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Cultivation Trial of *Pleurotus eous* and *Pleurotus ostreatus* Mushrooms on Rice Straw (*Oryza sativa* L.) in Daloa, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 valorization 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 *oryza 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

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substrates were moistened and packaged in heat-resistant bags. The various substrates were then sterilized and inoculated with spawn from *P. eous* and *P. ostreatus*. 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 colonization 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 efforts in the livestock and agriculture sectors (FAO, 2015). 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 (FAO, 2017).

Every year, people get rid of all kinds of agricultural waste. Rubbish accumulates over weeks and months, decomposing on the spot and becoming a source of disease (Amani et al., 2019). 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 (FIRCA, 2011). 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 burned. 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 (Pérez et al., 2002; Wongamthing et al., 2022; Mubasshira et al., 2020).

It is therefore essential to introduce effective methods for recovering these residues and transforming them into other useful products (Wathumbe & Mada, 2020). The development of new crops such as edible mushrooms can improve the quality of people's diets and reduce unemployment and poverty (Gévry et al., 2009). 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 (Urben, 2004 & Ngezimana et al., 2008). 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 (Zied et al., 2020). Yet, this cultivation is little practiced, and this aspect of Mycology remains under-explored in Côte d'Ivoire and more specifically in Africa (Soko et al., 2018).

Moreover, the genus *Pleurotus* is an edible mushroom with high nutritional value, easy growth on substrate, and good development in rustic conditions (Boulmerka & Laoufi, 2017). 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 (Boulmerka & Laoufi, 2017).

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 km² 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 millimeters per year (Coulibaly et al., 2021). 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% (N'Guessan et al., 2024) (Fig. 1).

2.2 Materials

2.2.1 Biological materials

Rice straw, rice bran and sawdust (organic plant matter) wereused 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 *Oryza sativa* were collected from a rice field on the study site after the harvest period, dried in the sun for two weeks, 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 different mushrooms. The substrates were moistened, immersed in a barrel, and boiled for 1 hour and 30 minutes. The substrates were drained using a metal drainer for 6 hours and packed into 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 a 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 into 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 sterilized in a steam barrel for 2 hours. After cooling the sachets for 24 hours, they were transferred to a room for inoculation.

2.3.3 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.

2.3.4 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

Table 1. Composition of fruiting substrates

	Formulations				
Compositions	**F0**	**F1**	**F2**	**F3**	**F4**
Sawdust	97%	0%	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 %	50 %	50%	50 %	50%
	60%	60%	60%	60 %	60%
pH	7-8	7-8	7-8	7-8	7-8

