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EFFECTS OF SIZE OF STIPE ON THE MYCELIA PERFORMANCE OF *Pleurotus florida* Jacq.

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ABSTRACT

The mycelia of a mushroom, is that part that is similar to the root system of any plant. It contains filament known as hyphae which takes in food, water and other things needed for the nourishment of a fungus, and there is a need to know the viability of a mushroom at different length size and how it affects the ramification process. An initial research was conducted to analyze the growth performance of *Pleurotus florida* mushroom culture using Potatoes Dextrose Agar (PDA), based on the stipe size of the mushroom. This research study is basically aimed to investigate how fast the mycelia of a mushroom can grow in a culture media plate in relation to the length of stipe. Studies discovered that the combined portion of cap and stipe formed dynamic mycelium growth in about three days after sprouting: the typical all-out growth was gotten from the shortest mushroom stipe size of about 4 mm. This shows that, the mycelium is more viable or active in a growing mushroom with short stipe of 4 mm than a fully grown mushroom with a longer stipe of about 9 mm. Different length of mushroom was used for the experiment. Each of the treatment was repeated thirteen times. The investigation resolved that there are more active mycelia with smaller stipe size of *Pleurotus florida* mushroom. Consequently, it speeds up the spawn preparation for the production of mushroom.

Keywords: Mycelia, stipe size, Potatoes Dextrose Agar (PDA), Spawn

Introduction

In the last decade fungal mycelium has grown much attention from academics and commercial enterprises due to its ability to up cycle agricultural and industrial wastes into viable composite materials (Jones *et al*., 2017). The process operates a natural, low-energy manufacturing process able to isolate carbon and create useful substitute materials (Jones *et al*., 2018). Mycelium composites involve links of filamentous hyphae that transforms into economically feasible and naturally pleasant supplies using organic progress instead of costly energy rigorous manufacturing processes Jones, *et. al.,* 2017. There are a few fungal species described in literature which can be used for composite manufacturing. Some frequently recycled mycological kinds are *Pleurotus florida* (Jones, 2017; and Travaglini, 2015).

P. *florida* is an edible, fast-growing, commonly accessible mushrooms belonging to the fungal collection (Webster *et. al.,* 2007). Fungi plays important ecological role in forest, such roles include mycorrhizal associations with vascular plants, decomposers of coarse organic material, pathogens of commercial tree species, and food resources for wildlife (Marcot, 2002). Fungi are eukaryotic and heterotrophic

organisms that causes diseases recycle nutrients in the ecosystem. They are often associated with food spoilage, food processing, as food and antibiotics like in the case of edible mushrooms (Agrios 2005; Trudell and Ammirati, 2019). Mushrooms are important components of the environment. These, are referred to as macro-fungi with distinctive fruiting bodies Chang and Miles (1992). There are high in demand for food and medicines apart from their ecological roles as decomposers and for bioremediations. Mushrooms have been used for treating insomnia, allergies, diabetes cholesterol reduction, stress, cancer and asthma, Bahl (1983).

They are high in proteins therefore are used as protein supplements in the underdeveloped world (Wani *et al.,* 2010). Fungi provide nourishment for the other organisms that live in the soil. Fungi are the leading causes of wood disintegration (Greaves, 1971). The mycelium of fungi releases strong extracellular enzymes and acids that are able to decompose lignin and cellulose which are essential components of plant wood fiber. Wood decay fungi play an integral role in forest nutrition cycling. Van der Wal, *et al*. (2015) reported that fungi also decompose oak stumps. White-rot fungi digest lignin by the secretion of enzymes and give a bleached look to wood due to undissolved cellulose. These enzymes have low substrate-specificity and can decompose various molecules that are similar to lignin

The term mushroom refers often fungi possessing stem (stipe), cap (pileus), hymenium (lamellae) including pores underneath its cap (Masarirambi *et al*., 2011).

