JPPIPA 11(5) (2025)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Characterization of Orchid Root Fungal Isolates as Leaflet Teaching Material to Improve Learning Outcomes of Grade X Vocational High School Students on the Subtopic of Kingdom Fungi

Fitriya Ayu Wulandari[1], Dwi Sucianingtyas Sukamto[1]*, Hanif Rafika Putri[1]

[1]Biology Education, Faculty of Teacher Training and Education, PGRI Argopuro University, Jember, Indonesia.

Received: January 03, 2025
Revised: March 21, 2025
Accepted: May 25, 2025
Published: May 31, 2025

Corresponding Author:
Dwi Sucianingtyas Sukamto
dwisucianingtyas2310@gmail.com

DOI: 10.29303/jppipa.v11i5.10934

Abstract: This study aimed to identify mycorrhizal fungi, primarily from the genera Rhizoctonia and Tulasnella, on orchid roots and develop leaflet-based teaching materials using the Research and Development (R&D) model with the 4D approach (Define, Design, Develop, Disseminate). Fungal identification was conducted through microscopic examination and molecular techniques (ITS rDNA sequencing) to ensure accurate classification. The 4D model involved defining student needs, designing leaflet content focused on fungal symbiosis, developing draft materials, and disseminating the final leaflet for classroom use. The effectiveness of the leaflet was evaluated with 40 students through pre- and post-tests measuring knowledge of fungal roles in orchid growth. Results showed the average pre-test score increased from 48.5 to 85.2 in the post-test, with an N-Gain score of 0.722 (72.29%), indicating moderate effectiveness. Improvements were most notable in students' understanding of fungal identification and symbiotic functions. These findings demonstrate that leaflet-based teaching materials developed from precise fungal identification can significantly enhance student comprehension of Kingdom Fungi concepts.

Keywords: Kingdom fungi; Learning outcomes; Orchid roots

Introduction

Orchids belong to the angiosperm group, comprising approximately 25,000 species, including both epiphytic and terrestrial types. Indonesia alone is home to around 5,000 orchid species. Their captivating beauty, characterized by a fragrant aroma and diverse shapes and patterns, makes orchids highly popular, especially in the ornamental plant industry (Purnama et al., 2016). One of the famous orchid species is *Dendrobium crenatum.* which not only has aesthetic value but also becomes an important object of scientific research.

Endophytic fungi, which inhabit the roots of orchids, play a vital role in enhancing the plant's ability to absorb water and nutrients, especially in environments where these resources are limited. These fungi also synthesize various bioactive compounds that contribute to the orchid's resistance against pests and diseases, thereby supporting the plant's overall health and survival. Despite their ecological and biological significance, the diversity and characteristics of endophytic fungi associated with dendribium orchids (*Dendrobium crenatum*) remain underexplored. This research aims to characterize the endophytic fungi found in the roots of dendrobium orchids, providing new insights into their morphology, taxonomy, and

How to Cite:
Wulandari, F. A., Sukamto, D. S., & Putri, H. R. Characterization of Orchid Root Fungal Isolates as Leaflet Teaching Material to Improve Learning Outcomes of Grade X Vocational High School Students on the Subtopic of Kingdom Fungi. *Jurnal Penelitian Pendidikan IPA*, *11*(5), 202-214. https://doi.org/10.29303/jppipa.v11i5.10934

potential applications in both biological research and educational contexts (Liao et al., 2025). The novelty of this study lies in its focus on moon orchid roots, a relatively understudied host, and the integration of fungal characterization with the development of innovative teaching materials for biology education, particularly in vocational high schools (SMK) (Salsabila, 2024).

In the context of biology education at the SMK level, the Kingdom Fungi is a challenging topic for many students. The abstract nature of fungal structures, reproductive mechanisms, and ecological roles often leads to disengagement and difficulty in comprehension. At SMK Darul Muklisin Mayang, Jember, biology teachers have reported that students struggle most with understanding fungal morphology and reproduction, which are critical components of the curriculum. Traditional teaching methods predominantly rely on textbooks and static modules, which lack interactive and visual elements that could facilitate deeper understanding. Previous attempts to improve learning outcomes have included lectures supplemented with textbook readings and occasional laboratory observations; however, these methods have not sufficiently addressed students' learning difficulties. Needs assessments and interviews with teachers at this institution reveal a pressing demand for more engaging, research-based teaching aids that can bridge the gap between theoretical knowledge and practical understanding (Kamil, 2023).

