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EFFECT OF MUSHROOM EXTRACT AND WATERING REGIMES ON CARPOGENESIS OF *Pleurotus tuberregium* (Fr) Singer

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ABSTRACT
Effect of mushroom extract using different watering regimes on the carpogenesis of *Pleurotus tuberregium* (Fr) Singer was investigated. The sclerotia were of scraped and soaked. *Pleurotus tuberregium* (20g) was sown each in 30 plastic plates using soil as substrate. The treatment was watered with mushroom extract while the control was ordinary water using different water regimes. The pileus diameter of the sporophore was highest 10.2 ± 2.767cm in Control 1(C_1) while the least value was in Treatment 2 (T_2) with a value of 5.5 ± 0.30cm. Stipe length was highest, 8.1 ± 1.45cm in Control 1 (C_1) and Treatment 1 (T_1) with a value of 8.1 ± 8.74cm while the least value was in Treatment 2 (T_2) of 4.6 ± 0.40cm. Mean fresh weight production by the mushroom was highest, 17.52 ± 3.91g in Control 1 (C_1) while the least value was in Treatment 2 (T_2) of 7.21 ± 1.44g. The results show that decrease in watering frequency and supplementation increase yield in the mushroom. This yield was however not appreciably higher than other controls and treatments.

KEYWORDS: Carpogenesis, *Pleurotus tuberregium*, Sporophore, Mushroom extract, Yield

INTRODUCTION

A Mushroom is a macrofungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand. (Chang and Miles, 2004).Their use as food and delicacy is now assuming greater importance in human diets world wide (Ogundana and Fagade 1982; Akpaja *et al*., 2003). They may be edible, non edible poisonous or non poisonous (Gray, 1970). Edible mushrooms are highly nutritious when compared with meat, egg and milk. They are known to be rich in proteins, sugars, lipids, amino acid, glycogen, vitamins (B, C & D) and mineral elements (Gosh and Chakravarty, 1990). Apart from their nutritive value, mushrooms also have potential medicinal benefits especially in antitumoral and hypocholesteromic agents (Fasadi and Kadiri, 1990). The Igbo people of Nigeria use it to treat heart problems, while it is used to treat asthma, cough and obesity among people of Edo State (Isikhuemhen and Okhuoya, 1995, Isikhuemhen *et al*., 2000a).

Species of *Pleurotus* are well known as edible mushrooms in different parts of the world. Indigenous people of Nigeria incorporate collected mushrooms including *P. tuberregium*, into their diet and medicine (Oso, 1977). Similarly, people in Ghana (Obodai *et al*., 2004). Cameroon (Kuyper *et al*., 2002) and Republic of Benin (Victor Ekun, personal communications). Also use mushroom as food and medicine unlike *Agaricus bisporus* which is generally used fresh or canned, *Pleurotus* can be dried and stored for long period without deterioration in culinary properties (Bahuklandi *et al*., 1989). In the view of rapid growth in production of *Pleurotus sp* world wide during the last few years, they can now be considered to be one of the more important mushrooms in cultivation.

Cultivation of edible mushrooms in the continent is limited. It is only common in Zimbabwe, Kenya and South Africa (Declaire; 1978). It is almost non existent in Nigeria. Although, many eat mushrooms, they collect them in their wild states. This practice is fraught with the danger of collecting poisonous species along with the edible ones.*(I believe the sentence is clear in that, people depend on wild mushrooms instead of the cultivated ones. This means there is the probability of picking poisonous mushrooms among the edible ones.)* These problems are highly minimized in developed countries of America, Europe and Asia, where mushroom farming is a lucrative business. In these areas much work has been done to develop the best growth conditions and the appropriate technology for growing these mushrooms to maximize profit (Zadrazil, 1974; Block *et al*., 1958, 1959).

