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Research Article

Diversity of Indigenous Fungi on the Roots of *Eucalyptus deglupta* in the Pelangi Forest at Ijen Geopark, Bondowoso

Evi Hanizar[1], Annisa Nur Imamah[2], Dwi Nur Rikhma Sari[3*]
[1] Departemen of Biology Education, Faculty of Teacher Training and Education, University of PGRI Argopuro Jember, East Java, Indonesia
[2] Ijen Geopark Executive Board for the Bondowoso Area, East Jawa, Indonesia
[3] Department of Biology, Faculty of Science and Technology, University of PGRI Argopuro Jember, East Java, Indonesia

[*]Email: rikhmasari.dnrs@gmail.com

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ABSTRACT
Eucalyptus deglupta, a native tree species known for its ecological and economic value, forms unique associations with root fungi that play essential roles in nutrient cycling and plant health. Therefore, this study aims to explore the diversity of indigenous fungi associated with the roots of Eucalyptus deglupta in the Pelangi Forest, located within the Ijen Geopark area, Bondowoso Regency. The methods employed included the isolation of fungal cultures and subsequent identification through macroscopic and microscopic examination of morphological characteristics. The isolation process yielded eight fungal isolates representing seven species: Microsporum gypseum, Aspergillus flavus, Mucor sp., *Trichoderma* sp., *Geotrichum* sp., *Saccharomyces cerevisiae, and Cladophialophora* sp. *The results indicated a considerable diversity among the isolated fungal species, with several taxa being reported for the first time in the study areaThese findings provide a valuable baseline for future ecological, biotechnological, and conservation studies, particularly in exploring the potential roles of these indigenous fungi in forest health, ecosystem resilience, and sustainable land management practices.*

Keywords: *Biodiversity; Diversity; Eucalyptus deglupta, Ijen Geopark; Indigenous Fungi.*

Introduction

Situated in the Bondowoso Region of East Java, the Ijen Geopark represents an integrated ecosystem comprising multiple geosites, biosites, and cultural heritage sites, all of which are characterized by high biodiversity in both flora and fauna [1]. This area has a close relationship with various geological processes. In the Ijen Geopark Bondowoso Biosite, there are several interesting sites, and it also has two main biological sites, namely the Bondowoso Coffee Biosite and the Pelangi Forest. Geopark development represents an integrated management approach aimed at preserving geological heritage (geoheritage), promoting cultural diversity, and safeguarding both geological (geodiversity) and biological (biodiversity) resources [2]. Biodiversity includes all living things on Earth that come from various sources, including terrestrial, aquatic, and marine ecosystems, and includes species variations among various species and their ecosystems [1] ,[3].

Eucalyptus deglupta, commonly known as the rainbow tree, is found in the Pelangi Forest Biosite of Ijen Geopark and

is recognized for its distinctive multicolored bark, making it a prominent ecotourism attraction in Bondowoso. Beyond its aesthetic appeal, *E. deglupta* plays an important ecological role due to its fast growth, extensive root system, and adaptability to various soil types. These characteristics support soil stabilization and nutrient cycling, making it an ideal model for studying plant–microbe interactions, particularly with indigenous root fungi that may contribute to its resilience and growth in a tropical ecosystem. Previous studies conducted in the Ijen Geopark area, primarily focused on the identification of seed plants (Spermatophyta) and macroflora diversity [1], providing limited insights into microecological aspects such as soil fungi. This focus on plant taxonomy and macro-level biodiversity has inadvertently left the rhizospheric microbial communities unexplored, despite their ecological significance.

This tree belongs to the Myrtaceae family, which consists of more than 700 species [4]. Its distinctive feature lies in the diverse colors of its trunk, earning it the name "rainbow tree." The color gradations on the bark yellow, pink, orange, green, and others vary from tree to tree, making each individual visually unique. The morphological structure of its roots includes a large and strong taproot that grows deep into the soil, functioning to anchor the tree and absorb nutrients. In addition to the taproot, it also develops finer lateral roots and fibrous roots that assist in water and nutrient uptake [4]. Given these unique morphological characteristics and its visually striking appearance, *Eucalyptus deglupta* was selected for this study not only for its ecological presence in the Pelangi Forest of the Ijen Geopark area, but also for its potential to harbor diverse and possibly unexplored fungal communities within its rhizosphere. Its role as a native species with both ecological and conservation value makes it an ideal candidate for studying indigenous fungal associations in tropical forest ecosystems.