In mycology, stipes are known to serve as a form of support for the cap of a mushroom. Similar to the tissues of mushrooms excluding the hymenium, the stipe is made up of composed of germ-free hyphal material. Most often, nevertheless, the productive hymenium spreads down the stipe around. Fungi having the stipes are said to be stipitate (Kuo, 2020)

In general, the advantages of a stipe are considered to be in facilitating spore distribution. An higher mushroom will more simply discharge its spores into wind flows or onto transitory animals (Kuo, 2020)

The maintenance and production of a reliable unadulterated culture mycelium with outstanding qualities is a key operation and is the first serious stage to the realization of spawn making and mushroom farming (Santosh *et al*., 2018)

Mushrooms contains non-starchy carbohydrates, having a significant level content of dietary fiber and moderate quantity of proteins, including most amino acids, minerals, and vitamins (Alam *et al*., 2001). At present, and most often, scientist extracts the mycelia of a mushroom not taking into consideration the length of the stipe, and or the size of the mushroom at harvest stage. Inoculations are done into the petri-dishes, and there are expectations of mycelia growth in a prepared culture. Therefore, there is a need to determine the effect the length of stipe of a mushroom can have on the rapid growth of the mycelia in a prepared culture medium.

Materials and Methods

This research was done at the Pathology Laboratory, Department of Forest Conservation and Protection, Forestry Research Institute of Nigeria, Jericho, Ibadan, Oyo State, Nigeria and it is located between

Latitude 7° 23' 20" to 7 °23' 40" North and longitude 3° 51' 23" to 3° 51' 52" East.

Culture preparation

A Potato Dextrose Agar (PDA) culture media: This agar media was prepared as described by (Chang and Hayes, 1978). 39 g of potato dextrose agar was added to 1 litre distilled water, placed in a boiling water bath to dissolve agar. It was autoclaved at 121^{o}C for 15 min. After cooling, it was then dispensed into Petri dishes (85 mm). The Pure culture of fleshy fungi/mushrooms was prepared through tissue culture. Different lengths of mushrooms of the species under study were collected from the wild, and their basidiocarp after alcohol sterilization is cut longitudinally into 2 splits and bits was transferred to pre sterilized PDA culture medium from collar region. It takes one week for an incubation of Petri-plates at 25C ± 2C in Biochemical Oxygen Demand (BOD). The flasks having media were sterilized in the autoclave at 15lb/sq. inch pressure for one hour and then poured in 90 mm Petri dishes under the laminar flow hood to avoid contamination. Media were cooled to 37^{o}C.

The mushroom was sliced into two halves form the pileus and longitudinally down the stipe. Tissues of the fresh mushrooms were inoculated on culture media. Circular growth of mycelium of diverse rations was noticed until the Petri dishes were complete with it. The plates were incubated at 37°C and observed for 15 days during which the mycelial vegetative growth of *Pleurotus florida* were recorded. A Completely Randomized design (CRD) was used with each of the treatment repeated thirteen times, therefore having total of eleven treatments.

Measurement of mycelia growth

For the separation of mushroom tissue, a germ-free cutting needle with adjustable spans was directly positioned on the outward of the exposed tissue and driven down carefully about 3 mm deep, using a reference line in the device that indicates the depth. The needle was then carefully removed. In most cases, cut tissues remained in the cutting hole, so that they could be released by simply pressing the cap of the movable needle, and were then seeded on Petri dishes containing the PDA medium.

In some cases, cut tissue remained on the original site and would be extracted using the transfer needle, before being positioned on the outward of PDA medium. To grow the mycelium of the quarantined tissues for *P. florida*, all injected PDA plates were grown in an incubator at 25 °C for 14 days. Colonies was measured in diameter and was measured along two perpendicular directions, and the average was calculated. Growth was obtained by deducting the original diameter of cut tissue from the measured value of the mycelial colony after cultivation.

Results and Discussion

Variance in stipe length (cm)

Mycelia growth and the length of stipes measured were presented in figure 1. This shows that stipe length of 4 mm grows rapidly to a full length in the petri dish, this was closely followed by stipe size 5.5 and 5.8. The ANOVA table for the length of stipe was presented in (Table 1). There was no significant difference (p = 0.05)

Difference in length of stipe (cm)

A culture medium is an enriched material which is often times derived from plant-based

sources that can promote and sustain the mycelial growth of the desired Mushroom (Reyes *et al.,* 2009a). Growth measurement of mycelia and the length of stipe were presented in figure 1. This shows that stipe with the least length grows rapidly to a full length in the petri dish; this was closely followed by sample stipe length 5.5 and 5.8. The ANOVA table for the stipe length was presented in (Table 1). There was no significant difference (p = 0.05) on stipe length (Table 1).

