EFFECT OF CARBON AND NITROGEN SOURCE ON THE MYCELIAL GROWTH OF *Ganoderma lucidum.*

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EFFECT OF CARBON AND NITROGEN SOURCE ON THE MYCELIAL GROWTH OF *Ganoderma lucidum.*

BY

Daniel Nwachuckwu DIBIE
LSC1907259

A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELORS OF SCIENCE DEGREE (B.sc) IN SCIENCE LABORATORY TECHNOLOGY (BIOTECHNOLOGY TECHNIQUES)

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CERTIFICATION

This is to certify that this research work was carried out by Daniel Nawachuckwu DIBIE in the department of science laboratory technology, life sciences, university of Benin, Benin city.

_____ _____

MR A.T. DANIA Date
(Project supervisor)

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DR. P.O. ALONGE Date
(Project Coordinator)

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PROF. E.O. OSHOMOH Date
(Head of Department, SLT)

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(External supervisor) Date

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DEDICATION

This work is dedicated to Almighty God, who is in His infinite mercy gave me knowledge and composure of thoughts.

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ACKNOWLEDGEMENTS

I would like to express my sincere and upmost gratitude to those who made this research work a success. Firstly, I am thankful to my supervisor, Mr A.T. Dania for his patience, love, guidance and support throughout this research.

My heartfelt gratitude goes out to my H.O.D., Prof. E.O. Oshomoh. My lecturers and the entire staff of the department of science laboratory.

I also want to thank my family; Mr and Mrs Benjamin Dibie my Siblings Angela and Obaze, My friends; Daisy, Blessing, Emmanuel, Courage, Patrick, Fetzz, Bobby, Agbonifo, Maysoma, for their constant encouragement, resources and support that facilitated the accomplishment of this project.

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ABSTRACT

The fungus *Ganoderma lucidum* is a medicinal mushroom that belongs to the family *Ganodermataceae* and mainly grows in the tropics. The effect of carbon and nitrogen sources on the mycelial growth of the fungus *Ganoderma lucidum* was investigated, and the best carbon and nitrogen sources were determined. Using standard potato dextrose agar (PDA) media as the growth medium, the mycelial growth of *Ganoderma lucidum* was observed. Subsequently, the sugar from PDA was then replaced with the selected nitrogen and carbon sources and used alternatively for each source inoculated with the fungus *Ganoderma lucidum*. The daily mycelial growth of each source was recorded. After 5 days of inoculation, the mycelial cells fully colonised the petri dishes. 10 days after inoculation, the fungus mycelial was harvested, dried, and the biomass recorded. Six selected carbon sources, which include glucose, lactose, starch, maltose, cellulose, and sucrose, were employed as nutrient supplements for the fungus *Ganoderma lucidum's* growth. All the sources promoted its growth, but maltose (66.88±1.44mm) and lactose (64.88±4.71mm) were most suitable for *Ganoderma lucidum* growth. Sucrose (55.0±1.87mm), and cellulose (37.88±1.44mm), was least suitable for *Ganoderma lucidum's* growth due to their growth rates. The most suitable carbon source for the biomass production of *Ganoderma lucidum* was sucrose (0.29±0.03g) and lactose (0.26±0.04g), while the least suitable was cellulose (0.15±0.02g) and glucose (0.15 ±0.06g). Six selected nitrogen sources were also employed as supplements in this study. They include ammonium sulphate, ammonium nitrate, potassium nitrate, sodium nitrate, yeast, and urea. All nitrogen sources promoted the fungus' growth with the exception of urea, which showed no growth. Among the selected nitrogen sources used, yeast (69.25 ± 2.33mm) and ammonium sulphate (61.25±3.01mm) were most suitable for *Ganoderma lucidum* growth. The least suitable were ammonium nitrate (36.35± 0.96mm), sodium nitrate (47.13±0.48mm), and urea, which promoted no growth. The most suitable nitrogen sources for the biomass production *of Ganoderma lucidum* were yeast (0.52± 0.03g) and sodium nitrate (0.83±0.16g) and the least suitable was ammonium sulphate (0.19 ±0.09g) and ammonium nitrate (0.16±0.02g). The morphological characteristics and density pattern of the mycelial of *Ganoderma lucidum* were also observed in the course of the study. Nutritional factors have been noted to be responsible for the growth and development of fungi. From this study, it is concluded that the most suitable carbon source for the growth of *Ganoderma lucidum* is maltose and lactose, while the most suitable carbon source for the biomass production of *Ganoderma lucidum* is sucrose and lactose.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Ganoderma lucidum (Fr.) Karst, a species of basidiomycetes which belongs to family Polyporaceae (or Ganodermaceae), order of Aphyllophorales, class Homobasidiomycetes, and division Basidiomycota is one of the most popular medicinal mushrooms in China, Japan, Korea, and other Asian countries (Wasser and Weis 1999). It has been under modern biochemical and pharmacological research during the last 30 years (Gao *et al.*, 2006). *Ganoderma lucidum* is an annual or perennial fungus; it is commonly known as "Ling Zhi" in China. It possesses tough and leathery to woody basidiocarps termed polypore. They grow either as biotrophs on live trees, or necrotrophs on dead trees, logs and stumps. *Ganoderma* species can survive under hot and humid environments such as sub-tropical and tropical regions. In the wild, *G. lucidum* mainly grows in subtropical and temperate climate regions such as Asia, Europe, Africa, and Americas (Siwulski *et al.*, 2015). *G. lucidum* has a kidney-shaped cap and its upper surface is russet, with a cloud-like, ring pattern, glossy exterior and woody texture. *Ganoderma* are white-rot fungi with the ability to decay lignin as well as cellulose (Adaskaveg and Gilbertson 1994). They are responsible for wood-decaying of conifers and hardwoods occurring throughout the world. Intensive studies showed that different bioactive components of *Ganoderma* have several medicinal effects, such as alkaloids, terpenoids, polysaccharides, steroids, fatty acids, and proteins (Mizuno *et al.*, 1995, Ihayere *et al.*, 2010). These compounds have immune-modulating, hypoglycemic, antimicrobial, antitumor, cardiovascular and other therapeutic effects (Wasser & Weis 1999, Gao *et al.*, 2005).

Ganoderma lucidum has a systematic theoretical background in traditional Chinese medicine, and research has now confirmed that it contains over 400 bioactive compounds, including polysaccharides, triterpenoids, steroids, fatty acids, amino acids, nucleosides, proteins, and alkaloids (Cör *et al.*, 2018). Polysaccharides and triterpenoids are the major bioactive compounds in *G. lucidum*. The active ingredients and relative pharmacological activities differ during the different growth stages of *G. lucidum*. Modern studies have shown that *G.*

lucidum contains many active compounds, including triterpenoids, polysaccharides, steroids, fatty acids, amino acids, nucleosides, proteins, and alkaloids. Several vitamins have been reported from *G. lucidum*, such as vitamins B1, B2, B6, β-carotene, C, D, and E (Yang *et al* (2019). Moreover, various minerals such as calcium, sodium, potassium, phosphorus, iron, carbon, magnesium, zinc, etc have been identified in *G. lucidum* (Ahmad 2018).

