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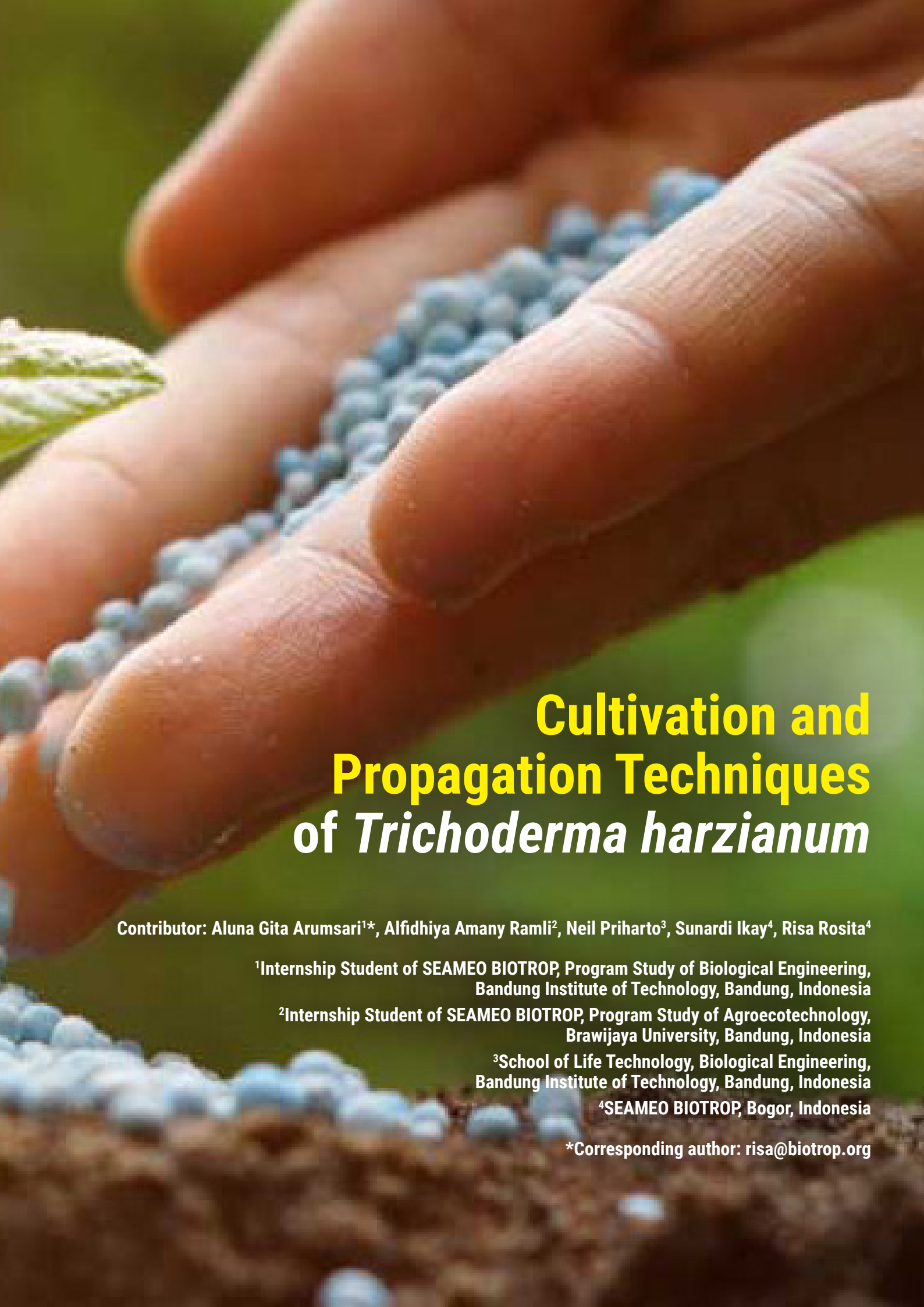

Cultivation and Propagation Techniques of *Trichoderma harzianum*

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Abstract

This research was conducted to determine the effect of temperature on the growth of *Trichoderma harzianum*. *Trichoderma harzianum* has functions to prevent the growth of diseases in plants, increase plant growth, and decompose organic matter. The research was conducted at the Phytopathology and Biosystem and Landscape Management Laboratory at SEAMEO BIOTROP, Bogor. The study was analyzed using a Completely Randomized Design (CRD) with different temperature treatments with five replications. The parameter observed was the diameter of the fungus growth after 6 days incubation period. Based on the research results, *T. harzianum* grew well when the fungus was incubated at 23 °C compared to 40 °C.

