

# Comparative Study on the Growth and Yield of *Ganoderma lucidium* Mushroom Cultivated on African Peach (*Nauclea diderrichii*) and Bush Mango (*Endospermum medullosum*) Sawdust as Substrates

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## Abstract

*This study was conducted to find out which substrate provides for the best artificial development of G. lucidium. Mango (Mangifera indica) leaves were added to the saw dusts of Nauclea diderrichii and Endospermum medullosum, which were both utilized as substrates for growing. The substrates were inoculated with Ganoderma spawn and then exposed and hydrated to start the fruiting process. Three replications of the process were carried out. It was observed that E. medullosum supported the growth and good yield of the mushroom by taking 7 days to commence ramification, 37 days to achieve complete ramification, and 68 days for harvesting. Due to the slowed mycelial growth, N. diderrichii was unable to promote the harvesting and ring formation of G. lucidium. As a result, it cannot be advised to use N. diderrichii sawdust as a substrate for the growth of G. lucidium. On the other hand, the mushroom proliferated well in the E. medullosum substrate producing fully grown Ganoderma mushroom in a short time compared to other studies that used different substrates other than E. medullosum. Considering the results of this study, E. medullosum supplemented with Mango (Mangifera indica) leaves is recommended as a suitable substrate for the cultivation of G. lucidium.*

**Keywords:** Growth, *Ganoderma lucidium*, medicinal mushroom, substrate, yield

## INTRODUCTION

A mushroom is a type of macro fungus that has an unusual fruitful body. Mushrooms are the fruit bodies of macroscopic, filamentous, and epigeal fungi, and they are classified as Basidiomycetes. On decaying organic matter, they develop saprophytically or parasitically [1]. In addition to being a wholesome, protein-rich food source, some kinds of mushrooms also generate chemicals that have medical use [2].

Since ancient times, macrofungi have been used across the world as a priceless food source and as traditional medicines. Both the mushroom's fruiting body and its mycelium contain substances with

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Received Date: December 28, 2024

Accepted Date: January 03, 2025

Published Date: January 04, 2025

**Citation:** S. Fakoya, T.J. Adedayo. Comparative Study on the Growth and Yield of *Ganoderma lucidium* Mushroom Cultivated on African Peach (*Nauclea diderrichii*) and Bush Mango (*Endospermum medullosum*) Sawdust as Substrates. International Journal of Fungi. 2025; 2(1): 39–45p.

diverse antibacterial action, and these substances may be extracted from a variety of mushroom species and be useful to humans [3]. The common mushrooms in Nigeria include: *Termitomyces*, *Pleurotus*, *Lentinus*, *Lenzites*, *Trametes*, *Ganoderma*, and others [4].

Mushrooms are recognized as nutraceutical because of their strong nutritional and functional benefits. According to Chang (2013) [5], in most parts of Africa, mushrooms are consumed based on their organoleptic properties, such as aroma, taste, flavor, and texture rather than for their nutritional and therapeutic [6]. For more than 4000 years, Far

Eastern nations have used the medicinal mushroom *Ganoderma lucidum*, and ancient writings have described its potent benefits [7].

Ling zhi is the Chinese name for *G. lucidum*, and Reishi is the Japanese name for the Ganodermataceae family [8].

Anti-tumor, anti-inflammatory, anti-microbial, hepatoprotective, hypotensive, anti-diabetic, antioxidant, immune system modulation, and bacteriostatic are just a few of the unique health advantages of Reishi mushrooms. Commercial herbal items made from *G. lucidum* include powders, dietary supplements, concentrated pills, syrup, and tea [9].

Due to the uneven distribution of *G. lucidum* in the wild and the growing demand for it as a medical product, attempts have been made to artificially cultivate it all over the world. The most popular artificial culture techniques for *G. lucidum* include the bottling procedure, sawdust bags, short wood segments, tree stumps, and wood logs [10].

## MATERIALS AND METHODS

### Experimental Site

The mushroom spawn was obtained from the Federal Institute of Industrial Research, Oshodi (FIRO) in Lagos. The sawdust used was obtained from a sawmill in Okitipupa, Ondo State, Nigeria (coordinates N 6° 27' 34.609" E 4° 45' 49.933"). The mango leaves were collected from the University's botanical garden mango tree. All practical was carried out in Olusegun Agagu University of Science and Technology (OAUSTECH) Mycology micro laboratory, Okitipupa, Ondo State, Nigeria.

### Cellular Expansion

To obtain a pure culture of *G. lucidum*, the potato dextrose agar method was used. The PDA plate was prepared according to the manufacturer's specifications. After preparation, the mixture was sterilized at 121 °C for 15 minutes. The mixture was then allowed to cool and was poured into plates under sterile conditions. A small grain of sorghum from a previous spawn was placed at the center of the plate and then properly stored at 25°C for 8 days for sufficient mycelial growth. This procedure was carried out in triplicates.

