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SUBSTRATE WITH LIGNOCELLULOSIC RESIDUES FOR *Pycnoporus sanguineus* CULTIVATION[1]

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ABSTRACT - Basidiomycete fungi that decompose wood produce substances with promising biological activity for the alternative control of plant diseases. The production of these substances can change according to the climatic conditions and the substrate used for fungal cultivation. The objective of this study was to develop a substrate with sawdust from *Eucalyptus* sp. and to verify its influence on biomass and cinnabarin production by *Pycnoporus sanguineus*. Sawdust was used in two particle sizes: less than 500 microns (G1) and between 500–841 microns (G2). Four isolates of *P. sanguineus* were plated on Petri dishes containing potato broth and agar media added with 0%, 1%, 5%, 10%, and 15% sawdust for each particle size. The largest final diameter of the colony and speed of mycelial growth were observed in the substrate with G1 particle size, with the Ps14 isolate showing the highest averages. For these variables, the sawdust concentration did not influence G1 granulometry and provided the highest values in G2 granulometry. Fresh mycelium mass and cinnabarin production showed the highest values in G2, with the isolated Ps13 and Ps08 showing the highest averages, whereas in G1, Ps14 had the best performance for the analyzed variables. These results indicate that sawdust from *Eucalyptus* sp., at concentrations of 10% and 15%, is an alternative for the *in vitro* cultivation of *P. sanguineus*, and that particle size influences the growth speed, fresh mass production, and cinnabarin content.

Keywords: Basidiomycetes. Fungal growth. Cinnabarin.

SUBSTRATO COM RESÍDUOS LIGNOCELULÓSICOS PARA CULTIVO DE *Pycnoporus sanguineus*

RESUMO - Fungos basidiomicetos decompositores de madeira produzem substâncias com atividade biológica promissora para o controle alternativo de doenças de plantas. A produção dessas substâncias é influenciada pela condição climática e substrato utilizado para cultivo do fungo. Dessa forma, objetivou-se desenvolver um substrato com serragem de *Eucalyptus* sp. para verificar a influência na produção de biomassa e cinabarina por *Pycnoporus sanguineus*. A serragem foi utilizada em duas granulometrias, inferior a 500 micra (G1) e entre 500 a 841 micra (G2). Quatro isolados de *P. sanguineus* foram repicados em placas contendo o meio de cultivo caldo de batata e ágar adicionados com zero, 1%, 5%, 10% e 15% de serragem para cada granulometria. O maior diâmetro final da colônia e velocidade de crescimento micelial foi observado no substrato com granulometria G1, sendo o isolado Ps14 com as maiores médias. A concentração de serragem, para essas variáveis, não influenciou na granulometria G1 e proporcionou maiores valores na granulometria G2. Massa fresca do micélio e produção de cinabarina apresentaram os maiores valores em G2, sendo dos isolados Ps13 e Ps08 as maiores médias, já em G1, Ps14 apresentou o melhor desempenho nas variáveis analisadas. A serragem de *Eucalyptus* sp., nas concentrações de 10% e 15% é uma alternativa para cultivo *in vitro* de *P. sanguineus*, e a granulometria influencia na velocidade de crescimento, produção de massa fresca e teor de cinabarina.

Palavras -chave: Basidiomiceto. Crescimento fúngico.Cinabarina.

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INTRODUCTION

Pycnoporus sanguineus is a fungus of the phylum Basidiomycota, usually found on dead trees in mild climate regions or in tropical forests; its basidiocarp is orange-colored to red, and forms horizontally on the stems of trees (TÉLLEZ-TÉLLEZ et al., 2016).

This fungus has attracted increasing biotechnological interest because of its ability to produce compounds with biological activity, such as cinnabarin and hydrolytic enzymes such as laccase, which have applications in the decomposition of agro-industrial waste and in nutrient cycling (SILVA et al., 2010). Further, the secondary metabolites produced by *P. sanguineus* have antiviral, antioxidant, antifungal, antibacterial, and antiparasitic activities and several applications (PINEDA-INSUASTI et al., 2017).

Cinnabarin is an orange pigment known for its antimicrobial action (BALDO et al., 2011). Therefore, it has the potential to be used in agriculture for the control of infectious diseases. Studies have shown the use of mycelium, basidiocarp, and extracts filtered from the culture medium of *P. sanguineus* to control fungal and bacterial diseases through their direct action against pathogenic microorganisms or by inducing plant defense responses, such as the synthesis of reactive oxygen species, plant defense enzymes, and phytoalexins (TOILLIER et al., 2010; VIECELLI et al., 2010; ARRUDA et al., 2012).

