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Influence of substrate formulation and *Pleurotus* spawn age on the growth and carpophore production in the locality of Allokoua (Côte d'Ivoire)

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Abstract
Since thousand years, the edible mushrooms are appreciated for their taste, their nutritional and therapeutic value by the populations. Besides, the trade of edible mushrooms is an important source of income for developing countries. The present work aims at improving the production of carpophore by the implementation of an adequate formulation of substratum of production of oyster mushroom. Seven substrata consisted of sawdust, its rice and some agricultural lime of variable composition were sterilized and inoculated with the spawn of 30 and 55 days old. The experimental design used is a device in two factors completely randomized with three repetitions. The parameters evaluated are the speed of growth of the mycelium on the substrata, the rate of survival primordia (TSP) and the weight of carpophores. The results showed that the substrata influence significantly the speed of growth of the mycelium. It is higher on the substrata being less than 5 % of sound of rice during the first weeks of incubation and decreases correlatively with the increasing quantities of sound of rice from the third week of incubation. The rate of survival of primordia is more important on the substratum F1 (33 %). Also the highest production was observed on the substrata F1, F2 and F3 containing less than 5 % of sound of rice.

Keywords: mushroom cultivation, substrate formulation, spawn age, *Pleurotus eous*

Introduction
Wild edible fungi are harvested and consumed by people for thousands of years (FAO, 2006) [5]. Mushrooms are appreciated for their taste, nutritional and therapeutic value. In all latitudes, people are used to collecting them in the wild (Oei, 1993) [13]. Like the peoples of Europe, Asia and America, the African people highly appreciate mushrooms. It is believed that mushrooms are an equivalent substitute for meat and have a food value comparable to that of many vegetables (FAO, 2006) [6]. As a result, mushrooms spawn is a very lucrative business in the local market. But seasonality in the appearance of fungi is a limiting factor for their availability, often random and concentrated on a few weeks per year, mainly in the rainy season. Therefore, the cultivation of mushrooms is proving to be a profitable activity for African farmers (Dibaluka *et al*., 2010) [4].
Although there is undoubtedly a demand and there is an overabundance of agricultural waste to produce the substrate for mushroom cultivation, very few mushrooms are produced in Africa (Oei, 1993) [13] and particularly in Côte d'Ivoire. Among the most popular mushrooms in the world, oyster mushrooms, commonly known as oyster mushrooms, are among the mushrooms that adapt better to tropical regions with a relatively hot climate whose temperature varies from 25 to 35 ° C (Lin, 2006) [9]. For many species of oyster mushrooms, culture in sterilized plastic bags is widely used. Depending on the availability of materials, the substrate formulation differs from one region to another (Oei, 1993) [13]. However, determining the optimum amount of additives is one of the major problems that significantly affects the yield of fungi, making mushroom cultivation difficult (Curvetto *et al*., 2002) [2]. Indeed, an overdose of additive supplement favors infections while the deficiency is responsible for low yields often recorded (Oei, 1993) [13]. Similarly, according to Oei (1993) [13] the storage time of seedling white can influence the growth vigor of the mycelium. The main objective of this work is to evaluate the effect of the substrate formulation and spawn age on the growth and carpophore production of the edible mushroom *Pleurotus eous* cultivated in recent years in Cote d'Ivoire.

2. Material and Methods
2.1Material
Biological material used consists of *Pleurotus eous* spawn produced in the laboratory of plant physiology of the University Jean Lorougnon Guede (Côte d'Ivoire).

2.2 Methods
2.2.1 Preparation of the culture substrate
The growing medium consists of sawdust (98 %), rice bran (1 %) and agricultural lime (1%). The set is stacked and moistened at the rate of 85 %. Flips were made on the job every four days until the substrate was completely cool. At each turn, the substrate is covered with a black plastic sheet to prevent water loss (Figure 1).