To accelerate mycelial growth and metabolite production, the major concerns were to find environmentally good and economically feasible compounds that stimulate mycelial growth and metabolite production of *G. lucidum* (Yan *et al*., 2000). Thus, it is necessary to find optimal nutritional and environmental conditions for culturing mycelia in the laboratory.

These experimental studies indicated that carbon and nitrogen sources are vital in the growth and biomass production of fungi, hence the need for this study.

1.2 Aim:

The aim of this study is to find the effect of carbon and nitrogen on the mycelial growth of *Ganoderma lucidum*.

1.3 Objective:

The objective of this study includes to;

i. determine the effect of carbon sources on the mycelial growth of *Ganoderma lucidum.*

ii. determine the effect of nitrogen sources on the mycelial growth of *Ganoderma lucidum*

iii. determine the effect of carbon sources on the biomass production of *Ganoderma lucidum*

iv. determine the effect of nitrogen sources on the biomass production of *Ganoderma lucidum*

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CHAPTER TWO

LITERATURE REVIEW

2.1 Gananoderma lucidim

Ganoderma lucidum is an annual or perennial fungus of the family Ganodermataceae (Campos Ziegenbein *et al.,*2006); it is commonly known as "Ling Zhi" in China. In the wild, G. lucidum mainly grows in subtropical and temperate climate regions such as Asia, Europe, Africa, and Americas (Siwulski *et al.,* 2015). G. lucidum has a kidney-shaped cap and its upper surface is russet, with a cloud-like, ring pattern, glossy exterior, and woody texture. *G. lucidum* has a systematic theoretical background in traditional Chinese medicine, and research has now confirmed that it contains over 400 bioactive compounds, including polysaccharides, triterpenoids, steroids, fatty acids, amino acids, nucleosides, proteins, and alkaloids (Cör *et al.,* 2018). Polysaccharides and triterpenoids are the major bioactive compounds in *G. lucidum*. The active ingredients and relative pharmacological activities differ during the different growth stages of *G. lucidum*. Modern pharmacology has shown that *G. lucidum* has antitumor (Kao *et al*., 2016), anti-inflammatory (Liu *et al.,* 2018), and antioxidation effects (Sarnthima *et al.,* 2017) and that it could regulate the respiratory, nervous, and immune systems (Kubota *et al.,* 2018). *G. lucidum* also has a hypoglycemic effect and can protect the liver (Wu *et al*., 2016). Nowadays, *G. lucidum* is used as a powder, tea, and dietary supplement. Therefore, it is extremely significant to study the pharmacological effects and safety of *G. lucidum*. *G. lucidum* plays a role in inhibiting tyrosinase activity and tyrosine-related protein expression, and thus, it may ameliorate pigmentation effect (Zhang *et al.,* 2011). It can also anti-aging by inhibiting ultraviolet B (UVB)-induced matrix metalloproteinase (MMP)-1 expression and increasing procollagen expression (Lee *et al.,* 2018). G. lucidum, a medical fungus, grows in subtropical and temperate climate regions such as Asia, Europe, Africa, and Americas in the wild (Siwulski

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et al., 2015). In Asia, G. lucidum mainly grows in China, Korea, and Japan. In Europe, it is distributed in Sweden, Denmark, and Poland. G. lucidum is distributed in Kenya, Tanzania, and Ghana in Africa (Wang *et al.,* 2012).

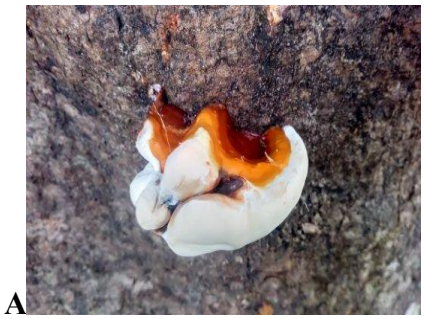
A  **B** 

Plate 2.1: Fruit bodies of *Ganoderma lucidum* (A) = young fruitbody; (B) Matured fruitbody

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In the family Ganodermataceae, the genus Ganoderma contains over 80 species of polypore fungi, many of which are native to tropical areas. They are utilized in conventional Asian treatments and have a wide genetic variety. Because Ganoderma possesses a double-walled basidiospore, it may be distinguished from other polypores. They may also be known as bracket fungi or shelf mushrooms. Large, woody, perennial brackets known as "conks" that are characteristic of Ganoderma are called basidiocarps. With or without a stem, they are lignicolous and leathery. On the trunks of living or dead trees, the fruit bodies generally develop in the shape of fans or hooves. They have truncate, double-walled spores with inner layers that are decorated in shades of yellow to brown. The term "shining" comes from the Greek word ganos, which also means "brightness" and "sheen."

A reddish species of Ganoderma called *Ganoderma lucidum* thrives on dead hardwood trees in Europe and parts of China, where it has a restricted range. Although there are wild populations in California and Utah, they were probably brought there by humans and

naturalized. Oriental medicine frequently uses the woody basidiomycotina mushroom *Ganoderma lucidum* to promote lifespan and well health. The common names for *Ganoderma lucidum* are "*Lingzhi*" in China, "*Youngzhi*" in Korea, *"Reishi*" in Japan, and "*Ganoderma*" in the United States.The annular mushroom known as *Ganoderma lucidum* can be found on a wide range of dead or decaying trees, including deciduous trees like oak, maple, elm, willow, sweet gum, magnolia, and locust, as well as coniferous trees like Larix, Ptea, and Pinus across North and South America, Europe, Asia, and the Middle East (in a temperate rather than subtropical region).

Purification, characterisation, and clinical evaluation of the components that are bioactive are the main areas of attention in current research. The fruiting bodies, spores, and mycelia of *Ganoderma lucidum* have all been chemically examined, and it has been found that they contain a variety of bioactive compounds. It has earned a reputation as the best herbal remedy in the East due to its alleged health advantages and lack of adverse effects. The Therapeutic Compendium and American Herbal Pharmacopoeia now include *Ling Zhi*. Both *Ganoderma sinense ("lingzhi*") and *Ganoderma lucidum ("lingzhi")* are used in Chinese medicine to cure cancer, decrease blood pressure, and boost immunity (Dai *et al.,* 2009; Sun *et al*., 2022).

