African Scientist Vol. 26, No. 2 June 30, 2025
Printed in Nigeria

1595-6881/2025 $80.00 + 0.00

https://africansciientistjournal.org

afs2025034/26211

Growth and Cultivation Studies of *Pleurotus tuberregium* (Fr. Singer) on Sawdust of *Brachystegia nigerica* Supplemented with Cassava Starch and Bambara Groundnut

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(Received June 20, 2025; Accepted in revised form June 26, 2025)

ABSTRACT: The effect of cassava starch and bambara groundnut powder on the growth of *Pleurotus tuberregium* (Fr. Singer) was investigated. The growth of *Pleurotus tuberregium* on sawdust of *Brachystegia nigerica* was supplemented with starch and bambara groundnut (*Vigna subterranean*) at 0%, 5%, 10%, 15% and 20% concentrations respectively. Mycelia extension, mycelia growth morphology and biomass were influenced significantly at $p > 0.05$ by these supplements. Starch supplemented medium had a faster growth rate (80.66 ±0.66 mm/day) at 5% but bambara groundnut-supplemented medium had its highest rate (78.33±1.67mm/day) at 10%. Bambara groundnut supplemented medium had the highest biomass (386.00±66.00 mg) at 10% and least biomass (188.00 ±3.00 mg) at 15%, while cassava starch supplemented media had its highest biomass (203.00±28.00 mg) at 5% and least (133±12.00 mg) at 15%. Half (50%) colonization was achieved after 11 days of colonization for 10% starch and it took 30 days to attain full (100%) colonization for 10% starch level. Accumulation of exudates suspected to be exopolysaccharide was observed as the mycelium grows in the substrate bags supplemented with starch. Lower concentration of local starch supplement supported a higher production of the exopolysaccharide. Cassava starch can be used to induce the production of exudates of *P. tuberregium.*

Keywords: Mushroom, *Pleurotus tuberregium*, Exudates, Mycelium

Introduction

Mushrooms are eukaryotic organisms that have a cell which possesses polysaccharide, chitin, alongside with lipids and proteins (Jonathan *et al*., 2013). A mushroom is the fleshy, spore-bearing fruit body of a fungus, typically produced above ground or on a substrate (Boa, 2004). Mushroom belong to the class Basidiomycetes, order Agaricales. Hence, the word "Mushroom" is most often applied to those fungi (Basidiomycota, Agaricomycetes) with a stem, a cap (pileus) and gills on the underside of the cap. The gills possess microscopic spores that help for dispersal across the surface of the ground or on its occupant surface (Chang and Miles, 2004).

The nutritional value of mushroom can be compared to those of eggs, milk and meat (Oei, 2003) and low crude fat content and a high proportion of polyunsaturated fatty (Fufa *et al*., 2021, Usman *et al*., 2022). The carbohydrate, fibre and protein content of *Pleurotus tuberregium* indicate that it is a healthy source of nutrients and as such can be included in diets of especially diabetic individuals (Ekute and Nwokocha, 2021). *Pleurotus tuberregium* is a good source of dietary macro-elements (K and Mg) essential for preventing osteoporosis, reducing kidney stones, and other health problems (Ekute and Nwokocha, 2021).

Mushrooms are popularly consumed as delicacy in Nigerians as in many other countries of the world (Fufa *et al*., 2021). The importance of edible mushrooms has increased in the recent times due to their gastronomic

value, nutritional potential, medicinal properties and ability to degrade and recycle agro-industrial residues. Mushrooms have been considered as a source of rich food because they contain proteins, sugars, glycogen, lipids, vitamins, amino acids and crude fibres.

Pleurotus tuberregium (Fries) Singer also known as the king tuber oyster mush-room is an example of an edible mushroom found in tropical and subtropical regions of the world (Obiaigwe *et al*., 2023). The sclerotium is often of a dark brown colour externally and white inside. *P. tuberregium*, also known as the "potato mushroom" or "Osu" in parts of Africa, is a unique white-rot fungus, known for its large, underground sclerotia. It is popularly used in Nigeria and other parts of the world as food and medicine (Aruwa *et al.,* 2021, Ekute and Nwokocha, 2021). *Pleurotus* species are characterized by a white spot print attached to the decurrent gills, often with eccentric (off centre) stipe or no stipe at all. They grow on wood in nature, usually on dead standing tree or on fallen logs. *P. tuberregium* is the only member of the *Pleurotus* genus that produce true sclerotium and also differs from other *Pleurotus* species in its non pleurotoid habit (Isikhuemhen and Nerud, 1999, Ekute and Nwokocha, 2021, Fasiku *et al*., 2023).