Indigenous fungi in plant roots are one group of microbes known to increase plant resistance to various diseases, both soil-borne and airborne [5]. These fungi function to stimulate plant growth, so they are included in the category of *Plant Growth Promoting Fungi* (PGPF) [5]. Rhizosphere fungi are one group of microbes that have been reported to induce plant resistance to various diseases, both soil-borne and airborne diseases [5]. Rhizosphere fungi help plant growth through various mechanisms such as increasing nutrient absorption, as a biological control against pathogen attacks, and also producing growth hormones for plants [6]. Endophytic fungi usually establish a mutualistic symbiotic relationship with their host plants. These fungi provide various benefits, including increased growth, resistance to pests, diseases, and drought. Among the soil fungal species, there are types of fungi that are beneficial to plants and those that can cause disease [7].

To identify the types of fungi present in the rhizosphere of *Eucalyptus deglupta* in the Pelangi Forest biosite of the Ijen Geopark area, a thorough process of fungal isolation and identification is essential. This is because many fungal species remain undocumented in both number and taxonomy. Such gaps in knowledge are especially prevalent in tropical regions like Indonesia, where fungal diversity is exceptionally high yet remains largely unexplored due to limited research and the complex nature of their ecological niches. Indonesia, which has a rich diversity of flora and fauna, is also estimated to have a high diversity of fungi, which is caused by the humid environment and tropical temperatures that support their growth [8].

Based on the aforementioned background, it is essential to explore indigenous fungi associated with the roots of *Eucalyptus deglupta* (rainbow trees) located in the Pelangi Forest within the Ijen Geopark area, Bondowoso Regency, East Java. These fungi are hypothesized to play

important ecological roles in supporting plant health, enhancing nutrient absorption, and contributing to ecosystem resilience. However, to date, no explicit research has investigated the indigenous fungal communities inhabiting the rhizosphere of *Eucalyptus deglupta* in this biosite. Previous studies have predominantly focused on macroflora diversity and geological attributes of the region, leaving the microecological interactions between native fungi and plant roots largely understudied. This research gap is especially critical considering the potential functions of these fungi as biofertilizers, biocontrol agents, and key players in biogeochemical processes. Thus, the present study aims to fill this gap by identifying and characterizing indigenous rhizospheric fungi, thereby laying a foundation for future ecological, biotechnological, and conservation-related endeavors in tropical forest systems.

Materials and Methods

This study was conducted using the method of isolation and identification of indigenous fungi on the roots of *Eucalyptus deglupta* plants located in the Ijen Geopark Pelangi Forest Area, Bondowoso, East Java. Fungal purification and identification were carried out at the Integrated Biology Laboratory of PGRI Argopuro University. The materials needed in this study are soil samples isolated from the roots of the Rainbow tree (*Eucalyptus deglupta*) in the Ijen Geopark Area of Bondowoso Regency, distilled water, PDA media, sterile distilled water, 95% alcohol, 70% alcohol, physiological salt.

This study employed a purposive random sampling method, wherein soil samples were collected randomly from the rhizosphere zone of *Eucalyptus deglupta* at various rooting points in the Pelangi Forest area, Ijen Geopark, Bondowoso. This approach was chosen to target root-associated fungi while maintaining randomization within the selected zones. Then the samples were mixed together and put into plastic bags [9]. A total of 10 grams of *Eucalyptus deglupta* plant rhizosphere was taken and suspended in 100 mL of sterile distilled water, then shaken for 20 minutes. Furthermore, 1 ml of the suspension was transferred into 9 mL of sterile distilled water in a test tube and shaken until homogeneous (dilution stage I/10^{-1}). The same dilution process was carried out until dilutions of 10^{-4} and 10^{-5} were achieved. The results of the 10^{-1} to 10^{-5} dilutions were taken 1 ml each and put into a sterile petri dish using a measuring pipette aseptically, then the PDA medium which was still in a liquid state (at a temperature of 45°C) which had been added with chloramphenicol was poured into the petri dish and homogenized by shaking the petri dish so that the suspension was evenly distributed in the media. Following this step, incubation was carried out at room temperature, ranging between 22°C and 25°C, for a duration of five to seven days. To obtain a pure culture, purification was carried out on the fungi obtained [9].