2.2 Scientific classification

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Kingdom	Fungi
Division	Basidiomycota
Class	Agaricomycetes
Order	Polyporales
Family	Ganodermataceae
Genus	Ganoderma
Specie	*G. lucidum*

2.3 History of genus Ganoderma

The history of the genus Ganoderma and the taxon *Ganoderma lucidum* are intertwined. In 1881, Karsten published the first description of the ganoderma, which only included the species *G. lucidum* (Curtis) Karst. *Boletus lucidus* Curtis (1781) and *Polyporus lucidus* (Curtis) Fr. (1821) were the previous names for this taxon (Karsten 1881). Murrill suspected that the characteristic of the species *P. lucidus*—a laccate (shiny or polished) pileus and stipe—was the cause of Karsten's division because only one species—*G. lucidum*—was included. In order to cover all species having pigmented spores, adherent tubes, and laccate crusted pilei, Patouillard altered Karsten's genus Ganoderma. It led to the classification of 48 species as belonging to the genus Ganoderma in his 1889 monograph. Prior research had only examined European species of Ganoderma, such as *G. lucidum, G. resinaceum* Boud. (1890), and *G. valesiacum* Boud .(1895), until Murrill looked into the genus in North America in 1902. Despite being an inedible mushroom, *ganoderma lucidum* is used to make tea that has a strong flavor. It is largely researched for its potential as medicine.

According to research on *G. lucidum*'s constituents, the mushroom has a 1.8% ash content, 26-28% carbohydrate content, 3-5% crude fat, 59% crude fiber, and 7-8% crude protein (Mau, Lin, and Chen 2001). Numerous compounds found in the mushroom, including triterpenoids, polysaccharides, and peptidoglycans, may be in charge of its therapeutic benefits. Among its many favourable health benefits are its ability to fight cancer, control blood sugar, prevent fatigue and depression, and protect the liver and stomach from harm. It also has antioxidant, antibacterial, and antiviral properties.

Additionally, laccate (shiny) Ganoderma species, often known as "reishi" or "lingzhi," have been recommended as a prophylactic anti-inflammatory treatment or to boost immunity by practitioners of Eastern traditional medicine (Wang *et al*., 2012; Hennicke *et al*., 2016). Reishi has been mentioned as a wonderful herb that improves human health as far back as

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100 B.C. (Cao *et al*., 2012).The reishi industry is quite lucrative, with a global trade value of more than $2.16 billion (Lai *et al*., 2004; Cao *et al*., 2012), and Eastern traditional medicine is also gaining popularity across the globe. Industry for dietary supplements, which includes vitamins, minerals, botanicals, etc., has $109 billion in global sales and is expected to nearly double those numbers by 2020 (Binns *et al*., 2017).

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Every type of fungus has particular dietary needs. The availability of nutrients, particularly carbon and nitrogen sources, is one of several variables that affect the mycelial growth of fungi in nature (Yassen *et al.,* 2013). According to Yassen *et al*. (2013) and Daza *et al*. (2016), nitrogen and carbon are, among other things, the vital and important nutrients for the growth, and reproduction of fungus. By employing mannose and galactose in *Ganoderma lucidum*, Peng *et al*. (2016) examined the impact of mixed carbon sources. Goh *et al.* (2016) studied the influence of different medium components (glucose, sucrose and fructose) on the growth of three different Ganoderma species namely *G. boninense, G. Lingzhi* and *G. australe*.

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Shih *et al.* (2019) also examined the influence of environmental and nutritional conditions on the mycelial growth of *G. boninense.* He reported that glucose and fructose were better sources for the mycelial growth of *Ganoderma boninense*. Pooja and Modi (2018) studied the cultural conditions of biomass production of *Ganoderma lucidum* and concluded that glucose and maltose as carbon sources produced optimal mycelial biomass, while yeast as a nitrogen source was most suitable for biomass production of *Ganoderma lucidum*.

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Xu *et al.* (2018) also concluded yeast and glucose as the best carbon and nitrogen sources for the biomass production of *Ganoderma lucidum* in his study on the improved production of mycelial biomass and ganoderic acid by submerged culture of *Ganoderma lucidum* SB97 using complex media. Krishna *et al.* (2021) studied optimization of growth conditions and

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biological activities of Nepalese *Ganoderma lucidum* and concluded sorbose and tehalose carbon as suitable source for the growth of *Ganoderma lucidum*. Lactose was least suitable for the growth of *Ganoderma lucidum*.

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Sources of materials: The fruiting body of *Ganoderma lucidum* used in this study was collected from a dead decaying wood in the wild at the Faculty of Life Sciences, Uniben, Benin City.

3.2 Medium for tissue culture of *Ganoderma lucidum*

Potato Dextrose Agar (PDA) was used for the cultivation of *Ganoderma lucidum* 200g of Irish potato (*Solanum tuberosum*) was peeled and boiled. The filtrate was then mixed with 20g glucose and 20g agar powder. The filtrate was made up to 100ml with distilled water. The medium was autoclaved at 121°C for 15min and poured inside sterile Petri dishes. The Petri dishes with uncontaminated medium were employed for further experiments.

3.3 Isolates collections and preparation of media

Fruiting bodies of *Ganoderma lucidum* were collected from dead decaying wood were obtained from a dead decaying wood in the wild at the Faculty of Life Sciences, Uniben, Benin City. The harvested fruiting body was washed under running tap water and then rinsed with sterile distilled water. Isolation of mycelium was done from fresh and cleaned basidiocarps. Small pieces of basidiocarps were cut and surface sterilized with 5% sodium hypochloride for 2 min, and then plated onto potato dextrose agar (PDA) medium supplied with traces of Streptomycin sulphate in the Petri dishes. The dishes were then incubated at normal room temperature of 27^0C for six (6) days to allow the mycelia to fully colonize the medium and the pure culture obtained which was used for the work. The developed pure mycelia were sub-cultured and maintained on PDA slants.

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3.4 Carbon Sources

Suitable carbon sources were screened by culturing the mushroom on mushroom minimal media (MMM: 20 g dextrose, 0.5 g $MgSO_4$, 0.46 g KH_2PO_4, 1 g K_2HPO_4, 2 g asparagine, 120 µg thiamine-HCl, 20 g agar, 1,000 *ml* distilled water) supplemented with one of 6 carbon sources (glucose, lactose, starch, fructose, cellulose, sucrose) at a concentration of 2%. The fungi were incubated in the dark for 7 days at 25℃, after which the mycelial growth, density and biomass of mycelia were evaluated. Tests were performed in the basal medium to screen carbon sources favourable for the mycelial growth of selected *G. lucidum* strains, supplemented with each of 6 carbon sources. To screen for the best carbon source favourable for the mycelial growth, each carbon source supplemented with 5 g of peptone to the basal medium at the concentration of 0.1M per 1000 *ml* and mixed thoroughly (Shim *et al.,* 1997). In both cases, the basal medium was adjusted to pH 6 and autoclaved for 15 minutes at 121℃, poured into plates. The inoculated dishes were replicated four times and incubated at 25℃ for 10 days under dark condition. Mycelia growth was measured as describe before.

