International Food Research Journal 20(6): 3387-3393 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Growth factors and cultivation of *Pleurotus tuber-regium* on selected plant wastes

[1*]Apetorgbor, A. K., [2]Dzomeku, M. and [3]Apetorgbor, M. M.

[1]*Department of Theeoretical and Applied Biology,KNUST, Kumasi, Ghana*
[2]*Food Research Institute, P. O. Box M 20, Accra, Ghana*
[3]*Forestry Research Institute of Ghana, P. O. Box 63 KNUST, Kumasi, Ghana*

Article history

Received: 23 March 2011
Received in revised form: 8 July 2013
Accepted: 12 July 2013

Keywords

Pleurotus tuber-regium
Sclerotia
Sawdust
Agricultural wastes
Medicinal values

Abstract

Pleurotus tuber-regium is a tuberous wild mushroom species, highly expensive, nutritious, very rich in proteins and known to possess medicinal values. The dwindling forests and the absence of commercial cultivation of this mushroom have resulted in scarcity of the sclerotia. The study was conducted to determine the optimum temperature, pH and light regime for the growth of *P. tuber-regium* and evaluate the use of water hyacinth (*Eichhornia crassipes*), plantain (*Musa sapiens*) leaves, millet (*Eleusine coracana*) stalk and composted 'wawa' (*Triplochiton scleroxylon*) sawdust as substrates for its cultivation. *Pleurotus tuber-regium* grew fastest at 35ºC, pH 6 and in continuous darkness. Only plantain leaf substrate produced fruitbodies and sclerotia whilst 'wawa' sawdust produced only sclerotia in bags. Plantain leaves yielded fruitbodies with a Biological Efficiency (B.E) of 54.47% while the sclerotia gave a B.E of 62.05%. The B.E value of 99.65% was obtained from sclerotia of 'wawa' sawdust. The sclerotium had highest crude protein content followed by the pileus and stipe in decreasing order. Knowledge of the growth requirements and the potential substrates will facilitate large scale production and commercialization of the fungus.

Introduction

Pleurotus tuber-regium (Fr.) Sing. is a tuberous wild species of white rot basidiomycete which produces fruitbodies from a unique globose sclerotium that is more like a giant truffle (Nwokolo, 1987). It is tropical and subtropical in distribution and found growing on many species of hard and soft woods like *Mangifera indica*, *Daniellia oliveri* and *Treculia africana*. *Pleurotus tuber-regium* is the only known *Pleurotus* species which produces true sclerotium, and also differs from all other *Pleurotus* species in its non-pleurotoid habit (Isikhuemhen and Nerud, 1999). The sclerotia are usually of various sizes, ranging from a few centimeters to several centimeters in diameter. They are spherical to oval in shape, dark brown on the outside and whitish on the inside (Okhuoya and Okogbo, 1991).

In Nigeria, *P. tuber-regium* is used as both food and medicine. The tuber is highly nutritional (very rich in proteins), expensive and considered a delicacy (Okhuoya and Okogbo, 1990). The hard sclerotium is peeled and ground while the fruitbody is chopped and used in vegetable soup (Oso, 1977). The tuberous sclerotium can be used as a partial replacement for melon (*Citrullus lanatus*) seed or groundnut (*Arachis hypogea*) cake in traditional preparation of sauces and soups. In many parts of tropical Africa, the sclerotium is milled with melon or groundnut seeds, seasoned and moulded into patties for cooking or baking. The groundnut cake patties are consumed as snacks while fruitbodies are cooked as cheap source of protein in tropical Africa (Nwokolo, 1987).

Pleurotus tuber-regium is known to possess medicinal properties and used by traditional medical practitioners in Nigeria (Okhuoya and Okogbo, 1990). In combination with various herbs, it has been used to cure headache, stomach ailments, colds, fever, asthma, smallpox, high blood pressure and as a tonic and treatment for coughs (Fasidi and Olorunmaye, 1994; Oso, 1997). Traditionally in some communities of Ghana, *P. tuber-regium* is known to possess medicinal values and have been used by herbal doctors to cure illnesses such as underweight in children, asthma and high blood pressure among others (Dzomeku, 2009). It has been reported that the pure culture of this fungus is able to kill and feed on nematodes (Hibett and Thorn, 1994). It is also able to ameliorate crude oil polluted soils (Isikhuemhen *et al*., 2003; Adenipekun, 2008) and the resulting soil sample supported the germination and seedling of *Vigna unguiculata*. Yongabi (2004) confirmed that the sclerotium of *P. tuber-regium* is a good coagulant and a disinfectant which can be used in natural and waste water purification. In China it is used in some folk recipes as a tonic and medicine for the treatment

*Corresponding author.
Email: *apetorgboralfred@yahoo.com*

of coughs and asthma (Chengua *et al.*, 2000).

