UNIVERSITY OF ZULULAND

Faculty of Science and Agriculture
Department of Biochemistry and Microbiology

Effects of Carbon, Nitrogen, Particle size and moisture on Oyster Mushroom Production in KwaZulu Natal - Cedara

Dissertation submitted in fulfilment of the requirements for the degree of Master of Science to the:

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Department of Biochemistry and Microbiology

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Submitted by:

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DECLARATION

I, Nkosinathi Jacob Tembe, declare that this dissertation is entirely my own work and has not been taken from the work of others, except where I have appropriately acknowledged and referenced the original source. This dissertation has never been submitted for any degree for examination in any University.

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Signed on the _____ day of _____, 2018

DEDICATION

To My wife: Thandiswa Tembe

My daughter: Afezeka Tembe

My mother: Rose Marry Ndelane

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I would like to thank the management and staff of Isikhowe Juncao Mushroom section for their continuous support throughout this study, without you this piece of work would have ended as a wish. Many thanks to the KwaZulu Natal Department of Agriculture and Rural Department as a whole for allowing me to use their resources to make this study a success.

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God I know you always with me, thank you for your guidance and protection all the time.

RESEARCH OUTPUT

This work has been presented at two annual research symposium

1. University of Zululand, Faculty of Science and Agriculture
2. Department of Agriculture and Rural Development

Two papers are intended to be published from this work

Most of the mushroom farmers from UMgungundlovu district (Pietermaritzburg) are already benefiting from this study since I always provide them with relevant advises as an Agricultural Advisor within their area.

ABSTRACT

Mushrooms are liked for their delicious flavour, high protein content and absence of cholesterol. Farmers require guidance in how they can manipulate the current existing mushroom production methods to fit in their cheaper, local ready available raw materials. Mushroom production can be optimized only if its substrate specific requirements are known and well maintained. Different potential sources of carbon and nitrogen such as wheat bran, teff, lucerne, bagasse, maize, sawdust, juncao and hominy chop were analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Analysis). Carbon and nitrogen content was used to set up five carbon/nitrogen (C/N) ratios (15:1, 25:1, 35:1, 70:1 and 100:1). Milling of the raw materials was done in three phases which were 8 mm, 12 mm and 25 mm using different sieves of the multi-purpose milling machine. The raw materials were mixed and the water was added to achieve 65% moisture. The raw materials were bagged and pasteurized at 90^0C for 14 hours. The substrates were inoculated with *Pleurotus ostreatus* spawn produced at Department of Agriculture and rural Development (DARD). The rate of the mycelium growth and contamination was monitored and recorded. When the substrates were fully colonised, they were cut on top and randomly placed on the wall and irrigated daily. Fresh mushrooms of each mushroom pack were harvested and weighed. The optimum substrate requirements were determined based on best C/N ratio, particle size, biological efficiency and contamination rate. The highest biological efficiency was achieved with the following substrate composition 31.3% bagasse, 6.3% wheat bran, 12.5% maize, 12.5% sawdust, 12.5% hominy chop, 12.5% juncao, 6.3% lurcerne and 6.3% teff with particle size of 8 mm and C/N ratio of 35:1. There are many agro-waste that can be successful used in production of oyster mushrooms, but its combination to form a required optimum C/N ratio is very important. This study showed that the particle size also played a crucial role in mycelium growth. The study concluded that particle size of the raw material when using plastic bag (150x300 mm) must be at least between 8 and 12 mm and C/N ratio should above 25:1 and below 35:1.

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LIST OF ABBREVIATIONS

BE - Biological efficiency

C/N - carbon nitrogen

$CaCO_3$ - Calcium carbonate

g - grams

kg - Kilograms

PDA - Potato dextrose agar

KZN - KwaZulu Natal

DARD - Department of Agriculture and Rural Development

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

Protein deficiency is fast becoming a world-wide phenomenon especially in developing countries due to agricultural unsustainability and water shortage. Marasmus and Kwashiorkor are some of the diseases caused by protein deficiency. Producing oyster mushrooms can be a suitable solution to this problem since they are rich in proteins (Yildiz *et al*., 2002).

Mushroom are liked for their delicious flavour, high protein content (20 to 40% on a dry weight basis), absence of cholesterol and are almost fat free. They have B group vitamins especially riboflavin and nicotinic acid and are also rich in inorganic salts like phosphorus, potassium, sodium, calcium and trace elements. *Pleurotus ostreatus* are also said to have a nutritional value similar to milk and meat which makes them a perfect meat substitute for vegetarians (Walde *et al*., 2006).

Fungi of the Pleurotus genus have an important place among the commercially employed basidiomycetes. They are eaten as a type of vegetable and may also be regarded as medicine (Gern *et al*., 2008, Pramanik *et al*., 2005) due to their ability to reduce high blood pressure, strengthen the immune system against pathogenic microorganisms, help recover from fatigue and constipation. They contribute to economic development and poverty alleviation due to their market demand (Guillamon *et al*., 2010).

Peoples' Republic of China is the major producer and consumer of oyster mushrooms, accounting for nearly 90% of total world production. Due to oyster mushroom effectiveness in poverty alleviation (Masarirambi *et al*., 2011), many developing countries are interested in

their cultivation today. The major challenge though is the lack of information or understanding of scientific methods used for their cultivation (Royse *et al*., 2004). This study will add value to agricultural scientific bodies and help farmers to improve mushroom cultivation and production.

Mushroom cultivation should be simple, low cost and environmental friendly. This may be achieved through the use of agricultural by-products like bagasse, sawdust, teef, maize stova, lucerne, rye grass, wheat bran and other agricultural waste which may contain lignin and cellulose (Oscar *et al*., 1999). The cultivation of oyster mushroom consists of four steps which need optimization. These are production of pure mycelium culture on agar, making of spawn, substrate production and fructification (Zharare *et al*., 2010).

Farmers in developing countries still have a challenge to search for alternative substrates that may be more readily available and cost effective. Optimizing available substrates to provide higher yield and better mushroom quality is another challenge. Thus, farmers are unable to use oyster mushroom production as a tool for poverty alleviation and to solve the problem of lack of proteins (Baysala *et al*., 2003). This research study was conducted at KwaZulu-Natal Department of Agriculture and Rural Development (KZNDARD). The current used method of oyster mushroom cultivation at KZNDARD was used as a control. The KZNDARD method was also modified for the purpose of this study. The modification of the method intended to make oyster mushroom production simple and help farmers to be able to modify any other method using local readily available raw materials.

1.2. Purpose of the Study

1.2.1 Problem statement

Protein deficiency is becoming a world-wide phenomenon especial in developing countries, therefore a cheaper source of protein like Oyster mushrooms is required. Farmers lack information on how they could use the local ready available raw materials for mushroom production.

1.2.2 Hypothesis

Oyster mushroom substrate production can be optimized only if its substrate specific requirements are known.

1.2.3. Aim

To determine the oyster mushroom optimum substrate requirements in order to achieve higher mushroom yields.

1.2.4. Objectives

- To investigate alternative sources of nitrogen and carbon for Oyster mushroom production.
- To determine the best particle size of the mushroom substrate pack using a plastic bag.
- To produce substrates and fresh mushrooms using different C/N ratios and particle size.
- To determine effects of C/N ratio and particle size on mycelium growth and biological efficiency on oyster mushroom production.

1.3. Literature Review

1.3.1. Introduction

Oyster mushrooms are a diverse group of saprotrophic fungi species that fall under the genus Pleurotus. Among cultivated species there is *P. ostreatus, P. salmoneo stramineus, P. populimus, P. djamor, P. eryngii, P. sajor-caju, P. cornucopae* and *P. cystidiousus*. All oyster mushrooms are edible and are highly nutritional (Zharare *et al*., 2010). Fungi are eukaryotic organisms that are neither plant nor animal but belong to realm of their own, although recent molecular studies have shown fungi to be more related to the animal kingdom than to the plant kingdom (Margulis and Chapman, 2009). In taxonomy, *Pleurotus ostreatus* belongs to Eumycota, Basidiomycetes, Agaricales, genus Pleurotus (Hearst *et al*., 2009).

Compared to other edible mushroom, *Pleurotus* is relatively simple to cultivate (Zadrazil, 1980). Oyster mushroom cultivation has its own specific production requirements. If the requirements are not met, it may result in failure or poor production. Increased level of nutrients available in a substrate provides more energy for mycelial growth and development. All the conditions have to be at an optimum level in order to obtain good yields of oyster mushroom. The main nutritional sources for oyster mushroom are cellulose, hemicellulose and lignin (Yildiz *et al*., 2002). These are organic carbons which may be divided into carbon and nitrogen source. Inorganic salts are also required by oyster mushroom as a source of nutrients (Viziteu Gabriel, 2004). Carbon nitrogen ratio (C/N) is an important factor for optimal substrate composition of oyster mushroom. Oxygen and water are also essential elements in mushroom cultivation (Kang, 2004).

Sugarcane bagasse (40%), hominy chop (30%), wheaten bran (15%), sawdust (10%), calcitic and hydrated lime (5%) are the raw materials used at KZNDARD for oyster mushroom

substrate pack production. These raw materials are regarded as the nutritional requirements of *P. ostreatus* (Macdonald *et al*., 2011). This formula can be adjusted by adding or removing other supplements depending of their availability at a rate of ± 3% in each raw material. The following formula was not included in the publication but it also used at DARD and it produce fairly higher yields. Sugarcane bagasse (37%), hominy chop (25%), wheaten bran (20%), sawdust (15%), calcitic and hydrated lime (3%). However, the exact amount of each nutrient source required per substrate to achieve the optimal mycelia growth and fruit body is unknown. Oyster mushroom can grow on any material type which contains lignin and cellulose regardless of the quality, yield and growth rate. Thus, it is for this reason that mushroom growers worldwide need to use different local available raw materials which suit their environmental condition (Poppe, 2004).

1.3.2. Morphology of *Pleurotus ostreatus*

The main body of a fungus is composed of enormous numbers of fine branching threads called hyphae, which together form a tangled mat or web called mycelium. Oyster mushroom grows in shelf-like cluster. It has whitish gills that run down a stubby, nearly-absent stem and has brownish cap which is open and flat fan shaped (Figure 1.1) (Zhanxi and Zhanhua, 2001). The colour of the cap is also related to the light intensity. Insufficient amount of light illumination may lead to pale colour. The spores produced by *Pleurotus ostreatus* are elliptical and egg white (Domondona *et al*., 2004).

Figure 1.1 Oyster mushroom (*Pleurotus ostreatus*) growing using trench method (Taken by me, NJ Tembe).

1.3.3. Life cycle of oyster mushroom

Naturally, fungi multiply by producing millions of spores. When a spore settles in a suitable environment it can germinate (Oei, 2005). The basidium (fruiting-body) produces basidiospores which germinate to form monokaryotic mycelium. Monokaryotic mycelium mate to form plasmogamy and then develop into dikaryotic mycelium. Basidioma is formed from dikaryotic mycelium (Figure 1.2). The optimum temperature for spore germination of *P. ostreatus* is between 24 and 28^0C. The hyphae can grow at temperature between 3 and 35^0C and the formation of fruit bodies requires between 12 and 22^0C. The hyphae of *P. ostreatus* can grow normal at pH between 3 and 7.5 (Jie, 2011). The modern tissue culture method simplifies the entire natural method. The tissue isolated from the fruiting body is inoculated into culture media and forms a mycelium which later grows into another fruiting body.

