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Influence of geographical location and growth condition on some morphological and nutritional parameters of *Pleurotus tuber-regium* (Sing) as revealed by morphometric and proximate analyses.

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Abstract

The investigation was conducted to assess the influence of geographical location and growth under two different light conditions on some morphological and nutritional attributes of *Pleurotus tuber-regium* mushroom. The mushroom sclerotium was collected from four different locations in Nigeria, planted in loamy soils and kept under complete darkness and 12 h daylight/12 h dark conditions respectively. The results showed that light condition significantly ($p<0.001$) influenced height and fresh weight ($p<0.01$). Mushrooms grown under complete darkness were taller compared to those grown under 12 h daylight/12h darkness. Furthermore, mushrooms collected from the South (Enugu 1) were the tallest as compared to those obtained from the North (Kano). The range in the values obtained for stipe length, pileus diameter and fresh weight under 12h day/ light condition was 5.6±1.47 - 8.0±1.44cm, 4.7±2.08 - 6.9±2.18cm and 18.4±3.79 - 22.3±3.79g respectively. Corresponding values for those produced under complete darkness were 6.8±0.44 – 9.2±0.47cm, 3.8±1.11 - 5.0±1.75 and 14.0±2.25 – 17.0±3.28g respectively. The level of protein, fat, fiber and moisture varied from locations. In view of the dietary and economic importance of mushrooms, it is apparent that location and growth conditions could be of importance when considering nutritional and morphological attributes of the fungus.

Key words – dendrogram – distribution – light – morphology – mushroom – nutrient – sclerotium

Introduction

Mushrooms constitute a very important and highly appreciated source of food, and are widely consumed for their nutritional and medicinal properties (Berne et al. 2008, Khan & Tania 2012). *Pleurotus tuber-regium* (Sing) is a tropical sclerotia mushroom common in the Southern part of Nigeria and is the only *Pleurotus* species in which fruiting bodies arise from a sclerotium (Isikhuemhen & Nerud 1999). Both the fungus and sclerotium are edible and their applications as food, condiments, in medicine and bioremediation have been documented (Okhuoya & Okogbo 1991, Fasidi & Olorunmaiye 1994, Isikhuemhen et al. 2000, Alobo 2003, Isikhuemhen et al. 2003, Adenipekun 2008, Badalyan et al. 2008). In recent years, different environmental conditions have been tested for their effect on sporophore formation and morphological attributes on mushrooms in artificial culture. Fruiting is a crucial developmental process in Basidiomycetes and the need for

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light to promote fruiting in *Pleurotus* was first noticed by Kaufert (1935). Requirement for light is dependent on mushroom species although most cultivated mushrooms require light for the initiation of fruiting and for normal developments (Chang & Quimio 1982). Aschan-Aberg (1960) reported that effective light for formation of basidiocarps by *Flammulina velutipes* (Physalacriaceae) is of wavelength of 435-470nm, *Coprinus cinereus* (Psathyrellaceae) requires a short exposure to light while for *Agaricus bisporus* (Agaricaceae) light is inhibitory. An 8hr light was reported to be adequate for *Pleurotus* species and *Lentinula edodes* (Oie 1990). Mushrooms especially *P. tuber-regium* has not enjoyed extensive commercial cultivation in Nigeria. In order to improve commercial production there is need for an understanding of the growing conditions that will enhance production of mature well formed fruit bodies. This study was carried out to investigate the effect of complete darkness and 12hr daylight/12hr darkness on the nutritional and morphological characteristics of *P. tuber-regium*.

Materials & Methods

Collection of materials

Sclerotia used in the study were procured from different locations in Nigeria, (Enugu, Anambra and Kano) while loamy soil was collected from Bells University of Technology, Ota, Ogun State. Plastic bowls used for seeding were procured from a local market, Ojuore market in Ota.

Substrate preparation & inoculation

Three small holes were made at the base and sides of the plastic bowls and 1kg loamy soil was weighed in to each bowl. The sclerotia were soaked in water for 18hrs and cut into about 30g pieces while the soil was watered to maintain a humid environment. Three samples of each sclerotium were seeded into the loamy soil and watered with 200mls of tap water. Subsequently, watering (200ml) was done at 48 hourly intervals. The plastic bowls seeded with sclerotia were kept at room temperature ($28^oC \pm 2^oC$) under two different light conditions, complete darkness (in a cupboard) and 12 h daylight/12 h dark conditions (on laboratory bench). Growth of the mushrooms under the two light conditions was monitored and the period of fruiting was 2-4weeks. The yield was determined in terms of height of stipe, diameter of pileus and fresh weight of harvested mushroom fruit body. The proximate analysis (% protein, fat, crude fiber & moisture) of both the sclerotia and harvested fruit bodies were carried out according to AOAC (1990) and the data were subjected to statistical analysis.

