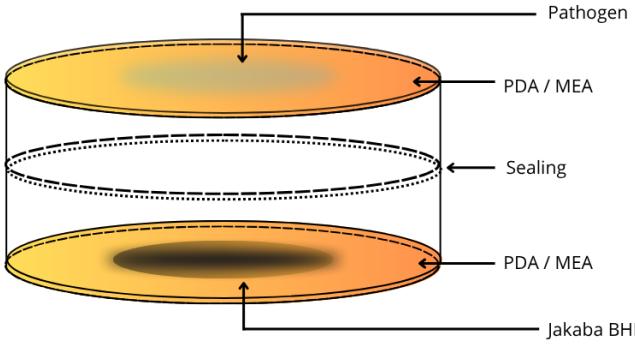

Figure 2. Volatile compound testing scheme. The diagram illustrates the paired-plate (dual-culture) setup, in which a Petri dish containing the Jakaba BHP01 isolate was stacked beneath a dish containing the *Colletotrichum* sp. pathogen and sealed with double-layered plastic film to prevent gas exchange with the surroundings. This scheme was used to evaluate the antagonistic effect of Jakaba BHP01 volatile organic compounds on pathogen growth when cultured on PDA and MEA media.

Incubation was carried out for 14 days, and the pathogen colony diameter was measured daily. The percentage of pathogen growth inhibition was calculated using the formula proposed by Shentu et al. [15] as using Equation 2.

$$\text{P (\%)} = \frac{\text{(DK-DP)}}{\text{DK}} \times 100\% \quad (2)$$

where DK represents the pathogen colony diameter in the control (without Jakaba BHP01) and DP represents the pathogen colony diameter in the VOC treatment.

This setup allowed for the quantitative assessment of the suppressive effect of VOCs produced by Jakaba BHP01 on pathogen growth under sealed, standardized conditions. All treatments were conducted concurrently under carefully controlled laboratory conditions to minimize environmental variations among experimental units during the observation period. This study represents a controlled preliminary laboratory-scale evaluation of Jakaba BHP01 under standardized experimental conditions.

Statistical Analysis

Data analysis was performed using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level using R Studio software. For the mycelial colony growth experiment, a three-way factorial ANOVA was applied with medium type (M; PDA, MEA, and MPA), pH (P; 4, 6, and 8), and temperature (S; 10, 28±1, and 35 °C) as fixed factors. The effect size was estimated using partial eta squared (partial η^2) to evaluate the magnitude of the treatment effects. The following linear model (Equation 3) was used by Mattjik and Sumertajaya [16]:

$$Yijkl = \mu + \alpha i + \beta j + \gamma k + (\alpha\beta)ij + (\alpha\gamma)ik + (\beta\gamma)jk + (\alpha\beta\gamma)ijk + \varepsilon ijkl \quad (3)$$

where:

Y_{ijkl} = Response of colony diameter growth under the treatment combination of media factor at level-*I,* pH factor at level-*j*, temperature factor at level-*k*, and replication *l*

μ = Overall mean

αi = Main effect of the media factor (*i* = PDA, MEA, MPA)

β_j = Main effect of the media factor (*j* = 4, 6, 8)

γ_k = Main effect of the media factor (*k*= 10°C; 28±1°C; 35°C)

$(\alpha\beta)_{ij}$ = Interaction effect between the media factor (level *i*) and the pH factor (level *j*)

$(\alpha\gamma)_{jk}$ = Interaction effect between the media factor (level *i*) and the temperature factor (level *k*)

$(\beta\gamma)_{jk}$ = Interaction effect between the pH factor (level *j*) and the temperature factor (level *k*)

$(\alpha\beta\gamma)_{jk}$ = Three-way interaction effect among media, pH, and temperature factors

ε_{ijkl} = Experimental error term, assumed to be normally distributed

Statistical analysis of the VOC assay was conducted using an independent two-sample t-test to compare pathogen growth between the control and VOC treatments. Prior to the analysis, the assumptions of normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene's tests, respectively. The results indicated that the VOC inhibition data satisfied both statistical assumptions. All statistical analyses were performed using the RStudio software. Because the VOC assay was conducted as a controlled preliminary laboratory-scale experiment within a single experimental run, the results should be interpreted with consideration of the limitations of temporal replication.

