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Macromineral consumption by *Pleurotus ostreatus* var. Florida in different straws

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Abstract: Organic agricultural waste is successfully used in the production of mushrooms as it promotes the reuse of resources and increases food production. For the formulation of the substrate for *Pleurotus spp*. production, the availability of nutrients must be rigorously analyzed to assess whether supplementation is necessary. The aim of this paper was to evaluate the agronomic behavior of *Pleurotus ostreatus* var. Florida when grown on different straws and to analyze nutrient dynamics at the beginning and end of cultivation. Straws of rice, wheat and brachiaria were used as substrates without any supplementation. The yields, biological efficiency, number and weights of mushrooms were evaluated in two crop cycles. Rice straw provided a higher yield, better biological efficiency and a greater number of mushrooms in both cultures. Wheat straw showed intermediate results for the same variables. None of the substrates showed a significant difference for mushroom weight. In the first crop, only Mg and the number of mushrooms exhibited a positive correlation; in the second crop, only K and the yield of the 1st flush showed a positive correlation. With this study, it was possible to demonstrate that agricultural residues with higher levels of K increase *P. ostreatus* yield, and that Mg management affects the number of mushrooms for harvesting. The results will be a useful guide for efficient and sustainable mineral supplementation of the substrate.

Keywords: nutrients; straw degradation; mushroom production; oyster mushroom

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INTRODUCTION

Pleurotus spp. are active in the decomposition process of different recalcitrant organic substrates and are among the most studied white-rot fungi [1-3]. As primary decomposers, they degrade complex structures such as lignin, cellulose and hemicellulose, thus playing a key role in nutrient cycling [4,5]. In addition to their environmental importance, mushrooms are considered a functional food, placing the genus *Pleurotus* as the second most produced mushroom in the world [6-8]. In Brazil, more than 15 tons of edible and medicinal mushrooms are produced annually, and *Pleurotus ostreatus* var. Florida with 7,475 tons represents 48% of this total, [9], which demonstrates its great economic importance for the national mushroom industry and the need to carry out new studies to seek alternative substrates.

Mushrooms benefit from an adaptable and flexible metabolism and their versatile biochemical capabilities are used in a variety of ways during morphogenesis [10]. Through its enzymatic apparatus, the fungus degrades the different compounds present in the substrate to obtain the elements required for its development [11]. The nutritional requirements of mushrooms can be grouped as follows [12]: (i) water, (ii) carbon (C), (iii) nitrogen (N), (iv) minerals and (v) vitamins. The production of *Pleurotus* spp. depends on the availability of these nutrients during the entire cultivation process. Thus, the substrate used influences the chemical, functional and sensory characteristics of mushrooms [7,13].

In the same way that C and N are the main nutrients and must be proportionally balanced [14], the

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cultivation of *Pleurotus* spp. also requires an ideal and balanced supply of other nutrients to increase yield and biological efficiency [15]. This is exemplified by the process of supplementing the substrate with cereal bran, which results in high efficiency for agronomic parameters [16]. During fungal growth and development, the available nutrients are entirely linked to the enzymatic metabolism of the microorganism [7]. Among the nutritional requirements, we also find minerals, essential elements that are required in lower concentrations than C and N; these are necessary for all kinds of physiological processes, and among them, we find S, P, K, Ca, Mg and the so-called trace elements [17].

In the production of *Pleurotus* spp., as a basis for the substrate agricultural residues are commonly used, which consist of biomass with an abundant source of C [18,19]. Different authors have compiled and characterized a wide range of materials used as a growing substrate for *Pleurotus*, including cereal straws, diverse types of sawdust, crop residues, agro-industrial byproducts, hays, legume straws, flours, and brans [17]. In addition to being a low-cost material, studies with *P. ostreatus* suggest that the straws of wheat, brachiaria and rice can be efficient substrates for the production of this genus [20-22].

We emphasize the importance of this topic, as changing the substrate source generates greater sustainability for production and efficient supplementation increases mushroom yield. Therefore, this study shows how different straws (wheat, brachiaria and rice) influence the harvest of *P. ostreatus* var. Florida, contributing towards an improved understanding of the dynamics and balance of elements (N, P, Ca, Mg, S and K) caused by the biodegradation of *P. ostreatus*.

MATERIALS AND METHODS

The experiment was carried out at the Centro de Estudos em Cogumelos (CECOG) of the Faculdade de Ciências Agrárias e Tecnológicas (FCAT), Campus de Dracena. A completely randomized design, in a simple factorial scheme, was adopted, with 3 different straws (rice, wheat and brachiaria) as substrate, with 8 repetitions, totaling 24 experimental units. The experiment was carried out in duplicate.

