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CULTIVATION OF SHIMEJII ON ELEPHANT GRASS SUBSTRATE SUPPLEMENTED WITH DIFFERENT KINDS OF BRAN

CULTIVO DE SHIMEJII EM SUBSTRATO CAPIM-ELEFANTE SUPLEMENTADO COM DIFERENTES TIPOS DE FARELOS

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ABSTRACT

The purpose of this work was to evaluate the cultivation of the strains of *P. ostreatus* in elephant grass substrate supplemented with different kinds of bran. The experiment consisted in the use elephant grass substrate supplemented with soy, wheat, rice or corn bran in concentrations of 0, 10 or 20% poured in flasks that were inoculated with spawns of BF24, DF33 and HF19 strains of *P. ostreatus* and incubated at room temperature (20 - 28 ºC). After the complete colonization of the substrate, the flasks were transferred to a fructification chamber with temperature between 20 and 26 ºC and average damp of 75 – 90%. The BF24 strain was found to be the most productive one in relation to the others and the supplementation of the elephant grass with wheat bran in concentrations of 10 and 20% favors higher productiveness and biological efficiency for the BF24, DF33 and HF19 strains of *P. ostreatus*.

Key-words: *Pleurotus ostreatus*; *Pennisetum purpureum*; mushrooms; productivity; biological efficiency.

RESUMO

O objetivo deste trabalho foi avaliar o cultivo de linhagens de *Pleurotus ostreatus* em substrato capim-elefante suplementado com diferentes tipos de farelos. O experimento consistiu no uso do substrato capim-elefante suplementado com farelos de soja, trigo, arroz ou milho em concentrações de 0, 10 ou 20%, colocados em frascos que foram semeados com as linhagens BF24, DF33 e HF19 de *Pleurotus ostreatus* e incubados a temperatura ambiente (20 - 28 ºC). Depois da completa colonização do substrato, os frascos foram transferidos para uma câmara de frutificação com temperatura entre 20 e 26 ºC e umidade relativa de 75 - 90%. A linhagem BF24 foi mais produtiva e a suplementação do capim-elefante com farelo de trigo em concentrações de 10 e 20% aumentou a produtividade e eficiência biológica das três linhagens de *Pleurotus ostreatus*.

Palavras-chave: *Pleurotus ostreatus*; *Pennisetum purpureum;* cogumelos; produtividade; eficiência biológica.

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INTRODUCTION

Pleurotus sp. presents a large variety of colors, extending from white to dark blue, brown, yellow and pink and varying according to the species, amount of light during fructification, nutritional needs and incubation period.

Many edible species are found in this genus, such as *Pleurotus ostreatus* (Jacq.) Quélet, *P. ostreatoroseus* Sing., *P. pulmonarius* (Fries) Quélet, *P. sajor-caju* (Fr.) Singer, *P. eous* (Berkeley) Saccardo, and others (Eira & Minhoni, 1997). Bononi et al. (1991) stated that mushrooms are excellent for dieting, for they are highly nutritious and non-caloric. This is due to their nutritional characteristics that resembles milk, presenting high level of proteins (27 – 48%), low values of lipids (2 – 8%), in addition to the presence of vitamins (Thiamin, riboflavin, niacin), minerals (calcium, iron, phosphorus), beta-glucans and compounds with antioxidant activities (Soto-Cruz et al., 1999; Savón et al., 2002; Zhang et al., 2002). Presently the existence of active therapeutic substances in fungi of this genus is under analysis. Mushrooms may play an important role in the reduction of cholesterol as well as hold anti-inflammatory, anti-viral, antimicrobial and antitumoral activities (Park et al., 2003).

Mushroom culture in Brazil is concentrated in the Southern and Southeastern regions, and the most important genus are *Agaricus bisporus* (Lange) Imbach, *A. brasiliensis* Wasser *et al*., *Lentinula edodes* (Berck) Pegler, *P. sajor-caju* and *P. ostreatus.* Until the 1980s, only *A. bisporus* was grown and, since then, the mushroom culture has been diversified by the introduction of other species of improved culture performance (Eira & Minhoni, 1997). In Brazil the consumption mushrooms is still insignificant - around 0.06 kg per year per capita – when compared to European countries with a consumption of 3.5 kg per year per capita. The decisive factor of this reality is the lack of tradition in the consumption of mushrooms and its relatively high prices (Dias et al., 2003).