Fig 1: Sawdust Substrate for growing mushroom

2.2.2 Formulation of substrate

The substrates consist of rice bran varying in content from 0 % to 21 % and sawdust ranging from 78 % to 99 %, to which 1 % agricultural lime is added (Table 1). Seven substrates were formulated and humidified at the rate of 85 % to 90 %. Sachets (18 cm x 10 cm) of polypropylene and heat-resistant type were filled manually with the various substrates at the rate of 1500 g / sachet (Table 1). The bags were covered with plastic wrap and closed with PVC hose rings and held with an elastic band before sterilization (Figure 2).

Tableau 1: Composition and substrate formulation

Substrats	F1	F2	F3	F4	F5	F6	F7
Son de riz	0 %	2 %	5 %	7 %	10 %	15 %	21 %
Sciure de bois	99 %	97 %	94 %	92 %	89 %	84 %	78 %
Chaux	1 %	1 %	1 %	1 %	1 %	1 %	1 %

Fig 2: Bags hermetically sealed for sterilization

2.2.3 Sterilization of the substrate

Sterilization of the substrate was steamed into drums (Figure 3). A wooden tripod stand is placed at the bottom of the drum and filled with water up to the height of the stand. The closed bags are arranged vertically on the support and stacked on one another until the barrels are filled. The barrels were closed with a lid drilled with a hole to allow a portion of steam to escape (Figure 3). The heat source is fed by firewood. The appearance of the first vapors indicates the beginning of the sterilization. Sterilization is complete after 2 hours 30 minutes of heating. The bags are removed from the barrels and transferred to the inoculation room.

Fig 3: Substrate Sterilization

2.2.4 Inoculation (lardage) of the substrate and incubation

Inoculation or "lardage" is performed under aseptic conditions in the inoculation room. It intervenes after total cooling of the sachets to avoid killing the mycelium. Seeded white compacted by colonization of the mycelium is decompacted using an iron rod previously sanitized. Two tablespoons *Pleurotus* spawn of each age were taken and sprinkled on the surface of the different substrates. The bags are closed again as before. The inoculated substrates are stored in a chamber called the incubation chamber. This dark room will favor the colonization of the substrate. The bags are arranged vertically next to each other on wooden shelves.

2.2.5 Fructification

After complete colonization, the bags are transferred to the fruiting room and placed horizontally on each other on wooden shelves designed for this purpose. The bags were opened with a knife and watered twice a day to promote the appearance of primordia.

2.2.6 Experimental design

Experimental setup is a completely randomized factorial device with two (2) factors. The first factor is the age of the spawn with two levels (30 days and 55 days) and the second consisting of the substrate with 7 levels (0%, 2%, 5%, 7%, 10%, 15%, 21% of rice bran). The different combinations give rise to 14 treatments divided into three repetitions. Each repetition consists of ten sachets per treatment.

2.3 Evaluated parameters

2.3.1 Colonization height of the mycelium

Height of colonization is the distance traveled by the forehead of the mycelium on the substrate. This height was measured using a ruler graduated from the point of inoculation at the forehead of the mycelium. This value made it possible to determine the colonization rate of mycelium (VCM) on substrate according to the following formula:

$$VCM(cm/day) = \frac{High\ colonization\ (cm)}{time\ (day)}$$

2.3.2 Survival rate of primordia (TSP)

The fruiting process begins with the appearance of the first buds that will later become mature mushrooms. During this

phase, primordia and mature fungi were counted and the survival rate of the primordia was assessed as

$$TSP\ [\%] = \frac{\text{number of mature mushrooms}}{number\ of\ primordia} \times 100$$

2.3.3 Evaluation of the carpophore weight (g / bag)

The fresh fruit-flavored mushrooms were weighed with a digital scale. The fresh weight of the resulting carpophore has been estimated.

2.4 Data analysis

The Statistica 7 software was used to tabulate the results, the descriptive statistics index calculations and the comparison of the means of different groups using the analysis of variance (ANOVA) at the 0.05 level. Curves, histograms and graphs are constructed using the Excel software

$$TSP\ [\%] = \frac{\text{number of mature mushrooms}}{number\ of\ primordia} \times 100$$

3. Results

3.1 Mean pH and relative humidity of substrates

Table 2 shows the pH and relative humidity of the different substrates before inoculation. It is found that substrates F1 and F2 are slightly acidic, those F5, F6 and F7 are slightly alkaline while F3 is neutral. The humidity of the substrates varies between 85% and 90%.