Pleurotus tuber–regium (Fr) Singer, an edible Basidiomycete, occur in both tropical and subtropical regions of the world (Zoberi, 1972, 1973). It is a common mushroom in the southern part of Nigeria and forms large spherical ovoid, subterranean sclerotia which sometimes measure up to 30cm in diameter (Oso, 1975). The sclerotia are dark-brown on the outside and whitish on the inside, and may be subterranean in the host (decaying wood). The local people who use this fungus for food and medicine usually collect the sclerotia from the wild, but it is getting difficult to find sclerotia due to the depletion of its forest habitat (Okhuoya, and Ajerio, 1988b*(The same authors have a paper this same year that is quoted above)*). It is believed by many Nigerians to be capable of curing ailments such as headaches, stomach pain, small pox, fever and chest pain (Oso, 1977; Nwoloko, 1987; Okhuoya *et al*., 1996; Oso (1975; 1977); Isikhuemhen and Okhuoya; (1995). It is used in Asaba area of Nigeria in herbal preparation for pregnant women to aid the development of foetus. In Ghana, the sclerotia are used mainly for fattening of malnourished babies and as one of the ingredients in the embalming of dead bodies (Okhuoya *et al*., 1998). Analysis of sclerotia of *Pleurotus tuberregium* has shown the presence of Calcium, Magnesium, Iron and Zinc (Okhuoya and Ajerio, 1888a). This makes it possible for the extract of the sclerotia of *Pleurotus tuberregium* to be used as a supplement in preparing the substrate for cultivation of this mushroom.

Successful domestication and biotechnological exploitation of this fungus requires that studies be done to determine the biological nature to know more about the physiology of this unique fungus. As a result of the scarcity of sclerotia of this fungus, the need arise for cultivation to constitute for or replace collection from the wild.

This study investigates the effect of Mushroom Extract and Watering regimes on carpogenesis of *Pleurotus tuberregium.*

MATERIALS AND METHODS

Substrates collection and preparation

Sclerotia of *Pleurotus tuberregium* were bought from Ekiosa market in Benin City, Edo State of Nigeria. They were scraped with knife to remove the brown outer cover and then cut into small sizes of (20g). The sclerotia of *Pleurotus tuberregium* (20g) of 30 pieces (600g) were soaked for 5 hours in a bucket of water and sown into 30 plastic bowls of uniform dimension (21cm in diameter and 7cm deep) perforated with 5 holes using iron smolder and filled with loamy soil gotten from a garden in Junior staff quarters of University of Benin, Benin City.

200g of sclerotia of *Pleurotus tuberregium* were blended and soaked with 4 litres of water for 48 hours to remove the extract of the mushroom using a sieve.

The first control was 20g of *Pleurotus tuberregium* soaked and sown in soil inside the perforated plastic bowls, watered once a day of at least 12 hours interval with ordinary water of 40ml. The second control was watered twice a day of at least 6 hours interval, each time with 40ml of ordinary water and the third control was watered thrice a day of at least 4 hours interval, each time with 40ml of ordinary water.

Then three experimental treatments was set up as in the three control experimental system but watered with mushroom extract as substitute for ordinary water for the control experiment.

Fruitbody induction

The plastic bowls containing the substrate (soil) in which the 20g of the sclerotia was planted were under a shade. This was followed by periodic watering in coherence with the watering regimes of the experimental setup. The following parameters were measured, recorded and analyzed; time of primordial emergence, cap diameter, stipe length, fresh weight, and dry weight

Data analysis

The results got were analysed using descriptive statistical parameters like mean and standard error. Analysis of variance (ANOVA) was also calculated.

RESULTS

The Sclerotia (20g) of *Pleurotus tuberregium* (Fr) Singer grew directly into sporophores irrespective of the different treatments. The different watering regimes did not prevent the growth of sporophore and mycelium.

The fastest primordial emergence of 15.00 ± 4.00 days was observed in Treatment 1 (T_1) watered once a day, at least 12 hours interval with 40ml of the mushroom extract of *Pleurotus tuberregium* (Table 1) while the least primordial emergence of 31.00 ± 7.00 was observed in treatment 2 (T_2) watered twice a day at least 6 hours interval with 40ml of the mushroom extract of *Pleurotus tuberregium* at each time of watering (Table 1). The difference in the time of primordial emergence between the different treatments and controls was found to be significant ($P < 0.05$).