One promising approach to enhance biology learning is the use of leaflets as creative and research-based teaching materials. Leaflets are concise, visually appealing information sheets that distill complex scientific concepts into accessible formats. They typically consist of brief texts supported by illustrations, diagrams, or photographs, which help clarify and reinforce key points (Susana, 2017). Unlike textbooks or comprehensive modules, leaflets offer a simplified yet informative medium that caters to students who benefit from visual learning and succinct explanations. The design elements of leaflets—such as color contrast, layout, and imagery—play a crucial role in capturing students' attention and sustaining their interest during study sessions. Research indicates that well- designed visual aids significantly improve information retention and motivation among learners (Lubis, 2024).

This study addresses the gap in biology education by combining the scientific characterization of endophytic fungi from moon orchid roots with the development of an educational leaflet tailored for SMK students. The research is important because it not only contributes to the scientific understanding of orchid-associated fungi but also translates these findings into practical teaching tools that can enhance student engagement and comprehension. By presenting fungal characteristics in a clear, visually supported format, the leaflet aims to make the Kingdom Fungi material more accessible and relevant to students' learning experiences. Furthermore, this research supports the broader educational goal of fostering scientific literacy and curiosity among vocational high school students, preparing them for further studies or careers in biological sciences (Aisha et al., 2023).

In summary, this research is significant for several reasons. First, it fills a scientific gap by providing detailed morphological and taxonomic data on endophytic fungi associated with moon orchids, a subject that has received limited attention. Second, it responds to the educational challenges faced by SMK students in learning about fungi by creating an innovative, research-based teaching aid. Third, it offers a model for integrating scientific research with educational practice, demonstrating how academic findings can be effectively translated into classroom resources. Ultimately, this study aims to enhance both scientific knowledge and educational quality, contributing to improved learning outcomes and greater student interest in biology (Kuway et al., 2023).

The ecological role of endophytic fungi extends beyond their symbiotic relationship with orchids. These fungi contribute to nutrient cycling, plant growth promotion, and stress tolerance, which are critical factors in maintaining healthy ecosystems. In orchids, particularly the dendrobium orchid, endophytic fungi facilitate the absorption of water and essential minerals in nutrient-poor substrates, enabling the plant to thrive in challenging environments (Grabka et al., 2022). This mutualistic interaction is not only vital for the survival of orchids but also offers a unique opportunity to study fungal biodiversity and function in specialized niches. Despite the recognized importance of endophytic fungi, research focusing specifically on their diversity and functional roles in moon orchids remains scarce, highlighting the need for comprehensive characterization studies (Rodriguez et al., 2009).

In the educational context, the Kingdom Fungi presents a multifaceted challenge for students, especially at the vocational high school level where practical applications of biological concepts are emphasized. The complexity of fungal life cycles, morphological diversity, and ecological significance often overwhelms students, leading to disengagement and poor academic performance. At SMK Darul Muklisin Mayang, Jember, biology educators have identified specific topics within the Kingdom Fungi curriculum—such as fungal reproduction, spore formation, and symbiotic relationships—as particularly difficult for students to grasp. Traditional pedagogical approaches, which rely heavily on didactic lectures and textbook-based learning, have proven insufficient in addressing these challenges. Attempts to incorporate laboratory exercises have been limited by resource constraints and lack of tailored instructional materials, resulting in suboptimal learning outcomes (Kamil, 2023).

The integration of research findings into educational materials represents a strategic approach to overcoming these learning barriers. Leaflets, as concise and visually engaging teaching aids, have demonstrated effectiveness in enhancing student comprehension and motivation (Maulana, 2017). Their format allows for the distillation of complex scientific information into manageable segments, supported by illustrations that cater to diverse learning styles. The use of leaflets in biology education aligns with constructivist learning theories, which emphasize active engagement and the use of multiple representations to facilitate understanding (Rindayana, 2024). Moreover, leaflets can be easily distributed and utilized in various learning environments, making them practical tools for resource-limited settings. The design principles underlying effective leaflets—such as clarity, visual appeal, and relevance—are critical to their success as educational interventions (Lubis, 2024).

This research is novel in its dual focus on both the scientific and educational dimensions of endophytic fungi associated with dendrobium orchids. While previous studies have explored fungal endophytes in various plant species, few have concentrated on the dendrobium orchid, a species of significant horticultural and ecological interest. By characterizing the morphology and taxonomy of these fungi, this study contributes to the broader understanding of fungal biodiversity and plant-microbe interactions (Prajapati et al., 2025). Simultaneously, the translation of these findings into a research-based leaflet addresses a critical gap in biology education at the SMK level, providing a tailored resource that supports student learning and engagement. This approach exemplifies the potential for research to inform and enhance educational practice, fostering a deeper connection between scientific inquiry and classroom instruction (Salsabila, 2024).

