PRODUCTION OF CONIDIA OF *Trichoderma harzianum* AS A FUNCTION OF FUNGAL STRAIN, SUBSTRATE HUMIDITY AND SOLID STATE FERMENTATION TIME

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ABSTRACT

Several formulations based on *Trichoderma* spp. are available in the Brazilian market for application as biocontrol agents or plant-growth promotion. However, only some studies are dedicated to improving the production of *Trichoderma* conidia in solid-state fermentation, this bioagent's main form of production. The objective of the work was to evaluate the effect of the moisture content of parboiled rice and the fermentation time on the production of conidia by *T. harzianum*, aiming to maximize efficiency and scale up production. The productivity of conidia of the *T. harzianum* isolates BRM 29675 and BRM 29274 was also compared as a requisite to develop new biofungicides. Solid-state fermentation was carried out on parboiled rice grains in 250 mL Erlenmeyer flasks, with treatments consisting of substrate humidity adjusted to 36.3, 46.1, 53.3, 58.8, 63.1 and 66.7%. In all treatments, linear models with R^2>0.90 were adjusted for the conidial production × substrate moisture relationship. Isolate BRM 29274 was the most productive. The optimal substrate humidity was 53.3 and 63.1%, and conidia production time could be reduced by 28% without differences (p>0.05) in yield.

Keywords: Scale-up process. Biocontrol. Sustainable agriculture. Industrial yield. Bioinputs.

1 INTRODUCTION

The Brazilian biological control industry has registered large growth rates in recent years thanks to good field results and the grain and fiber farmers' wide acceptance of this method. Combined with a millionaire market perspective and the concern with agricultural practices that are increasingly sustainable from an environmental and financial point of view, this positive scenario tends to grow in the following years.

Since the pioneering work of Weindling (1932) reporting the use of *Trichoderma* spp. in the biocontrol of plant diseases, the research in biologicals grew consistently. From the 1970s onwards, it gained prominence in the industry. Currently, hundreds of product formulations containing different species of *Trichoderma* are commercially available worldwide for many agricultural crop applications, emphasizing the biological control of plant diseases and stimulating plant growth promotion.

Meeting the growing market demands for biopesticides has become a challenge for bioindustries. Developing strategies to improve the production system is complex and demands a close relationship between industry, bioindustry, research and teaching institutions to develop new products and formulations. Optimizing the production process is challenging, as ideal conditions in biofactories are not always maintained or achieved, requiring the process to be constantly adjusted (Rezende et al., 2020).

The main method for mass production of conidia (asexually reproducing spores) of *Trichoderma* spp. is solid-state fermentation, where parboiled rice has been the main substrate used by the Brazilian bioindustries for this purpose. Among several factors, optimizing industrial production involves implementing techniques and strategies to ensure a consistent increase in conidia production, maintaining the quality and efficiency of biological material and basic research into the biology of microorganisms must be greatly expanded to improve these processes.

This work evaluated the effect of substrate moisture content on the production of conidia by *T. harzianum*, aiming to maximize efficiency and scale production. Optimized conidia production is requisite to produce new biofungicides, their registration and release in the Brazilian market.

2 MATERIAL & METHODS

The work was done at the Agricultural Microbiology Laboratory at Embrapa Arroz e Feijão. Two isolates of *T. harzianum*, BRM 29675 and BRM 29274, were used for evaluation. These isolates have fungicide and nematicide action and are part of this institution's Collection of Multifunctional and Phytopathogenic Microorganisms.

Solid-state fermentation was carried out according to the methodology described below to produce *Trichoderma* conidia by fermentation in rice grains. In 250 mL Erlenmeyer flasks, 20 g of parboiled rice (12% initial moisture) was added. The treatments consisted of adding 10, 15, 20, 25, 30 and 35 mL of distilled water to form the fungus colonization substrate, respectively equivalent to 36.3, 46.1, 53.3, 58.8, 63.1 and 66.7% humidity, respectively.

Then, the vials were autoclaved at 121 ºC for 40 min. After cooling at room temperature, each flask was inoculated with 100 µl of the suspension adjusted to 1.0×10^6 conidia mL^{-1}, suspended in 0.9% saline solution with Tween® 80 (NaCl P.A.: 9.0 g; distilled water: 1,000 mL; Tween 80: 1 mL). The bottles were incubated at 25 ± 1 ºC in continuous light for five and seven days until the evaluation. They were manually shaken every two days to break down fungal mycelia and promote aeration.

The experiment was evaluated after five and seven days of incubation. 50 mL of saline solution with Tween was added to each Erlenmeyer flask to promote washing and detachment of conidia from the rice grains. The suspension was then filtered through a double layer of fabric into 50 mL Falcon tubes. Spore counting and quantification were done using a Neubauer chamber under an optical microscope at 1000×.

The experimental design used was completely randomized with three replications in a 2 × 5 × 2 factorial scheme, with the factors being two *Trichoderma* isolates, five volumes of water and two incubation times. Each treatment had three replications. The data were subjected to ANOVA and regression analysis using the R software (version 4.3.1), with mean comparisons using the Tukey test ($p \leq 0.05$). The normality and homoscedasticity of the data were verified using the Shapiro-Wilk and Levene tests. The regression models were evaluated based on significance at $p<0.05$ and the coefficient of determination (R^2).