3.5 Nitrogen sources:

To screen for nitrogen source suitable for the mycelial growth of *G. lucidum* mushrooms were cultured on the mushroom minimal media supplemented with each of 6 nitrogen sources (ammonium sulphate, ammonium nitrate, potassium nitrate, urea, sodium nitrate, and yeast) at a concentra8tion of 0.2%. The basal medium which was used for screening the favourable nitrogen source was made of same additives as those used for carbon sources. Each nitrogen source with 20 g of glucose was added to the basal medium at the concentration of 0.02M (Shim *et al.,* 1997). A 5 mm diameter plug of inoculums of *G. lucidum* was placed in the centre of the Petri dish, which was then incubated in the dark for 10 days at 25℃. The mycelial growth, density and biomass of the mycelia were then examined.

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3.6 Inoculation and incubation of culture media

Each medium prepared with the carbon and nitrogen nutrition was then poured into the petri dish and inoculation of the media was carried out. Four replicate of each medium source was prepared and properly labeled with the names of each sources used. All steps of the experiment were carried out under aseptic conditions in a laminar flow chamber. Inoculated dishes were arranged for mycelia growth in a chamber with air circulation and controlled temperature at 28.0°C ± 0.1°C. For each replication, the mycelial growth was verified by calculating the average of four different measurements of the diameter (in mm) 10days after inoculation. Four replications were made to calculate the mycelial growth diameter average for each treatment.

3.7 Measurement of mycelial growth

The growth rate of *Ganoderma lucidum* on the different Nitrogen and Carbon sources were assessed by measuring the diameter of the mycelial in the petri dishes with a metre ruler. The daily measurement were recorded in milimeters.

3.8 Method to obtain dry weight (biomass)

Exactly ten days after Inoculation, the mycelial of *Ganoderma lucidum* in different carbon and Nitrogen sources were melted in hot water and put in filter paper labelled with their different sources and dried in an oven for 12hours.

3:9 Statistical analysis

All experiments were performed in four replicates to ensure reproducibility. All the data are expressed as mean ± SD (Standard deviation) of four replicates.

The parameters obtained were recorded, which includes the growth rate, daily mycelia growth, mycelial dry weight or biomass, mycelial density and morphology.

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CHAPTER FOUR

RESULTS

The addition of nutrient source, be it carbon or nitrogen source to the substrate promotes the growth of *Ganoderma lucidum* in different dimensions. The result shows mycelial growth, average growth rate, biomass, morphology and density of *Ganoderma lucidum* supplemented with the selected carbon and nitrogen sources provided.

The table below shows the mycelium growth extension of *Ganoderma lucidum* on selected carbon sources from day 1 to day 4. It was observed that *Ganoderma lucidum* fully colonized the petri dish 4 days after inoculation. The mycelial with the highest growth extension as at day 1 was lactose supplemented *Ganoderma lucidum* (11.00±0.82mm), while *Ganoderma lucidum* supplemented with cellulose had the least mycelial growth extension(6.75±0.96mm). In day 3, *Ganoderma lucidum* supplemented with lactose and maltose had same growth extension with cellulose being the least. In day 4 maltose supplemented had the highest growth extension of 66.88±1.44mm and cellulose had the least mycelial extension of 37.88±1.44mm

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Table 4.1. Mycelial growth extension of *Ganoderma lucidum* on different carbon sources.

Carbon Sources	mycelium growth extension (mm) 1DAI	2DAI	3DAI	4DAI
Glucose	10.25 ± 0.5	20.75 ± 1.94	36.38 ± 0.63	61.25 ± 1.19
Lactose	11.00 ± 0.82	22.38 ± 1.44	38.88 ± 2.93	64.88 ± 4.71
Starch	8.50 ± 0.82	19.50 ± 1.22	37.63 ± 1.55	61.88 ± 1.70
Maltose	9.63 ± 0.75	20.63 ± 1.89	38.88 ± 2.02	66.88 ± 1.44
Cellulose	6.75± 0.96	16.13 ± 1.25	27.25 ± 1.55	37.88 ± 1.44
Sucrose	10.13 ± 0.63	21.50 ± 1.68	35.75 ±1.50	55.00 ± 1.87

*Value= means ± standard deviation. DAI- Day after Inoculation.

The result on the table below showed that nitrogen sources foster the growth of *Ganoderma lucidum* as there is significant increase in the mycelium extension across the selected nitrogen sources used with the exception of urea which showed no mycelial growth. From day 1 to day 4, yeast source had the highest mycelial extension at each interval, while control had the least mycelial growth extension.

Table 4.2. Mycelial growth extension of *Ganoderma lucidum* on different nitrogen sources.

Nitrogen Sources	Mycelium growth extension (mm) 1DAI	2DAI	3DAI	4DAI
Ammonium sulphate	6.0 ± 1.58	19.13 ± 2.06	36.25 ±2.63	61.25 ± 3.01
Ammonium nitrate	6.25 ± 1.32	13.13 ± 1.65	23.5 ± 0.91	36.25 ± 0.96
Potassim nitrate	9.38 ± 2.06	21.63 ± 1.49	36.0 ± 1.08	57.0 ± 1.35
Sodium nitrate	6.75 ±1.19	17.25 ± 1.19	29.13 ± 0.95	47.13 ± 0.48
Yeast	11.25 ± 0.65	27.13 ± 1.80	47.0 ± 1.08	69.25 ± 2.33
Control	1.71 ± 0.13	3.06 ± 0.16	4.99 ± 0.24	7.56 ± 0.31
Urea	ND	ND	ND	ND

*Value =means ± standard deviation. Key: DAI- Day after Inoculation ND- Not determined

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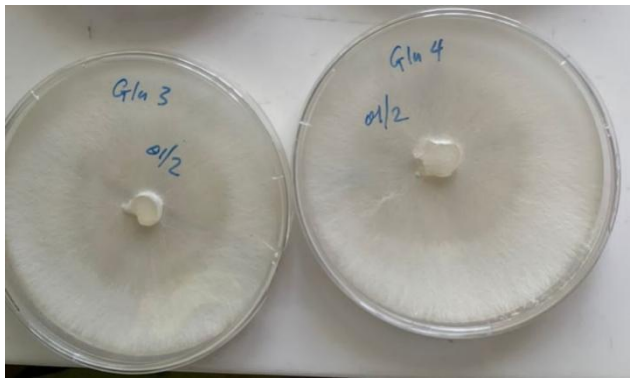

