EFFECTS OF FORMULATED CULTURE MEDIA ON THE VEGETATIVE GROWTH OF *Volvariella volvacea* (Bull. Ex Fr.) Sing.: USE OF SELECTED TUBERS FOR MEDIA FORMATION

BY

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

SEPTEMBER, 2023

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (BSc.) IN PLANT BIOLOGY AND BIOTECHNOLOGY

SEPTEMBER, 2023

CERTIFICATION

We certify that this research work was carried out by Oluwatobiloba Efe Ediagbonya of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Signed: ---------- ----------

Prof E. O. Akpaja Date

(Project Supervisor)

Signed: ---------- ----------

Prof E. D. Vwioko Date

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, my parents and my siblings.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to God almighty for his guidance and protection all through my life, I also want to thank my supervisor Prof. E. O. Akpaja, he helped me immensely at all times in my research and writing of this project. I would like to thank the Head of Department Prof. D. E. Vwioko and the entire staff of the great department of Plant Biology and Biotechnology. I wish to express my sincere gratitude to my parents Mr. and Mrs. Ediagbonya for their support throughout my course of study. I also wish to thank Mrs. Adeoti Gabriel, Mrs. Adeyinka Adejumo, Mrs. Stella Ediagbonya, Mr. Efosa Ediagbonya and Miss Omwenyeke Ediagbonya for always being there for me in my time of need. I want to thank my friends Ariyo Oluwafisayo, Adeyemi Adegboyega, Dare Pearl, Idon Godswill, Ekejiuba Winnifred, Afia Blessing, Nwabuidike Chidera, Ifemeje Jennifer, Onyenanu Rejoice, Usunobun Bartholomew, Akharagbon Vicnaomi and Ijeboi Sefi for their support throughout the cause of this research.

Special thanks to my sisters Kofoworola Ediagbonya and Moyinoluwa Ediagbonya, you are the best siblings i could have asked for.

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ABSTRACT

The purpose of this study was to determine the effect of selected tuber-based media on the vegetative growth of the fungus *Volvariella volvacea* (Bull. Ex Fr.) Sing. Pure culture of *V. volvacea* was gotten from the African Centre for Mushroom Research and Technology Innovations, University of Benin, Benin City, Nigeria. The pure culture was expanded aseptically on potato dextrose agar and stored until utilized. This evaluation was carried out by puncturing the pure culture with already sterilized cork borer then the inoculum was collected with an already sterilized loop and inoculated at the center of the formulated culture. The culture media were subjected to different orientations. This procedure was carried out for the five replicates of each formulated medium after which they were taken to the incubation room. The growth biometry was taken after 24 hours for 6 days. Potato dextrose agar recorded the highest mycelia growth on the different plate orientations while cocoyam dextrose agar and white yam dextrose agar recorded the lowest mycelia growth on plates with an upward orientation and plates with a downward orientation respectively. From this study it has been observed that the vegetative growth of *Volvariella volvacea* is influenced by the plate orientation and the tuber used to prepare the culture media. This study has shown that sweet potato and water yam can be considered as viable alternatives for potato in the preparation of culture media for the fungus.

Key words: Fungi, Plate orientation, Vegetative growth.

CHAPTER ONE

INTRODUCTION

Mushroom is often referred to as the "queen of vegetables" (Ponmurugan *et al.,* 2007). It is a macro fungus which has distinctive fruiting bodies that can either be epigeous or hypogenous. The lack of chlorophyll in mushrooms prevents them from producing their own food, therefore they must rely on dead and decayed organisms which makes them saprophytes (Nongthobam *et al*., 2021). The "mushroom capital of the world," Kennett Square, Pennsylvania, is where mushroom growing first began in America in 1896. Here, button mushrooms account for over half of the country's production (Roy *et al.*, 2014). The initial stage of growing mushrooms involves picking out the specific kind of mushroom you wish to cultivate. Then getting a culture of the mushroom, various types of mushrooms need different conditions such as the substrate they grow on, the temperature, the level of moisture, and other surrounding conditions. Agar plates; a standard medium for cultivating and isolating mushroom mycelium are then prepared and sterilized. Under aseptic conditions a tiny portion of the mushroom culture is placed on the agar plate and the mycelium is allowed to grow and expand on the agar plate. Once a well-developed mycelium culture is attained on the agar, a grain based spawn is prepared by transferring a small piece of mycelium is to a sterilized grain substrate, such as guinea corn. This grain will serve as the basis for the spawn. The grain-filled containers is sealed and the mycelium allowed to colonize the grains completely. This step usually takes a few weeks, during which the mycelium spreads and forms a network throughout the grain. When the grain has been fully colonized by the mycelium, the spawn is ready. The next step is to prepare the on which the mushrooms will develop. Mushrooms are primarily grown on waste products including sawdust, compostable materials, straw, and garbage (Gbolagade, 2006), and bagged depending on the preferred weight and then, sterilized or

pasteurized as needed. The grain spawn is then incorporated into substrate material and distributed evenly to ensure the mycelia spread throughout the substrate. The inoculated substrate is placed in a controlled environment with appropriate temperature and humidity till the mycelium colonizes the substrate over a period of time, typically a few weeks. Once the substrate is fully colonized, mushroom fruiting can be induced by opening the bags slightly or creating small holes on the sides of the bags. The cultivation of mushrooms depends on a variety of variables, including temperature, humidity, and the sterility of the substrates. From a nutraceutical perspective, mushrooms are also significant because they possess a variety of compounds, including tocopherols, unsaturated fatty acids, carotenoids, ascorbic acid and phenolic compounds (Ahlawat *et al.,* 2016). China produces over half of all grown mushrooms, and 1.4 billion people consume approximately 2.7 kilogram of mushroom per year per person (Rzymski *et al.*, 2017).

Because mushrooms contain all the different kinds of amino acids required by the human body and have a lower calorific value than other foods, they are suitable for people with heart disease (Koyyalamudi *et al*., 2009). The high protein, carbohydrate, essential mineral content, and low energy levels are comparable to meat, eggs, and milk (Colmenares-Cruz *et al*., 2017). In terms of dry weight, mushrooms are noted for having significant levels of vital minerals like phosphorus, potassium, calcium, iron, and copper as well as 56% carbohydrates, 30% proteins, 10% ash, and 2% fat. They also contain significant amounts of vitamins B and D (Nongthobam *et al*., 2021).

Mycophagists are those who gather mushrooms for human consumption, and the process of collecting them is known as mushroom hunting or mushrooming. Mushrooms are primarily utilized in a variety of cuisines across the world, and the majority of m ushrooms sold in supermarkets are commercially farmed on mushroom farms. A number of mushroom species are poisonous even though they may look like the certain edible species. Eating

mushrooms taken from the wild is risky and should only be undertaken by individuals knowledgeable in mushroom identification. Separating edible from poisonous species necessitates close attention to detail; there is no one attribute that can distinguish between edible and poisonous mushrooms.

Volvariella comes in a number of different species that are widespread around the world. These include *V. bombycine, V. coesiotincta, V. gloiocephala, V. hypopithys, V. iranica, V. jamaicensis, V. lepiotospora, V. peckii, V.sathei, V. speciosa, V.surrecta,* and *V.volvacea*. The *Volvariella volvacea* species is one of these widely employed for culinary purposes (Roy *et al.,* 2014). The mushroom is popularly known as paddy straw mushroom because it thrives on paddy straw, straw mushroom, or Chinese mushroom since artificial cultivation of this mushroom started in China (Chang, 1969). It also goes by the name "Nanhua mushroom" or "tributary mushroom". The Nanhua Temple of Chaohsi in China's northern Kwangtung Province is where the word Nanhua originated. (Roy *et al.,* 2014). *Volvariella volvacea,* is a quick-growing edible mushroom that is grown all over the world (Bisoyi and Chatterjee, 2020). Being the third largest farmed mushroom in the world, *Volvariella volvacea* is well renowned for its pleasing taste and flavorful nature (Thiribhuvanamala *et al*., 2012) and is a rich source of protein, fibers (chitin), vitamins (large amount of vitamin C, and also all water-soluble vitamins like riboflavin, biotin and thiamine), fats (5.7%), carbohydrates (56.8%), amino acids (all essential amino acids like alanine, arginine, glycine, serine etc.), unsaturated fatty acids, essential minerals (potassium, sodium and phosphorus) and has low calories values (Roy *et al.,* 2014). Fresh paddy straw mushrooms have a water content of roughly 90%, on a dry weight basis, it has 30-43% crude protein, 1-6% fat, 12-48% carbs, 4-10% crude fiber, and 5.13% ash on a dry weight basis. *V. volvacea* contains about 1.2 and 3.3 mg of thiamine and riboflavin per 100 g respectively (Maurya *et al.,* 2016). *V. volvacea*

is a fungus native to the tropics and subtropics and may be grown both indoors and outdoors. Despite being grown for 300 years, the paddy straw mushroom industry's growth has been severely constrained by a number of issues related to cultivation techniques. When compared to other cultivated mushrooms, the paddy straw mushroom's C: N ratio is quite high, needing to be in the ratio 40 to 60 (Bisoyi and Chatterjee, 2020). It is also an excellent candidate for adoption in year-round mushroom growing due to the high temperature necessities of 26°C to 30°C for mycelium development and 34°C to 37°C for fructification (Biswas and Layak, 2014). *Volvariella volvacea* as most edible mushrooms, are heterotrophic, they therefore have to get all the nutritive elements from the substrate. Its ability to break down cellulosic materials, the paddy straw mushroom may grow on a variety of cellulosic substrates, including cotton waste, sugar cane bagasse, rice straw, and stubble (Reyes, 2000).

Kingdom: Fungi

Phylum: Basidiomycota

Class: Agaricomycetes

Order: Agaricales

Family: Pluteaceae

Genus: *Volvariella*

Species *V. volvacea* (Bull. Ex Fr.) Sing.

Scientific classification of *Volvariella volvacea*

Paddy straw mushrooms, a relatively affordable source of food which can be produced in an array of conditions, may assist in combating malnutrition in developing nations (Kaushich *et al*., 2018).

Paddy straw mushrooms contains protein, carbohydrates, and fiber and are low in calories and fat, making them a good option for anyone seeking to lose weight or eat a heart-healthy diet (Kaushich *et al*., 2018), with a 100-gram meal delivering around 25% of the daily recommended intake of protein (Roy *et al.,* 2014). Additionally, it contains a sizable number of minerals and vitamins such folic acid, thiamine, riboflavin, biotin, pentathonic acids, and vitamin B12 (Maurya *et al.,* 2016). The mushroom also aids environmental sustainability as they may be grown on agricultural waste like rice straw that could have been thrown away. Paddy straw mushrooms also increase soil quality, which helps to prevent erosion and improve water quality (Kaushich *et al*., 2018).

Numerous animal and human research have been conducted to examine the health advantages of *V. volvacea*. It has been demonstrated that the mushroom contains anti-inflammatory effects as a result of Ergothioneine, a potent antioxidant that can help shield cells from harm (Roy *et al.,* 2014). Paddy straw mushrooms are a good source of antioxidants, which can aid in preventing cell deterioration brought on by free radicals (Zahid *et al.,* 2019). The mushroom's antioxidant characteristics make it a promising dietary supplement for the prevention and treatment of a wide range of ailments, including cancer, cardiovascular disease, and neurological disorders (Sudha *et al*., 2008). Also, under certain conditions the immune system, which is in charge of fending off illness, can be harmed by stress, a bad diet, and some medicines may lead to the damage of the immune system but the mushroom have compounds that may aid to strengthen the immune system (Zahid *et al.,* 2019). *Volvariella volvacea* is also a good source of potassium which is an essential mineral for heart health (Zahid *et al.,* 2019).