Spawn production

The strain used for the experiment was POS 19/01 of *P. ostreatus* var. Florida, which is deposited in the public culture collection of the CECOG. The spawn was prepared based on cooked sorghum grains and autoclaved at 121ºC for 4 h; 2% $CaCO_3$ was added to supply the Ca and adjust the pH [23]. The grains were inoculated with the strain of interest under aseptic conditions in a laminar flow chamber. The inoculated grains were incubated for 28 days at 27ºC for the mycelial run.

Substrate

Straws of rice (*Oryza sativa)*, wheat (*Triticum aestivum*) and brachiaria (*Urochloa brizantha*) were used as substrates. The material was harvested and dried. The straws were crushed until a particle size smaller than 1 cm was obtained in order to facilitate the degradation and use of the raw material by the mushroom. One by one, the substrates were moistened until a humidity of about 60% was attained. Based on their dry weight, the substrates were supplemented with 2% $CaCO_3$ and placed in high-density polyethylene bags at a proportion of 600 g of substrate per experimental unit. The experimental units were autoclaved at 121ºC for 4 h [24,23].

Substrate inoculation

After removal from the autoclave and spontaneous cooling, the experimental units were inoculated under sterile conditions at a proportion of 2% substrate wet weight (12 g of spawn). The spawned bags were incubated at 26ºC for 14 days [24].

Growing cycle

After the colonization period, the bags were cut at the top to induce primordia formation at 23°C. Harvesting was performed daily, twice a day, and started 33 days after inoculation. Three harvest flushes were obtained. Mushrooms were harvested with the pilei closed, counted and weighed.

Agronomic parameters

The parameters evaluated were yield, biological efficiency, number and weight of mushrooms. The yield

Table 1. Agronomic parameters of *Pleurotus ostreatus* var. Florida grown on different substrates.

Substrate	1st flush (%)	2nd flush (%)	3rd flush (%)	Yield (%)	Biological efficiency (%)	Number of mushrooms (Uni)	Weight of mushroom (g)
Crop 1							
Rice	8.51A	6.2 A	3.58	18.3 A	54.91 A	23.87 A	4.27
Wheat	2.79 B	6.45B	3.18	12.43 AB	37.31 AB	13.75 B	5.89
Brachiaria	6.22AB	0.95 B	0.73	7.91 B	23.73 B	9.75 B	6.73
Mean	5.84	4.53	2.49	12.88	38.65	15.79	6.63
DMS	4.81	5.24	3.37	6.79	20.37	9.39	3.08
Crop 2							
Rice	10.87	8.45 A	2.06	21.39 A	64.18 A	24.12 A	6.26
Wheat	5.47	7.91 AB	1.16	14.56 B	43.68 B	14.62 B	6.24
Brachiaria	5.32	2.66 B	1.85	9.84 B	29.52 B	8.75 B	8.11
Mean	7.22	6.34	1.69	15.26	45.79	15.83	6.87
DMS	6.06	5.61	2.70	6.29	18.87	8.74	3.76

Means followed by the same letters in the column do not differ statistically according to the T-test (LSD) at 5% probability level. 1F – 1st flush yield, 2F – 2nd flush yield, 3F – 3rd flush yield.

was calculated by dividing the fresh weight of mushrooms by the fresh weight of the substrate. Biological efficiency was obtained by dividing the fresh weight of mushrooms by the dry weight of the substrate. These two values are presented as a percentage. The number of mushrooms was obtained from the daily count and the sum of all mushrooms collected. The average weights of the mushrooms were obtained by dividing the total weight of fresh mushrooms by the number of mushrooms collected.

Mineral analysis

The contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) were measured in the substrates before and after the mushroom crop cycle. Three samples for each substrate were collected and analyzed following the methodology adapted previously [25]. To evaluate the total N, the titration method in a semi-micro-Kjeldahl distiller was used. To evaluate P and S, vanadate (VO_3^-) colorimetry and barium sulphate ($BaSO_4$) gravimetry were used in a spectrophotometer (at 420 nm). For K, Ca and Mg, the contents were obtained by $HClO_4$ (perchloric) + HNO_3 (nitric acid) digestion and analyzed by atomic absorption spectrophotometry (3300 AAS, Thermo Scientific™, USA). Data were expressed in g of mineral kg^{-1} of the sample.

Statistical analysis

Analysis of variance (ANOVA) was applied to the results, and the means were compared by least significant difference (LSD) tests at 5% probability, using SISVAR 5.7 software [26]. Pearson's correlation was also performed between the nutrient contents of the substrate and the agronomic parameters using the statistical Statgraphics Centurion software, and considered significant when the P value was less than 0.05.