The importance of this research is underscored by several factors. Scientifically, it advances knowledge of endophytic fungi in a specialized host, contributing to biodiversity documentation and potential biotechnological applications, such as natural pest resistance and growth promotion in orchids. Educationally, it responds to the urgent need for innovative teaching materials that can make abstract biological concepts tangible and accessible to students (Shakila et al., 2024). By improving the quality and relevance of teaching aids, this study supports the development of scientific literacy and critical thinking skills among vocational high school students, preparing them for future academic and professional pursuits in the biological sciences. Furthermore, the research promotes the integration of local biodiversity studies into education, fostering environmental awareness and appreciation among young learners (Aisha et al., 2023).

In conclusion, this study bridges the gap between scientific research and educational practice by providing a comprehensive characterization of endophytic fungi from moon orchid roots and developing an innovative leaflet as a teaching tool. This integrated approach not only enriches the scientific literature but also addresses the pedagogical challenges faced by SMK students in learning about the Kingdom Fungi. The expected outcomes include enhanced student engagement, improved comprehension of fungal biology, and the promotion of research-based learning materials in vocational education (Sari et al., 2023). Ultimately, this research contributes to the advancement of both biological sciences and education, demonstrating the value of interdisciplinary collaboration in addressing complex educational and scientific challenges.

Method

Time and Research Materials

This research was conducted at the Biology Education Laboratory of PGRI Argopuro University Jember from June to November 2024. The leaflet product trial took place at SMK Darul Mukhlisin, Mayang, Jember, in December 2024. The study utilized roots of *Dendrobium crenatum* orchids, sourced from the foothills of Mount Gumitir, Banyuwangi, where they grow epiphytically on host trees. Sample selection was carried out purposively, focusing on healthy orchid roots free from disease.

Isolation and Purification of Orchid Root Endophytic Fungi

The procedure for isolation and purification of endophytic fungi adopts the method by Michael et al. (2023), root samples are first cleaned with sterile running water to remove adhering dirt, after draining the sample is cut into small pieces measuring 2 x 2 cm. These sample pieces were then immersed in 70% ethanol for 30 seconds, followed by immersion in 12% NaOCl solution for 10 minutes after which they were rinsed using sterile distilled water 3 times.

The isolation of endophytic fungi was performed using the direct planting method. After sterilizing the orchid surface, the sample pieces were dried with sterile tissue for a few minutes. The orchid roots were then cut longitudinally into two parts and placed on PDA media, with the cut surface in contact with the agar. The Petri dish was incubated at room temperature (27–29 °C) for 7–14 days (Murdiyah, 2017). Fungal colonies that emerged were purified by aseptically transferring the mycelium to fresh Potato Dextrose Agar (PDA) medium. The incubation process continued at room temperature for 72 hours. Colonies that successfully separated and exhibited optimal growth were then subcultured on PDA media for macroscopic and microscopic identification (Aziz, 2017).

Identification of Endophytic Fungal Isolates

Endophytic fungi incubated for seven days were identified through macroscopic and microscopic analysis. Macroscopic observations involved examining various colony characteristics, including growth rate, color, shape, surface texture, elevation, and distinctive features. Meanwhile, microscopic identification focused on the presence of spores or conidia, hyphal type, and the morphology of spores and conidia using a microscope. The identification outcomes were then compared with reference materials from identification key books to determine the species of the observed endophytic fungi (Erliza, 2023).

Teaching Material Enhancement

The characterization outcomes of endophytic fungi obtained from orchid roots will be developed into teaching materials for the Kingdom Fungi subtopic. The enhancement process follows the 4D model, which consists of four stages: Define, Design, Develop, and Disseminate (Thiagarajan, 1974). The specific application of the 4D model in this study is presented in Table 1.