Ahlawat *et al.,* (2011) reported that their study, which assessed different strains of *Volvariella volvacea* for their production potential, disease resistance, and insect-pest tolerance utilizing composted substrate of paddy straw and cotton mill wastes. The reported that the strain OE-274

had an early harvest time, at 11.25 to 11.50 days after spawning. It also had the greatest yield followed by strain OE-272. In regards to disease/insect-pest resistance, strains OE-274 and OE-55-08 were the most resistant, while strain OE-272 was the most prone to insect-pest invasion. They concluded that strains OE-274 and OE-55-08 are the most viable strains for cultivation as they offer a substantial yield, a quick time to harvest, and strong resistance to diseases.

Belewu and Belewu, (2005) worked on a simple way of cultivating *Volvariella volvacea* on banana leaves. The authors of the study discovered that utilizing banana leaves as substrate greatly increased the amount of mushrooms produced compared to using alternative substrates, including paddy straw or sawdust. The mushrooms also grew better in the banana leaves, creating fruit bodies that were bigger and more uniform. The authors reached the conclusion that the procedure they outlined in their research is an easy and effective technique to grow mushrooms on banana leaves. The strategy employed for this work is appropriate for small-scale growers and easily suits local conditions.

Obodai and Odamtten, (2012) studied the mycobiota, physical and organic content of five waste products from agriculture utilized in the cultivation of the mushroom Volvariella volvacea (Bull. ex Fr.) Sing. The results revealed that the five wastes differed significantly in terms of their physical and organic contents. At 78.9% banana leaves had the greatest moisture content while cocoa shells had the lowest possible at 72.3%. Banana leaves also had the greatest ash level (12.8%), while cocoa shells were the lowest (8.5%) maize stover (10.6%), oil palm pericarp (9.9%) and rice straw (9.1%) had values in between respectively. Cocoa shells had the highest C/N ratio (24.5), followed by rice straw (21.7), maize stover (20.3), oil palm pericarp (17.5) and banana leaves had the lowest C/N ratio (14.3). The mycobiota of the five wastes also differed significantly and with 35 species, banana leaves had the highest fungal diversity. The authors concluded that

the physical and organic contents of the five wastes were appropriate for V. volvacea production. However, the mycobiota of the wastes differed significantly, and additional research is needed to establish the ideal waste for *Volvariella volvacea* cultivation.

Ukoima *et al*., (2009) investigated the growth of Volvariella volvacea on diverse agricultural wastes in Obubra, Cross River State, Nigeria. The study's goal was to find the best agricultural waste for growing *Volvariella volvacea* while reducing the environmental impact of farm waste burning. The researchers employed three distinct farm wastes: palm fiber, rice husk, and sawdust and they made the medium by combining agricultural manure with water and fermenting it for two weeks. The medium was then injected with the mushroom spawn and incubated at 28°C for 30 days. The study revealed that palm fiber is the best agricultural waste for cultivating straw mushrooms in Obubra, Cross River State, Nigeria. It was also determined that palm fiber is the best agricultural waste for cultivating straw mushrooms in the vicinity and they advised farmers in the region to cultivate straw mushrooms with palm fiber, since it is a valuable crop that can assist to lessen the environmental effect of farm waste.

Bao *et al.,* (2013) worked on the sequencing and analysis of the straw mushroom's genome (Volvariella volvacea). The straw mushroom genome was sequenced by employing a mix of Roche 454 GS FLX and Illumina Solexa sequencing technologies. The genome is 35.7 Mb and includes 11,084 predicted gene models. They researchers discovered that the straw mushroom genome has several genes involved in the decomposition of cellulose, hemicellulose, and pectin and the capacity to break down agricultural waste and produce mushrooms is attributed to these genes. The researchers also discovered that the straw mushroom genome has a number of genes engaged in the mating type system and how it responds to cold temperatures. The genome sequencing and study of the straw mushroom has revealed fresh information on the biology of this essential fungus.

The findings of this study will be valuable in increasing straw mushroom farming and generating new applications for this fungus in medicine and biotechnology.

Nannapaneni and Subbiah, (2016) researched the effects of organic nitrogen supplementation on the yield of Volvariella volvacea (Bull. Ex Fr.) Sing. Horse gram seed powder, tamarind seed powder, and silkworm pupal waste powder were all examined as organic nitrogen supplements. At 2%, 4%, and 6% concentrations, the supplements were applied to paddy straw-based mushroom beds and the results revealed that 2% horse gram seed powder and tamarind seed powder supplementation improved mushroom production as a maximum yield of 1226.3 g/5 kg of substrate was obtained with 2% horse gram and tamarind seed powder supplementation (1:1).They stated that the probable reasons why 2% horse gram seed powder and tamarind seed powder supplementation boosted mushroom yield are that the supplements are high in organic nitrogen, which is critical for mushroom development, they also include elements that help mushrooms develop, such as phosphorus, potassium, and calcium and also slowed the growth of pollutants like *Coprinus* spp. The addition of silkworm pupal waste powder at any quantity did not increase mushroom output as it encouraged the growth of *Coprinu*s spp., a fungus that is regarded as a contaminant. They came to the conclusion that combining tamarind seed powder and horse gram seed powder increased the production of V. volvacea strain CBE TNAU 1505.

Chen *et al.,* (2013) investigated the composition and expression of genes that codes for carbohydrate-active enzymes (CAZymes) in *Volvariella volvacea*. The researchers initially sequenced *V. volvacea*'s genome and discovered 285 genes that encode CAZyme. This is the seventh highest number of CAZyme genes among 15 biomass-degrading fungus with sequenced genomes. They next examined the expression patterns of these CAZyme genes in three strains of *V. volvacea*: two homokaryotic strain and a heterokaryotic strain. They discovered that 239

CAZyme genes were expressed in all three strains, independent of growing medium. However, the expression levels of several genes were much greater in the heterokaryotic strain. Additionally, the CAZyme gene composition of *V. volvacea* was compared to that of other fungi and they discovered that *V. volvacea* has a high concentration of members of the glycoside hydrolase families GH10 (hemicellulose degradation) and GH43 (hemicellulose and pectin degradation), as well as the lyase families PL1, PL3, and PL4 (pectin degradation). *V. volvacea*, on the other hand, lacks the families GH5b, GH11, GH26, GH62, GH93, GH115, GH105, GH9, GH53, GH32, GH74, and CE12 and understanding the CAZyme system of V. volvacea lays the groundwork for the generation of better strains of *V. volvacea* with higher enzyme production capacities. *V. volvacea* has a large number and diversity of CAZyme genes, indicating that it is well-adapted to degrade lignocellulose, a significant component of straw. This research has significant implications relating to the biotechnological application of *V. volvace*a as it has the potential to provide enzymes for the manufacture of biofuels and other bio products.

Thiribhuvanamala, G. *et al*., (2021) reported that their study entitled "Strategic Approaches for Outdoor Cultivation of Paddy Straw Mushroom (*Volvariella volvacea*) as Intercrop Under different Cropping Systems" with the aim of investigating the viability of growing paddy straw mushrooms as an intercrop in various cropping systems in India. They discovered that paddy straw mushrooms may be grown successfully as an intercrop in maize, banana, coconut, and coconut plus banana cropping systems with the strain Vv-19-06 of Volvariella volvacea performing best in all the cropping systems tested and the bioefficiency of the mushroom varied between 19.4% and 20.6% and the B:C ratio was satisfactory. The authors recommended a number strategic approaches for cultivating paddy straw mushrooms outdoors as an intercrop. Firstly, choosing the right Volvariella volvacea strain to ensure both high bio-efficiency and yield then properly

preparing the substrate for successful growth. The paddy straw needs to be soaked in cold water with 3% lime for 6 hours, then drained to achieve 60% moisture before using it to create the beds. These beds ought to be placed in a shaded area with effective ventilation. It's important to carefully monitor and adjust the temperature and humidity of the beds. Throughout the cropping period, the beds should be watered twice daily and harvesting the mushrooms should occur once they've reached full maturity. According to the authors, cultivating paddy straw mushroom should be encouraged as an intercrop in rice-growing areas since it offers farmers with additional revenue.

The aim of the study was to evaluate the effect of different tuber formulated culture media on the vegetative growth of *Volvariella volvacea* with a view to source for locally available substitutes for potato which is the major carbohydrate source in the medium.

CHAPTER TWO

MATERIALS AND METHOD

2.1 Source of Inoculum

Pure culture of the mushroom was obtained from the African Centre for Mushroom Research and Technology Innovations, University of Benin and expanded on potato dextrose agar. Thereafter it was stored in cupboards in the incubation room for seven days, several sub cultures were made until a pure culture was obtained and kept until utilized (Tudses, 2016).

2.2 Sample Collection

Cocoyam (*Colocasia esculenta*), Irish potato (*Solanum tuberosum*), Sweet potato (*Ipomoea batatas*), Water yam (*Dioscorea alata*) and White yam (*Dioscorea rotundata*) were obtained from Uselu market, Benin City, Edo State, Nigeria.

2.3 Preparation of Tuber-Based Culture Media

The media was prepared using the variety of tubers collected. Each tuber was weighed at 40g and boiled for an hour in 200 ml of water. The extract was filtered, stored in five different bottles and allowed to cool, then 4g of agar agar and 4g of dextrose sugar was added to each bottle (Fasidi, 1998). To stop bacterial growth, 0.8 ml of antibiotics (a combination of streptomycin and penicillin) was added.

2.4 Sterilization and Pouring of Petri dishes

The medium was sterilized in the autoclave for 1 hour. As the pressure increases, the temperature increases to 121^{o}C (Tudses, 2016). The temperature and pressure gradually decreases over time, and the autoclave is opened when the pressure reaches zero then the bottles are brought out. The bottles are placed in the laminar flow chamber to cool after being sterilized in the autoclave. The

petri dishes were sterilized by thoroughly wiping the petri dish's body with cotton wool filled with methylated spirit in one direction until the entire body was clean. The medium is then poured onto the plate and given time to cool and gel.

2.5 Inoculation and Incubation

Before inoculation, the laminar flow chamber is wiped with cotton wool filled with methylated spirit in one direction till the entire work space is covered, this process is replicated for the hands before working to prevent contaminating the entire work. The cork borer and loop used during inoculation is first sterilized by introducing it in fire till it turns red and then placing them in a conical flask containing methylated spirit. The pure culture is punctured with already sterilized cork borer and then the punctured part (inoculum) with a diameter of 5mm was carefully collected with the already sterilized loop and inoculated at the center of the culture media, after that the petri dish was covered round with a paper tape to seal the petri dish and labeled appropriately with a marker and the petri dishes were transferred to the cupboards in the incubation room. The plates were subjected to different directional orientation with some facing upwards and some facing downwards and the mycelia extension measurements were recorded after 24 hours for 6 days.

2.6 Daily Mycelia Extension

The measurements were taken by drawing two lines that intersects at 90^{o} on the center of the other side of the 90mm petri dish. The daily mycelia extension was taken along the two perpendicular lines using a meter rule (Ravimannan *et al*., 2014).