### Substrate Preparation

The procedure of producing mushrooms, as described by Wachtel-Galor et al. (2011) [11], was adopted in this study and it was carried out in triplicates. The major substrates for cultivation are lignocellulosic waste, while the minor substrates (additives) are nutritional supplements that were added in small quantities. The mango leaves were sun-dried for two weeks after which they were homogenized using an electric blender.

*Nauclea diderrichii* and *Endospermum medullosum* sawdust were mixed with blended *Mangifera indica* leaves (which served as additives) in a ratio of 6:1. They were soaked in water separately to enable them to have about 70% water content. Each substrate was then packed into 3 trials heat-resistant polythene bags. They were wrapped with aluminum foil and then sterilized using an autoclave at 121°C for 15 minutes. After sterilization, the bags were allowed to cool for 24 hours before inoculation.

### Substrate Inoculation

The different substrates in the bags were spawned with *Ganoderma* spawn by creating a hole inside the substrates using the spatula and adding 3 spoonsful of spawn into the hole under sterile conditions, then the bags were tied. They were then incubated (spawn running) at 25°C for six weeks after which they were exposed and hydrated to initiate fruiting. The polythene bags were then cut open to expose strips of the saw dust to give room for normal shaped fruit bodies.

The initial stages of *Ganoderma lucidum*'s development began on various days in various saw dusts. Fruiting bodies were harvested after the white margin on the caps vanished and the caps turned fully

red. All treatments' colonization times, days of primordial formation, and first harvest times were noted and compared.

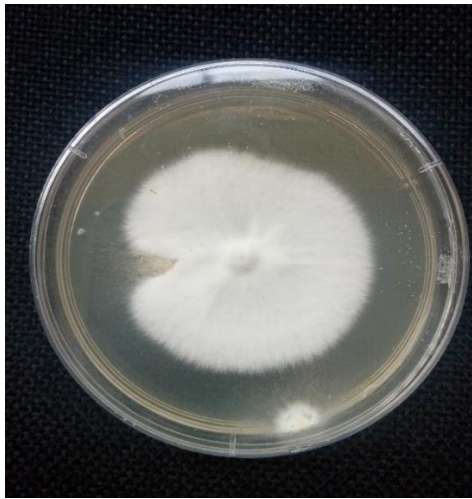
### Statistical Analysis

The experimental design was a Complete Randomized Block, and data were presented as mean  $\pm$  standard error means (SEM). The one-way analysis of variance (ANOVA) for means separation was carried out using Duncan Multiple Range (DMR) and SPSS 16.0 software.

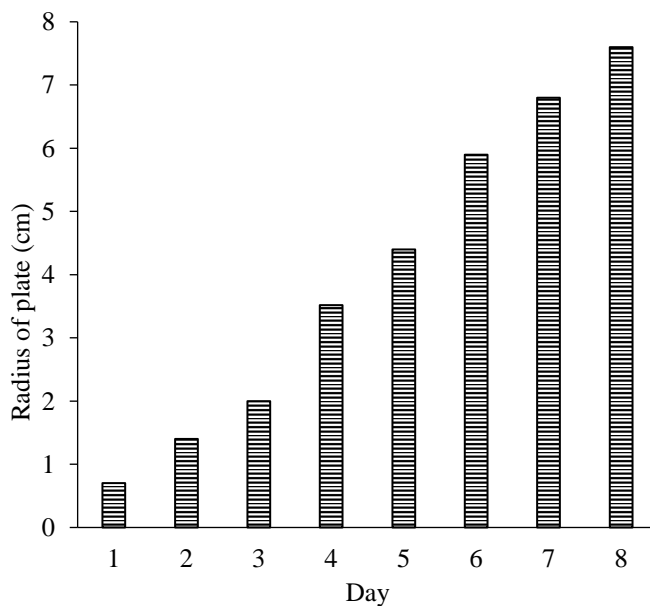
## RESULTS

### Mycelial Growth

The mycelial growth which is a cottony mass started from the center of the plate where the grain was inoculated and then spread evenly around the plate. The plate was monitored for three days, and it had a radius of 0.70 cm, 1.40 cm, and 2.00 cm on days 1, 2, and 3, respectively. Further monitoring of the plate for 5 more days showed a sufficient increase in the radius of the plate to 7.60 cm on the eighth day (Figures 1 and 2) showing the measurement of the cellular expansion on plates from the first to the eighth day.



**Figure 1.** Mycelial growth of *G. lucidum* mycelial on plate.



**Figure 2.** Daily radial mycelial growth measurements of *G. lucidum*.

### Ramification and Mycelial Colonization of Substrates

Ramification and substrate colonization were observed within 7 days of inoculation in the *Endospermum medullosum* substrate. Substrate colonization in the *Nauclea diderrichii* substrate was observed after 30 days of inoculation. *Endospermum medullosum* substrate was completely ramified after 28 days of spawn inoculation and had a whitish compact mass with cottony growth formed due to complete impregnation of mycelium into the substrate, as shown in Figure 3. *Nauclea diderrichii* substrate on the other hand was only partially ramified after 37 days of inoculation as shown in Figure 4.



