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RESEARCH ARTICLE

INFLUENCE OF SPAWN AGE (SEED) ON THE CARPOPHORE PRODUCTION AND NUTRITIONAL QUALITY OF THE EDIBLE MUSHROOM *PLEUROTUS EOUS* IN ALLOKOUA (CÔTE D'IVOIRE)

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ABSTRACT

Oyster mushroom cultivation is booming in Côte d'Ivoire and provides an additional source of income for farmers. However, many difficulties related to the quality and availability of spawn slow the activity of many producers. The objective of this study was to evaluate the effect of the *Pleurotus* spawn age on carpophore production and nutritional quality of oyster mushrooms grown in Côte d'Ivoire. The experimental design setup is a completely randomized Fischer block with three repetitions with a single factor (age of the spawn). Spawn age corresponding to 15 days, 30 days, 42 days, 48 days, 51 days, 58 days and 61 days were used to inoculate a substrate composed of 98% sawdust, 1% rice bran and 1% agricultural lime. The results showed that the rate of growth decreased during the course of mycelium evolution for all ages of spawn. The highest biological efficiency (EB) (16.89 ± 0.26) was observed for 61dayold spawn and the lowest (10.53 ± 0.25) at 15 days old. Dried oyster mushrooms are rich in protein (17.79 ± 2.04%) and carbohydrate (67.67 ± 0.12%), but with low fiber (0.55 ± 0.34%) and lipid (1.19 ± 0.4%). These conditions of deficit in these essential elements could be filled by a contribution in ingredient in the manufacture of a whole met.

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INTRODUCTION

Fungi are an integral part of most natural ecosystems, and contribute to the redistribution of food resources used by all organisms in the ecosystem. Moreover, because of the presence of certain chemical compounds appreciated for their medicinal virtues, certain species of edible fungi are suitable for cultivation. More than 2000 species are edible, and nearly 700 species have interesting pharmaceutical properties (Barros *et al.,* 2007). Indeed, these fungi are rich in protein and fiber, low in lipids and contain important vitamins and trace elements (Oei, 1993). Although about 300 mushroom species are edible, only about 30 are cultivable, such as *Lentinus edodes* in China. Of these, about ten are grown industrially, for more than two hundred years, are the *Agaricus spp* in the United States (D.L. Barney, 2009) and *Agaricus bisporus* in France. In Africa, the seasonal appearance of sporophores remains a limiting factor for their availability concentrated on a few weeks per year, mainly during the rainy season. In view of their great nutritional interest, mushroom cultivation is proving to be a timely and profitable activity for African farmers (Dibaluka *et al.,* 2010).

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Indeed, edible fungi are able to feed on several substrates and lignocellulosic waste (together in varying proportions of lignin, hemicellulose and cellulose) produced by forests, agriculture, animal husbandry and manufacturing (Sanchez, 2010). In all cases, one must first obtain pure mycelium, which can be done in many ways. One of the greatest interests of edible mushroom cultivation is to ensure the production and availability of spawn, especially since this production is demanding in terms of materials, expensive substances and, above all, expertise, particularly in the preparation culture media (Stanley, 2010; Diansambu *et al.*, 2015). It should be noted that the importation of spawn produced is also often hampered by customs bureaucracy, high transportation costs and the difficulty of keeping spawn at a low temperature during transport (S.M. Dibaluka *et al.*, 2010). These factors may delay the inoculation time of the substrate, impact the quality of the mycelium, and may result in a decrease in the production of carpophores. In Côte d'Ivoire, producers of oyster mushrooms (edible fungi) generally use sawdust as a substrate for mycelium growth. The culture of oyster mushrooms has grown in some localities of Côte d'Ivoire Coast (Abidjan, Bingerville, Abengourou etc.). But today, the major challenge faced by these spawn growers is in the choice of a good yielding spawn with good quality nutritional skills. The majority of the complaints registered are, among others, spawn (seed) growth and slow development resulting in a

decline in production. Our study proposes to evaluate the effect of the spawn age on the growth and development as well as the nutritional quality of the *Pleurotus* fungus under favorable eco-climatic conditions of the village of Allokoi (PK22) 4 km from Abidjan (Côte d'Ivoire).

MATERIAL AND METHODS

Material

Biological material: The biological material used consists of ***spawn*** of *Pleurotus* of varying ages and produced in the plant Physiology Laboratory of the University of Jean Lorougnon Guede, Daloa, (Côte d'Ivoire).

