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Original Research Article

Effect of Rice Bran Rice Husk Sawdust and Quicklime on the Production of Fungi (Pleurotus ostreatus)

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Abstract: The objective of the study is to evaluate the effect of rice bran, rice hull, sawdust and quicklime on the production of edible mushrooms, in particular Pleurotus ostreatus. The cultivation method used is that of the culture technique on pasteurized substrate. The experimental device adopted was that in complete random blocks with four treatments repeated three and made up of different quantities of the prepared substrate (T1= 0.5 kg T2 = 0.75 kg T3 = 1.0 kg and T4 = 1.25 kg). The results obtained revealed that the average diameters of the cap and the height of the stem of the mushrooms from the T2 treatment were greater than those obtained from the mushrooms of the T1 treatment. No significant difference between these parameters was observed under the different treatments. The highest mushroom production (800.0 ± 23.31g) and the lowest (490.8 ± 7.60g) are obtained respectively under the T3 and T1 treatments. Thus the one-kilogram substrate used under the T3 treatment was identified by its high production of Pleurotus ostreatus.

Keywords: Technique, substrate, pasteurization, Pleurotus ostreatus, yield.

INTRODUCTION

Myciculture is one of the most profitable and important economic activities for the household from the nutritional, medicinal and agronomic points of view. However in Togo it is very little practiced because of the lack of mastery of the techniques of its cultivation and the difficulties of finding a viable mycelium. Fungi feed by absorption. Their cultivation requires special and meticulous substrates. In countries with high population density and degraded soils, non-timber forest products are an alternative allowing rural populations to avoid food shortages and constitute a significant source of income. Among these products, mushrooms are particularly sought after because of their market value as well as for the many mineral salts and vitamins they contain (Mattila and *et al*. 2001). Edible mushrooms containing essential nutrients can be a solution to malnutrition especially in Africa where protein rich foods such as meat are expensive (Eyi 2008). Thus mushrooms, in particular oyster mushrooms, are of interest to more than one today because of their nutritional and therapeutic taste qualities (Chen 2010, Golan-Rozen 2011, Zhang 2011).

In Togo the collection of wild mushrooms is an ancient activity. People have developed several rudimentary mushroom reproduction techniques that do not use mycelial seeds. (Yaovi 2007). The harvesting of mushrooms in our bushes and forests is random and seasonal, because the formation of carpophores can only occur under certain conditions, namely high relative humidity, a favorable temperature and a lignicole environment (Willy and Tshimanga 2012). Also in recent years, the accelerated deforestation of the plant cover, the alteration of biodiversity and the effects of climate change have influenced the seasonal availability of carpophores (De Kesel and *et al*. 2002, Dibaluka and *et al*. 2010). These factors thus lead to the scarcity of mushrooms and influence harvests and sales on local markets. Faced with this observation, scientific studies have been undertaken on the cultivation of wild edible mushrooms in the world (Nieuwenhuijzen 2007). However, despite the nutritional virtues that mushrooms contain and the socio-economic advantages they bring, mushroom cultivation remains little practiced, especially in developing countries. This would be due to ignorance or insufficient knowledge of myscicultural practices. Thus once collected from the bush by people

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for personal use, mushrooms are now domesticated and cultivated for greater consumption (Royse 2002). In Togo the cultivation of mushrooms has begun to experience growing interest (Kalmis and *et al*. 2008, Atikpo 2008, Loss 2009). The most cultivated genus is the Pleurotus (Follykoé 2006) because it has a certain adaptation for tropical regions and allows it a wide geographical distribution to grow on different organic materials. The aim of the study was to evaluate the effect of rice bran, rice hulls, sawdust and quicklime on mushroom production.

1. MATERIALS AND METHODS

1.1. Experimentation site

The trial was conducted within the ANAJA farm specialized in the production of edible mushrooms. It is located in Zanguéra-Klémé 14 km west of Lomé in Togo. The site enjoys a tropical Guinean climate and is located in the Maritime region where the average temperature is 27°C. The amount of precipitation varies between 800 and 1100 mm per year. There is a Guinean equatorial climate in the area and it is characterized by two rainy seasons and two dry seasons. The long rainy season is from mid-March to mid-July. The short rainy season is from September to mid-November. The long dry season runs from mid-November to mid-March and the short dry season from mid-July to the end of August (Tchaniley and *al*. 2020). The soil of the site is a ferralitic soil formed from the continental deposit that covers part of the arable land of Ghana, Togo, Benin and Nigeria (Kadanga and Sogbédji 2017).

1.2. The mushroom farm

The mushroom house (Fig. 1) is made of wood covered with straw essential for the development and fruiting of the mycelium. It is protected from dust and insects. The temperature of the mushroom house during the experiment oscillated between 25 and 300C.