Keywords: Decomposition, fungal growth

Introduction

Trichoderma harzianum is commonly known as a fungus that has various benefits, especially for agricultural activities. This fungus is able to provide benefits that can prevent plants from disease and increase plant growth. Studies conducted by Fitria et al. (2021) stated that *T. harzianum* has several benefits such as producing the IAA (Indole Acetic Acid) hormone, which helps plant growth and development. In addition, according to Wardahni et al. (2022), the fungus produces secondary metabolites in the form of gliotoxin and glioviridin, which are toxic to the *bacterium Xanthomonas campestris* pv. *vesicatoria* causes bacterial spot disease on tomato plants. These metabolites will inhibit bacteria growth, thereby preventing plants from disease attack.

Besides being beneficial to plants, *T. harzianum* has good environmental benefits as a decomposer. According to Akmal et al. (2021), *T. harzianum* can be used as a decomposer in the composting process of empty palm oil bunches. As waste from the process of utilizing oil palm, the existence of empty fruit bunches that are not processed can pollute the environment through the resulting greenhouse gas (GHG) emissions. Composting with the help of the fungus *T. harzianum* can produce compost, which is used as an organic fertilizer.

The variety of benefits that can be obtained from using the fungus *T. harzianum* makes it essential to know how to cultivate and increase its number. However, environmental problems such as climate change, which causes an increase in temperature, can be one of the obstacles to the multiplication of the fungus *T. harzianum*. For this reason, it is necessary to study the impact of temperature on the growth and propagation of the fungus *T. harzianum*.

Method

This research was conducted from 1 to 30 June 2023 at the Phytopathology and Biosystem and Landscape Management Laboratory, SEAMEO BIOTROP, Bogor, West Java, Indonesia. Geographically, SEAMEO BIOTROP is located between 106º48' East Longitude and 6º26' South Latitude with an average annual rainfall of around 3,500-4,000 mm. The instruments used in this study consisted of analytical scales, spatulas, ruler (meter), Erlenmeyer flasks, autoclaves, test tubes, measuring cups, pipettes, ovens, Petri dishes, tweezers, cotton, paper, bunsen, loop needles, ovens, hot plates, Laminar Air Flow Cabinet (LAF), lighters and thermometers. The materials used in this study included nutrient agar media for PDA (Potato Dextrose Agar), 70% alcohol, distilled water, aluminum foil, cling wrap, spirits, sterile tissue, label paper, heat-resistant plastic, and paraffin. The research stages of this study are as follows:

Preparation of *T. harzianum* Culture Media

The culture and propagation media used were PDA (Potatoes Dextrose Agar) as an agar tube. The media volume made was 1000 mL; 39 grams of Potato Dextrose Agar media and 1000 mL Aquades were then put into the Erlenmeyer and covered with cotton. The solution is heated using a hotplate until it boils and mixes perfectly. A total of 4 ml of PDA solution was put into a test tube, covered with aluminum foil, then sterilized using an autoclave at 1 atm 121 ^{0}C for 15 minutes. After sterilization, the tube is placed on a slanted board until it solidifies. The oblique agar medium is ready to be used for culturing *T. harzianum*. Periodically check the condition of the fungal culture to ensure that the growth of the fungus is going well and is not contaminated by other fungi or bacteria. Making long-term cultures requires the addition of glycerol or paraffin to extend the shelf life. If the mushroom growth has reached the desired stage and is growing well, the fungus isolate can be collected and used for its purpose, in this case, for the use of fertilizers.

Propagation of *Trichoderma harzianum*

Petri dishes that have been filled with PDA media and have been sterilized are prepared. Fungal isolates that had been previously cultured were opened carefully under sterile conditions. The loop needle is used to transfer the

isolate from the tube to the prepared petri dish. Transfer the fungus isolate by sticking it on a flat surface. Make sure that the isolate is evenly distributed. The petri dish that was filled with *Trichoderma* was closed tightly and wrapped with cling wrap. Store the petri dish in an appropriate incubator at 40 °C and 23 ºC. Each treatment was repeated five times.

Observation Growth of *Trichoderma harzianum*

The diameter of the isolate is one of the parameters in observing the growth of the fungus. Diameter can predict how much the isolate has grown since it was first isolated. The wider the diameter, the more fungal biomass can be used as a parameter for fungal growth. Observation of fungal growth was carried out 1 day after cultivation. The isolate diameter measurements were repeated every day until 7 days after planting. The diameter of *Trichoderma* was measured using a ruler by making two diagonal lines across the petri dish by crossing them. Then the two diagonals were averaged.

Research Design

The research design used was a Completely Randomized Design (CRD) with 1 factor, namely temperature, which consisted of 2 levels, namely temperature was 23 °C to 40 °C. The experiment was repeated 5 times so that there were 10 experimental units. The independent variable in this study was room temperature, while the dependent variable influenced by the independent variable was the growth rate of the diameter of the fungus *T. harzianum*. Quantitative data from research results were analyzed using analysis of variance (ANOVA). If there is a significant difference between the treatments, a further test will be carried out using the Duncan Multiple Range Test (DMRT) at the alpha (α) level of 5%. Research data were analyzed using SAS software version 9.0.

