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ANTIFUNGAL ACTIVITY OF FERMENTATIVE BROTH FROM *Diaporthe schini* FOR PHYTOPATHOGEN INHIBITION

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ABSTRACT

The demand for food has been increasing over the past years due to the growth of the world population, requiring the agricultural sector to increase production to meet the planet's food needs. One of the major challenges faced by this sector is the control of microorganisms in crops. The objective of this study was to evaluate the inhibition on the growth of undesirable fungi in crops using a fermentative broth from *Diaporthe schin*i, using two techniques: the poisoned food method and the agar diffusion method. Antifungal activities were performed by measuring the growth diameter of phytopathogens on Petri dishes over 24-hour periods. The main results were obtained by inhibiting the growth of the fungi *Rhizoctonia solani*, resulting in a 10.3% growth inhibition, and *Macrophomina phaseolina*, did not presented inhibition effects and had a small growth benefit in the poisoned food method technique. The results suggest these fungi could be used to obtain efficient biocontrol agents for the control of these phytopathogens, after further studies.

Keywords: Antifungal activity. *Diaporthe schini*. Biocontrol. Phytopathogenic. Agriculture.

1 INTRODUCTION

The agricultural production has been intensified to meet food demands because the gradual increase in the world population. However, one of the main challenges in agriculture is the control of fungal microorganisms, which are responsible for production losses. The study of Da Costa et al. (2020)[1] observed that 88.1% of the samples presented diseases caused by the phytopathogen *Macrophomina phaseolina* in corn cultivation, resulting in a grain weight reduction of up to 47.6%, depending on the variety. Furthermore, the excessive use of chemical treatments for the control of these microorganisms has been generating problems of pest resistance and human health, as well as raising environmental concerns. As a solution, physical treatments, natural extracts, and biological control are used currently.

Consequently, there has been an increase in research related to the use of plant-derived products for the protection of agricultural plants, aiming to reduce environmental impacts and negative effects on human health. In this context, fungi and other microorganisms are capable of producing substances known as secondary metabolites, which influence the growth and behavior of agricultural and biological ecosystems. One of the major processes used for the production of fungal secondary metabolites is the fermentation process. The submerged fermentation stands out as it allows better control of parameters such as pH, temperature, agitation, and aeration, ensuring a more uniform production process.

The fungus of the genus *Diaporthe* has shown promising results for the production of secondary metabolites. Brun et al. (2020)[2] yielded positive results in the control of invasive species, such as weeds, prompting inquiries about the potential of this fungus in controlling invaders in different crops.

Therefore, this study aimed to evaluate the response to antifungal testing of the fermentative broth of the endophytic fungus *Diaporthe schini* against five different fungi causing diseases in plants, using two application methods, the poisoned food method and agar diffusion method.

2 MATERIAL & METHODS

The submerged fermentation of *Diaporthe schini* was conducted according the methodology described by Dos Reis et al. (2019)[3], performed in Erlenmeyer flasks containing 150 ml of medium, with agitation at 120 rpm at 28°C for 7 days. Once fermentation was complete, the cells and fermentation broth were separated. The cells were discarded and the fermentation broth was stored in falcon tubes at -20 °C until use. For this study, five phytopathogens were assessed: *Rhizoctonia solani* (RS), *Sclerotinia sclerotiorum* (SS), *Macrophomina phaseolina* (MP), *Fusarium oxysporum* (FO), and *Colletotrichum gloeosporioides* (CG).

The in vitro assays were performed in duplicate. For analysis of the fermentation broth, the culture medium was prepared with distilled water and potato dextrose agar (PDA) at a ratio of 39 grams of PDA per liter of water, and the samples were filtered using a syringe filter with a pore size of 0.22 µm. The Blank assay (only PDA) was conducted to control, and two analyzed methods, poisoned food method and agar diffusion method were performed. The poisoned food method consisted of incorporating the inhibitory agent into the culture medium according to the desired concentration[4], in this case, the culture medium was one-quarter of the fermentative broth of *Diaporthe schini* to three-quarters of distilled water. The agar diffusion method was carried out using 100 µl of fermentation broth onto the Petri dish with solidified PDA and a disposable Drigalski loop was used to assist in spreading until dry.

The plates were left in an incubator with light at 25°C, the diameters of fungal growth in control and sample plates were measured, and the antifungal effect was estimated by Equation 1[4].

$$Antifungal\ activity\ (\%) = \frac{D_B - D_A}{D_B} * 100 \quad (1)$$

Where D_B is the diameter of growth in blank plate and D_A is the diameter of growth in the plate containing fermentative broth from *Diaporthe schini.*