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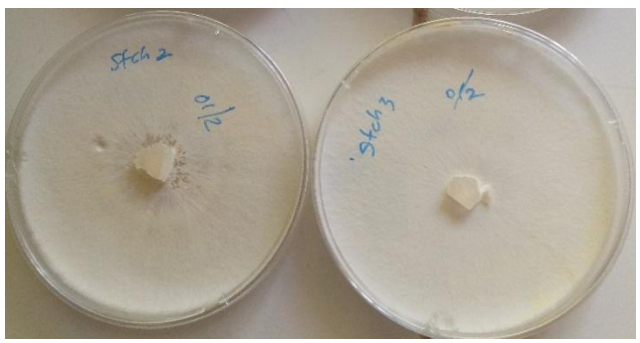

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Plate 4.1: The morphological features of *Ganoderma lucidum* in carbon sources (A. glucose, B. lactose, C. starch)

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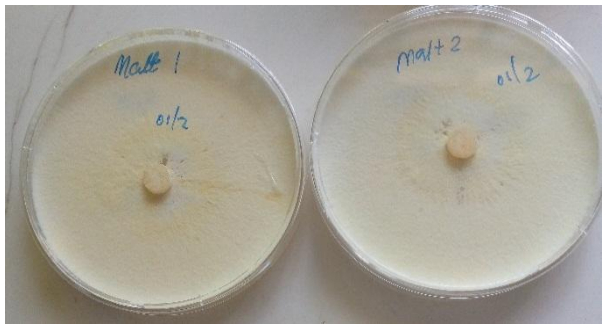

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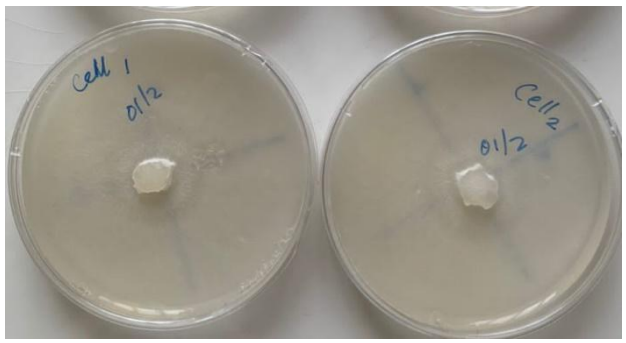

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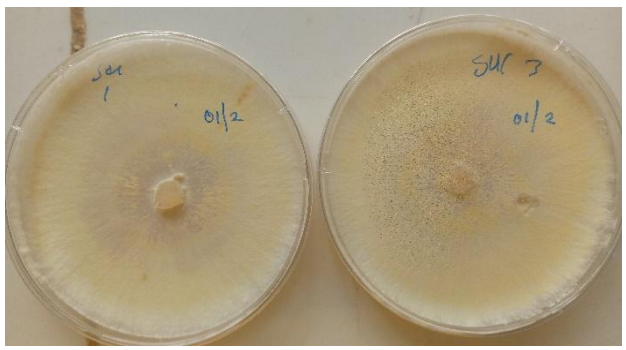

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Plate 4.2: The morphological features of *Ganoderma lucidum* in carbon sources:

A. Maltose B. cellulose C. sucrose

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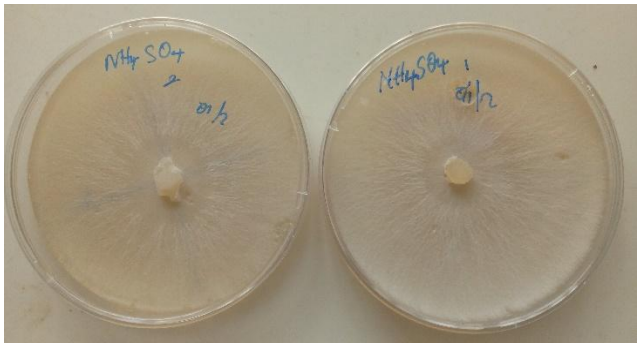

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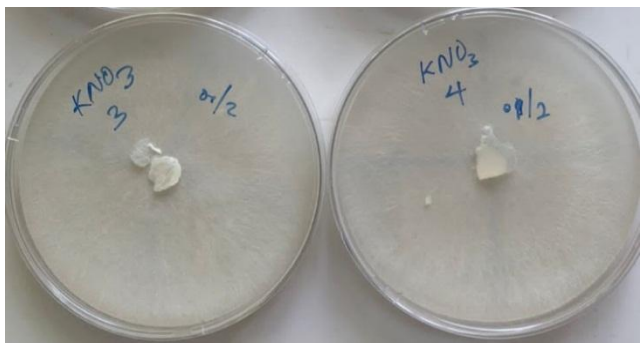

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Plate 4.3: The morphological features of *Ganoderma lucidum* in nitrogen sources A. ammonium sulphate, B. ammonium nitrate, C. potassium nitrate

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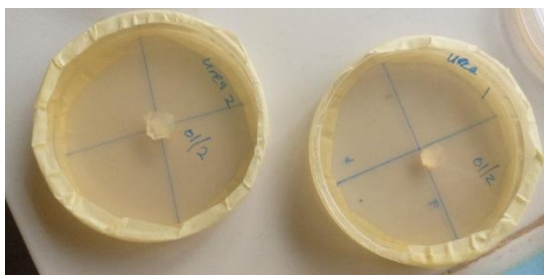

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Plate 4.4: The morphological features of *Ganoderma lucidum* in nitrogen sources: A. sodium nitrate, C. urea and, B. Yeast, D. control.

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The figure below indicates the average growth rate of the mycelium of *Ganoderma lucidum* on the different carbon sources used in this study 4 days after inoculation. The highest growth rate was recorded on maltose (16.7±1.44mm), closely followed by lactose (16.2±4.77mm), starch (15.5±1.70mm) and so on. The least mycelial growth rate was recorded on cellulose (9.5±1.44mm).

