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EVALUATION OF THE BIOCONTROL POTENTIAL OF THE *Trichoderma asperellum* URM6997/160821

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ABSTRACT

Biological biocontrol is a promising alternative to fungicides and antagonistic microorganisms of plant diseases. Since they have biocontrol mechanisms against phytopathogens, as well as induction of resistance and promotion of plant growth. Thus, dual cultures with phytopathogens and Trichoderma were conducted, followed by quantification of phosphate solubilization, siderophore production, and indole-3-acetic acid. The results obtained were promising for the dual culture test, with an inhibition rate above 50% and a considerable percentage of siderophores, requiring further evaluations to improve the quantification of phosphate solubilization and indol-3-acetic acid production.

Keywords: Trichoderma. Dual culture. Phosphate solubilization. Siderophore. Indole-3-acetic acid.

1 INTRODUCTION

Plants can be affected by biotic and abiotic factors, and species of Colletotrichum, according to Wang et al. (2024), are relevant phytopathogenic microorganisms that degrade plantations in temperate, tropical, and subtropical regions of various plant families worldwide. *Colletotrichum truncatum* is economically significant due to its vast host range, inciting diseases that cause significant economic losses[1]. Some of the crops from which *Colletotrichum truncatum* was isolated include peanut, cabbage, and bamboo[2].

Another phytopathogenic microorganism with a broad infectious aspect is *Sclerotinia sclerotiorum*, a necrotrophic fungus[3] that causes infections in stems, roots, and leaves, secreting reactive oxygen species in Brassica species (cabbage, radish, mustard, turnip, arugula), promoting host cell death[4]. Besides Brassica species, it can affect legumes such as common beans (Phaseolus vulgaris L.), leading to yield losses of 50 to 100%, posing a considerable threat to this crop due to climate change[3].

Therefore, biocontrol and biofertilizers are alternative resources for disease control and crop extension[5]. Endophytic microorganisms can also assist in plant growth, root stimulation, and disease control[6]. Since chemical signaling between the surrounding soil and plant roots preserves plant conditioning. In this way, micronutrients like phosphorus, when at low concentrations in plants, form thin, long, and shallow roots and[7] and arbuscular mycorrhizal fungi are used to absorb inorganic phosphorus[8] . Additionally, iron is a highly important component, and when deficient, microorganisms (fungi and bacteria) and plants produce an organic compound, siderophores, which are chelating agents secreted into the extracellular environment, showing high affinity for the element[9]. Similarly, small signaling molecules also aid in plant growth and development, known as phytohormones, such as auxin. And IAA (Indole-3-acetic acid) corresponds to the main auxin in plants[10].

The conventional method for controlling diseases caused by the phytopathogens *C. truncatum* and *S. sclerotiorum* is through chemical fungicides[11]. However, the use of these agents leads to fungicide-resistant strains and environmental toxicity, necessitating more economically viable, environmentally safe, and highly efficient alternatives[2]. In this regard, species of the Trichoderma genus serve as an alternative for fungal biocontrol agents, using mechanisms such as competition for nutrients or space, antibiosis through secondary metabolites, and mycoparasitism, providing an alternative to the use of agrochemicals. Additionally, it contributes to plant growth through signaling processes[12]. Thus, this work began with an assessment of the antagonistic capacity against phytopathogens, followed by microbial characterization with phosphate solubilization, siderophore production, and quantification of indole-3-acetic acid to promote plant development.

2 MATERIAL & METHODS

The strain of the fungus *Trichoderma asperellum* URM 6997/160821 was cultured on PDA (Potato Dextrose Agar) plates at 28°C in the dark for 7 days. In the confrontation assay, mycelial discs of the *Sclerotinia sclerotiorum* MMBF 03/18 and *Colletotrichum truncatum* MMBF 05/05 strains with a diameter of 1 cm were placed on PDA medium plates at the upper and lower ends, with Trichoderma as the antagonist. The plates were incubated at 28°C for 7 days, and replicated three times for each phytopathogen. The control was performed with the single culture of the phytopathogen. The inhibition efficiency (%) was represented by formula (1), where R1 corresponds to the radial growth of the control (single culture of the phytopathogen on BDA plate) and R2 with the radial growth of MMBF02/12 in the dual culture with Trichoderma[13].

$$(\%) = \left(\frac{R1 - R2}{R1}\right) x\ 100 \tag{1}$$

The fungus was characterized by its phosphate solubilization capacity, cultured in NBRIP medium at 28°C, 120 rpm for 5 days and quantified using the Murphy and Riley method (1962)[14]; siderophore quantification, cultured in potato and dextrose medium at 28°C, 150 rpm for 7 days, quantified using the Schwyn and Neilands method (1987)[15]; and determination of indole-3-acetic acid (IAA) production in TBS medium at 28°C, 100 rpm for 7 days, quantified using the Salkowski reagent[16]. Readings were taken using a spectrophotometer at wavelengths of 880 nm, 630 nm, and 530 nm, respectively.