2.7 Mycelia Growth Rate

The mycelia growth rate was calculated in order to analyze the impact of culture media and plate orientation on the rate of growth of *Volvariella volvacea*.

$$\text{Mycelia Growth Rate} = \frac{\text{(Final Mycelia Length - Initial Mycelia Length)}}{\text{Time}}$$

2.8 Mycelia Density Rating

This measurement was taken visually and keys allocated to show how dense the mycelia grew in the culture media (Fasidi, 1998).

5 =Very Dense

2=Dense

3= Medium

4= Sparse

1= Very Sparse.

2.9 Cultural Characteristics

Based on the visual appearance of the mycelia formed, the characteristics of the culture was determined to be either fluffy, tomentose, appressed or rhizomorphic.

2.10 Biomass

Whatman filter paper was air dried in an air drier at 70^0c for 24 hours and weighed thereafter, the grown mycelium on the media is removed from the petri dish and placed in a jar containing water and placed in a pot filled with water to boil till the medium melts and the mycelium is removed from the jar using forceps, it is then rinsed in warm water to ensure only the mycelium is left, thereafter, It is placed in the filter paper, labeled and air-dried for another 24 hours and the weight recorded.

2.11 Photograph

The Petri dish was photographed on a blue card board paper and a ruler just below it to show the exact length of the petri dish.

2.12 Analysis of Data

The results were expressed as mean values ± standard error. The data obtained from the study were subjected to Descriptive Statistics using Microsoft Office Excel 2013.

CHAPTER THREE

RESULTS

Volvariella volvacea colonized all of the selected mediums and plate orientations tested. The fungus' mycelia differed depending on the tuber-based growth medium and plate orientation. Although the majority of the treatment promoted speedy colonization of the mycelia, certain others did not support quick colonization and development.

For plates with an upward orientation (Table 3.1); the highest day of mycelia extension was found in potato dextrose agar (9.00 ± 0.00 at 5 days) as shown in plate 3.7, with the lowest in cocoyam dextrose agar (8.19 ± 0.81 at 6 days) while for plates with a downward orientation, the highest day of mycelia extension was found in potato dextrose agar (9.00 ± 0.00 at 6 days), with the lowest in white yam dextrose agar (6.24±0.09 at 6 days).

In (Table 3.1) the mycelia extension of the fungus on different days was recorded for the different mediums and their plate orientations. At day 1 sweet potato recorded the highest mycelia length on both plate orientations while cocoyam dextrose agar and white yam dextrose agar recording the lowest mycelia length on both plate orientation. The growth trend continued in this pattern until day 4 where there was a decline in mycelia growth in sweet potato dextrose agar accompanied with a growth increase in potato dextrose agar in both plate orientations and growth continued in this pattern. The plates with an upward orientation was fully colonized by the mycelia in three of the five mediums (potato dextrose agar, sweet potato dextrose agar, water yam dextrose agar) on the sixth day while only potato dextrose agar was fully colonized on the sixth day for plates with a downward orientation.

Figure 3.1 depicted the impact of the culture media and plate orientation on the mycelia growth rate of *Volvariella volvacea*. The highest growth rate was observed in potato dextrose agar with an upward plate orientation at a rate of 15.20 mm/day while white yam dextrose agar had the slowest growth rate at 8.72 mm/day.

There was a difference in the cultural characteristics of the mycelia in plates with the upward orientation and those with a downward orientation (Table 3.2); The mycelia in all the plates with a downward orientation appeared with different level of fluffiness while those with an upward orientation were either appressed or appressd and fluffy at the edge with the exception of white yam dextrose agar which appeared mildly fluffy. Also, potato dextrose agar had the highest mycelia density rating across both plate orientations at 5 while sweet potato dextrose agar had the lowest rating for both upwards and downwards plate orientation at 1 and 2 respectively.

(Figure 3.2) The biomass of *Volvariella volvacea* was correlated, the biomass of the fungus mycelia was weighed in grams and converted to milligrams. For plates with an upward orientation, potato dextrose agar had the highest biomass weighing 223.07 mg while the lowest biomass observed was obtained from white yam dextrose agar weighing 112.10 mg. White yam dextrose agar weighing 206.77 mg had the highest biomass for plates with a downward orientation while the lowest biomass recorded was obtained from sweet potato dextrose agar weighing 92.07 mg. depicts that the mycelia in plates with an upward orientation had a higher biomass than the plates with a downward orientation except for white yam dextrose agar.

Table 3.1: Effect of selected tuber-based media on the mycelial extension of *Volvariella volvacea*

MEDIUM	PLATE ORIENTATION	TIME AFTER INOCULATION (DAYS)					
		1	2	3	4	5	6
Cocoyam Dextrose Agar	a	1.08±0.03	2.12±0.05	3.24±0.06	4.93±0.05	6.72±0.15	8.19±0.81
	b	1.12±0.04	2.02±0.07	3.01±0.07	4.02±0.08	5.44±0.14	6.96±0.07
Potato Dextrose Agar	a	1.40±0.05	2.80±0.12	4.51±0.14	6.60±0.09	9.00±0.00	9.00±0.00
	b	1.58±0.04	2.78±0.08	4.35±0.25	6.32±0.19	7.60±0.25	9.00±0.00
Sweet potato Dextrose Agar	a	1.64±0.07	3.39±0.07	5.24±0.12	6.24±0.13	7.66±0.14	9.00±0.00
	b	1.63±0.06	3.50±0.13	5.33±0.20	6.22±0.14	7.23±0.08	8.67±0.15
Water yam Dextrose Agar	a	1.39±0.06	3.15±0.02	4.78±0.05	6.478±0.13	8.58±0.16	9.00±0.00
	b	1.43±0.03	3.18±0.08	4.64±0.03	5.67±0.11	6.70±0.11	8.41±0.04
White yam Dextrose Agar	a	1.12±0.05	2.20±0.05	3.50±0.08	4.71±0.08	6.72±0.15	8.87±0.13
	b	1.07±0.10	1.88±0.20	2.93±0.15	3.90±0.18	4.74±0.13	6.24±0.09

Values are mean of five replicates for mycelia extension (in centimeters) ± Standard error.

Keys:

(a): Culture media facing up

(b): Culture media facing down

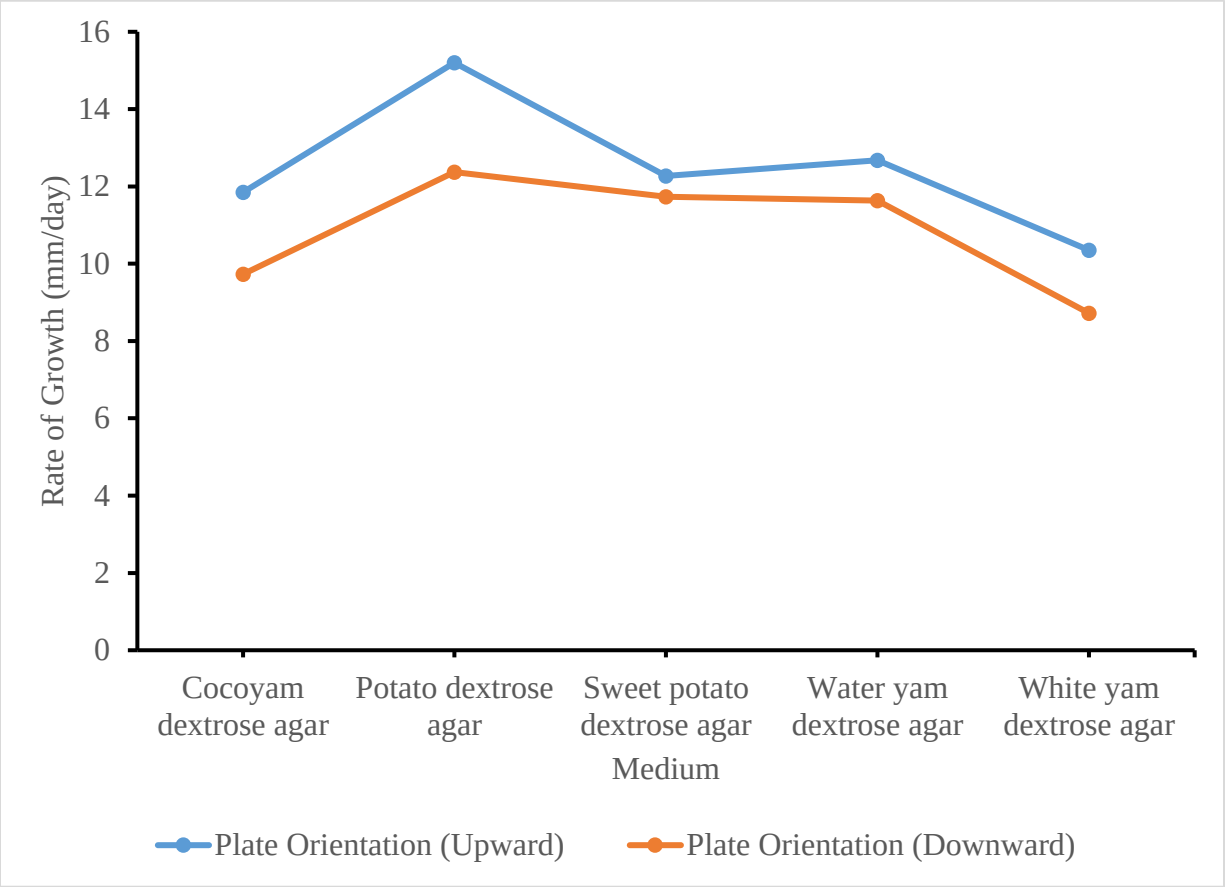

Figure 3.1: Effect of selected tuber based media on the mycelia growth rate of *Volvariella volvacea*.

Table 3.2: Mycelia density rating and cultural characteristics of *Volvariella volvacea* grown on selected tuber based medium.