Results and Discussion

Results

Macroscopic Characteristics of Jakaba BHP01 Mycelial Colonies

Jakaba BHP01 isolates were grown on three types of media PDA, MEA, MPA, and incubated at three temperatures: 10 °C, 28 ± 1 °C, and 35 °C. Based on macroscopic observations, each mycelial colony exhibited distinct morphological characteristics. Observations showed that the mycelium had a cottony (cotton-like) and granular texture due to differences in the thickness and density of the mycelium tissue. The underside of Jakaba mycelial colonies grown on PDA, MEA, and MPA media showed variations in color and the number of fruit body primordia (Figure 3). On PDA medium, the underside of the colony was white with only a few fruit body primordia, whereas on MEA medium, fruit body primordia were more abundant. On MPA medium, the underside of the colony displayed a dark brown center, with only a few fruiting bodies emerging.

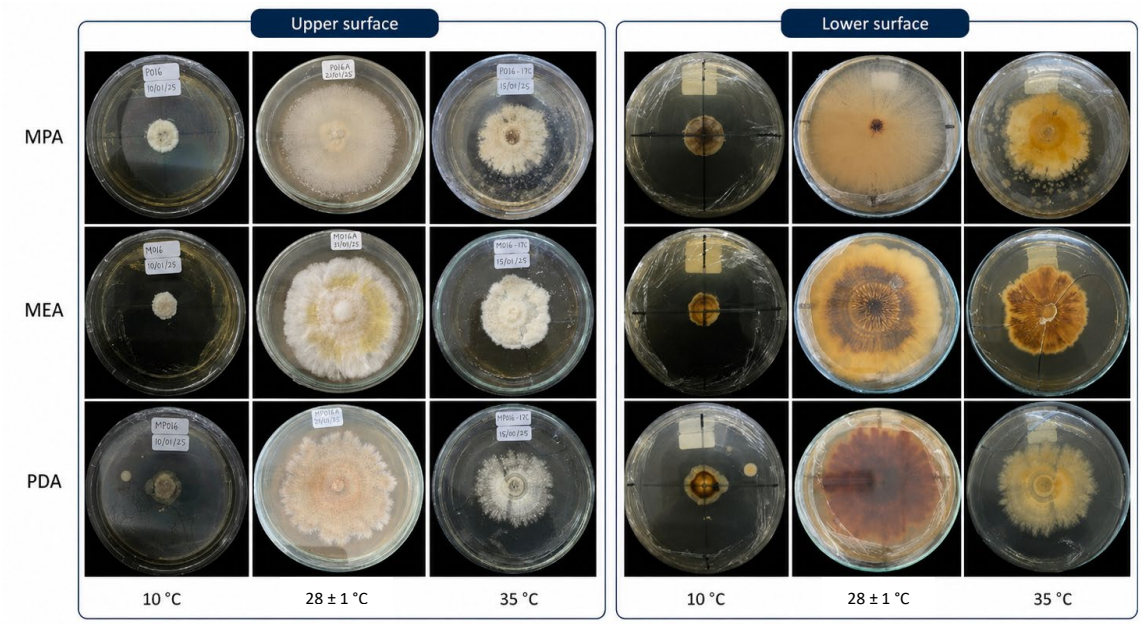

Figure 3. The upper (left) and lower (right) surfaces of the Jakaba BHP01 mycelium colony on day 17. The photographs compare colony morphology grown on PDA, MEA, and MPA media, highlighting differences in mycelial texture, color, and the number of fruit body primordia on the underside of each colony. On PDA, the underside was white with few primordia, whereas MEA produced more abundant primordia, and MPA showed a dark brown center with only a few fruiting bodies. These morphological differences illustrate how culture medium composition influenced the macroscopic development of the Jakaba BHP01 colony.

Growth of the Diameter of the Mycelium Colony of Jakaba BHP01

The graphic (Figure 4) shows that the treatment combinations had different effects on the growth of the mycelium colony diameter of Jakaba BHP01. The largest mycelium colony diameter growth on day 17, with a diameter of 7.63 cm, was produced by the M2P1S2 treatment combination (MEA medium, pH 4, and temperature 28 ± 1 °C). However, the treatment combination M1P1S1 (PDA medium, pH 4, and temperature 10 ± 0.1°C) showed the smallest diameter of the Jakaba BHP01 mycelium colony on day 17, with a diameter of only 1.55 cm.