There are reports that many compounds such as ß-D-glucans and their nonstarch polysaccharides (Chenghua *et al*., 2000; Zhang *et al*., 2001), and enzymes such as ribonuclease (Wang and Ng, 2001) with potential pharmacological benefits are found in the sclerotium of *P. tuber-regium*. Local people in Nigeria collect the sclerotium of *P. tuber-regium from the forest, bury it in warm humid soil to induce* growth of fruitbodies (Oso, 1997; Okuoya and Etugo, 1993; Isikhuemhen and Okhuoya, 1995) which appear within a relatively short period of 14–21 days (Patrabansh and Madan, 1997). This ensures a ready and regular supply of fresh mushrooms since rapid deforestation is destroying their natural habitat.

The dwindling forests and the absence of commercial cultivation of this fungus has resulted in the scarcity of sclerotia, thus making the few available very expensive. As a result of this the need arises for commercial cultivation to supplement collection from the wild. In Nigeria, most studies on *P. tuber-regium* have concentrated on food and the nutritional content of the sclerotium and fruitbodies (Okhuoya and Ajerio, 1988) and on factors influencing vegetative growth and fruitbody production in laboratory experiments (Okhuoya and Okogbo, 1991; Fasidi and Ekuere, 1993; Fasidi and Olorunmaye, 1994).

Research work on mushrooms in Ghana have been mainly on *Pleurotus saju-cajor*, *P. ostreatus* and *Volvariella volvacea* (Obodai *et al.*, 2000; Obodai *et al.*, 2003; Frimpong-Manso *et al*., 2011) but that of *P. tuber-regium* is limiting even though it is traditionally known and used for medicinal purposes. The present study was conducted to determine the optimum temperature, pH and light regime for the growth of *P. tuber-regium* and evaluate the possibility of using water hyacinth (*Eichhornia crassipes*) (an evasive species), plantain (*Musa sapientu*m) leaves, millet (*Eleucine coracana*) stalk (agricultural waste) and 'wawa' (*Triplochiton scleroxylon*) sawdust (wood residue) as substrates for the cultivation of the fruitbodies and sclerotia of *P. tuber-regium*.

Materials and Methods

Sample collection

Sclerotia of *P. tuber-regium* were collected from farms around Techiman in the Brong Ahafo Region of Ghana where the fungus grows in the wild. The Brong Ahafo Region has moist semi-deciduous forest, mostly in the southern and southeastern parts, and the Guinea savannah woodland which is predominantly in the northern and northeastern parts of the region (*http:/www.ghanaweb/ghanadistricts.com*).

Vegetative growth of Pleurotus tuber-regium on PDA at different temperatures, pH and light regimes

Petri dishes each containing 20 ml of Potato Dextrose Agar (PDA) (OXOID Ltd., Basingtoke Hampshire, England) were inoculated with 3 mm discs of 7-day-old cultures of *P. tuber-regium* (prepared from fruitbodies obtained from the sclerotia from the wild) and incubated at 25^{o}C, 30^{o}C and 35^{o}C. There were four replicates for each temperature.

To obtain the optimum pH for mycelial growth of *P. tuber-regium*, pH levels of 5, 6, 7 and 8 of PDA were made by using 1M NaOH and HCl to adjust the medium. Petri plates were inoculated with 3 mm mycelial discs of *P. tuber-regium* and incubated at 35ºC, the optimum temperature for growth of the mushroom.

Petri plates were inoculated with 3 mm mycelial discs of *P. tuber-regium*, covered with transparent polythene bags and exposed to continuous light of 350-500Lux while another set was covered with black polythene bags. Other sets of plates were exposed to alternating 4 hr light/4 hr darkness, 6 hr light/6 hr darkness and 12 hr light/12 hr darkness. There were four replicates for each set and they were all incubated for 10 days. Mycelium growth was determined by measuring the orthogonal diameters daily for six days.

