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Exploration and Identification of *Trichoderma* sp. with Baiting Method as a Biological Agent in Horticultural Lands

(Eksplorasi dan Identifikasi Jamur *Trichoderma* sp. Sebagai Agen Hayati pada Tanah Daerah Perakaran Hortikultura dengan Metode Baiting)

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Abstrak

Penggunaan agen hayati efektif untuk mencegah dampak negatif akibat penggunaan berlebih pestisida kimia sintetis. *Trichoderma* sp., jamur saprofit berperan sebagai agen biokontrol melawan berbagai kapang fitopatogen, lebih sering ditemukan pada tanah berakar dan daun daripada batang atau buah. Penelitian ini bertujuan mengidentifikasi karakteristik dan keberadaan *Trichoderma* sp. di lahan hortikultura menggunakan metode baiting (umpan). Isolasi dilakukan dengan mengambil jamur yang tumbuh pada umpan nasi, jagung, dan kelapa menggunakan jarum ose. Diperoleh tiga isolat *Trichoderma* sp. dari perakaran tanah cabai dan bambu dengan umpan nasi dan jagung. Hasilnya, ketiga isolat memiliki karakteristik serupa: koloni berwarna hijau tua, berbentuk circular, bertekstur powdery, dan bertepi entire. Secara mikroskopis, isolat memiliki konidiofor bercabang, tegak, tersusun vertikal, fialid pendek dan tebal, konidia bulat berwarna kehijauan, serta hifa bersekat menyerupai benang.

Kata kunci: Eksplorasi, Metode Baiting, *Trichoderma sp.*

Abstract

The use of biological agents is an effective way to prevent the negative impacts of excessive synthetic chemical pesticide use. Trichoderma sp., a saprophytic fungus, acts as a biocontrol agent against various phytopathogenic molds and is more commonly found in rooted soil and leaves than in stems or fruits. This study aims to identify the characteristics and presence of Trichoderma sp. in horticultural land using the baiting method. Isolation was carried out by collecting fungi grown on rice, corn, and coconut bait using an inoculation needle. Three Trichoderma sp. isolates were obtained from chili and bamboo root soil using rice and corn bait. The results showed that the three isolates had similar characteristics: colonies were dark green, circular in shape, powdery in texture, and had entire margins. Microscopically, the isolates featured branched and upright conidiophores arranged vertically, short and thick phialides, round greenish conidia, and septate hyphae resembling threads.

Keywords: Exploration, Baiting Method, Trichoderma sp.

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INTRODUCTION

Indonesia, a country with extensive archipelagos and abundant natural resources, is renowned for its agricultural produce, including rice, corn, tomatoes, and chilies. As population growth and food processing industries expand, the demand for agricultural products has risen, necessitating improvements in quality, quantity, and production. However, agricultural production has faced challenges. In 2023, rice production dropped to 53.63 million tons, a 2.05% decrease from 2022, while corn production fell to 14.46 million tons, a 12.50% decrease compared to the previous year [1]. These fluctuations are largely attributed to extreme weather delaying harvests in some regions [2].

Several factors contribute to declining agricultural yields, including poor seed quality, improper cultivation techniques, infertile soil, and pest attacks (OPT) from sowing to harvest [3][4]. Farmers often rely on synthetic chemical pesticides, which are frequently misused, leading to health risks and environmental damage [5]. A sustainable alternative is the use of biological agents, such as entomopathogenic fungi and antagonistic fungi, which are environmentally friendly, easy to produce, and less likely to induce resistance.

Trichoderma sp.**,** a saprophytic fungus, serves as a biocontrol agent against phytopathogenic molds. It is easily accessible and resilient, even in unfavorable environments, due to its ability to produce chlamydospores and withstand fungicides. With its cellulolytic activity and hyperparasitic nature, *Trichoderma* sp. effectively controls fungal pathogens like *Colletotrichum sp.* [7]. Studies show that *Trichoderma* sp. suppresses *Colletotrichum sp.* populations by producing chitinase enzymes that degrade the pathogen's cell walls [8][9]. It also combats pathogens such as *Phytophthora infestans*, causing late blight, and *Fusarium oxysporum*, responsible for tomato wilt [5].