The production of mushrooms presents many advantages in relation to other cultures such as a smaller cultivation area, a short life cycle and the use of agricultural residues as a medium for its growth. About 4 million tons of edible mushrooms are produced annually, and their major producers the United States, France, The Netherlands, Japan and China (Moda et al., 2005).

In the last few years various ligno-celullosic residues have been proposed as substrate for the growth of *Pleurotus* spp. such as the straw of various cereals, cotton residues, sugar-cane residues, sawdust, fruit pulp and peel, citric residues, banana leaves and coffee pulp (Li et al., 2001; Eira, 2003; Ragunathan & Swaminathan, 2003; Job, 2004; Moda et al., 2005). The use of different types of substrate by the fungus will depend on its capacity to secrete enzymes like cellulase, hemicellulase e ligninase, releasing nutrients for its growth. (Rossi et al., 2001; Mata et al., 2001).

According to Eira & Minhoni (1997) the techniques of mushroom production may involve natural substrates previously composted and pasteurized or may use axenic cultivation, which consists of using a sterile substrate. According to Felinto (1999) the technique of axenic cultivation is impracticable in a commercial scale due to the investment in equipment required. However, in developed countries this is the technique that presents the best results.

The production of *Pleurotus* depends on the genetic conditions of the mushroom species, on the nutritional quality and on the structure of the substrate. The culture conditions are a decisive factor in the efficiency of production (Sturion, 1994; Royse, 2002). According to Moda et al. (2005) the supplementation of the substrate is commonly used to raise productiveness, which is evaluated by the biological efficiency.

Among the most used cultivation supplements, cereal brans are sources of organic nitrogen (N), necessary to the growth of the mycelium mass, which may interfere in productiveness and biological efficiency of the fungus. The quantity and the kind of bran may vary according to the species or the strain under development as well as the growth stage.

This paper aimed to evaluate the cultivation of three strains of *Pleurotus ostreatus* in elephant grass substrate supplemented with different bran.

MATERIAL AND METHODS

The experiment was developed at the Experimental Laboratory of Mycology (LEMICO), of the Department of Microbiology and Parasitological (DEMP) of the Institute of Biology (IB), of the Federal University of Pelotas (UFPel), RS, Brazil. In this experiment the strains DF33, HF19 and BF24 of *P. ostreatus* were used, obtained from the Módulo de Cogumelos FCA/UNESP/Botucatu/São Paulo/Brazil, stored in the fungi collection of the LEMICO/DEMP/IB/UFPel. These strains, preserved in mineral oil, were inoculated to a medium of potato dextrose agar (PDA) and incubated at 28 ºC for 10 days until they were fully recovered and there was mycelium growth.

In order to prepare the spawn, sorghum grains (*Sorghum bicolor* L. Moench) previously boiled for 20 min, were used. After this, it was poured into glass flasks of 8.6 x 14 cm that were sealed with aluminum and plastic film and sterilized at 121 ºC (1,013 x 10^5 Pa) for 45 min with the substrate at room temperature, 10 mm discs of culture of each strain previously prepared in medium of culture of elephant grass + dextrose + agar (Donini et al., 2005), were transferred to the flasks containing the sorghum grains. Next the grains were incubated at 28 ºC until there was colonization of the grains by the fungus, making up the spawn.

Elephant grass (*Pennisetum purpureum* Schum), chopped in its vegetative stage, was used as substrate for cultivation. Later, it was fragmented in 2 cm slices and dried at room temperature. For the preparation of the material to be used in this

experiment, the substrate was previously humidified for 24 h and supplemented with soybean, wheat, rice and corn bran in concentrations of 0, 10 and 20% in relation to the humid mass of grass, making up 12 treatments to each strain.

The substrates used were stored in flasks of 9 x 16.8 cm, each treatment receiving a 13 cm height, corresponding to 250 g of substrate. The flasks were identified, sealed with aluminum paper and plastic film and sterilized twice at 121 ºC (1,013 x 10^5 Pa) for 60 min in a 48 h.

In a biosafety cabinet the substrate was inoculated with 1 per cent of the spawn. The flasks were incubated at room temperature (20 – 28 ºC) for 23 days and the colonization was complete in most treatments on the fourteenth day. However, the treatments remained under the same condition for another 9 days until the beginning of the formation of the fruiting bodies.