Substrate preparation

Triplochiton scleroxylon ('wawa') sawdust (collected from a sawmill at Ofankor in the Greater Accra Region) was mixed with 1% NPK (25: 15: 5) fertilizer, 10% rice bran and 1% $CaCO_3$ (w/w, based on dry weight of sawdust) and then moistened with water to a moisture content of about 70%. The mixture was then composted in a pyramidal heap (Obodai *et al*., 2002) for 28 days. The heap was turned every 4 days to obtain uniform composting and to avoid anaerobic fermentation in the core section of the heap. After composting, the heap was mixed again with 1% NPK (25: 15: 5) fertilizer and 0.5% $CaCO_3$ (w/w).

Dry plantain leaves, dry water hyacinth and millet stalk were cut into about 3 cm long pieces. The plantain leaves and water hyacinth were soaked in clean water containing 0.5% $CaCO_3$ (w/w) for 2 hrs while the millet stalk was soaked for 24 hrs after which the excess water was drained off. The prepared substrates were each put into heat resistant polypropylene bags (18 x 33 cm, with a thickness of 0.8 cm), filled to a weight of 1 kg and compacted (Obodai *et al.*, 2002). Four replicates were prepared for each substrate.

Growth of Pleurotus tuber-regium in bagged substrates

All bagged substrates were sterilized by steaming for 3 hrs. The substrates were aseptically inoculated with about 20 grains of *P. tuber-regium* spawn prepared with sorghum and incubated at 30 ± 2°C. Mycelial growth along the sides of the bag was measured weekly for three weeks. The bags were left to induce formation of fruitbodies and sclerotia. Data on yield performance of fruitbodies (weight and number) were collected during the 180-day cropping period.

Biological efficiency

Biological Efficiency (B.E) was worked out using the dry matter content of each substrate at bagging using the formula developed by Chang (1984):

$$BE = \frac{\text{Fresh weight of mushroom x 100\%}}{\text{Dry weight of substrate at bagging}}$$

Chemical constituents of substrates used, sclerotium and fruitbody of Pleurotus tuber-regium

Chemical analysis of all the substrates (composted sawdust, water hyacinth, millet stalks and plantain leaves), sclerotium and fruitbody of *P. tuber-regium* were carried out. Ash was determined as the residue of incineration of 1 g powdered sample in a crucible of known weight at 550°C in a muffle furnace (AOAC, 2002) while protein was determined by using the adjusted conversion factor (4.38) (Oei, 1991; Shashirekha *et al*., 2002). Cellulose and lignin were determined by the Acid Detergent Fibre (ADF) method of Van Soest and Robertson (1985). Total carbohydrate was determined by the anthrone method (Plummer, 1971). Serial dilutions of glucose stock (10 mg/100 g) solution were used and the absorbance was read at 620 nm against a reagent blank. Crude fibre was determined using petroleum spirit and sulphuric acid to extract 3.0 g of sampled substrate. The insoluble matter was washed with boiling water and sodium hydroxide solution after which the dried insoluble matter was incinerated with a dull red heat and the crude fibre content calculated (AOAC, 2002).

Statistical analysis

Data obtained from mycelial growth on PDA, proximate composition of substrates and fruitbodies were subjected to the one-way analysis of variance (ANOVA) in Excel at $P \leq 0.05$ and differences between means were determined using the least significant difference (LSD) statistics.

Results

Vegetative growth of Pleurotus tuber-regium on PDA at different temperatures, pH and light regimes

Radial mycelial growth of *P. tuber-regium* on PDA increased with increasing temperature (Fig.1). *P. tuber-regium* grew faster at 35ºC than at 25ºC and 30ºC. pH 6 was the best medium for mycelial growth followed by pH 7 and pH 8 in decreasing order (Table 1). Continuous darkness was the best for mycelial growth of *P. tuber-regium* followed by alternating 12 hr light/12 hr darkness. The poorest growth was observed in continuous light (Table 2).

Growth of Pleurotus tuber-regium on bagged substrates

The mycelia of *P. tuber-regium* on the bagged substrates colonized the water hyacinth fastest, followed by the plantain leaves and the millet stalk (Fig.2). On the water hyacinth the mycelia covered the whole bag in two weeks while on the plantain leaves, millet stalk and the composted sawdust the mycelia covered 68%, 66% and 65% of the substrates respectively within the same period. All the bags were fully colonized within 21 days.