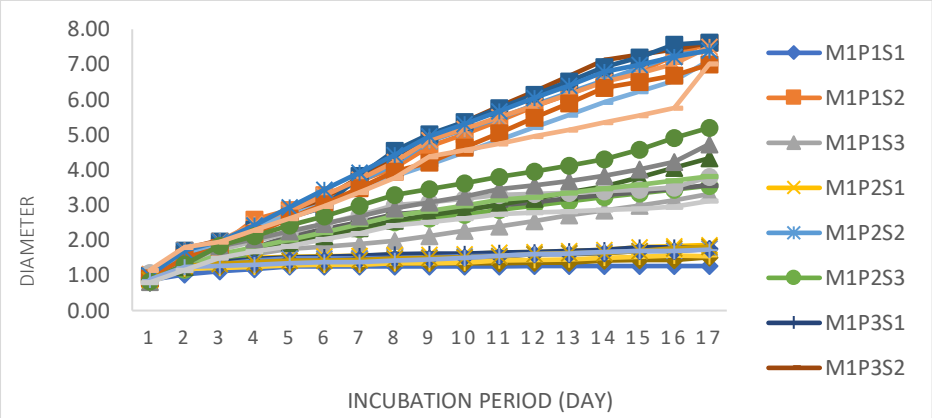

Figure 4. Growth of Jakaba BHP01 mycelium colony diameter under various treatment combinations. M1P2S2 as control. The graph plots colony diameter (cm) against incubation day for all 27 combinations of media, pH, and temperature. The M2P1S2 combination (MEA medium, pH 4, temperature 28 ± 1 °C) produced the fastest and largest diameter growth, reaching 7.63 cm by day 17, while the M1P1S1 combination (PDA medium, pH 4, temperature 10 ± 0.1 °C) showed the slowest growth, reaching only 1.55 cm on day 17. These results illustrate that incubation temperature was the main driver of differences in mycelial growth rate among treatments.

Table 1. Results of the analysis of variance test for each treatment on the growth of the diameter of the Jakaba BHP01 mycelium colony. The table presents the three-way ANOVA results for the main and interaction effects of media type, pH, and incubation temperature on Jakaba BHP01 colony diameter growth. Temperature was the only factor with a statistically significant effect ($p < 0.0001$), whereas media, pH, and all interaction terms were not significant ($p > 0.05$). These results indicate that, among the three tested factors, temperature was the dominant driver of Jakaba BHP01 mycelial growth.

Treatments	Df	Sum sq	Mean Sq	F Value	Pr (> F)
Media	2	0.6	0.28	0.634	0.5346
pH	2	1.5	0.76	1.723	0.1882
Temperature	2	443.8	221.91	504.951	<2e-16***
Media and pH	4	2.6	0.66	1.498	0.2156
Media and temperature	4	3.8	0.94	2.150	0.0871
pH and temperature	4	1.7	0.42	0.955	0.4397
Media, pH, and Temperature	8	3.6	0.45	1.025	0.2008
Residuals	54	23.7	0.44		

*Significantly influential at the 5% test level.

Based on the results of the analysis of variance at a 5% significance level (Table 1), it was found that the temperature factor had a significant effect on the growth of the mycelium colony diameter of Jakaba BHP01, while the media and pH factors did not show a significant effect. The interactions between the medium type of factor and the pH factor, the medium factor and the temperature factor, the pH factor and the temperature factor, as well as the interaction of all three factors did not have a significant effect on the growth of the mycelium colony diameter of Jakaba BHP01. Temperature also showed the largest effect size (partial $\eta^2 = 0.95$), indicating that temperature was the dominant factor influencing Jakaba BHP01 colony growth under the tested laboratory conditions.

The effect of temperature in this study can be determined more specifically by conducting further tests, namely the Duncan multiple range test or DMRT test. The Duncan test in this study was used to further test the results of the analysis of variance to determine the differences in the effects of each temperature on the growth of the diameter of the Jakaba BHP01 mycelium colony. The results of further analysis using the Duncan test showed significant differences between the temperature treatments on the growth of the diameter of the Jakaba BHP01 mycelium colony (Figure 5). The growth of the diameter of the Jakaba BHP01 mycelium colony at a temperature of 28 ± 1 °C yielded the highest results compared to temperatures of 10 °C and 35 °C. This shows that a temperature of 28 ± 1 °C in this study was the best temperature for the growth of the Jakaba BHP01 mycelium colony.