3 RESULTS & DISCUSSION

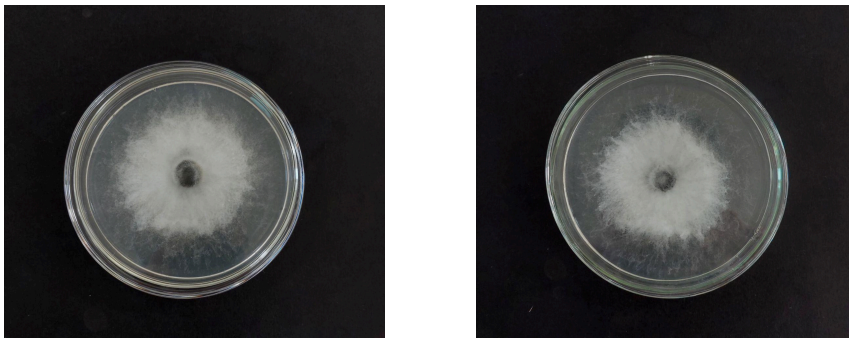
Table 1 shows the antifungal activity of fermentative broth from *Diaporthe schini* against five phytopathogens (*Rhizoctonia solani* (RS), *Sclerotinia sclerotiorum* (SS), *Macrophomina phaseolina* (MP), *Fusarium oxysporum* (FO), and *Colletotrichum gloeosporioides* (CG)).

Table 1 – Inhibitory effect of *Diaporthe schini* fermentative broth against plant pathogenic fungi.

	Phytopathogens fungi	Inhibitory effect (%)
Poisoned food method	*Rhizoctonia solani*	10.3 ± 0.13
	Macrophomina phaseolina	-5.1 ± 0.12
	Colleotrichum gloeosporioides	-18.0 ± 0.07
	Sclerotinia sclerotiorum	-18.9 ± 0.14
	Fusarium oxysporum	-5.3 ± 0.07
Agar diffusion method	*Rhizoctonia solani*	4.1 ± 0.15
	Macrophomina phaseolina	-0.3 ± 0.19
	Colleotrichum gloeosporioides	-20.0 ± 0.00
	Sclerotinia sclerotiorum	-8.1 ± 0.28
	Fusarium oxysporum	-4.0 ± 0.00

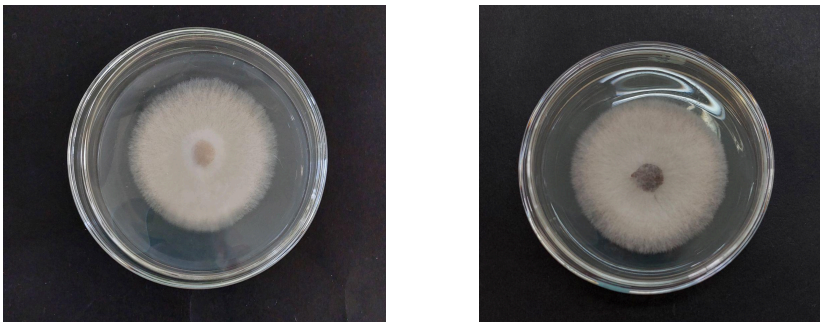
The metabolites presented in the fermentation broth had more impact in the fungus *Rhizoctonia solani*, resulting in a 10.3% growth inhibition compared to the control sample. On the other hand, the fungi *Colleotrichum gloeosporioides*, *Sclerotinia sclerotiorum,* and *Fusarium oxysporum* there were a positive impact with the growth acceleration by the fermentative broth. This can be attributed to the fact that there must be unconsumed micronutrients and some compounds that were in the degradation phase that favored their metabolism. The compounds which could have exhibited antifungal properties, did not demonstrate an effect against these phytopathogens. Finally, for the fungus *Macrophomina phaseolina* did not presented inhibition effects and had a small growth benefit in the poisoned food method technique.

Erhonyota et al. (2023)[5] compared different techniques for *in vitro* analysis of antifungal activity, the agar well diffusion and poisoned food method, the technique that uses dilution was generally more suitable for antifungal sensitivities as this protocol facilitates the better surface of area interaction of the antifungal agent to interact with the causative organisms. This study was in agreement with the results obtained in this work. The two methodologies used in this work aimed to spread the product throughout the cultivation medium, either on the surface (agar diffusion method) or diluted in the cultivation medium surface (poisoned food method). The agar diffusion method was better than the dilution method because it was not autoclaved, avoiding the degradation of the molecules, a lower amount of sample was required, and also the technique that creates a film between the cultivation medium and the fungus, which can inhibit its growth. This mechanism (creation of a film) is the same one that can be used in plantations by spraying products on the leaves. Thereby, the agar diffusion method was more suitable for these assays, in Figure 1 and 2 it is possible to observe the antifungal effect on the *Rhizoctonia solani* and *Macrophomina phaseolina*.



[1] [2]

Figure 1 *Macrophomina phaseolina*: [1] Control, [2] Agar diffusion method.



[1] [2]

Figure 2 *Rhizoctonia solani*: [1] Control, [2] Agar diffusion method.

4 CONCLUSION

The results obtained with these two phytopathogens, Rhizoctonia solani and Macrophomina phaseolina were promising and more detailed analysis could be performed to evaluated the concentrated broth. This increases the chances of utilizing biological agents for the treatment of agricultural pests, thereby reducing environmental impacts and health issues associated with the use of chemical treatments.

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