RESULTS

As regards agronomic parameters, a similar effect was observed in the two crop cycles (Table 1). Rice straw showed superior results, with the obtained values of 18.30% and 21.39% for the yield, 54.91% and 64.18% for the biological efficiency and 23.87 and 24.12 for the number of mushrooms (uni) in crops 1 and 2, respectively. Wheat straw produced 12.43% and 14.56% yields, 37.31% and 43.68% biological efficiency and 13.75 and 14.62 uni mushrooms. Brachiaria straw produced the lowest values, 7.91% and 9.84% yields, 23.73% and 29.52% biological efficiency and 9.75 and 8.75 uni mushrooms. The weight did not exhibit a significant difference for any parameter studied. Rice straw had the highest yield, which was mainly linked to the 1st flush (8.51% and 10.87% for crops 1 and 2). Wheat straw produced 2.79% for crop 1 and 5.47% for crop 2, and brachiaria straw 6.22% for crop 1 and 5.32% for crop 2.

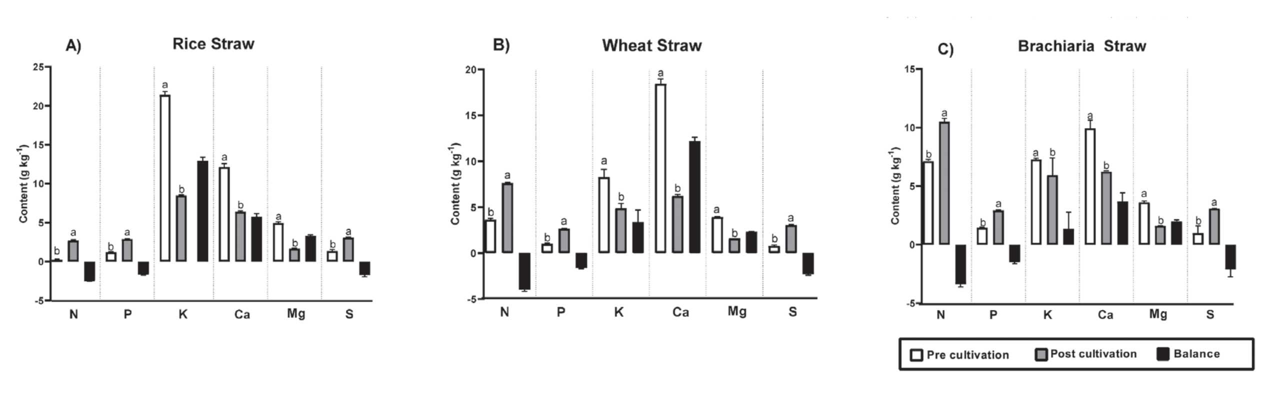


Fig. 1. Nutrient content and balance in substrates before and after cultivation of *Pleurotus ostreatus* var. Florida in rice (**A**), wheat (**B**) and brachiaria (**C**) straw. Means followed by the same letters in the graph do not differ statistically according to the T-test (LSD) at 5% probability.

All straws offered lower levels of N, P and S before the cultivation of *P. ostreatus* var. Florida and these values increased in the post-cultivation substrate (Fig. 1). The N content found in rice straw before (0.23 g kg^{-1}) and after (2.67 g kg^{-1}) cultivation was much lower than that found for wheat straw (3.64 g kg^{-1} for pre-cultivation and 7.63 g kg^{-1} for post-cultivation) and brachiaria straw (7.14 g kg^{-1} for pre-cultivation and 10.5 g kg^{-1} for post-cultivation). P and S behaved similarly in the treatments for the two nutritional evaluations.