MEDIUM	PLATE ORIENTATION	MYCELIA DENSITY	CULTURAL CHARACTERISTICS
Cocoyam Dextrose Agar	a	4	Appressed and raised at the edge
	b	4	Midly fluffy
Potato Dextrose Agar	a	5	Appressed at the centre and mildly fluffy at the edge
	b	5	Fluffy
Sweet potato Dextrose Agar	a	1	Appressed
	b	2	Fluffy,but more fluffy at the edge
Water yam Dextrose Agar	a	3	Appressed and raised at the edge
	b	3	Fluffy
White yam Dextrose Agar	a	2	Mildly fluffy
	b	4	Fluffy

Keys:

(a): Culture media facing up

(b): Culture media facing down

5: Very Dense

4: Dense

3: Medium

2: Sparse

1: Very Sparse

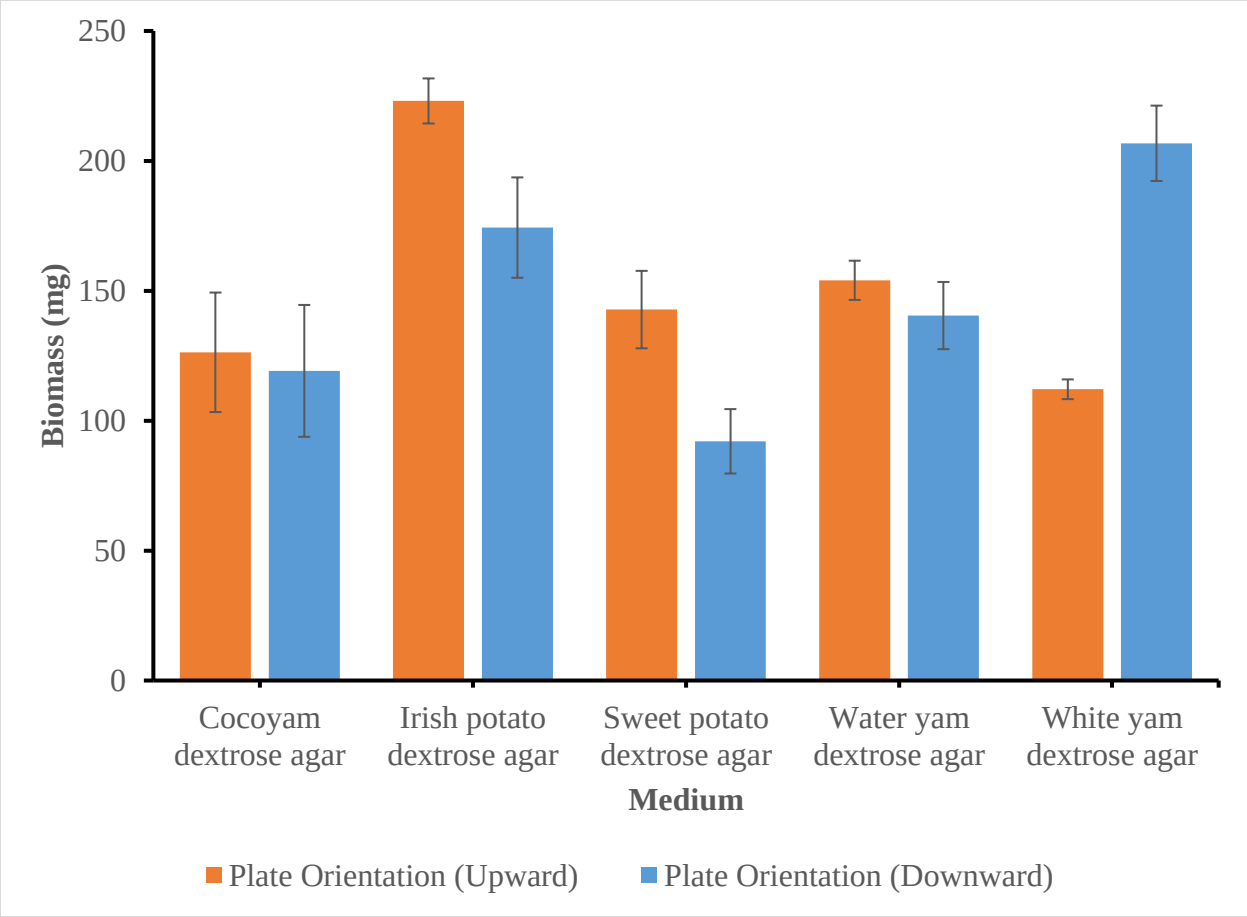

Figure 3.2: Effect of selected tuber based media on the biomass of *Volvariella volvacea*

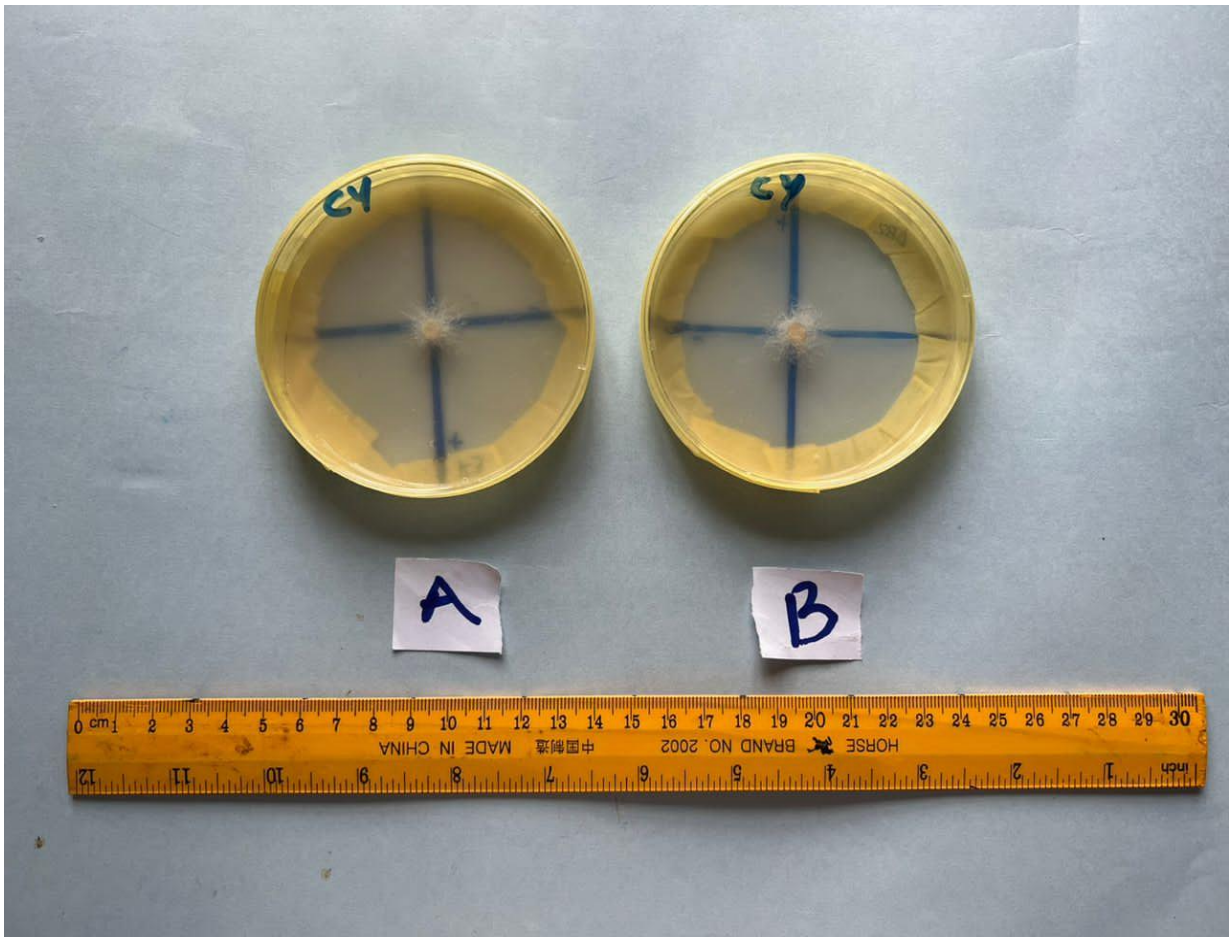

Plate 3.1: Pure cultures of *Volvariella volvacea* on cocoyam dextrose agar 2 days after inoculation

(A) Culture media facing up

(B) Culture media facing down

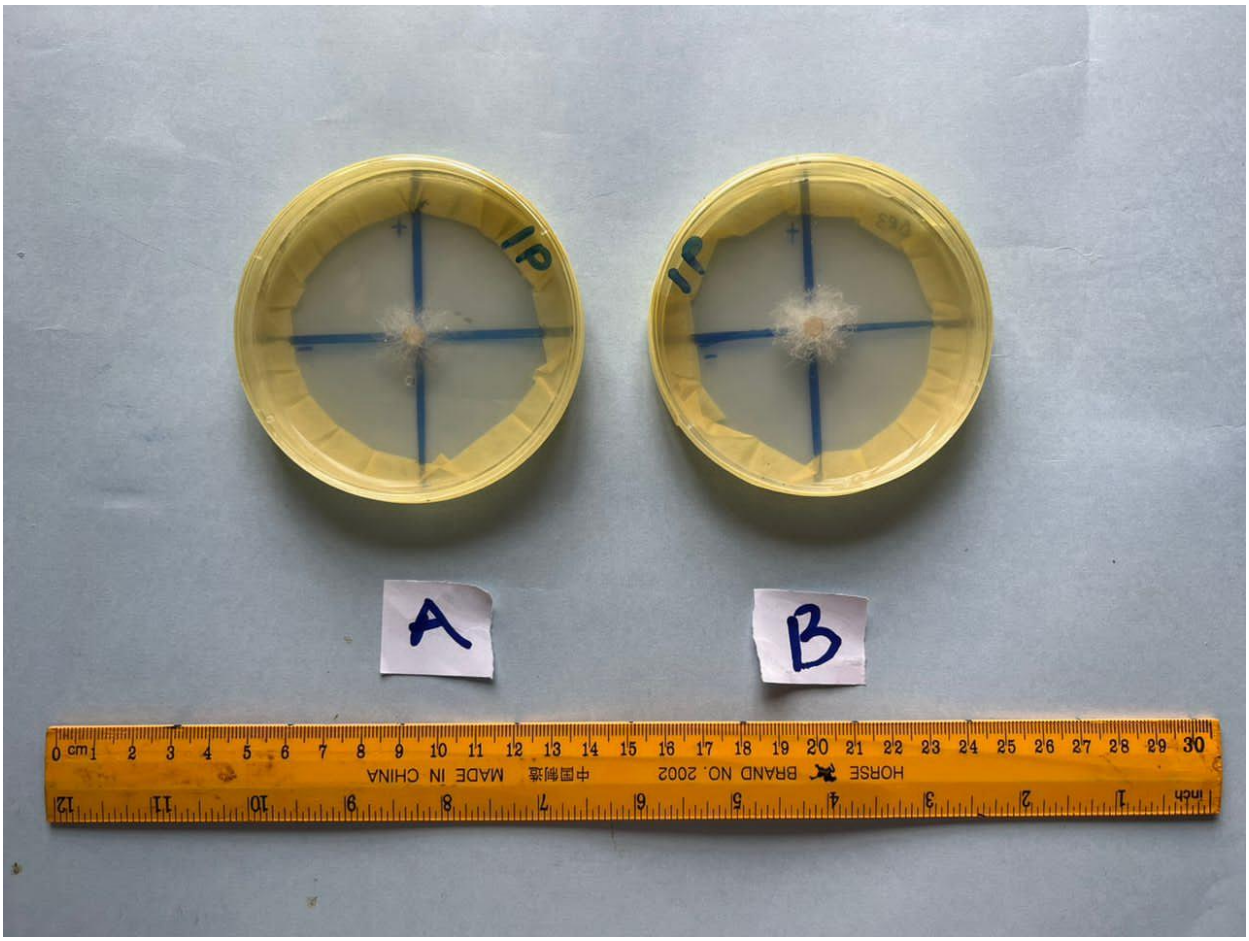

Plate 3.2: Pure cultures of *Volvariella volvacea* on potato dextrose agar 2 days after inoculation