Morphometric & proximate analyses

A total of seven dependent variables (protein, fat, fiber, moisture, and height of stipe, diameter of pileus & fresh weight of sporophore) and two independent variables (location and light conditions) were of interest in this study. With the aid of SPSS software package (Version 20), a summary statistics was obtained before performing one-way or two-way multivariate analysis of variance (MANOVA) to assess the effects of location and/or light condition on the dependent variables (nutrient content and morphometric characters). Simple pair wise correlation matrices were generated to determine possible inter correlations between dependent variables. Finally, discriminate and cluster analyses were performed to assess how well the morphological and nutritional characteristics discriminate between fungi from different locations. In all cases, $P<0.05$ was considered significant.

Results

The data on morphological characteristics of mushrooms obtained at different locations and growth under two different light conditions are shown in Figures 1 & 2. Sclerotia grown under 12h daylight/dark condition produced mushrooms that had shorter stipes with larger pileus diameter and weight than those cultivated under complete darkness (Plates 1 & 2). The range in the values

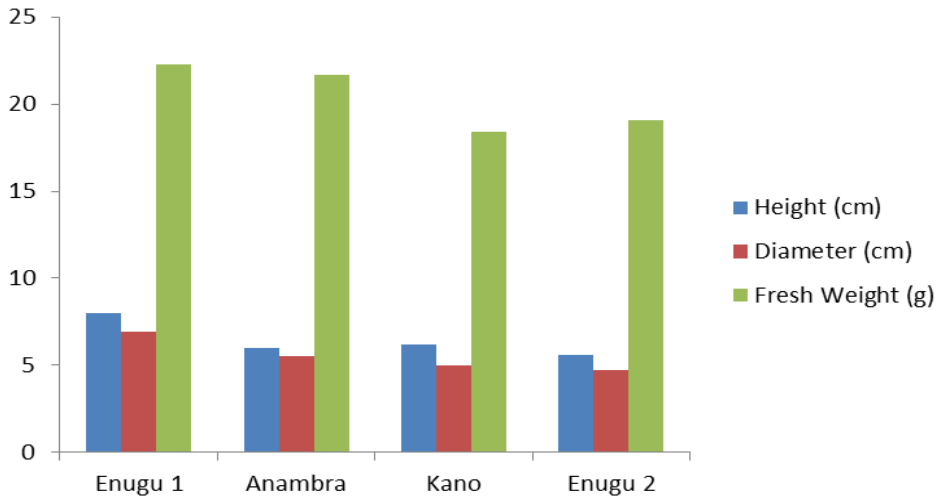

Fig. 1 – Morphological parameters of mushroom grown under 12 hr light/12 hr darkness

Plate 1 – Mushroom grown under 12hr light/12hr darkness

obtained for stipe height, pileus diameter and weight under 12h day/ light condition were 5.6±1.47 - 8.0±1.44cm, 4.7±2.08 - 6.9±2.18cm and 18.4±3.79 - 22.3±3.79g respectively. Corresponding values for those produced under complete darkness were 6.8±0.44 – 9.2±0.47cm, 3.8±1.11-5.0±1.75 and 14.0±2.25 – 17.0±3.28g respectively. As revealed by MANOVA, light condition significantly affected the morphological characteristics (Wilks' Lamda (3, 14) =0.009, $p<0.001$). Follow-up univariate ANOVA indicated that light conditions significantly influenced stipe height ($p<0.001$) and fresh weight ($p<0.004$), but did not significantly affect pileus diameter ($p=0.179$). Data (Table 1) showed that location significantly affected level of nutrients; however morphological characteristics were not affected. Pileus diameter was significantly correlated with fresh weight ($r=0.922$; $p<0.01$) and stipe height ($r=0.454$; $p<0.05$) while protein and fat contents were correlated with moisture and fiber respectively (Tables 2 & 3). Though the level of nutrients were similar in mushrooms and sclerotia, the fat content was significantly higher ($p<0.001$) in mushrooms (4.8 units) than in sclerotia (2.9 units). Dendrogram produced from hierarchical cluster analysis using furthest neighbor as the clustering method and Euclidean to assess proximity gave a three-cluster analysis: Cluster C could be defined as Enugu cluster while the other clusters (Clusters A and B) were not clearly defined (Fig. 3).