The habitat of *Trichoderma* sp. is more commonly found in rooted soil and leaves than in stems and fruits [10]. Soil, which hosts various microorganisms such as fungi, nematodes, bacteria, and others, is an ideal habitat for *Trichoderma* sp.. The root zones of horticultural plants like chilies and tomatoes provide suitable conditions due to sufficient nutrients and the ability of *Trichoderma* sp. to form a symbiotic relationship with plant roots, enhancing root growth and nutrient absorption [11].

In addition to horticultural plant soil, bamboo soil also serves as a habitat for *Trichoderma* sp., supported by nutrient availability, a moist environment, symbiotic interactions, and loose soil structure. Bamboo, being a plant with minimal disease susceptibility, tends to harbor a significant presence of *Trichoderma* sp. in its roots [12].

Soil fungi, such as *Trichoderma* sp., can be utilized as biological control agents by isolating pure cultures and identifying their characteristics through macroscopic and microscopic methods. The initial step in biological control techniques involves exploration, which includes field specimen collection, such as from bamboo soil and the root zones of horticultural plants like chilies and tomatoes. This study aims to determine the characteristics and presence of *Trichoderma* sp. in horticultural land.

METHODS

This study employed a descriptive-exploratory approach, beginning with field exploration to collect soil samples from agricultural land and bamboo areas in Sambikerep, Surabaya, to obtain biological control fungi. Field exploration was

conducted on March 7, 2024, in Sambikerep District, while laboratory activities, such as sterilization, isolation, inoculation, purification, and identification, were carried out at the Biological Agents Laboratory, UPT Food and Horticultural Crop Protection, East Java, located at Jl. Pagesangan II No. 58, Pagesangan, Jambangan, Surabaya, East Java.

Tools and Materials

This research requires tools like hovel, measuring tape, hoe, bottles, 3-way soil meter, markers, plastic bags, black cloth, trays, storage containers, knife, scissors, sieve, label paper, petri dishes, autoclave, laminar air flow (LAF), inoculation needle, Bunsen burner, tissue, plastic wrap, microscope slides, cover slips, microscope, and fungal identification books. Materials needed are water, soil samples, rice, corn, coconut, PDA medium, distilled water, 70% alcohol, and fungal isolates.

Sample Collection

Soil samples were collected from the root zones of chili plants (CB1 and CB2), tomatoes (T), and bamboo (B) in Sambikerep District, Surabaya. Soil was excavated near the roots to a depth of 20–30 cm using a trowel, with approximately 0.5 kg of soil per sample. Samples were placed in plastic bags, and soil properties such as pH, fertility, moisture, and light intensity were measured using a 3-way soil meter. Samples were stored in a cool, shaded location.

Baiting Method for *Trichoderma* sp. Exploration

The baiting method was used to isolate *Trichoderma* sp. from soil samples using rice, corn, and coconut as baits. Cleaned containers were filled with soil, then three rice balls (2 cm in diameter), two coconut pieces (3 × 3 cm), and circular corn pieces (3 cm in diameter) were added to separate containers. The containers were covered with black cloth and placed in an incubator for seven days. Fungal growth on the baits was isolated using an inoculation needle and transferred to sterile PDA medium in petri dishes.

Tools Sterilization

Sterilization consists of dry and wet methods. In this study, wet sterilization was performed using an autoclave. To prevent damage, tools were wrapped in heat-resistant paper or plastic before autoclaving at 121°C and 1 atm pressure for 1 hour.

Identification of *Trichoderma* sp.

Identification was conducted through macroscopic and microscopic observations. Macroscopic observation included colony shape, color, upper and lower colony surfaces, colony size, texture, and edge characteristics. Microscopic observation performed using a microscope, focusing on the presence of septa in hyphae, hyphal growth and color, and the presence, color, and shape of conidia, which could be round, oval, elliptical, or irregular.

RESULTS AND DISCUSSION

Exploration is an essential activity for obtaining antagonistic fungi that can be utilized as biological agents. Such exploration is typically carried out in fungal habitats, such as the root zones of healthy plants, bamboo surfaces, and fertile soil [13]. Based on the exploration and identification of fungi from soil samples collected from chili plant roots (CB1 and CB2), tomato plant roots (T), and bamboo soil (B) using rice, corn, and coconut as bait, the results shown in Table 1 indicated the presence of *Trichoderma* sp. in various soil samples. *Trichoderma* sp. is known for its adaptability, thriving in diverse habitats and environmental conditions.