Only the plantain leaf substrate produced fruitbodies of *P. tuber-regium*. Seven flushes with total fruitbodies weighing 1089.4 g were recorded. Sclerotia formation occurred in 'wawa' sawdust and plantain leaves, but not in the millet stalks or water hyacinth. The sawdust yielded heavier and more sclerotia than the plantain leaves. A total of 1,993 g and 1,241 g of sclerotia were harvested from the sawdust and the plantain leaves, respectively.

Biological efficiency (B.E) of fruitbodies and sclerotia

Plantain leaves yielded fruit bodies that gave a B.E of 54.47% while the sclerotia gave a B.E of 62.05%. The 'wawa' sawdust did not produce any fruit body but yielded sclerotia with a B.E of 99.65%.

Nutrient composition of substrates

The ash content of the substrates ranged from 6.02 g/100 g substrate in the millet stalk to 13.07 g/100 g in the water hyacinth (Table 3). The plantain leaves contained the highest amount of crude protein (15.92 g/100 g) followed by 'wawa' sawdust (12.50 g/100 g), millet stalk (6.73 g/100 g) and water hyacinth (1.34 g/100 g). Cellulose and hemicellulose were most abundant in 'wawa' sawdust (34.04 g/100 g; 15.71 g/100 g) and millet stalk (25.05 g/100 g; 21.41 g/100 g) respectively and least in water hyacinth (20.23

Table 1. Mycelial growth of *Pleurotus tuber-regium* at 35ºC on Potato Dextrose Agar at different pH's

Period of incubation (Days)	Mean diameter of fungal colony (cm) at pH*			
	5.0	6.0	7.0	8.0
1	2.7±0.12^a	4.0±0.13^b	2.7±0.11^a	2.3±0.16^a
2	4.5±0.15^a	6.2±0.09^b	5.0±0.19^b	4.3±0.13^a
3	5.2±0.07^a	7.5±0.14^b	5.4±0.12^a	5.0±0.12^a
4	5.8±0.09^a	9.0±0.05^c	6.5±0.11^b	6.1±0.13^b
5	6.3±0.03^a	-	7.8±0.08^c	7.0±0.06^b
6	7.6±0.11^a	-	9.0±0.11^c	8.2±0.07^b
7	8.7±0.07^a	-	-	9.0±0.04^b
8	9.0±0.04	-	-	-

→ overgrown the Petri plate.
*Values bearing the same letter in superscript in a row are not significantly different at the 5% significance level.

Table 2. Mycelial growth of *Pleurotus tuber-regium* on PDA in different light regimes

Period of incubation (Days)	Mean diameter* of fungal colony (cm) in				
	Continuous light	Continuous darkness	4 hr light/ 4 hr darkness	6 hr light/ 6 hr darkness	12 hr light/ 12 hr darkness
1	1.9±0.03^a	3.7±0.07^c	2.5±0.07^b	2.3±0.08^b	2.4±0.09^b
2	2.7±0.05^a	4.9±0.12^c	3.2±0.07^b	3.0±0.09^b	3.2±0.08^b
3	3.6±0.05^a	5.9±0.07^c	3.9±0.09^b	3.9±0.11^b	4.0±0.11^b
4	4.4±0.09^a	7.8±0.07^c	4.6±0.11^a	4.4±0.10^a	4.9±0.10^b
5	5.1±0.06^a	9.0±0.09^c	5.3±0.12^a	5.0±0.11^a	6.5±0.08^b
6	6.0±0.05^a	-	6.1±0.10^a	6.2±0.14^a	7.8±0.11^b
7	7.0±0.07^a	-	6.9±0.07^a	7.5±0.05^b	9.0±0.12^c
8	8.2±0.08^a	-	8.6±0.09^b	8.4±0.08^a	-
9	9.0±0.09^a	-	9.0±0.09^a	9.0±0.12^a	-

→ overgrown the Petri plate.
*Values bearing the same letter in superscript in a row are not significantly different at the 5% significance level.