(A) Culture media facing up

(B) Culture media facing down

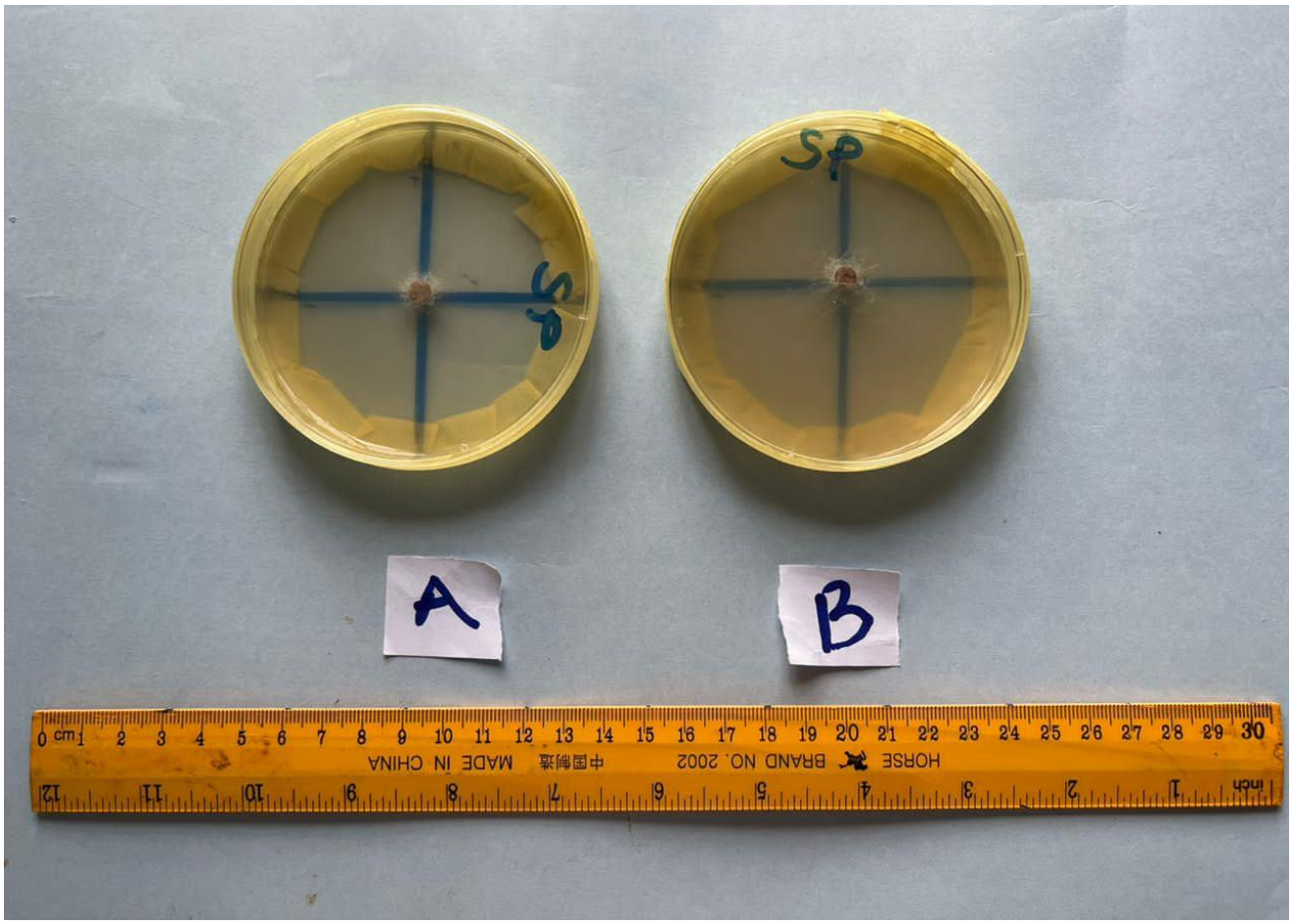

Plate 3.3: Pure cultures of *Volvariella volvacea* on sweet potato dextrose agar 2 days after inoculation

(A) Culture media facing up

(B) Culture media facing down

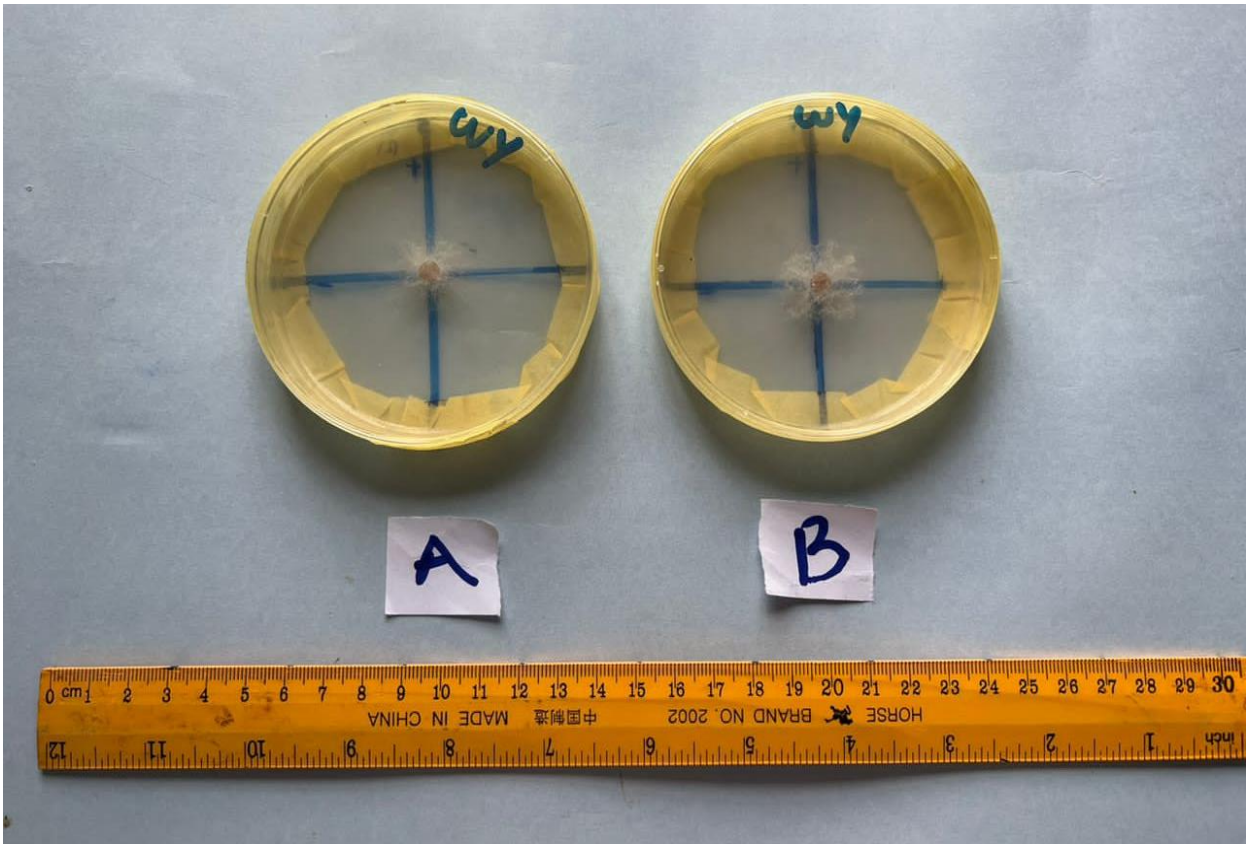

Plate 3.4: Pure cultures of *Volvariella volvacea* on water yam dextrose agar 2 days after inoculation

(A) Culture media facing up

(B) Culture media facing down

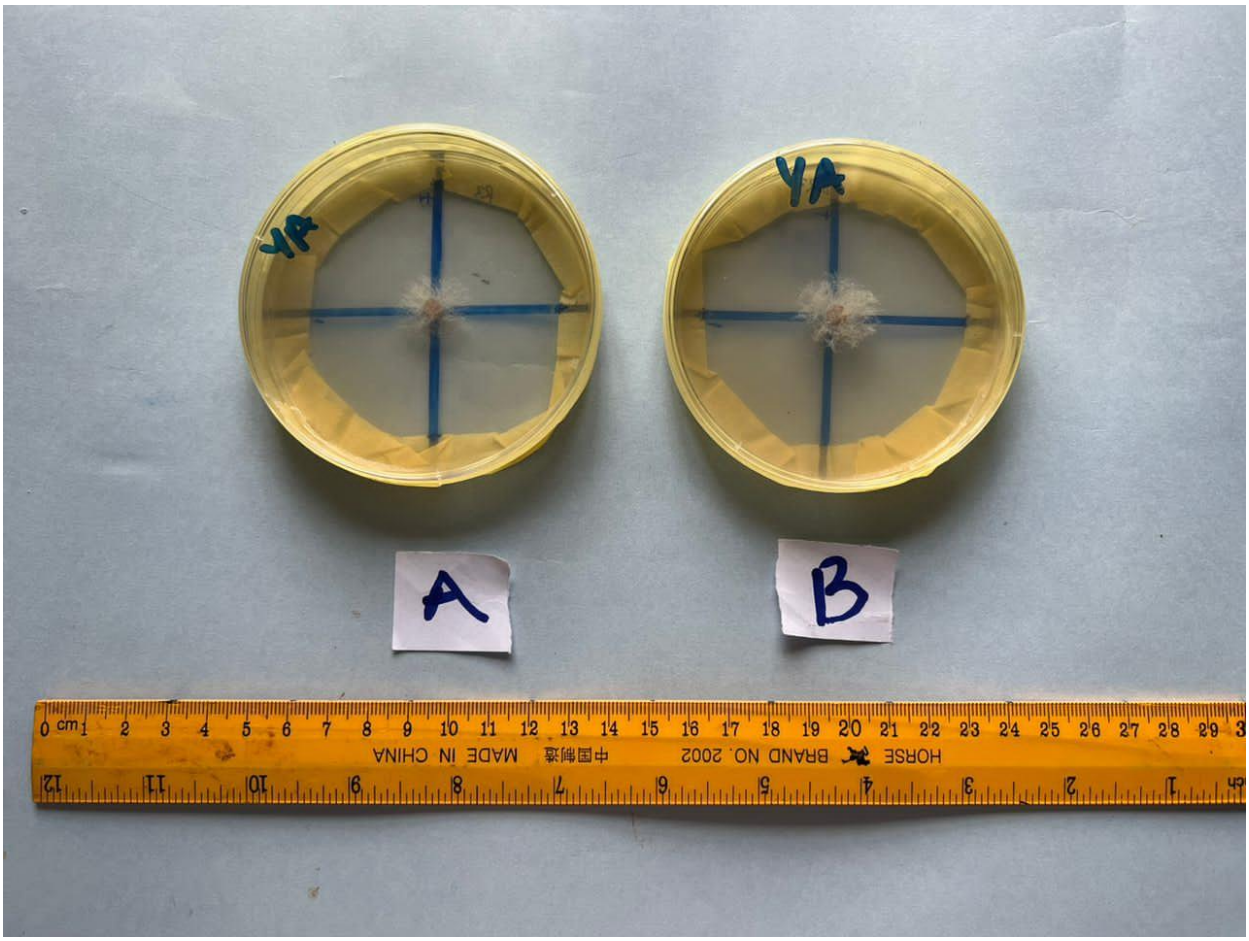

Plate 3.5: Pure cultures of *Volvariella volvacea* on white yam dextrose agar 2 days after inoculation