Method

Presentation of the study area: This study was conducted in a traditional mushroom farm, located 22 km from the exit of the city of Abidjan, on the northern highway. Its GPS location is 5 ° 23'39.79 " North latitude and 4 ° 9'1.19 " West longitude. The experimental site is located in the Guinean zone, which is characterized by a four-season subequatorial climate: a long dry season (december to march), a long rainy season (march to june), a short dry season (july to august) and finally a small rainy season (september to november). The annual rainfall exceeds 1,500 mm / year. Mean temperatures in the region range from 24 to 32 ° C.

Preparation of different spawn age: The carpophores of *Pleurotus* eous were harvested in a mushroom farm located in the Riviera III district in Abidjan (*Cote* d'Ivoire). Internal tissues were removed using a scalpel in aseptic condition deposited in Petri dishes containing a sterilized MEA (Malt Extract Agar) medium. After two weeks of incubation, the tissues collected produce numerous filaments constituting the pure culture.

The pure culture obtained is inoculated with the previously cooked grains of sorghum and sterilized by autoclave. The mycelial filaments invade the bottle containing the sorghum grains. This first generation of colonized bottle is the mother spawn from which will be produced the spawn used to inoculate the substrate. A new cooking of sorghum is carried out by variable time interval and inoculated with the white mother as previously to obtain spawn of ages corresponding to 15 days, 30 days, 42 days, 48 days, 51 days, 58 days and 61 days.

Formulation and substrate formulation: The formulation is the proportion of the different elements constituting the fruiting substrate. The substrate consists of sawdust, rice bran and hot agricultural or white mica in the respective proportions of 98%, 1% and 1%. The whole is carefully moistened at the rate of 85%, mixed, covered, with a black plastic cover. The substrate has undergone a series of turnarounds every 4 days for 6 months to obtain a compost ready for mushroom production.

Filling bags: The bags used are polypropylene and heat-resistant. The bags were filled at a rate of 1500 g of substrate / sachet in the proportions of 3 kg of rice bran, 300 g of agricultural lime and 183 kg of compost. Rice bran is a nitrogen source and agricultural lime decreases the acidic pH of the substrate to promote better growth and fructification of the mycelium. The bags were closed with a PVC pipe ring covered with a plastic film and held with a plastic strap.

Sterilization of substrate: Substrate sterilization was steamed in drums. A wooden support was placed in the bottom of the drum and 5 liters of water were added to the barrel. The bags of substrate were stored in the barrel until 4/5. The barrels were sealed with a lid and a greenhouse and then burned. As soon as the first steam appeared (Figure 1), the heating lasted 2h 30 min. The barrels were left for cooling for at least 24 hours of sachets. The bags cooled to room temperature were recovered and passed to the inoculation room**.**

Inoculation of the substrate: The inoculation consisted in removing the plastic film, the PVC pipe rings and the plastic bracelet on the bags to deposit about 170 g of spawn of each age. Once the inoculation ended, the bags were closed as before and then transferred to a room called incubation room to allow the mycelium to colonize the substrate.

Fructification: The fully colonized bags were arranged horizontally on top of one another in the fruiting room on specially designed shelves (Figure 2a). The bags were opened with a knife and watered 3 times a day. They are subsequently opened with a knife.

Experimental design: The experimental design setup is a completely randomized Fischer block with three repetitions. Each repetition consists of seven (7) completely randomized treatments. Each treatment consisted of ten (10) bags arranged vertically next to each other in the incubation room and then horizontally on top of one another in the fruiting room (Figure 2b).

Evaluated parameters

Spawn run and incubation time: This measurement corresponds to the time of complete invasion of the substrate by the mycelium.

Survival rate of primordial (TSP): The fruiting process begins with the appearance of the first buds that will later produce ready-to-eat mushrooms. During this phase, the primordia and the mature fungi were counted then the rate followed by the primordia evaluated as follows:

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

Stipelength (cm): The length of the stipe was measured using a graduated ruler (cm), from the attachment point of the mushroom to the substrate to the base of the cap.