1.3. Cultivation material

The substrate used consists of an unfair mixture of rice bran rice hull sawdust and quicklime (Fig. 2 3 4 5 and 6.

Fig-1: Mushroom farm

Fig-2: Quicklime

Fig-3: Sawdust

Fig-4: Rice bran

Fig-5: Rice hull

Fig-6: Mycelium

1.4. Experimental apparatus

The device adopted is that of completely random blocks of four treatments with three repetitions. The substrate used is a mixture of 2.25 Kg of rice bran 2.2kg of rice hull 41.66 Kg of sawdust and 0.180 Kg of lime and made it possible to define the following treatments: T1= 0.5kg T2 =0.75kg T3 = 1.0kg and T4=1.25kg.

The cultivation method used is that of the culture technique on pasteurized substrate. She understood:

- **Composting**

Composting consisted of mixing rice bran, rice hull, sawdust and quicklime according to the formulation of the mixture adopted above. Watering with water was lightly done to moisten the substrate. This substrate obtained is turned over every four (4) days in the open air to accelerate its decomposition. This work lasted a month. In this mixture the hull of the rice helped to retain water. Sawdust is the nutrient medium for future mushrooms. This nutrient medium is supplemented with rice bran for an additional nitrogen supply. Quicklime is used to soften and promote the decomposition of the mixture because of the diffusion of its heat in order to obtain good compost and create a buffer medium whose pH is close to 7.

After a month of composting, the bags are filled with the substrate prepared according to the formulation of the different treatments and the opening of the bags is narrowed by a plastic ring and the end is plugged with cotton (Fig. 7)

Fig-7: Bags filled with compost

Fig-8: Compost pasteurization

- **Pasteurization**

It consisted in sterilizing the bags filled with compost in order to destroy the undesirable microorganisms which are there. To do this, a barrel of 200 liters was needed, in which a circular shelf 10 cm high is laid at the bottom of it. Water is poured into the barrel up to the height of the shelf. Then the bags filled with prepared substrate are placed in this barrel on the shelf. Everything is brought to the fire on a hearth (Fig. 8). As soon as the water has started to boil and the steam escapes from the lid, the intensity of the fire in the hearth is kept constant for 2h 30min. After this time, the fire is extinguished and the pasteurized sachets are left to cool in the barrel for 24 hours.

- **Inoculation of pasteurized compost**

After sterilization and cooling of the bags, the rings and the cotton are removed to allow the mycelium to be inoculated. After this inoculation operation, the openings of the sachets are again narrowed by a plastic ring and the end is plugged again with cotton. The mycelium is then poured rationally on the pasteurized compost contained in each of the bags according to the proportion of 25.0 kg of compost for 1.0 kg of mycelium and according to the treatments carried out (Fig.9). To avoid contamination, hygiene must be strictly adhered to.

Fig-9: Inoculation of pasteurized compost

Fig-10: Sachets cut at the end after **at the end after 24 hours of incubation**

Fig-11: Incubation of mycelium

Fig-12: Appearance of mushrooms

Fig-13: Mushrooms formed

- **Fruiting and harvesting incubation**

The bags inoculated with mycelium are kept in the mushroom house on the shelves. After 24 hours, the end of the sachets is cut to destroy the ring and the cotton to allow the mycelium to breathe well and invade the substrate to produce future mushrooms (Fig. 10). However, the bags and the soil of the culture room are watered rationally if the need arises to maintain the humidity. The temperature of the culture room oscillates between 25 and 30°C. This incubation lasted 60 days. During this period the mycelium by invading the compost takes on an increasingly whitish color (Fig. 11).

When fruiting, small mushrooms are formed (Fig. 12) which evolve in 48 hours to give mushrooms ready to be harvested (Fig. 13). During fruiting, the light in the grow room should be dimly lit so that a newspaper can be read (Akutsè 2005). The harvest is done manually by slightly turning the foot of the mushroom on itself. Eight (8) days on average after the harvest other mushrooms appear and the harvest continues.

- **Mushroom production by treatment**

The production of fungi per treatment was calculated from the formula: I=PS/NS; PS is the sum of the total production over one treatment and NS is the total number of sachets per treatment.

- **Data analysis**

The software used for the data processing of the results obtained is the RStudio software; and the test used is the DUCAN test.

2. RESULTS

Table-1: Effect of the substrates used on the average height and diameter of mushroom caps and stems (cm)

Treatments	Cap height	Cap diameter	Stem height diameter	Stem
T1	5.090 ± 1.38	5.400 ± 1.85	4.42 ± 1.70	1.410 ± 0.40
T2	6.090 ± 1.65	7.670 ± 2.09	6.94 ± 2.04	2,860 ± 0.64
T3	5.515 ± 0.81	6.645 ± 0.51	6.02 ± 0.89	2.010 ± 0.45
T4	5.120 ± 1.14	6.560 ± 1.26	4.59 ± 0.60	1.615 ± 0.39

Duncan's test at the 5% threshold, Diameter = Mean ± Standard Error. T1 T2 T3 T4: the different weights of the substrate with T1 = 0.5Kg T2 = 0.75Kg T3 = 1.0Kg T4 =1.25Kg

2.1. Effect of the substrates used on the average height and diameter of the cap, the average height and diameter of the stem of mushrooms.

