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EFFECT OF CAFFEINE AND TANNINS ON CULTIVATION AND FRUCTIFICATION OF *PLEUROTUS* ON COFFEE HUSKS

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ABSTRACT

The objective of this work was to study the effect of caffeine and tannins on *Pleurotus* sp. cultivation and to evaluate the feasibility of using coffee husks as substrate for mushroom cultivation. Eight strains of *P. ostreatus* and two strains of *P. sajor-caju* were screened on a medium prepared from agar extract of coffee husk. Based on best mycelial growth and biomass production, the strain *P. ostreatus* LPB 09 was selected for detailed studies. With the increase of caffeine concentration, the mycelial growth and the biomass production decreased, and no growth was observed when concentration of caffeine was 2500 mg/L. Furthermore, *Pleurotus* did not degrade the caffeine, but absorbed it. Tannin under 100 mg/L in the medium stimulated the growth of mycelia, but above 500 mg/L it had a negative effect. When the concentration reached 1000 mg/L, the fungus still survived and showed a certain tolerance to it. No tannic acid was found in the mycelia, but its concentration decreased in the medium. This fact confirmed that *Pleurotus* had the capacity of degrading tannic acid. Fructification occurred after 20 days of inoculation and the biological efficiency reached about 97% after 60 days. Caffeine content in the husk after cultivation was reduced to 60.7% and tannins to 79.2%. The results indicated the feasibility of using coffee husk without any pretreatment for the cultivation of *Pleurotus*.

Key words: cultivation, *Pleurotus*, caffeine, tannin, detoxification

INTRODUCTION

Coffee is one of the most important beverages of the world, and its yearly production is about one million tons in more than 50 countries (13,21). Brazil is the largest producer of coffee in the world (13). In Brazil, coffee cherries are generally processed using the dry method, resulting in coffee husk, which is different from coffee pulp in composition of caffeine, tannin and polyphenois (8,9,28). The caffeine and tannin content was about 1.3 and 10%, respectively, in the coffee husk on dry weight basis (8,9,21,28). Due to the presence of these toxic compounds, this organic solid residue has lead to the problem of environmental pollution (5,8,9,21,28).

The edible mushroom *Pleurotus* sp. is considered a good alternative for protein rich food production in tropical countries (7,16). *Pleurotus* also shows good ability in producing fruiting body and simultaneously reducing or degrading toxic substances present in the substrate (7,11,29). Bermudez (3), Bernabe (4), Galzada (10), Lozano (14), Martinez (17,18), Mata (19), Soto (25,26) and Tango (26) studied the production of *Pleurotus* with coffee pulp, obtaining good results. Barbosa (1) and Maziero (20) used coffee husk for the production of *Pleurotus*, but had no success. It was reported that mycelia were initially strong and vigorous, but after some days the growth was interrupted. Apparently, the concentration of toxic compounds was more concentrated in the coffee husk than

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that in the coffee pulp. Beaux and Soccol (2) utilised the coffee husk for growing *Lentinus* and reported poor mycelial growth in comparison to other substrates due to its high concentration of toxic compounds.

Tan (27) studied the effect of tannic acid (concentrations 500 to 1000 mg/L) and caffeine (50 to 100 mg/L) on the growth of *L. edodes*. The results demonstrated that both of them, under experimental concentrations, exercised a toxic effect on the growth of the mushroom *L. edodes*. Wong (30) demonstrated that the mushroom *P. sajor-caju* was capable of degrading tannin in coffee spent ground. This work was attempted to evaluate the effect of caffeine and tannin on *Pleurotus* and evaluate the feasibility of using coffee husk as substrate to cultivate edible mushroom *Pleurotus* and possibility of degrading its toxic compounds.

MATERIALS AND METHODS

Microorganisms and screening

Eight strains of *Pleurotus ostreatus*, LPB 01, 03, 08, 09, 22, 23, 24, 31 and two *Pleurotus sajor-caju*, LPB 19 and 20 were used for screening purpose. The strains were maintained on potato-dextrose-agar (PDA) at 4ºC.

Radial growth

All the strains were grown in a medium containing the extract of coffee husk, which was prepared by cooking the coffee husks (40g.L^{-1}) in distilled water for one hour, filtering and completing the volume to 1 litter with distilled water. Its pH was adjusted to 7, and after mixing with Agar (2%), the medium was autoclaved at 121ºC for 15 minutes. 15 mL of the medium were distributed in Petri dishes (9 cm in diameter). The plate was inoculated with the fungal cultures as described by Soccol (24). The growth of mycelia was measured according to Soccol (24).

