# GROWTH POTENTIAL AND METABOLITE PRODUCTION OF THE FILAMENTOUS FUNGUS *TRICHODERMA* IN DIFFERENT SOLID CULTURE MEDIA

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## ABSTRACT

*Trichoderma* is a widely commercially employed filamentous fungus, notable for its utility in various industrial sectors, including pharmaceutical, agronomic, food, energy, and chemical industries. Its capacity to produce a variety of metabolites from different substrates contributes to this usefulness. This study aimed to evaluate the growth and production of secondary metabolites by a promising strain of *Trichoderma* in different culture media (Sabouraud Agar, Agar-Agar, Czapek Agar, Potato Dextrose Agar, Mannitol Agar, Tryptic Soy Agar, Yeast Extract Peptone Dextrose Agar, Yeast Extract Peptone Agar, and Malt Extract Agar). A disc containing *Trichoderma* spores and mycelium was inoculated in the center of each plate, with incubation at 25ºC for 5 days. In sequence, the treatments were compared by the diameter of the colonies. After extraction with ethyl acetate, the samples were subjected to HPLC for metabolite quantification. The *Trichoderma* strain showed better mycelial growth and sporulation in PDA and Malt Extract Agar. However, according to the chromatogram, the bioagent produced metabolites in the 16 - 20 minute interval, best represented by production in the Sabouraud medium. We concluded that the best substrate for metabolite production and identification is the Sabouraud culture medium.

**Keywords**: HPLC; Antagonistic fungus; Secondary metabolites; *Trichoderma*.

## 1 INTRODUCTION

The diversity of bioactive secondary metabolites produced by biological control agents in culture media can be vast. According to Muniz et al. (2018), antagonistic microorganisms should be easily cultivated in available culture media that meet their nutritional needs so that a large amount of inoculum can be easily obtained, preferably in a short period.

Teles et al. (2020) commented that antagonistic fungi could undergo various adaptations, pointing to almost unlimited potential for metabolic variations that facilitated colonization of the planet. This adaptability allows them to produce a series of secondary metabolites along with their penetrating and absorptive lifestyles, aiding their survival in stressful environments. According to these authors, the production of secondary metabolites by antagonistic fungi varies according to their life cycle and the environment in which they are inserted. Bernardi (2023) observed that fungi, as eukaryotic, have a more complex genome than prokaryotes and consequently encode the biosynthesis of natural products more abundantly and diversely.

Among the main antagonistic fungi is the genus *Trichoderma*. Many authors, such as Aguiar et al. (2013), reported that *Trichoderma* species are efficient biocontrol agents against a series of phytopathogenic fungi, acting both through the production of volatile and non-volatile metabolites. Thus, this study aimed to evaluate the growth and production of secondary metabolites from a promising strain of *Trichoderma* in different culture media.

## 2 MATERIAL & METHODS

The quantification of metabolites was performed by high-performance liquid chromatography (HPLC) using the methodology described below. The tested culture media included Sabouraud Agar, Czapek Agar, Potato Dextrose Agar (PDA), Agar-Agar, Mannitol Agar, Tryptic Soy Agar (TSA), Yeast Extract Peptone Dextrose Agar (YPDA), Yeast Extract Peptone Agar (YPA), and Malt Extract Agar. The preparation of culture media followed protocols established in the literature or provided by manufacturers and autoclaved at 121°C for 20 minutes. The colony growth diameter was measured on 90mm Petri dishes after 5 days of incubation to determine the best culture medium for fungal growth. For metabolite analysis, extraction was performed using ethyl acetate and methanol as organic solvents, followed by solvent evaporation and resuspension of metabolites in an acetonitrile and water solution. The solution containing metabolites was analyzed using the HPLC method.

The HPLC equipment used was the PerkinElmer Flexar model, with the following chromatographic parameters: The mobile phase was a gradient of water + TFA and Acetonitrile + TFA, starting with 85:15 for 5 minutes, followed by the same conditions for another 10 minutes (85:15), then shifting to (0:100) within 17 minutes, held for 10 minutes at these conditions (0:100), and finally returning to (85:15) over 3 minutes, using a C18 column, 5 µm particle size, 4.0 mm internal diameter, 250 mm length, a flow rate

of 1.0 mL/min, and a wavelength of 225 nm. These experiments aimed to understand *Trichoderma* behavior under different culture conditions and identify culture media favorable for its growth and production of biotechnologically relevant metabolites.

## 3 RESULTS & DISCUSSION

The *Trichoderma* spp. isolate grew to 90 mm colony diameter on media PDA and Malt Extract Agar, with no significant difference between them. The second highest growth was observed in Yeast Extract Peptone Agar and Yeast Extract Peptone Dextrose Agar, with a growth of 80 mm. The least favorable for colony development, below 50 mm, were Mannitol Agar, Agar-Agar, and Tryptic Soy Agar (Figure 1). These results were consistent with Mishra and Khan (2015), who studied the growth of *Trichoderma viride* in various culture media and observed that this fungus grew best in Sabouraud Malt Yeast Extract Agar, followed by Sabouraud Agar.