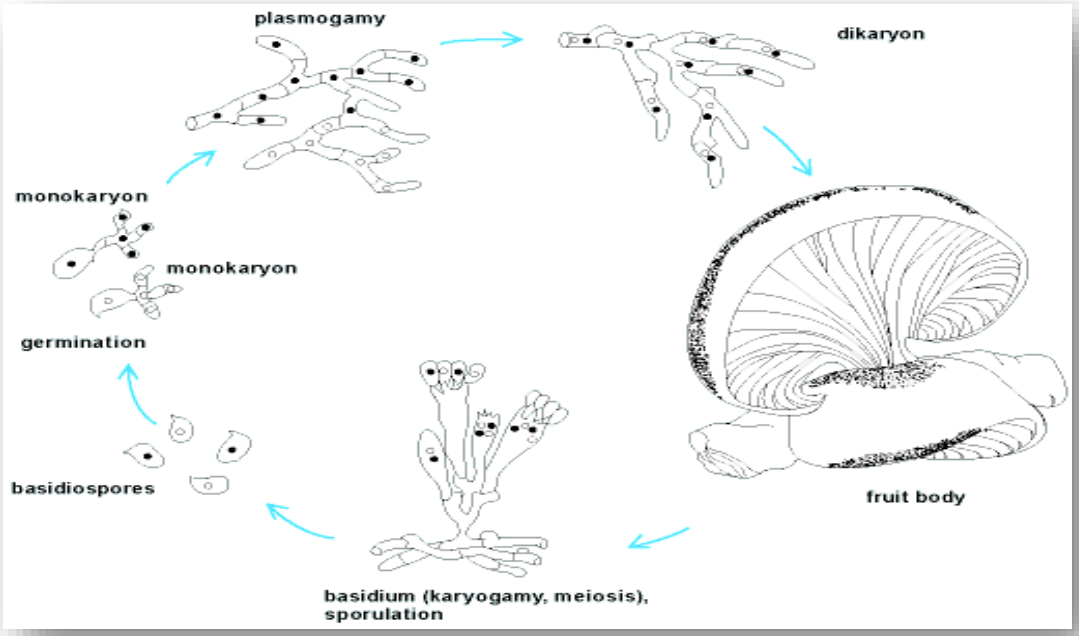

Figure 1.2 Life cycle of oyster mushroom (Adebayo and Martínez-Carrera, 2015)

1.3.4. Growth requirements of oyster mushrooms

As a fungus, *P.ostreatus* does not contain chlorophyll therefore cannot photosynthesize. It decomposes raw materials through hydrolase then absorb and spread nutrients through the mycelium. A suitable substrate for oyster mushroom should supply specific nutrients required for mycelium and fresh mushroom for growth (Viziteu, 2004). The following are the main nutritional requirements for mushroom growth:

1.3.4.1. Carbon source

Any nutrient source that provide carbon elements (carbon skeleton) for fungi are called carbon sources. Carbon is one of the most important nutritive factors for oyster mushrooms. It is composed of cellular material and supply energy to the mushroom. Fungi do not use inorganic carbon but rather organic carbon such as cellulose, hemicellulose, lignin, pectinic substance, monosaccharide, disaccharide, amylase, organic acid, and alcohols. Low molecular substances

such as monosaccharide, organic acid, and alcohols are directly absorbed and utilized by the fungi. Macromolecular organic substances are absorbed after enzymolysis (Jie, 2011).

1.3.4.2. Nitrogen source

Any nutrient sources that provide nitrogen element for oyster mushroom is called a nitrogen source. Nitrogen source is indispensable nutritive material for composition of protein and nucleic acids. Nitrogen sources required by oyster mushrooms are protein, peptone, amino acid, urea, ammonia, ammonium salt and nitrate. Low molecular substances such as amino acid, urea and ammonia are directly absorbed. Macromolecules like protein can only be absorbed after protease hydrolysis into amino acid (Tan and Wahab, 1997).

1.3.4.3. Carbon nitrogen ratio (C/N)

Carbon nitrogen ratio is the mole number ration between carbon atom in culture medium and nitrogen atoms in nitrogen source. It's an important factor for optimal substrate composition for *P. ostreatus*. The concentration of nitrogen source in the compost shall not be too high. Nitrogen excess may affect the degradation of lignin, which may prevent the mycelium growth (Bellettini *et al*. 2016). Most of the cellulosic materials such as cereal straw, cotton waste and saw dust need supplementation with a nitrogen source such as wheat or rice bran to reach optimal C/N ratio. The usual recommended C/N ratio is between 28:1 and 30:1 (Kang, 2004) and (Bellettini *et al*. 2016).

1.3.4.4. Inorganic salts

Inorganic salt provides *P. ostreatus* with the main elements other than carbon and ammonia. The concentrations of elements within mole per litre (mol/l) are called macroelements. These are K, P, Si, Fe, Mg, etc. These elements are used in the composition of cellular construction,

maintain enzyme functions, energy transfer, controlling colloidal state bioplasm and adjusting cellar osmotic pressure (Chang and Miles, 1989).

1.3.4.5. Water

Water is not a nutrient but plays a vital role in oyster mushroom life cycle and nutritive process. It is not only a component of cells but also the best solvent and medium to conduct biochemical reactions in cells. In addition, water maintains rigidity of cells. Thus, it is indispensable for oyster mushrooms. Water is needed both in vegetative growth and development phase of fruiting body. The recommended moisture content of the *P. ostreatus* substrate is between 60 – 65% (Stamets, 2000).

1.3.4.6. Absorption of nutritive materials

Nutritive materials enter cells of oyster mushrooms through cell membrane. Low molecular weight substances diffuse passively with the help of differential concentrations between the cells. They diffuse from higher concentration to the lower concentration for balance achievement. Water and gas molecule may pass in and out of the cell membrane more freely. Macromolecular nutrients can be only absorbed after being decomposed by enzymes into dissolvable micromolecular substance (Jie, 2011).

1.3. 5. Mushroom production systems

When choosing a suitable growing system, it is essential to consider labour availability as well as consideration of locally available substrates. Log cultivation is one of the methods used to grow oyster mushrooms where a part of the trunk or a large branch of a tree that has fallen or been cut off is used. It has been known as a traditional method and commonly assumed to be

of the best quality. However, recently there are new developed skills of shelf, bottle and bag cultivation which also produce quality mushrooms. Top opened boxes, bottles and bags are filled with the ideal substrates then watered to produce fresh mushrooms.

Log cultivation takes long time for first fruiting and shows low rate of productivity. Bag cultivation provides stable yields with relatively few failures. Shelf cultivation seems to be riskier when contamination occurs as it may easily spread through the whole substrate mass on the shelf. Bottle cultivation can be automated and requires a high investment (Kong, 2004).

1.3.6. Currently used method of oyster mushroom cultivation at KZNDARD

The following method was introduced at KZNDARD by Professor Lin and his team of the Juncao Institute in Fuzhou, China in January 2005. It has since been evaluated and adapted for use within the province as a means to support efforts to facilitate food security and sustainable job creation. This method was detailed discussed since it was used as a control and also modified for the purpose of this study. This method involves two major steps which are, spawn production and substrate production.

1.3.6.1. Preparation of primary spawn (master culture)

Spawn is the inoculum of the mushroom substrate extracted from the oyster mushroom fruiting body through tissue culture and cultivated in a culture medium. It must be kept pure/free from any contaminants. Spawn must be cultivated and stored under the appropriate temperature range. The spawn should be stored soon after the mycelia has grown. The temperature of the refrigerator should be kept at 4^0C. Too high or low temperature brings side effects. This practice should be well maintained to keep the spawn alive, keep the fine properties of spawn,

and keep the purity of the spawn. Slant test tubes are used to keep the spawn for a long period while petri dishes for short period, though spawn may need to be revived from time to time.

Potato dextrose agar (PDA) is the medium used for cultivation and storage of the mycelium after tissue culture. The composition of PDA is shown in Table 1.1. Thirty nine grams of PDA is suspended in 1 litre bottle of demineralised water. The bottle is shaken until it is completely dissolved. The bottle is autoclaved at 121^0C for 15 minutes, cooled in plates /slant test tubes then inoculated with a pure mycelium/tissue.

Table 1.1 Composition of Potato Dextrose Agar (PDA)

Components	**Weight (g)**
Potato extracts	4
Dextrose	20
Agar	15

1.3.6.2. Secondary spawn

The primary spawn in petri dishes or test tubes is inoculated in a bottle (secondary spawn) (Figure 1.3), for easy and fast growth then inoculated into a 300 mm long plastic bag (tertiary spawn) (Figure 1.4), which serves as an inoculum for large quantity substrate production (Zhanxi and Zhanhua, 2001).

Figure 1.3 Secondary spawn (Taken by me, NJ Tembe).

Raw materials	Quantity %
Bagasse	48
Horminy chop	30
Wheat bran	20
Lime	2

Figure 1.4 Tertiary spawn and its composition (Taken by me, NJ Tembe).

1.3.6.3. Substrate production process

The standard substrate composition used for oyster mushroom production at KZNDARD is as follows:

a) 40% sugar cane bagasse

b) 30% Hominy chop

c) 15% wheaten bran

d) 10% weathered, pine-tree sawdust

e) 2.5% agricultural lime

f) 2.5% calcium hydroxide (hydrated) lime

g) 100 litres of water

Bagasse need to be dried, making it easier for milling and eliminating some microorganisms which depend on moisture for their survival. Milled bagasse makes it easier to mix with other raw materials, easier access of nutrients and minimises damage to plastic bags used in production.

1.3.6.4. Mixing of raw materials

It is important that the dry raw materials are thoroughly mixed before the addition of clean water. This is achieved by a purpose-built 'ribbon' mixer (Figure 1.5). Once the raw materials are mixed, water is added and the standard should be 100 kg of dry material equivalent to 100 litres of water. The pH before bagging should be around 8 and 9.

Figure 1.5 Purpose built ribbon mixer (Taken by me, NJ Tembe).

1.3.6.5. Bagging

The mixed raw materials are bagged into convenient and manageable 1 kg sized plastic bags. The material used for the plastic bags must be non-porous to air, moisture and be heat resistant. Preferably a clear 60-micron plastic sleeves, 150 mm (width) x 300 mm (length) is used. Depending on the volume of substrate being handled, bagging is done by either hand or machine. In most cases, large amounts of substrate are needed to be produced therefore a purpose-built bagging machine (Figure 1.6) is used. Irrespective of the bagging method used, the density of a filled pack should be such that 1 kg of the moist substrate mixture is compressed into a 150 mm diameter cylinder to no more than 300 mm in length. The raw material within the pack is therefore firmly compressed to the level that when carefully handled, 'fingerprint indentations' are not left on the packs.

Figure 1.6 Purpose-built bagging machine (Taken by me, NJ Tembe).

1.3.6.6. Capping

Once the packs are adequately compressed, they are sealed or capped using a purpose-made plastic neck and cap. This is done manually (Figure 1.7). The cap is circular, 50 mm in diameter with a 45 mm sponge to filter air during mycelia growth, thus minimising the contamination.

Figure 1.7 Capping the substrates and packing in steel crates for pasteurisation (Taken by me, NJ Tembe).

1.3.6.7. Pasteurisation

The sealed packs are then placed into purpose-made, stackable steel crates approximately 450 mm long x 350 mm wide by 300 mm deep. Twelve (12) packs are placed in each crate. The crates are then stacked (4 higher, 6 wide and 11 long) and covered with a heavy duty plastic tarpaulin which is heat resistant. The steam generator (Figure 1.8) generate steam which is passed through a steel piping manifold between the crates to achieve and maintain a temperature of at least 90^0C for 14 hours within the individual pack (pasteurization).

Once the desired time and the maximum temperature have been achieved with the steam generator, the packs are allowed to cool slowly overnight to revert to room temperature without removing the tarpaulin cover.

Figure 1.8 Steam generator (Taken by me, NJ Tembe).

1.3.6.8. Inoculation

Inoculation is the process whereby mushroom spawn is introduced to the substrate (Figure 1.9). During inoculation hygiene protocols are maintained to avoid contamination with weed fungi which may affect the entire production process. It is absolutely essential that inoculation is carried out under sterile conditions. Disinfection of all instruments exposed to the air (hands, clothing sleeves, instruments and the outside of the mushroom packs) is essential. The sterilising agent for hands and instruments used is 70% alcohol and 0.5% solution of chlorine dioxide (TecsaChlor) liquid.