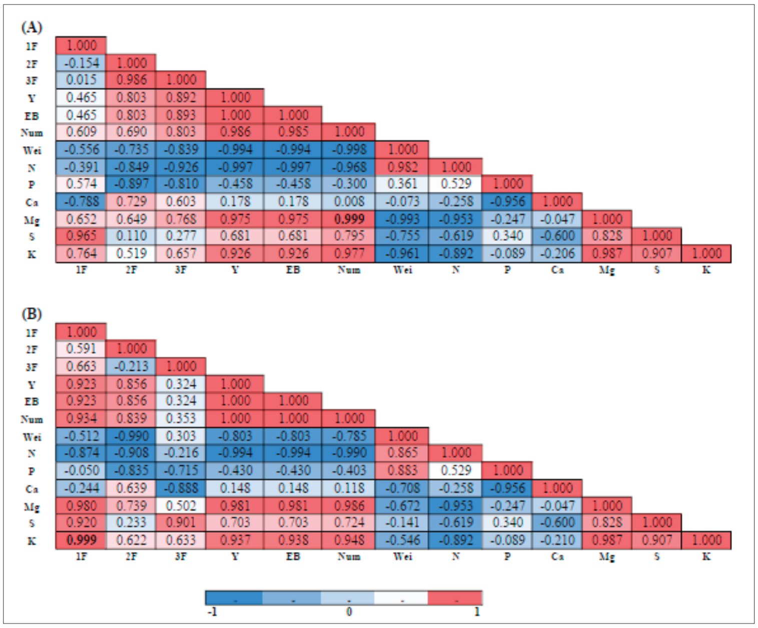
Fungal biodegradation under exposure to Ca, Mg and K cations was more intense for all substrates, which is presumably associated with the physiology and nutrition of the microorganism. Despite the lower N content, rice provided a high pre-cultivation K content (21.41 g kg^{-1}), which also resulted in high biodegradation (8.47 g kg^{-1}) and a 60.44% decrease in the K content of the substrate. It was assumed that K biodegradation was relevant to reach the highest yields (Table 1). A greater mineral decrease in *P. ostreatus* var. Florida was found in wheat straw for Ca (initially the straw contained 18.44 g kg^{-1} and after cultivation, the value was 6.23 g kg^{-1}, a reduction of 66.22%). However, biodegradation of Ca-containing substances in wheat straw does not correspond to the highest agronomic increments obtained (the yield obtained was 12.43% for wheat straw versus 18.3% for rice straw (Table 1)), demonstrating that Ca is less relevant than K.

Fig. 2 shows the values of the significantly negative correlation between the number of mushrooms and the weight of mushrooms (P=0.0408) for crop 1, and the positive correlation between the number of mushrooms and biological efficiency (P=0.0193) for crop 2. In both crop cycles, a positive correlation was found between yield and biological efficiency. A positive correlation between Mg and the number of mushrooms (P=0.0354) for crop 1 and a positive correlation between K and the 1st mushroom yield flush (P=0.0249) for crop 2 are reported.

DISCUSSION

The white-rot fungus *P. ostreatus* grows in various vegetable wastes from wood to straw. Considering the availability of straw from cereals and brachiaria, as well as the short period of growth compared to wood, we evaluated the production of oyster mushroom without the addition of supplements, considering the dynamics of nutrients at the beginning and at the end of cultivation. In order not to interfere in the degradation/absorption of these nutrients, we sterilized the substrate. Through an axenic production system, we verified which nutrients influence the agronomic parameters, aiming at a suitable substrate formulation.

The yield obtained in this research agrees with previous results [27] where the substrates based on rice straw and wheat straw were compared in the cultivation of *Pleurotus sajor-caju*, with the authors reporting yields for rice straw up to 10% higher than for wheat straw. In our study, rice straw produced a yield that was 47.22% higher than wheat straw.



(A)

	1F	2F	3F	Y	EB	Num	Wei	N	P	Ca	Mg	S	K
1F	1.000												
2F	-0.154	1.000											
3F	0.015	0.986	1.000										
Y	0.465	0.803	0.892	1.000									
EB	0.465	0.803	0.893	1.000	1.000								
Num	0.609	0.690	0.803	0.986	0.985	1.000							
Wei	-0.556	-0.735	-0.839	-0.994	-0.994	-0.998	1.000						
N	-0.391	-0.849	-0.926	-0.997	-0.997	-0.968	0.982	1.000					
P	0.574	-0.897	-0.810	-0.458	-0.458	-0.300	0.361	0.529	1.000				
Ca	-0.788	0.729	0.603	0.178	0.178	0.008	-0.073	-0.258	-0.956	1.000			
Mg	0.652	0.649	0.768	0.975	0.975	**0.999**	-0.993	-0.953	-0.247	-0.047	1.000		
S	0.965	0.110	0.277	0.681	0.681	0.795	-0.755	-0.619	0.340	-0.600	0.828	1.000	
K	0.764	0.519	0.657	0.926	0.926	0.977	-0.961	-0.892	-0.089	-0.206	0.987	0.907	1.000

(B)