Fungal colonies growing at dilutions of 10^{-1} to 10^{-2} were too numerous to be separated, so the purified colonies came from dilutions of 10^{-3} to 10^{-5}. Purification was carried out by transferring one fungal colony to a new sterile PDA medium. PDA media was prepared by adding 3.9 grams of PDA media into 100 mL of sterile aquadest (the PDA requirement is 39 g/L) and heating it on an electric stove until boiling to dissolve completely, so that it is ready to be used as a growth medium and testing medium [10].

After incubation, fungal colonies appeared on the PDA media. Then, the fungi that had different colony shapes and visuals were isolated and separated. Next, the fungi were purified, and the incubation period was carried out for 7-14 days. After the fungal isolate from the purification results had been obtained, observations were made without a microscope, namely the growth, color, shape, and arrangement of the colony tissue. The object glass was cleaned using alcohol and heated until clean

from fat and dust. Furthermore, lactophenol was dripped into the center of the object glass. The fungal culture was taken aseptically with an ose needle and placed on the object glass that had been dripped with lactophenol, then a little alcohol was added. Then the preparation was covered with a cover glass and heated over a fire before being observed under a microscope to obtain its microscopic characteristics. Identification was carried out by comparing the characteristics of the observed fungi with references from the identification book [10].

Results and Discussion

The Rainbow Forest (*Hutan Pelangi*) is located within the Ijen Geopark area, specifically in Bondowoso Regency, East Java Province, Indonesia (-7.8970° S,113.9230° E). The Rainbow Forest is renowned for the presence of *Eucalyptus deglupta*, commonly known as the "rainbow tree", whose bark displays striking color gradations. This site holds significant ecological and educational value and serves as an important location for studying local biodiversity, including soil microorganisms and endophytic/rhizosphere fungi.

This study is a descriptive study that aims to determine the diversity of indigenous fungi at several points in the Ijen Geopark area of Bondowoso Region, namely in the roots of the Rainbow Plant. The isolation results from the *Eucalyptus deglupta* plants rhizosphere in the Pelangi Forest of the Ijen Geopark Area of Bondowoso showed that there were six types or groups of fungal isolates, consisting of four identified fungal genera and two groups of fungi that could not be identified because they did not produce conidia (Table 1). All fungal isolates were identified morphologically, both microscopically and macroscopically, by referring to the identification book. The identified isolates showed different morphological characteristics both macroscopically and microscopically.

The seven fungal isolates displayed diverse morphological characteristics when cultured on PDA media (Table 1). Colony colors ranged from yellow-orange and greenish white to black, and surface textures varied from velvety to smooth, serrated, or rough and fuzzy. Conidial structures were present in most isolates, with shapes including oval, round, chain-like, and fusiform, and colors spanning from transparent to dark brown. Phialides were cylindrical where present, although some isolates lacked this feature entirely. All isolates exhibited growth zones and radial furrows, while hyphae were predominantly septate, except for one non-septate and another lacking hyphal structures. The presence of stolons and rhizoids was sporadic. Based on these traits, the isolates were taxonomically affiliated with genera including *Microsporum*, *Aspergillus*, *Mucor*, *Trichoderma*, *Geotrichum*, *Saccharomyces*, and *Cladophialophora*.

