



Exploration and Identification of *Gliocladium* sp as Biological Agent from the Rhizosphere of Tomato

Eksplorasi dan Identifikasi Jamur *Gliocladium* sp sebagai Agens Hayati dari Rhizosfer Tanaman Tomat

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Abstrak	Abstract
<p>Salah satu peran mikroorganismenya adalah sebagai agen hayati. Penelitian ini bertujuan mengeksplorasi dan mengidentifikasi agen hayati jamur <i>Gliocladium</i> sp. yang terdapat pada rhizosfer tanaman tomat. Eksplorasi dilakukan dengan mengambil sampel tanah perakaran tanaman tomat di lima lokasi menggunakan plot diagonal. Isolasi mikroorganismenya rhizosfer dilakukan menggunakan metode pengenceran bertingkat dan <i>spread plate</i> pada media PDA yang telah ditambahkan chloramphenicol. Isolat jamur diidentifikasi secara makroskopis dan mikroskopis. Hasil eksplorasi agen hayati jamur pada tanah rhizosfer tanaman tomat diperoleh 4 genus isolat jamur yaitu <i>Aspergillus</i>, <i>Trichoderma</i>, <i>Paecilomyces</i>, dan <i>Gliocladium</i>. Berdasarkan hasil identifikasi, isolat jamur <i>Gliocladium</i> sp. mempunyai warna koloni hijau kehitaman dengan koloni berbentuk <i>circular</i>, sedangkan secara mikroskopis mempunyai ciri morfologi konidiofor tegak, tumbuh lurus, dan bercabang.</p> <p>Kata kunci: <i>Gliocladium</i> sp; Rhizosfer; Tanaman tomat</p>	<p>One of the roles of microorganisms is as a biological agent. This study aims to explore and identify the biological agent of the fungus <i>Gliocladium</i> sp. found in the rhizosphere of tomato plants. Exploration was done by taking samples of tomato plant root soil in five locations using a diagonal plot. Rhizosphere microorganisms were isolated using the multilevel dilution and spread on PDA media added with chloramphenicol. Fungal isolates were identified macroscopically and microscopically. The results of exploring fungal biological agents in the rhizosphere soil of tomato plants revealed four genera of fungal isolates, namely <i>Aspergillus</i>, <i>Trichoderma</i>, <i>Paecilomyces</i>, and <i>Gliocladium</i>. Based on the identification results, the fungal isolate <i>Gliocladium</i> sp. has a blackish-green colony color with a circular colony. In contrast, microscopically, it has the morphological characteristics of upright conidiophores, growing straight, and branching.</p> <p>Keywords: <i>Gliocladium</i> sp; Rhizosphere; Tomato plant</p>

INTRODUCTION

Indonesia is an agricultural country where the agricultural sector provides the most significant contribution to the national economy. Horticultural agriculture is a promising commodity with high economic value for domestic market needs [1]. Indonesian farmers widely cultivate horticultural crops, which include vegetables, ornamental plants, fruit plants, and medicinal plants [2]. However, horticultural crop cultivation faces many challenges due to attacks from many plant pests. Plant pests consist of various organisms that can significantly reduce crop yields because they damage plants [3].

Farmers generally use synthetic chemical pesticides to control plant pests because they work faster, are readily available, and are easy to use [4]. However, farmers' synthetic chemical pesticides are often unwise and excessive [5]. The excessive and inappropriate use of artificial chemical pesticides has a negative impact on the environment [6].

To reduce the environmental impact of synthetic chemical pesticides, the government has recommended integrated pest management (IPM), which limits the use of artificial chemical pesticides in both types and doses [5]. One technique in IPM is using biological agents. Pest control with biological agents does not pollute the environment and does not affect non-target

organisms [7]. Biological agents include insects, bacteria, viruses, fungi, microplasma, protozoa, nematodes, and other organisms [8]. The diversity of biological agents in nature still needs to be explored. The rhizosphere is an area around plant roots suitable for developing soil microorganisms. The presence of antagonistic microorganisms in the rhizosphere acts as a protector of plants from pathogen attacks, so that it can prevent pathogens from spreading and infecting the roots [9].

Gliocladium sp. is a species of fungus that lives in saprophytic soil with the characteristics of upright conidiophores, round conidia, septate, and green in color [10]. *Gliocladium* sp. is an antagonistic fungus that lives well in healthy plant root soil, forming a mutually beneficial relationship with the plants it protects [11]. This fungus can reduce the number of soil pathogens by creating mechanisms of space competition, parasitism, antibiosis, and lysis [12]. *Gliocladium* sp. can produce antibiotic compounds, gliotoxin and glioviridin, and fungicidal, viridin, which are helpful biological agents [6].