(A) Culture media facing up

(B) Culture media facing down

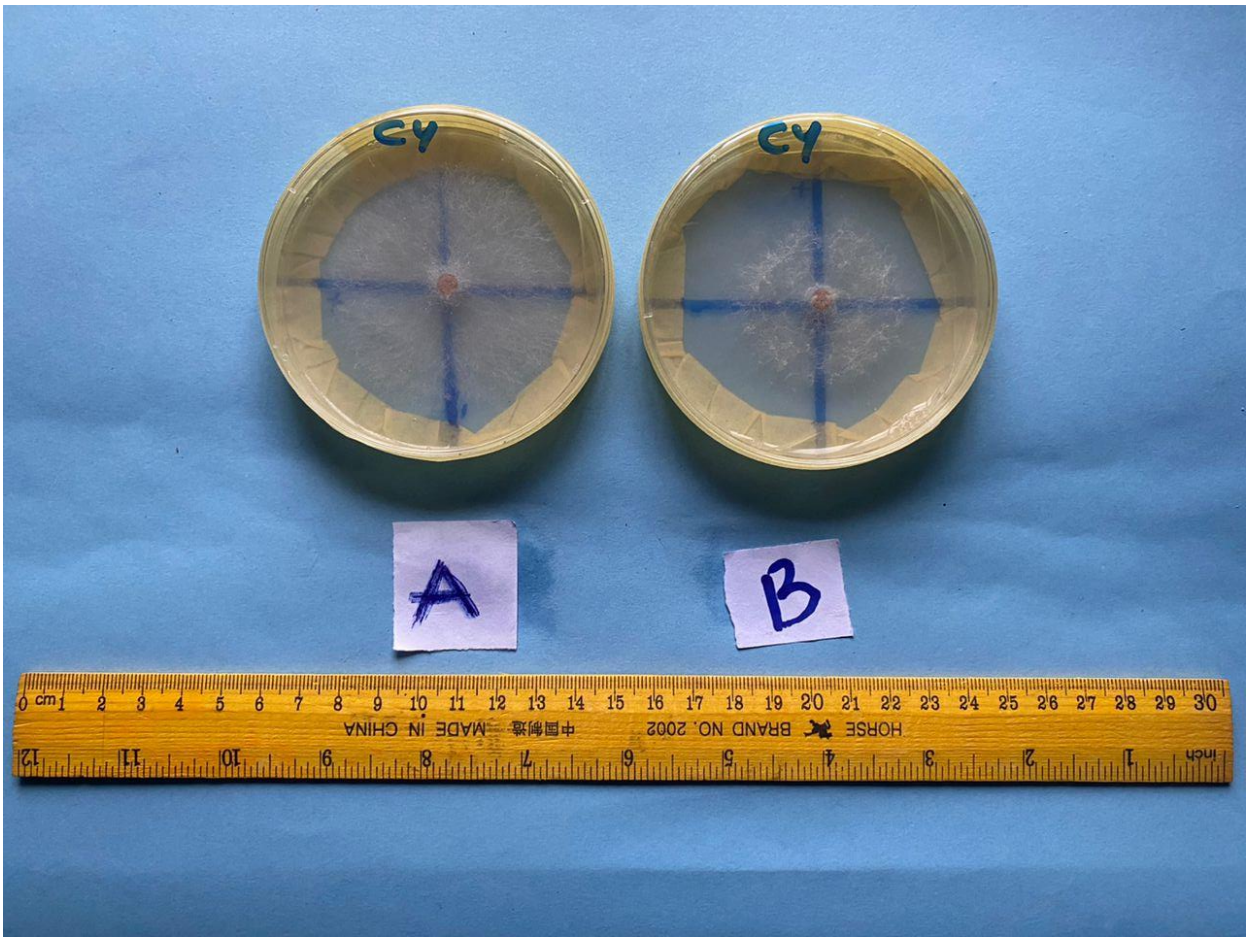

Plate 3.6: Pure cultures of *Volvariella volvacea* on cocoyam dextrose agar 5 days after inoculation

(A) Culture media facing up

(B) Culture media facing down

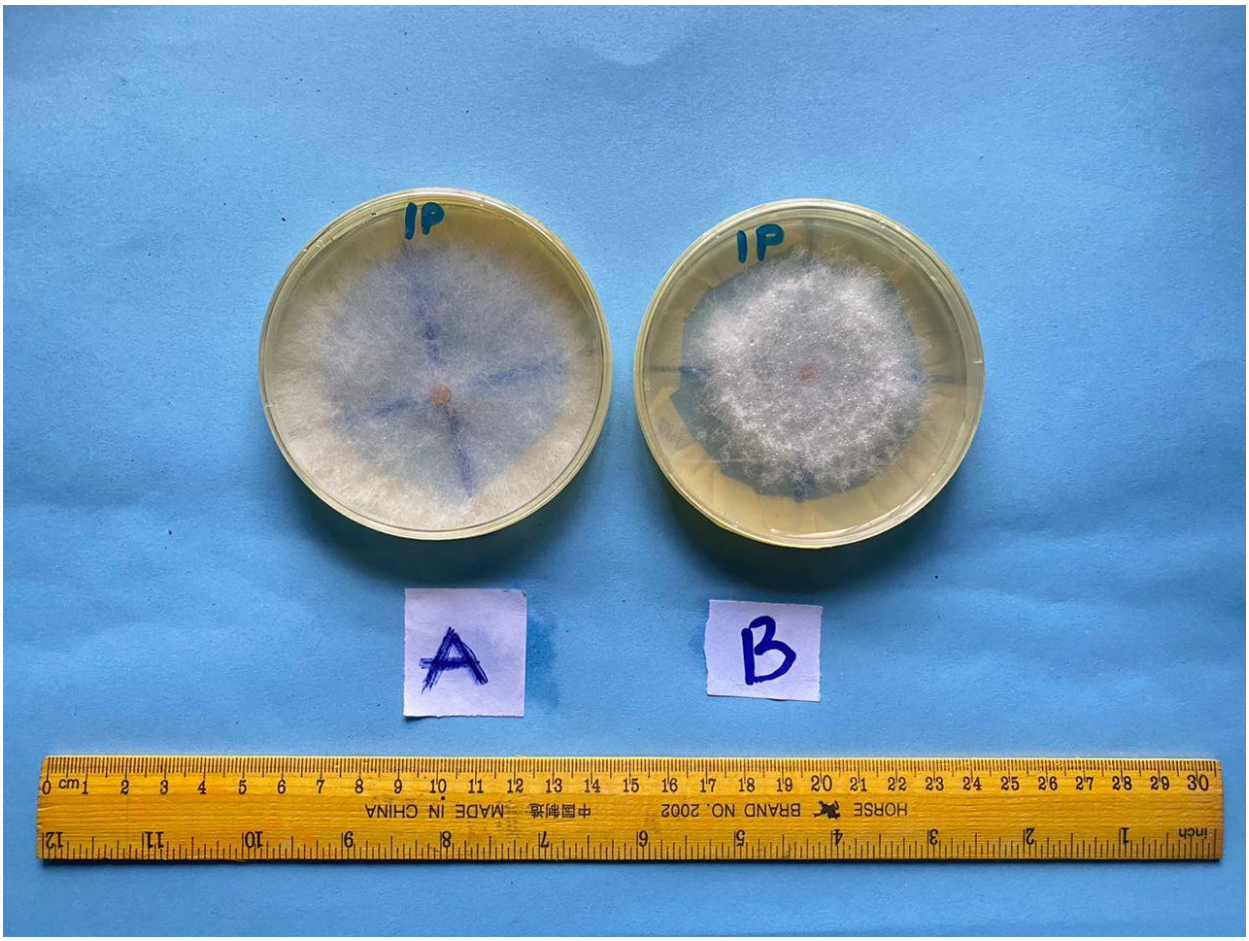

Plate 3.7: Pure cultures of *Volvariella volvacea* on potato dextrose agar 5 days after inoculation

(A) Culture media facing up

(B) Culture media facing down

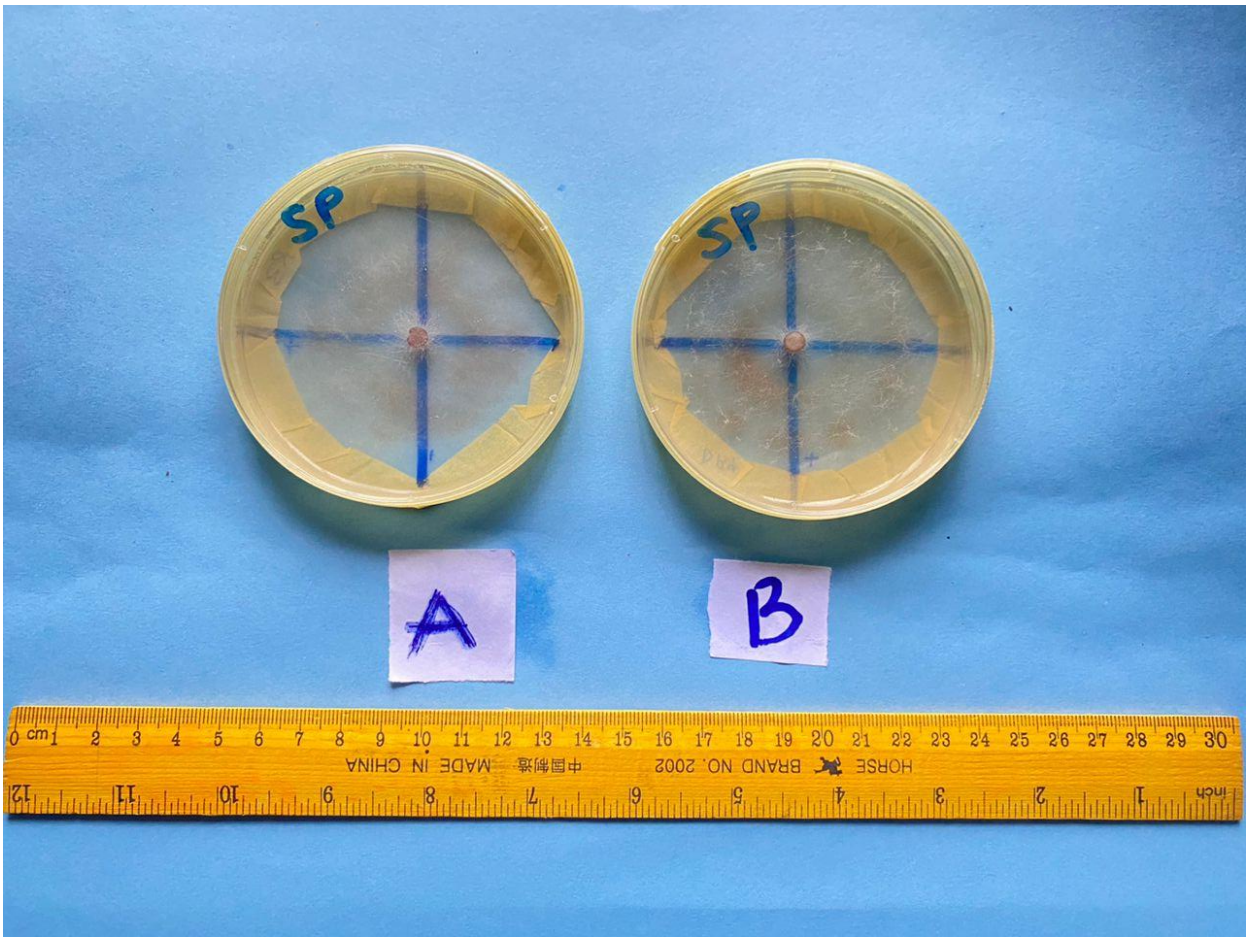

Plate 3.8: Pure cultures of *Volvariella volvacea* on sweet potato dextrose agar 5 days after inoculation

