Growth of *pleurotus tuber-regium* (Fries) singer on three substrates using hydrogen peroxide sterilization

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Abstract

The growth of the sclerotia of Pleurotus tuber-regium (an edible mushroom) on three substrates (loamy soil, sawdust and mixture of soil/sawdust) was studied for 10 weeks. 180 pieces of sclerotia (30g each) were inoculated into the sterilized and unsterilizd media, 0.45% hydrogen peroxide was the sterilizing agent. Each treatment was replicated three times with 10 pots in each of the 6 treatments.40.63% of the fruit body production occurred during the 11-20 day, period of the experiment. The highest number of mushrooms was produced from the sterilized soil (139 fruit bodies), unsterilized soil produced 100 fruit bodies and the sterilized mixture of soil/ sawdust produced 44 fruit bodies .The sawdust (sterilized and unsterilized) and the unsterilized mixture of soil/sawdust did not produce any mushroom. The study showed that loamy soil is the best substrate for the production of fruit bodies and that sterilized sawdust may be a good medium for spawn run in commercial mushroom production.

Introduction

Mushrooms are macroscopic fungi, which provide food for humans throughout the world (Oei, 1991). Evidences abound in other countries on cultivation success, where through improved technologies and pure culture spawns, mushrooms have become a source of foreign exchange (Ekpo and Aluko, 2002). *Pleurotus tuber-regium* (Fries) Singer, forms tubers (sclerotia) when the mycelium encounters unfavourable conditions. According to Oei (1991), so much energy is stored in the sclerotia such that addition of sufficient water will produce mushrooms. Most *Pleurotus* species are edible, fast growing and capable of rapid degradation of wood: Ekpo and Aluko(2002), Okhuoya and Etugo (1992), Oei (1991), Oso (1977), Zadzaril (1974), Block *et. al.* (1958).

The aim of the research was to find how well, the sclerotia of *Pleurotus tuber-regium* could grow on different substrates, such as, sterilized and unsterilized loamy soil, sawdust, and a mixture of loamy soil/sawdust.

Materials and methods

This experiment was carried out in the green house of the department of Forest Resources Management, University of Ibadan, Ibadan, Nigeria. The location falls on longitude 3^0 54' E and latitude 7^0 26'N of the equator at 750m above sea level. Duration of investigation was from November 2003 to January 2004 (10 weeks). This period was within the dry season in South-western Nigeria.

The sclerotia used in this study were purchased from the open markets in the Ibadan metropolis of Oyo state, Nigeria. The sawdust was collected (in jute bags) from a small –scale sawmill in the same area, while the soil was collected from the nursery of the project site. The soil/ sawdust mixture was prepared at a 1: 1 proportion.

Sterilization of substrates: There were six treatments, which consisted of 0.45% hydrogen peroxide sterilized and unsterilized: loamy soil, sawdust and loamy soil/sawdust mixture. The "Add and Stir" method described in Wayne (2001) was used for the sterilization procedure. Hence 25kg of sawdust, 115kg of soil and 85kg of soil/sawdust mixture were each mixed with 60 litres of 0.45% hydrogen peroxide solution. Each treatment was allowed to stay for 2 hours before draining the hydrogen peroxide solution. Each drained medium was put in 10 plastic containers (24cm x 16cm x 14cm) and replicated 3 times. Hence, there were altogether 180 containers.

Inoculation: The sclerotia were cut into 30g and soaked in water for 12hours (to improve their moisture content), following the method of Ekpo and Aluko (2002). The soaked inocula were sown in each of the substrates at a shallow depth (5-

8cm) in order to encourage early primordial emergence. Watering was done daily. Data were taken on time of sporophore emergence, measurements of stipe height and pileus diameter were taken daily until the measurements were constant; the sporophores were then harvested, cleaned, air dried and weighed. The experimental design used was a completely randomized design (one-way). In this design, all treatments were applied at random to the experimental unit. The statistical model used was

$$Y_{ij} = \mu + t_i + \sum_{ij}$$

where Y_{ij} = ijth observation under i treatment with jth experimental unit.

μ = General mean (i.e. parameter common to all treatments)

ti = Effects of treatment i

$\sum_{ij}$ =Random error due to treatment i in the jth experimental unit.

The data were subjected to descriptive statistics and analysis of variance.

Results

The results showed that all the substrates supported the growth of the mycelia (spawn rum) with sterilized sawdust performing as the best. However, only three of the substrates: sterilized soil, unsterilized soil and the mixture of sterilized soil/sawdust supported sporophore emergence (Table 1).

Out of these three, the highest number of Mushrooms was produced from the sterilized soil (139) followed by unsterilized soil (100) and the mixture of soil/sawdust (44). Most of the sporophore (Mushrooms) that emerged did so within the 11-20 days interval, though the first sporophore emerged on sterilized soil 7 days after inoculation.

The highest mean stipe height (4.58cm) was recorded in the sterilized soil during the 31-41 day period (Fig. I) while the sterilized mixture of soil/sawdust recorded the highest pileus diameter of 5.46cm during the 11-20 day period (Fig. II). The analysis of variance of the mean stipe height, pileus diameter, pileus height, total height and dry weight showed that significant differences occurred among the various treatments at 0.05% level of significance

Table I: Fruit body production by sclerotium of *Pleurotus tuber –regium* in different substrates

Substrates	Time intervals (days)							Total
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	
St soil	9	50	28	4	2	21	25	139
Unst soil	8	52	26	10	4	0	0	100
St mixt	3	13	0	8	0	8	12	44
Unst mixt	0	0	0	0	0	0	0	0
St sawd	0	0	0	0	0	0	0	0
Unst sawd	0	0	0	0	0	0	0	0
Total	20	115	54	22	6	29	37	283