The easy cultivation, availability, and economical production of the secondary metabolites from basidiomycetes make them promising for the control of plant diseases (SIVANANDHAN et al., 2017). Some of these factors may influence mycelial growth and the production of enzymes, polysaccharides, and bioactive compounds by these fungi. Among these, the fungal species and factors related to the cultivation such as nutritional content, source and amount of carbon and nitrogen, and presence of lignocellulosic material can be mentioned (ELISASHVILI; KACHLISHVILI; PENNINCKX, 2008; D'AGOSTINI et al., 2011; LEVIN; MELIGNANI; RAMOS, 2010; LEE; CHAO; LU, 2012).

Residues of plant or agroindustrial origin contain lignin and cellulose, which are used as substrates for the cultivating basidiomycetes (VALENCIA; CHAMBERGO, 2013). Cultivation of *P. sanguineus* in substrates based on sawdust of pine, eucalyptus, and rice husk was more efficient in the synthesis of cinnabarin compared to its cultivation in dextrose potato broth culture medium (BAUMER; MORGADO; FURIGO JÚNIOR, 2013).

The objective of this study was to develop a substrate with *Eucalyptus* sp. sawdust using different particle sizes and concentrations for *in vitro* cultivation of *P. sanguineus*, as well as to verify its influence on the fungal growth speed and on the production of mycelial biomass and cinnabarin.

MATERIAL AND METHODS

Production of *P. sanguineus* inoculum

The *P. sanguineus* inoculum was grown in solid PBA medium (200 mL of potato broth and 15 g of agar), in duplicate, for seven days in a biochemical oxygen demand incubator (BOD) at 25 ± 2 °C (SMÂNIA et al., 1997), and then transferred to Petri dishes containing the same medium for multiplication. The isolates Ps04, Ps08, Ps13, and Ps14 (Register SISGEN n° AF83853), were obtained from the Mycotheca of the Phytopathology Laboratory of Unioeste and were collected from the municipalities of Cascavel (Ps04 and Ps14) and Marechal Cândido Rondon (Ps08 and Ps13), both in Paraná State, Brazil; the isolates were morphologically identified as described by Nobles and Frew (1962).

Obtaining sawdust

Eucalyptus sp. sawdust was used as the substrate after drying in sunlight for 2 h, and then separating into two granule sizes using sieves of 32 Mesh and 20 Mesh, thus obtaining granulometry less than 500 microns (G1) and–500–851 microns (G2), respectively. Chemical analysis and carbon/nitrogen ratio determination were performed for both granulometries. The total organic carbon content was determined using the Nelson and Sommers (1982) method. The total nitrogen content was determined using the Kjeldahl method, as described by Tedesco, Volkweiss and Bohnen (1995). Reducing sugars were determined as described by Lever (1972), after adding sawdust at concentrations of 0.1%, 5%, 10%, and 15% in 10 mL of potato broth and autoclaving at 120 °C and 1 atm for 20 min.

In vitro assay

The experimental design was completely randomized, with a 4 × 2 × 5 triple factorial arrangement with six replicates. Four isolates of *P. sanguineus*, two granulometries (G1 and G2), and five concentrations of *Eucalyptus* sp. sawdust, totaling 240 samples were analyzed.

The isolates Ps04, Ps08, Ps13, and Ps14 were cultivated in PBA culture medium in Petri dishes of 90 mm diameter, with sawdust added to two granulometries (G1 and G2), separately, at concentrations of 0%, 1%, 5%, 10%, and 15%. In the center of the plate, a 0.5 cm disc containing the mycelium of *P. sanguineus* was removed from the colony edges after 14 days of cultivation in PBA medium. The Petri dishes were kept at 25 ± 2 °C in

the dark. The colony diameter was evaluated at 24 hour intervals. Fresh mycelial mass and cinnabarin pigment production were also evaluated.

Mycelial growth was evaluated by measuring the colony diameter through two transverse lines drawn outward on the plates (HENDGES et al., 2021). The 0.5 cm disc was deposited at the center of the plate, and over the PBA medium, at the intersection of the lines. Measurements were performed daily until some of the isolates reached the edge of the Petri dish. With averages obtained from colony growth every 24 h, the growth velocity was calculated using the formula described by Oliveira (1991):

$$MGSI = \Sigma \frac{(D - Da)}{N}$$

where MGSR = Mycelial growth speed index;

D = current average colony diameter;

Da = average diameter of the previous day's colony;

N = number of days after peaking.

The final diameter and growth speed data were adjusted to a second-degree polynomial regression model.

The fresh mass was determined by marking a circle with an area of 19.64 cm² in the center of the Petri dish, followed by scraping the aerial mycelium with a Drigalski handle and then weighing on an analytical scale. After weighing, the mass collected from the mycelium was packed in test tubes with 3 mL of methanol and stored for 72 h at 4 ± 2 °C. The cinnabarin content was quantified using spectrophotometry at 258 nm. The absorbance results were transformed according to the equation y = 0.7018x – 0.0077 (BAUMER; MORGADO; FURIGO JÚNIOR, 2013), where "x" represents the absorbance obtained from the spectrophotometer, whereas "y" represents the cinnabarin content.