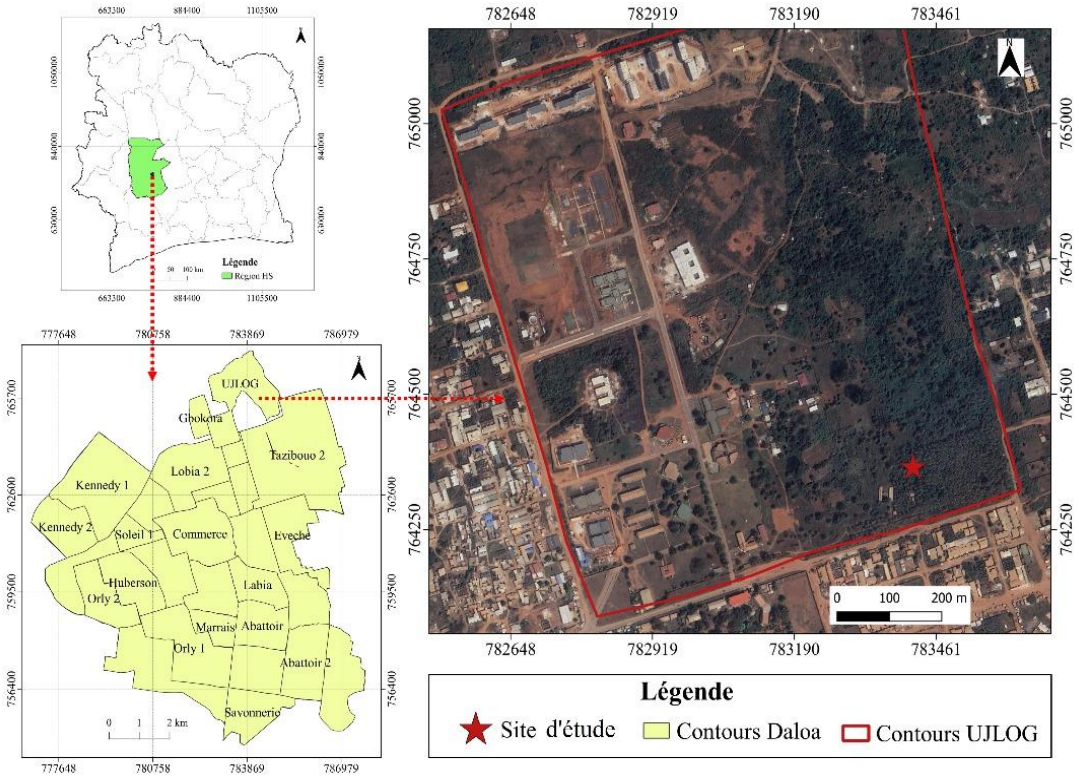

Fig. 1. Map of the study site (N'Guessan et al., 2024)

parameters were measured and others estimated, including:

- Colonization height: The colonization 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 colonization 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 colonized the entire bag,
- The colonization rate: The colonization rate (CT) was determined by the following formula

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

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

2.3.5 Fructification

Once the substrates had been fully colonized, 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 oeus* and *Pleurotus ostreatus* mushrooms in the different f ormulations 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.

Biological efficiency $(\%) = ($ fresh weight of the carpophores /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:

TSP (%) = (number of mature mushrooms / number of primordia)*100

2.3.6 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*

Results show growth of *P. ostreatus* and *P. eous* grown on rice straw substrate.

3.1.1.1 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 colonization height. On substrate F2, it was observed on day 9. On the other hand, it appeared earlier on the F1 substrate (the height of colonization 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 colonization heights are smaller than those obtained on the F0 substrate (sawdust, F0 = 21 cm) (Fig. 2a).

3.1.1.2 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 colonization 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 colonization obtained by growing *P. eous* on an F0 substrate (sawdust) was the greatest (h = 19 cm) (Fig. 2b).

3.1.2 Rate of colonization

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

3.1.3 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 runts (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.

3.1.4 Biological efficiency

The two mushroom species (*P. ostreatus* and *P. eous*) fruited on the different substrates (Fig. 5). Biological efficiency (BE) varied depending on the mushroom species and the fruiting substrates.

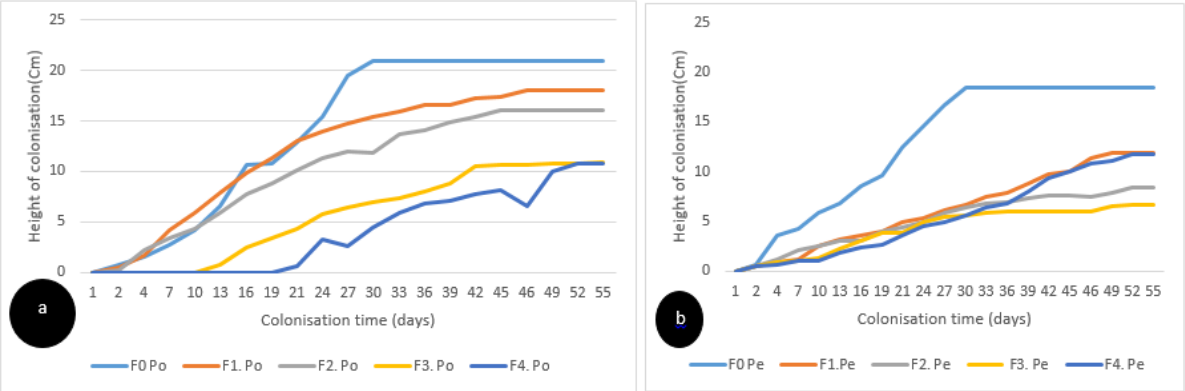

Fig. 2. Colonisation heights of *P. ostreatus* (a) and *P. eous* (b) on different straw formulations

(F0 = 97% Sawdust + 2% rice straw + 1% agricultural lime, 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)

