

# Cultivation trial of *Pleurotuseous* and *Pleurotustreatus* mushrooms on rice straw (*Oryza sativa* L.) in Daloa (Côte d'Ivoire)

## ABSTRACT

In Côte d'Ivoire, rice growing plays an important nutritional and economic role. However, rice fields also produce huge quantities of waste (rice straw) which is often not used or used very little and in some cases burnt; yet the valorisation of this agricultural waste can increase profitability. It is in this context that this study used *P. eous* and *P. ostreatus*, two edible mushroom, to bio-delignify rice straw and produce edible carpophores. To this end, the stems and leaves of oriza sp were sun-dried for a fortnight and cut into pieces (2-3cm). Agricultural lime and rice bran were added in varying proportions (1% = agricultural lime; 0-15% = rice bran) to obtain several formulations. The substrates were moistened and packaged in heat-resistant bags. The various substrates were then sterilised and inoculated with spawn from *P. eous* and *P. ostreatus* semis. The results showed that the mycelial filament of *P. eous* was observed three (3) days after inoculation. On the other hand, there were three dates (3rd, 9th and 21st day) of appearance of the mycelial front in *P. ostreatus*. The incubation period for *P. ostreatus* ranged from 51 to 57 days, while that for *P. eous* was 52 days. The addition of rice bran in increasing doses reduced the colonisation rate of both species of fungus. The lowest values of biological efficiency were obtained by growing *P. eous*. They ranged from 0% (F4) to 6% (F3). In the case of *P. eous*, the addition of rice bran in increasing doses increased the carpophore yield. But beyond 10%, the yield became zero. In the case of *P. ostreatus*, the yield became low when rice bran was added in increasing doses. This study confirms that rice field waste (rice straw) can be a raw material for the production of edible mushrooms. These results should be disseminated to the general public in order to increase the profitability of rice growers.

Keywords: *Pleurotus eous*, *P. ostreatus*, rice straw, substrate, carpophores, edible mushroom, biological efficiency

## 1. Introduction

In sub-Saharan Africa, people's food needs are far from being met despite the efforts made in the livestock and agriculture sectors [1]. To meet these demands, agriculture and food systems will have to adapt to the negative effects of climate change and become more resilient, productive and sustainable. This is the only way to guarantee the well-being of ecosystems and rural populations, while reducing greenhouse gas emissions [2]. Every year, people get rid of all kinds of agricultural waste. Rubbish accumulated over weeks and months ends up decomposing on the spot, a source of disease [3]. In Côte d'Ivoire, for example, several crops produce bio-waste, particularly rice. Côte d'Ivoire is the second largest producer of paddy rice in the UEMOA (West African Economic and Monetary Union), with more than 700,000 tonnes, and the third largest producer in the ECOWAS (Economic Community of West African States) region [4]. This production is accompanied by agricultural waste. Some straw is left in the rice field after harvesting; some is left to decompose, while others are burnt. This produces smoke, which contributes to the greenhouse effect. Very little of this straw is used to make traditional mattresses. The capacity to use these residues is limited [5]. It is therefore essential to introduce effective methods for recovering these residues and transforming them into other useful products [6]. The development of new crops such as edible mushrooms can improve the quality of people's diets and reduce unemployment and poverty [7]. In soilless cultivation, the fungus extracts nutrients from the substrate (grasses, wood and agricultural waste) through its mycelium to obtain the substances it needs for its development [8] & [9]. Mushroom cultivation is linked to the transformation of agricultural

and agro-industrial waste into food of high nutritional value. This metabolic capacity of mushrooms is achieved through microbiological processes which, to achieve their greatest economic variability, must be controlled by optimal physical, chemical, environmental and technical processes/conditions [10]. Yet this cultivation is little practised and this aspect of Mycology remains under-explored in Côte d'Ivoire and more specifically in Africa [11]. Moreover, the genus *Pleurotus* is an edible mushroom with high nutritional value, easy growth on substrate and good development in rustic conditions [12]. It is easily grown on a wide variety of agricultural residues, such as straw, grass, sawdust, coconut husk, maize seed, sugarcane bagasse and others of an organic nature. This excellent development is due to the production of certain lignocellulosic enzymes that allow easy degradation of lignin and cellulose from wood, as well as other plant substrates used for this particular crop [12]. The aim of this work is to improve the productivity of the fungi *P. eous* and *P. ostreatus*, through different formulations of rice straw.

