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Influence of light and spawn quantity on the growth of Nigerian mushroom *Pleurotus tuber-regium*

O.O. Kuforiji[1] and I.O. Fasidi[2]

[1]Bells University of Technology, P.M.B. 1015, Ota, Ogun State - 23401, Nigeria
[2]Department of Botany and Microbiology, University of Ibadan, Oyo State - 23402, Nigeria

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Abstract: *The effects of light and quantity of spawn on the sporophore and sclerotial yields of Pleurotus tuber-regium, cultivated on cotton wastes, rice straw, cocoyam peel, corncob, groundnut shell and sawdusts of Mansonia altissima, Khaya ivorensis and Boscia angustifolia were observed. The organism had sporophore and sclerotial yield values of 36.8 and 27.6 g kg^{-1} waste, respectively, in cotton waste, at light quantum of 695 lux. There was a highly significant increase in yield of sclerotia (188.0 g kg^{-1} waste), in total darkness , while malformed fruitbodies (sporophores) were produced in all the substrates under the same condition. Increasing the quantity of spawn from 5 to 30% reduced the period of spawn run from 13 to 6, 15 to 8 and 24 to 17 days, respectively, in P. tuber-regium fruitbodies grown in cotton waste, rice straw and sawdust of B. angustifolia, with yield values of 38.0 and 20.0 g kg^{-1} waste in cotton waste and rice straw. The optimal spawn levels for sclerotia formation in the two wastes were 10 and 5%, respectively. The mushroom did not produce sclerotia in corn cob and groundnut shell when exposed to light. However, maximal yield values of 286.8 and 288.4 g kg^{-1} waste were obtained in both substrates in total darkness.*

Key words: *Light, Spawn quantity, Sporophore, Sclerotia, Yield*
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Introduction

Pleurotus tuber-regium (Fr.) Singer (oyster mushroom) is an edible, tropical ,fast growing, sclerotium forming, rapid wood degrading fungus, used in Nigeria in various food and medicinal preparations(Braun *et al.*, 2000). The mushroom can be cultivated on a range of materials from forestry and agriculture, including hardwood sawdusts, paper, cereal, sugarcane baggase ,cocoyam and cassava peels and cotton wastes (Hall *et al.*, 2003; Kuforiji and Fasidi, 2003).The versality of substrates and the relative ease of cultivation coupled with the number of oyster mushrooms that are edible, have led to rapid increase in production in recent years . As a result, oyster mushrooms are now the second largest produced mushrooms in the world in terms of volume. Besides taste and nutritional appeal, they secrete a wide range of enzymes which can degrade all the three key categories of polysaccharides found in the biomass of agriculture and forestry residues, namely, cellulose, hemicellulose and lignin. Thus ,they are capable of growing on a wide range of substrates (Kuforiji *et al.*, 2003; Chang and Miles, 2004).One essential component in commercial mushroom production is the use of high quality spawn, which must be produced from a superior strain efficient in terms of time, energy cost and materials. The colonization of the substrates by the mushroom mycelia is known as 'spawn running'. Once this is completed, the substrate becomes suitable for fruiting or sporophore formation. In some groups of mushrooms, light is required to trigger fruiting, although fungi do not require light to produce carbohydrates, unlike green plants (Kuforiji and Fasidi, 2005). *Pleurotus tuber-regium* also form sclerotia so as to survive under adverse environmental conditions (Fasidi and Ekuere, 1993).

Thus, the present study was embarked upon in order to determine the effects of light and quantity of spawn on the sporophore and sclerotial yields of *P. tuber- regium* , on various agro wastes.

Materials and Methods

The inoculum used for this study was obtained from tissue culture of *Pleurotus tuber-regium* sporophores grown in the laboratory of the Federal Institute of Industrial Research, Oshodi, Nigeria.