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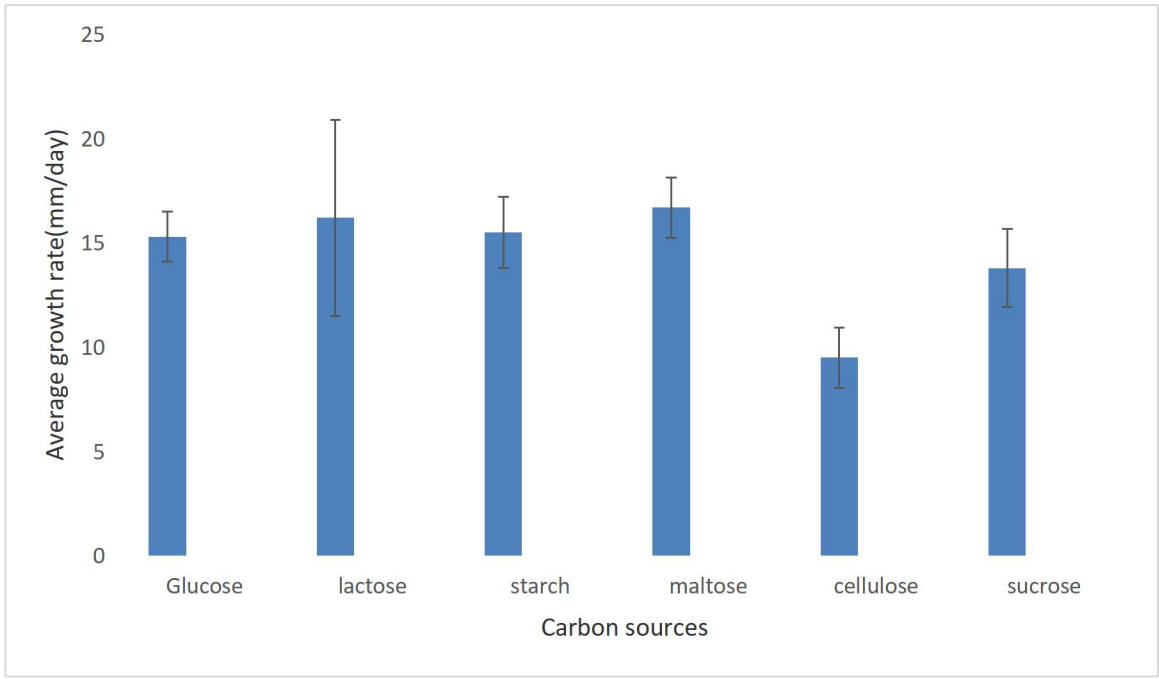

Fig. 1: Average mycelial growth rate of *Ganoderma lucidum* on different carbon sources four days after inoculation.

The figure below indicates the average growth rate of the mycelium of *Ganoderma lucidum* on the different nitrogen sources used in this study 4 days after inoculation . The highest growth rate was recorded in yeast (17.3±0.48mm), closely followed by ammonium sulphate (15.3±3.01mm).The least mycelial growth rate being control (1.9±0.31mm).

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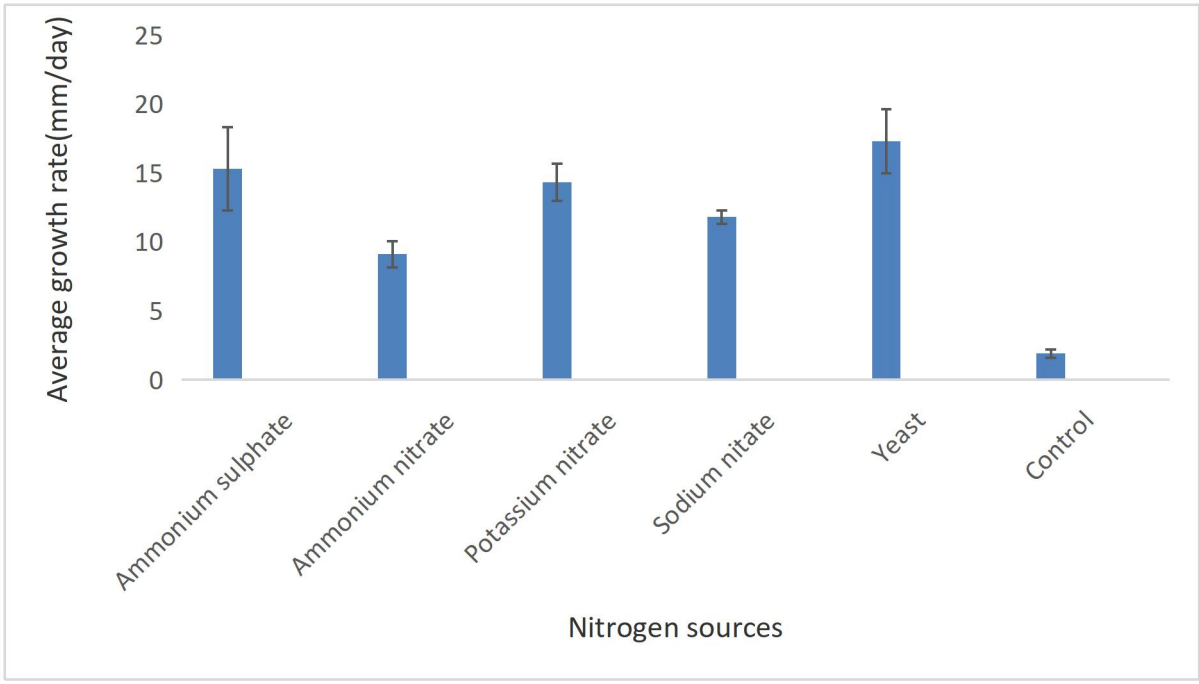

Fig. 2: Average mycelial growth rate of *Ganoderma lucidum* on different nitrogen sources 4 days after inoculation.

The table below shows the recorded biomass of *Ganoderma lucidum* on the selected carbon sources. The result shows that sucrose source had the highest biomass (0.24± 0.05g), closely followed by lactose (0.26±0.04g). The least biomass recorded was glucose (0.15±0.06g) and cellulose (0.15±0.02g) sources respectively.

Table 4.3. Effect of carbon on the biomass (g) of *Ganoderma lucidum*.

Carbon source	Biomass
Glucose	0.15 ± 0.06
Lactose	0.26 ± 0.04
Starch	0.20 ± 0.02
Maltose	0.24 ± 0.05
Cellulose	0.15 ± 0.02
Sucrose	0.29 ± 0.03

*Value= means ±standard deviation.

The table below shows the recorded biomass of *Ganoderma lucidum* on the selected nitrogen sources. The result shows that sodium nitrate source had the highest biomass (0.83±0.16g), closely followed by yeast (0.52±0.03g). The least biomass was recorded in ammonium nitrate (0.16±0.02g).

Table 4.4. The effect of nitrogen sources on the biomass (g) of *Ganoderma lucidum*

Nitrogen sources	Biomass (grams)
Ammonium sulphate	0.19 ± 0.09
Ammonium nitrate	0.16 ± 0.02
Potassium nitrate	0.37 ± 0.18
Sodium nitrate	0.83 ± 0.16
Yeast	0.52 ± 0.03
Control	0.32 ± 0.02

*Value= means± standard deviation

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Table 4.5. Morphological characteristics of *Ganoderma lucidum* on different carbon sources after 10days.