## **2. MATERIALS AND METHODS**

### **2.1 Study sites**

The department of Daloa lies between latitude 6°53'58" North and longitude 6°26'32" West. The department covers an area of 15,205 km<sup>2</sup> and has an estimated population of 705,378. The site is located at the Jean Lorougnon Guédé University and is subject to the same climatic characteristics as the study area. The University is located to the north-east of the town of Daloa. It lies between latitude north (6°54') and longitude west (6°26'), covering an area of around 415 hectares. It is influenced by a humid tropical climate, with rainfall ranging from 1,200 to 1,600 millimetres per year [13]. Temperatures range from 25°C to 28°C, with an average of 26.62 ± 1.02°C. Relative humidity varies from 73 to 84%, with an average of 79.83 ± 4.12% [14] (Figure 1).

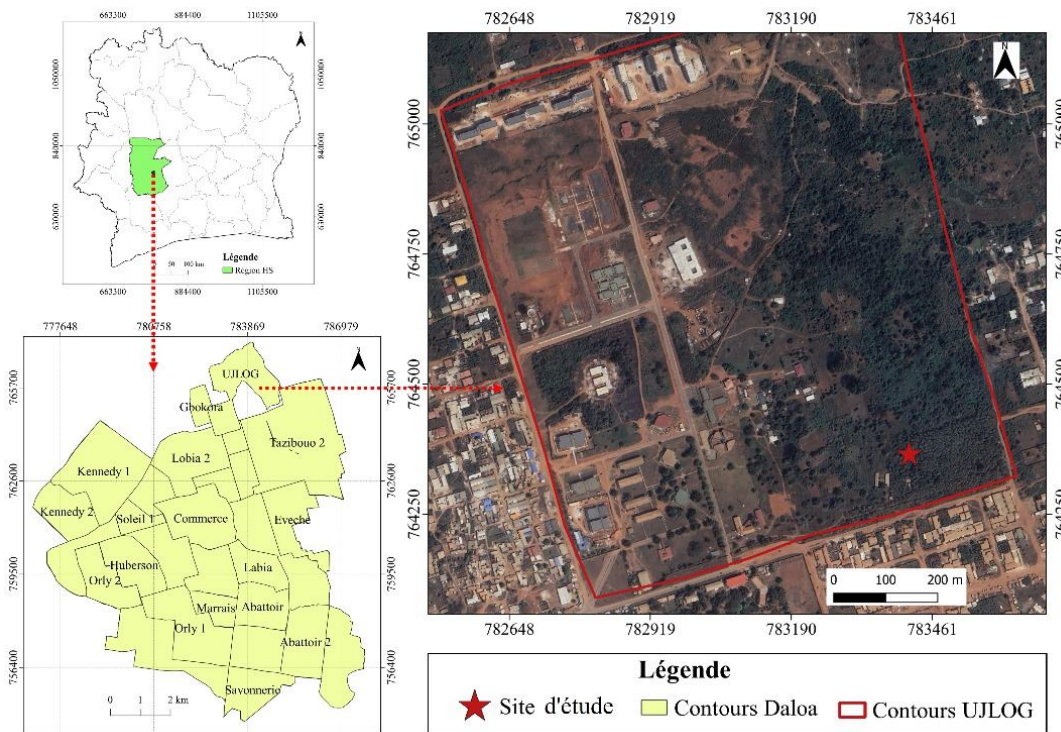


Fig. 1. Map of the study site [14]

## 2.2 Materials

### 2.2.1 Biological materials

Rice straw, rice bran and sawdust (organic plant matter) were used as substrates for fruiting carpophores. The spawns of *P. ostreatus* and *P. eous* were supplied by the Jean Lorougnon Guédé University in Daloa.

## 2.3 Methods

### 2.3.1 Preparation of the rice straw substrate

The stems and leaves of *Oriza* were collected from a ricefield on the study site after the harvest period, dried in the sun for a fortnight and cut into 2-3 cm pieces. Agricultural lime and rice bran were added in varying proportions (1% agricultural lime; 0-15% rice bran) to obtain several formulations of culture medium for the different mushrooms. The substrates were moistened, immersed in a barrel and boiled for 1h 30min. The

substrates were drained using a metal drainer for 6 hours and packed in heat-resistant bags (30 cm x 17 cm) at a rate of one kilogram of substrate per bag. The moisture content and pH of the substrate were determined using the THREE-WAY METER.

