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Cultivation of *Pleurotus* Mushroomsin Axenic Culture through the Use of Crop Residues

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Abstract: *The axenic method for cultivation presents the potential for regular and safe production as it provides for the sterilization of substrates, reduces the early period of production and maintains controlled and predictable conditions in the development of the mushrooms. This study aims to evaluate the axenic cultivation of Pleurotus sajor-caju, Pleurotus ostreatoroseus and Pleurotus citrinopileatus in three different substrates. The substrates of Ricinuscammunis L., Oriza sativa L. and Pennisetumpurpureum L. were rehydrated for 24 hours, drained and packaged. Each of the substrates were autoclaved, inoculated with spawn 3% (w/w) and incubated for colonization after cooling. For fruiting, the bags were stored in a fructification chamber (25°; RH: 75-90%). After harvesting, fresh weight, yield and biological efficiency were measured. P. citrinopileatus provided the highest average fresh mass (79.42g) and productivity (15.88%) when grown in agricultural residues of mamona. The highest biological efficiency (54.80%) was found in P. ostreatoroseus when grown in rice straw. The substrates of mamona residues and rice straw are the most appropriate for the cultivation of P. citrinopileatus and P. ostreatoroseus, respectively. The data presented in this study indicates that the cultivation of Pleurotus sp. in different substrates offers a cost-effective agricultural alternative for agriculture.*
Keywords: *biological efficient, elephant grass, mamona residues, rice straw,*

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I. Introduction

Humanity has known of the mushroom since antiquity. Due to the various nutritional properties attributed to mushrooms, they have been used as food and as medicine[1,2]. Over the course of time and in the search for different types of healthy food, mushrooms became part of the eating customs of people around the world [3.4].

In 1995, annual production of mushrooms was 2 million tons. Ten years later, in 2005, production had reached 3.3 million tons, an increase of 60% in 10 years [4].One of the most cultivated edible mushroom, *Pleurotus* sp. is a fungus with high ligninolytic capacity. The genus can be grown in a broad range of environments from a simple cereal straw to an elaborate ambient with a variety of residues and substrates[5-9]. Nunes et al. (2017) [9]emphasized the importance of using residues available in the region where farming is performed. In Brazil, for example, cultivation begun around 1980 as a way to use the sugarcane bagasse generated by the production of alcohol fuel [10].

The cultivation technology used depends on several factors ranging from availability and the types of substrates to the choice of cultivation technique, i.e. axenic or pasteurized, with the first offering a better energy consumption rate and increased technification[11]. The production of *Pleurotus* spp. in agricultural substrates grown using the axenic method, presents greater potential for regular and safe production. The process provides for the sterilization of substrates, thus reducing the early period of production, as well as the maintenance of controlled and more predictable conditions in the development of the mushrooms[12].

Therefore, the objective of this study was to evaluate the axenic cultivation of three species of *Pleurotus* spp. in different substrates through the analysis of the variables of fresh weight (g), yield (%) and biological efficiency (%) of the species.

II. Materials and methods

In this experiment, three species of *Pleurotus* sp. were examined: *P. sajor-caju* (PSC96/03), from the Module FCA/UNESP/Botucatu; *P. ostreatoroseus* (POR01/03) and *P. citrinopileatus* (PAM01/06).

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To recover the strains, they were expressed in medium agar + elephant grass + dextrose (AED) and incubated at 28°C over a period adequate for the growth of mycelial across the Petri dish (90 X 15mm).For inoculum preparation (‘spawn’), unpeeled and previously boiled (for 20 minutes) grains of rice were used. Next, they were placed into glass bottles (8.6 X 14cm), sealed with aluminum foil and cling film and autoclaved at 121°C (1 atm) for 45 minutes. With the grains at room temperature, discs of 10mm in diameter containing each strain culture were transferred to a separate bottle, and these were incubated at 28°C until the grains were colonized by fungus.The substrates used were elephant grass (*Pennisetumpurpureum*Schum), rice straw (*Oryza sativa* L.) and mamona residues (*Ricinuscommunis* L.), all of which were fragmented in size to 2-4cm and dried at room temperature. To prepare for the experiment, substrates were first moistened for 24 hours. Next, the water was drained, and the substrates packaged in polypropylene bags of 500 grams per bag and immediately autoclaved for 1 hour at a temperature of 121°C at 1 atm. In a laminar camera, 3% of inoculum (w/w) of each species was added to the substrates. The bags were incubated at 25 ±2°C until complete colonization of the substrates was achieved. Then, the bags were transferred to a fructification chamber (25 ±2°C) with a relative humidity of 75-90%.