Inoculation involves the transfer of spawn to cooled pasteurized packs. The use of spawn older than three months is not encouraged, since it could have lost energy. A ratio of 1:20 of spawn is used for quick colonisation of the inoculated pack. The temperature in the inoculation room ranges between 18^0C- 25^0C and the humidity must be less than 60%.

Figure 1.9 Inoculation process in sterile environment (Taken by me, NJ Tembe).

After inoculation, substrates are recapped, ensuring that the air spaces within the sponge on a cap are not closed to allow gaseous exchange. The packs are then placed into plastic crates and transferred to a clean dry mycelium growth room maintained at room temperature, where colonisation of the packs (spawn run) takes place.

1.3.6.9. Spawn run

Colonization rooms are kept dry, dark with the temperature maintained at 25^0C. The free flow of oxygen is maintained to ensure that no carbon dioxide accumulation takes place. Spawn run is completed within 6 to 8 weeks. Figure 1.10 shows different levels of mycelium growth. Once the mycelium has colonised the entire pack, it is then ready to be transported to the location where the fresh mushrooms are produced. It is important to note that exposure of the packs to direct sunlight should always be kept to a minimum (Macdonald *et al*., 2011).

Figure 1.10 Different mycelium growth patterns in the mushroom packs (Taken by me, NJ Tembe).

1.3.6.10. Structure construction for oyster mushroom cultivation

To meet the climatic condition requirements for oyster mushroom cultivation, a structure of your desirable size is constructed. It is advisable to use 80% shade cloth and treated poles. Juncao grass is planted around the structure for additional shade if there is no enough natural shade. The structure should be able to maintain at least 18 -25^{0}C and 80% relative humidity.

A number of factors need to be considered before building a structure. For instance, soil drainage, management of water run-off to prevent spilling into the mushroom trenches and availability of clean water supply. The existence of chicken houses within 250 m of the proposed mushroom trenches may attract pests which may affect mushroom cultivation.

1.3.6.11. Plantation

There are various methods of planting oyster mushroom. Wall and trench methods are two mostly used methods. These methods make the production of fresh mushroom much easier:

Trench method: Inside a 5 m x 5 m structure, two trenches are dug, each trench should be 40 cm deep, 1 m wide and 4 m long, 1 m apart from each trench and 0.5 m spacing around the edges of the trenches as indicated in Figure 1.11.

Five plastic conduits (1.5 m) are placed per trench to hold the plastic which covers the trench, forming a tunnel for mushroom protection and to ensure that the required temperature and humidity is maintained. Five kilograms of hydrated lime is spread on the trench floor to minimise contamination and if necessary1% solution Gamma BHC (Lindane/ Cypermethrin) is sprayed on the floor of each trench to minimise ants, insect and pests. Plastic bags are removed from the packs and are then placed vertically into the trench (80 per m^2).

Figure 1.11 Trench method of planting mushroom (Taken by me, NJ Tembe).

About 350 substrates are placed inside the trench in a range of 10 to 11 lines. Substrate packs must be on the same level to allow equal distribution of water and avoid water runoff, short packs are supported with treated soil underneath. Fifty substrates packs are crushed over the 350 substrates to cover the planted packs and close the gaps. As an alternative method, substrate packs may also be covered with 1 cm treated soil to retain moisture (3 kg hydrated lime to 20 kg soil), irrigate with 60 l of clean drinking water.

The trench is covered with plastic for four days without opening. From day 5, until the pins appear it is watered daily with 10 l of clean water. The amount of water may change, depending on the condition of the trench. When the trench is too dry 10 l might not be enough, one or two more (10 l) may be required. Once the fruit bodies (mushrooms) appear, the amount of water applied is reduced to 5 l per day. In spring, autumn (up to 1000 m above sea level) and winter (low altitudes along the coast) the tunnel is opened between 10:30 and 11:00 am (30 minutes) every day to avoid cold air of early morning hours. In early summer (October and November) it is opened between 8:30 and 9:00 am (30 minutes) every day to avoid hot air. During the mid-summer heat (December to February), it is opened between 8:30 to 9:00 am (30 minutes) and then again from 2:00 pm to 2:30 pm (30 minutes) to avoid heat in the trenches. If it is windy, time is reduced to 15 minutes at a time irrespective of season to minimise water evaporation. If it is raining the tunnel is closed for that day, irrespective of season. It always watered once a day, irrespective of the season.

Harvesting commence once the mushroom caps are 30 mm in diameter. The harvested mushrooms are cleaned then refrigerated as soon as possible. Oyster mushrooms may also be stored at room temperature for up to two days (Macdonald *et al*., 2007).

Wall method: Wall method is the simplest method of growing fresh mushrooms compared to trench method. It saves time and space. The top of the substrate is cut and placed on the wall as shown in Figure 1.12.

Figure 1.12 Wall method of planting mushroom (Taken by me, NJ Tembe).

Wall method also demand a clean environment with air flow, sometimes humidifiers and air conditioners are required to maintain temperature and humidity. Proper irrigation with humidifiers is required in a well-ventilated room to avoid dryness on the top of the substrate where the pins arise. With proper maintenance of the wall method, the first harvest may be obtained within 5 days.

1.3.7. Management of spent mushroom substrates (waste)

Mushroom production should be an environmental friendly activity as it utilizes organic wastes. The used substrates may become waste themselves if not correctly recycled. Spent substrate has been found to be a good fertilizer because of its nutrient-status (Table 1.2). It is rich in nitrogen, phosphorus and potassium, which act as a good growing medium for vegetables (Zhang *et al*., 2012).

Some countries use spent mushroom substrates on livestock feeding and as for biogas production. Contaminated spent mushroom substrates need to be treated or kept far from the farm in order to prevent re-contamination (Rinker *et al*., 2004).

Table 1.2 Comparing fertilizer value of compost from the edible *Pleurotus ostreatus* (Zhang *et al*., 2012).

Fertilizers	**N (%)**	**P_2O_3 (%)**	**K_2O (%)**
Pleurotus compost	1.7	0.61	1.13
Human manure and urine	0.3	0.16	0.3
Pig manure	0.6	0.6	0.5
Cow manure	0.59	0.28	0.14

1.3.8. Pathogens and pest affecting *P. ostreatus.*

Pathogens and pests affecting *P. ostreatus* may differ from country to country, provincially and even with districts mainly due to different environmental conditions. South Africa has a subtropical climate which is suitable for the cultivation of *P. ostreatus* and this climatic condition supports infectious agents as well. Mushroom cultivation conditions such as high humidity, warm temperature and limitation on chemical use are the major reasons for existence of many diseases and pest problems in mushroom cultivation (Cha, 2004).

A study conducted in a Gauteng farms has shown that *Cladobotryum mycophilum* and *Verticillium fungicola* are the two fungal pathogens that mostly attack the basidioma of the oyster mushrooms. During a three-year study of moulds present in pasteurised substrates a number of fungi were isolated on oyster mushrooms. These included *Trichodema viride*,

producing green mould on a substrate (Figure 1.13), caused by poor pasteurisation, poor air exchange and hygienic practices (Cohen, 2002).

Figure 1.13 Substrates contaminated with *Trichodema* spp (Cohen, 2002).

Trichurus spirales produce a small black hair like synnemata and *Chrysonilia sitiphila* which produce pink bread mould. This is mainly due to the use of contaminated spawn. Insects, pest and nematodes were also found to be more problematic in summer and autumn in South Africa. Beetles are sometimes found in growth rooms while wet substrates led to wide spread of flies (Elliott, 1995).

Brown blotch diseases are mainly caused by *Pseudomonas tolaasii* and *Pseudomonas agarici*. These bacteria have caused a significant crop loss in Korea. The most typical symptom is a brown spot on the caps and stipe. Viral disease - two isometric viruses were isolated from oyster mushroom showing viral disease symptoms. These viruses resulted in delay of fruiting body formation, shortening in stipe, abnormal shape and thin mushroom caps.

Pest - Sciarids (*Lycoriella mali*) are the most important pest of oyster mushroom. The larvae of scarids feed on mycelia, small pinheads and large mushrooms resulting in cuts in the

mycelium, less premodium formation and cavities. Scaptosids (*Coboldia fuscipes*), Scaptosids (*Coboldia fuscipes*), Cecids (*Mycophila* spp.), Phorids (*Megaselia tamiladuensis*) are the flies known to transfer mites and diseases. Larvae feed on the mycelium causing rotting of substrates and make cavities in mushroom fruiting bodies which results in yield loss. Mites - *Tarsonemus* spp. and *Histostoma* spp. are the major mushroom damaging mites. Mites feed on mycelia and fruiting bodies causing yield loss and decrease in mushroom quality (Cha, 2004).

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CHAPTER 2

DETERMINATION OF ALTERNATIVE SOURCES OF NITROGEN, CARBON AND MUSHROOM PACK PREPARATION TO DETERMINE CORRELATION BETWEEN PARTICLE SIZE AND AIR FILLED POROSITY (AFP) IN A 150 X 300 MM PLASTIC BAG

2.1. INTRODUCTION

Agro-waste such as sugar cane bagasse, wheat bran, hominy chop and sawdust have mostly been used for oyster mushroom production (Bellettini *et al*., 2016). Not much has been said about the use of grasses such as juncao, lurcerne and teff. These three gasses and maize were added at Department of Agriculture and Rural Department (DARD) formula to find other sources of C/N and optimum yields as Owaid *et al*. (2015) indicated that the mixture of different raw material may increase the biological efficiency. Mixing more than one raw materials could increase the protein content of the oyster mushroom. Kunjeku and Maliwichi, (2009), indicated that protein content of oyster mushroom depends on the substrate on which they are grown.

Juncao grass is a giant napier hybrid (*Pennisetum purpureum X P. typhoideum*). It is a tall growing (3 m +) grass with a strong fibrous root system. Juncao grass can be used as a feed for cattle, goats and poultry and may also be used for prevention of soil erosion (Macdonald *et al*., 2010). Professor Lin Zhanxi and his team of the Juncao institute in Fuzhou (China) have established the use of juncao as a mushroom substrate (Macdonald *et al*., 2011).

Teff (*Eragrostis tef*) is an annual tufted grass that reaches a height of 150-200 cm at maturity. It is valued for both grain and forage production. Farmers use teff straw as animal feed, especially during the dry season (Alemu, 2014). Teff is predominantly grown in Ethiopia. It has been introduced in South Africa in recent years and is cultivated as a forage crop (Refera,

2001). Some studies have shown that teff can be used as a substrate in mushroom production (Alemu, 2014; Tsegaye, 2015).

Lucerne is a perennial flowering plant in the legume family Fabaceae. The plant can grow up to about a metre in height. It is cultivated as forage used for grazing, hay and silage (http://www.lucerneaustralia.org.au; https://www.google.co.za).

Carbon and nitrogen (C/N) are important nutritive factor for optimal substrate composition for *P. ostreatus*. They supply energy required by oyster mushrooms to grow (Hoa *et al*., 2015). Different raw materials contain different amounts of carbon and nitrogen. A combination of different raw materials may form an ideal C/N ratio. Kang, (2004) recommended Carbon nitrogen ratio of 30:1 as the best for oyster mushroom production. Bellettini *et al*. (2016) have considered the range between C/N ratio 28:1 to C/N ratio 30:1 as the best for optimum yields.

The concentration of nitrogen source in the compost shall not be too high. Oyster mushrooms require less nitrogen and more carbon source. If the concentration is too high, the hyphae may grow at a rapid speed and the fruit will be retarded (Tsegaye, 2015).