### 2.3.2 Preparation of the sawdust substrate

In our trials, the sawdust substrate is the reference or control substrate (F0). The sawdust was collected from a local sawmill. It is the main substrate used by all oyster mushroom producers in Côte d'Ivoire. It is used in the following proportions (97% sawdust, 1% agricultural lime and 2% rice bran). The mixture is moistened to a level of 85-90%, placed in a pile and covered with black plastic. Every three days, using a shovel, the mixture is turned over to speed up the decomposition process and ensure that the substrate is completely cooled. The moisture content and pH of the substrate were determined at the end of the composting process. The substrate was packed in heat-resistant bags at a rate of one kilogram per bag (30 cm x 17 cm) using a digital scale (ScoutTM pro; model: ScoutTM pro spu602) and then sterilised in a steam barrel for 2 hours. After cooling the sachets for 24 hours, they were transferred to a room for inoculation.

Table 1. Composition of fruiting substrates

Compositions	Formulations				
	F0	F1	F2	F3	F4
Sawdust	97%	%	0%	0%	0%
Rice straw	0%	99%	94%	89%	84%
Rice bran	2%	0%	5%	10%	15%
Agricultural lime	1%	1%	1%	1%	1%
Moisture content	50 % 60%	50 % 60%	50% 60%	50 % 60 %	50% 60%
pH	7-8	7-8	7-8	7-8	7-8

#### 2.3.4. Inoculation of the various substrates

Inoculation of the substrates consisted of sprinkling two tablespoons of spawn of *P. eous* and *P. ostreatus* onto the substrates. The bags were then sealed with a ring of PVC tubing and covered with plastic film, then held in place with a plastic strap.

#### 2.3.5 Incubation

Incubation is the stage during which the mycelium invades the substrates. This stage takes place in a dark room. The bags are placed vertically on shelves designed for this purpose. During the colonisation process, a number of parameters were measured and others estimated, including:

- Colonisation height :

The colonisation height is the distance covered by the mycelium front on the substrate.

This height was measured using a graduated ruler from the point of inoculation to the front of the mycelium. This value was used to determine the colonisation rate of the mycelium on the substrates. The following formulae were used to calculate these parameters.

- Mycelium invasion time or incubation time

This measurement is determined after the mycelium has colonised the entire bag,

- The colonisation rate

The colonisation rate (CT) was determined by the following formula

$$TC = d \times 100 / LS$$

TC = colonisation rate, d = colonisation height and LS = bag length

#### 2.3.6 Fructification

Once the substrates had been fully colonised, they were transferred to the fruiting room. In this room, the bags were placed horizontally on top of each other on shelves and opened with a knife. The room was watered twice or three times a day to increase the relative humidity and encourage the appearance of primordia. A number of fruiting parameters were measured, including :

- Cap diameter

The diameters of the caps of the *Pleurotus oesus* and *Pleurotus ostreatus* mushrooms in the different formulations were measured using a graduated ruler.

- Biological efficiency (%)

Biological efficiency, also known as yield, was calculated by multiplying by 100 the ratio of the fresh weight of the carpophore or total harvest to the dry weight of the substrate.

$$\text{Biological efficiency (\%)} = (\text{fresh weight of the carpophores} / \text{dry weight of substrate}) * 100$$

- Survival rate of primordia (TSP)

The primordia and mature carpophores were counted and the survival rate of the primordia was assessed using the following formula:

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

### 2.3.7 Data processing

Curves and histograms were plotted using Excel. R software version 4.3.2 (2021-11-01) was used to perform the two-factor ANOVA test at the 5% threshold.