Table 1. 4D stages of enhancement

Stages	Analysis	Instrument
Define	Curriculum analysis	Interviews to fill out a needs questionnaire about learning problems and the use of teaching materials.
	Student analysis	Interviews and filling out a needs questionnaire about student characters during the learning process.
	Curriculum analysis	Learning curriculum, Competency Standards (SK) and Basic Competencies (KD), lesson plans, Pancasila profile, learning outcomes, learning objectives.
	Concept analysis	Learning curriculum, lesson plans, learning resources, teaching materials.
	Formulation of Teaching Material Objectives	Sub Material Kingdom Fungi Indicator.
Design	Initial Design Analysis	Draft leaflet product enhancement grids, draft validation sheet instruments, draft product practicality instruments, draft student science literacy instruments.
Develop	Product enhancement	Draft I of leaflet product
	Expert validation & revision	Draft II leaflet product
	Product trial	Draft III leaflet product
Disseminate	Product Deployment	Provided teaching materials to schools and compiled articles.

In Table 1, the Define stage begins with a needs analysis, which is carried out through distributing interviews to teachers and providing needs questionnaires to students. This analysis aims to identify core issues in the learning process and assess students' needs for the enhancement of teaching materials. Additionally, student characteristics were analyzed through teacher interviews and student questionnaires, covering aspects such as background, skills, cognitive enhancement, prior learning experiences, and science literacy levels.

The next stage is curriculum analysis, which involves reviewing learning documents such as Competency Standards (SK), Basic Competencies (KD), Learning Implementation Plans (RPP), the Pancasila Student Profile, Learning Outcomes, and Learning Objectives. This analysis ensures that the developed teaching materials align with applicable educational standards. Following this, researchers conducted a concept analysis based on the curriculum and relevant learning resources. This stage includes identifying subtopics and learning indicators related to the Kingdom Fungi, as well as analyzing existing teaching materials to ensure their continuity with student needs. The final stage in the Define phase is the formulation of teaching material objectives. This process involves translating basic competencies into more specific learning indicators, guided by the outcomes of the needs analysis, initial material review, and classroom observations.

The next stage is Design (Ramadhani, 2022), which is the product planning stage. At this stage, researchers compiled a draft of the leaflet product enhancement grid, as well as designed validation sheet instruments, product practicality instruments, and pre-test and post-test question instruments.

In the Develop stage, product enhancement was carried out starting with the making of the first draft of the leaflet. This product was then validated by material experts and media experts to test the quality of the content and design (Sari, 2019). If deficiencies were found, revisions were made to produce draft II leaflets. The validation and validity of the product was carried out by conducting a product validity test determined based on the validation score and assessment of lecturers and students with a Likert scale of 4 (strongly agree), 3 (agree), 2 (disagree), 1 (strongly disagree). After the leaflet is assessed by the expert, the percentage of product validity is then sought using the validation formula by Akbar (2013). Based on the validity value obtained, valid criteria are determined as listed in Table 2.

$$V = \frac{TSe}{TSh} \times 100\% \quad (1)$$

V = Validity
TSe = Total validation score from validators
TSh = Total maximum score

Table 2. Validity categories

Score (%)	Validity
85–100	Very valid
70–84	Valid enough
50–69	Less valid
01–49	Invalid

Based on the validation outcomes from material and media experts, any necessary revisions were made, outcoming in Draft III, which was then ready for testing on an experimental class at SMK Darul Mukhlisin, Mayang. The research subjects consisted of 20 students who had previously studied the classification of living organisms, particularly the Kingdom Fungi subtopic. Data collection was conducted using random sampling techniques in Biology classes. The main purpose of this trial is to get direct feedback from students and see student responses to Leaflet teaching materials. After obtaining the data, data processing was carried out which was analyzed using the percentage formula for student responses (Utomo, 2018) and categorized based on Table 3.

$$p = \frac{\sum X}{N} \times 100\% \quad (2)$$

p = percentage score
$\sum X$ = number of scores obtained
N =Maximum Number of scores

Table 3. Percentage of student response

Percentage (%)	Predicate
81–100	Very good
61–80	Good
41–60	Medium
21–40	Not good
0–20	Very unfavorable

To determine the effectiveness of leaflet teaching materials in improving student learning outcomes, it can be done by comparing the scores of the pretest and posttest questions calculated using the N-Gain formula (Kurniawan & Hidayah, 2020) and the effectiveness criteria of the N-Gain score can be seen in Table 4 (Wahab et al., 2021).

$$N - Gain = \frac{\text{postest score} - \text{pretest score}}{\text{ideal score} - \text{pretest score}} \quad (3)$$

Table 4. N-Gain score

Score	Criteria
< 40	Ineffective
40–55	Less effective
56–75	Effective enough
> 75	Effective

The last stage is Disseminate, which is the stage of disseminating teaching materials. At this stage, the teaching materials that have been developed are distributed to schools for use in learning. In addition, the research outcomes are also published in the form of scientific articles as part of academic dissemination.