Most organic matters containing cellulose, hemicellulose, lignin, starch, glucose, maltose, mannose, saccharose and pectin can be used as a source of carbon in mushroom substrate i.e. rice straw, wheat straw, cotton seed hulls, sugarcane, bagasse, sawdust, waste paper, leaves etc. For nitrogen sources, peptone, ammonium sulphate, asparagine, corn powder, bean cake powder etc. may be utilized by the *P. ostreatus* (Lin *et al*., 2018).

According to a study conducted by Mbassi Josiane *et al*. (2018), there is variation in proximate composition of the *P. ostreatus* grown from different substrates, which could be attributed to the nutritional composition of the substrate where they were cultivated and C/N ratio is one of the factors. In this chapter different raw materials such as wheat bran, teff, lucerne, bagasse,

maize, juncao and sawdust were analysed and its carbon nitrogen ratio content were used to set different C/N ratios.

The bag cultivation system offers some important advantages over the natural tree log production system. Bag production takes a shorter time, provides stable yields with relatively few failures (Royse *et al*., 2004). There are different types of bags that may be used for cultivation but, Rinsanka, (1980) recommended the polycarbonate gusseted bags that are 0.04 mm thick and have dimensions 20 × 27 cm when folded with a capacity to hold 1-1.5 kg of substrate. In this study 300 mm long and 150 mm in diameter polypropylene bag were used, as indicated in DARD method (Macdonald *et al*., 2011).

The coarse raw materials such as bagasse, lucerne and teff need to be milled in order to make it easier to mix with other raw materials, easier access of nutrients and minimises damage to plastic bags used when bagging the substrate. Too fine particles of raw materials may increase the compactness of the substrate pack, decrease air circulation and slowing the mycelium growth rate (Royse and Sanchez 2000; Yildiz *et al*., 2002). Nevertheless, the yield will be reduced if the substrate is packed too loosely (Lin *et al*., 2018).

The effect of rice and wheat straw particle size reduction by milling and chopping was determined by Zhang *et al*. (2002). The milled straw yielded higher (oyster mushrooms) than the chopped straw. However, it was found that when the straw was milled into particles that were too small, the mushroom yield decreased.

The study conducted by Patel *et al*. (2009) also indicated that one of the effects of reducing porosity of the substrate is the limitation of oxygen transfer, thus limiting the growth within the substrate. Therefore, in this chapter different particle sizes of the coarse raw materials (bagasse, lucerne, juncao and teff) were prepared. Mushroom packs were produced (DARD method) and the correlation of particle size and air filled porosity was determined. AFP was

defined as the best measure to determine the available porosity in a composting material, or in general, in an organic matrix by Ruggieri *et al*. (2009).

2.2. Aim and Objectives

2.2.1. Aim

To find alternative sources of carbon and nitrogen and to prepare substrate packs in order to determine the effects of particle size in mushroom production.

2.2.2 Objectives

- To determine carbon and nitrogen content of the raw materials
- To find different C/N ratios for the growth of oyster mushrooms
- To prepare different raw material particle size
- To produce substrate packs in a 150 x 300 mm plastic bags
- To determining air filled porosity
- To determine the correlation between particle size and AFP

2.3. Methodology

This study was conducted at KwaZulu-Natal Department of Agriculture and Rural Development (DARD) – Cedara. The project named Isikhowe juncao mushroom project was initiated in 2005 with the arrival of oyster mushroom expert team from China. The South African Government and Peoples Republic of China always had been partners in a number of projects and this is one of those.

Juncao Mushroom Technology was introduced to South Africa with an objective to eradicate poverty and ensuring food security and sustainable job creation in KwaZulu Natal rural communities. The Project has currently employed more than 20 permanent staff. This project has generated more than 50 food security gardens and 2 Satellite bases producing oyster mushrooms. This shows the impact of the project in South Africa and the huge need for research to improve the methods of production. DARD method of oyster mushroom was used as a control for this study. The current existing M.O.U. between the University of Zululand and DARD provided an opportunity to utilize all their available resources to make this study a success.

2.3.1 Analysis of carbon, nitrogen content and C/N ratio formation

Different cellulose rich raw materials such as wheat bran, teff, lucerne, bagasse, maize, and sawdust were analysed for carbon and nitrogen content using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Analysis). The carbon and nitrogen content were used to set up the following C/N ratios 15:1, 25:1, 35:1, 70:1 and 100:1. These C/N ratios were achieved by using Tom Richard and Nancy Trautmann formulae in a spread sheet program.

$$R = \frac{Q_1(C_1 \times (100 - M_1) + Q_2(C_2 \times (100 - M_2) + Q_3(C_3 \times (100 - M_3) + \ldots}{Q_1(N_1 \times (100 - M_1) + Q_2(N_2 \times (100 - M_2) + Q_3(N_3 \times (100 - M_3) + \ldots}$$

Where: R = C/N ratio of compost mixture Qn = mass of material n ("as is", or "wet weight") Cn = carbon (%) of material n Nn = nitrogen (%) of material n Mn = moisture content (%) of material n.

Milling of the dry raw materials (bagasse, lucerne, juncao and teff) was conducted in three phases: slight particles – 8 mm, medium particles – 12 mm and large particles - 25 mm. Different sieve sizes of the milling machine were used to achieve the desirable particle size.

Substrate preparation - Mixing of milled raw materials with hominy chop, wheat bran and fine sawdust was conducted on the floor using hand and spades. Water was added according to 1:1 ratio (1 kg: 1 l) to achieve the recommended 65% moisture. The rule of thumb method, whereby 1-2 drops of water are released when the substrate is squeezed was also used to ensure that there is enough water in the substrate (Mkhize *et al*., 2017).

When all the raw materials were well mixed, they were bagged in a 300 mm long and 150 mm in diameter polypropylene bag. The mixed substrate was equally compacted to make 1 kg bag and capped with a filtered cap. The capped substrate bags were placed in the steamer for pasteurization at 90^0C for 14 hours. After pasteurization, the substrates were cooled at room temperature. Cooled substrates were inoculated with approximately 3 g of *P. ostreatus* spawn, following the aseptic techniques under the lamina flow. Four substrates were isolate per treatment for AFP analysis. AFP was calculated by dividing drained volume of water by total volume recorded using a porometer.

2.4. Results and Discussion

Table 2.1 provide the analysis of the results of carbon and nitrogen content in different agro-wastes. Table 2.2 shows different formulae that form different C/N ratio using Tom Richard and Nancy Trautmann formula. A wide range of C/N ratios (15:1 to 100:1) were formed to accommodate most of the farmers as they may prefer different C/N ratios.

In preparation of raw materials, different particles sizes of the raw materials where prepared in order to determine the air filled porosity (AFP) in a mushroom pack. Different raw materials particle sizes were weighed accordingly to form the desired carbon nitrogen ratio. Mushroom packs for each combination of particle size and C/N ratio were produced. Air filled porosity

for each particle size and C/N ratio combination was analysed and the correlation between particle size and AFP was determined.

2.4.1. Sources of carbon and nitrogen

Carbon and nitrogen content of agro-waste will always vary even if there are of the same plant species. Carbon and nitrogen content are influenced by different growth methods and environmental factors. For example, when increasing N-uptake at inadequate N-supply by increasing rooting volume and density. The biomass and C/N ratio may increase (Lawlor, 2002). Therefore, if another analysis based on Table 2.1 can be done using the same agro-waste from different environment or growth method, it may provide different results. Furthermore, a study conducted by Ma *et al*. (2018) attested that plant C contents varied significantly among life forms.

Table 2.1 Carbon and nitrogen sources

C/N Source	C	N
Bagasse	41.3	0.5
Wheat bran	38.9	2.7
Maize	37.5	1.4
Sawdust	41.6	0.1
Hominy chop	39.9	1.6
Juncao	40.8	0.8
Lucerne	43.1	3.0
Teff	39.8	2.4

Bagasse and juncao (Table 2.1) shows the similarities in terms of C and N composition. The structural composition of the juncao and the sugar cane as well look alike in such a way that some people refer juncao as traditional sugar cane. In the absence of bagasse at DARD, juncao is used as the substitute (Macdonald *et al*., 2011). Considering the above similarities, even the mushroom yields using bagasse or juncao with different formulae should be approximately the same.

The carbon and nitrogen content of wheat bran and teff (Table 2.1) are approximately equal. The use of local readily available raw material is always encouraged in order to minimize the cost of mushroom production. Therefore, depending on the availability, farmers can use either teff or wheat bran.

Hominy chop is a by-product of dry corn milling when dry millers make corn grits, corn meal, corn flour and corn oil. Since it is a by-product, the nutrient content of hominy chop can vary from mill to mill (Lundquist, 2009) but is expected to be approximately the same with maize. Maize is expensive compared to hominy chop, therefore farmers will always opt for hominy chop if both maize and hominy chop are available.

2.4.2. Carbon and nitrogen ratio

Same C/N ratio can be formed by different substrate composition, for example, C/N ratio 35:1 and DARD C/N ratio 35:1 (Table 2.2). Different quantities of Juncao, teff, lucerne and maize was added in the DARD formula. However, the C/N ratio remained the same (35:1). Using Tom Richard and Nancy Trautmann formulae to calculate C/N ratio could be very beneficial to mushroom farmers since they can use any readily available raw material to achieve their desired C/N ratio.

The influence of wheat bran was noted in these formulas. As wheat bran decrease from 60.4 to 1%, the C/N ratio increased from 15:1 to 100:1. Wheat bran is a nitrogen source. Therefore, this could mean that as the nitrogen concentration increased, the carbon concentration decreased in the substrate. A review study conducted by Bellettini *et al*. (2016) concur with these results as it showed that during *pleurotus* spp. mycelium growth, wheat bran elemental composition decreased in its carbon content, then increased in nitrogen and oxygen content,

suggesting a preferential degradation by the fungal mycelium for certain polysaccharides than the others, and accumulation of proteins in the substrate.

At low C/N ratio (15:1) nitrogen rich raw materials (wheat bran, lucerne and teff) are higher and carbon reach raw material (bagasse, sawdust and juncao) are low while at higher C/N ratio (100:1) carbon rich raw materials are higher and nitrogen rich raw material are low (Table 2.2). Therefore, the mushroom farmers must know the carbon and nitrogen content of their raw material in order to easily obtain their desired C/N ratio.

Table 2.2 Different Carbon nitrogen sources forming different C/N ratios ($CaCO_3$ excluded).

C/N source %	**15:1**	**25:1**	**35:1**	**70:1**	**100:1**	**35:1(DARD)**
Bagasse	1.1	9.1	31.3	59.3	49.5	40
Wheat Bran	60.4	22.7	6.3	1.7	1	15
Maize	2.2	4.5	12.5	16.9	1	0
Sawdust	0.1	4.5	12.5	16.9	44.6	10
Hominy chop	1.1	22.7	12.5	0.8	1	30
Juncao	2.2	27.3	12.5	16.9	1	0
Lucerne	16.5	4.5	6.3	1.7	1	0
Teff	16.5	4.5	6.3	1.7	1	0

2.4.3. Particle size

The sieves used in a milling machine are designed to allow most of the desired particle sizes to pass through. However, in practical, when doing sieving analysis of a particle collective, you may find that it cannot produce a sharp cut between particle size classes. In this study it was found that some smaller particles have passed through the sieves but the majority of the particle were representing the desired size. Therefore, the desirable particle size of 8, 12 and 25 mm was fairly achieved.

2.4.4. Substrate packs production and AFP analysis

Substrate packs were successful produced using DARD method. The mixed raw materials were equally compressed manually by hand up to a marked level on the plastic bag and weighed (1 kg) in order to avoid variations. AFP was calculated by dividing drained volume of water by total volume recorded using a porometer. Table 2.3 shows the AFP results of different particle sizes. The control has C/N ratio of 35:1 and 8 mm particle size, although the raw material used for experimental formula of 35:1 and 8 mm particle size are different, the obtained AFP was almost the same (Table 2.3). These observations do not necessarily mean that the same C/N ratio will have the same AFP.