The data of fresh mass of mycelium and cinnabarin content were transformed using the equation √x+1. They were then subjected to analysis of variance (ANOVA) and the means were compared using Tukey's test and regression analysis, both with levels of 5% significance, using Sisvar software (FERREIRA, 2011).

RESULTS AND DISCUSSION

The carbon/nitrogen ratio of *Eucalyptus* sp. sawdust was 262:1 for granulometries less than 500 microns (G1) and 245:1 for granulometries between 500–841 microns (G2) (Table 1). The values of reducing sugars ranged from 0.67–0.9 µg mL^{-1} for the two sawdust granulometries and was similar for all sawdust concentrations (Figure 1). Thus, it was not possible to correlate the content of reducing sugars found in the substrate with differences in the mycelial development of *P. sanguineus* isolates.

Table 1. Chemical analysis of the *Eucalyptus* sp. sawdust used for *Pycnoporus sanguineus* cultivation.

Granulometries (microns)	N[1]	P	K	Cu	Zn	Mn	Fe	C[2]	Ratio C/N
	g kg^{-1}			mg kg^{-1}				%	
< 500	1.75	0.16	1.05	5.00	6.00	82.00	911.00	45.88	262/1
500-841	1.75	0.09	0.90	2.00	5.00	66.00	690.00	42.88	245/1

[1]Total nitrogen. [2] Organic carbon.

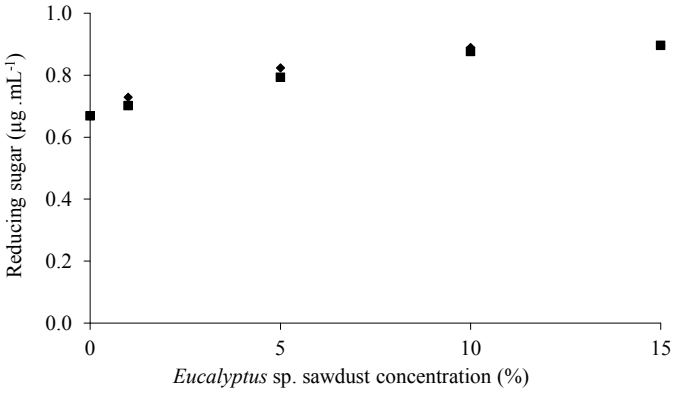

Figure 1. Reducing sugar content (µg mL^{-1}) in potato broth with *Eucalyptus* sp. sawdust. Values were transformed using the equation: y = 0.0306 + 0.0244x. (♦) refers to granulometry less than 500 microns; (■) refers to granulometry between 500–841 microns.

The colony diameter was evaluated for eight days. Upon *in vitro* cultivation, the colony of *P. sanguineus* in PBA medium containing sawdust of *Eucalyptus* sp. with granulometry less than 500 microns (G1) presented a significantly higher final diameter compared to that grown on medium containing sawdust with a granulometry between 500 –841 micra (G2), for all isolates.

Considering the mean of both granulometries, the isolate Ps14 showed higher growth compared to the other isolates, with a diameter of 3.41 cm, whereas the isolate Ps04 presented the smallest growth, with a diameter of 2.97 cm (Table 2). In medium containing sawdust with granulometry between 500–841 microns, mycelial growth was decreased, and though an increase was observed in the final diameter at concentrations of 10% and 15%, but this was still significantly lower than that observed in G1 (Figure 2).

Table 2. Final diameter (cm) of the colonies of *Pycnoporus sanguineus* isolates cultivated on different sized granules of *Eucalyptus* sp. sawdust added to the culture medium.

P. sanguineus isolates	Final diameter (cm)					
	Granulometries					
	< 500 microns		500 - 841 microns		Means	
04	3.58	c A	2.35	b B	2.97	c
08	4.09	a A	2.10	c B	3.10	b
13	3.77	b A	2.38	b B	3.07	bc
14	4.22	a A	2.59	a B	3.41	a
Means	3.91	A	2.36	AB		
	CV (%) 8.21					

Different lowercase letters in the columns and uppercase letters in the rows indicate significant differences among the means, by Tukey's test at $p < 0.05$. CV = coefficient of variation.