Result and Discussion

The isolation of orchid roots revealed six generals of endophytic fungi, each differing in shape, color, colony characteristics, conidia, and hyphae. Fungal identification was conducted by examining macroscopic characteristics on PDA media and microscopic features using a binocular microscope. The identification process was guided by identification key books and relevant literature (Aulya, 2020).

Morphology refers to the physical characteristics of fungi, including variations in hyphae, mycelium, and conidia. The macroscopic characteristics of endophytic fungal isolates from orchid roots are presented in Table 5. Based on the outcomes of Table 5, this study identified and characterized the fungal isolates based on macroscopic and microscopic morphology. Isolation was carried out on PDA (Potato Dextrose Agar) media, and the identification outcomes showed the presence of several genus of fungi, namely *Rhizoctonia sp.*, *Aspergillus flavus*, *Aspergillus niger*, *Trichoderma sp.*, and *Rhizopus sp*.

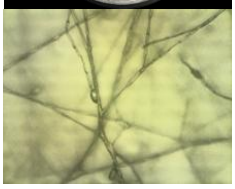
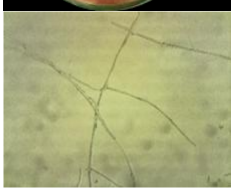
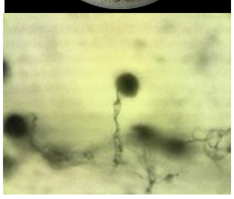
Rhizoctonia sp. has white to orange colonies with an irregular shape and a flat, cottony surface. This fungus shows rapid growth with a radial spreading pattern. Microscopically, *Rhizoctonia sp.* has branched hyphae with an angle close to 90°, thick hyphal walls and fused, but no conidia or asexual spores were found (Hamzah et al., 2021).

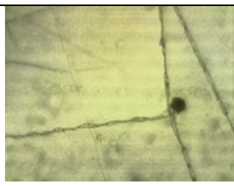
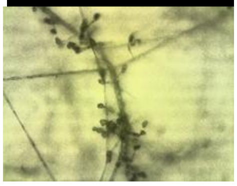
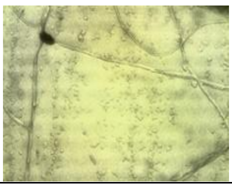
Aspergillus flavus forms green colonies with a granular textured surface and rapid growth. Microscopcally, it shows concentrated hyphae with irregular branches round vesicles at the end of conidiophores, and greenish-yellow round conidia (Saldan et al., 2018).

Aspergillus niger has solid black, more regularly shaped colonies with smooth edges and a feather-textured surface. The underside of the colony is yellow to golden brown. Microscopically, *Aspergillus niger* has concentrated hyphae with irregular branches, round vesicles at the end of conidiophores, and small conidia arranged in long chains in solid black (Mizana et al., 2016).

Trichoderma sp. formed ash-black colonies, irregularly shaped with smooth edges and fur-textured surfaces. Microscopically, the hyphae were concentrated with many branches, conidiophores branched like trees, and dark green round conidia arranged in short chains (Nurliana & Anggraini, 2018).

Table 5. Identification outcomes of endophytic fungi of orchid root

Isolate Code	Genus	Characteristics of
F1	*Rhizoctonia sp.*  	Macroscopic: White colonies; Has an irregular shape; The surface of flat colonies is cottony in texture; and Its rapid growth spreads radially throughout the PDA media. Microscopic: Hyphae branch at an angle of almost 90° (perpendicular); Hyphal walls are thick with clear partitions; Transparent hyaline hyphal color; and Oval-shaped conidia.
F2	*Rhizoctonia sp.*  	Macroscopic: Orange colored colonies; Has a regular shape; Flat, cottony-textured colony surface; and Rapid growth spreads radially throughout the PDA medium. Microscopic: Hyphae branch at an angle of almost 90° (perpendicular); Hyphal walls are thick with clear partitions; Transparent hyaline hyphal color; and It has no conidia or asexual spores.
F3	*Aspergillus flavus*  	Macroscopic: Green colonies; Has an irregular shape; Flat colony surface with granular texture; and Its rapid growth spreads radially throughout the PDA media. Microscopic: Hyphae are septate and irregularly branched; Hyphal walls are thin to medium with a slightly colored hyaline structure; Vesicles are round at the end of the conidiophores; Conidiophores are erect and unbranched; Conidia are round in shape; and Conidia color is greenish yellow.
F4	*Aspergillus niger* 	Macroscopic: Deep black colonies; Has a regular shape with smooth edges; Hairy textured colony surface; Rapid growth spreads radially throughout the PDA medium; and The underside of the colony is yellow to golden brown. Microscopic: Concentrated hyphae and irregular branches; Hyaline hyphae of medium thickness; Vesicles are round at the tip of the conidiophores; Conidiophores are erect, unbranched, and blackish brown in color; Conidia are round, small, and arranged in long chains; and Deep black conidia