## 3. RESULTS AND DISCUSSION

### **3.1 RESULTS**

#### **3.1.1 Assessment of the growth height of *P. ostreatus* and *P. eous***

#### **3.1.2 Growth height of *P. ostreatus* on rice straw substrate**

Mycelial growth of *P. ostreatus* occurred in several ways. On culture media F1, F2 and F4, exponential growth was observed from the beginning to the end of incubation. However, there was a variation in the date of appearance of the colonisation height. On substrate F2, it was observed on day 9. On the other hand, it appeared earlier on the F1 substrate (the height of colonisation was observed just after the 3rd day of incubation). On the other hand, the mycelium front appeared late on culture medium F4 (after the 21st day of incubation). In addition, an exponential growth phase followed by a stable growth phase was observed on substrate F3. The exponential growth phase (between the 6th and 36th) is longer than the stable growth phase (between the 39th and 51st). All these colonisation heights are smaller than those obtained on the F0 substrate (sawdust, F0= 21 Cm) (Figure 2a).

#### **3.1.3 Growth height of *P. eous* on rice straw substrate**

On culture media F1, F2, F3 and F4, the mycelium fronts of *P. eous* evolved in the same way. An exponential growth of the mycelium had been observed during incubation. For all these substrates, the height of colonisation appeared from the third day of incubation. However, on culture medium F4, mycelial growth occurred in two phases. These phases are the exponential growth phase and the stable growth phase. The exponential growth phase occurs between day 3 and day 30 of incubation. Finally, the stable growth phase begins after the 30th day and ends on the 52nd day. Furthermore, the height of colonisation obtained by growing *P. eous* on an F0 substrate (sawdust) was the greatest (h= 19 Cm) (Figure 2b).

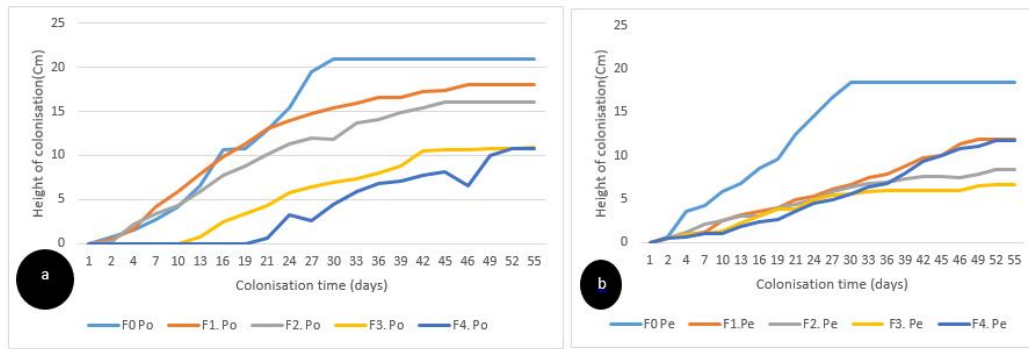


Fig. 2. Colonisation heights of *P. ostreatus* (a) and *P. eous* (b) on different straw formulations

(F1= 99% rice straw + 1% agricultural lime + 0% rice bran, F2= 94% rice straw + 1% agricultural lime + 5% rice bran, F3= 89% rice straw + 1% agricultural lime + 10% rice bran and F4= 84% rice straw + 1% agricultural lime + 15% rice bran).

### 3.1.4 Rate of colonisation

The rate of colonisation varies according to the substrate and the species of fungus. The highest colonisation rates are obtained by *P. ostreatus*. These rates vary from 50 to 100%. On the other hand, the lowest colonisation rates were observed with the species *P. eous*. These colonisation rates for *P. eous* ranged from 90% to 30%. Generally speaking, for these two species of fungus, the colonisation rate drops when the quantity of rice bran is increased. Thus, adding rice bran in increasing doses to a rice straw substrate reduces the colonisation rate of *P. ostreatus* and *P. eous*. But 5% rice bran is the dose needed to increase the rate of colonisation of *P. eous* on the rice straw substrate. Beyond this quantity, the addition of rice bran reduces the colonisation rate of *P. eous*. However, rice bran is not recommended when growing *P. ostreatus* on a straw substrate (Figure 4). *P. ostreatus* colonises the sawdust substrate better than *P. eous*. The best colonisation rates in this study were obtained when growing *P. ostreatus* and *P. eous* on sawdust substrate. Figures 3 and 4 illustrate this information.

