



REVIEW ARTICLE

COMPARATIVE ANALYSIS OF THE PRODUCTIVITY OF TWO EDIBLE MUSHROOMS (PLEUROTUS EOUS AND PLEUROTUS OSTREATUS) ON TURF SUBSTRATE (FESTUCA OVINA) IN THE HAUT-SASSANDRA REGION (CÔTE D'IVOIRE)

*KOMENAN N'guessan Romain, SOKO Dago Faustin and KOTCHI Valère

Plant Production Improvement Laboratory, Agroforestry Department, Jean Lorougnon Guédé University
(Côte d'Ivoire)

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*Corresponding author:

KOMENAN N'guessan Romain

ABSTRACT

The use of various techniques for waste recovery in order to obtain value-added products is one of the objectives of sustainable development. One of the best ways to recover agricultural waste is to use it in mushroom cultivation. The objective of this study was to determine the colonisation and fruiting capacity of *Pleurotus eous* and *Pleurotus ostreatus*, cultivated on substrates based on grass clippings (*Festuca ovina*) and sawdust (control). The grass clippings were cut and dried in the sun for two weeks, then cut into pieces (2-3 cm). Agricultural lime and rice bran were added in varying proportions (1% agricultural lime and 0-15% rice bran) to obtain several formulations. These mixtures were then moistened (50-60% moisture content). The sawdust substrate consisted of 97% sawdust, 1% agricultural lime and 2% rice bran. This mixture was moistened to 85% moisture content and composted for two months. These different substrates were placed in heat-resistant bags and pasteurised for 2 hours and 30 minutes in a barrel. After cooling, the substrates were inoculated with *P. eous* and *P. ostreatus* mycelium. The parameters measured were mycelial growth and carpophore fruiting. The results show that both mushroom species completely colonise the sawdust substrate after 30 to 35 days. However, colonisation remains partial on the grass substrate after 50 to 60 days. In *Pleurotus eous*, production on the grass substrate varied from 4,199 to 5,268 g, compared to 1724 g for the sawdust substrate. The highest production was obtained with substrate F2, with a biological efficiency of 42%. In *Pleurotus ostreatus*, production varied from 1622 to 1936 g on the grass substrates, compared to 2625 g for the control. The highest yield was obtained on the turf formulations, with F2 producing 1936 g. Analysis of variance showed that the turf formulations had a significant effect on the fresh weight and diameter of the fruiting bodies. Disseminating these results to farmers is a way of adding value to agricultural waste and increasing their income.

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INTRODUCTION

Environmental protection is one of the pillars of sustainable development and is a major challenge for the future of humanity and the planet, considering that this protection is in the interests of future generations. It is also important for the well-being of the current generation. It is necessary to recycle the waste produced (Amrane & Belkacemi, 2017)¹. Waste is the residue of materials that are useless for life and can also be harmful to human health and the environment (Rania *et al.*, 2020)². The use of various recovery techniques aimed at mitigating risks and recovering value-added products (Amrane & Belkacemi, 2017)¹ must be the common objective. One of the best ways to recover agricultural waste is to use it in mushroom cultivation. This is because mushrooms play a key role in maintaining the ecological balance of biotopes and actively contribute to the formation of humus, which is essential for plant growth (Senn-Irlet *et al.*, 2012)³. As a result, they are considered to be the main recyclers of organic matter from plant materials (Lutzoni *et al.*, 2004)⁴. In addition, mushrooms are one of the largest producers of protein per unit of surface area and time (Kortei, 2011)⁵. In fact, in soilless cultivation, mushrooms extract nutrients from substrates (grass, wood and agricultural residues) through their mycelium to obtain the substances necessary for their development (Urban, 2004⁶; Ngezimana *et al.*, 2008⁷). This metabolic capacity of mushrooms operates through microbiological processes which, in order to achieve their greatest economic variability, must be controlled by optimal physical, chemical, environmental and technical processes/conditions (Zied *et al.*, 2020)⁸. Mushroom cultivation is an ecologically sustainable option. However, it is not widely practised and this aspect

of mycology remains under-explored in Côte d'Ivoire, and more specifically in Africa (Soko *et al.*, 2018)⁹. Despite the abundance of agricultural waste locally and the problem of malnutrition, large-scale mushroom production methods are incompatible for agricultural producers who lack the money to set up such businesses (Boa, 2006)¹⁰. However, these mushrooms are prized for their taste, nutritional value and therapeutic properties.