Effect of light on growth of *P. tuber-regium:* One hundred gram of the sterilized agro - wastes; cotton waste, rice straw, corn cob, groundnut shell, cocoyam peel and sawdusts of *Mansonia altissima, Khaya ivorensis* and *Boscia angustifolia* were inoculated with 10 g cotton waste spawn of *Pleurotus tuber-regium*. A set of the experiment was left in the open laboratory and the other set kept in a cupboard in total darkness. The light intensity was measured using a light meter Guarda Fx-101 luxmeter. The period of fruiting (sporophore formation) was 8-12 weeks. For sclerotia formation, the inoculated substrates were incubated for 4 months. The yields of sporophores and sclerotia were determined at temperature and relative humidity of 28$\pm$ 2^{o}C and 68$\pm$2%, respectively in both set ups (Kuforiji and Fasidi, 2007).

Effect of spawn quantity on growth of *P. tuber-regium:* One hundred gram each of the above named sterilized wastes, was

Table - 1: Effect of light (695 lux) on sporophore and sclerotia yield in *P. tuber-regium*

Substrates	Yield of fruitbodies (g kg^{-1} waste)		Yield of sclerotia (g kg^{-1} waste)	
	Light	Continuous darkness	Light	Continuous darkness
Cotton waste	36.8 ± 0.5^{a}	Malformed fruitbodies	27.6 ± 0.5^{d}	188.0 ± 0.9^{d}
Rice straw	20.3 ± 1.0^{c}		-	160.0 ± 1.2^{e}
Corn cob	19.4 ± 0.6^{c}	produced in all	-	286.8 ± 0.5^{a}
Groundnut shell	-		-	288.4 ± 03^{a}
Cocoyam peel	9.5 ± 1.0^{de}	the substrates	36.0 ± 0.6^{bc}	199.4 ± 0.5^{c}
M.altissima	22.8 ± 15^{c}		34.6 ± 1.3^{c}	184.4 ± 0.6^{d}
K. ivorensis	32.2 ± 1.5^{b}		57.4 ± 0.5^{a}	212.2 ± 1.5^{b}
B. angustifolia	12.5 ± 2.0^{d}		39.2 ± 0.9^{b}	135.0 ± 0.6^{f}

$F_{0.05}$ = 3.20, $F_{0.01}$ = 5.18. Means ± S.E difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and 1% levels, respectively. Column value followed by the same letters are not significantly different by Duncan's multiple range test (p<0.05)

inoculated with 5-30% cotton waste spawn of *P. tuber-regium*. These were then incubated at 28^{o}C and relative humidity of 68±2%. The light intensity was 695 lux. The period of fruiting was 8-12 weeks, while for sclerotia formation, the substrates were kept in the dark for 4 months. The yields of sporophore and sclerotia in various wastes were weighed. Each experiment was carried out in triplicate (Fasidi,1996).

Statistical analysis: Samples were in three replicates and the means were quoted with their standard error (S.E.).Statistical analyses were carried out using analysis of variance and the Duncan multiple range test (Sokal and Rohlf, 1969).

Results and Discussion

The yields of fruitbodies and sclerotia produced in the wastes are as shown in Table 1.The highest yield of 36.8 g kg^{-1} was in cotton wastes followed by 32.2 g kg^{-1} waste in *Khaya ivorensis*, while the least was in cocoyam peel at light quantum of 695 lux. This shows the importance of light for sporophore formation in *P. tuber-regium*. No sporophores were produced in groundnut shell and in continuos darkness, the fruitbodies were not well developed as sporophores with no caps were produced in all the wastes. Stamets and Chilton (1983), reported that lighting is required for *Agaricus bitorquis* and *A. brunnensence*, while natural daylight or glowlight on a 12 hr. cycle on /off is necessary for the cultivation of *Coprinus comatus* and *Flammulina velutipes. Pleurotus oestreatus* is known to be phototropic and most responsive to an exposure of 2000 lux hr^{-1} for 12 days, while Chang (1977) reported that light intensity produced no significant effect on growth, unlike temperature and humidity. Jandick and Kapoor (1977) observed that presence or absence of light did not affect production of fruitbodies in *Pleurotus* sp.

Sclerotia development, however is enhanced in total darkness as high yields of 288.4 and 286.8 g kg^{-1} waste were obtained on groundnut shell and corn cob, respectively. At light quantum of 695 lux, no sclerotia developed on rice straw, corn cob and groundnut shell, while the yield was low in cotton waste, cocoyam peel and sawdusts of *Mansonia altissima, K. ivorensis* and *Boscia angustifolia* (Table 1). This result was collaborated by Stamets (1993) for *Psilobe mexicana* and *P. tampensis* which produced sclerotia on grain seeds six weeks and six months, respectively after inoculation when incubated in total darkness.