Next, the flasks were opened - removing the aluminum - and transferred to a fructification chamber with temperatures of 20 - 26 ºC and average damp of 75 - 90%. The harvest of the mushrooms was performed in a 25-day period and began 28 days after inoculation. The mushrooms were manually harvested and weighted to obtain the humid mass.

The variables under analysis were productiveness in humid mass (P% = MUC/MUS x 100 where MUC = mushroom humid mass and MUS = substrate humid mass) and biological efficiency (EB% = MUC/MSS x 100 where MUC = mushroom humid mass and MSS = substrate dry mass) (Nascimento & Eira, 2003).

The analysis of total carbon and nitrogen of the substrates was performed in the Soil Departament/FAEM/UFPel, using the Walkey-Black method to organic carbon and Semi-micro-Kjeldahl method to nitrogen (Tedesco et al., 1995), and the C/N ratio is described in Table1.

The experiment consisted of an A x B x C factorial (A = strain, B = bran, C = concentration of bran). Experiment design was a completely randomized design with four replications per treatment, being each flask an experimental unit. For media comparison the results obtained were submitted to variation analysis and Duncan's. Bran concentrations values were submitted to regression analysis using SANEST statistics program (Zonta & Machado, 1984).

TABLE 1 – C/N ratio of the elephant grass substrate supplemented with brans in different concentrations.

Substrate	Ratio C/N
Elephant grass	162:1
Elephant grass + 10% soybean bran	43:1
Elephant grass + 20% soybean bran	21:1
Elephant grass + 10% wheat bran	60:1
Elephant grass + 20% wheat bran	55:1
Elephant grass + 10% rice bran	52:1
Elephant grass + 20% rice bran	47:1
Elephant grass + 10% corn bran	71:1
Elephant grass + 20% corn bran	63:1

RESULTS AND DISCUSSION

The analysis of the media by the Duncan test showed that the BF24 strain had the highest productivity and biological efficiency (Figure 1), practically twice as high as that of HF19.

As to productivity and biological efficiency variables treatments with the addition of wheat bran, rice bran and corn bran in concentrations of 10 and 20% in the strains BF24, DF33 e HF19 were considered to be superior to the treatment without bran addition (0%). In the HF19 strain the corn bran was efficient only at 20%. The supplementation with soy bran presented an inferior effect with reference to productivity and biological efficiency in relation to the other treatments for the three strains (Tables 2, 3 and Figure 2). For brans in which the concentration of 10% was superior, there was no deleterious effect when the concentration was raised to 20%, nor was there a raise in productivity and biological efficiency.

In general, the treatments with elephant grass supplemented with wheat bran presented the best results in productivity and biological efficiency in the three strains. We observed that the two concentrations of wheat bran (10 and 20%) did not present differences, which indicates that the use of 10% was sufficient to obtain the maximum productivity and biological efficiency, bearing the advantage of the reduction of the production costs. We also observed that the treatments with elephant grass supplemented with soy bran presented the lowest averages for the two variables in all strains which were statistically inferior to all substrates, including the treatment without the addition of this bran (0%) (Tables 2, 3 and Figure 2).