Cap diameter: The hat of the mushroom *Pleurotus eous* has a circular shape. The measurement of its diameter was made at maturity using a graduated ruler. The diameter of the hat has been divided into two categories: diameter less than 3 cm (D <3 cm) and diameter greater than 3 cm (D ≥3 cm). Evaluation of the carpophore weight (g/bag) and biological efficiency (BE): The resulting carpophores were weighed with a digital scale. The fresh weight of the carpophore obtained was estimated in grams. Biological efficiency is still called yield. It was calculated by multiplying by 100 the ratio "fresh weight of

the carpophore or total harvest to the fresh weight of the substrate" (Oei, 1993) reported as a percentage.

$$Biological\ efficiency\ (\%) = \frac{mushroom\ wight}{substrate\ wight} \times 100$$

Spent mushroom substrate (SMS)evaluation: The Spent Mushroom Substrate (SMS) represents the amount of substrate remaining after production of the carpophores. After harvest, the substrate is weighed to quantify the amount of substrate used by the mycelium for producing the carpophore.

Chemical analysis and determination of nutrient composition: The assays were performed according to the recommended official methods AOAC (1990). Proteins were assayed by the Kjeldahl method. The lipids are extracted with an immiscible solvent (n-hexane) in a Soxhlet extractor (UnidTracator, HT2 System 1045, sweden). After evaporation of the solvent and weighing in an oven at 105 °C for 30 minutes, the difference in weight gives the lipid content of the sample.The ashes are obtained after carbonization of the sample on a bunsen burner and then incinerated inan oven at 600 °C for 6 hours.Carbohydrates are calculated by the difference according to the equation below (AOAC, 1998).

% glucides =100 - (% protéines+% lipides+% cendres +% fibres)

Data analysis: The various variables were analyzed on the computer using Excel software for plotting the evolution and speed curves, then histograms and finally the Statistica 7.1 software used to tabulate the results, the statistical index calculations. Descriptive and comparison of the averages of different groups using ANOVA at the 5% threshold. In the case where there was at least one pair of averages different from the others, the Least Significant Difference (LSD) test was used to compare the averages.

RESULTS

Spawn run and incubation time: This measurement corresponded to the time between inoculation and complete colonization of the substrate. The invasion time of the mycelium of the seven ages of spawndepends on sawdust-based substrate. From the analysis of (Figure 3), it appears that the mycelium of the various ages selected has successfully invaded the support based on sawdust with a variable colonization time.

The incubation time of these seven white *Pleurotus* seedlings was 0-48 days. 51-day-old spawn invaded the substrate after 42 days of incubation (Figure 3). As for the 58- and 61-day spawn, they complete their colorization on the 44th day (Figure 3). Those at 42 and 48 days end at day 46 and finally spawn at 15 and 30 days at day 48 (Figure 3).

Carpophore production: *Pleurotus* spawn ages were used to seed the fruiting substrate which is sawdust. After 48 days of incubation, the substrate bags were put in the fruiting room. Thus, after 2 days, we saw the appearance of the first buds which later became mature mushrooms.

Stipe length: Variance of analysis of stem growth averages showed that there are significant differences in stipe length between the sporophores of different spawn age. The average stalk length of 42 and 61dayold seedlings was longer than other seedling blanks (3.26 cm and 3.24 cm) (Table 2). The length of the 51 dayoldspawn squirrel carpophore stipes is the smallest (2.56 cm).

Cap diameter: Number of carpophore with a diameter less than 3 cm (D <3) The age of the spawn has a significant influence (P <0.05) on the diameter of the fungi cap. The 30day spawn produces on average more carpophore with a diameter of less than 3 cm in diameter than the other spawn

Tableau 1. Effect of spawn age on the number of carpophore and stipe lenght

Spawnage (days)	Number of carpophore Diameter < 3cm	Number of carpophore Diameter ≥ 3cm	Longuestipelenght(cm)
15 days	4,43±0,15d	8,40±0,40b	2,92±0,06a
30 days	7,66±0,66b	9,36±0,47c	2,92±0,06a
42 days	7,26±0,37ab	8,20±0,43ab	3,26±0,15c
48 days	7,13±0,30ab	8,36±0,41b	2,81±0,13ab
51 days	6,33±0,45c	7,43±0,47a	2,56±0,26b
58 days	6,73±0,65ac	7,30±0,45a	2,81±0,15ab
61 days	7,20±0,10ab	10,43±0,83d	3,24±0,16c
F	18,18988	13,35162	7,52972
P	0,000007	0,000044	0,000940

Tableau 2. Effect of spawn age on primordia survival rate, carpophore production, biological efficiency and spent mushroom substrate (SMS)