Exploration of the biological agent of the fungus *Gliocladium* sp. in strawberry plantations and Dasong Forest found microscopic fungal isolates in the rhizosphere from the following genera: *Fusarium*, *Penicillium*, *Scopulariopsis*,

Epicoccum, Trichoderma, Mucor, Aspergillus, Verticillium, Rhizopus, and Gliocladium [13]. Several studies on the role of the fungus *Gliocladium* sp. as a biological agent showed that the biological agent *Gliocladium virens* was able to control *Fusarium oxysporum* disease in chili plants [6]. In addition, based on research, the biological agent *Gliocladium* sp. inhibited *Colletotrichum* spp. with a percentage of 70.33% [14].

Tomato (*Lycopersicon esculentum* Mill) is one of the seasonal vegetable plants belonging to the Solanaceae family, which has high economic value and is widely cultivated in Indonesia [15]. Pests and diseases often attack tomato cultivation. *Gliocladium* sp. in the rhizosphere of plants can be a biological control agent against soil-borne pathogens and strengthen plant resistance to unfavorable environmental conditions [16].

Gliocladium fungi produce several enzymes, including exoglucanase, endoglucanase, cellobiose, and chitinase, which can suppress the development of pathogens [17]. In addition, *Gliocladium* sp. in the rhizosphere area around the plant roots also contains more beneficial microorganisms. The rhizosphere of horticultural plants such as tomatoes is a suitable environment for the life of *Gliocladium* sp. because there is dynamic activity between root exudates and

soil microorganisms, as it produces organic matter, which is beneficial for developing soil microorganisms [18]. This study aimed to explore and identify biological agents of *Gliocladium* sp. fungi found in the rhizosphere of tomato plants.

METHODS

This study is a descriptive exploratory study using a purposive sampling method by taking samples found in tomato horticultural land, exploring the presence of biological agents, namely rhizosphere fungi (*Gliocladium* sp.). This study describes descriptively the exploration and identification of fungi. Exploration of biological agents in the rhizosphere of tomato plants was carried out in the tomato horticultural land, Jl. Bringin Indah, Sambikerep District, Surabaya City. Biological agents were isolated at the Biological Agent Laboratory of the East Java Food Crop and Horticulture Protection UPT on Jl. Pagesangan II No. 58, Pagesangan Village, Jambangan District, Surabaya City, East Java from March to May 2024.

Materials and Equipment

The equipment used in this study was Laminar Air Flow Abl-laf 100, semi-automatic autoclave, gas stove, petri dish, ose needle, bunsen, stirring rod, microscope, cover glass, object glass, measuring cup, test tube, test tube rack, syringe, Erlenmeyer

flask, ruler, analytical balance, plastic wrap, plastic bag, pH meter, hoe, shovel, tray, plastic box, vortex, L glass rod, black cloth. The materials used in this study were tomato rhizosphere soil samples, distilled water, denatured alcohol, Potato Dextrose Agar (PDA) media, cotton, 70% alcohol, waste paper, and chloramphenicol.

Sampling Technique

Soil samples were taken from the tomato horticultural land. Exploration was carried out at five different spots. Sampling tomato plant roots using a 20-30 cm depth diagonal plot. A 500 g soil sample was taken from each plot, put into a plastic bag, and taken to the Biological Agent Laboratory - the technical implementation unit for Food Crop Protection and Horticulture of East Java. Here, the biological agents contained in the samples were isolated and identified.

Isolation of Rhizosphere Microorganisms

Rhizosphere microorganisms were isolated using the multi-stage dilution method and spread on potato dextrose agar (PDA) media mixed with chloramphenicol. The multi-stage dilution method aims to reduce the density of microorganisms suspended in the sample fluid. A 25 g sample of air-dried tomato soil was dissolved in 250 mL of sterile distilled water. Furthermore, multi-stage dilution was carried out until a dilution level of 10^{-7} was reached. The mixture at a dilution level of 10^{-3} to 10^{-7} was diluted to 0.5 mL and then inoculated on PDA media. The

PDA media inoculated with the sample were then incubated at room temperature for 3 days. Purification was carried out after the isolated fungal colonies grew on PDA media. Purification aims to obtain pure fungal isolates without contaminating microbes using the streak for single colony technique on new PDA media and then re-incubating.