	1F	2F	3F	Y	EB	Num	Wei	N	P	Ca	Mg	S	K
1F	1.000												
2F	0.591	1.000											
3F	0.663	-0.213	1.000										
Y	0.923	0.856	0.324	1.000									
EB	0.923	0.856	0.324	1.000	1.000								
Num	0.934	0.839	0.353	1.000	1.000	1.000							
Wei	-0.512	-0.990	0.303	-0.803	-0.803	-0.785	1.000						
N	-0.874	-0.908	-0.216	-0.994	-0.994	-0.990	0.865	1.000					
P	-0.050	-0.835	-0.715	-0.430	-0.430	-0.403	0.883	0.529	1.000				
Ca	-0.244	0.639	-0.888	0.148	0.148	0.118	-0.708	-0.258	-0.956	1.000			
Mg	0.980	0.739	0.502	0.981	0.981	0.986	-0.672	-0.953	-0.247	-0.047	1.000		
S	0.920	0.233	0.901	0.703	0.703	0.724	-0.141	-0.619	0.340	-0.600	0.828	1.000	
K	**0.999**	0.622	0.633	0.937	0.938	0.948	-0.546	-0.892	-0.089	-0.210	0.987	0.907	1.000

Fig. 2. Correlation between nutrients present in the straw (pre cultivation) and agronomic parameters for crop 1 (**A**) and crop 2 (**B**). 1F – 1st flush yield), 2F – 2nd flush yield, 3F – 3rd flush yield, Y – total yield, EB – biological efficiency, Num – number of mushrooms, Wei – weight of mushrooms, N – nitrogen, P – phosphorus, Ca – calcium, Mg – magnesium, S – sulfur, K – potassium. The data presented correspond to the value of r (correlation). *Data had a P value (probability) <0.05. Values between 0.6 and 0.8 have high correlation, values between 0.8 and 1.0 have extremely high correlation.

S is necessary for certain S-containing amino acids such as cysteine and methionine, the vitamins thiamine and biotin and for certain products of metabolism. The presence of P in adenosine triphosphate (ATP), nucleic acids and phospholipids of membranes point to the fundamental importance of this element in the living fungal cell. K has a role as a cofactor in some enzyme systems, it is involved in carbohydrate metabolism and is important in the maintenance of ionic balance. Mg is essential to all fungi since many enzymes are activated by Mg and it is also important in ATP metabolism [12]. Ca is required by green plants and is also a macronutrient relevant to fungi as it is involved in the transport of chemical, electrical and physical stimuli, in addition to exerting a protective and antagonistic effect [12,28,29].

The low availability of K considerably impairs yield and development, even when the availability of P and Mg is within normal limits, as it is the main mineral in the agricultural residues/wastes commonly used in the production of mushrooms [7]. According to the obtained results for the use of brachiaria straw as substrate, it is necessary to carry out a K-based supplementation for *P. ostreatus* var. Florida production, as was done [23] for substrates with peanut production residues. A positive correlation between the K present in the supplement and the weight of mushrooms was observed.

P is the most abundant mineral in *P. ostreatus* when using a sawdust-based substrate [13]. In a study with different solid residues for the cultivation of *P. ostreatus* var. Florida with substrates based on grass straw, K was predominant [28], similar to the results obtained herein for rice and wheat straw. However, after cultivation, K was the predominant nutrient only for rice straw, while for wheat and brachiaria straw, N was the most abundant nutrient.

Grass-based substrates have high mineral availability of Ca and K for oyster mushrooms, but they are not as efficient sources of P and Mg as sawdust-based residues [13,30]. Several recent works report that the combination of different residues for the formulation of substrates is the most suitable alternative for the best nutrient supply to the mushroom [31-33]. In Europe, the base substrate is wheat straw, while in Brazil, a mixture of brachiaria and sugarcane bagasse is used.

Considering the best yield obtained (rice straw), it was observed that the nutrient with the highest pre- and post-cultivation values was K, which is evidence of its importance in mushroom nutrition. K sources are commonly used in supplementation, both in spawning and in substrate formulation after wetting [34-36].

Finally, considering the biological efficiency, a positive point to be mentioned for rice straw is its lower environmental impact during post-cultivation (disposal of the spent mushroom substrate), since when cultivated in this substrate, *P. ostreatus* var. Florida degrades on average by 59.54% of the initial dry substrate, as compared to wheat and brachiaria straw which degrade on average by 40.49% and 26.62%, respectively; rice straw is more efficient, as corroborated by previous results [27].

CONCLUSIONS

Rice straw provided superior agronomic performance in relation to the analyzed variables, followed by wheat straw with intermediate values and brachiaria with the lowest values. Mineral analysis and Pearson's correlation demonstrated that Mg is an important nutrient for the number of mushrooms and K is important for the 1st flush yield in the cultivation of *P. ostreatus* var. Florida.

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Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: All data underlying the reported findings have been provided as part of the submitted article and are available at: https://www.serbiosoc.org.rs/NewUploads/Uploads/Iossi%20et%20al_8281_Data%20Report.pdf

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