The sclerotia of *Pleurotus tuberregium* contains food reserves which is in form of a compact mass of hardened fungal mycelium and it help the fungi survive environmental extremes (Thorn *et al*., 2000). The sclerotium is dark brown on the outside and white on the inside. The need for sclerocia all over the world remains high on demand while its harvest from the wild is reduced with the constant deforestation and conversion of the forests into agricultural fields, (Ishikuemhen, 2004). There is therefore urgent need for the cultivation of this mushroom for nutrition and economic empowerment. Various types of agricultural wastes have been used as substrates for the cultivation of mushroom (Josephat *et al*., 2020).

The aim of the study is to determine the effect of cassava starch and bambara groundnut on the growth of *Pleurotus tuberregium* (Fr. Singer) using sawdust of *Brachystegia nigerica.*

Materials and methods

Preparation of culture media: Potato Dextrose Agar (PDA) was the medium used in the course of study. The potato infusion is made by boiling 200 g of sliced potato (washed but unpeeled) in 1 L of distilled water for 30 min and then poured out gently through a loosely woven cotton cloth called cheese cloth. Distilled water was added such that volume makes a suspension of 1 L. Twenty (20) g dextrose and 20 g agar powder were added and the medium was sterilized by autoclaving at 15 psi, at 121 ℃ for 15 min.

Preparation of mushroom pure culture: Sterilized medium was dispensed into Petri-dish and allowed to cool and gel. Solidified media was inoculated with agar block of a 7-day old culture of *P. tuberregium*. Inoculated cultures were incubated at room temperature for mycelia growth until pure culture was gotten. Pure culture of P. *tuberregium* was used to inoculate agar plates supplemented with cassava starch and bambara groundnut flour at 0, 5, 10, 15 and 20 % levels to investigate the vegetative growth of the mushroom. Carbon source was added to trigger mycelia growth. The glucose level varied with the percentage of the supplement. Three (3) replicates were done for each level. Measurement of the mycelial growth using meter rule, was done daily after 24 h of inoculation.

Mycelial density rating and biomass determination: The mycelia density of each plate was done and rated according to the categories described as: very sparse, sparse, moderate, dense, and very dense. Mycelial biomass (dry weight) was determined by heating the Petri dishes and removing the suspending mycelia from the melted agar. The mycelia mat is dried and weighed in a balance.

Preparation of spawn: Sorghum grains were used to prepare spawn using standard methods

Preparation of substrate: The sawdust was collected from a saw mill factory and dried. The sawdust was mixed wheat bran, calcium carbonate at 78:20:2 ratio. Afterwards, the mixture was mixed thoroughly with water to achieve 65 % moisture content.

Bagging and pasteurization: At different concentrations (0, 5, 10, 15, 20 %), the two supplements were added into the polypropylene bag, measuring 6 x 12 cm. The loaded bags were fitted with PVC neck which now served as the opening and plug with cotton and wrapped with foil. The bags were pasteurized for 4 h. After pasteurization, the bags were allowed to cool and then inoculated with 7-day old spawn. After the inoculation, the bags were then incubated in a dark room at temperature of 26 ℃ to induce mycelia growth.

Analysis of data: The mean values and standard error of the three (3) replicates were recorded. Differences among means were analyzed with SPSS to determine analysis of variance and test of significance were done by Duncan's multiple range tests.

Results

Mycelia growth was higher and faster for culture supplemented with 10 %, 20 % of Bambara groundnut flour and less for 0% supplementation (Tables 1,2). The mycelium biomass was higher for 10 % and 20 % supplementation (Table 3). The density and morphological characteristics of the mycelium also varied, ranging from sparsely dense to very dense (Table 4). For the cassava starch, it shows that a fast rate of growth of *P. tuberregium* occurred and it took eight days for the colonization of the entire medium supplemented at different levels. 20 % supplementation had the highest growth rate, followed by 5 % supplementation. The least growth was observed in 15 % (Table 5).