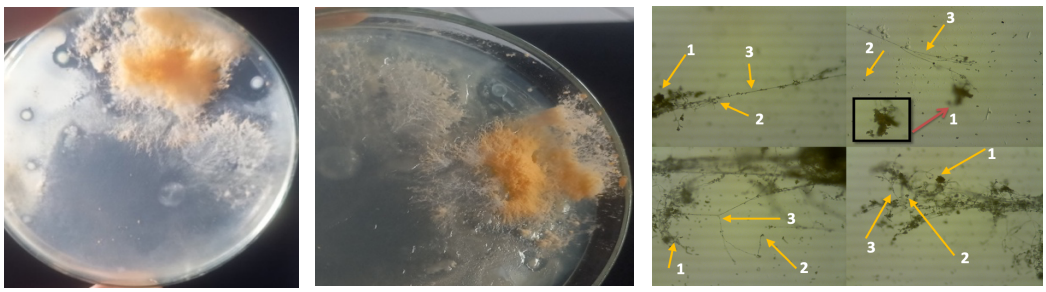

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Figure 1. (a) *Microsporum gypseum* colonies on PDA medium after 7 days of incubation; (b) Reserve of *Microsporum gypseum* colonies on PDA medium after 7 days of incubation; (c) Microscopic morphology of *Microsporum gypseum* (indicated by red and yellow arrows); Notes (1) Sporangium; (2) Phialid; (3) Hyphae.

Table 1. Morphological identification of fungi from *Eucalyptus deglupta* roots

Observation Character	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolat 7
Colonies on PDA media							
Colony Color	Yellow Orange	Greenish White	White gray	White	Cream White, Pale Yellow	Cream white	Black
Reserve Colony	Orange	Greenish	White gray	White	White, Pale Yellow	Cream white	Black
Colony Surfacei	Velvety	Velvety	Velvety	Smooth, slippery, neatly rounded	Smooth serrated	Smooth serrated	Rough, Fuzzy
Conidia/ Sporangium	Indicated	Indicated	Indicated	Indicated	Arthroconidia	None	Indicated
Form	Oval/Elongated	Grouped	Round	Round Oval	Chain cylinder	None	Fusiform; Chain-like
Color	Yellow Orange	Greenish	Transparent	White	Transparent	None	Drak Brown
Phialide	Indicated	Indicated	None	Indicated	Indicated	None	Indicated
Form	Cylinder	Cylinder	-	Cylinder	Small cylinder	None	Cylinder
Additional Properties							
Growing Zone	Indicated	Indicated	Indicated	Indicated	Indicated	Indicated	Indicated
Radial Furrow	Indicated	Indicated	Indicated	Indicated	Indicated	Indicated	Indicated
Hyphae	Septate	Septate	Non-septate	Septate	Septate	None	Septate
Stolons and Rhizoids	Nothing	Stolon	None	None	None	None - Budding	Indicated
Genus	*Microsporum*	*Aspergillus*	*Mucor*	*Trichoderma*	*Geotrichum*	*Saccharomyces*	*Cladophialophora*

Microsporum gypseum fungus on PDA media, showed rapid growth and reached maturity within 6 to 10 days with a white colony color and changed to orange yellow (Table 1 and Figure 1). *Microsporum gypseum* produces hyphae, macronidia, and micronidia. Macronidia are widespread, fusiform and symmetrical with rounded ends, while micronidia are fewer in number, usually clustered and found along the hyphae [11]. *Microsporum gypseum* is a geophilic fungus commonly found in soil, particularly in warm and humid environments. Its presence in the rhizosphere indicates the diverse fungal community structure in the area and reflects the adaptability of certain fungal species to soil based ecosystems. It is important to note that *Microsporum gypseum* is a fungus that causes skin infections, decomposes keratin, and can damage nails and hair. This fungus have the ability to digest skin keratin because it is keratinophilic, so that infections can attack various layers of the skin, from the stratum corneum to the stratum basalis. *M. gypseum* is a geophilic dermatophyte that lives in soil and can cause moderate inflammation in humans [12].