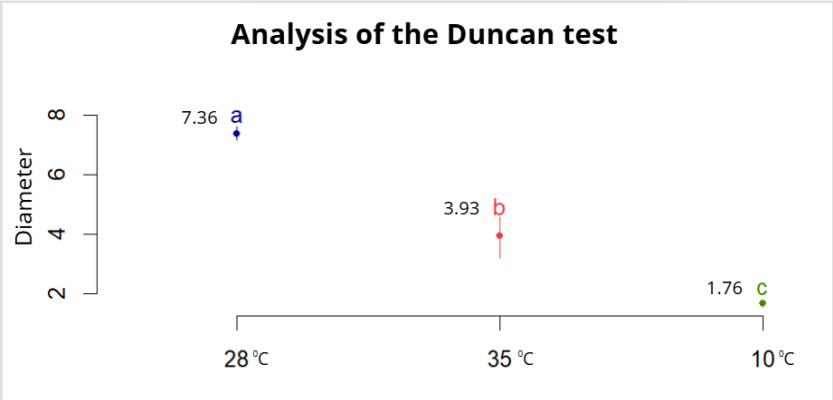

Figure 5. Results of Duncan's test on the effect of temperature on the growth of Jakaba BHP01 mycelium colony diameter. Numbers followed by different letters in the columns indicate significantly different treatments at the 5% test level; The X-axis represents incubation temperature (°C), while the Y-axis represents mycelial colony diameter (cm). The bar chart shows mean colony diameter (cm) at each incubation temperature (10 °C, 28 ± 1 °C, and 35 °C), with different letters above the bars indicating statistically significant differences based on Duncan's Multiple Range Test at the 5% level. The colony diameter at 28 ± 1 °C was significantly larger than at 10 °C and 35 °C, confirming that 28 ± 1 °C was the optimal temperature for Jakaba BHP01 mycelial growth.

Pathogenicity Test of Jakaba BHP01 on Sengon Seeds using Two Types of Media

Pathogenicity tests were conducted to evaluate the effect of Jakaba BHP01 on the germination and early growth of Sengon seed. The results showed that seeds treated with Jakaba BHP01 germinated normally under the tested laboratory conditions. No visible disease symptoms or growth abnormalities were observed in the treated seedlings during the study period. In addition, the treated seeds exhibited better early growth performance than the untreated control. These findings indicate that Jakaba BHP01 did not show pathogenic characteristics toward sengon seedlings under controlled laboratory conditions (Figure 6).

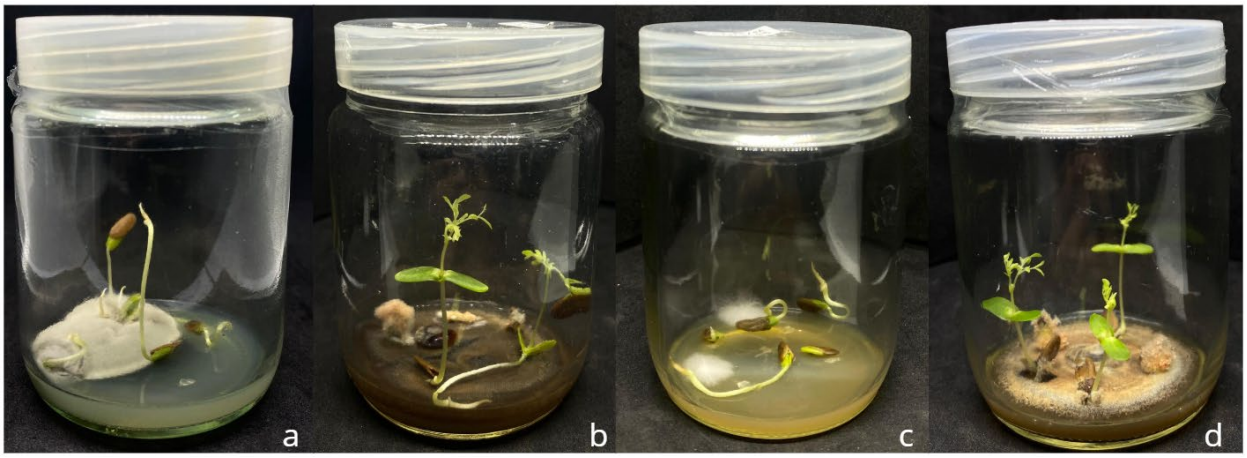

Figure 6. Pathogenicity test of Jakaba BHP01 on PDA and MEA media. a: control (PDA); b: treatment (PDA); c: control (MEA); d: treatment (MEA).