Table 1: Effect of Bambara groundnut flour supplement on daily mycelia growth of *Pleurotus tuberregium*

Days	Mycelia Diameter (mm)					Basal
	0%	5%	10%	15%	20%	
1	5.33±0.33 a*	6.33±1.20a	4.67±0.33^{a}	6.00±0.57^{a}	5.67±0.67^{a}	5.00±0.57^{a}
2	13.67±0.67^{a}	12.33±0.88^{a}	10.67±0.89^{a}	11.67±1.20^{a}	12.33±0.89^{a}	11.33±1.20^{a}
3	18.00±1.00^{a}	17.33±0.88^{a}	17.33±1.76^{a}	16.67±1.76^{a}	19.00±1.00^{a}	16.00±1.52^{a}
4	24.33±2.67^{a}	24.00±2.00^{a}	27.00±2.65^{a}	20.67±1.33^{a}	24.00±2.31^{a}	21.00±2.00^{a}
5	32.33±5.69^{ab}	36.00±2.89^{ab}	41.00±2.89^{b}	29.67±1.33^{ab}	34.67±3.33^{ab}	27.67±2.67^{ab}
6	47.67±5.81^{bc}	44.33±6.00^{bc}	57.00±3.05^{c}	41.67±3.76^{ab}	49.00±4.16^{bc}	33.67±2.33^{a}
7	63.33±7.05^{b}	49.33±4.41^{b}	71.00±5.03^{b}	59.00±4.51^{b}	68.00±3.51^{b}	40.00±2.65^{a}
8	71.67±5.24^{b}	66.00±5.13^{b}	78.33±1.67^{b}	69.33±5.69^{b}	75.67±2.96^{b}	48.00±2.65^{a}

** mean of three replicates ± standard error. Mean followed by the same letter along rows are not significantly different at P>0.05

Table 2: Effect of Bambara groundnut powder and cassava starch on growth rate (mm/day) of *P. tuberregium* mycelia

Level	Bambara groundnut	Cassava starch
0%	8.96±0.65**	10.08±0.22
5%	9.63±0.26	10.08±0.08
10%	9.79±0.21	9.95±0.04
15%	9.08±0.30	9.75±0.21
20%	9.46±0.37	10.00±0.00
Basal	6.00±0.33	

**= Means of three replicates ± standard error

Table 3: Effect of Bambara groundnut flour and cassava starch on the mycelia biomass (mg) of *P. tuberregium*

Percentage supplementation	Bambara flour	Cassava starch
0%	237.00±32.00**	171.00±9.00
5%	199.00±20.00	203.00±28.00
10%	386.00±66.00	164.00±12.00
15%	188.00±3.00	133.00±12.00
20%	273.00±43.00	173.00±1.00
Basal	50.00±2.00	50.00±2.00

** = means of three replicates ± standard error

Table 4: Mycelia density and cultural characteristics of *P. tuberregium* on supplemented media.

Percentage supplementation	Bambara flour	Cassava starch
0%	++++ AR C	+++++ AR
5%	++++ AR C	++++ AR
10%	+++++ AR	+++++ AR C
15%	+++ AP	++++ AR
20%	++++ AP	+++++ AR
Basal	++ AP	++ AP

Key: - No growth, + very sparse, ++ Sparse, +++ Moderate, ++++ Dense, +++++ Very dense. AP- Appressed, AR-Aerial, C- Centric

Table 5: Effect of cassava starch on the daily mycelia extension of *P. tuberregium*

Days	Mycelia Diameter (mm)					Basal
	0%	**5%**	**10%**	**15%**	**20%**	
1	9.33±0.33^{b}	10.33±0.67^{b}	9.33±0.33^{b}	5.67±0.67^{a}	9.33±0.33^{b}	5.00±0.57^{a}
2	16.33±1.20^{c}	19.00±0.57^{d}	13.67±0.33^{ab}	14.00±0.58^{bc}	15.67±0.33^{bc}	11.33±1.20^{a}
3	25.33±0.67^{c}	25.00±2.52^{c}	22.67±0.33^{bc}	20.00±0.57^{b}	24.33±0.67^{c}	16.00±1.52^{a}
4	31.00±2.52^{b}	35.00±2.5^{b}	32.33±1.45^{b}	24.00±0.57^{b}	35.33±2.33^{b}	21.00±2.00^{a}
5	50.33±0.67^{cd}	52.00±0.12^{d}	47.33±0.88^{c}	36.33±1.45^{b}	52.67±0.88^{d}	27.67±2.67^{ab}
6	59.33±1.45^{c}	62.00±0.58^{cd}	58.00±1.15^{c}	51.33±1.20^{b}	63.33±0.88^{d}	33.67±2.33^{a}
7	74.67±0.88^{c}	74.00±3.05^{c}	73.67±0.67^{c}	65.00±2.00^{b}	77.33±0.33^{c}	40.00±2.65^{a}
8	81.33±1.76^{b}	80.66±0.66^{b}	79.66±0.66^{b}	79.66±0.33^{b}	80.00±0.00^{b}	48.00±2.65^{a}