The pileus diameter of the sporophore was highest in control 1 (C_1) with a diameter of 10.20 ± 2.77cm when the substrate was watered once a day with ordinary water while the least cap diameter was recorded in treatment 2 (T_2) with a diameter of 5.50 ± 0.30cm when the substrate was watered twice a day with 40ml of mushroom extract (Table 2). The cap easily expanded and increased with time. However, the pileus starts curving at the periphery after some time.

The height of the fruiting bodies (Stipe length) was highest in control 1 (C_1), 8.10 ± 1.45cm and Treatment 1 (T_1) 8.10 ± 8.74cm and least in Treatment 2 (T_2) with a value of 4.60 ± 0.40cm (Table 2).

The significant difference between the different treatments and control resulting to the growth of sporophores was significant at 95percent probability level. Plate 3 shows the developmental stages of *Pleurotus tuberregium*.

Mean fresh weight production by the mushroom was highest, 17.52 ± 3.91g in control 1 (C_1) and least in Treatment 2 (T_2). With a value of 7.21 ± 1.44g. The differences in weight in all the treatments, between the different Treatments and controls were significant ($P < 0.05$) (Table 3).

Mean dry weight production by the mushrooms was highest, 2.32 ± 0.46g in control (C_1) while the least value was obtained in Treatment 2 (T_2) with a value of 0.89 ± 0.18g (table 3).

There was significant difference between the Treatments and Controls as per dry weight at 95% probability level.

DISCUSSION

Pleurotus tuberregium (Fr) singer grew successfully with sporophore production. The various treatments and control gave different results. This may be due to effective substrate utilization (Cannon, 1986; and Laborde, 1992). *Pleurotus tuberregium* has been successfully grown for the production of fruit bodies (sporophores) on diverse substrate (Okhuoya & Etugo, 1993). Probably, mycelia growth absorbed water into the sclerotia for use in the conversion and transport of stored nutrient to the growing sporophores. The nutrient used in the formation of the sporophore is not likely to originate only from the sclerotium inoculum, but also from the surrounding substrate. It is generally believed that sclerotia serve as reservoirs and for the ecological purpose of survival through harsh and unfavourable environmental conditions (Amir 1992; 1994; and Buscot 1993).

In control 1 (C_1) the highest sporophore yield was recorded with a fresh weight of 17.52±3.91g, and a dry weight of 2.32±0.45g in Table 3; the cap diameter 10.20± 2.77cm and stipe length 8.10 ± 1.46cm as seen in (Table 2). More so in treatment 1 (T1) the highest sporophore yield was recorded with a fresh weight of 14.74 ± 1.00g, dry weight, 1.80 ± 0.12g in table 3; the cap diameter 6.70 ± 0.65cm and stipe length 8.10 ± 0.87cm (table 2). Frequent watering of the sporocarps may have water logged their tissues and result in the slightly poor development. Thus, *Pleurotus tuberregium* may not need too much water for proper growth and development.

The fastest primordial emergence of 15.00± 4.00 days was observed in treatment 1(T_1) while primordial emergence was slightly delayed in other treatment. This is due to supplementation with the mushroom extract leading to an additional nutrient which may delay the colorization of the substrate by the mycelia and growth of the sporophores (Mizuno, 1999).

The fastest primordial emergence of 19.00±4.18 days was observed in control 1 (C_1) While primordial emergence was delayed in other controls. This may be due to leaching of the soil nutrients by the subsequent watering in other controls which will take the *Pleurotus tuberregium* time to adapt to the new condition.

Further studies would have to be carried out to really ascertain why there was a delay in primordial emergence in treatments and controls; More also, to ascertain why the difference in supplementation lead to delay in primordial emergence.