Tableau 2: pH mean and relative humidity

Substrate	F1	F2	F3	F4	F5	F6	F7
pH mean	6,8	6,9	7	7,3	7,3	7,7	8
Relative Humidity(%)	90	90	90	90	85	85	85

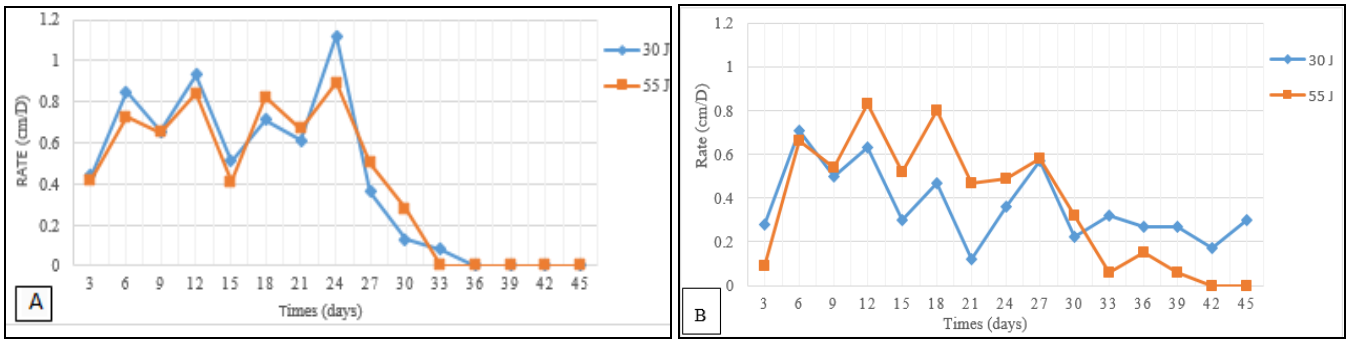
3.2 Rate of colonization of the mycelium