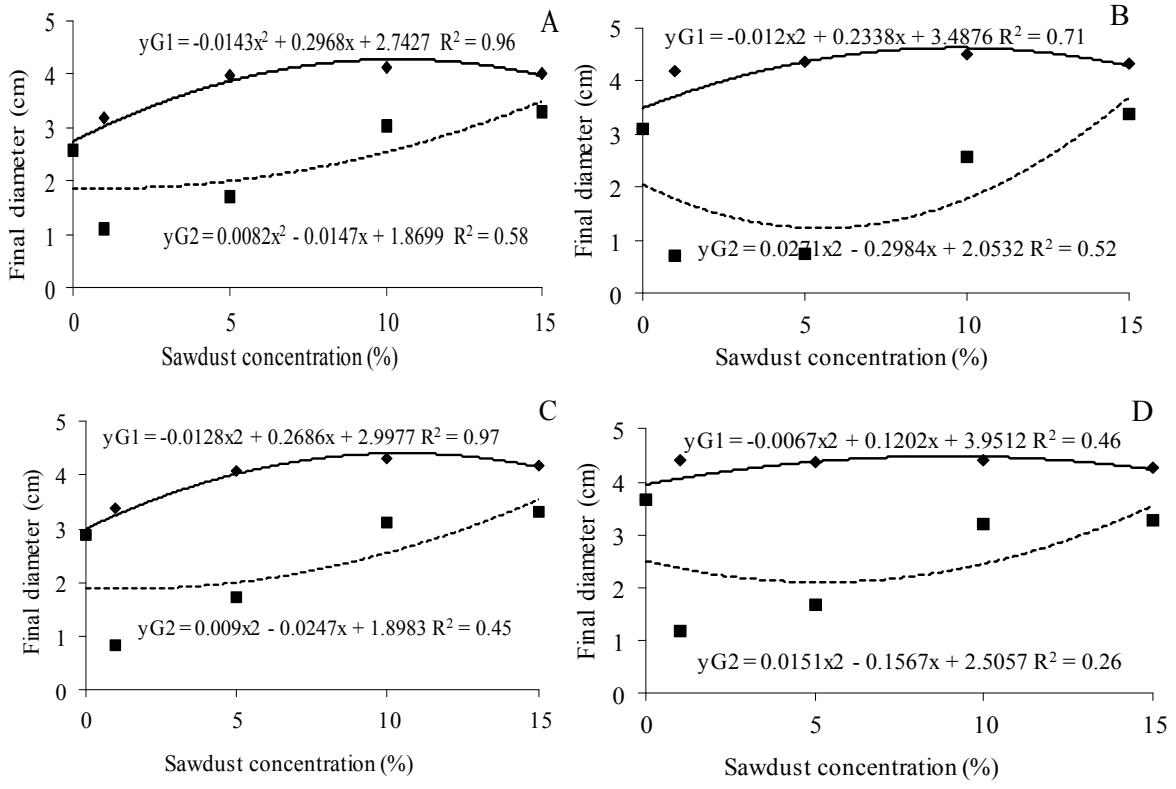

Figure 2. Final diameters of *Pycnoporus sanguineus* colonies (A: Ps04; B: Ps08; C: Ps13; D: Ps14) in relation to the sawdust concentration of *Eucalyptus* sp. added to the culture medium. (♦) with the continuous line and equation yg1 refers to granulometry less than 500 microns; (■) with the dashed line and equation yg2 refers to granulometry between 500–841 microns.

In this study, we observed that the C/N ratio varied between the granulometries. The production of enzymes by white rot fungi, such as *P. sanguineus*, is dependent on the strain and conditions of the culture medium, and the carbon/ nitrogen ratio is one of the most important nutritional factors (VANCE; CHAPIN, 2001; LEVIN; MELIGNANI; RAMOS, 2010). D'Agostini et al. (2011) showed that the C/N ratio influenced laccase production, and that the lowest ratio of 5 showed higher enzyme production compared to that with the ratios of 10 to 30, by the fungi *Pleurotus ostreatus*, *Lentinula edodes*, and *Agaricus blazei*.

The nitrogen source can influence enzyme production, whereas the source and amount of carbon influence colony growth and enzyme production (LEVIN; MELIGNANI; RAMOS, 2010; D'AGOSTINI et al., 2011; RAJPUT; KHANZADA; SHAHZAD, 2014). In this study, the substrate with the highest carbon source showed a higher colony diameter and growth rate of *P. sanguineus*. The mycelial growth of white-rot fungi *P. ostreatus*, *L. edodes*, and *A. blazei* was increased according to the highest C/N ratio, that is, colony growth was directly proportional to the C/N ratio (D'AGOSTINI et al., 2011).

The final diameter of *P. sanguineus* colonies was measured until the 8th day of incubation, when the first colony reached the edge of the plate. This growth period corroborates the results of Wille (2007), who observed the growth of *P. sanguineus* in a culture medium based on *Eucalyptus grandis* and *Acacia meansii*. Negrão et al. (2014) found variation in the mycelial growth of *P. sanguineus* in cultivation medium enriched with sawdust according to the environmental temperature, reaching maximum colony growth in 6, 4, and 4 days, at temperatures of 23, 27, and 31 °C, respectively.

The average mycelium growth rate of *P. sanguineus* showed differences between the sawdust granulometries. The growth rate in granulometry less than 500 microns was significantly higher than that in granulometry between 500–841 micra for all isolates. In relation to the isolates, in granulometry less than 500 microns, Ps14 and Ps08 showed the highest values with growth speeds of 5.09 mm day^{-1} and 4.83 mm day^{-1}, respectively, whereas in the granulometry between 500–841 micra, the isolate Ps14 differed from the others with a growth speed of 3.26 mm day^{-1} (Table 3).