CONCLUSION

This experiment showed that soil could be used for the commercial cultivation of *Pleurotus tuberregium*. The cultivation as reported in this experiment is very simple which could be easily done by any amateur mushroom grower since local people across the western coast of Africa use this fungus for food and medicine. However, the experiment has shown the best method and watering regime to adapt in boosting *Pleurotus tuberregium*. Researchers and local grower of mushrooms should use low cost technology as analysed in this research work to increase production of this mushroom.

REFERENCES

Akpaja E. O., Isikhuemhen, O. S. and Okhuoya, J. A. (2003): Ethno mycology and uses of edible and medicinal mushrooms among the Igbo people of Nigeria. *International Journal of Medicinal Mushrooms* 5:313 – 319.

Amir R., Levanon, D. Hader, Y. and Chet, I. (1992). Formation of scherotia by *Morchella esculenta*: relationship between media composition and tugor potential in the mycelium. *Mycological Research*. 96:943 – 948.

Amir, R. (1994). The role of source – sink relationships in translocation during sclerotial formation by *Morchella esculenta. Mycological Research*. 98:1409 – 1414.

Bahukhandi, D., Munjal, R. L. and kapoor, J. N. (1989). Cultivation trials of *Pleurotus opuntiae* (Dur. and lever) Sacc. *Current Science*. 58:856 – 857.

Block, S. S., Tsao, G. and Han, L. (1958): Production of mushrooms from sawdust. *Journal of Agriculture and Food Chemistry*. 6:923 – 927.

Block, S. S., Tsao, G. and Han, L. (1959): Experiments in the cultivation of *Pleurotus ostreatus*. *Mushroom Science* 4:309 – 325.

Buscot, F. (1993). Mycelial differentiation of *Morchella esculenta* in pure culture. *Mycological Research*. 97:136 – 140.

Cannon, P. F. (1986) International Commission on the Taxonomy of fungi (ITCF): Name changes in fungi of microbiological, industrial and medicinal importance. Part 1. *Microbiological Science.* 3:168-171.

Chang, S.T and Miles, P.G (2004). Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environment Impact. CRC Press. Boca Raton. 415p.

Declaire, J. R. (1978): Economics of cultivation of edible mushrooms. In: The biology and cultivation of edible mushrooms Chang, S. T. and Hayes, W. A. (eds). Academic Press. New York. 819p.

Fasadi, I. O. and Kadiri, M. (1990). Changes in nutrient contents of two Nigerian mushrooms, *Termitomyces robustus* (Beeli) Heim and *Lentinus subnudus* Berk, during sporophore development. *Die Nahrung*, 34:415 – 420.

Gray, W.D. (1970). The use of Fungi as food in processing. Butterworts, London. 113p

Ghosh, N. and Chakravarty, D. K. (1990). Predictive analysis of the protein quality of *Pleurotus citrinopeleatus*. *Journal of Food Science Technology* 27:236 – 238.

Isikhuemhen O. S., Moncalvo, I. M., Nerud, F. and Vilgalys, R (2000). Mating compatibility and phylogeography in *Pleurotus tuberregium*. *Mycological Research.* 104:732 – 737.

Isikhuemen, O. S., and Okhuoya, J. A. (1995). A low cost technique for the cultivation of *Pleurotus tuberregium* (Fr) singer in developing tropical countries. *Mushroom Growers News letter* 4:2 – 4.

Kuyper T. W., Van Dijk J. F. W and Onguene, N. A. (2002). Knowledge and utilization of edible mushrooms by local populations of the remain forest of South Cameroon, Oslo, Norway, 115p.

Larborde, J. (1992) Required qualities of indoor composition. *Mushroom World* 3:23-30.

Mizuno, T. (1999). The extraction and development of antitumoractive polysaccharides from medicinal mushrooms in Japan (review) *International Journal of Medicinal Mushrooms.* 1: 9-29.

Nwokolo, E. (1987), Composition of nutrients in the sclerotium of the mushroom *Pleurotus tuber–regium*. *Plant foodsfor Human Nutrition.* 37:133 – 139.