This is why selling fresh mushrooms is a very lucrative activity in local markets (Dibaluka *et al.*, 2010)¹¹. This study was conducted to contribute to the practice of sustainable agriculture. Its objective is to promote the use of waste from green spaces in the city of Daloa. According to Kouassi (2020)¹² green spaces in the city of Daloa are found in: primary schools, secondary schools, churches, mosques, vocational training centres, roundabouts, public and private administrations, garages, restaurants, hotels, the university, cemeteries, health centres, the municipal stadium, the public garden, homes and roadside areas. The maintenance of these areas produces enormous quantities of bio-waste such as grass clippings and other waste from public gardens. This can be used to produce edible mushrooms. At Jean Lorougnon Guède University, for example, grass clippings are usually cut, collected, discarded and burned, which contributes to greenhouse gas emissions. The aim of this study is to improve the productivity of edible mushroom species (*Pleurotus eous* and *Pleurotus ostreatus*) by assessing their ability to colonise and fruit on grass substrates, which constitute a significant amount of waste on the university campus.

MATERIALS AND METHODS

Study area: The study was conducted at Jean Lorougnon Guédé University (UJLoG) in Daloa. The city of Daloa is the capital of the Sassandra-Marahoué District in the Haut Sassandra region. The Daloa department is located approximately 410 km from Abidjan (economic capital) and 141 km from Yamoussoukro (political capital) (Anonymous, 2015¹³; Kouassi, 2020¹²). The municipality of Daloa has an average altitude of 273 m above sea level. It is located between 6°44' and 7° north latitude and between 6°40' and 6°48' west longitude (Figure 1). Precipitation data collected from the Airport, Aeronautical and Meteorological Operations and Development Company (SODEXAM) for the last fifteen (15) years indicate that the current climate is characterised by two seasons. These seasons are equatorial in nature. There is a rainy season that begins in March and ends in October, and a dry season that runs from November to February. The rainiest month is September, with an average rainfall of 148.02 mm, while the least rainy month is January, with an average rainfall of only 10.09 mm. Over the last 15 years, the current average rainfall has been 92.82 mm and the average temperature has been 26.5 °C, with a minimum of 24.77 °C in August and a maximum of 28.02 °C in February (Kouassi, 2020)¹².

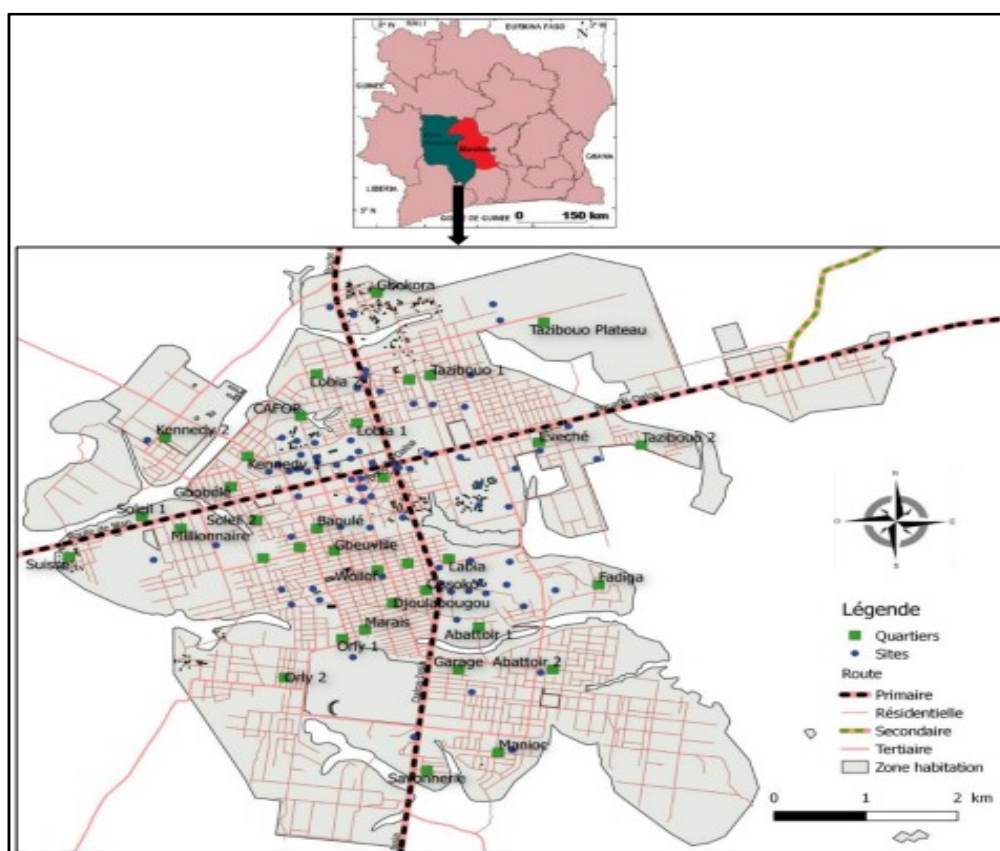


Figure 1. Geographical location of the city of Daloa (Kouassi, 2020)¹²

Materials: The fungal material consists of seeds or spawn from two species of edible mushrooms (*Pleurotus eous* and *Pleurotus ostreatus*) supplied by the plant improvement and production laboratory at Jean Lorougnon Guédé University. The plant material consists of sawdust substrate collected from sawmills in the city and grass clippings from the university grounds.