** Mean of three replicates + standard error. Mean followed by the same letter(s) along rows are not significantly different at P > 0.05

The sawdust supplemented waste had varying effects on the rate of mycelia growth and mycelia colonization. The effect of the local starch and bambara groundnut flour supplementation was observed for a period of 50 days. The time for 50%, 75% and 100% colonization were recorded. Evaluation of sawdust mixed with the two supplements for the colonization of *Pleurotus tuberregium* showed mycelia growth in the substrate supplemented with local starch while that supplemented with bambara groundnut flour, showed no growth. The fastest mycelia colonization was observed on 10% cassava starch supplementation with 30 days full colonization after inoculation. It took 11 days for 50% colonization, 25 days for 75% colonization and 30 days for 100% colonization. 20% supplementation had the slowest growth rate and was not colonized up to 100%. It had 50% level of colonization after 36 days and 75% colonization after 48 days. However, it was observed that a clumplike structure identified as polysaccharide was formed in the substrate bags (Table 6).

Table 6: Time (days) of mycelia colonization and exudate emergence on cassava starch supplemented sawdust.

Supplementation	Percentage Colonization			Exudate Emergence
	50%	70%	100%	
0%	18.3	30.6	47	40
5%	17	29.5	39	46
10%	11	25	30	29
15%	19	30	37	39
20%	36	48	-	70

Discussion

The vegetative growth of *Pleurotus tuberregium* in culture prepared with sawdust extract was enhanced by cassava starch and Bambara groundnut powder. Tavarwisa *et al.,* (2021) had reported on the use of baobab fruit shell as growth substrate for P. ostreatus. Increased concentration of supplementation promotes vegetative growth and polysaccharide formation. Media supplemented with both cassava starch and bambara groundnut powder supported the growth *P. tuberregium.* This work agrees with the report of Ali *et al.,* (2024) on *Volvoriella volvacea*. The fast utilization of local starch by *P. tuberregium* could be attributed to the ease with which it metabolizes the material to produce cellular energy for the growth.

Bambara groundnut flour supplemented media produced a higher biomass than that obtained in cassava starch supplemented media. The high yield may be the nutrients composition of the bambara groundnut flour. This agrees with the report of Chikwendu *et al.,* (2021) who reported that the yield of sporophore of *Pleurotus* species is dependent on the nature or nutrient composition of the substrate. The mycelia density of the mushroom was dense for the supplements tested compared with control that was sparse. The sawdust tested supports the mycelia growth of *Pleurotus tuberregium.* This is in line with the findings of Kadiri and Fasidi (1990), who reported sawdust as the best substrate for mycelia growth and fructification. Cassava starch induced exudate formation in *Pleurotus tuberregium* as well as fruit body and sclerotia formation. *Pleurotus* species are recognized for producing polysaccharide like β-glucans with important medical properties as a constituent of the fruitbodies or the mycelium (Morais *et al.,* 2002). Yang and Liau, (1998) and Wu *et al*., (1998), had reported that polysaccharide formation and mycelium growth are greatly affected by culture conditions. Higher supplementation inhibits polysaccharide formation. Gern *et al.* (2006) had reported that a higher glucose concentration also presents a negative effect on the polysaccharide concentration. It can be

inferred that the mycelia could not produce fruit body due to the fact that the fungus was storing up food in form of polysaccharide for survival and also for the formation of fruit body

Conclusion

Sawdust supplemented with cassava starch or bambara groundnut flour enhanced vegetative growth of *P. tuberregium* and also induced the formation of exudates in the mushroom. Further study is therefore necessary to determine the effect of these supplements on the nutritional composition and medicinal value of *P. tuberregium.*

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