Table 2.3 AFP analysis results

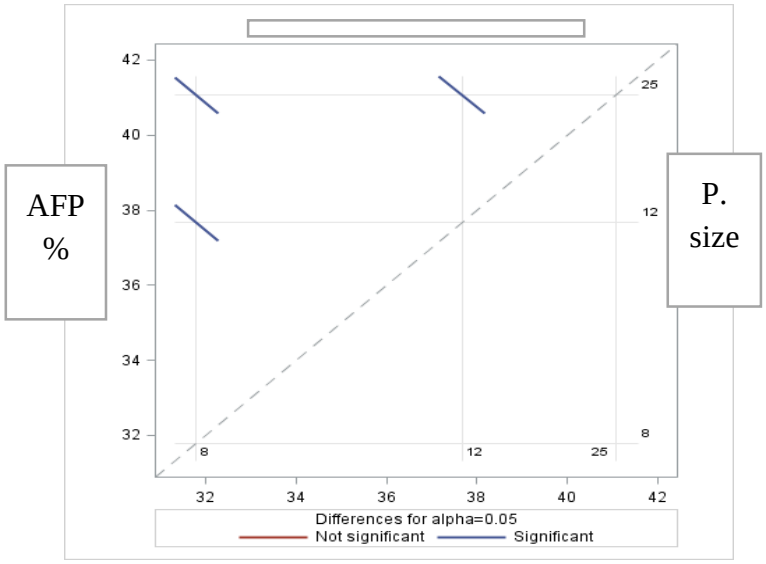
C/N ratio	Particle size(mm)	AFP (%)
15:1	8	37
15:1	12	38
15:1	25	44
25:1	8	42
25:1	12	43
25:1	25	45
35:1	8	38
35:1	12	40
35:1	25	47
70:1	8	23
70:1	12	32
70:1	25	34
100:1	8	21
100:1	12	38
100:1	25	39
Control	8	37

Different C/N ratios were formed by different amounts of raw materials (Table 2.2). This variation seems to impact the AFP, since there was variation in AFP of the same particle size (Table 2.3). Physical composition of the raw material might have contributed to these variations. Considering the physical composition, juncao is the coarsest raw material since it is

even hard to break than sugar cane. The highest percentage of juncao is 27,3% on C/N ratio of 25:1 (Table 2.2), this formula produced relatively higher AFP even on the smallest particle size of 8 mm (Table 2.3). This observation could mean that the formulae with higher percentage of coarse raw material are expected to have higher percentage of AFP.

2.4.5. The effect of particle size on AFP

The average AFP was calculated and the graph (Figure 2.1) was plotted to analyse the correlation between the particle size and AFP. There was a positive correlation between particle size and AFP (Figure 2.1). As the particle size increased the AFP also significantly increased. The raw material particle size of 8 to 12 mm and 12 to 25 mm both showed the significant difference in terms of AFP (p =0.05). Understanding of the correlation between particle size and AFP will help farmers in determining the best particle size for their raw material. For example, if there is no enough oxygen in the mushroom pack, farmers will have to increase the particle size of the raw material in order to increase the space between the particles (AFP) thus improving the oxygen transfer (Bellettini *et al*.,2016)



Particle size(mm)	AFP (MEAN)	Standard Error	Pr > |t|
8	31.7988506	0.3263678	<.0001
12	37.6632997	0.3532797	<.0001
25	41.0649819	0.3658113	<.0001

Figure 2.1 Mean AFP comparisons for particle size

2.5. Conclusion and Recommendations

Alternative sources of carbon and nitrogen may be obtained through analysing the available raw material using ICP-OES. If the amount of carbon and nitrogen are known, desirable C/N ratio can be achieved using Richard and Nancy Trautmann formula. This formula may be very useful to emerging mushroom farmers. It may help farmers to calculate C/N ratio for any local readily available raw materials, giving them a choice to avoid costly raw material. Using the local readily available raw material may reduce the production costs, thus allowing farmers to make good returns. The farmers must know their local readily available sources of carbon and nitrogen and try to use them in mushroom production. The newly introduced sources of carbon and nitrogen (juncao, lucerne, maize hominy chop, teff and maize) in DARD formula seems to have a good potential for mushroom production since they contributed to achieve the desirable C/N ratios. In chapter 3 of this study, detailed discussion on performance of each formula in terms of mycelium growth and biological efficiency was provided. Particle size has an influence in AFP. The higher the particle size the higher the AFP. These results concur with a review study conducted by Bellettini *et al*. (2016), which indicated that particles with large size causes an increase in space between particles (AFP). The type of raw material and cultivation system are the one which may inform the farmers about the suitable particle size of the raw material. Farmers must know the nature of their raw material, either it requires to be too fine or larger in other to be productive. The trial and error method using the local available raw material to find the best particle size could be the best option. DARD uses 150 x 300 plastic bags. Larger and sharp particles cannot be easily packed and compressed in such small plastic bag. They could tear or prick the bag, exposing the substrate to contaminants. Therefore at least 1 – 12 mm could be the suitable particle size for the raw material. In case the particle size need to be increased above 12 mm, the size of the plastic bags also has to be increased.

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CHAPTER 3

EFFECTS OF C/N RATIO AND PARTICLE SIZE ON MYCELIUM GROWTH AND BIOLOGICAL EFFICIENCY ON OYSTER MUSHROOM PRODUCTION

3.1. INTRODUCTION

Mushroom production may be influenced by many factors, which may act individually or have interactive effects among them. Chemical composition, environmental factors (temperature, humidity and luminosity), water activity, ratio of carbon to nitrogen, minerals, surfactant, pH, moisture, sources of nitrogen, particle size, and amount of inoculum, antimicrobial agents and the presence of interactions between microorganisms are considered as chemical, physical and biological factors that are linked to mushroom production (Bellettini *et al*., 2016; Uddin *et al*., 2011). According to Curvetto *et al*. (2002), mushroom yield and Biological efficiency are closely linked to nutrient type and growth conditions. In this study the main focus was to study the effects of C/N ratio and particle size on mycelium growth, biological efficiency and mushroom yield.

When a compact white mass (mycelium) is formed all over the mushroom pack, it may be planted to produce fresh mushrooms (Sales-Campos *et al.*, 2011; Okwulehie and Okwujiako, 2008). The mycelium growth period may vary, since mycelium growth depend upon different factors such as the type of inoculum used, type of raw material and conditions of inoculation place. Environmental factors such as temperature, relative humidity, light, gas exchange and water availability within the mushroom pack may trigger the fruiting. These induction factors may also have impact on the quality of fresh mushrooms (Zadrazil and Grabbe, 1983). For

Pleurotus spp. in general it takes 20-30 days to reach full growth (Turković, 2015; Tsegaye, 2015).

Mushroom fruiting usually occurs in flushes, also named rhythmic cycles (Chang, 2008). Under proper fruiting conditions, the additional flushes may occur without any new inductions but the flushes can be controlled by heating the blocks/bags followed by reducing the temperature (Sales-Campos *et al.*, 2011). Another method of inducing flushes is the sprinkling of water over the bags (Przybylowicz and Donoghue, 1990).

Mushroom may be harvested by simple grasping each mushroom stalk individually and twisting the mushroom until it is pulled out of the substrate (Khare *et al*., 2006). The mushroom harvesting period may vary but usually ranges from 6-12 weeks and they can be harvested on a number of flushes (Przybylowicz and Donoghue, 1990). The proper size for mushrooms to be harvested may depend on the mushroom species, market value and consumer preferences (Chang and Chiu, 1992; Chang, 1998). Harvesting of mushrooms should be carefully carried out before gills open in order to avoid a decreased market value and quality of mushroom (Singh and Mishra, 2008). However, it all depends to the farmer's preference and the market requirements. A farmer at uMsunduzi municipality (Pietermaritzburg) grows oyster mushrooms with the purpose of drying them for processing. The farmer mostly prefers the big mushrooms (over grown) as they have high dry weight.

3.2. Aim and Objectives

3.2.1 Aim

To determine the best physical and chemical composition required by oyster mushroom using a plastic bag method for DARD formula.

3.2.2 Objectives

- To determine the effects C/N ratio in mycelium growth and biological efficiency of oyster mushrooms.
- To determine effects of particle size in mycelium growth and biological efficiency oyster mushrooms.
- To establish the best combination of particle size and C/N ratio against mycelium growth, biological efficiency and contamination rate in oyster mushroom production.

3.3. Methodology

The rate of mycelium growth and time of full colonisation of a substrate was measured in 10 days' interval using a 30 cm ruler. Three points were made on 3 different sides of the mushroom pack. In 10 days' interval, a ring was made at the end point of the mycelium growth mark. The rate of the mycelium growth was calculated through measuring the distance from the top, down to each of the rings at three equally spaced points around the mushroom pack.

Fully colonised substrates were moved to fresh mushroom growth room, which provided the following fruiting requirements: temperature of 15 to 20^0C, a humidity of 80 to 95%, a good ventilation, light and sanitation. The mushroom packs were cut on the top and placed horizontal

against the wall on a steel rack. The irrigation was done daily, approximately two litres per 50 substrates using a spray bottle.

The planted mushroom packs were monitored and harvested for the period of three months. When the mushroom cap reached an approximately 30 mm diameter, it was harvested and weighed. The Biological Efficiency (BE) was calculated according to Royse *et al*. (2004) and Stamets, (2000) equation: BE = [weight of fresh mushroom harvested (g)/ dry substrate weight (g)] × 100.

During mycelium growth measurement, the number of contaminated bags for each treatment were recorded in order to determine which treatment was less or more attractive to foreign microorganisms. The data was analysed using Statistical Analysis System (SAS) software.

3.4. Results and Discussion

The effects of C/N ratio and particles size on biological efficiency and mycelium growth are discussed in details. The best combination of C/N ratio and particle size was also determined. The best formula was the one with the higher rate of mycelium growth, low contamination and higher biological efficiency.

3.4.1. Biological efficiency

The biological efficiency (BE) is used as a measure of substrate conversion into fresh mushrooms. It basically provides the yield potentials (Oseni *et al*., 2012). The BE was worked out against the dry weight for each mushroom pack. Table 3.1 shows the average BE of each combination of C/N ratio and the particle size in order to determine the most

effective combination. For each combination of C/N ratio and particle size, 50 mushroom packs were prepared.

Table 3.1 Average BE (%) on each C/N ratio and particle size (mm) of the raw material.

C/N ratio	Particle size	BE
15:1	8	0
15:1	12	0
15:1	25	16.98
25:1	8	39.37
25:1	12	38.02
25:1	25	40.09
35:1	8	112.68
35:1	12	80.02
35:1	25	70.45
70:1	8	58.27
70:1	12	55.76
70:1	25	60.11
100:1	8	38,07
100:1	12	40.09
100:1	25	49.22
Control	8	99.27

For the combination of C/N ratio 15:1, particle size 8 and C/N ratio 15:1, particle size 12 mm, its mushroom packs were not planted. The mycelium growth rate was low, in 40 days the mycelium in most of the mushroom packs could not reach the 50% of the mushroom pack. After 40 days', the mushroom packs began to rot and as a result they were discarded. Hence, the BE was zero (Table 3.1). On the same C/N ratio (15:1) with a particle size of 25 mm, 16.98% BE was recorded. This could be the effect of particle size since it is the only factor that brought the difference. The highest BE (112.68) which was even more than the control was achieved at C/N ratio 35:1, with particle size of 8 mm.

3.4.2. Effects of C/N ratio on mycelium growth and biological efficiency

Analysis based on Figure 3.1 and Figure 3.2 assisted to determine whether it is C/N ratio or particle size which immensely contributed to achieve the higher BE. The effect of each factor (C/N ratio and particle size) was studied against mycelium growth and biological efficiency. The Mycelium growth analysis of oyster mushroom, will help inform the farmers about the average duration (days) that the mushroom packs may take to be fully colonised from inoculation day. This further helps to estimate the duration of the entire mushroom production process.