Table 3. Growth speed of *Pycnoporus sanguineus* isolates cultivated on culture medium with different granulometries of *Eucalyptus* sp. sawdust added.

P. sanguineus isolates	Growth speed (mm day^{-1})					
	Granulometries					
	< 500 microns		500 - 841 microns		Means	
04	4.06	c A	2.86	b B	3.46	c
08	4.83	a A	3.00	b B	3.91	b
13	4.20	b A	2.91	b B	3.56	c
14	5.09	a A	3.26	a B	4.17	a
Means	4.54	A	3.01	AB		
	CV (%) 10.38					

Different lowercase letters in the columns and uppercase letters in the rows indicate significant differences among the means, by Tukey's test at $p < 0.05$. CV = coefficient of variation.

The variable growth speed demonstrated a quadratic adjustment of the curves for the sawdust concentrations added to the culture medium. The highest values were observed for G1 granulometry; however, in both granulometries, the concentrations of 10% and 15% showed the highest values. The growth speed of the mycelia varied among the isolates. The isolate Ps08 ranged from 1.86 mm day^{-1} (1% of sawdust) to 5.57 mm day^{-1} (15% of sawdust) (Figure 3).

The growth rate is closely linked with several factors such as pH, temperature, luminosity, carbon/ nitrogen ratio, and nutrient availability (ALEXOPOULOS; MIMS; BLACKWELL, 1996). This variable is measured by the diameter of the colony on the plate; however, growth is also related to the mycelial density. The rapid expansion of a thin mycelium may be related to poorer culture medium (LEVASSEUR et al., 2014). Silva et al. (2010) observed an average growth speed of 12.8 mm day^{-1} for *P. sanguineus* in culture medium with eucalyptus sawdust of 0.42 cm granulometry, that is, 67% higher than that observed for the Ps14 isolate in this study at (4.17 mm day^{-1}. Different granulometries seemed to influence the growth speed of the fungus.