Effect of caffeine and tannin

The experiments were conducted in PDA plates with addition of different concentrations of caffeine and tannin using the selected strain. The concentrations of caffeine and tannin tested were: 30, 50, 100, 500, 1000, 2500 mg.L^{-1} and 100, 500, 1000, 5000, 10000 mg.L^{-1}, respectively.

Fructification and detoxification

The moisture content of coffee husk solid medium was adjusted to 55 - 60%, filled in plastic bags (20 x 35 cm in size) and was autoclaved at 121ºC for 1.5 h. The bag was inoculated with the spawn and incubated in the dark at 24ºC. After 15 days, the bag was transferred to a lighted environment chamber (90% relative humidity) and plastic was removed to allow the aeration and lightning in order to facilitate fruiting body development.

Spawn preparation

A mixture of sawdust of *Eucalyptus* sp (80%) and rice bran (20%) was used for spawn preparation. The humidity was adjusted to 60% and then the mixture was filled in a glass jar of 500 mL capacity. After 20 days of incubation, the spawn was ready to be used for the inoculation of the solid substrate when the mixture turned totally to white.

Biomass estimation

Biomass was estimated by drying the biomass and measuring its dry weight according to Soccol (24).

Biological Efficiency

Biological efficiency (BE) is the ratio of the weight of fresh fruiting bodies and the initial weight of dry substrate, multiplied by 100. It indicates the fructification ability of the fungus.

Analysis of caffeine and tannin

The spectrophotometric method, suggested by the manual of the Lutz Adolfo Institute (12), was used to analyse caffeine, using chloroform as solvent. The concentration of tannins was determined according to the method described in the manual of the Ministry of Agriculture (15). The concentration of caffeine and tannin in the mycelia and fruiting body was also analysed.

RESULTS AND DISCUSSION

Selection of strains of *Pleurotus*

Table 1 shows the behaviour of ten strains of *Pleurotus* sp. on the extract of coffee husk in relation to the radial growth of the

Table 1. Growth of *Pleurotus* strains on coffee husk extract medium*.

Strains	Velocity of radial growth of mycelia (mm.day^{-1})	Biomass (mg per dish)
P. ostreatus LPB 09	9.68 ± 1.67	43.40 ± 0.14
P. ostreatus LPB 01	9.50 ± 1.38	40.10 ± 1.84
P. ostreatus LPB 22	9.34 ± 1.53	35.70 ± 0.00
P. ostreatus LPB 08	9.03 ± 2.85	31.90 ± 4.80
P. sajor-caju LPB 20	8.98 ± 2.07	24.50 ± 2.30
P .ostreatus LPB 23	8.95 ± 1.80	25.60 ± 6.00
P .ostreatus LPB 24	8.82 ± 1.25	18.80 ± 0.56
P. ostreatus LPB 31	8.80 ± 1.22	15.80 ± 0.56
P. sajor-caju LPB 19	8.12 ± 1.81	22.30 ± 2.00
P. ostreatus LPB 03	6.55 ± 1.73	14.20 ± 0.35

*Temperature of incubation: 24ºC; initial pH: 7.0., results are the average of three measures.

mycelia and the biomass production for nine days. Although all the strains grew well on this medium, *P. ostreatus* LPB 09 showed the best growth with high-density of mycelia growth, resulting in the highest radial growth velocity of 9.68 mm.day^{-1} and a biomass of 43.4 mg.plate^{-1} in nine days. Table 2 presents the analysis of variance for radial growth velocity. This parameter did not show significant differences for all strains at 95% level. It is possible to say that the daily variation of radial growth was equal to all strains. After the Shapiro Wilk and F test (data not shown), it was possible to verify that the variances were not significantly different at 95% and 99%. Consequently, it is possible to say that there are significant different at level 99% for biomass production (Table 3). The Tukey test showed (Fig. 1) that LPB 09 has significant differences from other strains. Further investigations were then carried out using the strain *P. ostreatus* LPB 09.

Table 2. Analysis of variance of radial growth of *Pleurotus* strains.

Summary growth AV	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Strains	9	21.513	2.390	0.9953	0.9953
Residuals	20	48.031	2.402		

Table 3. Analysis of variance of biomass production of *Pleurotus* strains

Summary growth AV	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Strains	9	27779.68	308.85	42.557	4.218e-11***
Residuals	20	145.15	7.26		

Codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' '.