The quantity of spawn affected the duration of spawn run as well as the yield of fruitbodies and sclerotia as shown in Table 2, 3. The period of spawn run decreased from 13 to 6, 15 to 8, 20 to 14 and 25 to 18 days in cotton wastes, rice straw, cocoyam peel and sawdust of *M. altissima*, respectively, with increase in yield from 34.8 to 38 g kg^{-1} waste, 18 to 20 and 15 to 18.6 g kg^{-1} waste on inoculating with 25% spawn in cotton waste, rice straw and sawdust of *M. altissima*, respectively. There was a 5% level significant increase in yield from 36 to 39.5 g kg^{-1} waste in *K. ivorensis* which inferred that this substrate was best suited for the cultivation of *P. tuber-regium* unlike the sawdusts of other plants. Although the period of spawn run was faster with increased quantity of spawn in *B. angustifolia* and cocoyam peels, there was no significant increase in the yield of sporophores, while, in the formation of sclerotia, different quantities of spawn gave optimal yields, suggesting that there is no correlation between the amount needed for sporophore and that of sclerotia formation (Table 2, 3). Stamets (1993) reported that spawn quantities of 3 and 3.5- 5% were adequate for corn cob and straw at moisture contents of 70 and 75%, respectively and spawn run of 10-14, 30-60 and 10-14 days were reported for *Pleurotus oestreatus, Psilocybe cyanensence* and *P. tampensis* in wheat straw, sawdust and rice bran (ratio 4:1) and rye grass seed, respectively. The difference in quantity of spawn required from the above may be due to different strains used in this study. Also it is advantageous to use high spawning rates as these lead to more rapid colonization of the substrates. This must proceed as fast as possible to prevent other organisms from becoming established. Other factors like moisture content and supplementation of the substrates with rice bran have been reported to increase the yield of this mushroom. *Pleurotus tuber-regium* cultivated on cotton waste, rice straw, cocoyam peels and sawdusts of *Khaya ivorensis*, *Mansonia altissima* and *Boscia angustifolia* gave maximum yields of fruitbodies when moisture contents of substrates were 70.6, 75, 61.3, 76.6, 69.7 and 72.5%,

Table - 2: Effect of quantity of spawn on yield of *P. tuber-regium.* Spawn run (days) and yield of fruitbodies (g kg^{-1} waste)

Quantity	Cotton waste		Rice straw		*M. altissima*		*K. ivorensis*		*B. angustifolia*		Cocoyam	
of spawn (%)	Spawn run	Yield	Spawn run	Yield	Spawn run	Yield	Spawn run	Yield	Spawn run	Yield	Spawn run	Yield
5	13	34.8 ± 4.0^{a}	15	18.0 ± 2.9^{de}	25	15.0 ± 2.3^{f}	21	36.0 ± 4.2^{b}	24	15.5 ± 2.6^{ef}	20	9.0 ± 0.6^{g}
10	11	34.8 ± 3.6^{c}	13	18.0 ± 2.3^{de}	25	15.0 ± 2.5^{f}	17	36.5 ± 4.5^{b}	24	15.5 ± 2.6^{ef}	16	9.0 ± 1.3^{g}
15	9	36.6 ± 4.5^{bc}	10	18.5 ± 2.9^{d}	23	16.5 ± 3.3^{e}	15	37.5 ± 4.5^{b}	24	16.5 ± 2.9^{e}	15	9.5 ± 0.6^{g}
20	7	38.0 ± 4.6^{ab}	10	20.0 ± 2.6^{d}	20	18.6 ± 3.9^{d}	15	39.5 ± 4.6^{e}	20	16.5 ± 2.6^{e}	15	9.5 ± 0.3^{g}
25	6	38.0 ± 4.6^{b}	8	18.5 ± 2.5^{d}	20	18.6 ± 3.5^{d}	13	39.5 ± 4.3^{a}	18	16.5 ± 2.6^{e}	15	9.5 ± 0.6^{g}
30	6	37.0 ± 4.4^{b}	8	18.0 ± 2.3^{de}	18	18.3 ± 3.0^{d}	13	38.5 ± 4.0^{a}	17	16.0 ± 2.3^{ef}	14	9.5 ± 0.6^{g}