Statistical analysis of the data in Table 1 shows that there is no significant difference between the average heights obtained from the caps of Pleurotus ostreatus under the different treatments. The data from the test results reveal that the greatest average height (6.090 cm) of the caps is obtained from the mushrooms of the T2 treatment followed respectively by those obtained from those of the T3 (5.515 cm) and T4 (5.120 cm) treatments. The lowest average height (5,090 cm) is obtained under the T1 treatment.

2.2. Effect of substrates used on the average diameter of mushroom caps

Statistical analysis of the data in Table 1 reveals that the highest mean diameter (7670 cm) of mushroom caps is recorded under treatment T2 and the smallest mean diameter (5400 cm) is obtained under treatment T1. This same analysis also reveals that there was not a significant difference with regard to the average diameter of the hat of the mushrooms between the results obtained from the T3 and T4 treatments, on the other hand there was some between the data of the results obtained T1 and T2 treatments.

2.3. Effect of substrates used on the average height and diameter of mushroom stems

The great average height (6.94 ± 2.04 cm) of the mushroom stems is obtained from the T2 treatment followed by that of the mushrooms from the T3 treatment. The weakest one (4.42 ± 1.70cm) is obtained from the mushrooms of the T1 treatment. However, there was no significant difference between the average height obtained from the feet of mushrooms harvested from treatments T1 and T4. But the statistical analysis of the data in table 1 shows that there is a significant difference between the average diameters of the cap and the foot of the mushrooms obtained under the different treatments. The highest mean mushroom diameter (21.860 ± 0.64cm) is obtained from mushrooms harvested under the T3 treatment.

2.4. Effect of substrates used on mushroom production

Table-2: Effect of substrates used on mushroom production

Traitements	Average mushroom weight per Treatment (kg)
T1	490.8 ± 7.60
T2	750.6 ± 5.90
T3	800.0 ± 23.31
T4	640.2 ± 14.25

Statistical analysis of the data from the results in Table 2 shows a significant difference between the production of Pleurotus ostreatus obtained under the different treatments. The same results show that the greatest production (800.0 ± 23.31g) is obtained from the T3 treatment and the lowest (490.8 ± 7.60g) is obtained from the T1 treatment.

3. DISCUSSION

3.1. Effect of the substrate used on the average height and diameter of the cap the average height and diameter of the stem of mushrooms harvested under the different treatments

Statistical analysis of the data in Table 1 shows that there was no significant difference between the average cap height and also between the average stem height of the mushrooms. But there were some on the

average diameters of the hat and the foot of these. The larger average diameter of the cap (7.670 cm) obtained from the mushrooms harvested on the T2 treatment whose weight of the substrate was 0.75 Kg could be explained by a good invasion of this substrate by the mycelium. This result can also be explained by a sufficient content of nutrients, in particular nitrogen provided by rice bran (Akutsè, 2005). The average cap diameter of the mushrooms harvested under the T3 and T4 treatments gave 6.645 ± 0.51 cm and 6.560 ± 1.26 cm respectively. The lowest average diameter (5.400±1.85 cm) is obtained from the T1 treatment. According to the conclusions of Gunde and Cinerman (1995) conclusions taken up in their work on the effect of rice bran and sawdust on the production of edible mushrooms (Pleurotus ostreatus) in 2022 by Dogbé and Kpedzroku the oyster mushroom has a hat covering a diameter of 5 to 25 cm at maturity. Our results located in the interval from 5,400 cm to 7,670 cm are consistent with those found by these authors. It could be that in the substrate of the T1 treatment the invasion of the substrate by the mycelium was not total. This suggests that the degradation of this substrate did not provide much nutrients for the good development of the fungi compared to the fungi of the other treatments (T4 T2 T3).