(A) Culture media facing up

(B) Culture media facing down

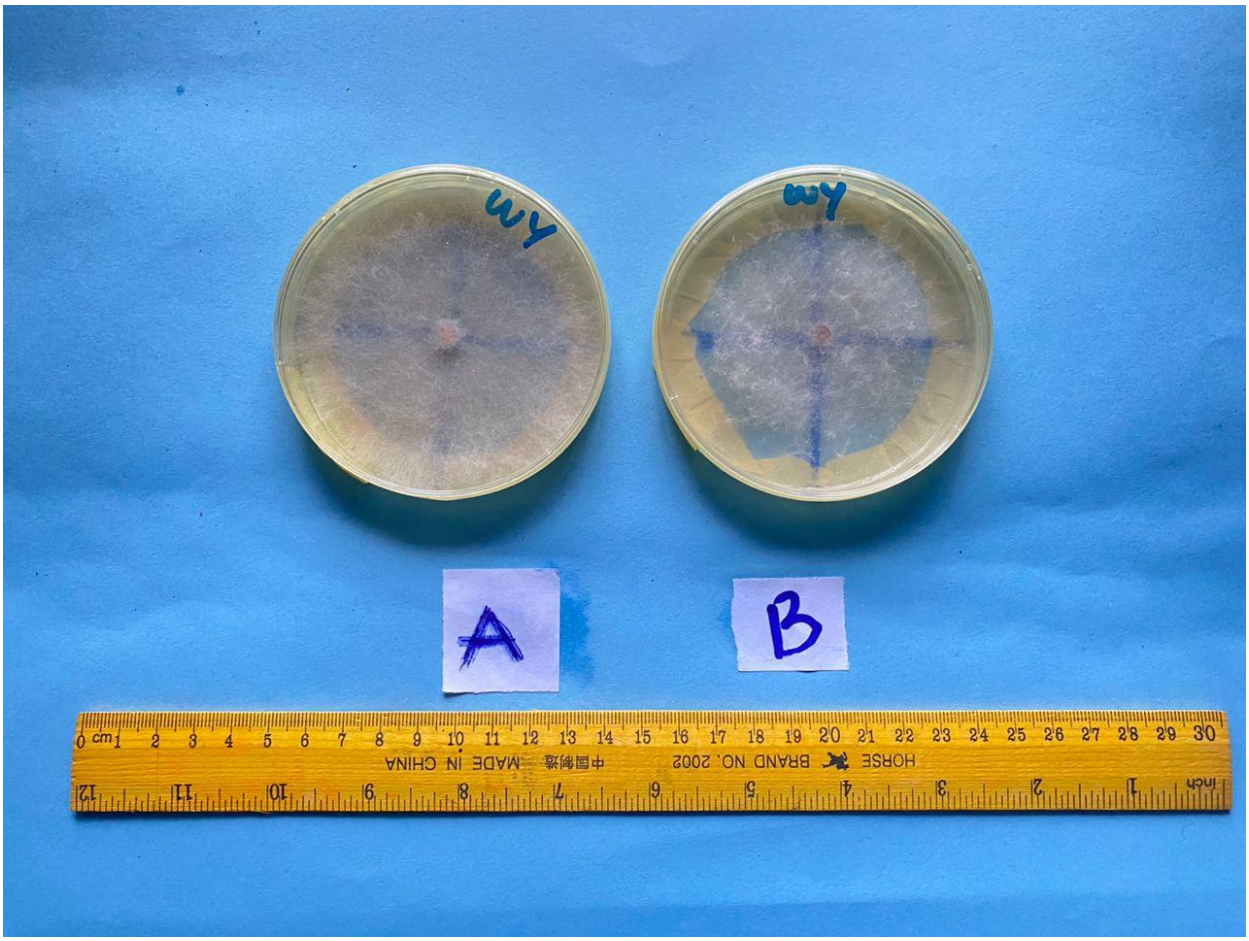

Plate 3.9: Pure cultures of *Volvariella volvacea* on water yam dextrose agar 5 days after inoculation

(A) Culture media facing up

(B) Culture media facing down

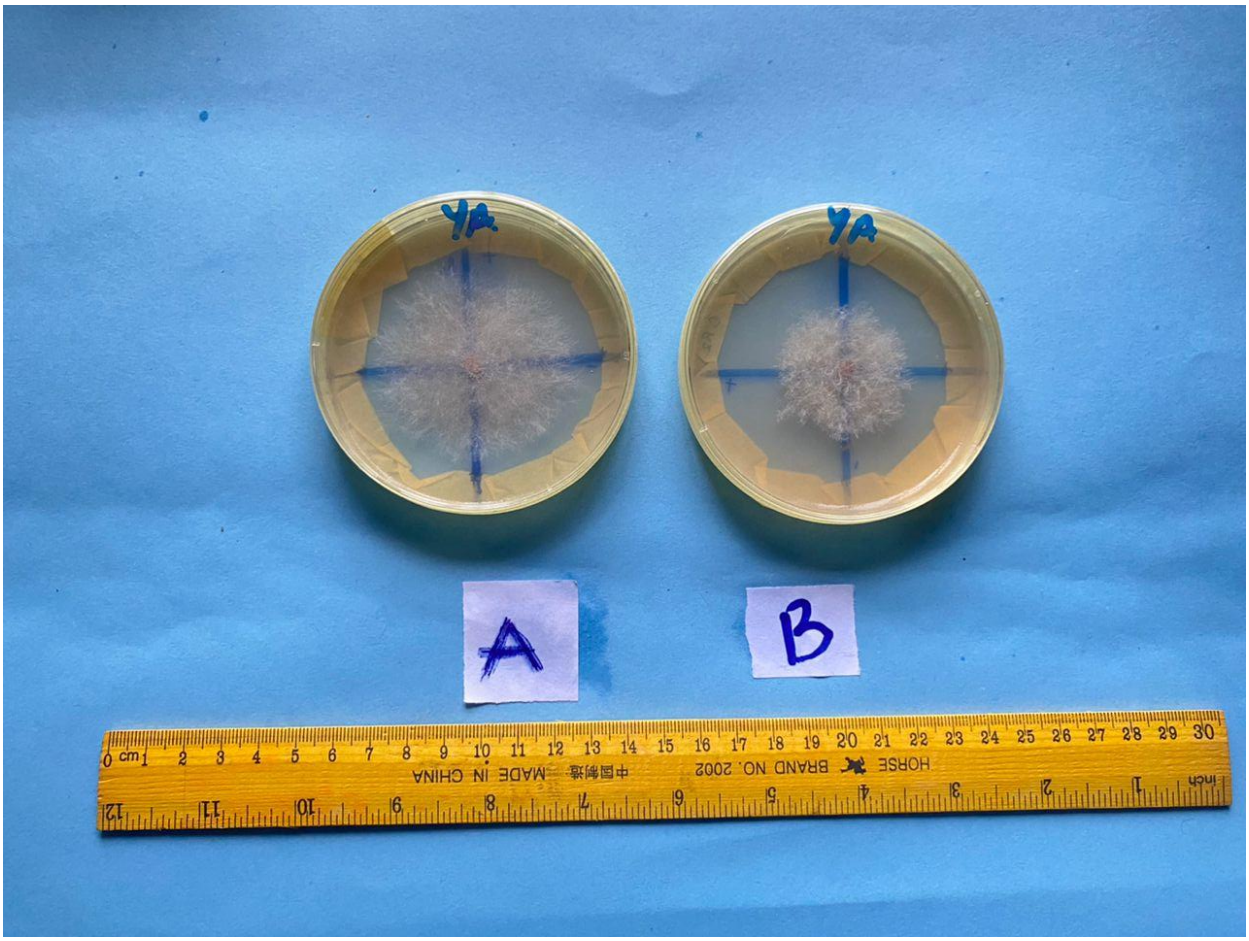

Plate 3.10: Pure cultures of *Volvariella volvacea* on white yam dextrose agar 5 days after inoculation

(A) Culture media facing up

(B) Culture media facing down

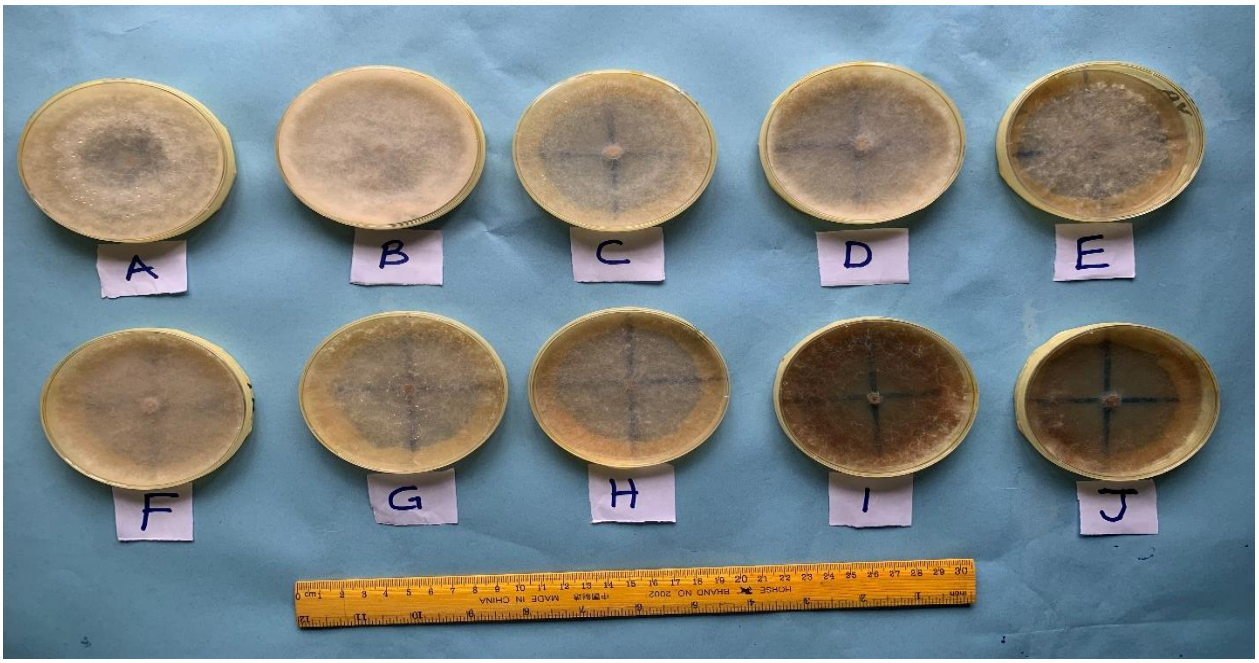

Plate 3.11: Mycelia density rating of *Volvariella volvacea* grown on selected tuber based medium

Keys: (A): Potato dextrose agar (culture media facing up) (B): Potato dextrose agar (culture media facing down) (C): Cocoyam dextrose agar (culture media facing down) (D): Cocoyam dextrose agar (culture media facing up) (E): White yam dextrose agar (culture media facing down) (F): Water yam dextrose agar (culture media facing up) (G): Water yam dextrose agar (culture media facing down) (H): White yam dextrose agar (culture media facing up) (I): Sweet potato dextrose agar (culture media facing down) (J): Sweet potato dextrose agar (culture media facing up).

A=B: Very Dense

C=D=E: Dense

F=G: Medium

H=I: Sparse

J: Very Sparse.

CHAPTER FOUR

DISCUSSION

In this study it was observed that in all of the prepared tuber formulated medium, impressive mycelia development was observed, but to various degrees. This suggests that these media compositions are highly conducive to the vegetative growth of the fungus *Volvariella volvacea*. This finding aligns with a research by (Amadi and Moneke, 2012), which suggested that tubers can be utilized as raw materials to create media for the development of microorganisms, especially fungi, because they contain a large amount of carbohydrates.