In this study the mycelium growth was expressed in percentages (%) where by a fully colonised mushroom pack is equivalent to 100%. The change of mycelium from one marked point to another defines the mycelium growth. Mycelium grows from top where the inoculum was place to bottom and each movement marks certain percentage of growth within the mushroom pack.

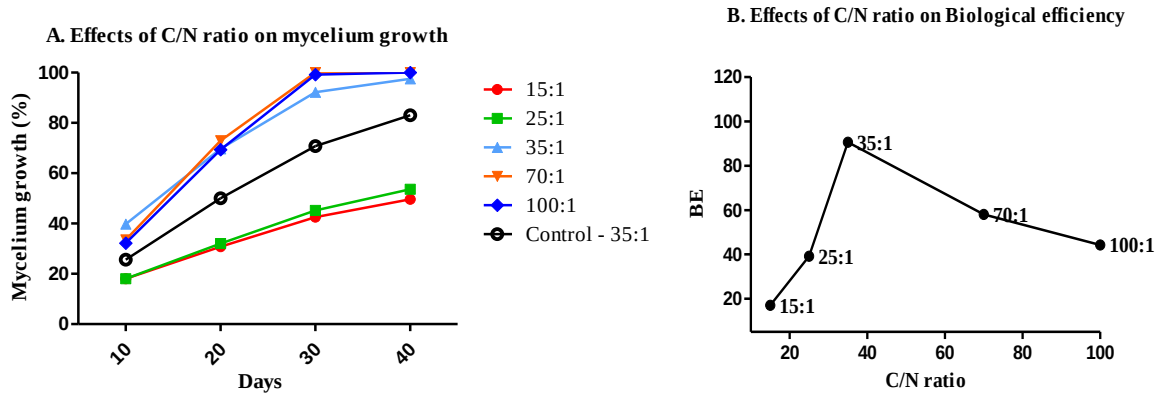

Figure 3.1 Effects of C/N ratio on mycelium growth and biological efficiency

The average percentages of each C/N ratio were calculated, regardless of its particle size. All three particle sizes where included in order to eliminate its effect in this regard. The average BE for each C/N ratio was also calculated. The results are presented in Figure 3.1. At C/N ratio 15:1, the mycelium growth on most of the mushroom packs could not reach 50% in 40

days. As the result, most of the packs were not planted. This affected the average biological efficiency (BE) as indicated on Figure 4.1 that C/N ratio 15:1 has the lowest BE.

The mycelium growth in C/N ratio 25:1 is slight above the C/N ratio 15:1. In 40 days, the mycelium growth in most of the mushroom packs were on average around 50%. Although most of the mushroom packs were planted, but some could not reach 60% marked point of the mushroom pack. A slight increase on the BE was also observed (Figure 3.1).

C/N ratio 35:1, 70:1 and 100:1, its mycelium reached 60% growth mark on the mushroom pack in 20 days, and eventual most of the mushroom packs reached 100% in 30 days. Turković, (2015) and Tsegaye, (2015) they also indicated that *pleurotus spp* usual takes 20 to 30 days for mycelium growth. The mycelium growth rate for these C/N ratios were quicker than the control. These observations may trigger the temptation to conclude that the higher the C/N ratio the higher the mycelium growth rate. But other factors like particle size still need to be considered.

In average, C/N ratio 35:1 had the highest BE, followed by C/N ratio 70:1. The BE gradually increased with increasing C/N ratio up to the point where it reached its highest peak (C/N ratio 35:1) and thereafter started to decrease as the C/N ratio gradually increased. These observations could mean that there are limitations in terms of C/N ratio, a certain C/N ratio range is required for the growth of *pleurotus* spp.

The total carbon value in the C/N ratio represents the carbon content, therefore increase in C/N ratio means increase in carbon content and decrease in nitrogen (Ryu *et al*., 2015). Relating the findings of Ryu *et al*. (2015) to these results could mean that C/N ratio 15:1 is the lowest and has higher a nitrogen content. The C/N ratio 100:1 is the highest and has a lower nitrogen. As the C/N ratio increased (15:1 - 35:1), the nitrogen decreased up to optimum amount required

by *pleurotus* spp since the BE increased and reached its peak at C/N ratio 35:1. After a 35:1 C/N ratio, the nitrogen was becoming low and as a result, the BE also began to decrease.

Bellettini *et al*. (2016) have cited Silva *et al*. (2007) in his review study indicating that the supplementation with nitrogen can increase crop productivity, but to a certain level, as high nitrogen values can inhibit fruiting of mushrooms. The study also indicated that there was growth inhibition when *P. ostreatus* was cultivated in hydrolysed sugarcane bagasse without added nitrogen. However, the addition of different nitrogen sources in levels greater or equal to 1.5% nitrogen also inhibited the growth of *P. ostreatus*. These analyses indicated that nitrogen concentration was required at a specific concentration since high or low affected the productivity. This study (Figure 3.1 B) to concurred with the previous reported findings that the centred C/N ratio (35:1) poses higher BE.

According to the results obtained in this study the optimum C/N ratio required by *P. ostreatus* to achieve the highest BE could be between 26:1 to 35:1, since as from C/N ratio 25:1 the BE peak went straight up and from the C/N ratio 35:1 it decreased. The findings can be related to the studies conducted by Bellettini *et al*. (2015), Naraian *et al*. (2009) and Urben (2004) as cited by Bellettini *et al*. (2016), that C/N ratio 28:1 to 30:1 is an important condition for mushroom production.

3.4.3. Effects of particle size on mycelium growth and BE

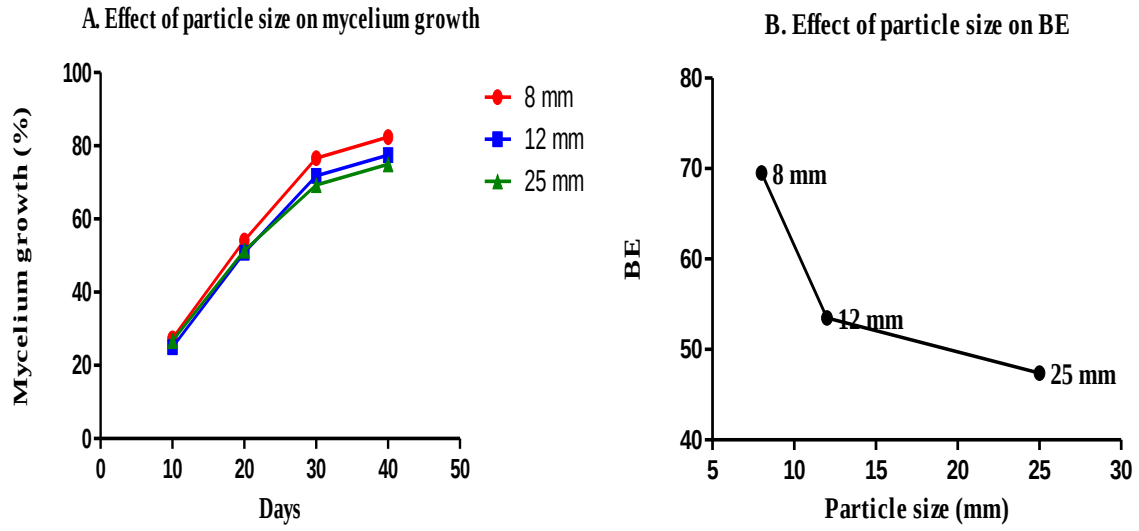

Figure 3.2. Effects of particle size on mycelium growth and BE

The average mycelium growth over 40 days was used in order to determine the effects of particle size (Figure 3.2 A). The particle sizes in all C/N ratios was used to determine the average mycelium growth. Therefore, in this case C/N ratio has no effect on the results illustrated on figure 3.2. In every 10 days' interval that the mycelium growth was measured, a significant difference was observed ($p = 0.05$). On day 30 the mycelium growth of particle sizes 8 and 12 mm was above 70% average, and most of the mushroom packs were fully colonised and ready to be planted. The 25 mm particle size was below 70% at day 30. All three particle sizes though, seemed to have a significant impact on the mycelium growth ($p = 0.05$). The 8 mm particle size had the highest impact, reaching above 80% in average in day 40 ($p = 0.05$). The 25 mm particle size had the lowest growth rate, reaching a maximum of 74% at day 40. These results only indicated the effect of particle size on the mycelium growth. Its relation with other factors like C/N ratio and the best combination of particle size and C/N ratio is discussed later. On average the results showed that the higher the particle size, the lower the mycelium grow. The 8 mm was the smallest particle size and had a higher rate of mycelium

growth. These results concur with Yildiz *et al*. (2002) who indicated that the smaller the particles provide a larger surface area used by mycelium, thus increasing in its biomass.

The findings by Yildiz *et al*. (2002) also make sense to this study considering that in nature the oyster mushroom grows on wood which may be defined as compact with a very low air filled porosity (AFP). The kind of compactness on wood though, may be impossible to achieve using plastic bag. In Chapter 3 it was indicated that the higher the particle size, the higher the AFP. Therefore, this could mean that the *pleurotus spp* require the particle size factor lower than 12 mm for mycelium to grow faster using plastic bag (150 x 300 mm) method. Nevertheless, it will be interesting to know the average effect of the particle size on biological efficiency as well, since farmers are more interested on the produce.

The average of biological efficiency achieved on each particle size is shown in figure 3.2 B. Particle size of 8 mm had the highest biological efficiency and 25 mm has the lowest. Based on these results, a conclusion that the higher rate of mycelium growth and higher BE can be achieved with the mushroom pack particle size of 8 mm in the plastic bag (150 x 300 mm) can be made.