		color.
F5	*Thricoderma sp.*  	Macroscopic: Blackish ash colored colonies; Has an irregular shape with smooth edges; Hairy textured colony surface; Its rapid growth spreads radially throughout the PDA media; and Bottom of the colony is solid black. Microscopic: Has hyphae with many branches and fibers; Hyaline and thick hyphae; Tree-like branched conidiophores; The phialid is oval with a tapered tip; and Conidia are round, dark green in color arranged in short chains.
F6	*Rhizopus sp.*  	Macroscopic: Colonies are yellowish white, slightly brown when ripe; Irregular shape with denser edges; The surface of the colony is hairy like cotton, but more compact; Growth spreads radially but slower; and the underside of the colony is yellowish to brownish in color. Microscopic: Hyphae are non-concentrated and denser; Hyaline hyphae with thick walls; Sporangiophores are erect, rarely branched; The sporangium is round, sparse, and grayish in color; and Sporangiospores round to oval, arranged in small clusters.

Rhizopus sp. has yellowish white colonies, irregularly shaped with denser edges, and a texture that resembles cotton but is more compact. This fungus grows radially with a slower growth rate than other isolates. Microscopically, *Rhizopus sp.* has non- concentrated hyphae with high density, erect and rarely branched sporangiophores, and round sporangium containing sporangiospores arranged in small groups (Jagat et al., 2021).

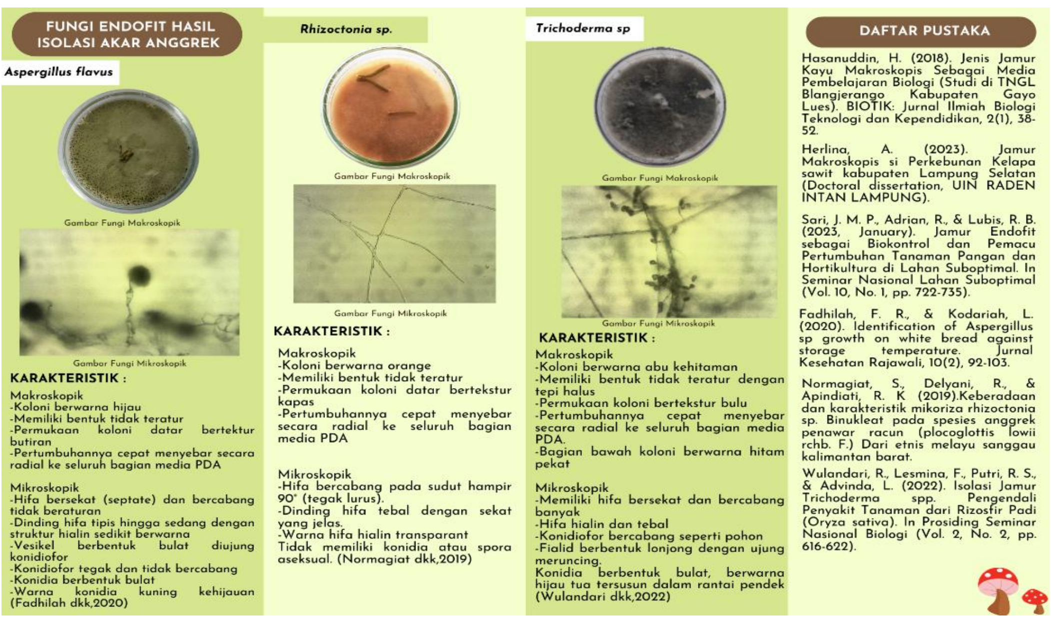
This characterization shows that the fungi found have a variety of morphological characteristics that can be used as material in the enhancement of teaching materials. Information on the macroscopic and microscopic characteristics of fungi is expected to improve students' understanding of the concept of Kingdom Fungi more visually and systematically (Munarti et al., 2023).