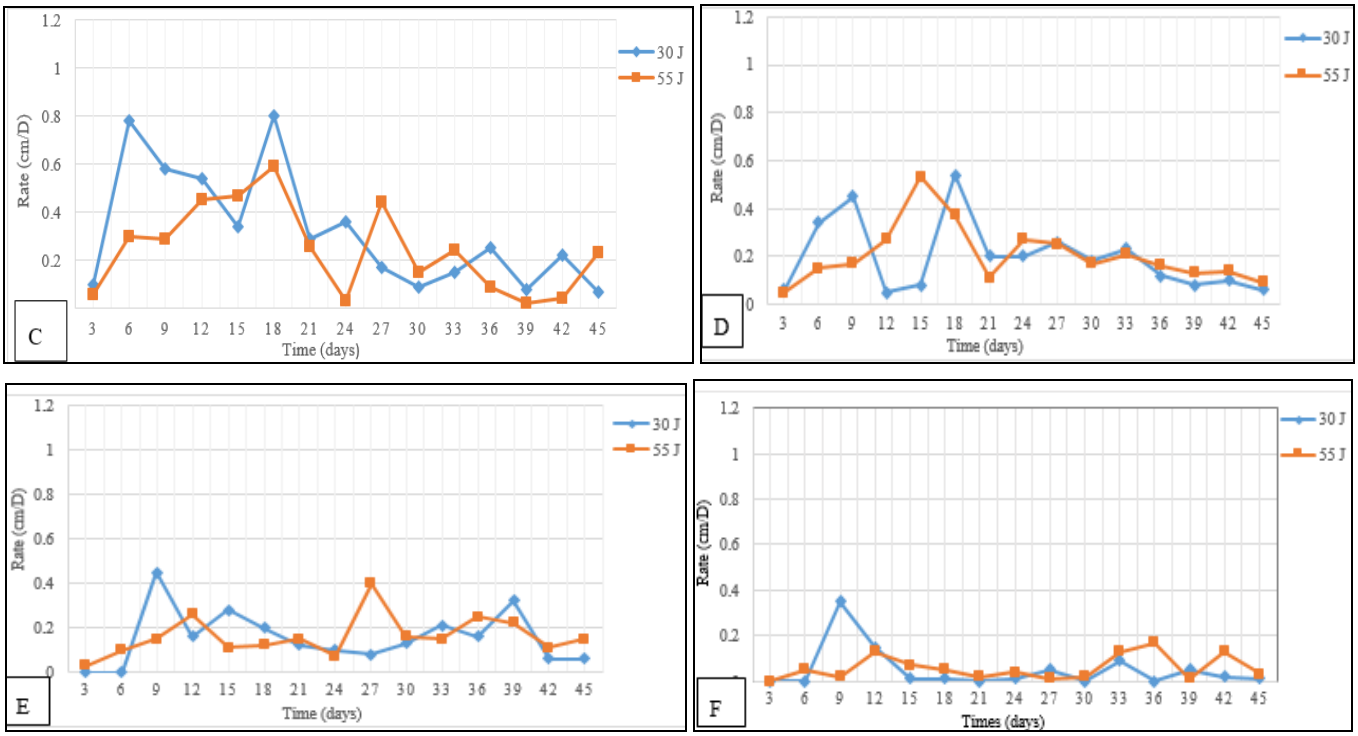
The rate of mycelial colonization varies according to the different substrates (Figure 4). In general, it is more important during the first weeks of colonization and then decreases after the third week of incubation. It decreases with increasing doses of rice bran. Analysis of variance shows that the age of spawn does not significantly affect the rate of colonization. However, a significant effect ($p < 0.05$) of the substrate as well as a significant interaction is observed on the colonization rate (Table 3). On the F1 substrate (0% rice bran), before the 24th day of incubation, the average colonization rate is 0.73 cm/day. After the 24th day, the rate of colonization gradually decreases to vanish from the 33rd day (Figure 4A). Before the 27th day of incubation, the average colonization rate is 0.55 cm / day on the substrate F2 (2 % of rice bran). After the 27th day, the rate of colonization decreases gradually and vanishes from the 42nd day for the 55-day-old white (Figure 4B). On the substrate F3 (5 % rice bran), the average colonization rate is 0.5 cm / day before the 21st day. After the 27th day, the average colonization rate is 0.15 cm / day (Figure 4C). On the F4 substrate (7 % rice bran), the average colonization rate is 0.3 cm / day before the 21st day. The rate of colonization decreases, but is more or less constant (0.2 cm / day) between the 21st and the 33rd day. After the 33rd day, the colonization rate decreases until the 45th day of incubation (Figure 4D). On the F5 substrate (10 % rice bran), the colonization rate is low throughout the incubation period. It varies between 0.45 cm / day and 0.05 cm / day with an average speed of about 0.2 cm / day (Figure 4E). The colonization rate is very low on the substrate F6 (15 % of rice bran). It varies between 0 and 0.35 cm / day with an average speed of less than 0.1 cm / day throughout the incubation time (Figure 4F). No colonization of the mycelium was observed on the F7 substrate (21% rice bran).

Tableau 3: Analysis of variance of colonization rate of mycelium

Source of variation	df	SS	MS	F Test.	P
Age	1	0,000	0,000	0,000	0,991
Substrate	6	1,757	0,293	175,467	0,000***
Age*Substrate	6	0,041	0,007	4,138	0,004**
Error	28	0,046723	0,00167		
Total	41	1,845			

ddl; degree of freedom; SS; sum of square; MS; mean square ; Ftest; Test of Ficher-Snedecor; P; probability; significant; **; high significant ; ***; very high significant.





A; substrate F1; B; substrate F2; C; substrate F3; D; substrate F4; E;substrate F5; F; substrate F6

Fig 4: Colonization rate of mycelia on the different substrate

3.3 Survival rate of primordia (TSP)

The survival rate of the primordia varies with the different substrates and the age of the spawn. It ranges from 0 % to 27 % with spawn age of 30 days and from 0 to 34% spawn age of 55 days (Figure 5). Analysis of variance indicated spawn age and substrate significantly influenced survival rate of primordia ($p < 0.05$) (Table 4). The average survival rate of 30 day old of primordia is lower than that of 55 day old F1, F2 F3 and F6 substrates. No primordia were observed on the F7 substrate regardless of the age of the seed. The F1 substrate provided the highest survival rates of primordia with an average of 30.36%.