3.4.4. Effects of the combination of the particle size and C/N ratio on mycelium growth

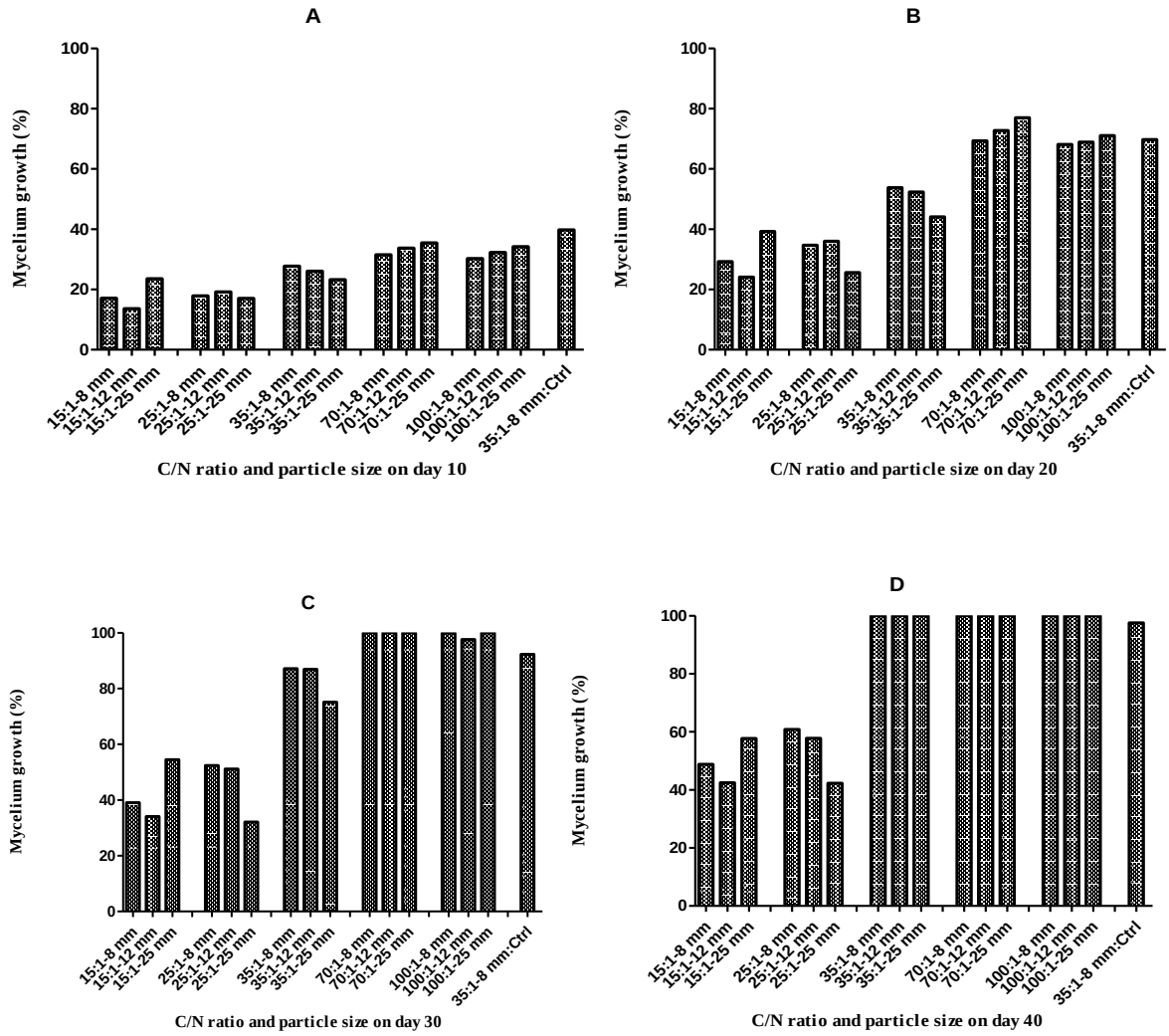

Figure 3.3 Effects of particle size and C/N ratio on mycelium growth in 10 days' intervals

The mycelium growth in the mushroom packs were measured in 10 days' intervals. Four intervals were recorded as indicated by letters A, B, C and D on Figure 3.3. At 10 days' intervals, the mycelium growth rate was significant different ($p = 0.05$). This meant that 10 days' interval was also a good interval to analyse the clear patterns of mycelium growth. Mkhize *et al*. (2016) used 7 days' intervals and managed to achieve the major objective which was to analyse the mycelium growth rate. From day 10 to 40, there was a clear pattern indicating that the mycelium was colonising the mushroom pack from top where the inoculum

was placed to bottom. When the mycelium growth reached the bottom of the mushroom pack, it meant that the mushroom pack was fully colonized, thus reached 100% growth (C and D). There was no clear mycelium growth correlation between most of the particle sizes as indicate on 10 days' interval (A and B). The mycelium growth of the same particle size (25 mm) was the highest on C/N ratio 15:1, low at C/N ratio 25:1 and 35:1, then higher on C/N ratio 70:1 and 100:1.

The C/N ratio significantly influenced the mycelium growth rate when compared to the particle size. In all four intervals (A, B, C and D) there was a positive correlation between C/N ratio and mycelium growth. The higher the C/N ratio, the higher was the mycelium growth. The mycelium in mushroom packs with C/N ratio 35:1, 70:1 and 100:1 reached 50% in 20 days. In 30 days most of the mushroom packs were on 100%, meaning they were fully colonised. At 40, days the three highest C/N ratios (35,70 and 100:1) were fully colonised and ready for planting. The mycelium growth of C/N ratio 70:1 and 100:1 were quicker than the control. Most of the mushroom packs on C/N ratio 15:1, the mycelium could not reach the 50% at day 40. Only few mushroom packs were full colonised and planted on C/N ratio 15:1 and 25 mm particle. Clearly, this was mainly due to particle size instead on C/N ratio since low C/N ratio had low BE in this study as discussed of sub-section 3.4.2.

3.4.5. Determining the best C/N ratio and particle size for oyster mushrooms (*Pleurotus* spp).

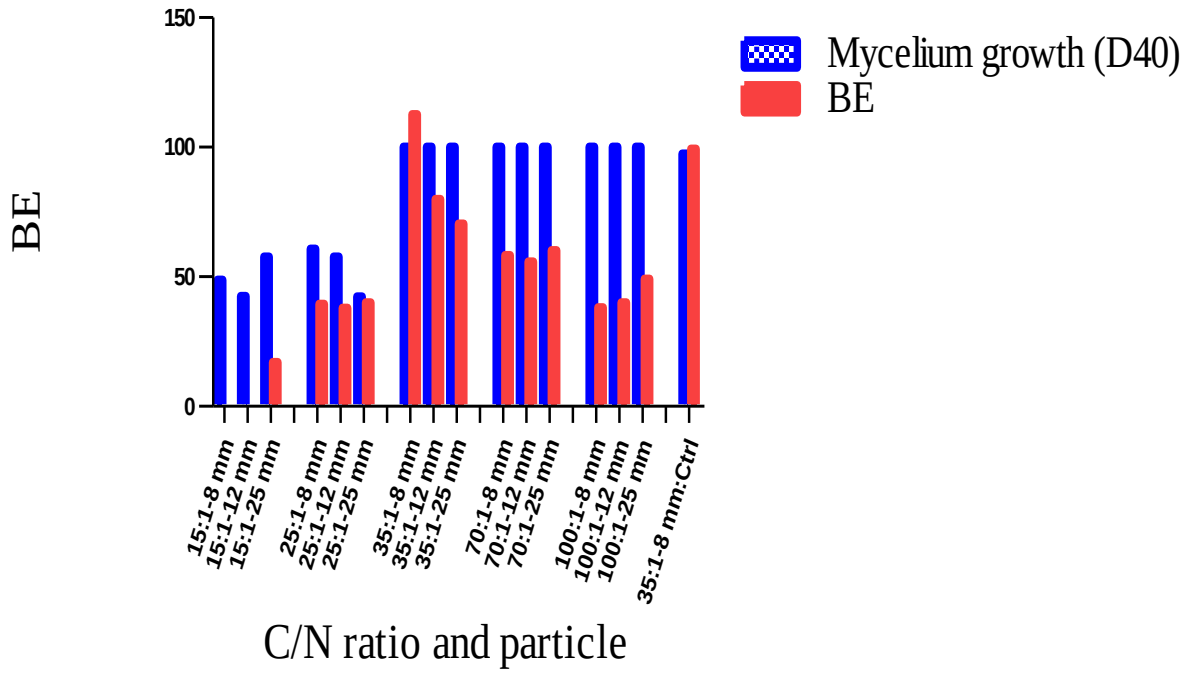

Figure 3.4 Optimum C/N ratio and particle size for Oyster mushrooms.

As previous mentioned, the C/N ratio and particle size had an effect on BE. Figure 3.4 indicate the best combination of C/N ratio and particle size in order to achieve the optimum BE in shorter period. The optimum BE was achieved at 35:1 C/N ratio with 8 mm particle size and the mushroom packs were fully colonised in ±30 days. The control had the second best BE, followed by C/N ratio 70:1, particle size 25 mm. The lowest BE was observed in the lowest C/N ratio (15:1). Thus these results indicate that the faster the mycelium growth cannot be translated to the highest BE. It also means that the mushrooms required a limited amount of carbon and nitrogen. The mycelium growth of C/N ratio 70:1 and 100:1 was the fastest but the best BE was obtain at 35:1 C/N ratio which was the second best in terms of mycelium growth.

Control (DARD formula) had the same C/N ratio (35:1) and the same particle size with the experimental C/N ratio but the BE obtained was different. The difference between the C/N ratios was due to the type of raw materials used. These observations could mean that C/N ratio

alone cannot define the BE potential of the mushroom pack but other factors like the type of raw materials needed to be considered. One may argue that C/N ratio 70:1 is the best since the mushroom packs were quickly fully colonized and it gave a fairly good BE of ±60%. Based on our analyses the mushroom farmers must make a choice of either to consider the period it takes to first harvest or yields (BE).

3.4.6. Moisture

The optimum moisture content for mycelium and mushrooms growth varies from the different mushroom species and the type of raw material used. However, low moisture content may result in the death of the fruiting body. Higher moisture may reduce the porosity of the substrate, thus limiting oxygen transfer within the mushroom pack (Patel *et al*., 2009). Therefore, farmers have to know the moisture content required for their mushrooms. In this study 65% moisture was used. The results indicated that moisture wasn't anyhow a negative factor, thus 65% moisture could be used in this type of raw materials to grow oyster mushrooms. These observations concurred with Ryu *et al*. (2015); Chang and Miles (2004) as they indicated that 50% to 70% moisture is suitable for the growth of oyster mushrooms.

3.4.7 Contamination

Mushroom packs can be attacked by fungal pathogens and other foreign organisms due to a number of reasons, including poor pasteurisation and not following the aseptic techniques during inoculation. Contaminants may compete with mycelium resulting in low yields (Cohen, 2002).

Table 3.2 Contaminated bags percentage per each combination of C/N and particle size

C/N ratio	Particle size	Contaminated bags(%)
15:1	8	8
15:1	12	4
15:1	25	2
25:1	8	2
25:1	12	2
25:1	25	2
35:1	8	0
35:1	12	0
35:1	25	0
70:1	8	0
70:1	12	0
70:1	25	0
100:1	8	0
100:1	12	0
100:1	25	0
Control	8	2

In this study the contamination was very low (Table 3.2). The highly contaminated combination was 15:1 C/N ratio and 8 mm particle size with only 4 mushroom packs out of 50 which represented 8%. Comparing these results with Mkhize *et al*. (2016) who reported 12.5% contamination in his study, the low contamination might have occurred due to a high substrate quality used in this study. The contaminated bags, mainly had some green patches which were suspected to be *Trichodema* or penicillin since they both could form green mold.

3.5. Conclusion and Recommendations

For the successful production of *Pleurotus* spp, a number of factors, which may act individually or have interactive effects among them need to be considered. The combination of C/N ratio, particle size, type of raw material and other factors that were not detailed discussed in this

study such as moisture, temperature etc. may play a vital role for the success of the mushroom project, which may be defined as producing higher yields (BE) and quality mushrooms at a very short period thus avoiding loss. The quality of the substrate should also be considered since the level of nutrients provided in the substrate is likely to affect the quality of the mushroom. Based on the saying “You get what you gave”, if the substrate had very low nutrients, the mushroom is likely to have low nutrients as well. In this study the C/N ratio and particle size indicated to have influenced both mycelium growth and BE. Therefore, a depth understanding of physical, chemical and biological properties is required for the success of the mushroom farm.

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CHAPTER 4

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1. GENERAL DISCUSSION AND CONCLUSION

The importance of carbon and nitrogen in oyster mushroom production cannot be anyhow be ignored, otherwise that would be like planning for failure. Oyster mushrooms can grow in any agro-waste with lignin and cellulose but it is good to have agro-waste that mushrooms like to grow on. For mushrooms to grow optimally they determine their own growth environment and conditions. Carbon nitrogen ratio (C/N) is one of the important need for mushrooms to grow at optimum level. The following authors (Ryu *et al*., 2015; Singh and Mishra, 2008; Curvetto *et al*., 2002; Royse and Sanchez, 2000) all emphasized the importance of carbon and nitrogen on their studies based on mushroom production. Different C/N ratios can be obtained using different raw materials. However, the same C/N ratio may also be obtained using different raw materials.

Some raw material may need some preliminary treatment, depending on the type of cultivation system used (Bellettini *et al*., 2016). Larger particle size cannot be well bagged and compressed in the plastic bag, therefore without even considering the importance of milling to increase the surface area for mycelium to grow, it is obvious that one may have to think of how the raw materials may fit into the plastic bag. Air filled porosity (AFP) in the mushroom pack have mainly influenced by the particle size of the raw material. The bigger the particle size, the higher the AFP. Particle size or AFP must not be too big or too small.