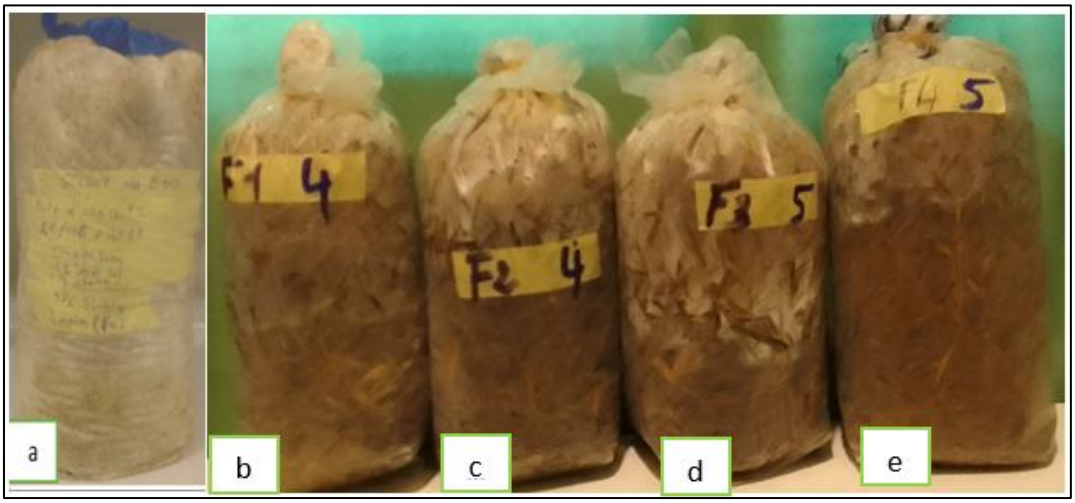

Fig. 3. Colonization of different substrates

(a :F0= 97 % 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)

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***
Formulation Vs variety	0,463	0,755	0,003**	0,001***	0,001***	0,001***	0,7407
residuel	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: fresh weight, dm: average diameter, ls: stipe length, TVS: primordial follow-up rate and Pr: probability of occurrence

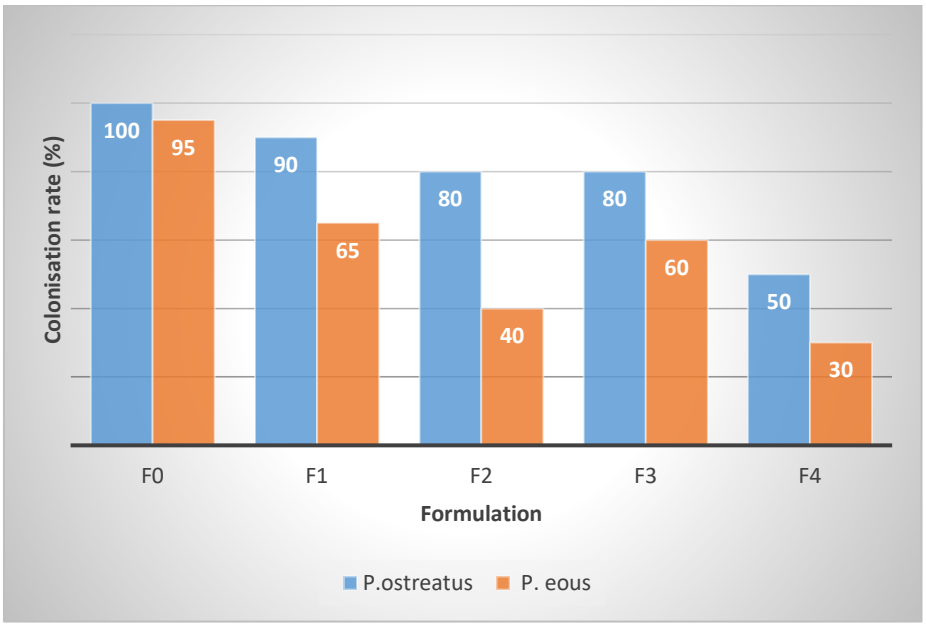

Fig. 4. Colonization rates of *P. eous* and *P. ostreatus* on rice straw formulations

(Tc : Colonisation rate of P.ostreatus, Tc : P.eous colonisation rate,¨ F0= 97 % Sawdust+1% Agricultural Lime+ 2% Rice Bran F1= 99% Rice Straw +1% Agricultural Lime+ 0% Rice Bran, F2 = 94% Rice Straw + % 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)