$F_{0.05} = 1.92_{1}$, $F_{0.01} = 2.50$, Means ± S.E difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and 1% levels, respectively. Column value followed by the same letters are not significantly different by Duncan's multiple range test ($p<0.05$)

Table - 3: Effect of quantity of spawn on yield of sclerotia (g kg^{-1} waste) in *P. tuber-regium*

Quantity of spawn %	Cotton waste	Rice straw	*M. altissima*	*K. ivorensis*	*B. angustifolia*	Corn cob	Cocoyam peel	Groundnut peel
5	165.0 ± 1.6^{n}	173.0 ± 2.0^{m}	185.0 ± 1.3^{k}	205.0 ± 2.6^{h}	135.0 ± 0.9^{r}	285.6 ± 2.5^{ab}	195.3 ± 1.6^{j}	289.0 ± 2.3^{a}
10	185.3 ± 1.6^{k}	155.3 ± 1.6^{o}	184.8 ± 0.9^{k}	223.3 ± 1.9^{g}	140.1 ± 1.3^{q}	286.8 ± 2.5^{a}	195.4 ± 1.6^{j}	286.3 ± 2.0^{a}
15	180.1 ± 1.3^{l}	130.2 ± 1.6^{s}	156.2 ± 1.5^{o}	205.4 ± 1.6^{h}	153.0 ± 1.3^{o}	280.3 ± 2.6^{c}	200.1 ± 1.9^{i}	255.2 ± 2.6^{e}
20	150.0 ± 1.6^{p}	105.2 ± 1.3^{u}	153.0 ± 1.6^{o}	123.3 ± 1.5^{t}	135.2 ± 1.5^{r}	270.3 ± 2.3^{d}	205.2 ± 1.3^{h}	235.1 ± 2.5^{f}
25	125.3 ± 1.5^{t}	100.1 ± 1.5^{v}	153.3 ± 1.5^{o}	155.1 ± 1.6^{o}	135.5 ± 0.9^{r}	255.1 ± 2.6^{e}	205.3 ± 1.5^{h}	200.3 ± 2.6^{i}

$F_{0.05} = 2.28$, $F_{0.01} = 3.23$, Means ± S.E difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and 1% levels, respectively. Column value followed by the same letters are not significantly different by Duncan's multiple range test ($p<0.05$)

respectively. For sclerotia, groundnut shells and corncob were the most suitable ones at moisture levels of 63.7 and 63.1%, respectively. Supplementation of the substrates did not significantly improve the yields in cotton waste and rice straw, while sawdusts of *M. altissima*, *K. ivorensis* and *B. angustifolia* gave maximum yields of 30.5, 36.5 and 15.5 g kg^{-1} waste when supplemented with 20, 25 and 15% rice bran, respectively. Although there was mycelial run on groundnut shells and cocoyam peels when supplemented with rice bran, no fruitbodies were formed and no sclerotia were produced on supplemented substrates (Kuforiji and Fasidi, 2007). Philipousis and Balis (1995) reported that sclerotium formation and maturation in *Morchella* sp is greatly affected by the nutrient status of the substrate, while Singh and Verma (2000) mentioned that the sclerotia of *Morchella* can be produced under both nutrient rich and nutrient poor conditions. Cultivation of edible mushrooms on agro industrial wastes must be encouraged so as to reduce the environmental pollution posed by indiscriminate dumping of these materials. In addition, an edible food that is relatively rich in high quality protein is produced, thus alleviating the problem of malnutrition in the society (Kuforiji and Fasidi, 2008). Huge amount of biodegradable biomass generated as waste is available for utilization as a source of energy which can be treated for pollution abatement as well as energy generation (Narsi *et al.*, 2006).

Thus, it can be concluded that light and quantity of spawn affect the sporophore and sclerotial yield of *Pleurotus tuber-regium* in the agrowastes with *Khaya ivorensis* as the most suitable substrate, giving the highest yield.

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