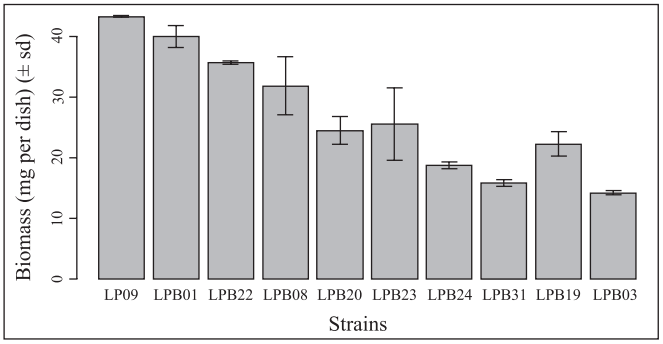

Figure 1. Biomass production of different strains of *Pleurotus*.

Effect of caffeine

Table 4 demonstrates the effect of different concentrations of caffeine on the cultivation of *P. ostreatus* LPB 09 in relation to the mycelial growth and biomass production on PDA after six days of culture. The mycelia growth and the biomass production decreased with the increase of caffeine concentration in the medium. Growth was completely inhibited when the concentration of caffeine in the medium was 2500 mg.L^{-1}. It was also possible to observe that *Pleurotus* degraded caffeine partially. *Pleurotus* was not capable to degrade completely the caffeine, but absorb it partially.

The absorption of caffeine by *Pleurotus* mycelia was not reported in the literature. Tan (27) cultivated *L. edodes* using different concentrations of caffeine in the medium. Caffeine concentrations of 50 to 100 mg.L^{-1} had positive effect on mycelial growth, but a concentration of 300 mg.L^{-1} or above inhibited mycelial growth and fructification.

Effect of tannins

Table 5 shows the effect of different concentrations of tannic acid on the mycelial growth of *P. ostreatus* LPB 09 in PDA after seven days of culture. Above 500 mg.L^{-1} it had a negative effect. When the concentration reached 1000 mg.L^{-1}, the fungus still survived and showed a certain tolerance to it. No tannic acid was found in the mycelia, but decreased in the medium, confirming that the fungus had the capacity of degrading tannic acid.

Wong *et al*. (30) mentioned that the *P. sajor-caju* was capable to degrade the tannins in coffee spent ground. Cai *et al*. (6) observed that the derived monomers of lignin in low concentration (100 mg.L^{-1}) were capable to stimulate the growth of *Pleurotus*. Tan (27) studied the effect of tannic acid (from 500 to 1000 mg.L^{-1}) on the growth of *L. edodes*. The results showed that these concentrations exerted toxic effects.

Table 4. Caffeine effect on the mycelial growth of *P. ostreatus* LPB 09 in PDA after 6 days and its accumulation in the mycelia.

Concentration of caffeine (mg.L^{-1} of medium)	Radial growth of mycelia (mm.day^{-1})	Biomass (mg per dish)	Concentration caffeine in mycelia (mg.g^{-1})	Final concentration (mg.L^{-1})
0	13.12 ± 0.21	55.32 ± 1.43	0	0
30	12.56 ± 0.43	53.44 ± 2.36	0.0657	0
50	12.23 ± 0.26	52.76 ± 1.28	0.1342	4.02
100	11.02 ± 0.32	47.29 ± 1.55	3.7866	58.00
500	8.13 ± 0.42	32.56 ± 2.35	9.1625	399.49
1000	4.52 ± 0.41	12.37 ± 2.44	13.8527	860.02
2500	0	0	/	/

"/": no analysis.

Table 5. Effect of tannic acid on the mycelial growth of *P. ostreatus* LPB 09 in PDA after 7 days and its degradation.

Concentration of tannic acid ($mg.L^{-1}$)	Radial growth of mycelia ($mm.day^{-1}$)	Biomass (mg per dish)	Concentration tannic acid in mycelia ($mg.L^{-1}$)	Final concentration of tannic acid in the medium ($mg.L^{-1}$)
0	11.29 ± 0.11	50.12 ± 1.55	0	0
100	11.76 ± 0.23	52.84 ± 1.76	0	29.63
500	8.71 ± 0.00	43.06 ± 1.21	0	277.09
1000	2.72 ± 0.24	10.20 ± 1.12	0	429.45
5000	1.58 ± 0.34	3.27 ± 1.56	/	/
10000	0.82 ± 0.11	1.33 ± 0.12	/	/

"/": no analysis.