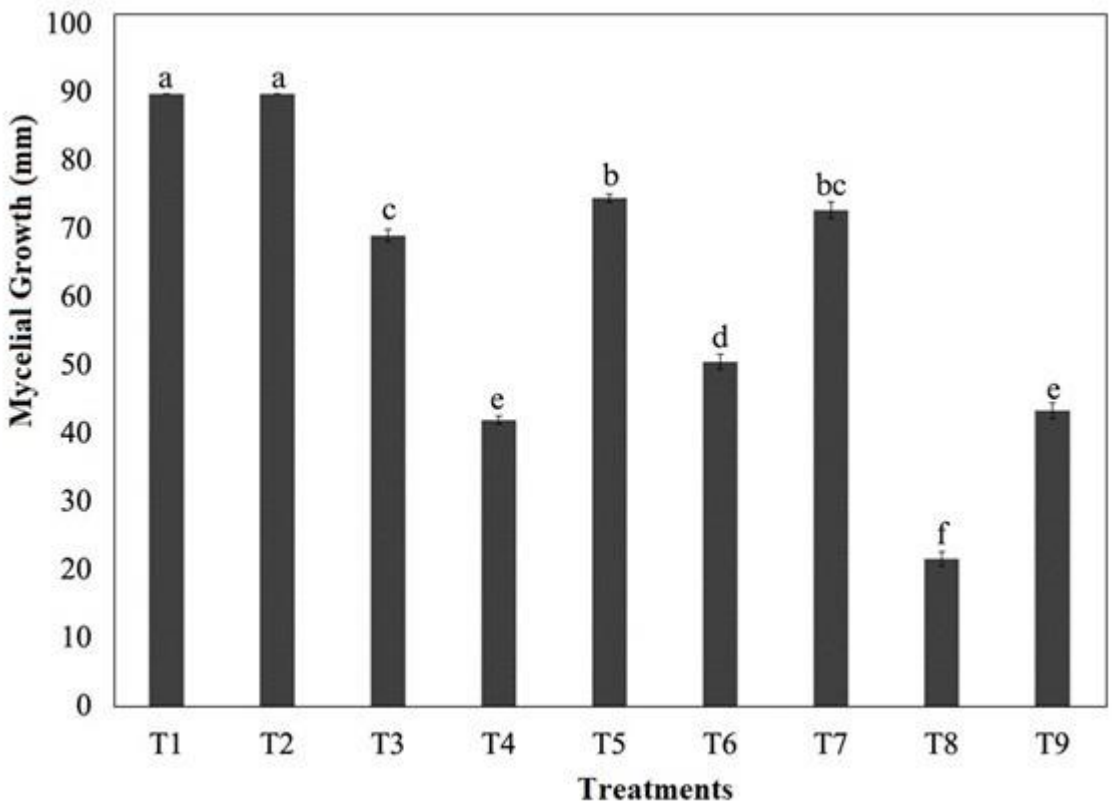


**Figure 1.** Colony diameter of a *Trichoderma* sp. isolate cultivated in different solid culture media: T1-Potato Dextrose Agar (PDA), T2-Malt Extract Agar, T3-Sabouraud Agar, T4-Mannitol Agar, T5-Yeast Extract Peptone Agar (YPA), T6-Czapek Agar, T7-Yeast Extract Peptone Dextrose Agar (YPDA), T8-Tryptic Soy Agar (TSA), and T9-Agar-Agar.

Figure 2 shows an example of the HPLC analysis of metabolites produced in culture media after the growth of *Trichoderma* spp. The Sabouraud, PDA, Malt Extract, and YPDA media revealed diverse secondary metabolites in the range of 16 to 20 minutes of analysis. However, the chromatogram from the Sabouraud medium notably showed peaks with higher optical densities at around 17 and 19 minutes.