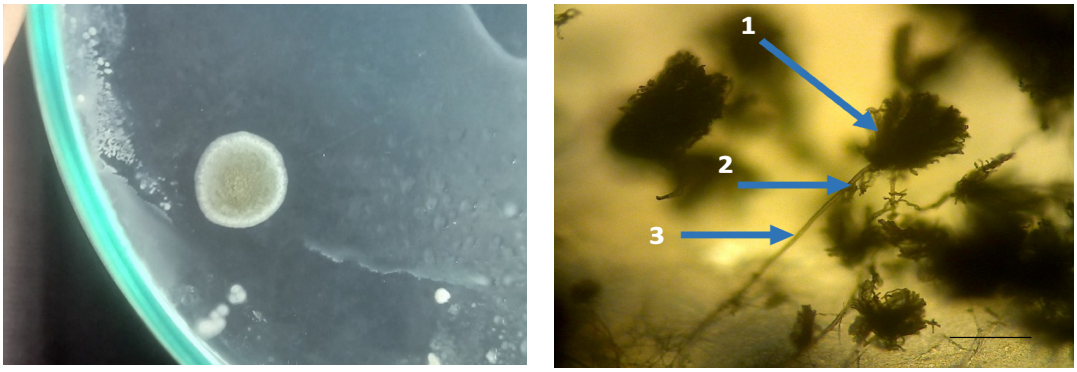

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Figure 2. (a) *Aspergillus flavus* colonies on PDA medium after 7 days of incubation; (b) Microscopic characteristics of *Aspergillus flavus* on PDA medium after 7 days of incubation (indicated by blue arrows); Notes (1) Sporangium; (2) Phialid; (3) Hyphae.

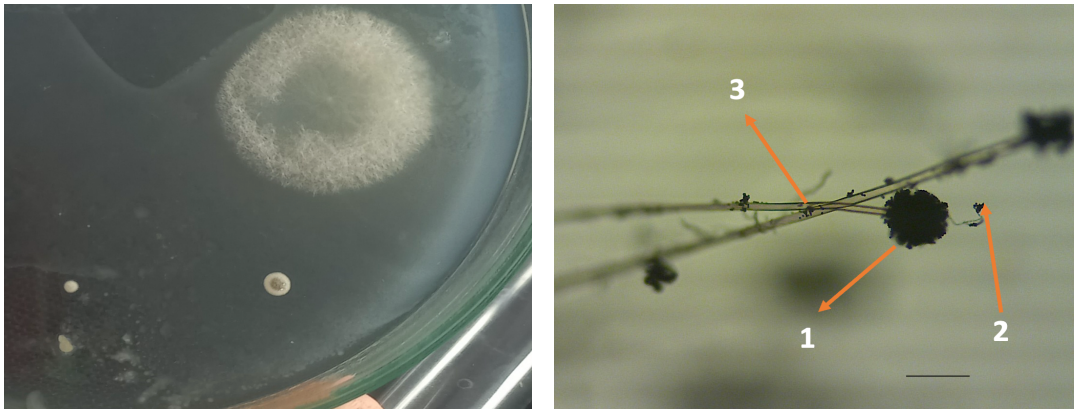

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Figure 3. (a) *Mucor* sp. colony on PDA medium after 7 days of incubation; (b) Microscopic character of *Mucor* sp. on PDA medium after 7 days of incubation (indicated by orange arrows); Notes (1) Sporangium; (2) Spores; (3) Hyphae.

Microscopically, *Aspergillus flavus* showed the presence of conidiophores, vesicles, and spores/conidia that were round and bluish green in color (Table 1 and Figure 2). Microscopic analysis revealed that the conidiophores were short and smooth with a greenish color, while the conidial heads (vesicles) were club-shaped

(clavate) and could change to oblong (columnar) as the colony aged [9]. Sterigmata were seen covering most of the vesicles upper part. Spores/conidia were round, greenish in color, and their surfaces had a serrated texture (echinulate) [13]. *Aspergillus flavus* initially grew white, this colony changed to yellowish green with a white edge, while the lower surface showed a yellow to brown color. Macroscopically, the *Aspergillus flavus* colony appeared yellowish green with a yellow to brown underside. On microscopic observation, conidiophores are clearly visible, not pigmented, have a rough surface, and are less than 1 mm long [14].