Methods: Two types of fruiting substrate were used to conduct this study. These substrates were grass clippings (lawn waste) collected from the university grounds and sawdust (control substrate) from the sawmill in the city of Daloa.

Preparation of the sawdust substrate: The sawdust was mixed with rice bran and agricultural lime in a ratio of 97% sawdust, 1% agricultural lime and 2% rice bran (Table 1). The mixture was then moistened to 60% humidity and piled up. This pile was covered with a black plastic sheet and left to compost for two months. Heat-resistant bags (30 cm x 17 cm) were filled with this substrate and weighed, at a rate of one kilogram of substrate by weight (1 kg), using a digital scale (ScoutTMpro; model: ScoutTM prospu602).

Table 1. Formulation of the different fruiting substrates

Formulations				
	F0	F1	F2	F3
Turf	0 %.	99 %.	94 %	84%
Sawdust	97 %	0 %.	0 %.	0 %.
Lime	1%	1%	1%	1%
Rice bran	2 %	0 %	5%	15 %
Moisture content	50-60	50-60	50-60	50-60
pH	7-8	7-8	7-8	7-8

Preparation of the turf substrate: The turf sheaves were cut and dried in the sun at a moisture content of 8% for two weeks. They were cut into pieces (2 to 3 cm long). This mixture was combined with 1% agricultural lime and rice bran in proportions ranging from 0 to 15% to obtain different formulations (Table 1). The different substrates were moistened and pressed to remove excess water. Heat-resistant bags (30 cm x 17 cm) were filled with this substrate and weighed, at a rate of one kilogram of substrate by weight (1 kg), using a digital scale (Scout TMpro; model: Scout TM prospu602). Finally, a multimeter (THREE-WAY METER) was used to measure the pH and moisture content of the different substrates (Table I). The bags were closed with a PVC pipe ring, covered with plastic film and secured with a plastic band.

Sterilisation and inoculation of substrates: The bags were sterilised with steam in barrels for 2 hours and 30 minutes at 100°C. Twenty-four hours after sterilisation, the bags were retrieved and placed in the inoculation room. The substrates were inoculated with the spawn of *P. eous* and *P. ostreatus* at a rate of two teaspoons per bag.

Experimental setup: The trial was conducted using a completely randomised factorial design. The first factor was the mushroom species, with two levels (*P. eous* (P1) and *P. ostreatus* (P2)). The second factor was the substrates, with four levels (F0, F1, F2 and F3). The combinations of different mushroom species and substrates resulted in 10 treatments, each consisting of 10 inoculated bags, for a total of 200 bags for the trial.

Data collection: The data collected relates to the chemical composition of the culture substrates on the one hand, and the growth and fruiting data of the fungi on the other.

Determination of the chemical composition of the substrates: The chemical analysis of the substrates was carried out using the ICPMS EDP (Inductively Coupled Plasma Mass Spectrometry EDP) method. This method involves determining the minerals using Agilent 5800 air-argon ICP OES (inductively coupled plasma optical emission spectroscopy). The values of macronutrients and micronutrients were calculated in mg/kg. Nitrogen was measured using the Kjeldahl method (AOAC, 1990) ¹⁴. The lignocellulosic fraction (cellulose, hemicellulose, and lignin) was determined using NDF (Neutral Detergent Fibre), ADF (Acid Detergent Fibre), and ADL (Acid Detergent Lignin). A pH meter (calibrated with a buffer solution at pH = 7 and pH = 4) was then introduced into each substrate mixture to determine the pH of the substrates (AFNOR, 1981) ¹⁵.

Parameters measured during incubation and fruiting: The bags were resealed and placed in incubation. During this stage, data such as the distances travelled by the mycelium front were measured every two days using a graduated ruler. After total colonisation of the substrates, they were transferred to the fruiting room. The bags were placed horizontally on aluminium shelves. The bags were opened with a knife and sprayed two to three times a day. The primordia and mature carpophores were counted and the survival rate of the primordia was assessed using the following formula:

$$TSP = ((\text{number of mature mushrooms} / \text{number of primordia}) \times 100)$$

The carpophores obtained were weighed using a precision balance (Scout TMpro; model: Scout TM prospu602). The biological efficiency (yield) of each substrate was assessed by multiplying by 100 the ratio of the fresh weight of the carpophores harvested per substrate to the dry weight of the substrate (Oei, 1993) ¹⁶, reported as a percentage. According to the following formula:

$$\text{Biological efficiency} = (\text{fresh weight of mushrooms (g)} / \text{dry weight of substrate (g)}) \times 100$$

The diameters of the carpophore caps and the lengths of the stipes obtained with different formulations were measured using a graduated ruler.

Data processing: The curve and histogram plots were created using Excel. R software, version 4.1.2 (2021-11-01), was used to perform a two-factor ANOVA test and Tukey's post-hoc test at a 5% significance level to highlight the differences between the parameters measured for the different formulations.