Table 4: Analysis of variance for survival rate of primordia

Source of variation	df	SS	MS	Test F.	P
Age	1	180,46	180,46	8,0161	0,00849*
Substrate	6	4108,00	684,67	30,4125	0,000***
Age*Substrate	6	645,54	107,59	4,7791	0,00181*
Error	28	630,36	22,51		
Total	41	5564,36			

ddl; degree of freedom; SS; sum of square; MS; mean square ; Ftest.; Test of Ficher-Snedecor ; P; probability; *; significant; **; high significant ; ***; very high significant.

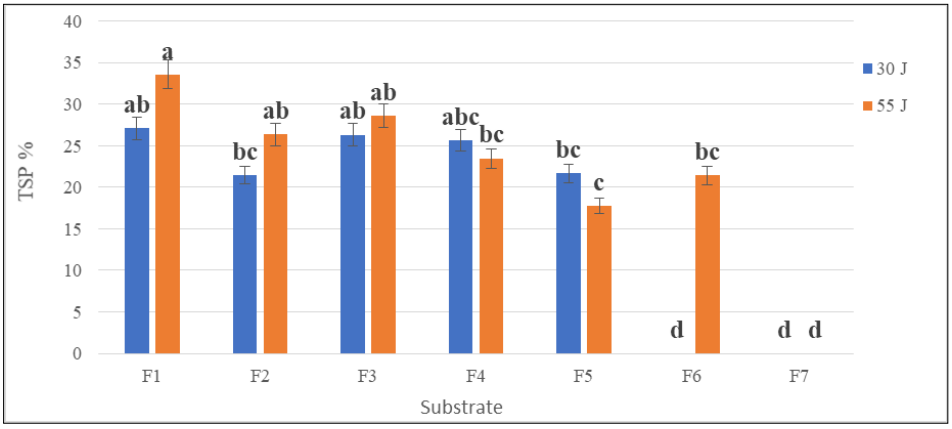


Fig 5: Survival rate of primordia according to the spawn age and substrate

3.4 Average Wight of carpophore

The average weight of the carpophore varies with the substrate (Figure 6). The highest yield of carpophore is observed on substrates F1, F2, F3 with an average of 148g / bag / cycle of 2 months, a biological efficiency (EB) of 9.82. Above 5 % of rice bran, the production of carpophore decreases significantly. F7 substrate does not produce carpophore. The analysis of the variance of the weight of the carpophore shows that the age of the seedling does not significantly affect the production, however a very significant effect of the substrate is observed. The interaction of seedling age and substrate has no effect on the production of carpophore (Table 5).

Tableau 5: Analysis of variance for carpophore wight

Source of variation	df	SS	MS	Test F.	P
Age	1	51,3	51,3	0,0413	0,840516
Substrate	6	158366,2	26394,4	21,2147	0,000***
Age*Substrate	6	2313,2	385,5	0,3099	0,926453
Error	28	34836,3	1244,2		
Total	41	195567,0			

ddl; degree of freedom; SS; sum of square ; MS; mean square ; Ftest .; Test of Ficher-Snedecor ; P; probability ; *; significant ; **; high significant ; ***; very high significant.

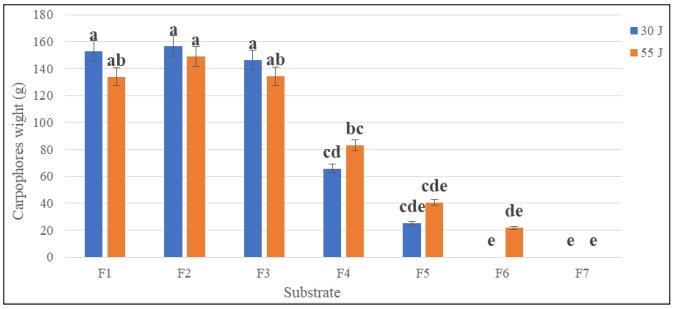


Fig 6: Weight of the carpophores according to the spawn age and the substrate. *The means followed by the same letter are not significantly different at 5 % level*

