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Department of Biochemistry and Microbiology

PERFORMANCE OF THREE OYSTER MUSHROOM SPECIES GROWN ON MAIZE STALK SUPPLEMENTED WITH WHEAT BRAN AND MAIZE FLOUR

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

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Supervisor: Prof. G.E. Zharare

Department of Agriculture (University of Zululand)

Co- Supervisor: Prof. A.K. Basson

Department of Biochemistry and Microbiology (University of Zululand)

Submitted by:

Senzosenkosi Surprise Mkhize

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DECLARATION

I, Senzosenkosi Surprise Mkhize, 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 _____, 2016

DEDICATION

To My Parents Mr. and Mrs. Mkhize,

Mcebo, Thulile, Khosi, Siyabonga, Mbali, Nkosinathi and Manqoba.

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RESEARCH OUTPUT

Three papers were written from this research. While the first one (Chapter 3) has been published in, *Food Science and Technology* (*Campinas*), the second paper (Chapter 4) has been accepted in the same journal. The third paper (Chapter 5) has been submitted to *Scientia Horticulturae*.

- **Published Research paper**

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ABSTRACT

The process of mushroom cultivation adds value to the economy, environmental restoration and food security (provision) worldwide. However, there is paucity of information to the mushroom growers about the strategy of improving yield and production of mushrooms at minimal costs with reduced contamination rate. Therefore, understanding the amendments, supplements, and pre-treatment requirements for the local substrate to make them optimally suitable for mushroom cultivation is an essential step towards developing ways of improving yield and production at minimal costs with reduced contamination. The study was conducted to determine or evaluate the performance of three selected *Pleurotus* mushroom species (*Pleurotus ostreatus, P. pulmonarius,* and *P. salmoneostramineus*) grown on maize stalk which was supplemented with varying increasing levels of maize flour (MF) and wheat bran (WB) supplements. The results obtained in this study indicated that the mushroom species were not highly affected by contamination. However, there were levels of supplementation that resulted in higher rates of contamination, such as 20% WB (75% rate) for *P. pulmonarius* and 20% MF (75% rate) for *P. salmoneostramineus*. The overall results showed that all the mushroom species were affected by the addition of both supplements into the maize stalk substrate. It was observed that the higher levels of supplementation resulted in lower mycelial growth rate (MGR) and days to full colonisation of substrate were delayed whereby the lower levels of supplementation (WB and MF) resulted in significantly faster mycelial growth rate and shorter colonisation period. The time it took for mushrooms to start pinning (TP) was shorter within the first flush for all the mushroom species compared to the second and third flushes. Although, higher levels of supplementation negatively affected the MGR and days to full colonisation, there were however, some advantages of high supplementation. The higher levels of supplementation improved the productivity (biological efficiency and yield) for all mushroom strains. The biological efficiency (BE) and yield increased up to certain levels of higher

supplementation after which they both decreased or dropped after reaching peak. In addition, a higher rate of contamination was observed under supplement levels beyond the optimum level. It can be recommended that for quick production, low supplementation or no supplementation may be required. This action may save production costs, nonetheless for an improved productivity (BE and yield), high levels of supplementation should be used (12% WB and 14% MF for all mushrooms, and for *P. salmoneostramineus* 18% WB is also recommended). However, supplementing beyond certain limits will result in a decrease in productivity due to contamination.

Key words: *Pleurotus*, productivity, yield, maize stalk, supplement.

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LIST OF ABBREVIATIONS

BE	Biological efficiency
$CaCO_3$	Calcium carbonate
g	grams
kg	Kilograms
MF	Maize flour
MSY	Mushroom yield
MGR	Mycelial growth
PDA	Potato dextrose agar
TP	Time for pinning
WB	Wheat bran

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

In South Africa, poverty is mostly dominant in the rural areas (May, 2000; Rahman & Westley, 2001), probably because of poor education emanating from deprivation of education in the colonial times (Woolard, 2002). Mushroom production is one of the strategies that can be used for poverty interventions (Imtiaj & Rahman, 2008) and also for combating malnutrition (Eswaran & Ramabadran, 2000). Among the edible mushrooms, the most popular mushrooms grown for poverty alleviations are oyster and shiitake mushrooms. In South Africa, the production of these mushrooms is still in its infancy (Ambi *et al.*, 2011), and their cultivation has to adapt to local conditions and resources. In particular, the production of these mushrooms requires adapting to locally available substrates.