RESULTS

Chemical composition of production substrates

Chemical component of turf: The hydrogen potential value (pH = 6.90 ± 0.620) indicates that the turf substrate is almost neutral. The chemical composition of the turf substrate indicates that it contains polysaccharides, notably lignin (14.70 ± 3.24 mg/kg), cellulose (3.98 ± 0.23 mg/kg) and hemicellulose (14.70 ± 3.24 mg/kg). The high total carbon and nitrogen content resulted in a C/N ratio of 16.37. The substrate also contains macroelements and microelements in varying concentrations. Among the macroelements, the highest levels are found in calcium (80.13 mg/kg) and potassium (85.91 mg/kg), while the lowest level is found in phosphorus (2.00 mg/kg). As for microelements, the levels are very low, ranging from 0.07 mg/kg for iron to 1.32 mg/kg for zinc, except for manganese, which has a very high level (85.75 mg/kg). The macronutrients present in this substrate are in the following order: K > Ca > Mg > Na > P. The trace elements are in the following order: Mn > Zn > Fe. No heavy metals were detected in the turf substrate (**Table 2**).

Chemical composition of sawdust: Chemical analysis indicates that the pH of the substrate is neutral (pH 7.4) with a moisture content of 36% at the time of collection. Sawdust contains polysaccharides, including cellulose, lignin and hemicellulose in varying proportions. The C/N ratio is high (47%). Chemical analysis also reveals that sawdust contains many minerals, including macroelements and trace elements in varying concentrations. Among the macroelements, the sodium (Na) content is higher (126.54 mg/kg) but the phosphorus content is very low (2.25 mg/kg). For trace elements, the manganese content is higher (6.64 mg/kg) compared to 0.16 mg/kg for iron; however, no traces of heavy metals were found (**Table 2**).

Table 2. Chemical composition of grass clippings and rice bran

Lignocellulosic elements	Compounds	Turf	Sawdust	Rice bran
	Cellulose	3,98 ± 0,23 (mg/kg)	8,32 ± 0,23 (mg/kg)	11,89 ± 0,37 (mg/kg)
	Hemicellulose	14,70± 3,24 (mg/kg)	31 ± 1,29 (mg/kg)	9,12 ± 0,53 (mg/kg)
	Lignin	14,70 ± 3,24 (mg/kg)	3 1± 1,29 (mg/kg)	9,12 ± 0,53 (mg/kg)
chemical characteristics	Total carbon (TC)	41,25 ± 1,28 %	62, 047 ± 1,599 (%)	18,19 ± 0,10 %
	Organic carbon (OTC)	28,84 %	22,001 ± 0,023 (%)	47,42 ± 0,07 %
	Nitrogen (N)	2, 52 %	0,466 ± 2,590 (%)	1,68 %
	C/N ratio	11,44	47, 212	26,34
	pH	6,90 ± 0,62	7,47 ± 0,62	5,90
	Moisture	8 ± 0,47 %	35,66 ±13,57	8,94 ± 0,52
Macroelements	Magnesium (Mg)	59,59 ± 0,47 (mg/kg)	46,63 ± 0,47 (mg/kg)	42,241 ± 12,40 (mg/kg)
	Sodium (Na)	25,11 ± 9,92 (mg/kg)	126,54 ± 54,32 (mg/kg)	24,27 ± 1,07 (mg/kg)
	Potassium (k)	85,91 ± 0,04 (mg/kg)	57,91 ± 0,00 (mg/kg)	81,49 ± 0,00 (mg/kg)
	Phosphorus (P)	2,00 ± 0,00 (mg/kg)	2,25± 0,40 (mg/kg)	1,10 ± 0,36 (mg/kg)
	Calcium (Ca)	80,13 ± 0,47 (mg/kg)	51,55 ± 1,15 (mg/kg)	54,70 ± 1,34 (mg/kg)
Trace elements	Zinc (Zn)	1,32 ± 0, 20 (mg/kg)	1,57 ± 0,00 (mg/kg)	0,46 ± 0,30 (mg/kg)
	Copper (Cu)	0,00 ± 0,00 (mg/kg)	0,00 ± 0,00 (mg/kg)	0,00 ± 0,00 (mg/kg)
	Iron (Fe)	0,07 ± 0,01 (mg/kg)	0,16 ± 0,01 (mg/kg)	0,131 ± 0,11 (mg/kg)
	Manganese (Mn)	85,76 ± 9,92 (mg/kg)	6,64 ± 0,36 (mg/kg)	7,20 ± 0,00 (mg/kg)
Heavy metals	Lead (Pb)	-0,00 ± 0,00 (mg/kg)	0,000 ± 0,000 (mg/kg)	-0,00 ± 0,00 (mg/kg)
	Cadmium (Cd)	-0,00 ± 0,00 (mg/kg)	-0,002 ± 0,000 (mg/kg)	0,00 ± 0,00 (mg/kg)
	Mercury (Hg)	-0,00 ± 0,00 (mg/kg)	0,000 ± 0,000 (mg/kg)	0,00 ± 0,00 (mg/kg)
	Chromium (Cr)	-0,00 ± 0,00 (mg/kg)	0,000 ± 0,000 (mg/kg)	-0,00 ± 0,00 (mg/kg)
	Nickel (Ni)	0,00 ± 0,00 (mg/kg)	0,00 0 ± 0,000 (mg/kg)	-0,00 ± 0,00 (mg/kg)