Identification of Rhizosphere Microorganisms

The explored biological agents grown on PDA media and purified are then identified macroscopically and microscopically. Identification includes observation of the fungal colony's color, the colony's shape, mycelium texture, and the colony's edge macroscopically. Microscopic identification is carried out by observing fungal isolates with parameters including the shape of conidia, vesicles, conidiophores, hyphae, and the presence or absence of septa using a microscope [27]. Identification is carried out referring to the fungal identification book "Illustrated Genera of Imperfect Fungi" by Barnett & Hunter (1998) [19].

Data Analysis

The data obtained are presented in pictures, which are analyzed and described in a narrative based on macroscopic characters such as colony color, colony shape, microscopic shape, and conidia shape.

RESULTS AND DISCUSSION

Exploration and Macroscopic Identification of Biological Agent Fungi

The results of exploring and identifying fungal isolates of biological agents are presented in Table 1. Based on Table 1, the results of macroscopic morphological identification of color, shape, texture, and colony edges obtained four genera of fungal isolates from tomato plant rhizosphere soil, namely the genera *Aspergillus*,

Trichoderma, *Paecilomyces*, and *Gliocladium*. The biological agent *Gliocladium* sp, in Table 1, was found in tomato root soil samples diluted at 10^{-4} . Based on the results of the exploration and identification of *Gliocladium* sp. fungal colonies, macroscopically (Figure 1), blackish green colonies with a thin texture resembling cotton are observed. The shape of the *Gliocladium* sp. fungal colony is circular with a flat colony edge (entire).

Table 1. Result of exploration and identification of fungi using serial dilution method

Soil types	Genera	Color	Shape	Texture	Edge	Dilution level
Tomato 5 plots Composite Diagonal	<i>Aspergillus</i> sp.	Black	<i>Circular</i>	<i>Powdery</i>	<i>Entire</i>	10^{-7}
	<i>Trichoderma</i> sp.	Deep green	<i>Circular</i>	<i>Powdery</i>	<i>Entire</i>	10^{-7}
	<i>Paecilomyces</i> sp.	Green	<i>Circular</i>	<i>Cotton</i>	<i>Entire</i>	$10^{-3}, 10^{-5}$
	<i>Gliocladium</i> sp.	Blackish green	<i>Circular</i>	<i>Cotton</i>	<i>Entire</i>	10^{-6} 10^{-4}

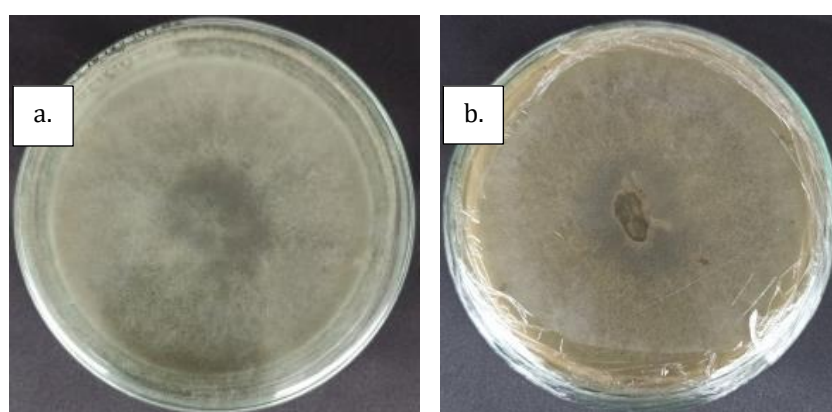


Figure 1 *Gliocladium* sp. macroscopic fungal; (a) *Gliocladium* sp. colony top view (top), (b) *Gliocladium* sp. colony bottom view (reverse)

The results of this exploration are obtained isolates of *Gliocladium* sp. from exploration

results in the tomato production center land in Solok Regency and Agam Regency, West

Sumatra, using the multi-stage dilution method [17]. However, the antagonistic fungal colony of *Gliocladium* sp. found had a macroscopic morphology of dark green that faded with white edges and smooth, dense, velvet-like fungal mycelium. This differs from the color of the *Gliocladium* sp. colony from the results obtained, which was blackish green and had thin mycelium that spread to fill the petri dish. In addition, another study also showed similar results, where the antagonist *Gliocladium* sp. with multi-stage dilution was also found in tomato horticulture centers in Kupang Regency, East Nusa Tenggara [20]. One crucial aspect affecting the rhizosphere's diversity and population of microorganisms is organic compounds in sap produced by plant roots. Tomato plant roots release organic compounds that can attract nematodes and stimulate the germination of antagonistic fungal spores [21]. *Gliocladium* sp. fungus in tomato plant roots' soil increases plant growth and protects plants from soil-borne pathogens (*Fusarium oxysporum*). Soil-borne pathogens often attack horticultural plants, including tomato plants [22].