The mushrooms were manually collected and weighed, and data was gathered regarding the parameters of fresh weight (g), yield (%) and biological efficiency (%) and calculated according to Bernardi et al. (2007) [13]. The carbon/nitrogen relationship of the substrates was measured by the Departamento de Solos/ERS/UFPel according to the Walkey-Black and Semi-micro-Kjeldahl method, respectively [14]. The results were analyzed by the Tukey test to compare averages using the statistical program SANEST [15].

III. Results And Discussion

The highest values for fresh weight (g) and yield (%) of the mushrooms produced during this study were obtained by the *P. citrinopileatus* (PAM01/06) in a substrate of residue culture of mamona, which were 82.5% and 82.3% higher, respectively, than when grown in rice straw. The combination of *P. citrinopileatus* with mamona residues was 108.7% and 163.2% higher in fresh weight (g) and yield (%), respectively, than that obtained with the combination of *P. ostreatoroseus* (POR01/03) and *P. sajor-caju* (PSC96/03) in elephant grass (Table 1). For both these variables, the ranking of the types of mushrooms, in order of decreasing significance, was *P. citrineopileatus*, *P. ostreatoroseus* and *P. sajor-caju*. In relation to the substrate, this was higher when mushrooms were grown in mamona residues. The *P. citrineopileatus* species stood out with results 163.2% higher in fresh weight and 13.4% higher in yield than the lowest recorded figures for these variables, which were 30.18g and 6.03%.

In relation to the biological efficiency of each species of mushroom cultivated in relation to the substrate,the results for *P. ostreatoroseus* (POR01/03) were 134.8% higher than the lowest value, 23.34% found for *P. sajor-caju* (PSC96/03), as shown in **Table 1**. The better results achieved with *P. ostreatoroseus* are also illustrated in Fig 1. The best performance among the substrates was obtained when rice straw used, since this substrate showed a biological efficiency 134.8% higher than the lowest percentage, which was recorded for elephant grass. The elephant grass yielded low harvests, 134.8%, 86.2% and 42.5% lower than the highest biological efficiency (54.8%) obtained for *P. citrinopileatus* (PAM01/06), *P. ostreatoroseus*(POR01/03) and *P. sajor-caju* (PSC96/03), respectively.

The results obtained in this study are similar to those obtained by Yu et al. (2008) [16], which produced *P. ostreatus* in a substrate based primarily upon sterile rice straw and achieved a maximum biological efficiency of 46.68%, which is somewhat higher those that obtained with *P. sajor-caju* (PSC96/03) and *P. citrinopileatus* (PAM01/06) in this study. However, the value is close to that achieved with *P. ostreatoroseus* (POR01/03), 54.80%, considering both studies were conducted in the same substrate and using the same axenic mode of cultivation. Other results that corroborate those obtained with regard to *P. ostreatoroseus* (POR01/03) grown in rice straw during this study can be found in Castro et al. (2007) [17], who obtained biological efficiency of around 55% through the cultivation of *P. sajor-caju* in residues of cotton textile processing.

Some results involving the use of rice straw are described by Rout et al. (2016) [18], who observed biological efficiency of 85.83% when using a substrate composed of rice straw in a yield trial with the mushroom *P. sajor-caju*. Moreover, Bernardi et al. (2007) [13] present results of biological efficiency of 58.64% for *P. citrinopileatus* (PAM01/06) when grown in elephant grass (*Pannisetumpurpureum*), which is 34.40% higher than the biological efficiency obtained in this work using the same strain of mushroom and substrate, though adopting pasteurized cultivation. Thus, when taking into account the results obtained by the aforementioned authors and comparing these with the results of this experiment. lower profitability for pasteurized axenic cultivation can be inferred.

The results concerning the carbon/nitrogen (C/N) relationship (Table 2) reveal the highest relationship of C/N in the treatment with elephant grass, which was approximately 3.06 higher than that found for rice straw and 4.38 higher than that recorded for mamona residues (37:1). Therefore, as illustrated in Tables 1 and 2, differences are evident in the C/N relationships required by each mushroom tested in the experiment. For two of the species, *P. sajor-caju* and *P. ostreatoroseus*, the high C/N relationships were unsatisfactory as cultivation in elephant grass showed the poorest results across all variables. *P. sajor-caju* (PSC96/03) and *P. ostreatoroseus* (POR01/03) were observed to have the worst results for the three variables. For *P. citrinopileatus* (PAM01/06), the cultivation in a substrate with a high C/N relationship (elephant grass) showed average results from values measured in the residues of mamona and rice straw substrates. In contrast, the low C/N relationship in mamona residues formed the substrate for greater productivity for *P. citrinopileatus* (PAM01/06) and *P. sajor-caju* (PSC96/03). However, *P. ostreatoroseus* (POR01/03) obtained the best results with rice straw and with a substrate with a median C/N relationship.