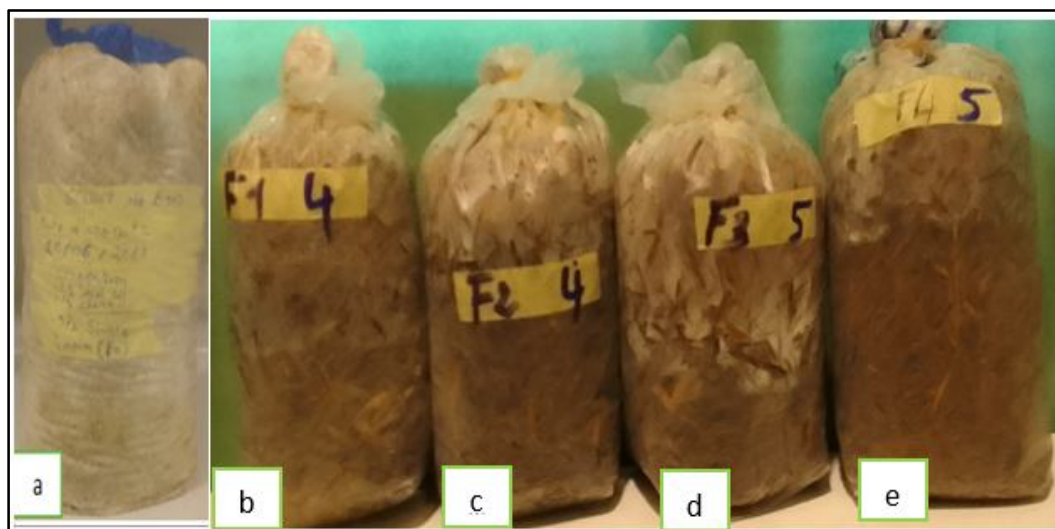


Fig.3.Colonisationof differentsubstrates

(a :F0= 99% Sawdust+1% Agricultural Lime+ 2% Rice Bran ; b : F1= 99% Rice Straw +1% Agricultural Lime+ 0% Rice Bran ; c : F2 = 94% Rice Straw + 1% Agricultural Lime + 5% Rice Bran ; d : F3= 89% Rice Straw + 1% Agricultural Lime + 10% Rice Bran and e : F4= 84% Rice Straw + 1% Agricultural Lime + 15% Rice Bran).

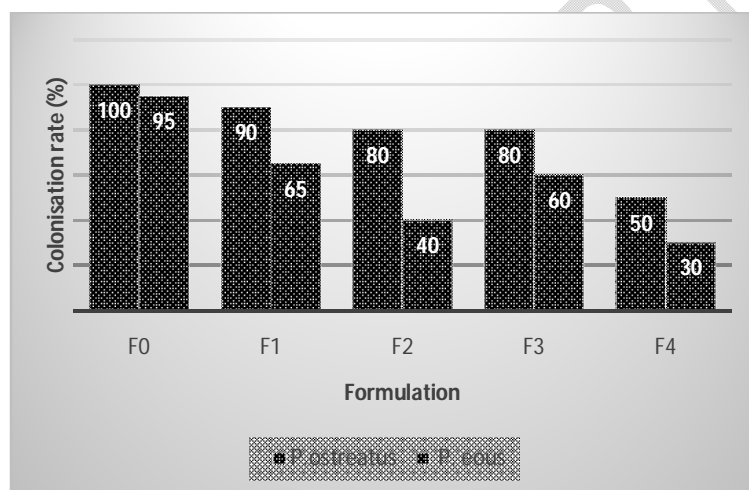


Fig. 4. Colonisation rates of *P. eous* and *P. ostreatus* on rice straw formulations

(Tc : Colonisation rate of *P.ostreatus*, Tc : *P.eous* colonisation rate," F0= 99% Sawdust+1% Agricultural Lime+ 2% Rice Bran F1= 99% Rice Straw +1% Agricultural Lime+ 0% Rice Bran, F2 = 94% Rice Straw + 1% Agricultural Lime + 5% Rice Bran F3= 89% Rice Straw + 1% Agricultural Lime + 10% Rice Bran and F4= 84% Rice Straw + 1% Agricultural Lime+ 15% Rice Bran).

### 3.1.5 Combined effect of formulation and mushroom variety on fruiting parameters

The two-factor ANOVA test performed at the 5% threshold gives the following information:

Apart from the number of carpophore harvested (Ncr), substrate formulation and mushroom variety have a significant effect on all other fruiting parameters. However, the combination of these two factors (Formulation Vs Variety) had no significant effect on the number of primordia (Pri) and runs (Avo). On the other hand, this combination does have a significant influence on the other fruiting parameters (number of carpophore harvested, stipe length, carpophore diameter, follow-up rate of).