Table 1. Macroscopic Identification Results of Rice Baiting Fungi

No	Soil Type	Genus	Color	Type	Texture	Edge
1	T	*Aspergillus* sp.	Black	*Circular*	*Powdery*	*Entire*
2	B	*Aspergillus* sp.	Black	*Circular*	*Powdery*	*Entire*
		***Trichoderma* sp.**	**Dark green**	***Circular***	***Powdery***	***Entire***
3	CB 1	*Tricladium* sp.	Green	*Filamentous*	*Powdery*	*Filamentous*
		Aspergillus sp.	Black	*Circular*	*Powdery*	*Entire*
4	CB2	*Aspergillus* sp.	Black	*Circular*	*Powdery*	*Entire*
		Gliocladium sp.	Deep dark green	*Circular*	*Cotton*	*Entire*

According to Table 1, baiting with rice yielded several fungal species, including *Aspergillus* sp., *Trichoderma* sp., *Tricladium* sp., and *Gliocladium* sp. *Trichoderma* sp. was found in the rice baiting from bamboo soil. Rice was chosen as a bait because *Trichoderma* sp. tends to grow on carbohydrate-rich media. Carbohydrates are essential for *Trichoderma* sp., providing energy, cell-building materials, and electron acceptors for energy production [14]. As an energy source, rice acts as an effective bait to attract *Trichoderma* fungi, increasing the chances of finding *Trichoderma* sp. flourishing in the environment [15].

Table 2. Macroscopic Identification Results of Corn Baiting Fungi

No	Soil Type	Genus	Color	Type	Texture	Edge
1	T	*Aspergillus* sp.	Black	*Circular*	*Powdery*	*Entire*
		Phialophora sp.	White	*Circular*	*Cotton*	*Entire*
2	B	***Trichoderma* sp.**	**Dark green**	***Circular***	***Powdery***	***Entire***
		Phialophora sp.	White	*Circular*	*Cotton*	*Entire*
3	CB 1	*Phialophora* sp.	White	*Circular*	*Cotton*	*Entire*
		***Trichoderma* sp.**	**Dark green**	***Circular***	***Powdery***	***Entire***
4	CB 2	*Phialophora* sp.	White	*Circular*	*Cotton*	*Entire*

Table 2 indicates that corn baiting identified several fungal species including *Aspergillus* sp., *Phialophora* sp., and *Trichoderma* sp. *Trichoderma* sp. was specifically found in bamboo soil (B) and chili soil 1 (CB1) baited with corn. Corn was chosen as bait due to its high carbohydrate content, which supports fungal growth. As noted by [16], *Trichoderma* sp. depends heavily on carbohydrates and proteins as key macronutrients for its metabolism. These macronutrients are transported into fungal cells through complex diffusion mechanisms aided by carrier molecules [14].

Table 3. Macroscopic Identification Results of Coconut Baiting Fungi

No	Soil Type	Genus	Color	Type	Texture	Edge
1	T	*Aspergillus* sp.	Black	*Circular*	*Powdery*	*Entire*
		Periconia sp.	White	*Filamentous*	*Cotton*	*Undulate*
2	B	*Aspergillus* sp.	Black	*Circular*	*Powdery*	*Entire*
		Periconia sp.	White	*Filamentous*	*Cotton*	*Undulate*
3	CB1	*Aspergillus* sp.	Black	*Circular*	*Powdery*	*Entire*
		Periconia sp.	White	*Filamentous*	*Cotton*	*Undulate*
4	CB 2	*Aspergillus* sp.	Black	*Circular*	*Powdery*	*Entire*
		Periconia sp.	White	*Filamentous*	*Cotton*	*Undulate*

Table 3 indicates that coconut baiting identified *Aspergillus* sp. and *Periconia* sp., but no *Trichoderma* sp. was detected. This is likely due to coconut's low carbohydrate content, which is insufficient for *Trichoderma* sp. growth. Carbohydrates are essential nutrients for microbial growth, with rice and corn having significantly higher levels [10]. White rice contains 31.76% glucose and corn 42.28% glucose [17]. In contrast coconut has only 4% carbohydrates [14] but a higher fat content of 32.24% [18].