Negative values indicate values below the device's detection limit, detection limit (0.0001) ppm

Chemical composition of rice bran: Rice bran is an acidic substrate (pH = 5.9) used as an additive to the main substrate (grass or sawdust). Rice bran is very rich in nitrogen (1.68%) and total organic carbon (47.42 ± 0.07%). The C/N ratio of rice bran is estimated at 26.34. Rice bran also contains polysaccharides, particularly cellulose (11.89 ± 0.37 mg/kg), which has a higher content than lignin (9.12 ± 0.53 mg/kg) and hemicellulose (9.12 ± 0.53 mg/kg). It also contains macroelements and microelements in varying amounts. The highest macroelement contents are observed for potassium (81.49 ± 0.00 mg/kg) and calcium (54.70 ± 1.34 mg/kg), with a particularly low phosphorus content (1.10 ± 0.36 mg/kg). The macroelements present in rice bran are in the following order: K > Ca > Mg > Na > P. As for microelements, the order of magnitude is Mn > Zn > Fe > Cu. No heavy metals were identified in rice bran (Table 2).

Height and growth rate of *P. ostreatus* and *P. eous* on turf substrates: There is variation in the date of appearance of the different mycelia. The different mycelia gradually colonise the substrates. Generally, the mycelium of *P. ostreatus* is observed from the fourth day of incubation. However, the mycelium of *P. eous* is observed from the seventh day of incubation. In *P. eous*, the greatest height of colonisation is observed on substrate F0 (20 cm for a colonisation period of 30 days). On this substrate, mycelial growth is regular and rapid. However, growth is relatively slow on grass-based substrates. Thus, *P. eous* had the lowest growth height on the F1 substrate (9 cm for a colonisation period of 60 days). Furthermore, in *P. ostreatus*, the greatest

colonisation height was observed on the F2 substrate (17.5 cm for a colonisation period of 60 days). The lowest growth height was obtained on substrate F4 (15 cm for a colonisation period of 60 days). It was also observed that these two species of fungus reach their highest colonisation heights after 30 days of incubation. After this date, colonisation is continuous (Figure 2).

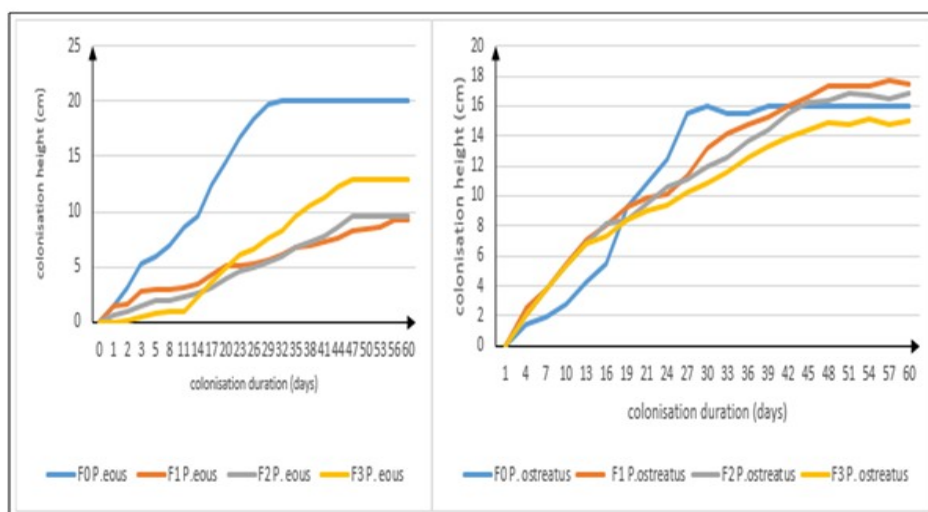


Figure 2. Evolution of the colonisation height of *P. ostreatus* and *P. eous* on different turf formulations

Effect of turf formulation on fruiting parameters: The two-factor ANOVA test conducted at a 5 % significance level indicates that the formulations have a significant effect on the fresh weight of carpophores (fw) and the average diameter of carpophores (dm). However, this effect is more significant on the diameter of carpophores (dm). Apart from these two parameters, the different formulations do not significantly influence the other fruiting parameters (Table 3).