4. Discussion

The production of edible mushrooms including pleuroculture has appeared in Côte d'Ivoire in recent years. This work analyzes the effect of the cultivation substrate formulation and seed age or seedling white on the production potential of oyster mushroom (*Pleurotus eous*) in a context of improved production and subsequent producing populations. The results show the importance of the composition of the culture substrate on the colonization capacity of the substrate by the mycelium. A higher intake of 5% rice bran weakens the growth capacity of the mycelium which reduces the production capacity of the fungus by the substrate. According to Chandy (1997) [1], mushroom vigor is largely related to the richness of the substrate in essential nutrients available. The results of the study conducted by Manirakiza *et al*. (2014) [10], stress that the variation in yield is influenced by the composition of the substrate. Of the seven (7) substrate formulations used in this study, only the F7 formulation (21% additive rice bran supplement) could not be colonized by the mycelium. This formulation is not suitable for growing oyster mushrooms. In general, we find that the average rate of mycelium invasion decreases gradually as the additive supplement of rice bran increases in the substrate formulation. Indeed, the results reveal that the colonization rate of the mycelium is higher for slightly acidic substrates (F1 and F2) than the neutral substrate (F3) and slightly alkaline (F4, F5, F6 and F7). Indeed, according to Oei (1993) [13] most cultivated fungi prefer to grow in slightly acidic environments. Also, Dibaluka *et al*. (2010) [4] adds that the rate of invasion of the substrate by oyster mushroom mycelium is close to 0.5 cm per day. In this study, the continuous addition of rice bran additive increases the pH of the substrate, leading to a decrease in the colonization rate. Beyond 5% additive supplement in rice bran, the colonization rate drops below 0.5 cm / day. The increase in the pH of the substrate causes a drop in the rate of colonization of the mycelium following an excess of nutrients in the substrate. According to Mondo *et al*. (2016) [11] and Oei (1993), the more nutrient such that the available nitrogen in the substrate plus the mushroom yield is high. Most species of oyster mushrooms develop optimally on substrates with a C / N ratio of 50 (INERA, 1995) [8]. As part of this study, nitrogen intake is provided by the additive supplement of rice bran. However, the results obtained show that the yield of carpophore decreases when the percentage of additive supplement in rice bran is high in the formula of the substrate. For the same basic substrate (sawdust), the best carpophore yields were recorded when the rate of invasion of the mycelium was high. This finding is supported by Mushagaluza *et al*. (2017) [12] who states that mycelial growth rate is related to yield or biological efficacy. But this finding contradicts the studies conducted by De Kesel *et al*. (2002) [3] and Dibaluka *et al*. (2010) [4], who found that Marasmiellus invasion of stems and mycelial growth rate is not directly related to yield or biological efficiency. The results obtained in our study show that the survival rate of the primordia decreases as the percentage of rice bran increases in the formulation of the substrate. Conversely, the excess additive supplement of rice bran causes an increase in the abortion rate. According to Mushagalusa *et al*. (2017) [12] in oyster mushrooms, intra and interspecific competition leads to a high abortion rate due to excess nutrients in the substrate. In this same logic, Flegg *et al*. (1985) [6] states that the higher the number of feet (high survival rate) the lower the production of carpophore. There is therefore a survival rate that is favorable to a better production that remains itself related to the additive intake in its sound.

5. Conclusion

In general, the cultivation of oyster mushrooms uses agricultural waste (sawdust and rice) which pose a problem of storage in the processing companies to make them substrates. It therefore appears as a tool for combating environmental pollution by organic waste. However, to obtain a good yield in mushroom mushrooms, it is important to know how to dose the various elements that go into the formulation of the substrate. Of the two age of spawn of *Pleurotus eous* (30 days and 55 days), the best yields were obtained on substrates with an additive supplement of rice bran less than or equal to 5%. But beyond this threshold, the yield drops by half or the substrate does not produce fungi. So, it is recommended to

growers to add 2% additive supplement of rice bran in the formulation of the substrate to limit the risks of contamination and to have good yield.

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