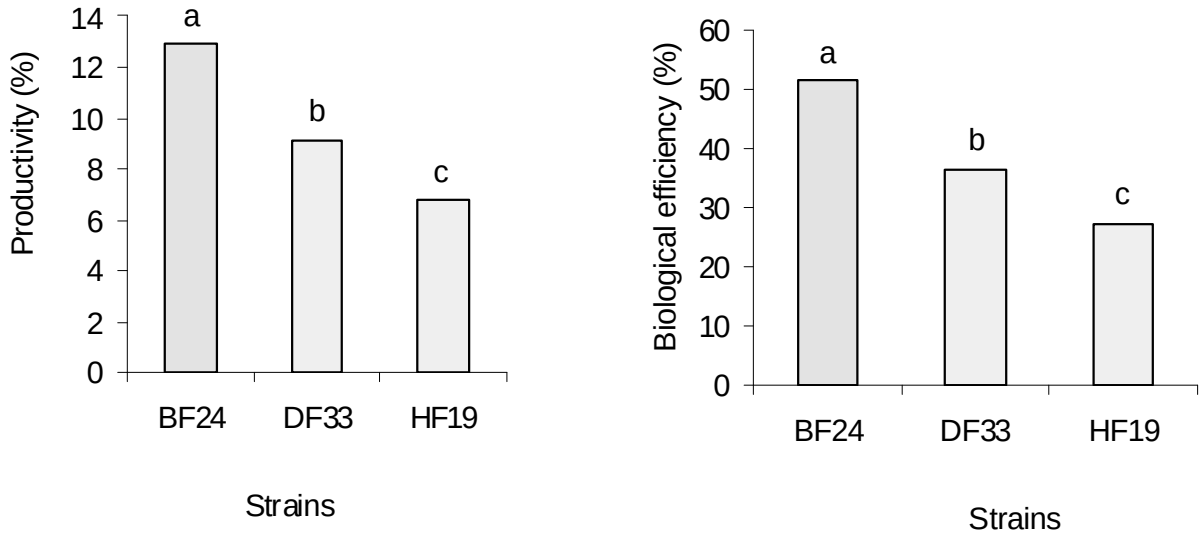

FIGURE 1 – Average productivity and biological efficiency of BF24, DF33 and HF19 strains of *P. ostreatus* cultivated in elephant grass substrate supplemented with bran in different concentrations. Averages followed by the different letters differ from each other by the Duncan's test at 5% probability.

TABLE 2 – Productivity average(%) of the BF24, DF33 and HF19 strains of *Pleurotus ostreatus* cultivated in elephant grass substrate supplemented with soybean, wheat, rice and corn brans in different concentrations.

Strains	Brans	Concentration(%)		
		0	10	20
BF24	Soybean	9.30 a A	2.20 c B	0.00 b B
	Wheat	9.30 a B	23.59 a A	22.40 a A
	Rice	9.30 a B	17.00 b A	18.69 a A
	Corn	9.30 a B	15.00 b A	18.70 a A
DF33	Soybean	3.60 a A	0.60 c A	0.00 c A
	Wheat	3.60 a B	17.50 a A	21.60 a A
	Rice	3.60 a B	15.10 ab A	15.80 b A
	Corn	3.60 a B	10.00 b A	14.10 b A
HF19	Soybean	2.00 a A	0.00 c A	0.00 b A
	Wheat	2.00 a B	16.70 a A	12.40 a A
	Rice	2.00 a B	14.20 a A	10.40 a A
	Corn	2.00 a B	6.00 b B	13.80 a A

Averages followed by the same small letters in the columns and capital letters in the lines do not differ from each other by the Duncan's test at 5% probability.

According to Sturion (1994), in the fruit body development phase the occurrence of a lower C/N ratio in the cultivation substrate is more favorable, which did not happen in this experiment in the substrates supplemented with soybean bran with a lower C/N ratio (Table 1).

It is thought that the substrates excessively enriched with bran, as well as the nature or some component in the soybean bran, influenced productivity and biological efficiency. Besides affecting the formation of fruit bodies, the excess of nitrogen may have affected the degradation of lignin, which may prevent the mycelium from developing, but this was not the case in this research.

According to Zanetti & Ranal (1997), if on the one hand the low content of N decreases productivity, on the other hand high content of this nutrient also affects the production of fruit bodies

TABLE 3 – Biological efficiency average(%) of the BF24, DF33 and HF19 strains of *Pleurotus ostreatus* cultivated in elephant grass substrate supplemented with soybean, wheat, rice and corn brans in different concentrations.

Strains	Brans	Concentration(%)		
		0	10	20
BF24	Soybean	37.20 a A	8.80 c B	0.00 b B
	Wheat	37.20 a B	94.39 a A	89.59 a A
	Rice	37.20 a B	68.00 b A	74.79 a A
	Corn	37.20 a B	60.00 b A	74.80 a A
DF33	Soybean	14.40 a A	2.40 c A	0.00 c A
	Wheat	14.40 a B	70.12 a A	86.40 a A
	Rice	14.40 a B	60.40 ab A	63.20 b A
	Corn	14.40 a B	40.00 b A	56.40 b A
HF19	Soybean	8.00 a A	0.00 c A	0.00 b A
	Wheat	8.00 a B	66.80 a A	49.59 a A
	Rice	8.00 a B	56.79 a A	41.59 a A
	Corn	8.00 a B	24.00 b B	55.20 a A

Averages followed by the same small letters in the columns and capital letters in the lines do not differ from each other by the Duncan's test at 5% probability.

negatively where there is an optimum concentration of N to mycelia phase and production. Divergences in methodology and in calculation to the identification of this value difficult.