Table 3. Effect of turf formulation on fruiting parameters

	Pri	avo	Ncr	pf	ls	dm	TSP
F0	27.63 ± 27.03	18.64 ± 18.61	8.55 ± 7.50	64.63 ± 64.14	4.41 ± 1.55	6.59 ± 2.57	39.18 ± 26.63
F1	34.55 ± 34.43	25.15 ± 25.04	9.46 ± 8.96	55.40 ± 47.24	4.04 ± 1.47	6.14 ± 2.44	39.18 ± 29.98
F2	30.82 ± 30.30	23.07 ± 23.00	7.82 ± 6.43	40.85 ± 34.90	3.87 ± 1.36	5.79 ± 2.76	39.83 ± 34.90
F3	24.99 ± 24.55	17.18 ± 17.05	7.36 ± 5.31	56.81 ± 37.00	3.95 ± 1.30	9.12 ± 2.84	37.12 ± 18.02
residual	1252	963.7	55.42	3962	2.114	6.91	891.9
Pr	0.24	0.376	0.345	0.049 *	0.05	0.001 ***	0.976

Meaning codes: 0 **** 0.001 *** 0.01 ** 0.05 * , 0.1 " " 1 avo: abortive fruit, Ncr: Number of carpophores harvested, Pf: fresh weight, dm: average diameter (cm), ls: stipe length (cm), TSP: primordium tracking rate and Pr: probability

Effect of mushroom variety on fruiting parameters: Analysis of variance at the 5% threshold indicates that both mushroom species have a significant effect on the number of primordia (Pri), the number of abortions (Avo), the number of carpophores harvested (Ncr), the diameter of carpophores (dm) and the survival rate of primordia (TSP). However, the mushroom variety has no significant effect on the fresh weight of carpophores (fw) and the length of stipes (lst) (Table 4).

Table 4. Effect of mushroom variety on fruiting parameters

	Pri	avo	Ncr	pf	ls	dm	TSP
<i>P. eous</i>	42.48 ± 38.92	29.24 ± 28.26	9.77 ± 8.55	52.63 ± 40.02	4.10 ± 1.61	5.65 ± 2.08	43.15 ± 34.28
<i>P. ostreatus</i>	21.43 ± 21.24	17.05 ± 13.89	7.28 ± 5.90	89.58 ± 59.23	4.10 ± 1.22	7.68 ± 3.11	35.51 ± 25.61
Residual	1222	970	57.1	4309	2.151	6.6	875
Pr	0.001 ***	0.001 ***	0.001 **	0.375	0.996	0.001 ***	0.0233 *

Meaning codes: 0 **** 0.001 *** 0.01 ** 0.05 * , 0.1 " " 1 avo: abortive fruit, Ncr: Number of carpophores harvested, Pf: fresh weight, dm: average diameter (cm), ls: stipe length (cm), TSP: primordium tracking rate and Pr: probability

Combined effect of formulation and mushroom variety on fruiting parameters: The two-factor ANOVA (substrate formulation* mushroom variety) performed at the 5% threshold shows that substrate formulation and mushroom variety significantly influence the fresh weight of carpophores (fw), the length of carpophore stipe (ls) and the survival rate of primordia (TSP). Furthermore, the combined action of these two production factors does not influence the number of primordia (avo), the number of abortions (avo), the number of carpophores harvested (Ncr), or the diameter of the carpophores (dm) (Table 5 and Figure 3).

Table 5. Effect of formulation and mushroom variety on fruiting parameters

	Pri	avo	Ncr	pf	ls	dm	TSP
residual	305028	389639	17766.5	1304165	646.36	1962.62	267288
Pr For -Variété	0.98	0.90	0.12	0.009465 **	0.005792 **	0.121	0.006201 **

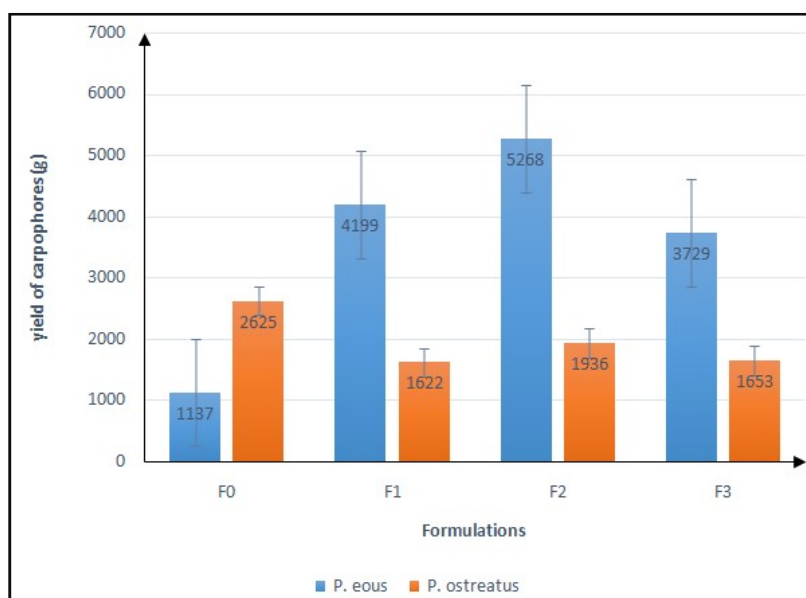
Meaning codes: 0 **** 0.001 *** 0.01 ** 0.05 * , 0.1 " " 1 avo: abortive fruit, Ncr: Number of carpophores harvested, Pf: fresh weight, dm: average diameter (cm), ls: stipe length (cm), TSP: primordium tracking rate, For : Formulation and Pr: probability