Based on observation data adjusted to the identification book, isolate 11 belongs to the genus Mucor, class Zygomycetes (sexual reproduction with zygospores, namely the fusion of two gametangia and asexual with spores produced by sporangia), order Mucorales, family Mucoraceae. The genus *Mucor.sp* has special characteristics, namely non-septate hyphae and forms sporangia, unbranched sporangiophores (Figure 3). The color of the colony is transparent brownish. Macroscopically, this fungus is like *Rhizopus* sp. Its mycelium is like cotton but its color is whiter compared to *Rhizopus* sp. and microscopically this fungus has stolons but does not have rhizoids and its sporangiophores are shorter [14]. *Mucor* sp., has white colonies and eventually gray, yellow and smooth, non-septate hyphae sometimes forming branches, sporangiospores grow on all parts of the mycelium [15], [16], round columella, and does not form stolons (Table 1 and Figure 3).

Trichoderma sp. belongs to the class Deuteromycetes, order Moniliales, and family Moniliaceae. These types of *Trichoderma* have conidia with smooth walls. The colonies are initially hyaline, then turn slightly greenish white, especially in areas rich in conidia (Table 1 and Figure 4). Conidiophores can branch in a pyramid-like pattern, with lateral branches appearing at the bottom, while branches towards the tip become shorter. Phialide are slender and long, especially at the top of the branches. Conidia have a semi-spherical to short oval shape [17].

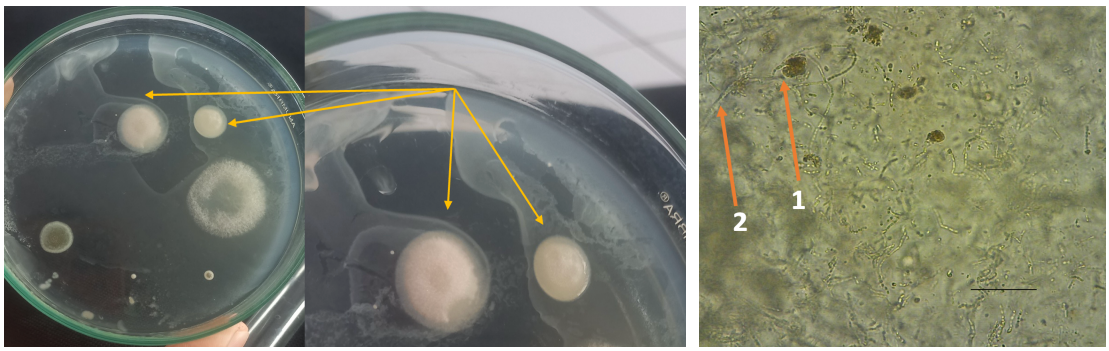

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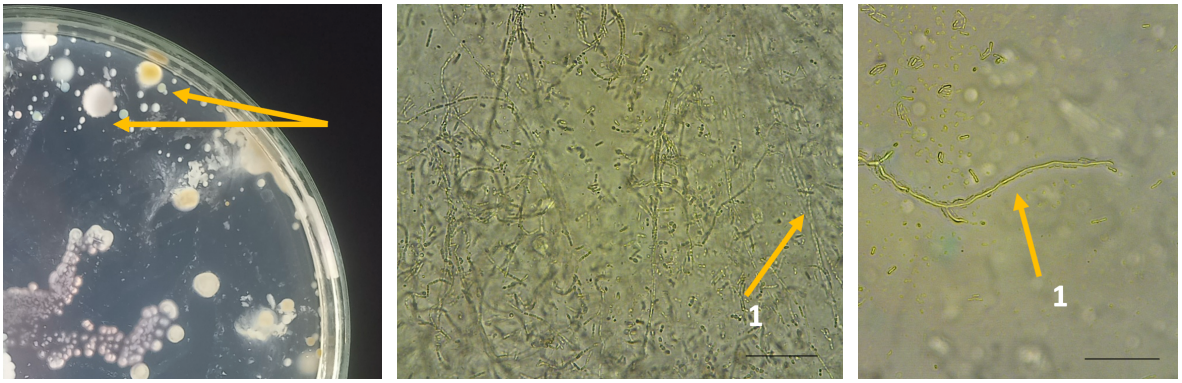
Figure 4. (a) *Trichoderma* sp. colonies on PDA medium after 7 days of incubation; (b) Microscopic morphology of *Trichoderma* sp. (indicated by orange arrows); Notes (1) Conidia; (2) Conidiophores.