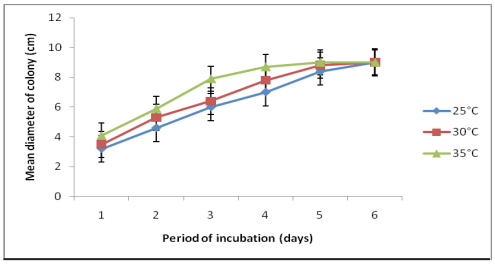
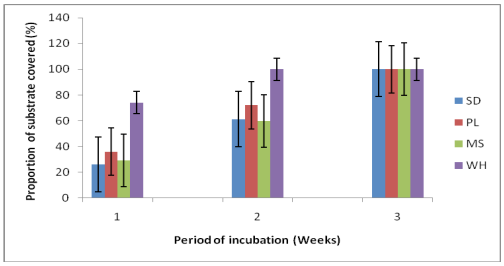


Figure 1. Mycelial growth of *Pleurotus tuber-regium* on PDA at different temperatures



SD: Sawdust PL: Plantain leaves MS: Millet straw WH: Water hyacinth

Figure 2. Mycelial growth of *Pleurotus tuber-regium* on bagged substrates

g/100 g; 15.71 g/100 g). Lignin was most abundant in 'wawa' sawdust (20.43 g/100 g) and lowest in water hyacinth (12.22 g/100 g); plantain leaves had 14.45 g/100 g while millet stalk had 13.53 g/100 g.

Nutrient composition of sclerotium and fruitbody of Pleurotus tuber-regium

The nutrient composition varied from the part of *P. tuber-regium* used (Table 4). The ash, fat and carbohydrate compositions were higher in the sclerotium than in the fruitbody while the crude protein and moisture contents were higher in the fruitbody than in the sclerotium. The crude protein and the ash compositions were very high in the pileus (cap) compared to those in the stipe (stalk) but those of the fat and carbohydrate did not differ much.

Discussion

Mycelium growth of *P. tuber-regium* on the PDA was fastest at 35ºC, whilst 25ºC supported the least growth of the mycelium. Oso (1977) and Isikuemhem *et al.* (2000) found the optimum growth temperature for this fungus to be between 30 and 35ºC.The pH value 6.0 supported the best growth of *P. tuber-regium* with pH 5.0 recording the least growth. This could be due to the fact that extracellular enzymes that are extruded by the mushroom for degrading the complex macromolecules (cellulose and hemicelluloses) into simple soluble compounds to be taken up by the mushroom are very active at pH 6.0 and subsequently inactive at pH 5.0. Poppe and Hofte (1995) reported that most enzymes have their optimum range of activity, and that ranges below or above the optimum may render the enzymes inactive or slow down their activities.

Continuous darkness supported the fastest growth of *P. tuber-regium* mycelia whilst continuous light exposure of 350-500Lux supported the least growth. Light was therefore not needed for the mycelial growth of *P. tuber-regium*. Oei (1996) noted that mycelia growth of mushrooms can take place in the dark, but for fruiting light must be present. Continuous darkness is needed for good growth of the mycelia. In continuous light the mycelium grows but at a very slow rate.

All the bags were fully colonized within 21 days of incubation. Mycelial growth in the water hyacinth was the fastest followed by that in the plantain leaves and the millet stalk. Xiujin *et al.* (2001) observed that *Pleurotus* spp. had greater capability to digest lignin and this degradation played an important role in the development of the mycelium. Sawdust yielded heavier and more sclerotia (1,993 g) than the plantain leaves (1,241 g). The sawdust substrate which has the highest cellulose value of 34.04% is more likely to be similar to that obtained in decaying logs, the natural habitat of the fungus. Many lignocellulosic substrates can support growth of *P. tuber-regium* (Isikhuemhen and Nerud, 1999).

The fact that the sawdust did not produce fruitbodies was due to the fact that the fungus was still forming mycelia and storing food reserves in the sclerotia. The food reserves stored in the sclerotia would later be used in the formation of fruitbodies. However, when Okhuoya and Etugo (1993) seeded

Table 3. Proximate composition of fresh and spent substrates (in parenthesis) used in the cultivation of *Pleurotus tuber-regium*