a = carpophores of *P. eous* that have fruited on a substrate of grass clippings; **b** = carpophores of *P. eous* that have fruited on a substrate of sawdust; **c** = carpophores of *P. ostreatus* that have fruited on a substrate of grass clippings; **d** = carpophores of *P. ostreatus* that have fruited on a substrate of sawdust

Figure 3. Fruiting of *P. eous* and *P. ostreatus*

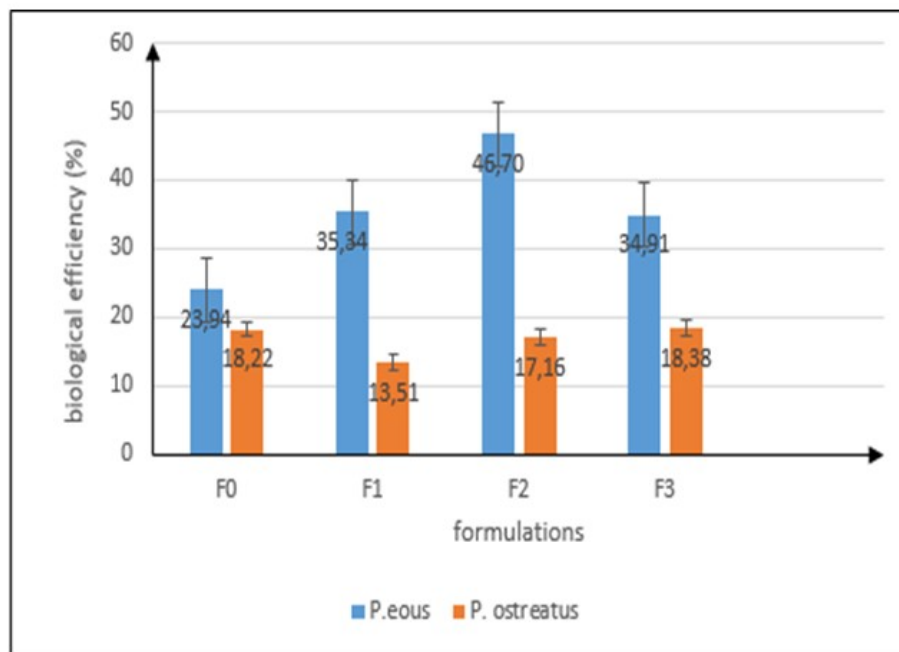
Total yield in fresh weight of carpophores for each substrate: The total yields in fresh weight of *P. ostreatus* obtained on the control substrate (F0) are higher than those of *P. eous*. On turf substrates, the production of *P. eous* is greater than that of *P. ostreatus*. These yields vary from 4,199 to 5,268 g. In contrast, the yields of *P. ostreatus* vary from 1,622 to 1,936 g. When 5% rice bran is added to the turf substrate, the highest yields are obtained for *P. eous* and *P. ostreatus*. However, production is higher for *P. eous*. The lowest production on turf substrates is observed on F1 (97% sawdust + 1% agricultural lime + 2% rice bran) for *P. ostreatus*. Whereas in *P. eous*, the lowest yield on grass substrates is obtained on substrate F0 (97% sawdust, 1% agricultural lime, 2% rice bran) (Figure 4).



F0 = 97% sawdust + 1% agricultural lime + 2% rice bran, **F1** = 99% turf + 1% agricultural lime + 0% rice bran, **F2** = 94% turf + 1% agricultural lime + 5% rice bran, **F3** = 89% Turf + 1% Agricultural lime + 10% Rice bran

Figure 4. Change in fresh fruit body yield of *P. eous* and *P. ostreatus* depending on the different substrates of turf and sawdust

Biological efficiency (BE): Biological efficiency is a parameter that is directly related to the fresh weight of the mushroom. It varies depending on the substrate formulation and the mushroom species. In *Pleurotus eous*, adding 1% to 5% rice bran improves the fresh weight production of carpophores. However, beyond a 10% addition of rice bran, production declines. The biological efficiency obtained with *Pleurotus eous* mushrooms grown on turf substrate varies from 35.34% to 46.70%, compared to 23.94% for the F0 sawdust control (97% sawdust, 1% agricultural lime, 2% rice bran). In general, biological efficiency (BE) remains lower (13.51% to 18.38%) on all substrates cultivated with *P. ostreatus* (Figure 5).