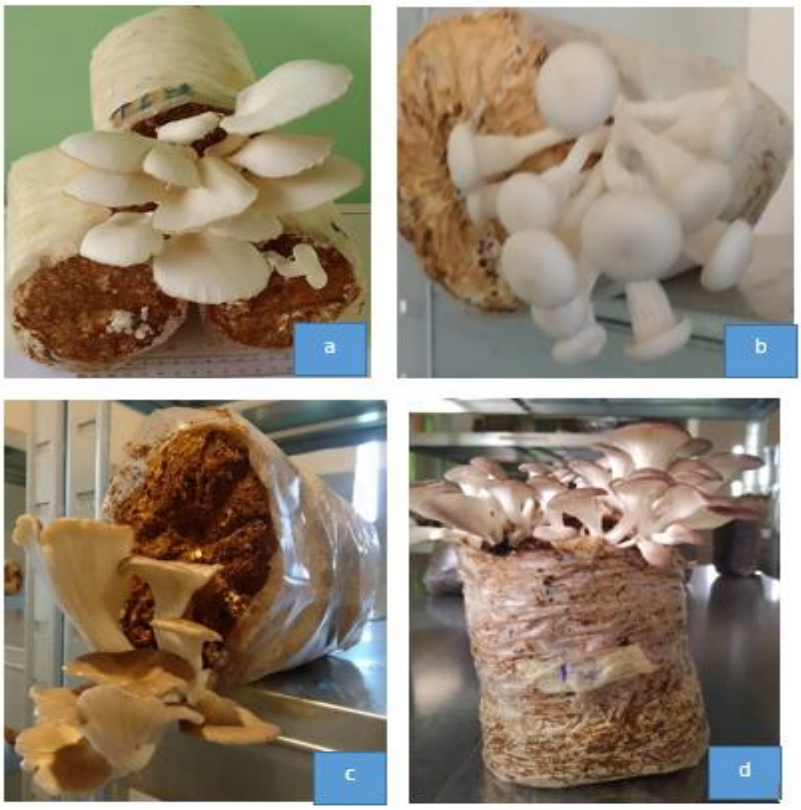

Fig. 5. Fructification of *P. eous* and *P. ostreatus* on differents substrates

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

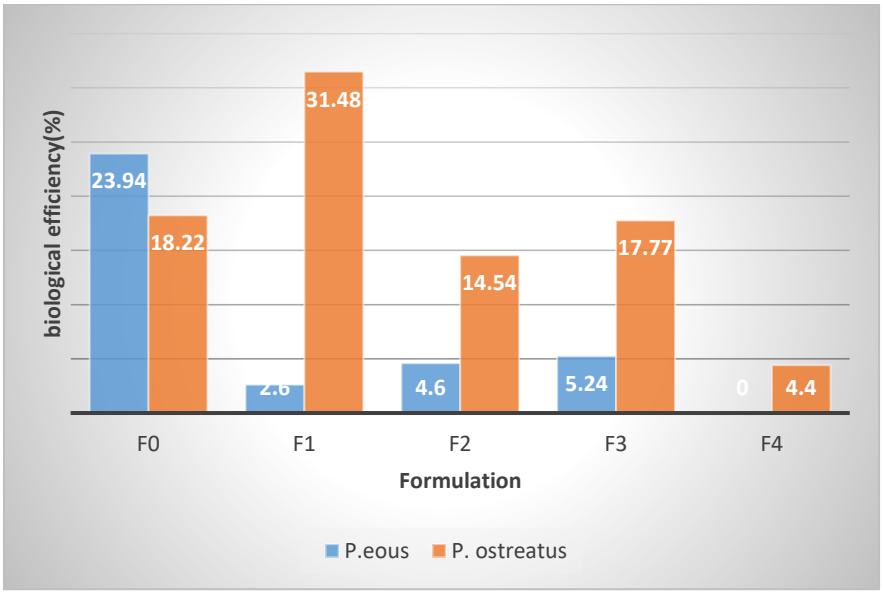

Fig. 6. Biological efficiency values for *P. eous* and *P. ostreatus* grown on different substrates
(F0= 97 % saw dust + 1% agricultural lime + 2% rice bran, F1= 99% rice straw + 1% agricultural lime + 0% rice bran, F2 = 94% rice straw + 1% agricultural lime + 5% rice bran, F3= 89% rice straw + 1% agricultural lime + 10% rice bran and F4= 84% rice straw + 1% agricultural lime + 15% rice bran)