3 RESULTS & DISCUSSION

The treatments produced high concentrations of *T. harzianum* conidia, compatible with those found in fermentative processes in rice grains (Muniz et al., 2018). Figure 1 shows that isolate BRM 29274 was the most efficient in producing conidia in five of the six humidity levels, producing 1.2×10^9 conidia mL^{-1} after seven days of incubation. The isolate BRM 29675 was more efficient in low substrate humidity conditions, producing 2.8×10^8 conidia mL^{-1}. The highest productivity was observed in both isolates in treatments with 53.3 and 63.1% substrate moisture, respectively.

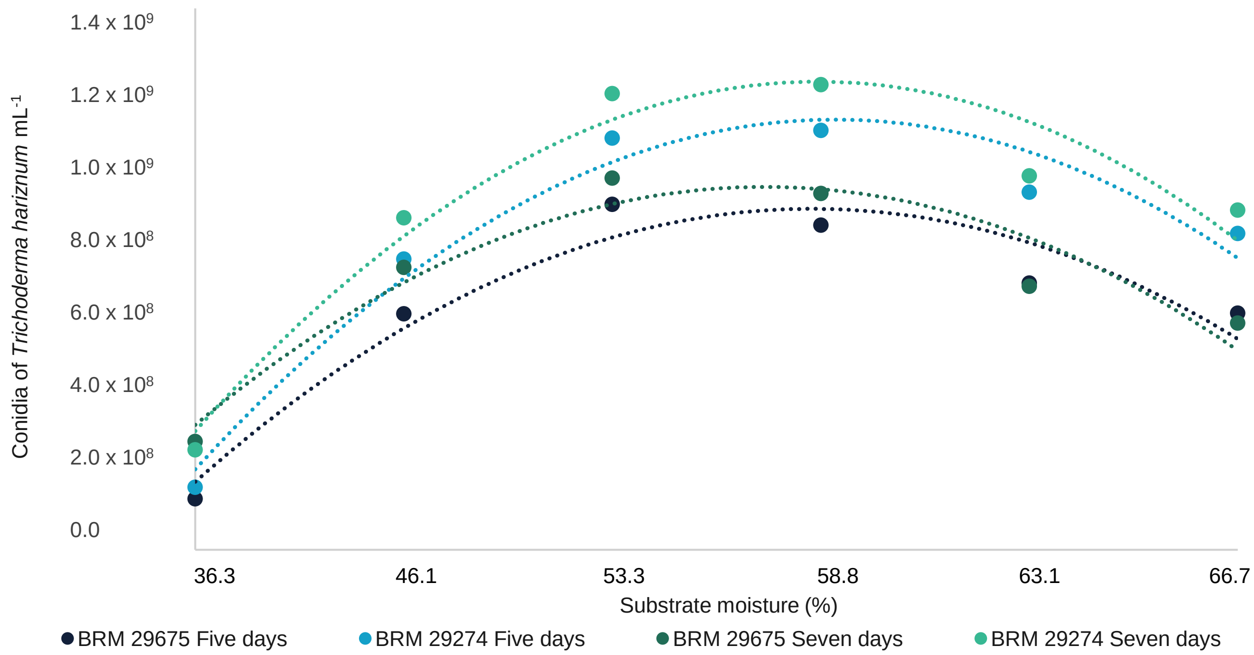

Figure 1 Production of conidia by *Trichoderma harzianum* (BRM 29675 and BRM 29274) as a function of substrate moisture (autoclaved parboiled rice) and days of cultivation.

ANOVA demonstrated significant differences between two of the three-factor levels studied (isolate and humidity) concerning the dependent variable (conidial production). The *T. harzianum* isolates differed from each other (p=0.003), and the moisture concentration of the substrate also substantially affected the results (p<0.001). The normality and homoscedasticity tests demonstrated no differences in variances between the groups for this data set; therefore, analyses were carried out that assumed homogeneity of variance.

In all cases, response curves for conidial productivity were obtained regardless of substrate humidity and incubation time. The linear regression models presented in Figure 1 with respective coefficients of determination were satisfactory, with $R^2 = 0.91$ and $R^2=0.92$ for the isolate BRM 29675 incubated for five and seven days, respectively. For isolate BRM 29274, the coefficients of determination were $R^2 = 0.96$ for five days and R^2 and $R^2 = 0.94$ for seven days.

Solid-state fermentation may be laborious, but it still has advantages over liquid-state fermentation of *Trichoderma* spp. The latter may facilitate automation and cost reduction, but it normally has drawbacks such as spore retrieval and poor storage stability. Therefore, solid-state fermentation is still the most important method to produce conidia, with opportunities for improvement. Our results showed that the industrial yield of *Trichoderma* conidia can be improved with fast-growing isolates and the optimization of the substrate humidity. The decrease from seven to five days of incubation represents a 28% reduction in time to yield conidia and, possibly, 53.3% of parboiled rice grain moisture facilitates the harvest compared to higher substrate humidity.

4 CONCLUSION

The isolate BRM 29274 was the most efficient in producing conidia regardless of substrate humidity. Moisture levels of 53.3% and 63.1% of parboiled rice grains showed the best conidia yields in the five- and seven-day incubation periods, respectively. There was no difference in conidial productivity of the two *T. harzianum* isolates in the incubation periods evaluated.

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ACKNOWLEDGEMENTS

The authors acknowledge EMBRAPA, who funded the research project linked to the present study. We also thank FUNARBE, CNPq, and CAPES for the graduate scholarships for our research team.