F0 = 97% sawdust + 1% agricultural lime + 2% rice bran, **F1** = 99% turf + 1% agricultural lime + 0% rice bran, **F2** = 94% turf + 1% agricultural lime + 5% rice bran, **F3** = 89% Turf + 1% Agricultural lime + 10% Rice bran

Figure 5. Biological efficiency of *P. eous* and *P. ostreatus* obtained on different substrates

DISCUSSION

Given the issue of the increasing use of sawdust as a substrate for oyster mushroom production, waste from cutting grass, which is present in large quantities on university grounds, could provide an additional substrate for mushroom cultivation and reduce the negative impact of waste on the environment. A possible solution is being explored by conducting trials to cultivate two species of oyster mushroom on turf substrates. To understand the behaviour of the two species of mushroom, *Pleurotus eous* and *Pleurotus ostreatus*, a chemical analysis of the substrates was carried out, revealing that the grass substrate has an almost neutral pH (6.90) and contains polysaccharides, notably cellulose, hemicellulose and lignin. According to the work of Mustin (1987)¹⁷, The polysaccharide content varies depending on the different plant groups. The various polysaccharides present in plant walls are sources of carbon that provide nutrition for fungi Tuomela *et al.* (2000)¹⁸ and Vane (2003)¹⁹ indicate that various microorganisms, particularly basidiomycetes, are involved in the degradation of these molecules in order to release simple compounds that can be assimilated by fungi. These authors believe that various enzymes are involved in the degradation of these polysaccharides, particularly manganese peroxidases and peroxidases. Our work reveals that the sawdust used as a control is composed of significant amounts of macroelements, including magnesium (62.78%), sodium (67.33%), potassium (78.46%), calcium (81.67%) and phosphorus, which has a low content. On this substrate, both species of fungus developed correctly with an incubation period of approximately 30 days. Our results are consistent with those of Kurzman & Zadrazil (1982)²⁰. These authors have shown that the chemical composition of the substrate affects the mycelial growth of various fungal species. According to Ceci *et al.* (2009)²¹, the type of substrate, environmental conditions and fungal species affect mycelial growth.

In general, macroelements (P, K, Mg and S) are necessary for the growth of many fungi (Miles & Chang, 1997)²² Molena (1986)²³ also emphasises the importance of calcium in mycelial growth Kurzman and Zadrazil (1982)²⁴ indicated that the mineral elements Na, Mg and Ca stimulate mycelial growth and carpophore formation Chang and Miles (1989)²² also indicate that potassium is very important for fungi because it is a cofactor in many enzyme systems. Microelements (Fe, Zn, Mn and Cu, with the exception of manganese) are present in trace amounts in both substrates and are also important for mycelial growth. In our study, *P. ostreatus* mycelium is generally observed from the fourth day of incubation, and *P. eous* mycelium is observed from the seventh day of incubation. This variability can be explained by the time it takes for the mycelium to transform certain compounds in the substrate into nutrients. Indeed, Zied *et al.*, (2020)⁸ showed that oyster mushrooms produce strands of mycelium that quickly attack plant tissue and break it down through the action of various hydrolytic and oxidative enzymes. For these two species of mushroom, as the amount of rice bran increases, the height of colonisation and carpophore production decreases. Furthermore, the growth rate remains significantly higher for the species *Pleurotus ostreatus* cultivated on different grass substrates. The addition of rice bran ranging from 0% to 10% is favourable for mycelial growth and carpophore production. Biological efficiency varies according to the substrate and the mushroom species. In *Pleurotus eous*, efficiency (EB) varies from 35.34% to 46.70%, while it varies from 13.16% to 18.28% in *Pleurotus ostreatus*. These results show that carpophore production is not linked to mycelial growth rate, but rather results from complex interactions between various environmental parameters, including temperature, mushroom species, humidity, etc. These values differ from those obtained by Bangala & Mpadi (2019)²⁵. These authors used banana flower stalks as the base substrate and wheat bran as an additive. This difference in results can be explained by the difference in the nature of the fruiting substrate.

CONCLUSION

This study shows that turf substrate contains numerous polysaccharides, notably cellulose, hemicellulose and lignin. It also contains macroelements and microelements in varying quantities. No heavy metals were detected in this substrate. This study has shown that the turf covering almost the entire Jean Lorougnon GUEDE University area is an excellent substrate for cultivating the mushroom species *Pleurotus ostreatus* and *Pleurotus eous*. The incubation period for both species is approximately 60 days, compared to 30 days when cultivated on sawdust. The *P. eous* species is more suitable, with biological efficiencies ranging from 35.34% to 46.70%, compared to 13.16% to 18.28% for *P. ostreatus*. The best formulation selected is the combination of *P. eous* cultivation on the F2 formulation (94% grass, 1% agricultural lime, 5% rice bran).

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