Spawn age (days)	Survival rate ofprimordia (%)	Carpophore production (g/bags)	Biological efficiency (%)	Spent mushroom substrate (SMS)
15 days	20,43±0,68a	157,98±16,40c	10,53±0,25a	622,53±11,50b
30 days	17,54±1,31d	249,69±23,43ab	16,64 ±0,07e	727,83±8,27c
42 days	28,49±0,60e	230,33±13,31a	15,35±0,13c	649,36±22,70b
48 days	24,88±0,94c	238,68±9,06ab	15,91±0,04d	562,60±26,32a
51 days	22,96±1,66bc	228,05±4,78a	15,20±0,45d	554,63±19,20a
58 days	20,38±1,58a	184,74±5,09d	12,31±0,29b	568,90±7,10a
61 days	20,87±1,33ab	253,43±6,09b	16,89±0,26e	562,63±7,26a
F	25,69442	22,88255	241,51	46,08550
P	0,000001	0,000002	0,000000	0,000000

Tableau 3. Nutritional value of oyster mushrooms

	% Humidity	% dry mater	% carbohydrate	% protein	% Lipid	% Fiber	% ashes
Dry oystermushroom	13,78 ± 0 ,14	86,2±1,03	66, 67±0,12	17,79±2,04	1,19±0 ,18	0,55±0,34	11,05±2,03

(7.66 carpophores). As for the 15dayold seedling, it produces on average less carpophore with a diameter of less than 3 cm (4.43 carpophores). Number of carpophore with a cap diameter greater than or equal to 3 cm (D ≥3):

The age of the spawn has a significant influence (P <0.05) on the diameter of the mushroom caps (Table 1). The 61 dayold spawn produces on average more carpophore with a hat 3 cm or more in diameter than the other seedling white (10.43 carpophores), while the 51 and 58 day-old spawn fruit produce on average less than carpophore with a hat diameter greater than or equal to 3 cm (7.43 and 7.30 carpophores) (Table 1). In the same colum, means followed by the same letters are not significantly difference at 5 % level

Survival rate of primordia(%): The age of the spawn has a significant influence (P <0.05) on the diameter of the mushroom caps (Table 2). The 42 dayold spawn primordia of *Pleurotuseous* survival the most (28.49%) compared to other spawn. As for the 30 day spawn, it was marked by a low survival rate of 17.54%.

Average wight of capophore(g/ bag) and biological efficiency(EB): The age spawn has a significant influence (P <0.05) on the production and biological efficiency of the carpophores (Table 2). The 61day spawn average produced more carpophore than the other spawn ages with an average of 253.43g of the carpophore. The lowest production of carpophore was represented by the 15-day-old spawn with 157.98g of carpophore production. With respect to biological efficiency, the greatest value was obtained with the 30 and 61day old spawn ages (Table 2). The lowest biological efficiency (10.53 %.) was achieved by the 15-dayoldspawn

Effect of spawn age on spent mushroom substrate (SMS) production: The age of spawn has a significant influence (P <0.05) on spent mushroom substrate (Table 2). The residual substrate or SMS of Spent Mushroom Substrate has decreased. It goes from 1500g to an average of 562.60g, an average decrease of 37.50%. This value is higher when the substrate is colonized with the 30-day old mycelium (48.52%). The lowest SMS were observed for different old spawn at 48, 51, 58 and 61 days. In the same colum, means followed by the same letters are not significantly difference at 5 % level

Figure 1. Substrate sterilization

(a)

(b)

Figure 2. Disposition (a) and opening (b) of the bags

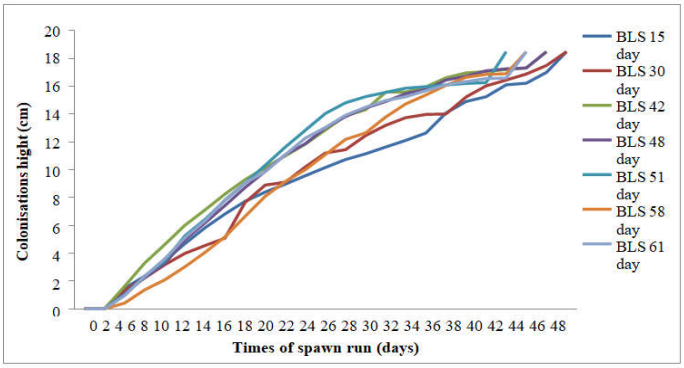

Figure 3. Evolution of the colonization height of the mycelium according to time

Chemical analysis of dry carpophore of *Pleurotuseous*: The analyzed oyster mushrooms are rich in proteins and carbohydrates with respectively 17.79 ± 2.04 g / 100 g / 100 gMS g / 100 gMS and 66.67 ± 0.12 g / 100 g MS, in addition we observe a low lipid content (1.19 ± 0.18) and fibers (0.55 ± 0.34).