Table 2. Combined effect of formulation and mushroom variety on fruiting parameters

	Fruiting parameters						
	pri	avo	Ncr	ls	dm	TVS	pf
formulation	0,001 ***	0,001***	0,177	0,001 ***	0,001***	0,007 **	0,001***
variety	0,023 *	0,014 *	0,293	0,001 ***	0,001 ***	0,516	0,001*** Pr
Formulation Vs variety	0,463	0,755	0,003 **	0,001**	0,001 ***	0,001 ***	0,7407
residual	82092,26	46891,26	14871,72	1138,82	1152,14	184829, 26	167245,26

Meaning codes: 0 \*\*\*\* 0.001 \*\*\* 0.01 \*\* 0.05 \* 0.1 \*\* 1

avo: runt, Ncr: number of carpophore harvested, Pf: freshweight, dm: averagediameter, ls: stipe length, TVS: primordial follow-up rate and Pr: probability of occurrence.

### 3.1.6 Biological efficiency

The two mushroom species (*P. ostreatus* and *P. eous*) were fruiting on the different substrates (Figure 6). Biological efficiency (BE) varies according to the mushroom species and the fruiting substrates. *P. eous* produced more carpophores than *P. ostreatus* on the F0 substrate. The lowest biological efficiency values were obtained by growing *P. eous* on the different substrates containing rice straw. These ranged from 0% substrate (F4) to 5% substrate (F3). For this mushroom, the addition of rice bran increases biological efficiency. But above 10% of rice bran, biological efficiency was zero. In the absence of rice bran in the rice straw substrate, EB reaches a maximum value of 30%. The addition of rice bran in

increasing doses significantly reduces EB, down to a value of 4% for an addition of 15% rice bran (Figure 7).

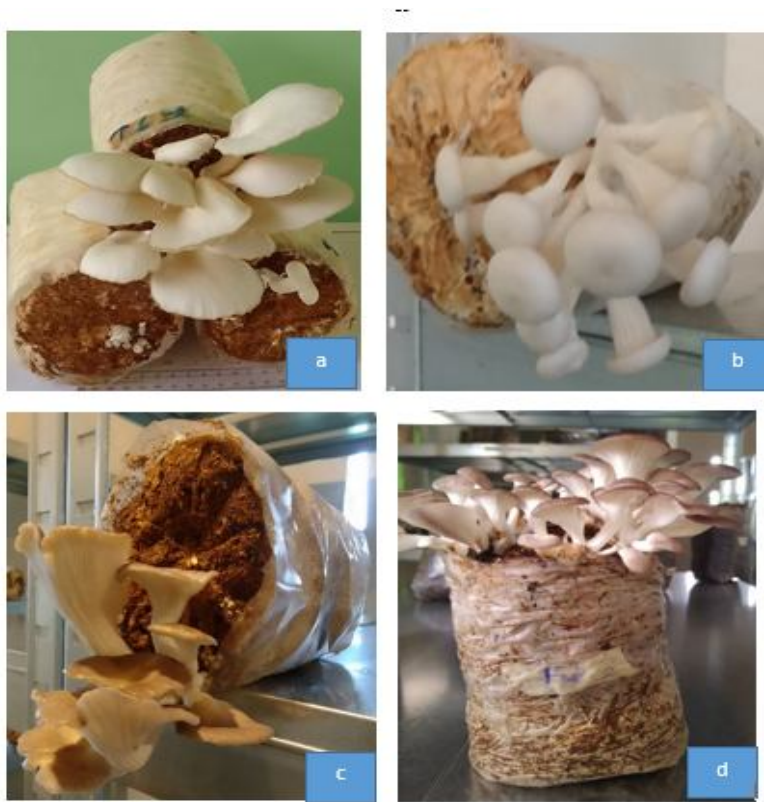


Fig. 5. Fructification of *P. eous* and *P. ostreatus* on different substrates

(a= photo of *P. ostreatus* on sawdust b= photo of *P. ostreatus* on rice straw C= photo of *P. eous* on sawdust d= photo of *P. eous* on rice straw)