3 RESULTS & DISCUSSION

In the assays, the antagonistic microorganism, URM6997/160821, grew considerably, demonstrating significance with possible suppression of the phytopathogens MMBF 03/18 and MMBF 05/05. The test was conducted (Figure 1) with Trichoderma inoculation at the same instance as the phytopathogens. Thus, the inhibition efficiency was 77.6% and 77.2% for MMBF 03/18 and MMBF 05/05, respectively. Strain URM6997/160821 showed an inhibition rate above 50%. Kamaruzzaman et al. (2021)[17] showed that *T. asperellum* efficiently reduced the growth of *Botritis cinerea* and *S. sclerotiorum* in dual culture plates. Similarly, Wang et al. (2024)[18] achieved inhibition rates of 78.7%, 97.7%, and 70.3% for *Rhizoctonia solani, Fusarium oxysporum*, and *S. sclerotiorum*.

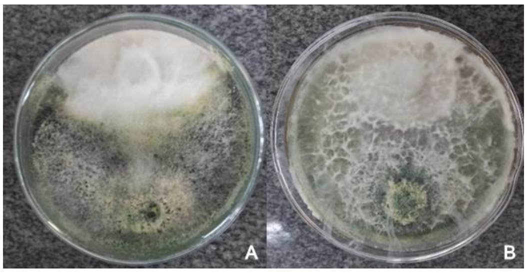

Figure 1: Plates showing antagonistic activity of Trichoderma strains (inoculated at the bottom) against the phytopathogens Sclerotinia and Colletotrichum (inoculated at the top) in dual culture assays. In sequence, (A) dual culture plate between *Trichoderma asperellum* URM 6997/160821 and *Sclerotinia sclerotiorum* MMBF 03/18; (B) dual culture plate between *Trichoderma asperellum* URM 6997/160821 and *Colletotrichum truncatum* MMBF 05/05.

The biocontrol mechanisms extend to mycoparasitism, based on the intertwining of the antagonist's hyphae with phytopathogens, through chemotropism, obtaining nutrients, and enzyme production (chitinase, glucanase, protease); antibiosis, which involves inhibiting the growth of the phytopathogen through the release of secondary metabolites, whether volatile or not (polyketides, terpenoids, pyrones, and anthraquinones)[19], and the production of other antagonistic properties including cellulase, xylanase, pectinase, and trichodermin[20].

Phosphate solubilization values, siderophore production, and indole-3-acetic acid quantification corresponded to 3.08×10^{-3} mg/ml, 100.73 PSU (%), and 0.088 mg/mL, respectively. The values obtained for strain URM 6997/160821 regarding phosphate solubilization were low compared to Prasad et al. (2023)[21], who obtained solubilization values of 0.0048 to 0.12256 mg/mL, and Bader et al. (2020), with results of 0.2158 to 0.2882 mg/mL. This could be addressed by lowering the pH, as suggested by previous mentioned studies with pH 3.8. Regarding siderophore production, based on the percentage of siderophores found, it can be considered that the strain exhibited the chelating organic compound, with values potentially elevated due to complexation with other essential elements. The studied strain showed indications for IAA production, as per Bader et al. (2020), who reported values of 0.0072 to 0.0211 mg/mL. Previous studies by Syamsia et al. (2015) [22] reported higher values, suggesting the addition of tryptophan as an alternative.

4 CONCLUSION

The results of this study demonstrated the beginning of an evaluation of the Trichoderma strain as a potential promising microorganism for biocontrol agents, in the presence of the phytopathogens *C. truncatum* and *S. sclerotiorum*. Furthermore, improved fermentation conditions, pH adjustments, and tryptophan addition should be tested for better quantification of phosphate solubilization and IAA production, to establish more concise bases for formulations and applications of the antagonistic endophyte in disease control.

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