Results and Discussion

The results of the variance of the increase in the diameter growth of *T. harzianum* after being given a heating treatment in the oven at different temperature levels showed a very significant difference in the diameter of the growth of *Trichoderma* (P>0,05). Based on the research results, after 6 days incubation period, *T. harzianum* grew well when the fungus was incubated at 23°C compared to 40°C (Figure 1). Meanwhile, *Trichoderma* colony is shown in Figure 2.

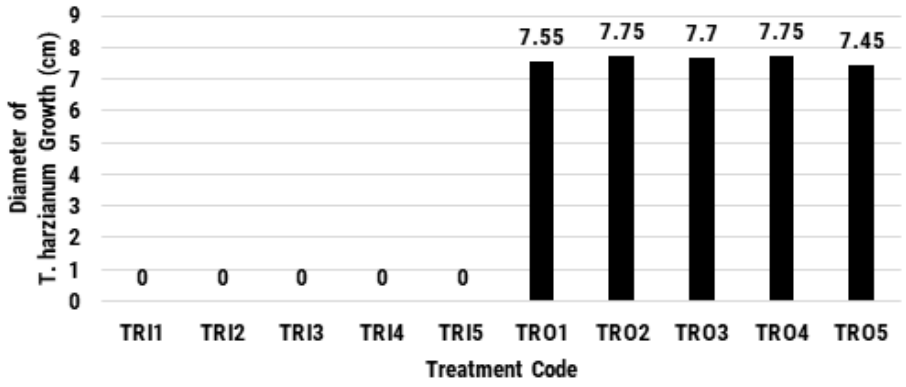

Figure 1. Diameter growth of *Trichoderma harzianum* after treatment (TR= *Trichoderma harzianum*; I= temperature 40°C; O= temperature 23°C)

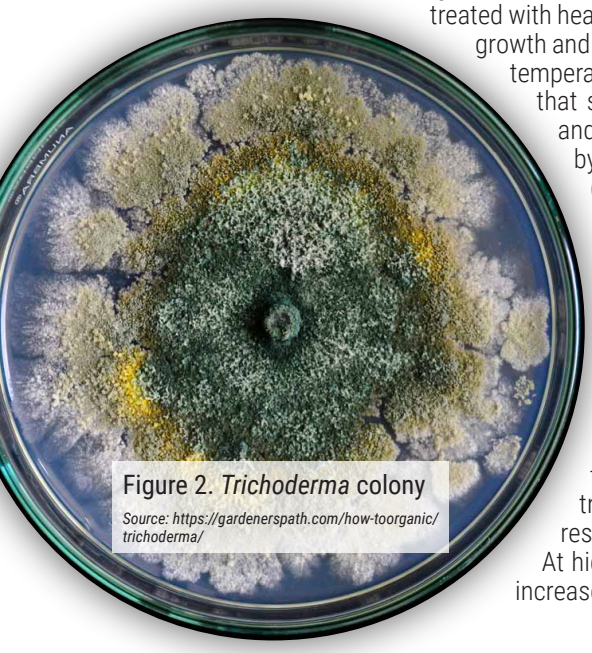

Figure 2. *Trichoderma* colony

Source: https://gardenerspath.com/how-toorganic/trichoderma/

Figure 1 showed a significant difference in the growth of diameter *T. harzianum* that had been treated with heating treatment at 23°C and 40°C. Based on the result, in the experiment at 40°C, no growth and diameter increased in *T. harzianum*. It happened because it had passed the optimal temperature limit for growing fungi. The results are supported by Zali & Purdiyanto (2011) that stated high temperatures could inhibit the production of carboxymethylcellulose and xylanase enzymes, whereas, in the treatment at 23°C, the diameter was increased by 7.55 cm, 7.75 cm, 7.7 cm, 7.75 cm, and 7.45 cm. In relation to Zali & Purdiyanto (2011), which stated that the growth of *Trichoderma* has a balanced quality to grow well at room temperature (28°C). According to Prabowo et al. (2006), apart from the increase in the diameter of *T. harzianum*, there is also an increase in the length of the hyphae if placed at an optimal temperature. The results of the long hyphae analysis after heating in the oven at different temperature levels showed a very significant difference in the length of the stem (hyphae) of *Trichoderma* (P>0,01). The highest growth in length of the stem (hyphae) of *Trichoderma* was achieved by treatment at room temperature (28°C). In addition to the increase in diameter and length of the stem (hyphae), several surviving spores also exist. The number of spores that survive at room temperature is better than at higher or lower temperatures. The results of the temperature treatment variance had a significant effect (P>0.05). It shows that the resistance of sports within certain temperature limits is very influential. At high temperatures, the spores could not develop properly or could not increase the number of spores present in each treatment.

Conclusion

According to the study, the fungus *T. harzianum* showed better growth and increased diameter size in a petri dish at 23°C. However, at 40°C, *T. harzianum* was unable to grow properly.

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