Obodai, M. Vowotor, K. A. and Marfo, K. (2004). Performance of various strains of *Pleurotus species* under Ghanaian conditions. Available at http://www.mushword.com

Ogundana, S. K. and Fagade, O. E. (1982). Nutritive value of some Nigerian Mushrooms, *Food Chemistry*. 8:263 – 268.

Okhuoya, J. A. and C. A. Ajerio, (1988a). Analysis of Sclerotia and Sporophores of *Pleurotus tuberregium* (Fr) Singer, an edible mushroom in Nigeria. *Korean Journal of Mycology*. 16:204 - 206.

Okhuoya, J. A. and C. A. Ajerio, (1988b). Sporocarp development of *Pleurotus tuberregium* (Fr.) Singer under different watering systems. *Korean Journal of Mycology*. 16: 207 – 209.

Okhuoya, J. A. and Etugo J. E. (1993). Studies on the cultivation of *Pleurotus tuberregium (*Fr) Singer. In various farm wastes. *Proceedings of the Oklahoma Academy of Sciences* 71:1 – 3.

Okhuoya, J. A., Eboigbe O. E. and Isikhuemhen, O. S. (1996). Growth interactions between *Pleurotus tuberregi*um (Fr.) Singer and *Sclerotium rolfsii* Sacc. *Folia Microbiogical*. 41:433 – 435.

Okhuoya, J. A., Isikhuemhen, O. S. and Evue, G. A. (1998). *Pleurotus tuberregium* (Fr.) Sing: sclerotia and sporophore yield during cultivation on sawdust of different woody plants. *International Journal of Mushroom Sciences* 2:41 – 46.

Oso, B. A. (1977). *Pleurotus tuberregium* from Nigeria, *Mycologia.* 69:271 – 279.

Oso, B. A., (1975), Mushroom and the Yoruba people. *Mycology.* 67:311 – 319.

Zadrazil, F. (1974): The ecology and industrial production of *Pleurotus ostreatus, P. florida, P. cornuciepia and P. eryngii*. *Mushroom Science* 9:621 – 652.

Zoberi, M. H. (1972) Tropical Macro fungi, Macmillan, London: 158p.

Zoberi, M. H.(1973) Some edible mushrooms from Nigeria. *Nigerian Field*. 38, 81 – 90.

Table 1: Effect of Mushroom Extract and Watering Regimes on Primordial Emergence of *Pleurotus Tuberregium*

Treatments	Time of Primordial Emergence (Days)
C_1	*19 ± 4.18
C_2	24 ± 0.00
C_3	25 ± 2.00
T_1	15 ± 4.00
T_2	27 ± 4.53
T_3	31 ± 7.00

* Time of primordial emergence (days) = Mean of 5 replicates),± Standard error, C_1 = Control 1, C_2 = Control 2, C_3 = Control 3, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3

Table 2: Morphometric Measurement of *Pleurotus tuberregium*

Treatment	Cap Diameter (cm)	Stipe length (cm)
C_1	*10.2 ± 2.77	8.1 ± 1.45
C_2	9.2 ± 0.00	5.3 ± 0.00
C_3	6.9 ± 0.45	6.5 ± 0.95
T_1	6.7 ± 0.65	8.1 ± 0.87
T_2	5.5 ± 0.30	4.6 ± 0.40
T_3	6.7 ± 0.60	5.4 ± 1.15

* Mean of 5 replicates ,± Standard error, C_1 = Control 1, C_2 = Control 2, C_3 = Control 3, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3

Table 3: Sporophore Yield of *Pleurotus tuberregium*

Treatment	Fresh Weight (g)	Dry Weight (g)
C_1	17.52 ± 3.90	2.32 ± 0.46
C_2	10.80 ± 0.00	1.61 ± 0.00
C_3	15.70 ± 2.90	1.96 ± 0.20
T_1	14.74 ± 1.00	1.80 ± 0.12
T_2	7.21 ± 1.44	0.89 ± 0.18
T_3	10.95 ± 4.35	1.30 ± 0.50

* Mean of 5 replicates, ± Standard error, C_1 = Control 1, C_2 = Control 2, C_3 = Control 3, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3

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