SUBSTRATE		COMPOSITION* OF							
Common name	Scientific name	Ash (g/100 g)	Crude protein (g/100 g)	Cellulose (g/100 g)	Hemicellulose (g/100 g)	Lignin (g/100 g)	Crude fibre (g/100 g)	Organic matter (g/100 g)	pH
Plantain leaves	*Musa sapientum*	10.50±0.03^{b} (6.11±0.13^{b})	15.92±0.06^{d} (18.49±0.01^{d})	22.68±0.10^{a} (13.0±0.15^{a})	20.67±0.03^{b} (10.25±0.21^{b})	14.45±0.04^{a} (8.29±0.10^{a})	25.69±0.14^{b} (12.12±0.11^{b})	86.81±0.13^{a} (83.81±0.13^{a})	6.70±0.05^{c} (7.91±0.07^{c})
Water hyacinth	*Eichhornia crassipes*	13.07±0.05^{c} (10.90±0.15^{c})	1.34±0.11^{a} (1.12±0.14^{a})	20.23±0.06^{a} (5.17±0.10^{a})	15.71±0.09^{a} (6.97±0.15^{a})	12.22±0.05^{a} (9.16±0.05^{a})	12.53±0.22^{a} (5.41±0.22^{a})	84.12±0.17^{a} (80.12±0.17^{a})	5.97±0.05^{b} (6.60±0.12^{b})
Millet stalk	*Eleusine coracana*	6.02±0.09^{a} (2.10±0.03^{a})	6.73±0.04^{b} (5.50±0.07^{b})	25.05±0.05^{b} (19.11±0.05^{b})	21.41±0.05^{b} (11.27±0.05^{b})	13.53±0.03^{a} (8.00±0.13^{a})	32.11±0.17^{c} (10.09±0.09^{c})	90.37±0.11^{b} (85.37±0.11^{b})	5.49±0.07^{a} (6.34±0.09^{a})
'Wawa' sawdust	*Triplochiton scleroxylon*	9.80±0.11^{b} (4.59±0.10^{b})	12.5±0.15^{c} (11.98±0.03^{c})	34.04±0.09^{c} (25.91±0.17^{c})	15.71±0.10^{a} (13.18±0.13^{a})	20.43±0.04^{b} (10.76±0.22^{b})	40.01±0.15^{d} (20.16±0.07^{d})	88.02±0.17^{b} (84.02±0.17^{b})	7.01±0.09^{c} (8.27±0.04^{c})

*Values bearing the same letter in superscript in a column are not significantly different at the 5% significance level.

Table 4. Nutrient composition of sclerotium and fruitbody of *P. tuber-regium*

Constituent	Nutrient constituent (mg/100 mg)* in		
	Sclerotium	Fruitbody	
		Pileus	Stipe
Moisture	41.03±0.85^{a}	52.3±0.09^{b}	60.1±0.09^{c}
Ash	5.6±0.64^{c}	2.0±0.05^{b}	0.4±0.02^{a}
Fat	1.03±0.08^{b}	0.5±0.09^{a}	0.6±0.11^{a}
Crude protein	0.55±0.32^{a}	9.9±0.06^{c}	2.4±0.06^{b}
Carbohydrate	43.13±0.73^{b}	39.5±0.09^{a}	38.1±0.10^{a}

*Values bearing the same letter in superscript in a column are not significantly different at the 5% significance level.

various media including sawdust with sclerotium of *P. tuber-regium*, the sawdust medium did not produce fruitbodies until it was cased with river sand. When the sawdust was seeded with grain spawn, fruiting bodies were only produced when it was supplemented with oats. Casing, using soil, appears to be necessary in the cultivation of this fungus as observed by Okhuoya and Okogbo (1991).

Cellulose content of the substrates used ranged from 20.23 g to 34.04 g/100 g substrate. 'Wawa' sawdust had the highest cellulose value of 34.04 g/100 g; this accounted for the high yield of sclerotia produced by the 'wawa' substrate. Cellulose content of the spent substrates ranged from 5.17 g to 25.91 g/100 g. The most degraded substrate was water hyacinth whose cellulose content reduced from 20.23 g to 5.17 g/100 g substrate. This implies that more cellulose was used up during the cultivation of the mushroom. The ability to degrade cellulose was attributed to the synergetic action of three different types of hydrolases called cellulases (Datta and Chakravarty, 2001). pH of the substrates increased during cultivation of the fungus and was within the range of 6.34 and 8.27. This occurred because the mushroom produced metabolites that affected the hydrogen ion concentration of the medium (Zadrazil and Kurtzman, 1982).

Within the period of study only the plantain leaves produced fruitbodies and this could be due to the ability of the fungus to quickly utilize the nutrients for mycelium formation and food storage (sclerotium formation) and later convert them into fruitbodies. The physiology of fruitbody morphogenesis requires rapid and steady supply of soluble nutrients such as glucose (Fasidi and Olurunmaiye, 1994). The millet stalk and water hyacinth produced neither fruitbodies nor sclerotia because the fungus was still forming mycelia.