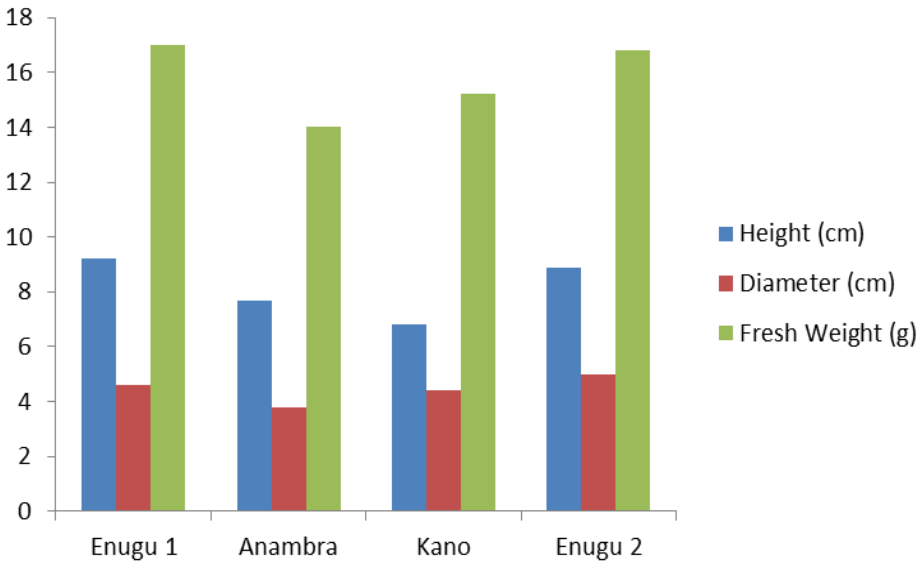

Fig. 2 – Morphological parameters of mushroom grown under complete darkness

Plate 2 – Mushrooms grown under complete darkness

Discussion

Light is known to be one of the signals triggering fungal development in model systems (Richartz & Maclellan 1987, Ku¨ es 2000, Corrochano 2007, Bayram et al. 2008) and photoperiod is used in industrial production of some mushrooms as part of the protocol for triggering flushing.

Signs of mycelium growth were observed after 7days of cultivation and primordia were formed two and four days later under complete darkness and light/dark condition respectively. Sclerotia grown in complete darkness developed elongated stipes and smaller pileus when compared with the ones grown under light/dark regime. Kuforiji & Fasidi (2009) reported that malformed fruit bodies of *P. tuber-regium* were formed in agricultural wastes under complete darkness. The requirement of light for fruiting is known to differ in different mushrooms (Chang & Quimio 1982). *Flammulina* and *Coprinopsis cinereus* require light while for *Agaricus bisporus* light is reported to inhibit stipe elongation and pileus expansion. J-Y Wu et al (2013) obtained the highest mycelia biomass (9.89 g L^{-1}) of *Pleurotus eryngii* in submerged culture under dark conditions.