Statistical analyses of the results of caffeine and tannins concentrations effect on *Pleurotus* growth were conducted. However, it was not possible to apply the analysis of variance to the data in order to compare the differences between them. The study was based in progressive concentrations of caffeine and tannins and the results are not correlated with the applied concentrations.

Fructification of Pleurotus on coffee husk

The first fructification started after 20 days of inoculation (5 days after opening of the plastic bag); the second, third, and four flashes were harvested during 60 days of cultivation. The fructification is shown in Fig. 2. The total biological efficiency was 96.5%. Martinez (16) obtained a biological efficiency of 175.8% on drained coffee pulp in Mexico. The results on Brazilian coffee husk were lower than those obtained by Martinez.

Figure 2. Fructification of *P. ostreatus* LPB 09 on coffee husk.

Coffee husk detoxification

Table 6 shows that the concentration of caffeine in the coffee husk was reduced to 60.7% after colonisation and fructification of *Pleurotus*. Analysis of fruiting body of this mushroom showed a concentration of 0.197 $mg.g^{-1}$ of dry fruiting body. It confirmed that *Pleurotus* did not completely degrade the caffeine, but it was partially accumulated in the mushroom. The tannin concentration was also reduced significantly in the husk, reaching a reduction of 79.17%. But in the fruiting bodies, no tannin was found, which suggested that the mushroom was capable of completely degrading the tannin present in the coffee husk.

Table 6. Change of caffeine and tannin content before and after growing of *P. ostreatus* LPB 09 in coffee husk.

Component	Initial content ($mg.g^{-1}$)	Final content ($mg.g^{-1}$)	Reduction %
Caffeine	0.65 ± 0.12	0.197 ± 0.093	-60.69
Tannin	3.65 ± 0.21	0.76 ± 0.14	-79.17

CONCLUSIONS

This study demonstrated that the strain *P. ostreatus* LPB 09 was capable to produce the fruiting body on coffee husks, even with high concentrations of caffeine and tannin in the medium. Coffee husk was found to be an excellent substrate for mushroom cultivation, especially *P. ostreatus*. Fructification of mushroom reduced 60.69% of caffeine present in the coffee husk. However, the fungus was not capable of completely degrading caffeine, which was partially accumulated in the mycelia and in the fruiting bodies. On the contrary, tannin was degraded, with a reduction of 79.19% in the coffee husk. The final solid residue, after the fructification of the mushroom, could be an excellent product for animal feeding, due to its content of protein (9.62%).

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RESUMO

Efeito da cafeína e taninos no cultivo e frutificação de *Pleurotus* em casca de café

O objetivo do presente trabalho foi estudar o efeito da cafeína e taninos sobre o desenvolvimento do fungo *Pleurotus* sp. e avaliar a possibilidade do uso da casca de café como substrato no seu cultivo. Oito cepas de *P. ostreatus* e duas cepas de *P. sajor-caju* foram selecionadas em um meio à base de extrato aquoso de casca de café e Agar. A cepa *P. ostreatus* LPB 09 foi escolhida para estudos posteriores com base na velocidade de crescimento do micélio e produção de biomassa. O estudo do desenvolvimento do cogumelo em meio com diferentes concentrações de cafeína mostrou que o aumento da concentração da mesma influencia negativamente crescimento. A partir de uma concentração de cafeína de 2500 mg/L não foi observado crescimento. Observou-se ainda que o *Pleurotus* não degradou a cafeína, mas a absorveu. Os taninos em concentração abaixo de 100 mg/L no meio estimularam o crescimento do micélio, no entanto acima de 500 mg/L mostraram efeito negativo. Quando a concentração de taninos atingiu 1000 mg/L, o cogumelo ainda sobreviveu e mostrou uma certa tolerância. O ácido tânico não foi encontrado no micélio, o que confirma que o *Pleurotus* possui a capacidade de degradá-lo. A frutificação ocorreu após 20 dias de inoculação e a eficiência biológica atingiu 97% em 60 dias. O conteúdo de cafeína da casca de café foi reduzido para 60.7% e o de taninos para 79.2%. Os resultados mostram boas perspectivas para o cultivo de *Pleurotus* utilizando casca de café como substrato sem a necessidade de qualquer pré-tratamento.

Palavras-chave: cultivo, *Pleurotus*, cafeína, taninos, detoxificação

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