Exploration of *Trichoderma* sp. using the baiting method revealed its abundance in bamboo soil. Bamboo soil serves as a habitat for antagonistic fungi and bacteria that can suppress pathogens. Bamboo plants have fibrous roots and strong rhizomes, which stabilize soil and retain water, creating an ideal ecological environment for microbial communities, particularly beneficial microorganisms like *Trichoderma* sp. [19]. *Trichoderma* sp. is commonly found in bamboo soil due to its suitable pH range (4–7), high organic matter content, and nutrient availability. Bamboo soil has a higher C-organic content compared to other soils [10]. Measurements in bamboo soil from Sambikerep showed a pH of 7, aligning with the optimal habitat for *Trichoderma* sp. High C-organic content in soil enhances the total population of bacteria, fungi, and functional microbes, driven by the availability of ideal organic matter [20].

Trichoderma sp. can thrive in various habitats. Exploration revealed that *Trichoderma* sp. was found not only in bamboo soil samples but also in chili soil sample 1 (CB1). The presence of *Trichoderma* sp. in CB1 is attributed to its favorable conditions for fungal growth [21]. pH measurements showed that CB1 soil has a pH of 7, which is optimal for *Trichoderma* sp. growth. Additionally, CB1 soil typically contains high organic matter. As a saprophytic fungus, *Trichoderma* sp. can adapt to diverse habitats [22]. In CB1 soil, it acts as a biocontrol agent and competes effectively with pathogens [23].

The exploration results showed that *Trichoderma* sp. was not found in tomato soil or chili soil sample 2 (CB2). This absence may be due to a decline in *Trichoderma* sp. populations influenced by abiotic soil factors such as temperature, humidity, and pH. Additionally, high levels of plant pathogens in the soil could inhibit the presence of *Trichoderma* sp. [24]. The absence of *Trichoderma* sp. can result from environmental factors, competition with other microorganisms, or unsuitable soil

conditions for its growth [25]. Furthermore, heavy metal contamination in soil can lead to the development of metal-resistant *Trichoderma* sp. strains, potentially affecting its distribution across different soil types [26].

Trichoderma fungi are commonly found in fertile soils rich in organic matter and free from heavy metal contamination. *Trichoderma* is crucial for biological control. The presence of various microorganisms in the soil indicates the availability of organic materials necessary for their survival. The exploration yielded three *Trichoderma* sp. isolates. Macroscopic observations of *Trichoderma* sp. from bamboo and chili soil sample 1 (CB1) focused on colony color, morphology, colony edge, and texture on Petri dishes. Microscopic observations examined the presence of septa in the hyphae, hyphal growth patterns, presence of conidia, and conidial shape. The macroscopic morphology confirmed that the isolates from bamboo and CB1 soil were *Trichoderma* sp. fungi.

Macroscopic and microscopic observations of *Trichoderma* sp. from rice and corn baiting showed similar morphological characteristics across three fungal isolates. These isolates exhibited dark green, circular colonies with a powdery texture and entire edges. These characteristics match the findings of [27], which noted that *Trichoderma* sp. colonies are initially white, turning dark green after 7 days. The fungal isolates on PDA medium are shown in Figure 1, where the dark green color of *Trichoderma* sp. is visible. Macroscopic observations revealed that *Trichoderma* sp. colonies were round, greenish, with a circular pattern in the center and a smooth surface [15].

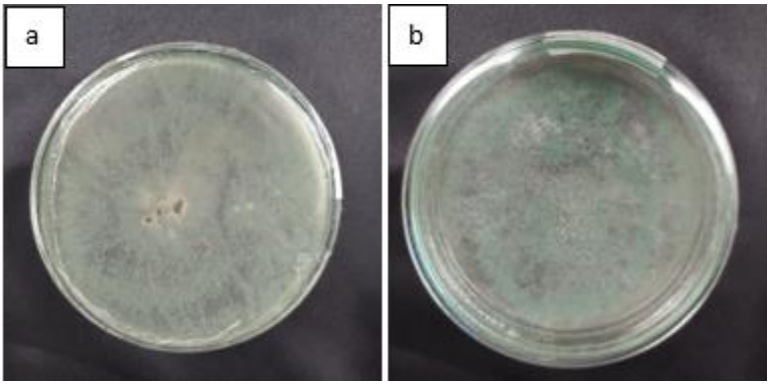

Figure 1. Macroscopic appearance of *Trichoderma* sp. (a) reverse side (b) top side