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	0.96	0.16	1				
Ncr	0.35	0.45	0.34	0.56	1			
pf	-0.14	**0.80**	0.13	**0.83**	0.59	1		
Pri	**0.80**	0.12	0.25	0.21	0.76	0.20	1	
TVS	-0.30	**0.78**	0.08	**0.82**	0.49	**0.72**	0.03	1

avo: runt, Ncr: number of carpophore harvested, Pf: fresh weight, dm: average diameter, ls: stipe length, TVS: primordial follow-up rate and EB: biological efficiency

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

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

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

P. eous produced more fruiting bodies (carpophores) than *P. ostreatus* on the sawdust substrate (F0). However, the lowest biological efficiency values were obtained by growing *P. eous* on substrates containing rice straw. These ranged from 0% for the substrate with the highest rice bran content (F4) to 5 % for the one with 5% rice bran (F3). For *P. eous*, adding rice bran

initially increased biological efficiency, but adding more than 10% resulted in zero efficiency.

In contrast, the absence of rice bran in the rice straw substrate (F1) resulted in the highest BE for *P. eous* (30%). However, adding rice bran in increasing doses significantly reduced BE, dropping to 4% for a substrate with 15 % rice bran (F4) (Fig. 6).

3.1.5 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 measured variables. However, 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, indicating a correlation between these variables. The strongest correlation was observed between the ls-dm pair (0.96).

This suggests that a higher number of primordia is associated with a higher number of runts. Additionally, longer stipes of the carpophores were correlated with wider caps, larger masses, and a higher follow-up rate of primordia.

3.1.6 Correlation between different fructification parameters of *P. eous*

Pearson's correlation coefficient (r) was used to estimate the degree of association between the different variables studied. Strong correlations were observed between the measured variables. 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. This indicates a correlation between these variables.

This means that a higher number of primordia is associated with a higher number of runts and a higher number of carpophores harvested. Additionally, a longer stipe is correlated with a larger cap diameter and a greater mushroom mass. Furthermore, a higher survival rate of primordia is associated with longer stipes and broader caps. Table 4 provides more details.

In summary, the pairs ls-dm, pf-ls, pri-avo, TVS-dm, TVS-ls, and TVS-pf showed strong correlations for both *P. eous* and *P. ostreatus*. However, for *P. ostreatus*, the r coefficient indicates that a higher number of primordia is associated with a higher fresh weight of the carpophore. In contrast, for *P. eous*, a higher number of primordia is associated with a higher number of carpophores. This suggests a diversity of correlations between these two fungal species.

3.2 Discussion

The colonization and fruiting capacity of two edible mushrooms, *Pleurotus ostreatus* and *P. eous*, were studied on different formulations compared to sawdust, used as a reference substrate 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 different substrates was observed. The mycelial filament of *P. eous* was observed on all substrates three days after inoculation. In contrast, there were three different dates of appearance of the mycelial front for *P. ostreatus* (3rd, 9th, and 21st days).

Thus, the colonization of rice straw substrates depended on the mushroom species and the substrate. This diversity could be explained by enzyme production during the incubation phase. This assertion is corroborated by Dibaluka (2012), 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, Oei (1993) and Curvetto et al. (2002) 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, which reduces the colonization speed of the fungus, hence the observed delay.

Incubation times for *P. ostreatus* ranged 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 Makanua et al. (2015). These authors state that invasion times vary between 15-30 days. This difference could be explained by the choice of species and substrates. 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 favorable to mycelial growth. This point of view is also confirmed by Kiyuku et al. (2020).

Biological efficiency (BE) varied according to the 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 Makanua et al. (2015). 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 primary objective of this study was to explore the feasibility of using rice straw as a substrate for cultivating edible mushrooms, specifically *Pleurotus ostreatus* and *P. eous*.

Key Findings:

- **Mycelial Growth:**
  - *P. eous* exhibited faster colonization, appearing on all substrates within three days.
  - *P. ostreatus* showed varying colonization rates, with the mycelium front appearing on the 3rd, 9th, and 21st days on different substrates.
- **Fruiting and Yield:**
  - *P. eous* generally had lower yields compared to *P. ostreatus*.
  - *P. ostreatus* produced more carpophores when grown on rice straw substrates with low rice bran content, compared to the control (sawdust).
  - *P. eous* had low carpophore production on rice straw substrates, regardless of rice bran content.
- **Correlation Analysis:**
  - A strong positive correlation was observed between the number of primordia and the fresh weight of carpophores in *P. ostreatus*.
  - In *P. eous*, a high number of primordia led to a high number of carpophores.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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