Furthermore, the 4D modeling procedure was carried out. At the define stage (Waruwu, 2024), researchers obtained the following outcomes. First, the needs analysis: Students of class X SMK Darul Mukhlisin have difficulty in understanding the material of the Diversity of living things, especially in the Kingdom Fungi submaterial, due to the limited teaching materials that are interesting and interactive, as well as learning methods that still focus on lectures without sufficient visual exploration. Second, student analysis: Students tend to be less focused when learning takes place, often talk outside the context of the lesson, and so far, only use teaching materials in the form of PowerPoint and textbooks that do not support in-depth understanding of concepts (Sufa, 2023). Third, curriculum analysis: Kingdom Fungi material in the applicable curriculum includes classification, characteristics, roles, and examples of fungi in everyday life. Fourth, concept analysis: The material compiled in the teaching materials includes macroscopic and microscopic morphological characteristics of fungi, classification of fungi based on their reproductive structure and ecological role, as well as the benefits and impact of fungi in various fields of life (Magdalena et al., 2020). Fifth, the formulation of objectives: Improve student learning outcomes by clarifying the concept of the Fungi Kindom, helping students understand the characteristics of fungi visually and systematically, and improving critical thinking skills in categorizing fungi based on their characteristics and functions.

At the design stage, the outcomes of the design include the design of leaflets as visual-based teaching materials, the preparation of teaching material grids, as well as the design of validation instruments, student response instruments, and instruments to measure the effectiveness of teaching materials on improving student learning outcomes (Apriana, 2023).

At the develop stage, the outcomes of making the initial draft of the leaflet are presented in Figure 1. This leaflet is designed in an attractive and informative format, consisting of several main sections. The opening section contains the title of the leaflet, the topic or submaterial discussed, the author's name, and an introduction that explains a little about the material. The content section includes a brief description of the Kingdom Fungi, classification, macroscopic and microscopic morphological characteristics of fungi, and their role in everyday life. The final part of the leaflet is equipped with reference sources used in the preparation of teaching materials.

(a)



(b)

Figure 1. Examples of developed *leaflet* sections. (a) Title and introduction to the material. (b) Image of the content section of the research outcomes

After developing the leaflet teaching materials, the next stage is validation. Media experts assess various aspects, including the use of design variations that are engaging but not excessive, appropriate color selection, a balanced ratio of text to images, and the alignment of visuals and tasks with the content. Additionally, validation evaluates the inclusion of engaging tasks, layout consistency, design creativity, visual appeal, ease of use, and the extent to which the media supports independent and active learning (Jalil, 2021).

Meanwhile, material experts focus on aspects such as content accuracy and quality, depth and coverage of the material, language appropriateness, the inclusion of learning plan headings, linguistic precision and conciseness, clarity of objectives, and the relevance of various learning components within the leaflet. The validation outcomes from media and material experts are presented in Table 6 and Table 7.

Table 6. Material expert and media expert validation outcomes

Validator	Percentage of outcomes (%)	Category
Media	86	Very valid
Material	90	Very valid

Table 7. Revisions based on media expert validator suggestions

Advice	Revision outcome
	
The writing of the title should be centered and the font color on the author and agency name is not clear enough.	Revised the title and improved the font color on the author and institution name.

After making revisions and producing Draft 2 of the leaflet, the next stage involved testing students to evaluate their response to the teaching materials. Student responses were analyzed by converting their answers into percentage scores categorized under feasibility. The student response sheet included three assessment categories: material feasibility, presentation feasibility, and language feasibility. The outcomes of the student response trial are presented in Table 8.

Table 8. Student response outcomes

Component	Percentage (%)	Criteria
Content feasibility	90.83	Very feasible
Presentation feasibility	93.22	Very feasible
Language feasibility	91.56	Very feasible
Average	91.87	Very feasible

Students rated the leaflet teaching materials as very feasible, with the highest rating for presentation feasibility (93.22%). This suggests that the visual and structural design of the leaflets effectively enhances student interest and motivation for independent learning. Therefore, leaflet-based teaching materials were classified as a highly feasible instructional resource.