**Figure 3.** Fully ramified *E. medullosum* substrate.



**Figure 4.** Partially ramified *N. diderrichii* substrate.

When the substrate bags were fully colonized with mycelia, they were opened to start fruiting, adding water to the bags to increase moisture content. Only the *E. medullosum* substrate produced mushrooms while the *N. diderrichii* substrate did not go past the ramification stage even after incessant hydration.

The ramified *E. medullosum* substrate first developed into a white *Ganoderma* mushroom after 36 days of spawn inoculation before it began to develop reddish rings at regular intervals until the number

of rings became constant on the fully grown *Ganoderma* mushroom as shown in Figure 5. The number of reddish rings was counted and recorded at regular intervals as shown in Table 1. The mushroom developed into a fully grown *Ganoderma* mushroom with a maximum number of rings after 68 days of inoculation. Table 1 compares and displays the colonization period, primordial formation days, and first harvest period for the two substrates while Table 2 shows and illustrates the whole development of red rings on the mushroom.



**Figure 5.** Fully grown *Ganoderma* mushroom on *E. medullosum* substrate.

**Table 1.** Effect of different substrates on growth parameter of *Ganoderma lucidum*.

Substrates	Initial Ramification (Days)	Colonization Period (Days)	Premordial Formation (Days)	Days of the First Harvest
<i>Endospermum medullosum</i>	7.30 ± 0.70	28.00 ± 0.00	36.30 ± 0.50	68.20 ± 0.20
<i>Nauclea diderrichii</i>	3.50 ± 0.70	–	–	–

**Table 2.** Development of red rings on *Ganoderma* mushroom.

Number of Rings	Formation Days
1	56.60 ± 0.60
3	58.20 ± 0.40
5	61.60 ± 0.20
7	64.40 ± 0.60
10	68.20 ± 0.20

Note: Mean values ± standard error of the mean (SEM).

## DISCUSSION

All the *G. lucidum* cellular expansion plates showed healthy mycelia growth, especially between the 6th to 7th day of inoculation and a short growth period (8 days) which, according to Yang et al. (2012) [12], are traits that validate their use in the exploitation of ganoderic acids and other biological principles. Although it is possible to suppose that these plates showed greater substrate-independent vegetative agility, the actual cause of this is still unknown [10]. Connected environmental nutrient uptake and biodiversity effects to optimal mycelium development.

The ramification process (early stage of mycelial growth) was initiated for *E. medullosum* and *N. diderrichii* in 7 days and 30 days, respectively. Complete ramification (completion of mycelial growth) was attained in 28 days for *E. medullosum* while partial ramification was attained in 37 days for *N. diderrichii*. The primordial formation and first harvest of *G. lucidum* in *E. medullosum* were in

36 days and 68 days, respectively. In the study conducted by Kamra et al. (2013) [8], the first harvest of *G. lucidum* took up to 92 days.

With *Shorea robusta* sawdust supplemented with gram flour, wheat bran, corn flour, and rice bran, the colonization period of *G. lucidum* was 35 days in Nepal [7]. In this study, the colonization period of *G. lucidum* supplemented with mango leaves was 28 days. The variation may result from variations in the nutritional value of the various sawdusts. This contrast seems to result from the type of supplement used as well as the ratio of supplements.

Due to extremely low mycelial development, the colonization period, primordial formation period, and first harvest days for *N. diderrichii* in this experiment could not be determined. *N. diderrichii* could not give any yield due to very poor mycelial growth because the sawdust of *N. diderrichii* was finer than that of *E. medullosum*, suggesting that *N. diderrichii* has a lower water holding capacity and poorer aeration.

The results are consistent with those of some authors who claimed that broad-leaved hardwood sawdust was favored for industrial manufacturing. However, for increased aeration and water-holding capacity, coarse sawdust or wood chips must be combined with fine sawdust so that mycelium can therefore grow quickly and thickly.

Thus, the *N. diderrichii* sawdust sample cannot be suggested to be considered as a substrate for the cultivation of *G. lucidum*.

## CONCLUSIONS

The effect of two kinds of sawdust on the growth and yield of *G. lucidum* was investigated in this study. The type of sawdust and additives used greatly influenced the output of *G. lucidum*, as previously mentioned as opposed to *N. diderrichii* which recorded stunted growth. Therefore, it is crucial to select the right substrate when producing *G. lucidum* for commercial purposes. Among both treatments, *E. medullosum* sawdust with mango leaves had the fastest colonization period and best yield, it is important to use the proper substrate for the artificial production of *G. lucidum*.

Considering the results of this study, *Endospermum medullosum* sawdust supplemented with *Mangifera indica* leaves is recommended as a suitable substrate for the artificial production of *G. lucidum*. Supplementation showed a positive role in mycelia growth and yield of mushrooms. So, supplementation is required for the commercial production of *Ganoderma lucidum*. Supplements that can be recommended for further research on artificial cultivation of *G. lucidum* include rice bran, wheat bran, corn flour, and gram flour amongst others.

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