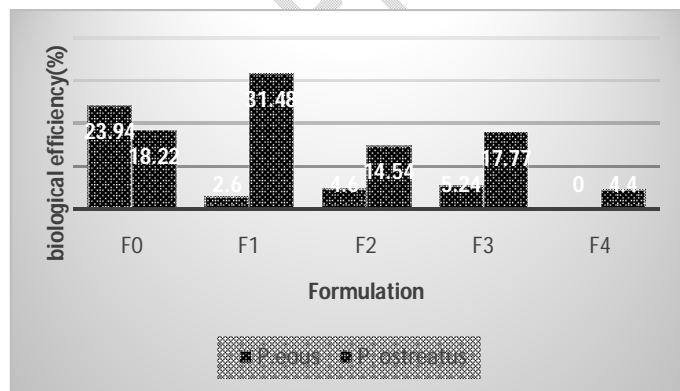


Fig. 6. Biological efficiency values for *P. eous* and *P. ostreatus* grown on different substrates

(F0= 99% sawdust + 1% agricultural lime + 2% rice bran, F1= 99% rice straw + 1% agricultural lime + 0% rice bran, F2 = 94% rice straw + 1% agricultural lime + 5% rice bran, F3= 89% rice straw + 1% agricultural lime + 10% rice bran and F4= 84% rice straw + 1% agricultural lime + 15% rice bran).

### 3.1.7 Correlation between different fructification parameters of *P. ostreatus*

Pearson's correlation coefficient  $r$  was used to assess the degree of association between the different variables studied. Some strong correlations were observed between the variables measured. It was noted that most of the correlation coefficients were low. Only the pairs pri-avo (0.80), ls-dm (0.96), pf-dm (0.80), TVS-dm (0.78), pf-ls (0.85) and TVS-ls (0.82) showed a strong positive value. This means that there is a correlation between these variables. The strongest correlation was obtained by the ls-dm pair (0.96) (Table III). Thus, the higher the number of primordia, the higher the number of runts. Also, the longer the stipes of the carpophores, the wider their caps and the larger the masses of the carpophores. Finally, the longer the stipes of the carpophores, the higher the follow-up rate of the primordia.

Table 3. Matrix of correlations between the eight variables measured in *P. ostreatus*

	avo	dm	EB	ls	Ncr	pf	pri	TVS
avo	1							
dm	-0.19	1						
EB	0.10	0.17	1					
ls	-0.13	<b>0.96</b>	0.16	1				
Ncr	0.35	0.45	0.34	0.56	1			
pf	-0.14	<b>0.80</b>	0.13	<b>0.85</b>	0.59	1		
pri	<b>0.80</b>	0.12	0.25	0.21	<b>0.76</b>	0.20	1	
TVS	-0.30	<b>0.78</b>	0.08	<b>0.82</b>	0.49	<b>0.72</b>	0.03	1

avo: runt, Ncr: number of carpophore harvested, Pf: freshweight, dm: averagediameter, ls: stipe length, TVS: primordial follow-up rate and EB: biological efficiency.

### 3.1.8 Correlation between different fructification parameters of *P. eous*

The degree of association between the different variables studied was estimated using Pearson's  $r$  correlation coefficient. Strong correlations were observed between the variables measured. The pairs ls-dm (0.96), pf-dm (0.80), pf-ls (0.85), pri-avo (0.85), pri-Ncr (0.76), TVS-dm (0.78), TVS-ls (0.72) and TVS-pf (0.78) have strong positive  $r$  values. There is a correlation between these variables. This means that the higher the number of primordia, the

higher the number of runts and the number of carpophores harvested. Also, when the mushroom has a long stipe, the diameter of its cap is long and the mushroom will have a large mass. In addition, when the survival rate of the primordia increases, the mushroom has a long stipe. Finally, when the survival rate is high, the mushroom has a broad cap. Table IV provides more details.

In summary, the pairs ls-dm, pf-ls, pri-avo, TVS-dm, TVS-ls, TVS-pf showed a strong correlation for both *P. eous* and *P. ostreatus*. But in *P. ostreatus* the r coefficient shows that the greater the number of primordia, the greater the freshweight of the carpophore. In *P. eous*, on the other hand, when the number of primordia is high, the number of carpophore is high. There is therefore a diversity of correlations between these two species of fungi.