Fungi of the genus *Geotrichum* produce smooth, unicellular hyaline chains, with sub globose to cylindrical shapes, called arthroconidia (Table 1 and Figure 5) slimy (ameroconidia) through the holoarthric fragmentation process of undifferentiated hyphae. These arthroconidia vary in size and can germinate at one end of a branch to form a bud, which then develops into a septate mycelium [18]. In PDA, *Geotrichum* colonies grow rapidly, forming flat, white to cream-colored colonies, with a dry, smooth texture that appears unpigmented

[18], [16]. The hyphae produced are hyaline, have septates, branch, and fragment into smooth, unicellular hyaline chains, with sub globose to cylindrical arthroconidia. Its size is about 6-12 x 3-6 µm and is separated by a double septum separation [18], [19].



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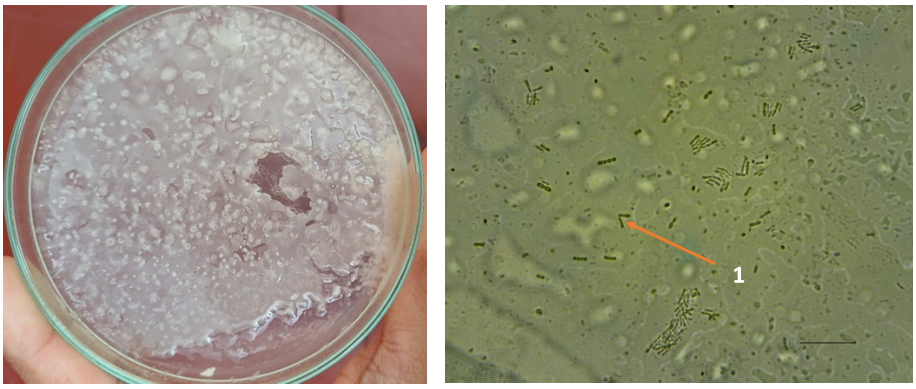
Picture 5. (a) *Geotrichum* sp. colonies on PDA medium after 7 days of incubation; (b) Microscopic characteristics of *Geotrichum* sp. on PDA medium; (c) Characteristics after 4 days of incubation (indicated by yellow arrows); Note (1) Arthroconidia.

The characteristics of *Saccharomyces cerevisiae* are similar to *Candida tropicalis*, characterized by white color, protruding shape, coccus shape, and smooth, shiny, and slippery surface [20]. The morphology of yeast is different from bacteria, because *Saccharomyces cerevisiae* has a much larger size, which is 5–20 µm, while bacteria range from 1–10 µm. In addition, yeast has a distinctive shape (Figure 6) with buds (Table 1 and Figure 6) which function as a means of regeneration, different from bacteria that divide themselves.



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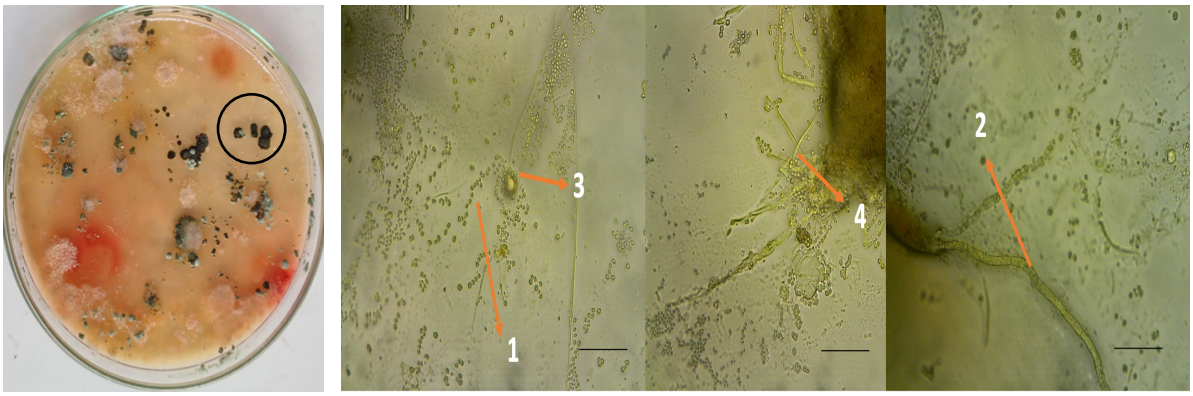
Picture 6. (a) *Saccharomyces cerevisiae* colonies on PDA medium after 7 days of incubation; (b) Microscopic characteristics of *Saccharomyces cerevisiae* on PDA medium after 7 days of incubation (indicated by orange arrows); Note (1) Multilateral Budding.