Table 9. Pre-Test and post-test scores with N-Gain analysis

Measure	Score
Mean Pre-Test Score	48.5
Mean Post-Test Score	85.2
Gain (Post-Test - Pre-Test)	36.7
Ideal Gain (100 - Pre-Test)	51.5
N-Gain Score	0.722 (72.29%)
N-Gain Category	Moderate effectiveness (56–75%)

Explanation of N-Gain Score Calculation

The Normalized Gain (N-Gain) score measures the improvement in student learning relative to the maximum possible improvement:

$$\mathrm{N-Gain} = \frac{\mathrm{Post-test\ score} - \mathrm{Pre-test\ score}}{100 - \mathrm{Pre-test\ score}} \quad (4)$$

Pre-test score: The average initial score before using the leaflet (48.5), indicating a moderate initial understanding.
Post-test score: The average score after using the leaflet (85.2), showing significant improvement.
Gain: The actual increase in score (36.7).
Ideal gain: The maximum possible increase (100 - 48.5 = 51.5).

An N-Gain score of 0.722 (72.29%) falls in the "Moderate Effectiveness" category according to established criteria (Wahab et al., 2021), reflecting substantial learning improvement attributable to the leaflet teaching materials. The N-Gain results demonstrate that the leaflet teaching materials moderately improved student understanding of Kingdom Fungi concepts. This moderate gain aligns with high student satisfaction in content, presentation, and language clarity as evidenced in Table 8. The significant rise from a pre-test average of 48.5 to a post-test average of 85.2 illustrates the educational effectiveness of these visually rich materials.

The effective presentation and clear language likely contributed to enhanced motivation and engagement, which are critical for meaningful learning. The leaflet's visual design, supported by student feedback, appears to facilitate better conceptual grasp through the inclusion of morphological characteristics and ecological roles of fungi.

Unlike previous studies focusing on different educational topics or cognitive frameworks (Basir & Rohmawati, 2023; Winarso & Yuliyanti, 2017), this study specifically addresses student learning in Kingdom Fungi using research-based content tailored to vocational high school students, adding relevant and contextual evidence to the literature. This study successfully developed and implemented leaflet-based teaching materials derived from fungal isolates on orchid roots, yielding a moderate improvement in student learning outcomes with an N-Gain score of 0.722. Specifically, students showed increased understanding of fungal morphology, classification, and ecological functions, as reflected by significantly improved post-test scores.

While the results are promising, areas for improvement include enhancing the leaflet's interactivity and visual appeal to further boost student engagement and learning retention. Future iterations might integrate digital elements or interactive tasks to increase effectiveness. Overall, leaflet teaching materials based on authentic research content hold potential as alternative instructional tools in biology education, especially for topics requiring visual and innovative approaches.

Conclusion

This study successfully identified six genera of endophytic fungi isolated from orchid roots, namely *Rhizoctonia sp.*, *Aspergillus flavus*, *Aspergillus niger*, *Trichoderma sp.*, and *Rhizopus sp*. The morphological characteristics of these fungi, including colony shape, color, growth pattern, and microscopic structures, were systematically characterized and used as foundational content for developing leaflet-based teaching materials. The teaching materials were developed using the Research and Development (R&D) model with the 4D approach (Define, Design, Develop, Disseminate). The identified fungi have ecological importance, especially in symbiotic relationships that support orchid growth and nutrient absorption, making them valuable as authentic, research-based instructional content. Incorporating these fungal characteristics into leaflets enhances biology education by providing concrete, visual, and contextual examples that help students better understand the abstract concepts of Kingdom Fungi. Effectiveness analysis using the N-Gain Score showed a score of 0.722 (72.29%), categorized as "Moderately Effective," indicating a significant improvement in students' understanding after using the leaflet materials. Student responses also reflected high feasibility in content, presentation, and language, further supporting the instructional value of the leaflets. Overall, fungi from orchid roots present strong potential not only as a source of scientifically accurate, research-based teaching materials but also as objects for further biological study and exploration. The leaflets developed in this study contribute meaningfully to the enhancement of biology learning by clarifying complex fungal concepts and motivating student engagement. Future improvements should focus on enriching visual and interactive content to maximize learning outcomes and further capitalize on the educational potential of fungi.

Acknowledgments
I sincerely appreciate everyone who contributed to this research and hope it proves beneficial for biology education in Indonesia.

Author Contributions
Conceptualization, F.A.W., D.S.S., and H.R.P.; methodology, formal analysis, data curation, and writing—original draft preparation, F.A.W.; investigation, F.A.W. and D.S.S.; resources, writing—review and editing, and validation, D.S.S. and H.R.P.; Visualization, H.R.P. All authors have read and agreed to the published version of the manuscript.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest
The authors declare no conflict of interest.

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