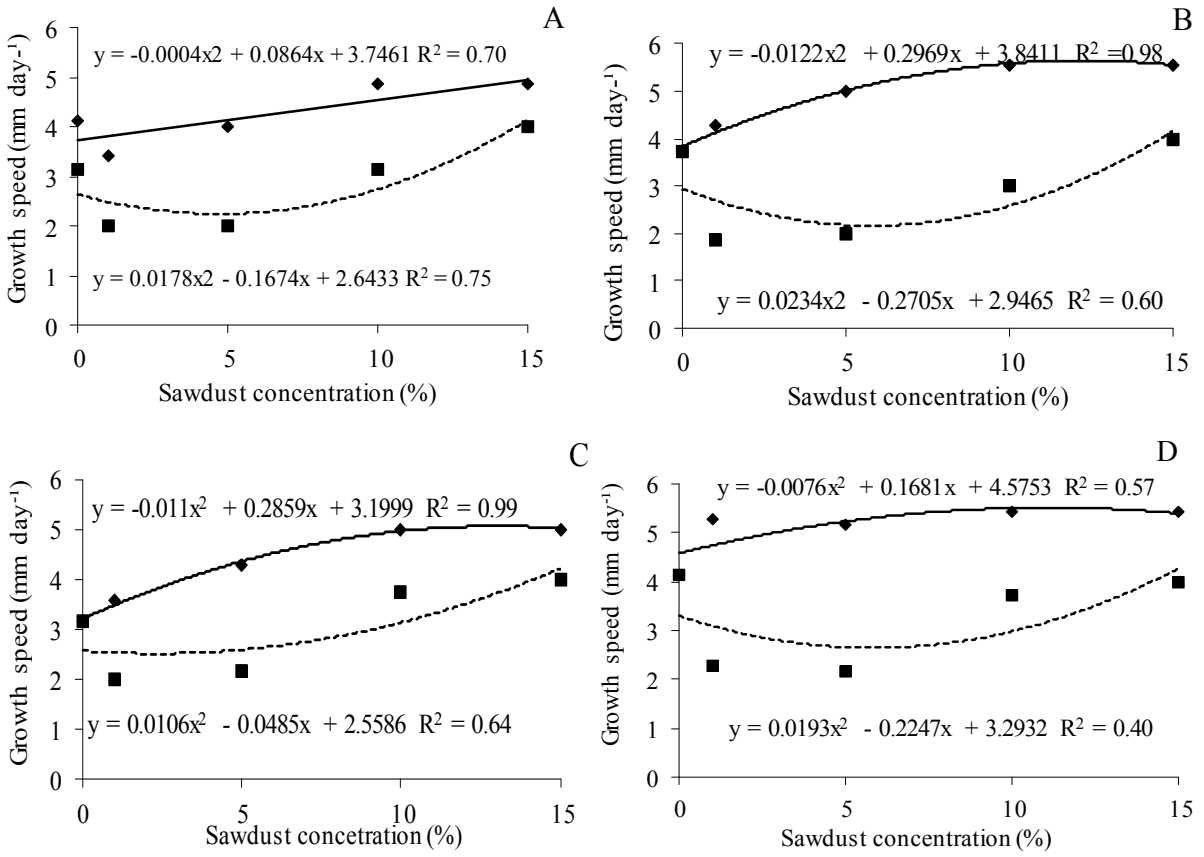

Figure 3. Growth speed of *Pycnoporus sanguineus* isolates (A: Ps04; B: Ps08; C: Ps13; D: Ps14) in relation to the sawdust concentration of *Eucalyptus* sp. added to the culture medium. (♦) with the continuous line and equation yg1 refers to granulometry less than 500 microns; (■) with the dashed line and equation yg2 refers to the granulometry between 500–841 microns.

In this study, the fastest fungal expansion occurred in the granulometry below 500 microns; however, the highest fresh mass was observed in the case of fungal growth in medium with sawdust of granulometry between 500–841 microns. According to Marino (2007), growth speed is inversely correlated with mycelial density. Dense mycelium may be formed due to the release of substances that stimulate mycelial growth (GUTIERREZ et al., 1995). The authors reported that during the growth phase of *Pycnoporus cinnabarinus*, it released polysaccharides related to increased mycelial density, contributing to the resistance of the fungus to stresses in field conditions.

The fresh mycelial mass of *P. sanguineus* was higher in medium containing sawdust with granulometry between 500–841 microns, with 2.24 mg cm^{-2}, compared to that in medium containing sawdust with granulometry below 500 microns, at 1.75 mg cm^{-2}. In the granulometry below 500 microns, Ps13 presented the highest mean, differentiating from Ps04 and Ps14, whereas in the granulometry from 500–841 microns, Ps08 and Ps13 had the highest fresh mass and were differentiated from the others. The average fresh mass of Ps08 and Ps13 isolates was higher than that of the others at 2.21 mg cm^{-2} and 2.37 mg cm^{-2}, respectively (Table 4). Regarding sawdust concentrations, the mycelial fresh mass in granulometry less than 500 microns did not differ statistically among the fungal isolates, based on the t-test ($p < 0.05$). With the granulometry between 500–841 microns, there was a continuous increase in the concentrations of 5%, 10%, and 15%, and the isolate Ps08 showed a higher fresh mass of mycelium among the isolates (Figure 4). The mycelial mass exhibited quadratic adjustment of the regression curve. The minimum coefficient of determination (R^2) of the samples was 0.931, with a satisfactory adjustment of the data obtained in the assay.

Table 4. The fresh mass of mycelium of *Pycnoporus sanguineus* isolates (A: Ps04; B: Ps08; C: Ps13; D: Ps14) cultivated on different granulometry of *Eucalyptus* sp. sawdust added to the culture medium. (♦) with the continuous line and equation yg1 refers to granulometry less than 500 microns; (■) with the dashed line and equation yg2 refers to the granulometry between 500-841 microns.

P. sanguineus isolates	Fresh mass of mycelium (mg cm^{-2})					
	Granulometries					
	< 500 microns		500 - 841 microns		Means	
04	1.51	c B	1.86	bcA	1.68	b
08	1.84	abB	2.58	abA	2.21	a
13	2.15	abB	2.59	acA	2.37	a
14	1.52	bcB	1.94	bcA	1.73	b
Means	1.75	abB	2.24	a A		
CV (%) 24.44						

Different lowercase letters in the columns and uppercase letters in the rows indicate significant differences among the means, by Tukey's test at $p < 0.05$. CV = coefficient of variation.