The soybean bran was important in the formulation of the cultivation medium and during the colonization phase but it presented low productivity and biological efficiency. These results differ from the ones obtained by Zanetti & Ranal (1997) cultivating *Pleurotus* sp. 'Florida' in bags with 1 kg of sugarcane bagasse supplemented with guandu (*Cajanus cajan*), on which occasion they were able to verify higher productivity of mushroom and biological efficiency (94.73%) when the supplementation was 15%. Vogel & Salmones (2000) added soybean flour in a 5.5% rate to the dry mass of substrate of wheat straw in the cultivation of three different strains of *Pleurotus* spp. and verified a biological efficiency of 58.8, 64.8 and 80.47% respectively for the IE - 227, 1314 and IE - 226 strains. In another experiment, the same authors used a commercial supplementation instead of the soybean flour and verified that the biological efficiency of the strain IE-226 increased to 99.3%.

According to Curvetto et al. (2002) the supplementation of sunflower seed skin with NH_4^+ for the production of *P. ostreatus* raises the productivity of this species in up to 50%, as it promotes the mycelium development through the adjustment of the C/N ratio of the substrate used. That was also verified in this paper, especially when wheat bran was used as supplement. One of the hypothesis discussed by Royse (2002) is the C/N ratio adequacy, which relates the supplementation of the substrates with different nutrients as a determinant factor to the production of *P. cornucopiae* (Paulet : Pers.) Rolland.

Wang et al. (2001) upon researching the cultivation of *P. ostreatus* found that the supplementation of the barley straw substrate with wheat bran up to a 45% ratio promoted an increase in biological efficiency of the mushroom. A decrease in the values of the biological efficiency variable in response to the increase in the values of the supplementation was found. Therefore these results can be associated to the ones obtained in this experiment, where the addition of the same bran provided an increase in productivity and biological efficiency for the three strains used.

Dias et al. (2003) verified that *P. sajor-caju* cultivated in pure corn straw substrate obtained 51% of biological efficiency with pure straw, whereas cultivation with 10% of wheat bran supplement raised this value to 83%. However, when these authors cultivated it in pure bean straw or with the same supplementation, meaningful differences were not found, so it is not necessary to supplement the bean straw for the cultivation of this species. For elephant grass, the increase in the supplementation with wheat bran from 10 to 20% did not increase productivity or biological efficiency. For the composition of the substrates used in the cultivation of mushroom, the availability of nutrients can be altered, showing different results between the supplementation with soybean and wheat straw for productivity and biological efficiency, as occurred in this experiment.

Yildiz et al. (2002) mentioned 79.4% of biological efficiency to *P. ostreatus* when it was cultivated in pure wheat straw. Banik & Nandi (2004) described the increase of the biological efficiency of *P. sajor-caju* cultivated in rice straw supplemented with dung in the proportion of 1:1. But as this ratio was increased, there was a decrease in the variable analyzed. On the other hand Moda et al. (2005) said that the cultivation of