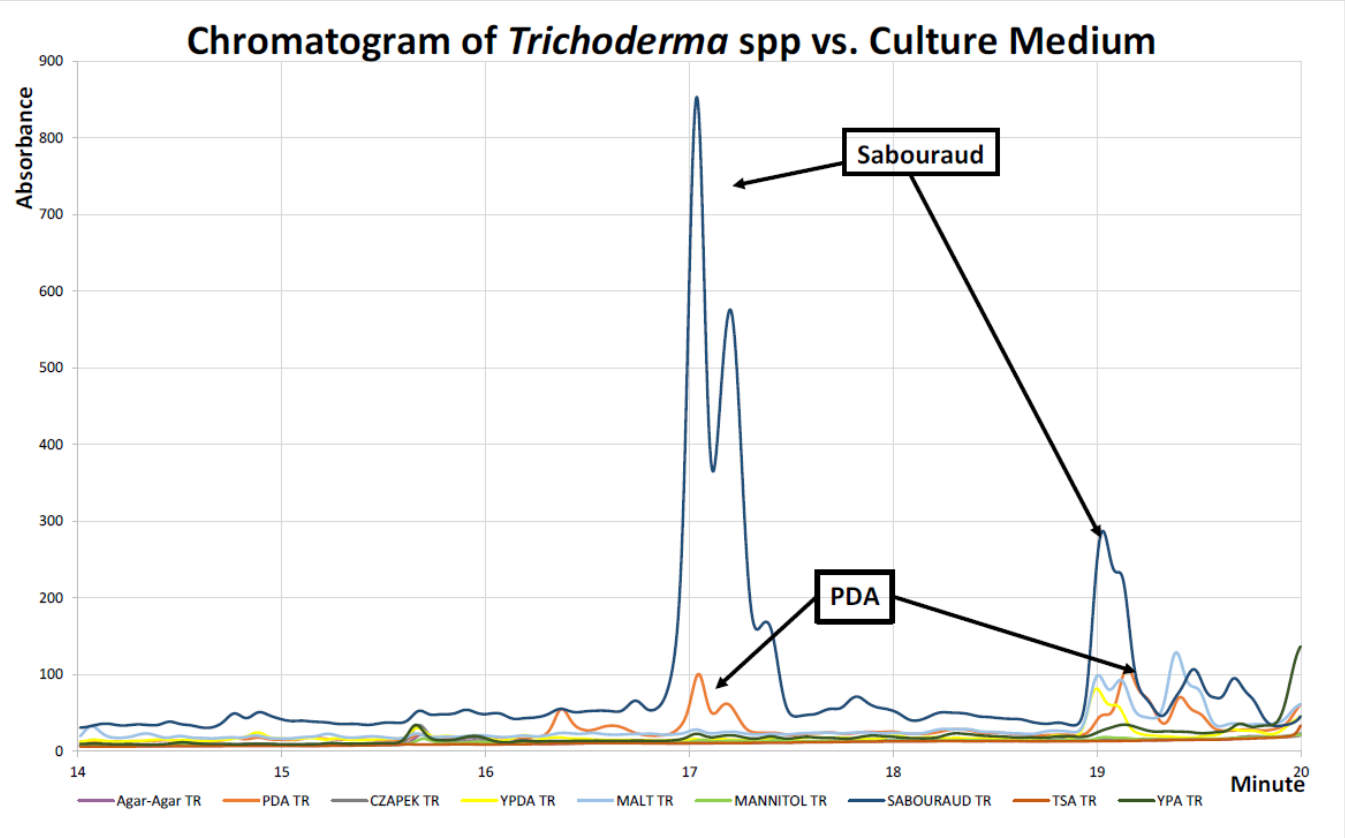


**Figure 2**. Chromatogram of a *Trichoderma* spp. isolate cultivated in different culture media. T1-Potato Dextrose Agar (PDA), T2-Malt Extract Agar, T3-Sabouraud Agar, T4-Mannitol Agar, T5-Yeast Extract Peptone Agar (YPA), T6-Czapek Agar, T7-Yeast Extract Peptone Dextrose Agar (YPDA), T8-Tryptic Soy Agar (TSA), and T9-Agar-Agar.

## 4 CONCLUSION

This study demonstrated that Trichoderma's mycelial growth varies significantly depending on the substrate used for fungal growth, indicating that the medium composition directly influences its development. Among the tested media, PDA and Malt Extract Agar were the most effective in producing mycelia and spores after five days of incubation.

In the quantitative analysis of secondary metabolites by HPLC after extraction with organic solvent, Sabouraud Agar excelled, showing chromatograms with peaks of optical density superior to the other tested media.

We concluded that although Sabouraud Agar did not show excellent mycelial growth, it was the best for producing secondary metabolites. Therefore, its use is recommended for studies on the production of secondary metabolites by *Trichoderma* spp.

## REFERENCES

[1] AGUIAR, ARde; AGUIAR,D; TEDESCO, SB; SILVA, ACFda. Antagonismo a fungos assoiados às sementes de Paspalum notatum Flügge por *Trichoderma*. Enciclopédia Biosfera, Centro Científico Conhecer-Goiânia, v.9, n.17, p.197, 2013.

[2] BERNARDI, D.I. Metabólitos secundários bioativos produzidos por fungos filamentosos de ambientes extremos. Tese (Doutorado em química orgânica e Biológica). Instituto de Química de São Carlos, Universidade de São Paulo, 2023, 201p.

[3] MISHRA, P.K.; KLAN, F.N. Effect of different growth media and physical factors on biomass production of *Trichoderma* viride. People's Journal of Scientific Research, Jul 2015; v.8, issue 2. 11-16p.

[4] MUNIZ, PHPC; PEIXOTO, GHS.; TEIXEIRA, MPM; MELO,SCM; CARVALHO. DDC. Produção de conídios em substrato sólido e colonização superficial por *Trichoderma harzianum*. Revista de agricultura Neotropical, Cassilândia-MS, v.5, n.4, p 40-44, out/dez. 2018. INSS 2358-6303.

[5] TELES, AM; EVERTON. GO; MOUCHREK, AN; TELLIS, CJM; BEZERRA, GFdeB; SOUZA, FA. Triagem fitoquímica de extrato obtido do fungo *Penicillium purpurogenum* advindo de ambiente marinho poluído do Maranhão. Research, Society and Development, v.9, n.8, e539986109, 2020 (CC BY 4.0) ISSN 2525-3409/Doi://http://dx.doi.org/10.33448/rsd v.9i8.6109.

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