Note that: St = sterilized, unst = unsterilized,mixt = mixture, sawd = sawdust

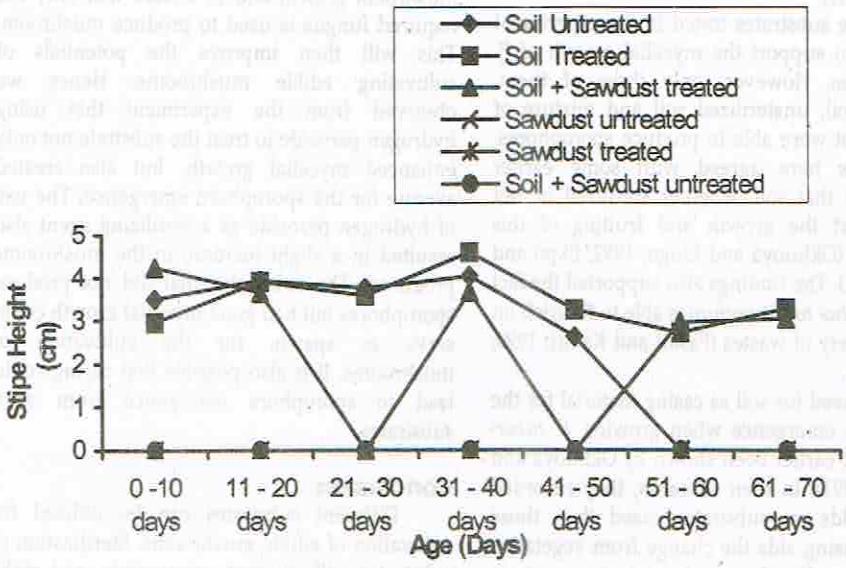

Fig.I: Mean stipe height of Pleurotus tuber-regium in three rooting media.

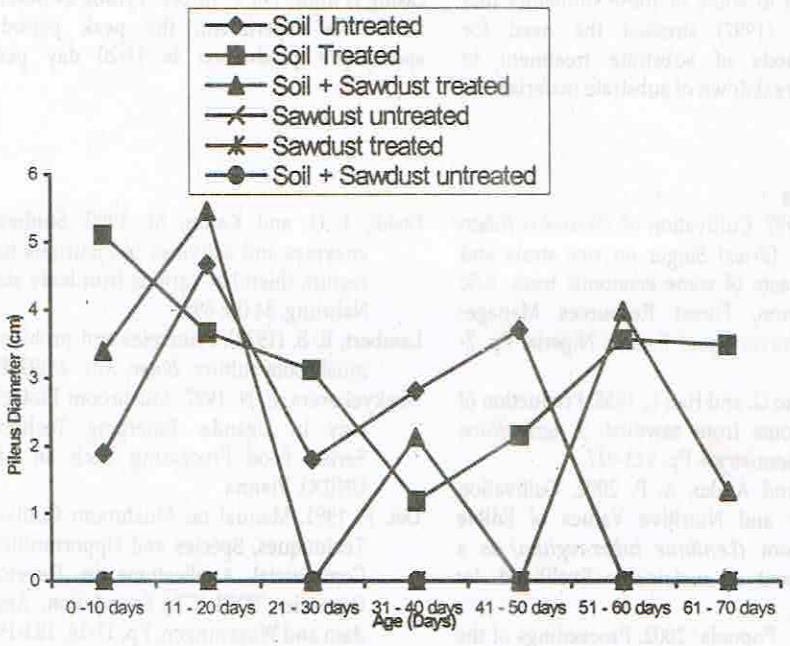

Fig.II:Mean pileus diameter of Pleurotus tuber-regium on three rooting media.

Discussion

All the substrates tested in the experiment were able to support the mycelial growth of P. *tuber-regium*. However, only three of them, sterilized soil, unsterilized soil and mixture of soil/sawdust were able to produce sporophores. The results here agreed with some earlier observation that soil, whether sterilized or not will support the growth and fruiting of this mushroom (Okhuoya and Etugo, 1992; Ekpo and Aluko, 2002). The findings also supported the fact that *Pleurotus tuber-regium* is able to flourish on a wide variety of wastes (Fasidi and Kadiri, 1990; Ajayi, 1997).

The need for soil as casing material for the sporophore emergence when growing *P. tuber-regium* had earlier been shown by Okhuoya and Okogbo (1991). In their research, they recorded higher yields on substrates cased than those without. Casing aids the change from vegetative phase (mycelium) to reproductive phase (Lambert, 1938). The untreated soil and sawdust that did not produce sporophores may have had some contaminants that prevented the sprouting as there was mycelial growth.

It is due to some of these inhibitors that Nkakyekorera (1997) stressed the need for various methods of substrate treatment to facilitate the breakdown of substrate materials for mushroom growth and to ensure that only the required fungus is used to produce mushroom. This will then improve the potentials of cultivating edible mushrooms. Hence we observed from the experiment that using hydrogen peroxide to treat the substrate not only enhanced mycelial growth, but also created avenue for the sporophore emergence. The use of hydrogen peroxide as a sterilizing agent also resulted in a slight increase in the mushrooms produced. The substrates that did not produce sporophores but had good mycelial growth could serve as spawn, for the cultivation of mushrooms. It is also possible that casing could lead to sporophore emergence from such substrates.

Conclusion

Different substrates can be utilized for cultivation of edible mushrooms. Sterilization of substrates will eliminate competitors and make substrates more suitable for mushroom growth. Sawdust can be used for spawn production for mushroom cultivation. Substrates such as sawdust can only produce sporophores when casing is done. For *P. tuiber-regium*, as observed from this experiment, the peak period of sporophore production is 11-20 day period.

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