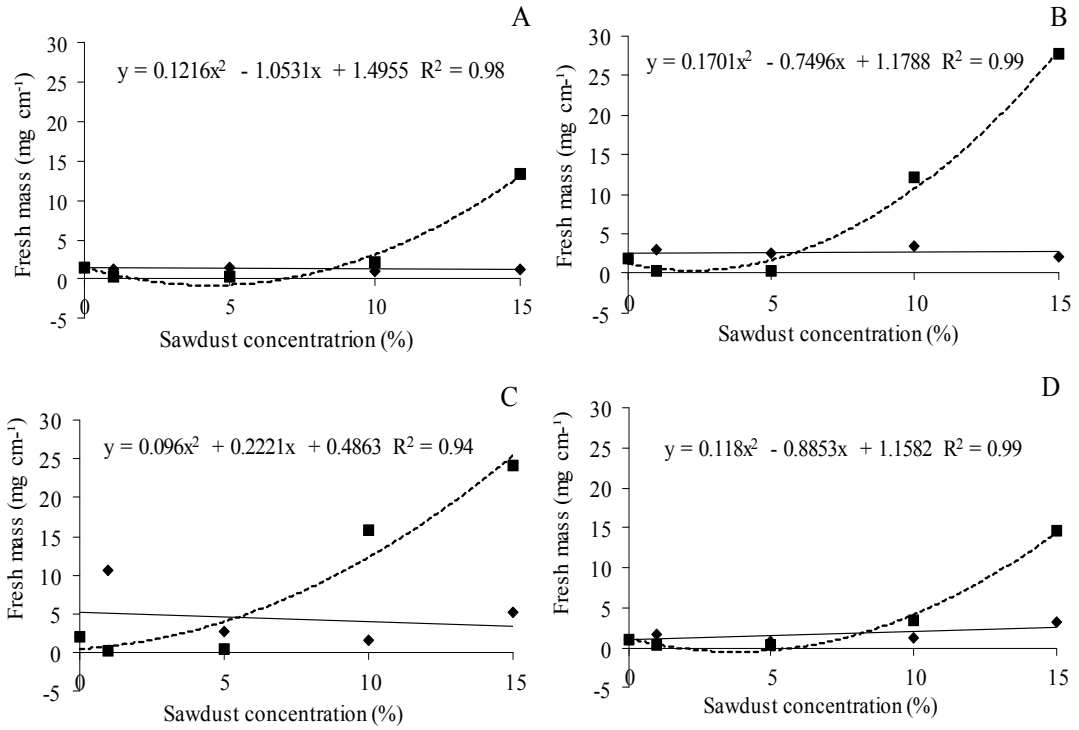

Figure 4. Fresh mass of the mycelium of *Pycnoporus sanguineus* isolates (A: Ps04; B: Ps08; C: Ps13; D: Ps14) in relation to the concentration of *Eucalyptus* sp. sawdust added to the culture medium.

The structure of the culture medium, with smaller sawdust particles, provided the fungus with greater expansion capacity, whereas the larger particles allowed the formation of a greater mycelial mass of *P. sanguineus*. The ability of the fungus to colonize and produce basidiocarps in lignocellulosic substrates is related to the mycelial vigor and the ability to activate physiological mechanisms necessary for the use of nutrients in the culture medium (MATA; DELPECH; SAVOIC, 2001). In this study, the nutritional and environmental factors necessary for developing *P. sanguineus* basidiocarps were not evaluated; therefore, it was not possible to affirm that granulometry less than 500 microns is the

most appropriate for formulating substrates based on sawdust for cultivating this fungus.

In this study, larger granulometry resulted in increased mycelial mass and higher cinnabarin content. The opposite was reported by Baumer, Morgado and Furigo Júnior(2013), who claimed that there is no direct relationship between biomass production and cinnabarin.

The cinnabarin content of *P. sanguineus* isolates presented a significantly higher mean sawdust granulometry between 500–841 microns at 1.32 mg mL^{-1}, compared to 500 microns at 0.83 mg mL^{-1} (Table 5). The isolates Ps13 and Ps14 had the highest averages.

With granulometry less than 500 microns, the isolate Ps14 produced the highest cinnabarin, whereas with granulometry between 500–841 microns, Ps13 showed the highest average cinnabarin, being statistically similar to Ps04 and Ps14, and different from Ps08. Regarding sawdust concentrations, cinnabarin content showed a difference only for granulometry between 500–841 microns and dose-dependent behavior, promoting increases in values from the 5% concentration. There was a variation in values according to the isolates, with a maximum value of up to 3.82 mg mL^{-1} for Ps13 (Figure 5).

Table 5. Cinnabarin content produced by *Pycnoporus sanguineus* isolates grown on *Eucalyptus* sp. sawdust granulometry added to the culture medium.

P. sanguineus isolates	Cinnabarin content (mg mL^{-1})				
	Granulometries				
	< 500 microns		500 - 841 microns		Means
04	0.77	b B	1.11	ab A	0.94
08	0.54	b A	0.84	b A	0.69
13	0.51	b B	1.94	a A	1.23
14	1.49	a A	1.38	ab B	1.44
Means	0.83	B	1.32	A	
	CV (%) 13.67				

Different lowercase letters in the columns and uppercase letters in the rows indicate significant differences among the means, by Tukey's test at $p < 0.05$. CV = coefficient of variation.