Jakaba BHP01 has the potential to enhance seed germination (Table 2). Jakaba BHP01 treatment produced varying effects depending on the type of medium and parameters measured. A significant effect was only observed in sprout length on MEA, indicating that this medium better supports Jakaba activity than PDA. For other parameters, such as fresh weight and germination percentage, no significant differences were found between the control and treatment groups. Nevertheless, some trends of improvement suggest the initial potential of Jakaba BHP01 in promoting plant growth. PDA treatment resulted in a lower germination percentage than the control, indicating that certain culture conditions may have influenced seed germination responses. Despite this reduction, the treated seedlings that successfully germinated developed normally during the observation period. Therefore, Jakaba BHP01 did not exhibit clear pathogenic characteristics toward sengon seedlings under the tested laboratory conditions.

Table 2. Result of analysis of variance by comparing a treatment with a control (95% confidence level overall).

Parameter	Media	Treatments		P-Value
		Control	Jakaba BHP01	
Sprout Length (cm)	PDA	3.39	4.77	0.275
	MEA	2.58	4.44	0.045*
Fresh weight of sprout (gr)	PDA	0.069	0.085	0.103
	MEA	0.067	0.107	0.325
Germination percentage (%)	PDA	86.66	46.66	0.055
	MEA	46,66	60	0.67

*Significantly influential at the 5% test level.

The table compares sprout length, fresh weight, and germination percentage of sengon seeds treated with Jakaba BHP01 against untreated controls on PDA and MEA media. A significant treatment effect ($p = 0.045$) was observed only for sprout length on MEA medium, while the other parameters showed no significant differences between treatment and control. These results indicate that Jakaba BHP01 did not exhibit pathogenic effects on sengon seeds and showed a preliminary growth-promoting effect on MEA medium.

The Ability of Jakaba BHP01 to Produce Volatile Compounds

Volatile organic compounds (VOCs) are increasingly recognized as important biochemical signals that enable fungi to suppress pathogens without direct physical contact, and several studies have demonstrated their

strong inhibitory potential in agricultural and forestry systems [17]. Given this relevance, we evaluated whether Jakaba BHP01 produces VOCs that can interfere with the growth of *Colletotrichum* sp., a common plant-pathogenic fungus. Assessing VOC activity across different media provides insights into how nutrient composition influences the quantity and type of volatiles emitted by antagonistic fungi. This evaluation is essential for determining the biocontrol potential of Jakaba BHP01 under nursery conditions, where non-contact suppression mechanisms are particularly valuable. The visual comparison below illustrates the differential pathogen responses across PDA and MEA media when exposed to the VOCs produced by Jakaba BHP01 (Figure 7).

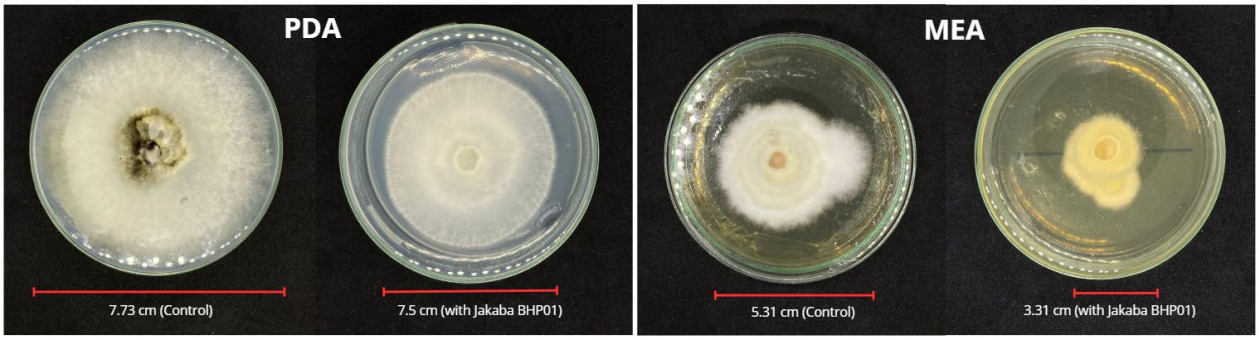

Figure 7. Antagonistic activity of Jakaba BHP01 using volatile compounds in inhibiting the growth of *Colletotrichum* sp. on PDA and MEA Media. The photographs show the paired-plate assay results, comparing *Colletotrichum* sp. colony growth in the control (pathogen alone) versus treatment (pathogen exposed to Jakaba BHP01 volatile compounds) on PDA dan MEA media. Volatile compounds produced by Jakaba BHP01 inhibited *Colletotrichum* sp. growth by 2% on PDA and 38% on MEA, indicating that the culture medium influenced the strength of the antagonistic effect. These results demonstrate that Jakaba BHP01 has potential as a biocontrol agent against *Colletotrichum* sp., with MEA medium supporting a stronger volatile-mediated inhibitory effect.