Species of *Cladophialophora* sp. exhibits colonies that are black or dark brown due to the presence of melanin pigments produced by the hyphae and conidia (Table 1; Figure 7). The colonies of *Cladophialophora* sp. typically appear clustered with somewhat irregular edges and may feature radial furrows formed by the growth of hyphae from the center to the periphery [5]. The colony surface often appears rough and fuzzy, with the fuzzy texture being more prominent in younger colonies, where conidia (asexual spores) are just beginning to form. The colony

generally grows radially, spreading from the center to the edges, and forms structures resembling chains of conidia that are clearly visible. *Cladophialophora* sp. is septate, meaning there are partitions that divide the hyphae into separate cellular sections. The hyphae are black or dark brown and often appear branched, bearing conidia that are oval or fusiform (tapering at the ends), typically arranged in chains or clusters at the tips of the hyphae [21]. These conidia are black or dark brown due to the presence of melanin in their cell walls. The hyphae of *Cladophialophora* sp. are septate, with septa dividing the hyphae into individual cells, and phialides are cylindrical or tubular in shape, functioning as sites for conidia formation [21].

Fungal communities in soil contribute significantly to ecological stability and nutrient cycling, serving as biofertilizing agents for various plants, one example being *Eucalyptus deglupta*. The species *Microsporum gypsum* has geophilic properties, is widely found in soil, and can be isolated worldwide, the distribution is global [22]. This species can be categorized as cosmopolitan or limited to certain geographic areas and has a role in the soil as an agent for improving soil conditions, especially clay or a mixture of clay-sand with a pH between 7–7.5 [22], [12]. *Aspergillus flavus* is a cosmopolitan fungus that can be found in various types of land and environments [23]. This fungus has the ability to decompose organic matter, remediate pollutants, and function as a biocontrol agent for certain disease-causing pathogens, its ability to produce various secondary metabolites, including organic acids and various enzymes [24]. In addition, *Aspergillus flavus* can increase the growth of tomato plants in soil contaminated with heavy metals such as cadmium (Cd) and chromium (Cr), and overcome plant poisoning by adjusting plant physiology [25].



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Picture 7. (a) *Cladophialophora* sp. colonies on PDA medium after 7 days of incubation; (b) Microscopic characteristics of *Cladophialophora* sp. on PDA medium after 7 days of incubation (indicated by orange arrows); Notes (1) Conidia; (2) Hyphae; (3) Sporangium; (4) Conidiophore.

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In addition, *Aspergillus flavus* and *Trichoderma* sp. based on the results of the research that has been done, both also have a role as biofertilizer agents in several cultivated plants with saline soil [5], [26]. According to the research outcomes, *Geotrichum* sp. demonstrated the ability to serve as an effective microorganism in plastic waste decomposition [9]. And the fungus *Mucor* sp. can also be used as a fertilizer to fertilize the soil because it plays a role as a provider of plant nutrients [27]. The *Cladophialophora* sp. fungus isolate is a species from the Eurotiomycetes class, where several species are pathogenic to specific host plants and cause spots on the leaves of *Hosta plantaginea* [27].

Conclusion

The number and types of fungi successfully isolated from the rhizosphere of *Eucalyptus deglupta* plants in the Pelangi Forest Area, Ijen Geopark Region, Bondowoso Regency, East Java, produced six fungal isolates, namely *Microsporum gypseum, Aspergillus flavus, Mucor* sp., *Trichoderma* sp., *Geotrichum* sp., *Saccharomyces cerevisiae*, and *Cladophialophora* sp..

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Conflict of interest

We declare that there is no conflict of interest.

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