Carbon source	morphology
Glucose	fluffy
Lactose	wooly
Starch	wooly
Maltose	yellow ring wooly
Cellulose	slight fluffy
Sucrose	yellow ring wooly

The table above displays the morphology of *Ganoderma lucidum* on selected carbon species. Most of the mycelia were seen to be wooly with the exception of lactose and cellulose which appeared fluffy.

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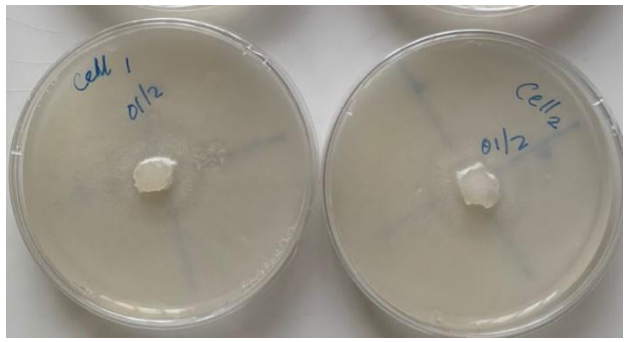

Plate 4.5: Fluffy mycelial growth of *Ganoderma lucidum* on a carbon source.

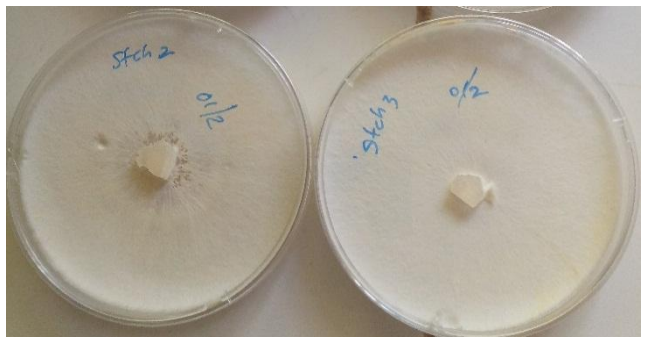

Plate 4.6: Wooly mycelial growth of *Ganoderma lucidum* on carbon source

Table 4.6. Morphological characteristics of *Ganoderma lucidum* on different nitrogen sources after 10days.

Nitrogen sources	Morphology
Ammonium sulphate	fluffy
Ammonium nitrate	fluffy
Potassium nitrate	fluffy
Sodium nitrate	fluffy
Yeast	wooly
Urea	ND
Control	wooly

Key: ND- not determined

The table above shows the morphology of *Ganoderma lucidum* on different nitrogen sources. Most of the nitrogen sources mycelia appeared fluffy with the exception of yeast and control being wooly. Urea source morphology was not determined.

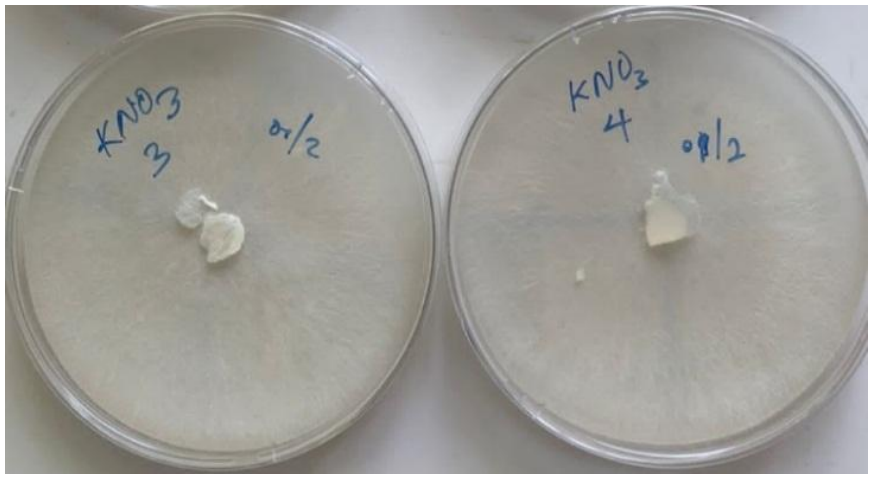

Plate 4.7: The fluffy mycelia of *Ganoderma lucidum* in nitrogen source.

Plate 4.8: The wooly mycelia of *Ganoderma lucidum* in nitrogen source.

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Table 4.7. Density of *Ganoderma lucidum* on different carbon sources

Carbon source	Density
Glucose	+
Lactose	+
Starch	++
Maltose	+++
Cellulose	+
Sucrose	+++

Key: + = slightly dense , ++ = dense, +++ = very dense

The table above shows the density pattern of *Ganoderma lucidum* on different carbon sources. The density pattern of maltose and sucrose sources was very dense. Starch was dense, while glucose, lactose and cellulose was slightly dense.

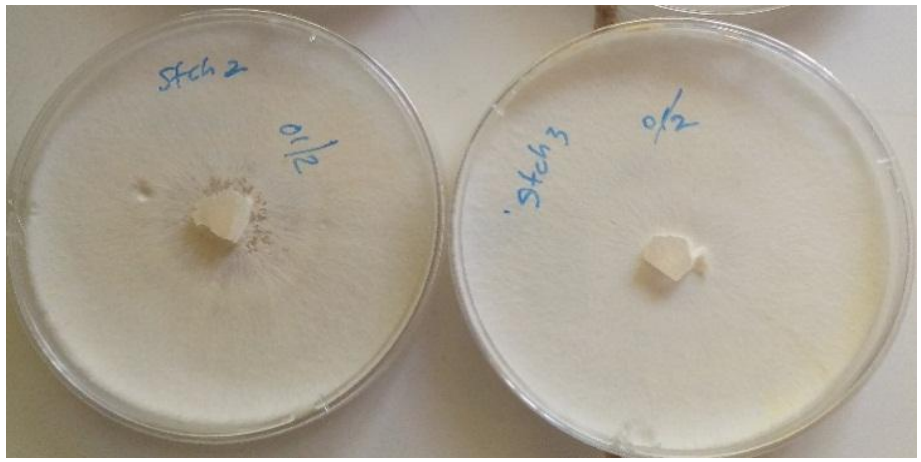

Plate 4.9: Dense pattern of *Ganoderma lucidum* in carbon source.

Plate 4.10: Very dense pattern of *Ganoderma lucidum* in carbon source

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Table 4.8. Density of *Ganoderma lucidum* in different nitrogen sources

Nitrogen source	Density
Ammonium sulphate	+
Ammonium nitrate	+
Potassium nitrate	+
Sodium nitrate	++
Yeast	+++
Urea	ND
Control	+++

Key: +=slightly dense, ++=dense , +++=very dense, ND -not determined.

The table above shows the density pattern of *Ganoderma lucidum* on different nitrogen sources. The density pattern of yeast source and control was very dense. Sodium nitrate was dense, while ammonium sulphate, ammonium nitrate and potassium nitrate was slightly dense.