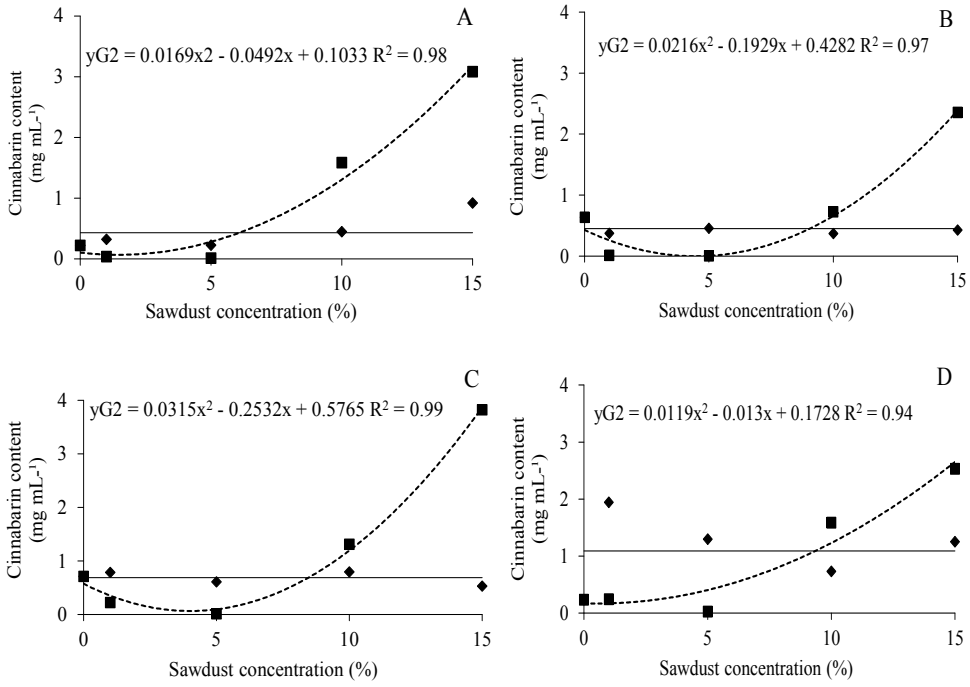

Figure 5. Cinnabarin content of *Pycnoporus sanguineus* isolates (A: Ps04; B: Ps08; C: Ps13; D: Ps14) cultivated on different granulometries of *Eucalyptus* sp. sawdust added to the culture medium. (♦) with the continuous line and equation yg1 refers to granulometry less than 500 microns; (■) with the dashed line and equation yg2 refers to the granulometry between 500–841 microns.

Fungi decompose organic waste using enzymes to extract nutrients for growth and development (SHARMA; JAITLY, 2017). *P. sanguineus* produces ligninolytic enzymes such as laccase, and its production depends on carbon and nitrogen sources, as well as on the concentration of nitrogen in the culture medium (EUGENIO et al., 2009). Some authors have indicated that a low concentration of nitrogen in the growth substrate is important for laccase production by white rot fungi (POINTING; JONES; VRIJMOED, 2000; ELISASHVILI; KACHLISHVILI; PENNINCKX, 2008; D'AGOSTINI et al., 2011).

The low nitrogen concentration in the substrate is a prerequisite for laccase production and lignin degradation, which is considered a critical element for microorganisms, as it participates in the biosynthesis of cellular components essential for the development of microorganisms such as proteins, enzymes, and nucleic acids (GIANFREDA; XU; BOLLAG, 1999; KUYPERS; MARCHANT; KARTAL, 2018). Soluble components may be related to higher mycelial growth rate in granulometry less than 500 microns; however, enzymatic studies are needed to prove this possibility.

The potential of white rot fungi to produce enzymes such as cellulase, xylanase, laccase, and manganese peroxidase is attributed to the fungal species, lignocellulosic substrate, and cultivation method (ELISASHVILI; KACHLISHVILI; PENNINCKX, 2008). The C/N ratios found in *Eucalyptus* sp. sawdust in this assay were higher than those found by Abreu et al. (2007), which had a ratio of 103:1, in study of the degradation of *Eucalyptus* sp. wood by white rot basidiomycetes.

Fungi of the genus *Pycnoporus* are capable of producing secondary metabolites such as the pigment cinnabarin, lignolytic enzymes, and polysaccharides, which have biotechnological importance in the degradation of industrial waste, control of pathogens in agriculture, production of enzymes, and pharmaceutical compounds (LEE; CHAO; LU, 2012; PINEDA-INSUASTI et al., 2017).

The production of secondary metabolites is influenced by the sources of carbon and nitrogen (EUGENIO et al., 2009). High N values can stimulate mycelial growth, but decrease the synthesis of enzymes (D'AGOSTINI et al., 2011). Therefore, the highest synthesis of cinnabarin in the 500–841 micron group can be justified by the carbon/nitrogen ratio being lower than that with the granulometry of less than 500 microns. However, nutritional factors, which were not evaluated here, may be more involved in the synthesis of cinnabarin compared to physical factors, providing as a hypothesis for future studies.

CONCLUSIONS

Eucalyptus sp. sawdust can be used in the *in vitro* cultivation of *P. sanguineus* for the production of compounds with biological activity such as cinnabarin. The granulometry and concentration of sawdust influenced the parameters of growth and synthesis of substances. Thus, the genetic characteristics of the isolates influenced the growth and production of cinnabarin.

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