Conclusion

Vegetative growth of *P. tuber-regium* on PDA was fastest at 35ºC, followed by 30ºC, with 25ºC supporting the least growth. The best pH for mycelial growth was 6. Continuous darkness supported the fastest mycelial growth while continuous light exposure supported the least growth. Among the substrates used only plantain leaves produced fruitbodies. Sclerotia formation occurred in 'wawa' sawdust and plantain leaves only. Sawdust yielded heavier and more sclerotia than the plantain leaves. The biological efficiency of the sclerotia was higher than that of the fruitbodies. The nutrient composition of *P. tuber-regium* shows that the fruitbodies are good sources of nutrients for man.

Acknowledgements

We gratefully acknowledge all staff of the Mycology Unit of the Food Research Institute, especially Messrs. Richard Takli and William K. Amevor for their immense assistance during the field and laboratory work. We are also grateful to the Director of the Food Research Institute, Dr Wisdom A. Plahar for his continuous encouragement and support.

References

Adenippekun, C. O. 2008. Bioremediation of engine-oil soil by *Pleurotus tuber-regium* Sing., a Nigerian white-

rot fungus. African Journal of Biochemistry 7:55-58.

AOAC 2002. Official Methods of Analysis. 17th ed. Maryland: Association of the Official Analytical Chemists.

Chang, S. T. 1984. Conversion of agricultural and industrial wastes into fungal protein. Conservation and Recycling 7(2-4): 175-180.

Chenghua, D., Xiangliang, Y., Xiaoman, G., Yan, W., Jinggyan, Z. and Huibi, X. 2000. A ß-D-glucan from the sclerotia of *Pleurotus tuber-regium* (Fr.) Sing. Carbohydrate Research 328: 629-633.

Datta, S. and Charkravarty, D. K. 2001. Comparative utilization of lignocellulosic components of paddy straw by *Tricholoma lobyense* and *Volvariella volvacea.* Indian Journal of Agricultural Science 71: 256-261.

Dzomeku, M. 2009. Studies on occurrence, ethnomycology and cultivation of *Pleurotus tuber-regium* (Fr.) Sing. Kumasi, Ghana: Kwame Nkrumah University ofScience and Technology, M.Phil. thesis.

Fasidi, I. A. and Ekuere, U. U. 1993. Studies on Pleurotus tuber-regium: Cultivation, proximate composition and mineral content of sclerotia. Food Chemistry 48: 255-258.

Fasidi, I. A. and Olorunmaye, K. S. 1994. Studies on the requirements for vegetative growth of *Pleurotus tuber-regium*, a Nigerian mushroom. Food Chemistry 50: 397- 401.

Frimpong-Manso, J., Obodai, M., Dzomeku, M. and Apetorgbor, M. M. 2011. Influence of rice husk on biological efficiency and nutrient content of *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer. International Food Research Journal 18:249-254.

Hibbett, D. S. and Thorn, R. G. 1994. Nematode trapping in Pleurotus tuber-regium. Mycologia 86 (5): 696-699.

*http:/www.ghanaweb/ghanadistricts.com*Regions. Assessed on 01/09/2006.

Isikhuemhem, O. S., Moncalvo, J.-M., Nerud, F. and Vilgalys, R. 2000. Mating compatibility and phylogeography in *Pleurotus tuber-regium*. Mycological Research 104:732-737.

Isikhuemhen, O.S., Anoliefo, G. and Oghale, O. 2003. Bioremediation of crude oil polluted soil by the white rot fungus *Pleurotus tuber-regium* (Fr.) Sing. sclerotia. Environmental Science Pollution 10:108-112.

Isikhuemhen, O. S. and Okhuoya, J. A. 1995. A low cost technique for the cultivation of *Pleurotus tuber-regium* (Fr.) Singer in developing tropical countries. Mushroom Grower's Newsletter 4: 2-4.

Isikhuemhen, O. S. and Nerud, F. 1999. Preliminary studies on the ligninolytic enzymes produced by the tropical fungus *Pleurotus tuber-regium.* Antonie van Leeuwenhoek 75: 257-260.