Table 4. Matrix of correlations between the eight variables measured in *P. eous*

	avo	dm	EB	ls	Ncr	pf	pri	TVS
avo	1							
dm	-0.19	1						
EB	0.10	0.17	1					
ls	-0.13	<b>0.96</b>	0.16	1				
Ncr	0.35	0.45	0.34	0.56	1			
pf	-0.14	<b>0.80</b>	0.13	<b>0.85</b>	0.59	1		
pri	<b>0.80</b>	0.12	0.25	0.21	<b>0.76</b>	0.20	1	
TVS	-0.30	<b>0.78</b>	0.08	<b>0.82</b>	0.49	<b>0.72</b>	0.03	1

(avo: runt, Ncr: number of carpophore harvested, Pf: freshweight, dm: averagediameter, ls: stipe length, TVS: primordial follow-up rate and EB: biological efficiency)

### 3.2 Discussion

The colonization and fruiting capacity of two edible mushroom *P. eous* and *P. ostreatus*, were studied on different formulations in comparison with sawdust used as reference substrates in Côte d'Ivoire. Several formulations of rice straw-based substrates were inoculated with the spawn of *P. eous* and *P. ostreatus*. A variation in the date of appearance of the mycelium front on the different substrates was observed. The mycelial filament of *P. eous* was observed on all substrates three (3)

days after inoculation. On the other hand, there were three dates (3rd, 9th and 21st day) of appearance of the mycelial front in *P. ostreatus*. Thus, colonization of rice straw substrates depended on the species of mushroom and the substrate. Indeed, this diversity could be explained by enzyme production during the incubation phase. This assertion is corroborated by [15] who argue that, in some cases, high levels of additives are toxic to mycelial growth. In addition to the toxicity of the nutrients supplied, there is also nutrient competition. Contrary to these authors, [16] and [17] assert that the more nutrients are added to a substrate, the greater its susceptibility to infection, and the greater the competition between oyster mushrooms and contaminants; this competition then reduces the colonization speed of the fungus, hence the delay observed. Incubation times for *P. ostreatus* range from 51 to 57 days, and for *P. eous* from 52 days on all culture substrates. These mycelial invasion times differ from those obtained by [18]. These authors state that invasion times vary between 15-30 days. This difference could be explained by the choice of species and substrates. For these authors had grown *Lentinus sajor-caju* and *Pleurotus florida* on substrates based on Acacia pods, *Terminalia superba* sawdust, and sugarcane bagasse (*Saccharum officinarum*). The addition of rice bran in increasing doses to the rice straw substrate reduced the colonization rate of *P. ostreatus* and *P. eous*. This finding shows that the addition of rice bran was not at all favorable to mycelial growth. This point of view is also confirmed by [19]. Biological efficiency (BE) varies according to fungus species and growing medium. The lowest values of biological efficiency were obtained when growing *P. eous* on different substrates containing rice straw. These values ranged from 0% (F4) to 6% (F3). These values, of biological efficiency are different from those obtained by [18]. According to them, the yield is between 10 % and 12%. In fact, these authors had grown *Lentinus cladopus* on substrates based on sugarcane bagasse (*Saccharum officinarum*). These differences are simply a consequence of the choice of mushroom species and growing media.

#### 4. Conclusion

The aim of this study was to demonstrate the use of rice straw for the cultivation of edible mushroom. The mycelial filament of *P. eous* was observed on all substrates three (3) days after inoculation. In contrast, there were three dates (3rd, 9th and 21st day) for the appearance of the mycelial front in *P. ostreatus*. The lowest yields were obtained by growing *P. eous*. When rice bran content is low, carpophore production in *P. ostreatus* is higher than in the control (F0). On the other hand, in the same condition, *P. eous* carpophore production is very low compared with the control. The correlation coefficient  $r$  shows that the greater the number of primordia, the greater the fresh weight of the carpophore. In *P. eous*, on the other hand, a high number of primordia results in a high number of carpophores. There is therefore a diversity of correlations between these two species of fungi. In the context of this research, it is quite satisfactory to produce edible carpophores of *P. ostreatus* and *Pleurotus* from rice straw, thus adding value to yields. For this study, only *Pleurotus* and *P. ostreatus* were seeded on these different substrates. It would be wise to extend the study to other mushroom species. It would be wise to carry out biochemical analyses to determine the intrinsic quality of carpophores produced on rice straw substrates.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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