The results showed that the Jakaba BHP01 fungus had the ability to produce volatile compounds that could suppress the growth of *Colletotrichum* sp. pathogens on PDA and MEA media with inhibition percentages of 2% and 38%, respectively (Figure 7). The higher inhibition observed on MEA medium suggests that the medium composition may influence the production or activity of volatile compounds produced by Jakaba BHP01. In contrast, VOC-mediated inhibition on PDA medium was relatively low under the same experimental conditions. This indicates that the Jakaba BHP01 fungus produced more volatile compounds on MEA media.

Discussion

Macroscopic observations of the Jakaba BHP01 fungal isolate focused on physical growth characteristics, such as mycelial development, color changes, colony shape, and growth patterns. Generally, fungal mycelial growth on culture media appears as the emergence of thread-like filaments [18]. Macroscopic observations include the color of the colony, edge shape, surface topography, and overall growth pattern [19]. The physicochemical properties of fungi depend on the composition of the culture medium, pH, temperature, light, water availability, and the surrounding atmospheric gas mixture [20]. The difference in mycelium texture can be considered an indicator of Jakaba productivity, both in terms of its ability to form active biomass and to produce spores. Culture optimization through media selection, pH balance, and temperature regulation is critical for maximizing fungal mycelial development and metabolite production. Previous studies have demonstrated that growth factor manipulation substantially affects enzymatic activity and sporulation in various fungi [21]. Jakaba BHP01 mycelium on MEA medium at normal temperature and pH produced a thick and uniform aerial cottony texture compared to other media. This indicates an increase in biomass accumulation and a higher sporulation production potential compared to other media. These conditions support the role of Jakaba as a bioactivator, making it a suitable reference point for optimizing the production of Jakaba-based liquid organic fertilizer starters.

The Jakaba isolate exhibited the distinctive feature of fruit body primordia growing on the underside of the colony. These fruit body primordia are elongated and resemble coral structures. Macroscopic forms of Ascomycota include spherical, elongated, bowl-shaped, stalked, sponge-like, and coral-like structures. The ability to form more fruiting bodies is an indicator of reproductive success and higher spore production potential. This is related to the survival and effectiveness of microbes in liquid organic fertilizers. However,

this effect is still indicative and needs to be supported by quantitative data related to the growth of the isolate diameter and further secondary or enzymatic metabolite content.

Microorganism growth, particularly fungi, can be defined as the process of increasing the volume of an organism, accompanied by an increase in its biomass. Fungal colonies tend to form circular lines on solid media. One parameter of fungal growth is the radial growth rate of fungal mycelial colonies. Generally, the growth of fungal mycelium colony diameter is influenced by several factors, such as the medium, pH, and temperature. This aligns with Xiong et al. [22], who stated that the main factors influencing fungal growth include environmental acidity (pH), the type of substrate used as a growth medium, oxygen concentration, and environmental temperature.

The largest and smallest colony diameters on day 17 were produced by combinations of treatments with different media and temperatures. However, the pH values of the two treatment combinations did not differ. This indicates that Jakaba BHP01 has a fairly wide tolerance to acidic environmental conditions, enabling it to grow and develop well within that pH range. This finding is highly advantageous for developing liquid organic fertilizer production, as it provides high flexibility in selecting raw materials for fermentation substrates. Smith and Onions [23] explained that fungal filaments can vary across different pH levels.