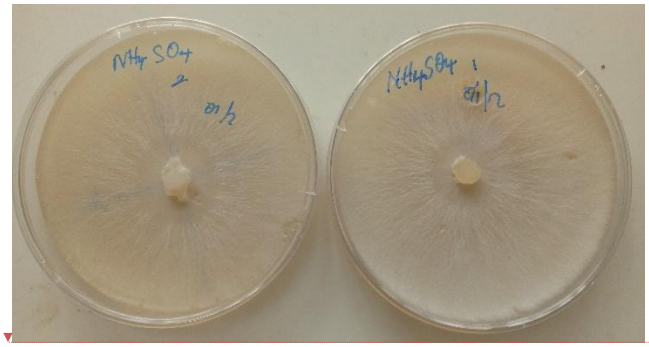

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Plate 4.11: Slightly dense pattern of *Ganoderma lucidum* in nitrogen source.

Plate 4.12: Dense pattern of *Ganoderma lucidum* in nitrogen source.

Plate 4.13: Very dense pattern of *Ganoderma lucidum* in nitrogen source.

CHAPTER FIVE

DISCUSSION

Carbon and nitrogen sources have been confirmed to supplement the growth of mushrooms. The need to identify the most effective carbon and nitrogen sources led to this study. The data obtained demonstrated the effect of carbon and nitrogen sources on the growth of *Ganoderma lucidum.* Research findings have revealed that disaccharides undergo hydrolysis into monosaccharides before entering various metabolic pathways. The range of carbon sources utilized for the mycelial growth of different fungi is broad. Monosaccharides, disaccharides, and polysaccharides serve as suitable carbon sources, with fructose and glucose among monosaccharides, and maltose among disaccharides, proving most effective for *Auricularia auricula (*Luna *et al*., 2004*)*.

Monosaccharides play a crucial role in fungal carbohydrate metabolism. Complex carbohydrates are typically broken down into monosaccharides or their derivatives before entering different metabolic pathways. These sugars exist freely in fungal structures and as components of oligosaccharides and polysaccharides. Many fungi hydrolyze sucrose into glucose and fructose, assimilating it through hydrolytic pathways.

According to the experimental results, there is a significant difference in the mycelial growth extension of *Ganoderma lucidum* on different carbon sources. Table 4.1 demonstrates a steady increase in mycelial extension from days 1–4. On day 1, lactose recorded the highest mycelial extension, closely followed by glucose, while cellulose exhibited the least growth. By day 4, maltose had achieved the highest mycelial growth extension, closely followed by lactose. Starch and glucose supported moderate mycelial growth, whereas cellulose exhibited minimal development. The growth rate of *Ganoderma lucidum* on different carbon sources (Fig. 2) revealed that maltose had the highest growth rate, followed by lactose, while starch and glucose had moderate growth rates. Cellulose showed the least growth.

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This outcome aligns with the findings of Krishna *et al.* (2021), who reported that *Ganoderma lucidum* hardly utilized cellulose and lactose as carbon sources. Chandana *et al.* (2008) noted that sucrose exhibited moderate mycelial development, whereas lactose and glucose supported lower growth levels. Differences in fungal growth patterns may be attributed to variations in fungal strains, culture conditions, and environmental factors, including temperature. Pooja *et al.* (2018) reported that maltose produced an excellent to moderate amount of mycelial biomass in Ganoderma lucidum. Akpaja and Okhuoya (2017) also stated that lactose, starch, and cellulose enhanced the mycelial growth of *Daldinia concentrica*.

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The biomass production of *Ganoderma lucidum* on selected carbon sources is shown in Table 4.3. Among the carbon sources tested, sucrose significantly supported the highest biomass production (dry weight), followed by maltose and starch. Conversely, glucose and cellulose resulted in the lowest biomass production. This contradicts the findings of Pooja *et al*. (2018), who reported that glucose had the highest biomass production, followed by maltose. *Aysun et al*. (2016) found that all carbon sources encouraged the growth of *Macrolepiota procera* mycelial biomass, with dextrose being the most effective, while sucrose, lactose, and glucose showed no statistically significant differences. The variations in results may be attributed to differences in *Ganoderma lucidum* strains or environmental influences.

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Nitrogen is an essential element for fungi, serving structural and functional roles. Chitin, the main component of fungal cell walls, is a linear polymer of D-glucosamine. Similarly, proteins, the basis of protoplasm, are nitrogenous substances. Other nitrogen-containing compounds include purines, pyrimidines, certain vitamins, and essential metabolites. While fungi can utilize both organic and inorganic nitrogen sources, their metabolic potential varies. Some fungi use atmospheric nitrogen, many utilize nitrate nitrogen, and an even greater number depend on ammonium nitrogen. Generally, ammonium nitrogen is assimilated (Lilly and Barnett, 1951).

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The experimental results (Table 4.2) revealed a daily increase in *Ganoderma lucidum* growth when exposed to nitrogen sources. Yeast and ammonium sulfate supported the best mycelial growth. Excluding urea, which failed to support mycelial development, potassium nitrate, sodium nitrate, and ammonium nitrate exhibited moderate growth. These findings align with those of Chandana *et al.* (2008), who found that Ganoderma lucidum did not develop mycelia when exposed to urea but showed moderate growth with potassium nitrate. Similarly, Shih *et al.* (2009) reported that *Ganoderma boninense* did not develop mycelia on urea. Woo-sik *et al.* (2009) identified yeast and maltose extract as optimal nitrogen sources for cultivating *Ganoderma applanatum*. These findings suggest that *Ganoderma lucidum* thrives better on organic nitrogen sources (e.g., yeast) than inorganic nitrogen sources.

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Table 4.4 presents the biomass production of *Ganoderma lucidum* on selected nitrogen sources. Sodium nitrate yielded the highest mycelial biomass production, followed by yeast. Potassium nitrate resulted in moderate biomass production, while ammonium sulfate and ammonium nitrate produced the least biomass. Aysun *et al.* (2018) reported that ammonium nitrate and yeast extract resulted in the lowest biomass production in *Macrolepiota procera*. These findings highlight species-specific variations in optimal nitrogen sources for mycelial biomass production.

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CONCLUSION

Nutritional factors have been noted to be responsible for the growth and development of fungi. From this study, it is concluded that the most suitable carbon source for the growth of *Ganoderma lucidum* is maltose and lactose, while the most suitable carbon source for the biomass production of *Ganoderma lucidum* is sucrose and lactose. Yeast and ammonium sulphate are the most suitable nitrogen sources for the growth of *Ganoderma lucidum*, while yeast is the most suitable nitrogen source for the biomass production of *Ganoderma lucidum.*

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