Effect of stipe length on mycelia growth (cm)

Analysis of variance (ANOVA) for the growth of mycelia based on the stipe length was presented in table 1. Chang and Miles (2004) observed that Maximizing of growth rate and yield for growth of mushroom are influenced by pH of the media as well as inoculum size and nutrient composition. Despite the stipe length size of 4.0 mm having the fastest rate of growth, no significant difference observed on mycelia growth as influenced by different stipe size (Figure 1) suggesting that the growth on mycelia in Petri plates cannot be completely related to the stipe size of the mushroom. This finding was observed in an experiment on stipe size that ranges between 4.4 and 6.1 (Kortei *et al.,* 2018). This result was also corroborated by De Leon *et al.,* (2017), when they affirmed that the largest stipe diameter and longest stipe was recorded on substrate supplemented with 5% rice hull with mean value of 7.24 mm ± 0.75 and 43.01 mm ±5.09, respectively. However, statistical analysis revealed no significant difference in the size of stipe among different treatments.

It was experimental that there was higher mycelia growth in stipe length size of 4.0 mm with the mean mycelia growth of 2.5 cm this was followed by stipe length size of 5.5 mm having a mean mycelia growth of 2.00cm and the lease amongst them is stipe length size of 8.6 mm having a mycelia growth length of 1.73cm. The influence of stipe size on mycelia growth was experimental for 14 days after inoculation. The difference in mycelia growth with size of stipe maybe as a result of feasibility difference in composition (Shah *et al*., 2004). Elisashvili *et al.,* (2008) reported that the low hydrolase activity that was reported to have during substrate colonization may be the cause of the uniformity in the beginning of mycelial growth in the petri dish. The implication is that there is little to no breakdown of substrate materials for the release of nutrients during this brief period. As a result, it's possible that the nutrients in the additives were not released, weren't made available to the mycelia, and didn't have an impact on the mycelia's initial growth.

Table 1: Analysis of variance (ANOVA) for the growth of mycelia based on the stipe length

SV	df	SS	MS	F value	P value
Stipe length	10	24.65	2.47	1.2353	0.274435
Error	132	263.41	1.99		
Total	142	288.06			

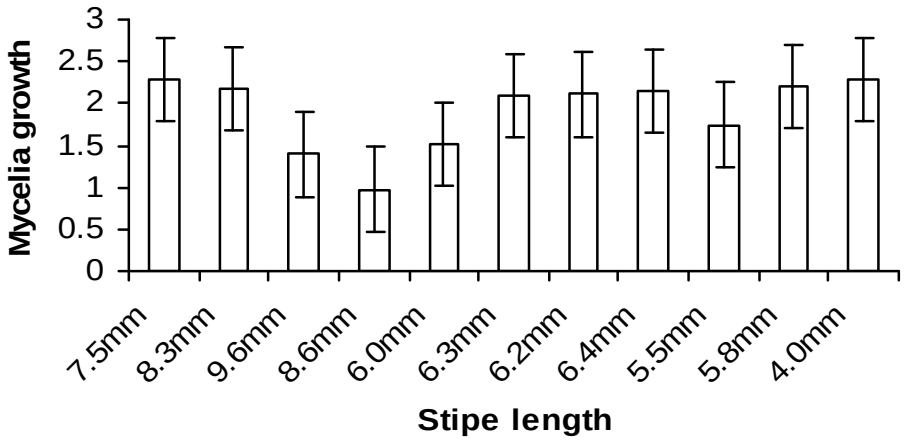

Figure1: Growth dimension of mycelia and the length of stipe obtained by measuring the length with a ruler

Conclusion and Recommendation

In this study, the researchers establish the fact that the size of stipe on the mycelia performance of *pleurotus florida* does not have a significant difference in the ramification of mycelia when inoculated in a prepared culture plate/petri dish. The overall ramification gotten in the petri-dish based on the size of stipe was an indication that different length of stipe of a mushroom is a viable spot for culture preparation.

With this study, more mycelia from mushrooms can be obtained at faster rate and the need put to use. Therefore, there is the need to advance more on the technology that can better the production of *P.florida* and other species of mushroom from the culture stage.

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