This could be explained by the low quantity of the substrate of the T1 treatment (0.5kg) which could not have provided a sufficient quantity of nutrients to the mushrooms in culture. The average height and diameter of the stem of mushrooms harvested from treatments T2 and T3 (Table 1) are the highest. These results could be explained by a good invasion of the mycelium of the substrate of these treatments and a rational ventilation of the culture chamber. In fact, the smallest average diameters of the mushrooms were obtained from the T1 and T4 treatments. This could be explained by the fact that the substrate of the T1 treatment (1.0 kg) was much lighter in terms of quantity and that of the T4 treatment (1.25 Kg) was much denser. On the one hand, the mycelium would have lacked nutrients on the T1 treatment for the invasion well. On the other hand, the mycelium would have had great difficulty on the substrate of the T4 treatment to colonize it normally because of the density of its weight. According to Akutsè (2005) the substrate should not be too airy or too dense. If the substrate is too dense or too airy, the mycelium will hardly colonize it. The same author concludes that if the substrate is not compacted enough, the mycelium will need more energy to reach the next strand of sawdust or straw and if it is too compacted the mycelium cannot breathe properly. Another factor influencing the height of the mushroom stem is the environment in which the mushrooms grow. According to Islami and *et al*. (2013) there are two important components which are very influential in the growth of fungi, namely oxygen and carbon dioxide. So the T3 treatment substrate was normally aerated. This result corroborates that of Akutsè (2005) who states that good ventilation in the culture room is essential for the development of fungi. But these conditions were not the same in the case of the T4 treatment mushrooms. We can say that the amount and proportion of substrate ingredients used in the T3 treatment improved the average stem height and diameter of the cultivated mushrooms. The results obtained justify the effectiveness of sawdust, lime, rice bran and rice hull in the production of Pleurotus ostreatus.

3.2. Effect of the substrate used on the yield of mushrooms harvested under the different treatments

The results from the statistical analysis reveal an increase in mushroom production respectively under treatments T3 (800.0 ± 23.31g) and T2 (750.6 ± 5.90g) compared to the production obtained on other treatments. These results could be explained by good colonization of the substrate by the mycelium and confirm those of Pokhrel and *al*. in 2009 who concluded that exceptional mycelial growth is a vital factor in mushroom cultivation. In addition, our results obtained could be explained by the fact that factors such as good aeration of the culture medium and a sufficient content of the substrate in nutrients would have favored the normal colonization of the mycelium on the substrate used. This idea corroborates that of Chang and Miles (1989) who asserted that the production of fungi is influenced by the composition of the substrate and the degree of aeration. According to Kimenju and *al*. (2009) a highly nutritious substrate also improves the maintenance of vegetative growth of the mycelium. The T4 treatment, although the weight of its substrate was greater (1.25 Kg) gave a low production of mushrooms (640.2 ± 14.25g) compared to that of the mushrooms obtained from the substrates used under the T2 and T3 treatments (Table 2). This result would be due to the high density of its substrate which should have prevented the mycelium from invading it properly and would thus have reduced the enzymatic activity of the fungi on the normal degradation of the substrate in which the lignin, cellulose and cellulose are found. Hemicellulose in order to release the nutrients necessary for their normal absorption. This idea corroborates that of Oei (1993) who states that when the substrate is too dense, the mycelium will colonize it with difficulty. According to Suparti and Marfuah (2015) the density level of the planting medium affects the growth rate of the mycelium. Other authors, namely Soniya and *al*. (2013) approaching their discussions along the same lines confirmed that the low degradation of ligno-cellulosic substances in sawdust by Pleurotus ostreatus could also be another factor affecting the overall values of the production of fungi on sawdust. Tajudeen et al, 2012 in their study on the effect of wheat bran supplement on the growth and yield of oyster mushrooms (Pleurotus Ostreatus) on sawdust substrate concluded that the ligno-cellulosic materials contained in sawdust are generally low in protein and

therefore insufficient for growing mushrooms and therefore require supplemental nitrogen, phosphate and potassium. This is in direct agreement with the composition of the substrate used in our experiment insofar as rice bran was provided as a source of nitrogen for the mushrooms in culture.

CONCLUSION

In Togo, mushroom cultivation benefits from several advantages, including the abundance of substrates (agricultural waste such as rice bran, rice hulls, sawdust, etc.). The simple culture method on pasteurized substrate requires little investment and is an important method for producing mushrooms accessible to the population. Different species of fungi can colonize the same substrate and a given fungus can colonize different kinds of substrates. However, for this colonization to be perfect so that the fungi can grow well, it is necessary to provide a suitable additive to the substrate, in this case rice bran to provide nitrogen to the rice hull to maintain the Humidity of the culture medium sawdust which brings significant elements to the fungi and lime which constitutes a buffer element to control the pH of the medium close to neutrality. The present study showed that sawdust supplemented with rice bran from the rice hull and lime improved the production of Pleurotus ostreatus. However, the use of rice bran, rice hulls, sawdust in mushroom production is only effective if the weight of the substrate of a treatment is neither too dense nor too light. The results obtained from the study showed that the mushrooms harvested under the T3 treatment were well identified by their weight (800.0 ± 23.31g) by the average diameter of their caps (6.645 ± 0.51cm) and the average height of their feet. (6.02 ± 0.89) and by the average diameter of their feet (2.010 ± 0.45cm). Thus the T3 treatment substrate consisting of one kilogram of ingredients used with their well-defined proportions could be recommended to producers of Pleurotus ostreatus.

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