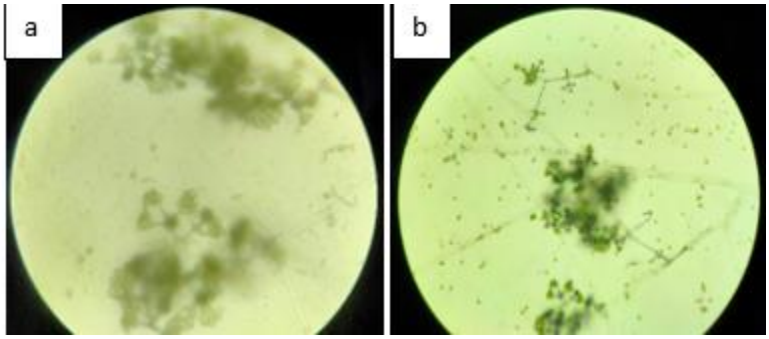

Figure 2. Microscopic appearance of *Trichoderma* sp. (a) 100x magnification (b) 40x magnification

The observations indicate that the microscopic characteristics match those of *Trichoderma* sp. The microscopic identification results are shown in Figure 2.

The identification was conducted using a binocular microscope, focusing on the shape of the conidia, conidiophores, and the presence of septa. The identification was further confirmed using the identification key from the book Illustrated Genera of Imperfect Fungi by Barnett and Hunter (1986) [28].

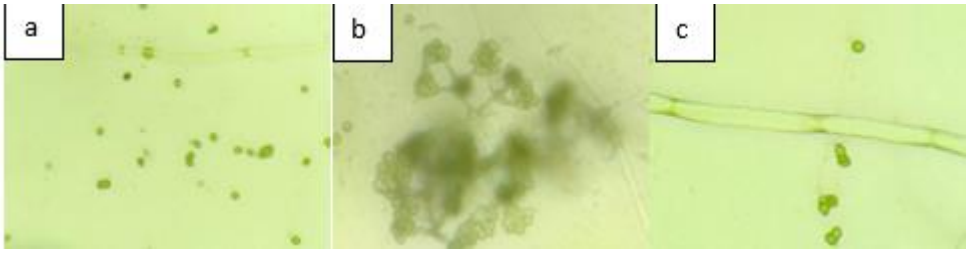

Figure 3. Microscopic characteristics of *Trichoderma* sp. (a) Conidia shape, 40x magnification (b) Conidiophore shape, 100x magnification (c) Septate hyphae, 40x magnification

Microscopic observations of the three *Trichoderma* sp. isolates revealed that they have branching conidiophores, upright conidiophores, and vertical arrangements. This is consistent with the observations of [29], who noted that *Trichoderma* sp. features upright, branching, and vertically arranged conidiophores. *Trichoderma* sp. also has thick, short phialides, round conidia, and green-colored conidia. This is supported by [27], who described *Trichoderma* sp. as having short, thick phialides and green, round conidia. According to [30], *Trichoderma* has hyaline conidiophores in varying sizes, with phialides typically branching and arranged singly or in groups. The conidiophores are unicellular, egg-shaped, and form small clusters. Additionally, *Trichoderma* has thread-like hyphae with septate structures.

CONCLUSION

The baiting method using rice and corn successfully identified *Trichoderma* sp., which displayed consistent characteristics. However, the baiting method using coconut did not yield any *Trichoderma* sp. isolates. *Trichoderma* sp. was explored in the root soils of chili 1 (CB1), chili 2 (CB2), tomato, and bamboo in the Sambikerep area, resulting in three isolates: one from CB1 soil and two from bamboo root soil. The macroscopic characteristics of the three *Trichoderma* sp. isolates from bamboo and CB1 soils included dark green color, circular shape, powdery texture, and entire edges. Microscopically, *Trichoderma* sp. was characterized by branching, upright conidiophores, arranged vertically. The fungus also had thick, short phialides, round conidia, and green conidia. Additionally, *Trichoderma* sp. exhibited thread-like, septate hyphae.

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