In this study, Figure 3.1 shows that potato dextrose agar, sweet potato dextrose agar and water yam dextrose agar supported the largest growth rate of *Volvariella volvacea*, and this supports the claim by (Sawiphak *et al.*, 2021), who observed that the growth of fungi on water yam dextrose agar was comparable to the growth on standard potato dextrose media. In fact, the growth of some fungi, such as the mushroom *Pleurotus ostreatus* was significantly higher on water yam dextrose agar than on potato dextrose agar. Also, according to a study by (Wongjiratthiti and Yottakot, 2017), sweet potato can serve as an effective alternative to potato as nutritional replacements in the production of fungal media. Additionally, Table 3.1 shows that potato dextrose agar, sweet potato dextrose agar, and water yam dextrose agar had the highest mycelia extension on both plate orientations. In contrast, the slower mycelia growth on cocoyam dextrose agar and white yam dextrose agar media suggests that cocoyam and white yam are not viable alternatives for potato for the growth of fungal media. One reason cocoyam is unviable is that it is opaque, making growth measures difficult to read. This study complements to the findings of a prior study by (Omodara and Adebolu, 2017).

Comparing the rate of extension of the culture media facing up with those facing down, as shown in Table 3.1. It was observed that the mycelia fully colonized the culture media facing up more quickly than those facing down. It could be suggested that the orientation of the plates may have a significant impact on the mycelia extension rate of the fungus. The capacity of the mycelium to colonize the agar surface may be impacted by the downward orientation. This outcome aligns with the idea that mycelia growth is influenced by gravity (Moore, 1996).

Based on the results shown in Table 3.2, there was a clear distinction between the cultural traits of the plates depending on their orientation. This finding implies that the plates' orientation has a big impact on the cultural characteristics of *Volvariella volvacea*. The difference in growth patterns observed may be attributed to the fungal response to gravity, which can impact the distribution of mycelia and how it develops. Further research is required to explore the specific mechanisms underlying this orientation-dependent effect.

Since fungi have the ability to flexibly rearrange their mycelium in response to changes in the availability of nutrients, the mycelia density of a fungus is highest where resources are most abundant (Olsson, 1995). This is in line with this study as shown in Table 3.2.

Looking into the biomass in Figure 3.3, the biomass of *Volvariella volvacea* on plates with an upward orientation is influenced by Irish potato dextrose agar. While white yam dextrose agar affects the fungus with a downward plate orientation, all of the media showed that the plates with an upward orientation had a larger biomass than the plates with a downward orientation with the exception of white yam dextrose agar, it has been suggested that the plates' orientation may have an effect on the biomass of *V. volvacea*. A reason for this being that the upward plate orientation allows the mycelium to grow more evenly, resulting in a higher biomass.

CONCLUSION

In a general purpose culture medium, various starch containing tubers can be used in place of the potato. As a consequence of this research, sweet potato and water yam are viable alternatives for potato in the creation of culture medium for fungal growing. The plate orientation also influences the growth of the fungal mycelia, as the upward plate orientation assisted the development of *V. volvacea*. The results of this study can be used to develop more efficient and cost-effective techniques of growing *V. volvacea*.

REFERENCES

Ahlawat, O. P., Manikandan, K. and Singh M. (2016). Proximate composition of different mushroom varieties and effect of UV light exposure on vitamin D content in *Agaricus bisporus* and *Volvariella volvacea*. *Mushroom Research*, **25(1)**: 1-8.

Ahlawat, O. P., Singh, R. and Kumar, S. (2011). Evaluation of *Volvariella volvacea* strains for yield and diseases/ insect-pests resistance using composted substrate of paddy straw and cotton mill wastes. *Indian Journal of Microbiology*, **51(2)**: 200–205.

Amadi O. C. and Moneke A. N. (2012). Use of starch containing tubers for the formulation of culture media for fungal cultivation. *African Journal of Microbiology Research*, **6(21)**: 4527-4532.

Bao, D., Gong, M., Zheng, H., Chen, M., Zhang, L. *et al*., (2013). Sequencing and comparative analysis of the straw mushroom (*Volvariella volvacea*) genome. *Plos One*, **8(3)**: 1-12.

Belewu, M. A. and Belewu, K.Y. (2005). Cultivation of mushroom (*Volvariella volvacea*) on banana leaves. *African Journal of Biotechnology*, **4(12)**: 1401-1403.

Bisoyi, S. K. and Chatterjee, S. (2020). A review on paddy-straw mushroom production and incidence of contestant moulds. *International Journal of Current Microbiology and Applied Sciences*, **11**: 1612-1621.

Biswas, M. K. and Layak, M. (2014). Techniques for increasing the biological efficiency of paddy straw mushroom (*Volvariella volvacea*) in Eastern India. *Food Science and Technology*, **2(4)**: 52-57.

Chang, S. (1969). A cytological study of spore germination of *Volvariella volvacea*. *Botanical Magazine Tokyo*, **82**: 102-109

Chen, B., Gui, F., Xie, B., Deng, Y., Sun, X. *et al*., (2013). Composition and expression of genes encoding carbohydrate-active enzymes in the straw-degrading mushroom *Volvariella volvacea. Plos One*, **8(3)**: 1-9.

Colmenares-Cruz, S., Sanchez, J. E. and Valle-Mora, J. (2017). *Agaricus bisporus* production on substrates pasteurized by self-heating. *AMB Express*, **7(1)**: 130-135.

Fasidi, I. O. (1998). Studies on *Volvariella esculenta* (Mass) Singer: cultivation on agricultural wastes and proximate composition of stored mushrooms. *Food Chemistry*, **55(2)**: 161-163.

Gbolagade, J. S. (2006). Bacteria associated with compost used for cultivation of Nigerian edible mushrooms *Pleurotus tuber-regium* (Fr.) Singer, and *Lentinus squarrosulus* (Berk.). *African Journal of Biotechnology*, **5(4)**: 338-342.

Kaushik, S., Ipsita, D. and Kumar, S. (2018). Paddy straw mushroom (*Volvariella* spp.): a natural scavengers who help in malnutrition and environment protection. *African Journal for Microbiology research*, ***10(5)***: 10983-10985.

Koyyalamudi, S. R., Jeong, S., Song, C., Cho, K. Y. and Pang, G. J. (2009). Vitamin D2 formation and bioavailability from *Agaricus bisporus* button mushrooms treated with ultraviolet irradiation. *Journal of Agricultural and Food Chemistry*, **57(8)**: 3351-3355.

Maurya, A. K., Kumar P., Singh V. and Kumar, S. (2016). Evaluation of substrates and supplements for enhancing the productivity of paddy straw mushroom (*Volvariella volvacea*), **9(6)**: 717-720.

Moore, D. (1996). Graviresponses in fungi. *Advances in Space Research*, **17(6/7)**: 73-82.

Nannapaneni, K. K. and Subbiah, K. A. (2016). Influence of organic nitrogen supplementation on yield of paddy straw mushroom, *Volvariella volvacea* (Bull. Ex Fr.) Sing. *International Journal of Green Pharmacy*, **10(4)**: 237-241.

Nongthombam, J., Kumar, A. and Patidar, S. (2021). A review on study of growth and cultivation of oyster mushroom. *Plant Cell Biotechnology and Molecular Biology*, **22(5and6)**: 55-65.

Obodai, M. and Odamtten, G.T. (2012). Mycobiota and some physical and organic composition of agricultural wastes used in the cultivation of the mushroom *Volvariella volvacea.* Journal *of Basic and Applied Mycology*, ***3(2012)***: 21-26.

Olsson, S. (1995). Mycelial density profiles of fungi on heterogeneous media and their interpretation in terms of nutrient reallocation patterns. *Mycological Research*, **99(2)**: 143-153.

Omodara, T. R. and Adebolu, T. T. (2014). Effect of formulated culture media on growth of some fungal species. *International Journal of Botany and Research*, ***4(5)***: 29-34.

Ponmurugan, P., Sekhar, Y. and Sreesakth, T. R. (2007). Effect of various substrates on the growth and quality of mushroom. *Pakistan Journal of Biological Sciences* **10(1)**: 171-173.

Ravimannan, N., Arulanantham, R., Pathmanathan S. and Niranjan, K. (2014). Alternative culture media for fungal growth using different formulation of protein sources. *Annals of Biological Research*, **5(1)**: 36-39.

Reyes, R. G. (2000). Indoor cultivation of paddy straw mushroom, *Volvariella volvacea*, in crates. *Mycologist*, **14(4)**: 174-176.

Roy, A., Prasad, P. and Gupta, N. (2014). *Volvariella volvacea*: A macrofungus having nutritional and health potential. *Asian Journal of Pharmacy and Technology*, **4(2)**: 110-113.

Rzymski, P., Mleczek, M., Niedzielski, P., Siwulski, M. and Gąsecka, M. (2017). Cultivation of *Agaricus bisporus* enriched with selenium, zinc and copper. *Journal of Science, Food and Agriculture*, **97(3)**: 923-928.

Sudha, A., Lakshmanan, P. and Kalaiselvan, B. (2008). Antioxidant properties of paddy straw mushroom (*Volvariella volvacea* (Bull. ex Fr.)) Sing. *International Journal of Applied Agricultural Research*, **3(1)**: 9-16.

Sawiphak, S., Wongjiratthiti1, A. and Saengprasan, C. (2021). *Dioscorea alata* as alternative culture media for fungal cultivation and biomass production, *Pertanika Journal of Tropical Agricultural Science*. **44(2)**: 317-336.

Tharmila, S., Jeyaseelan, E. C. and Thavaranjit, A. C. (2011). Preliminary screening of alternative culture media for the growth of some selected fungi. *Archives of Applied Science Research*, **3(3)**: 389-393.

Thiribhuvanamala, G., Krishnamoorthy, A. S., Kavitha, C., Kamal, S., Kumar, A. *et al*., (2021). Strategic approaches for outdoor cultivation of paddy straw mushroom (*Volvariella volvacea*) as intercrop under different cropping Systems. *Madras Agricultural Journal,* **107**: 10-12.

Thiribhuvanamala, G., Krishnamoorthy, S., Manoranjitham, K., Praksasm V. and Krishnan, S. (2012). Improved techniques to enhance the yield of paddy straw mushroom (*Volvariella volvacea*) for commercial cultivation. *African Journal of Biotechnology*, **11(64)**: 12740-12748.

Tudses, N. (2016). Isolation and mycelial growth of mushrooms on different yam-based culture media. *Journal of Applied Biology and Biotechnology*, **4(5)**: 33-36.

Ukoima, H. N., Ogbonnaya, L. O., Arikpo G. E. and Ikpe, F. N. (2009). Cultivation of mushroom (*Volvariella volvacea*) on various farm wastes in Obubra local government of Cross river State, Nigeria. *Pakistan Journal of Nutrition* **8(7)**: 1059-1061.

Wongjiratthiti, A. and Yottakot, S. (2017). Utilisation of local crops as alternative media for fungal growth. *Pertanika Journal of Tropical Agricultural Science*, **40(2)**: 295 – 304.

Zahid, A., Ramzan, M., Fozia, Ahmed, S. (2019). Nutritional and medicinal significance of paddy straw mushroom (*Volvariella volvacea*). *Science letters*, **7(3)**: 104-107.