Nwokolo, E. 1987. Composition of nutrients in the sclerotium of the mushroom *Pleurotus tuber-regium*. Plant Foods and Human Nutrition 37: 133-139.

Obodai, M., Cleland-Okine, J., Awotwe, B., Takli, R. and Dzomeku, M. 2002. Training manual on mushroom cultivation in Ghana, pp16-19. Technical manual of the CSIR-Food Research Institute.

Obodai, M., Cleland-Okine, J. and Johnson, P.-N.T., 2003. Use of agricultural wastes as substrate for the mushroom *Volvariella volvacea*. Tropical Science 43:121-124.

Obodai, M., Sawyerr, L.C.B. and Johnson, P.-N.T. 2000. Yield of seven strains of oyster mushrooms (*Pleurotus* spp.) grown on composted sawdust of *Triplochiton scleroxylon*. Tropical Science 40:95-99.

Oei, P. 1991. Manual on mushroom cultivation: techniques and opportunity for commercial applications in developing countries, p.21-26: CTA.

Oei, P. 1996. Mushroom cultivation with special emphasis on appropriate techniques for developing countries, p.15-17, Leiden: Tool Pubications.

Okhuoya, J. A. and Ajeirio, C. 1988. Analysis of sclerotia and sporophores of *Pleurotus tuber-regium*, an edible mushroom in Nigeria. Korean Journal of Mycology 16: 204-206.

Okhuoya, J. A. and Etugo, J. E. 1993. Studies on the cultivation of *Pleurotus tuber-regium* (Fr.) Sing., an edible mushroom. Bioresource Technology 44:1-3.

Okhuoya, J. A. and Okogbo, F.O. 1990. Induction of edible sclerotia of *Pleurotus tuber-regium* in the laboratory. Annals of Applied Biology 117: 295-298.

Okhuoya, J. A. and Okogbo, F.O. 1991. Cultivation of *Pleurotus tuber-regium* (Fr.) Sing. on various farm wastes. Proceedings of Oklahoma Academy of Science 71: 1-3.

Oso, B. A. 1977. *Pleurotus tuber-regium* from Nigeria. Mycologia, 69 (2): 271-279.

Patrabansh, S. S. and Madan, M. 1997. Studies on cultivation, biological efficiency and chemical analysis of *Pleurotus sajor-caju* on different biowastes. Acta Biotechnology 17 (2): 107-122.

Plummer, D. T. 1971. An introduction to practical biochemistry, p. 112-113.New York: McGraw Hill.

Poppe, J. and Hofte, M. 1995. Twenty wastes for twenty cultivated mushrooms. Mushroom Science Journal SIV (1): 171-179.

Shashirekha, M. N., Rajathnam, S. and Bano, Z. 2002. Enhancement of bioconversion efficiency and chemistry of mushroom *Pleurotus sajor-caju* produced on spent rice straw substrate supplemented with oil seed cakes. Food Chemistry 76: 27-31.

Van Soest, P. J., and Robertson, J. B. 1985. Analysis of forages and fine foods. A laboratory manual for Animal Science, p. 613. Ithaca, New York: Cornell

Wang, H. X. and Ng, T. B. 2001. Isolation of pleuturegin, a novel ribonuclease-inactivating protein from the sclerotia of the edible mushroom *Pleurotus tuber-regium.* Biochemistry and Biophysics Research Communications 288:718-721.

Xiugin, L., Yunzhi, P. and Ruihong, Z. 2001. Compositional changes in microstructural changes of cotton seed hull substrate during *Pleurotus ostreatus* growth and the effects on feed value of the spent substrate. Transactions of CSAE 17: 96-100.

Yongabi, K. A. 2004. Studies on the potential use of medicinal plants and microfungi (lower plants) in

water and waste water purification. Downloaded from *http://www.plant.com/pages/moringayongabi.htm.*

Zadrazil, F. and Kurtzman, R. H. 1982. The biology of Pleurotus cultivation in the tropics. In Chang, S. T. and Quimio, T. H. (Eds.). Tropical mushrooms, biological nature and cultivation methods. Hong Kong: The Chinese Press.

Zhang, M., Cheung, P. C. K. and Zhang, L. 2001. Evaluation of mushroom dietary fiber (nonstarch polysaccharides) from sclerotia of *Pleurotus tuber-regium* (Fries) Singer as a potential antitumour agent. Journal of Agriculture and Food Chemistry 49:5059-5062.