DISCUSSION

In this work, the aim was to evaluate the impact of spawn age on the growth and development of carpophores of the edible fungus *Pleurotuseous*, as well as its nutritional value. The results of the statistical analysis showed that the age of the spawn influenced almost all the parameters studied. The general finding is that spawn age greater than 30 days is much faster than spawn of 30 days or less. The rapid growth observed by spawn older than 30 days is thought to be related to high mycelial concentration. Righelator (1975) has shown that the specific growth rate, which is the amount of organism produced per unit of time and per unit of organism, is a constant characteristic of the strain and medium. In other words, the growth rate is proportional to the concentration of the organism. Thus, the higher the concentration of the mycelium in the medium, the better the invasion of the substrate by the mycelium. Also, we found that a spawn kept for 2 months (61 days) had a time of invasion and evolution faster than a spawn of one month old. Proust (2017) showed that most of the mother spawn of cultivated mushrooms can be kept for 6 months. Moreover, it was observed by Proust (2017) that a spawn stored for a very long time decreased the survival rate of the *Pleurotusprimordia* by 50%.The highest survival rates (28.49%) were observed for 42day spawn. Statistical analysis revealed a significant difference at the 5% level. Note that in oyster mushrooms, intra or interspecific competition leads to a high abortion rate of primordia due to an excess of nutrients in the substrate. The high rate of survival at 42days old spawn is explained by the fact that they would develop a survival capacity linked to the maturity of the spawn.

Also, the work carried out by P.B. Flegg (1985) revealed that the higher the number of feet on the substrate, the longer the height of the stipe, the smaller the diameter of the sporophores. However, our study showed a high survival rate of primordia, a high stipe length with the 42-day spawn including that of 61 days, a diameter of cap in carpophore between 7.26 and 8.20 cm respectively greater than diameters (D≥3 cm) between the smallest value and the largest. Our results confirm that of Flegg (1985). These results reveal a significant difference at the 5% threshold for production or biological efficiency (EB) of the different spawn ages. The 30 and 61old spawn are more effective for a better production of carpophores. The quality of the mushrooms produced is related to the length of the stipes and the diameter of the hats. Sturdy mushrooms, so cap diameter greater than 3cm are significantly larger than those of diameter less than 3cm. The age of the spawn significantly influences the length of the stipe and the cap diameter of the carpophores. These two parts of the carpophore are determinant in the expression of the yield. The analytical study of dried oyster mushrooms obtained has shown good nutritional disposition. Indeed, this mushroom is high in protein with 17.79 ± 2.04g / 100gMS and 66, 67 ± 0.12 g / 100gMS. These values are higher than the conventional rates required by FAO (2003) for a balanced dish that is between 12 and 15%. In addition, these oyster mushrooms are very poor in lipids with 1.19 ± 0.18 g / 100gMS whereas the official recommendation of the FAO (2002) is between 30% and 35%. As for carbohydrates, they are represented at a value of 66.67 ± 0.12 g / 100 gMS, which is considerably higher than the levels required by FAO (2003), which varies between 50 and 55%. Carbohydrates are the main potential substrate for high activities, while lipids are the preferred substrate for moderate activities (Anonymous, 2001). The analyzes show that *Pleurotuseous* is very poor in fiber with 0.55 ± 0.34 g / 100gMS compared to other cooked dishes which is between 3 and 5 g / 100gMS (M. N'dong *et al.*, 2007). Indeed, these prepared mushroom dishes in Africa could be accompanied by other ingredients much richer in fiber and fat. All dishes prepared with these oyster mushrooms could especially help to overcome all protein-energy diseases. These dishes based on oyster mushrooms supplemented with fiber-rich ingredients would be recommended. Fiber is very important because it protects the body against bowel cancer, diabetes and cardiovascular disease (Ponka *et al.,* 2005). They especially facilitate cellular hydration (Afass, 2002).

Conclusion

This study showed that 30 and 61-day spawn are more effective in the production of carpophores because they give a higher biological efficiency than other spawn with a value of 16.76%. A 15day spawn significantly reduces the production of the carpophores. The *Pleurotus* mushroom has good nutritional quality resulting in a good protein and carbohydrate content. The low levels observed in fiber and fat could be filled in the preparation of dishes by a contribution of other ingredients richer in these nutrients.

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