Both C/N ratio and particle size have significantly influenced the mycelium growth rate and the biological efficiency. Depending on the type of raw materials, mushrooms may require a specific C/N ratio and particle size to grow (Tsegaye, 2015). The higher rate of mycelium

growth does not translate to the higher BE. Thus mushroom pack may quickly be fully colonised by the mycelium but still produce low yields.

Naturally oyster mushrooms grow on dead logs where the C/N ratio may be around 100:1. Therefore, expectations to consider logs to be the best substrate may arise, but the fact is that most of the researchers found more substrates which produce higher yield and quality mushrooms. According to the findings of this study the recommended combination of C/N ratio and particle size is 35:1 C/N and 8 mm particle size. The substrate composition was 31.3% bagasse, 6.3% wheat bran, 12.5% maize, 12.5% Sawdust, 12.5% hominy chop,12.5% juncao, 6.3% lurcerne and 6.3% teff. This formula may be considered as costly but since the main purpose is ensuring that the highly nutritious mushrooms are given to the community in helping to eradicate protein deficiency diseases and hidden hunger it will be too relevant for KZNDARD and the rest of the mushroom farmers in general. Kunjeku and Maliwichi, (2009) have indicated that mixing the raw materials may improve the protein content in mushrooms.

Contamination has been reported by most of the authors as a major treat in mushroom production. In this study a very low contamination rate was observed. This means the correct procedures were followed for the entire production process. The DARD formula may still provide higher yields, but the challenge is sometimes on the quality of available raw materials. The quality of bagasse varies with the source. At sometimes, a very fine bagasse is delivered. The standard procedure is to mill before mixing, which more often than not results in a very fine substrate affecting aeration, leading into lower yields. Flexibility in terms of adjusting the standard procedure to suit the condition of the raw material is sometimes essential. Avoiding milling the bagasse could save time, save electricity, less labour, avoid dust from bagasse and reducing the mushroom production costs.

4.2. Recommendations

In order to avoid the higher costs of production, farmers must know their local readily available sources of carbon and nitrogen and play around them through trial and error in order to find a combination that may provide them with better yields. The higher yields of oyster mushrooms may be obtained at C/N ratio higher than 25:1 and C/N ratio below 35:1. Farmers must know the physical composition of their raw material in order to decide the cultivation system that they can use. They must also consider the availability of the resources before they decide the type of raw material to be used. For example, juncao grass cannot be useable without a milling machine but wheat straw can be used without any preliminary treatments.

Farmers must always consider the combination of both C/N ratio and particle size as important factors in mushroom production. When using the small plastic bags for raw material (150 x 300 mm) plastic bags, at least 1 – 12 mm could be the suitable particle size for the raw material. Hygienic practices are highly recommended in all steps of production in order to avoid the contamination and health risk in all the products. In future fresh mushroom nutrient analysis need to be done in order to know the contribution of the nutritional rich raw material. Nutrients in fresh mushrooms may vary depending on the quality of raw material.

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APPENDIX A

A.1 Preparation of raw material

Figure A.1.1 Juncao grass

Figure A.1.2 Multi-purpose milling machine

Figure A.1.3 Three different particle sizes of bagasse

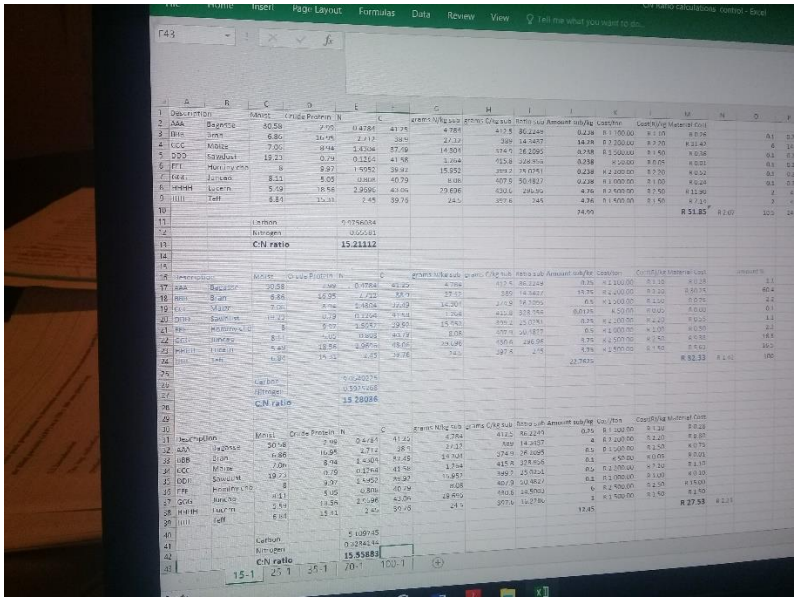

Figure A.1.4 Application of Tom Richard and Nancy Trautmann formula to calculate C/N ratio in Excel

A.2 Mushroom packs production

Figure A.2.1 Mixing and bagging of mushroom packs

A.3 Inoculation and mycelium growth

Figure A.3.1 Mushroom packs inoculation

Figure A.3.2 Mycelium growth measurement

A.4 Growing fresh mushrooms

Figure A.4.1 Growing fresh mushrooms using wall method

A.5 Results of analysed data

Hint: A =C/N ratio 15:1, B=25:1,C=35:1, D=70:1,E=100:1, Control =DARD formula, D =days, BE=Biological efficiency

Table A.5.1 Effects of C/N ratio on mycelium growth in 10 days intervals

The MEANS Procedure

Ratio=A

Variable	Mean	Std Dev	Variance	Coeff of Variation
D10	18.0794123	5.9842064	35.8107267	33.0995629
D20	30.8274543	8.1982559	67.2114002	26.5940089
D30	42.5169960	11.4543483	131.2020957	26.9406341
D40	49.6170894	10.2616287	105.3010237	20.6816418

Ratio=B

Variable	Mean	Std Dev	Variance	Coeff of Variation
D10	18.0015151	4.9916996	24.9170653	27.7293307
D20	32.0627484	7.8930972	62.3009842	24.6176564
D30	45.2013496	12.4390751	154.7305890	27.5192560
D40	53.6024250	12.4511070	155.0300659	23.2286263

Ratio=C

Variable	Mean	Std Dev	Variance	Coeff of Variation
D10	39.767040	5.2685563	27.7576858	20.5300662
D20	69.711050	8.7434057	76.4471430	17.4776559
D30	92.226770	14.6997820	216.0835915	20.7737985
D40	97.508970	14.5039408	210.3642979	17.4698591

Ratio=Control

Variable	Mean	Std Dev	Variance	Coeff of Variation
D10	25.662640	9.6532100	93.1844637	24.2743976
D20	50.026190	13.3993083	179.5414619	19.2212110
D30	70.761170	11.4508270	131.1214389	12.4159476
D40	83.022650	6.2042416	38.4926135	6.3627393

Ratio=D

Variable	Mean	Std Dev	Variance	Coeff of Variation
D10	33.5199079	6.2783620	39.4178295	18.7302484
D20	72.9666939	10.8203583	117.0801545	14.8291745
D30	100.0000000	0	0	0
D40	100.0000000	0	0	0

Ratio=E

Variable	Mean	Std Dev	Variance	Coeff of Variation
D10	32.2353845	7.1259097	50.7785892	22.1058623
D20	69.3439353	8.5507954	73.1161026	12.3309925
D30	99.1730908	6.2226865	38.7218273	6.2745715
D40	100.0000000	0	0	0

Table A.5.2 Effects of particle size and C/N ratio on mycelium growth in 10 days intervals

Level of Ratio	Level of Psize	D10		D20		D30		D40	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
A	**12mm**	13.5980315	4.37981820	24.0802200	4.7275938	34.050413	4.9189525	42.445054	5.7462641
A	**25mm**	23.5478906	4.26214988	39.1831846	6.4037436	54.469898	10.4317613	57.659580	11.1769976
A	**8mm**	17.0923147	4.41273514	29.2189582	4.5414747	39.030676	5.8723269	48.746635	6.5321793
B	**12mm**	19.1504425	5.34745210	35.9472871	5.5273660	51.133417	5.6997508	57.770858	5.7852971
B	**25mm**	17.0284553	5.06491657	25.5949876	6.8468295	32.105951	7.5999876	42.251935	10.0864786
B	**8mm**	17.8256475	4.36009904	34.6459705	6.8491984	52.364680	10.8085595	60.784482	11.5756182
C	**12mm**	26.0269440	5.82584069	52.3250637	9.8571295	86.890424	16.4468732	100.000000	16.3690296
C	**25mm**	23.2111110	3.66073981	44.0234619	6.8905493	75.069459	11.7630187	100.000000	13.9319334
C	**8mm**	27.7498556	5.11814682	53.7300500	5.5965369	87.108080	8.4920075	100.000000	8.9256815
Control	**8mm**	39.7670425	9.65321002	69.7110511	13.3993083	92.508970	11.4508270	97.508970	6.2042416
D	**12mm**	33.6808553	7.28902845	72.6772817	7.9821168	100.000000	0.0000000	100.000000	0.0000000
D	**25mm**	35.4150742	5.18298052	76.9513041	13.4925362	100.000000	0.0000000	100.000000	0.0000000
D	**8mm**	31.4637942	5.62301796	69.2714960	8.9066964	100.000000	0.0000000	100.000000	0.0000000
E	**12mm**	32.2574264	8.55581373	68.8824487	9.1037313	97.591736	10.6463964	100.000000	0.0000000
E	**25mm**	34.1982487	5.65259365	71.0392505	8.6911640	100.000000	0.0000000	100.000000	0.0000000
E	**8mm**	30.2504785	6.40141005	68.1101067	7.6632267	99.927536	0.5613019	100.000000	0.0000000

Table A.5.1 Optimum C/N ratio and particle size required by oyster mushrooms

The MEANS Procedure

CNRatio=A Partsize=25mm

Variable	Mean	Std Dev
MY	0.0670900	0.0246527
BE	16.9848101	6.2411826

CNRatio=B Partsize=12mm

Variable	Mean	Std Dev
MY	0.1410590	0.0752454
BE	38.0212938	20.2817805

CNRatio=B Partsize=25mm

Variable	Mean	Std Dev
MY	0.1487252	0.0731726
BE	40.0876614	19.7230635

CNRatio=B Partsize=8mm

Variable	Mean	Std Dev
MY	0.1393686	0.0419724
BE	39.3696529	11.8566198

CNRatio=C Partsize=12mm

Variable	Mean	Std Dev
MY	0.2115406	0.1030039
BE	80.0190278	27.7638503

CNRatio=C Partsize=25mm

Variable	Mean	Std Dev
MY	0.1755704	0.0786964
BE	70.4481986	19.9231447

CNRatio=C Partsize=8mm

Variable	Mean	Std Dev
MY	0.4696863	0.1199952
BE	112.6797502	33.8969605

CNRatio=Control Partsize=8mm

Variable	Mean	Std Dev
MY	0.3514324	0.1289678
BE	99.2747031	36.4315864

CNRatio=D Partsize=12mm

Variable	Mean	Std Dev
MY	0.2179960	0.0735282
BE	55.7590366	19.8189170

CNRatio=D Partsize=25mm

Variable	Mean	Std Dev
MY	0.2887955	0.0849792
BE	60.1127769	21.5137199

CNRatio=D Partsize=8mm

Variable	Mean	Std Dev
MY	0.1673363	0.0819051
BE	58.2701324	23.1370276

CNRatio=E Partsize=12mm

Variable	Mean	Std Dev
MY	0.1487252	0.0731726
BE	40.0876614	19.7230635

CNRatio=E Partsize=25mm

Variable	Mean	Std Dev
MY	0.1944120	0.0749670
BE	49.2182278	18.9789846

CNRatio=E Partsize=8mm

Variable	Mean	Std Dev
MY	0.1347503	0.0870740
BE	38.0650536	24.5971814