Jakaba isolates grown on various media (PDA, MEA, and MPA) under pH 4, pH 6, and pH 8 conditions had relatively similar average diameters. This indicates that all three types of media can meet the basic nutritional requirements for the growth of Jakaba BHP01 mycelium colonies, thus not affecting the growth rate based on colony diameter size. Additionally, this also demonstrates that Jakaba BHP01 can grow within an acidic to alkaline (weakly alkaline) pH range without being significantly affected by pH levels. Therefore, based on the analysis of variance test at the 5% level, the three types of media with a pH range of 4–8 were found not to affect the growth rate of the mycelium colony diameter of the Jakaba BHP01. The absence of a significant pH effect suggests that Jakaba BHP01 may possess relatively broad ecological plasticity under the tested laboratory conditions.

The growth of Jakaba BHP01 mycelial colonies under different media and pH combinations showed similar results and did not differ significantly. This finding suggests that PDA, MEA, and MPA can support the basic mycelial growth requirements of Jakaba BHP01 under the tested laboratory conditions. The absence of a significant media effect indicates that differences in nutrient composition among the tested media were not the primary factor controlling the colony diameter in this isolate. Previous studies have demonstrated that fungal growth responses can differ depending on the medium composition. As reported by Gwon [24], MEA and PDA were the most suitable for mycelial growth, while there was no growth on SDA. These findings suggest that Jakaba BHP01 may possess broader adaptability to nutrient conditions, indicating its potential for further development and large-scale cultivation. Temperature appeared to be the dominant factor affecting mycelial growth. Further studies involving nutrient profiling and biomass measurement are required to clarify whether specific carbon or nitrogen sources influence the physiological performance of Jakaba BHP01.

Differences in incubation temperature had a significant effect on the growth of the Jakaba BHP01 mycelium colony. Temperature plays an important role in the production of lignin peroxidase enzymes. Generally, each type of fungus has different optimum, minimum, and maximum temperatures for its growth. Differences in incubation temperature have a significant effect on the growth of the Jakaba BHP01 mycelium colony. Temperature plays a crucial role in the production of lignin peroxidase enzymes. Generally, each type of fungus has different optimal, minimum, and maximum temperatures for its growth [25].

The mycelium colony of Jakaba BHP01 under three different temperature conditions (10 °C, 28 ± 1 °C, and 35 °C) showed different diameter growth. The diameter growth of the mycelium colony of Jakaba BHP01 during the 17-day incubation period tended to stagnate at 10 °C. This indicates that 10 °C can inhibit the diameter growth of the Jakaba BHP01 mycelium colony. The inhibition of diameter growth of the Jakaba BHP01 mycelium colony at 10 °C is suspected to be due to disrupted enzyme activity caused by low temperature conditions. In line with Griffin [26], who stated that temperature during fungal growth can affect enzyme activity, the composition of fungal cell organelles, the composition of the plasma membrane and lipid content. Fungal mycelium colony growth is associated with enzyme release. One factor influencing enzyme production is the temperature [27]. Temperature may influence fungal growth by regulating enzymatic activity and metabolic processes associated with nutrient utilization and mycelial.

Thermotolerance for fungal growth is quite broad; however, most species grow well at temperatures around 25 °C [28]. The growth of the mycelium colony diameter of Jakaba BHP01 at 35 °C did not yield better results

than at 28 ± 1 °C. Fungi at 35 °C can still grow and develop, but their growth is quite slow. The slow growth of the mycelium colony diameter of Jakaba BHP01 is likely due to temperature conditions that nearly cause environmental stress to the fungus. High-temperature stress (heat shock) in fungal cells causes cellular damage by disrupting hydrogen bonds and hydrophobic interactions, leading to protein and nucleic acid denaturation. Such conditions are highly likely to occur because fungi lack the ability to regulate their internal temperatures.

In addition to analyzing the suitability of growing conditions, it is necessary to determine the biological safety of using Jakaba BHP01 on plants to avoid negative effects. Under the tested laboratory conditions, no visible disease symptoms of tissue necrosis were observed in seedlings treated with Jakaba. According to this method, fungi are non-pathogenic to plants if seeds can germinate normally (or similarly to the control) and do not cause necrotic symptoms [27]. Conversely, in the control treatment without Jakaba BHP01, contamination was observed in the medium, likely originating from pathogens carried by the seeds. These results add value to the aspects of safe seed production, as they leave no traces of contamination. These results also support the potential of Jakaba BHP01 as a biocontrol agent and growth enhancer for early stage sengon plants.