Comparing biological efficiency and the C/N relationship in substrates, Liang et al. (2009) [19], working with substrates of corn straw and sawdust (45:45), *Panicumrepens* and sawdust (45:45) and corn husks and sawdust (60:30), verified these as the most appropriate for *P. citrinopileatus* production since they showed a C/N relationship of between 46:1 and 51:1. These findings differ from those described in this work, where the mamona residues culture showed higher biological efficiency for *P. citrinopileatus* (PAM01/06) but a lower C/N relationship (37:1).

An increase in biological efficiency can be achieved with the assistance of certain supplements added to pure substrate. Loss et al. (2009) [20] used corn grains and concluded, using mathematical models, that higher biological efficiency (91.12%) can be obtained with the use of 50% of the effluent. The increase in biological efficiency by substrates was verified by Özçelik and Peksen (2007) [21] whose mixtures of pieces of beech, wheat straw and wheat meal (3:1:1) enhanced biological efficiency in *Lentinulaedodes* cultivation by 87.73% compared with a substrate composed of hazelnut peels. Similarly, this experiment demonstrates the possibility of an association between substrates and biological efficiency, offering promising results concerning the use of novel substrates for growing mushrooms, such as mamona agricultural residues.

IV. Conclusions

In conclusion, the cultivation of *Pleurotus* sp. in different substrates offers a cost-effective alternative for agriculture. In this study, *P. citrinopileatus* (PAM01/06) displayed higher fresh mass and productivity and lower biological productivity in axenic cultivation compared with *P. sajor-caju* (PSC96/03) and *P. ostreatoroseus* (POR01/06). Regarding the substrates, agricultural mamona residues and rice straw are the most appropriate for the cultivation of *P. citrinopileatus* (PAM01/06) and *P. ostreatoroseus* (POR01/06), respectively.

V. Figures And Tables

Table 1. Fresh weight (g), yield (%) and biological efficiency (%) of three species of *Pleurotus* spp. grown axenically.

Mushroom	Substrates	Fresh weight	Yield	Biological efficiency
Pleurotus sajor-caju (PSC96/03)	Rice straw	46.80 cd	9.36 cd	37.44 bc
	Mamona	51.39 bcd	10.28 bcd	30.83 cd
	Elephant grass	30.18 e	6.03 e	23.34 d
Pleurotus ostreatoroseus (POR01/03)	Rice straw	68.50 ab	13.70 ab	54.80 a
	Mamona	58.38 bc	11.67 bc	35.03 bcd
	Elephant grass	38.05 de	7.61 de	29.43 cd
Pleurotus citrinopileatus (PAM01/06)	Rice straw	43.52 cde	8.71 cde	34.82 cd
	Mamona	79.42 a	15.88 a	47.65 ab
	Elephant grass	49.73 cd	9.95 cd	38,47 bc

CV (%)	**14.20**	**10.20**	**13.82**

Means followed by the same letter in the columns do not differ according to the Tukey test (α=0.05).

Table 2. Carbon and nitrogen (C/N) relationship of substrates used during the axenic cultivation of *Pleurotus* spp.

Treatment	**C/N relationship**
Rice straw	53:1
Mamona residues	37:1
Elephant grass	162:1

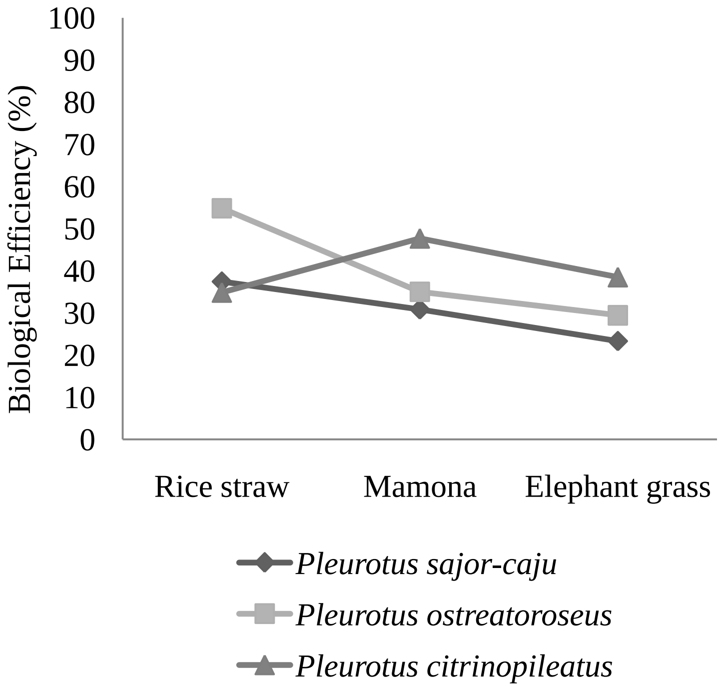

Figure 1. Biological efficiency (%) of *Pleurotus sajor-caju* in three substrates: rice straw, mamona residues and elephant grass.

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