Based on the identification results, it is known that the *Gliocladium* sp. fungal isolates that grow on PDA media in petri dishes show macroscopic morphological characteristics of blackish green with circular colonies (Figure 1). *Gliocladium* sp.

fungal colony is initially white, then becomes blackish green or pale after 3-5 days, and the colony is round [23]. The mycelium of the *Gliocladium* sp. fungus is thin and fills the entire petri dish. Based on another study, the mycelium of the *Gliocladium* sp. fungus appears thin and grows spread out on the petri dish [20], and the macroscopic characteristics of the *Gliocladium* sp. fungal colony have a cottony texture [24]. In this study, the *Gliocladium* sp. fungal colony has a cotton texture with a flat colony edge (entire).

Microscopic Identification of Biological Agent *Gliocladium* sp.

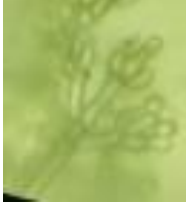


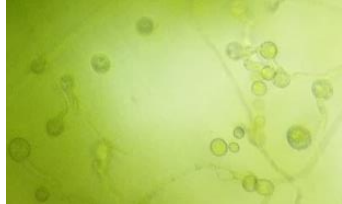
The results of microscopic observations of the morphological characteristics of the *Gliocladium* sp. fungus have upright and straight conidiophores with a length of around 6.5-14.6 μm [20]. The *Gliocladium* sp. fungus at 40x magnification is seen to have branched and septate conidiophores [25]. In addition, another study revealed that the conidiophores of the *Gliocladium* sp. fungus branch upwards and the ends form a brush-like structure [26].

Based on the results of microscopic identification, the *Gliocladium* sp. fungus has round conidia [24]. Conidia or spores are the asexual reproductive organs of fungi [27]. Another part of the *Gliocladium* sp. fungus is the phialide. Based on Table 2, it can be seen that the phialide part of the *Gliocladium* sp. fungus is branched with a pointed tip, and

there are conidia at the top. According to another study [28], the phialides of *Gliocladium* sp. are branched and have pointed tips. The phialides of *Gliocladium* sp.

are arranged on the main branches of the conidiophores and have conidia at the tip of the phialide with a size of 7.2-12.0 μm x 2.2-4.5 μm [20].

Table 2. Result of microscopic identification of *Gliocladium* sp. fungi

Characteristics of <i>Gliocladium</i> sp. fungal isolate at magnification of 100 \times			
Shape	Conidiophore	Phialids	Conidia
			

The antagonistic fungus *Gliocladium* sp. found in rhizosphere soil samples can affect plant growth and fertility. Rhizosphere microorganisms acting as biological control agents can increase plant resistance to various diseases and be used as biological fertilizers [18]. Microorganisms around the rhizosphere are essential in maintaining plant root health. Microorganisms found in the rhizosphere play a role in stimulating plant growth by fixing nitrogen, helping to dissolve phosphate, and absorbing nutrients in the soil [29]. The fungus *Gliocladium* sp. can break down the C chain in organic matter into simple chains that plants use to make plant growth fertile [30].

The existence and diversity of rhizosphere microorganisms can be caused by several factors, including nutrients, acidity levels (pH), and chemical compounds in the soil environment [31]. Various soil types have different organic and inorganic mineral content, which can affect the existence of

Gliocladium, *Nigrospora*, *Penicillium*, *Aspergillus*, *Rhizopus*, and *Fusarium* [33]. Meanwhile, the exploration of antagonistic fungi from the rhizosphere of mung bean plants obtained several fungal isolates from the genera *Trichoderma*, *Aspergillus*, *Chrysonilia*, *Mortierella*, *Gliocladium*, *Penicillium*, and *Acremonium* [34]. Organic compounds released by plant roots to stimulate the growth of rhizosphere fungi are influenced by plant species, plant age, and environmental conditions around the plant [21]. In addition, environmental factors such as temperature, soil moisture, and soil conditions can affect the types of microbes in the rhizosphere soil [29].

CONCLUSION

The results of exploring and identifying *Gliocladium* sp. fungi as biological agents were found in tomato plant rhizosphere soil samples using a multi-stage dilution method at a dilution level of 10^{-4} . *Gliocladium* sp.

fungi identified macroscopically have blackish green colonies, circular in shape, cotton colony texture, and entire or flat colony edges. Meanwhile, the results of microscopic identification show that *Gliocladium* sp. fungi have upright and branched conidiophores, round conidia, and have branched phialids with pointed tips.

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