The analysis results showed that only MEA media provided a significant difference in seedling length between the control treatment and Jakaba BHP01 (Table 2). This indicates that MEA media is more responsive to Jakaba BHP01 treatment in stimulating seedling growth. This difference may be due to the interaction between volatile compounds or metabolites from Jakaba and the nutrient content of MEA. Therefore, the use of Jakaba BHP01 in MEA medium has the potential to enhance the initial growth vigor of the plants. The fresh weight of the seedlings increased compared to that of the control on both PDA and MEA media, but the increase was not statistically significant. The germination percentage on MEA media increased by 13.34%, but this increase was not statistically significant. The response of seeds to Jakaba BHP01 treatment was likely influenced by the type of medium. It is likely that the nutrient content and physicochemical properties of the medium influence the biological activity of Jakaba BHP01 fungus during germination. Therefore, further research is recommended to optimize the dose and application and explore the interaction between the medium and fungus in greater depth.

Volatile organic compounds are compounds consisting of a mixture of complex lipophilic compounds with low molecular weights, produced via various biosynthetic pathways [28]. These compounds are produced by microbial metabolic activity and serve various beneficial functions for survival. Volatile compounds released by microbes can enhance plant growth and increase plant resistance to infections by various pathogens [29]. Fungal volatile organic compounds (VOCs) are increasingly recognized as potent non-contact antimicrobial agents capable of inhibiting pathogen growth through the release of metabolic vapors [29]. Research on cellulase activity in Jakaba BHP01 has resulted in the formation of a clear zone on CMC medium [30]. The Jakaba BHP01 colony in that study had a diameter of 30 mm and formed a mycelium colony of 40 mm, resulting in cellulase activity of 0.33 mm. Although the inhibitory activity observed in this study suggests that Jakaba BHP01 may produce bioactive enzymes or volatile metabolites, these mechanisms remain hypothetical because they were not directly assessed in this study. Similar fungi are known to secrete hydrolytic enzymes and volatile organic compounds that suppress pathogens, and these processes may also contribute to the antagonistic effects detected here. However, to avoid overinterpretation, we acknowledge that the specific biochemical pathways involved in Jakaba BHP01 are unknown.

The present study demonstrated that Jakaba BHP01 was able to maintain stable mycelial growth across several culture conditions, with temperature being the dominant factor influencing colony development. Under these laboratory conditions, Jakaba BHP01 also exhibited VOC-mediated antagonistic activity against fungal pathogens, while showing no visible pathogenic symptoms on Falcataria moluccana seedlings. Collectively, these findings suggest that Jakaba BHP01 possesses multiple biological characteristics relevant to preliminary microbial-based nursery applications under controlled laboratory conditions.

Conclusions

This study concluded that the optimal growth of Jakaba BHP01 mycelium occurred on malt extract agar (MEA) medium at pH 4 and an incubation temperature of 28 ± 1 °C, with temperature being the most influential factor in fungal development. The isolate's ability to produce volatile compounds that inhibited *Colletotrichum* sp. by up to 38% and its non-pathogenic response toward *Falcataria moluccana* seedlings confirmed its potential as a safe and effective bioactivator and biocontrol agent. Targeted follow-up studies,

such as enzyme activity assays (e.g., chitinase, cellulase, and β-1,3-glucanase), GC–MS-based VOC profiling, and quantitative metabolite analysis, are required to confirm the mechanisms underlying the biocontrol potential of this strain. These investigations will help clarify whether the observed inhibition is driven by enzymatic activity, volatile chemistry, or a combination thereof. The ability of Jakaba BHP01 to maintain growth under different environmental conditions and exhibit VOC-mediated antagonistic activity may provide preliminary relevance for sustainable nursery management approaches in forestry systems.

Author Contributions

RAK: Conceptualization, Methodology, Investigation, Formal analysis, Writing; **ENH**: Conceptualization, Supervision, Writing – Review & Editing; **S**: Methodology, Supervision, Writing – Review & Editing; **AM**: Supervision, Writing – Review & Editing.

AI Writing Statement

During the preparation of this manuscript, the authors used Paperpal to enhance the grammar and overall writing quality. Following the use of this service, the authors carefully reviewed and revised the manuscript as necessary and assume full responsibility for the final content of the publication.

Conflicts of interest

There are no conflicts to declare.

Acknowledgement

This research is funded by the Indonesian Endowment Fund for Education (LPDP) on behalf of the Indonesian Ministry of Higher Education, Science, and Technology and managed under the EQUITY Program (Contract No: 4297/B3/DT.03.08/2025 and No: 42011/IT3/HK.07.00-4/P/B/2025).

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