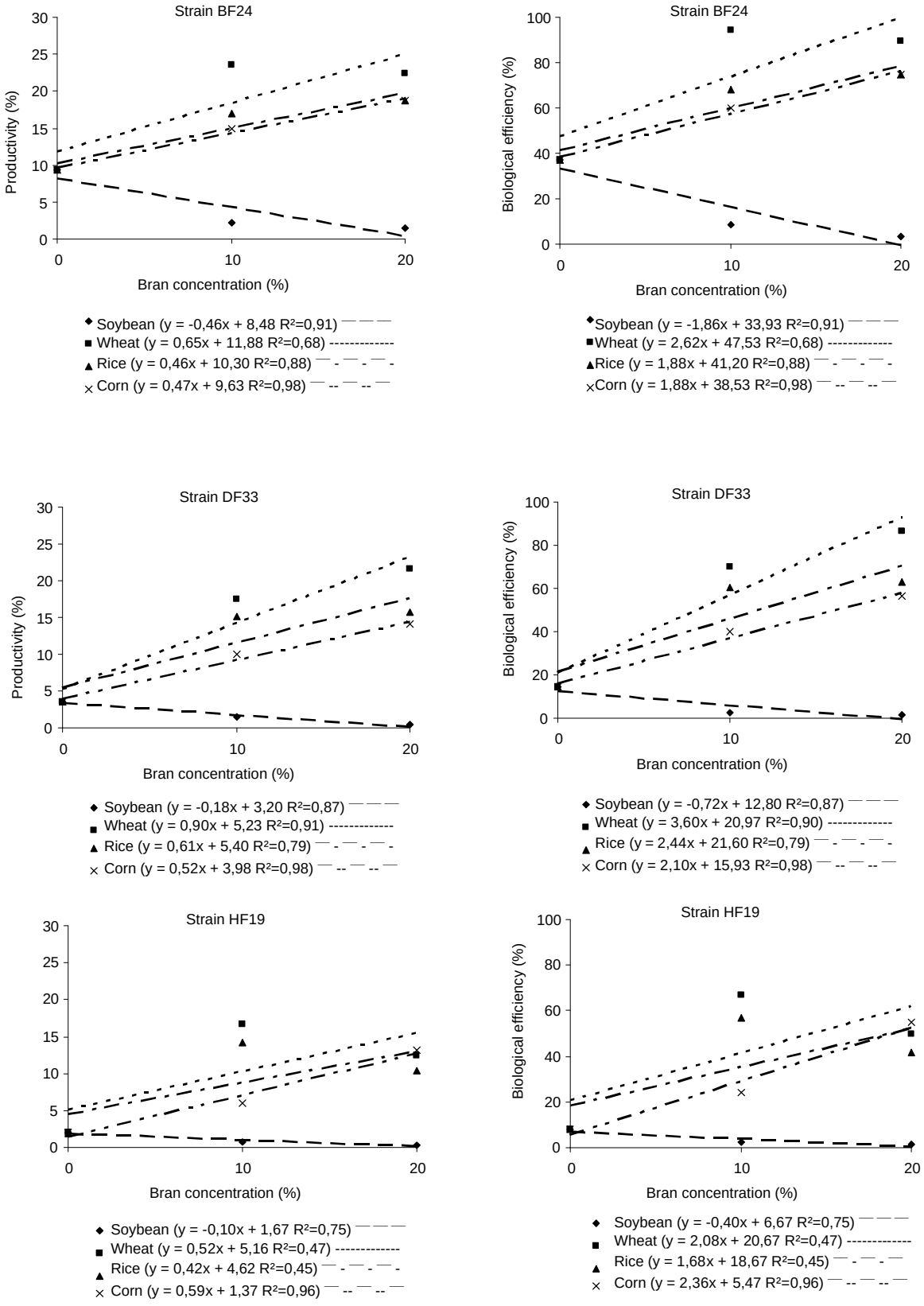

FIGURE 2 – Polynomial regression for productivity and biological effectivity of the lines mushrooms BF24, DF33 and HF19 of *Pleurotus ostreatus* grown on the substrate elephant grass enriched with soy, wheat, rice or corn bran in different concentrations.

this mushroom in crushed sugar-cane supplemented with corn bran decreases biological efficiency. That did not happen in our experiment where the corn bran did not affect the speed of the mycelium growth but raised productivity and biological efficiency.

The supplementation of paper residues with rice bran is described by Baysal et al. (2003), these authors obtained an increase in the biological efficiency with the increase in concentration (10 and 20%) of bran concentration during the production of *P. ostreatus.* This fact can be related to our research where the increase in supplementation of elephant grass with the same type of bran resulted in an increase in productivity and biological efficiency, except for the HF19 strain which had a decrease in these variables as the concentration of bran was increased (20%).

Salmones et al. (2005), while working with *P. ostreatus*, mentioned a differentiated biological efficiency for the two strains of this mushroom – IE38 and IE49 – 50.2% and 54.2% respectively. These data confirm the results obtained in our research, where a variation in the biological efficiency for the different strains of the same species of fungi was verified, proving this way the different requirements concerning the composition of the substrate used.

CONCLUSIONS

1) According to the results obtained in the present research it can be concluded that the BF24 strain of *Pleurotus ostreatus* presents higher productivity and biological efficiency when produced with elephant grass substrate supplemented with soybean bran, wheat, rice and corn bran.

2) The supplementation of elephant grass with wheat bran in the concentrations of 10 and 20% favors higher productivity and biological efficiency for the BF24, DF33 and HF19 strains of *P. ostreatus*, followed by rice and corn bran.

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