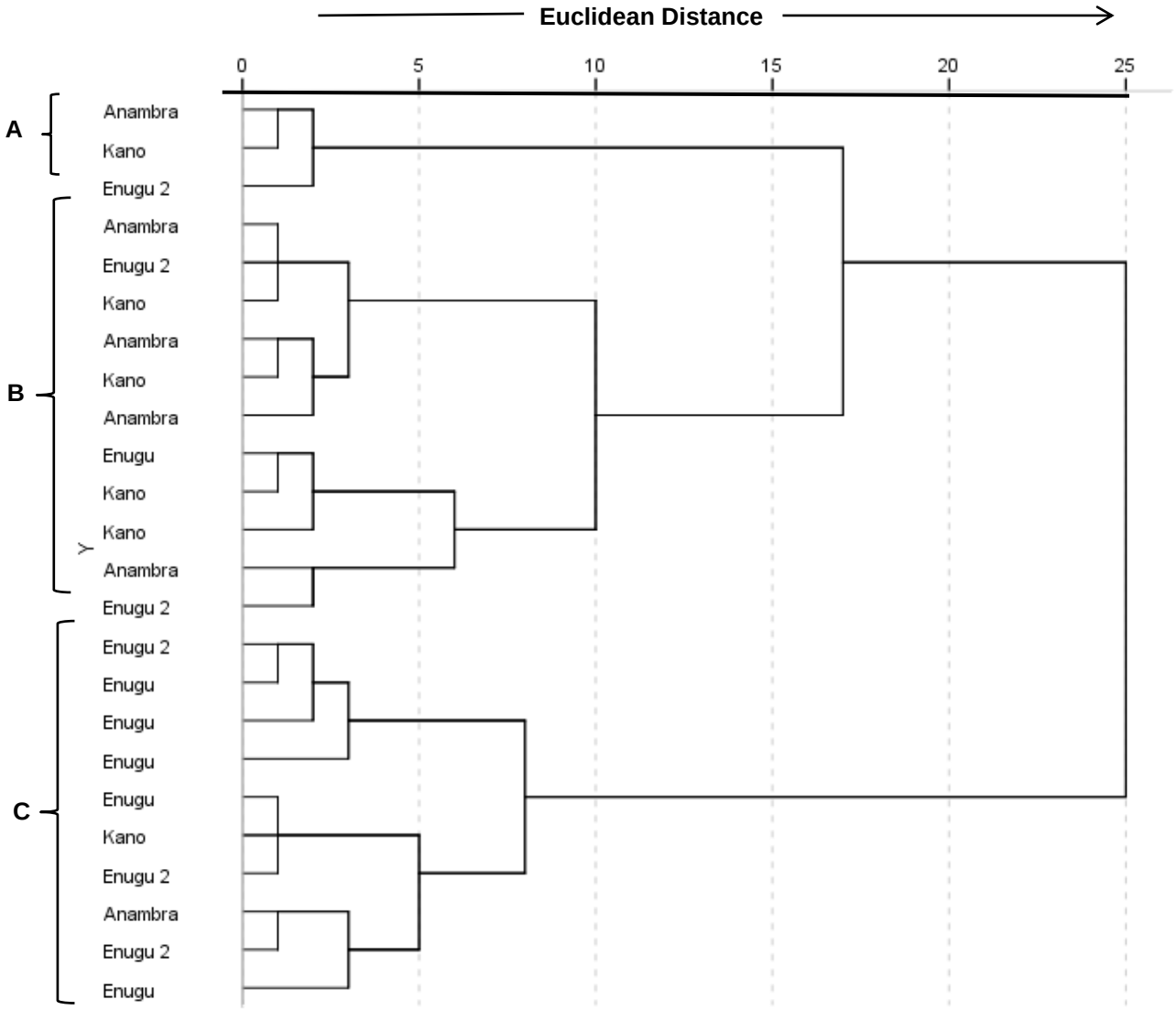

Fig. 3 – Dendrogram showing clustering of mushroom from different locations using height as the grouping variabl

While the effect of light condition was more pronounced on stipe height and weight that of location was significant at $P<0.01$ and at $P<0.05$ for pileus diameter. Santelices et al. (2012) observed that geographical location had significant effect on growth on *Suillus luteus* and suggested that edaphoclimatic condition could play a role in growth of mushrooms.

The diameter of pileus was shown to be highly correlated to the weight which implies that the size of the diameter contributes to the overall weight of the fruit body. This is evident as the fruit bodies with large pileus diameter weighed more than those with fewer diameters hence the mushrooms produced under light/dark regime were more robust and heavier than those cultivated in complete darkness. This observation was contrary to the work of Arjona et al. (2009), who reported no fruiting under complete darkness; he suggested that the inducing signal for fruiting is blue light or very low light intensities. The discrepancy could be attributed to the substrate used for cultivation; the above author used a defined synthetic medium while loamy soil was employed for this work.

Geographical location had influence on some nutritional properties of the sclerotium. Sclerotia from the Northern part of the country had lower moisture content compared to those from the South. This could be attributed to the different climatic condition existing in the two areas. The Northern part (Kano) has higher temperature (29 ^{o}C-38 ^{o}C), lower rainfall 690mm/yr and lower

humidity (75% relative humidity) while the Southern part usually has higher rainfall (1694.5mm), lower temperature (29.5^{o}C-34.9^{o}C) and humid environment (94% relative humidity) (Anon 2014). The dendrogram showed clustering of mushroom from different locations using stipe height as the grouping variable. From the hierarchical cluster analysis using furthest neighbor as the clustering method and Euclidean to assess proximity, three clusters A, B and C were observed. Cluster C could be defined as Enugu cluster while the other clusters (Clusters A and B) were not clearly defined.

Table 1 Effect of location on the proximate composition of sclerotia collected at different locations

Proximate	**Location**			
Parameters	**Enugu 1**	**Anambra**	**Kano**	**Enugu 2**
Moisture (%)	12.8±0.13	14.1±0.13	11.8±0.13	15.3±0.13
Fibre (%)	21.7±0.23	12.9±0.12	16.2±0.22	9.8±0.03
Protein(%)	7.9±0.20	9.02±0.20	6.5±0.20	8.2±0.20
Fat(%)	3.2±0.20	2.5±0.20	2.7±0.20	3.2±0.20

Results are presented as mean±SD

Table 2 Simple bivariate correlation matrix of association between the morphological parameters

	Height	**Diameter**	**Fresh Weight**
Height	1	0.479*	0.208
Diameter		1	0.922**
Fresh Weight			1

*P<0.05; **P<0.01

Table 3 Simple bivariate correlation matrix of association between different types of nutrients

	Moisture	**Fibre**	**Protein**	**Fat**
Moisture	1	0.187	0.725**	0.402
Fiber		1	-0.277	0.623**
Protein			1	0.288
Fat				1

*P<0.05; **P<0.01

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