Oyster mushrooms are saprophytes that decompose agricultural plant by-product (Saidu *et al.*, 2013) as they have the ability to use cellulose, hemicellulose and lignin materials as source of their nourishment (Sofi *et al.*, 2014). This ability, therefore, enables them to be grown on a wide range of plant residue, and hence, their suitability for poverty alleviation. Mushroom production also provides an environmentally friendly and economical way of converting agricultural and forest wastes into nutritious food (Ragunathan *et al.*, 1996; Wood & Smith, 1987). In the coastal areas of KwaZulu-Natal (KZN) in South Africa, there is a high proportion of sugar cane and maize biomass on the farms that is wasted by burning. In addition to this waste, there is also a Mondi paper plant in Felixton (South Africa), which produces large amount of pulp waste from its paper recycling plant. All of these plant wastes from the coastal areas of KZN are potential substrates for the cultivation of oyster mushrooms. There is, however, an information gap as to the amendments, supplements and pre-treatment

requirements of these local plant substrates/wastes to make them optimally suitable for mushroom cultivation.

As much as the substrate is the key component in mushroom cultivation, there are some considerations that should be met with for optimal mushroom cultivation. Tisdale (2004) have outlined three conditions. Firstly, the substrate must be suitable for the growth and fruiting of the mushroom fungus. Secondly, the substrate should be available locally in sustainable quantities and must be low in cost. Thirdly, the climate should be suitable for the growth and fruiting of the fungus. The current research focusses on adapting the locally abundant lignocellulosic material for oyster mushroom production. Although mushrooms can grow on different lignocellulosic materials, they do not grow optimally if the lignocellulosic materials are provided alone (Choi, 2004). This is because the lignocellulosic materials are usually deficient in other important nutrients, such as proteins and mineral elements. Thus, the lignocellulosic materials require supplementation with materials that have sufficient amounts of nitrogen, potassium, phosphate, and vitamins for improved growth and yield of the mushrooms (Mangat *et al.*, 2008). In addition, other carbon sources are required (Moore-Landecker, 1982). However, high supplementation of the lignocellulosic substrate, especially with nitrogen-containing materials, may increase the incident of contamination (Miller & Jong, 1987; Przybylowicz & Donoghue, 1990). Thus this present study aimed at assessing the supplementation required to make the waste cellulosic material (maize stalk) commonly found in the coastal region of KZN, suitable for the production of three oyster mushrooms species (*Pleurotus ostreatus*, *Pleurotus pulmonarius* and *Pleurotus salmoneostramineus*).

1.2 Research aim

The aim of the study was to determine the potential or performance of three oyster mushroom species (*P. ostreatus*, *P. pulmonarius* and *P. salmoneostramineus*) when cultivated on maize

stalk residues (base substrates) supplemented with various concentration levels of wheat bran (WB) and maize flour (MF).

The three main questions that the study sought to answer in relation to the above aim were:

(i) What is the yield potential or performance of *P. ostreatus, P. pulmonarius* and *P. salmoneostramine*us grown on maize stalk (residues)?

(ii) How do WB (a source of nitrogen) and MF (an additional source of energy) affect the performance of *P. ostreatus, P. pulmonarius* and *P. salmoneostramineus* grown on maize stalk residues?

(iii) How much of WB and MF optimize the growth and yield of each of the three test mushroom species?

1.3 Objectives

The objectives of the study were**:**

(i) To determine how MF and WB as a supplement affects the yield and growth performances of three oyster mushrooms grown on maize stalk residues.

(ii) To determine the performance of each of test mushroom species (three oyster mushroom) grown on supplemented maize stalk residues.

(iii) To determine the level of supplementation that would optimize the performance of each mushroom species.

(iv) To determine which mushroom species would respond best compared to the other mushrooms species.

1.4 Hypotheses

(i) There is an optimal amount of both MF and WB that is below and above by which mushroom performance would be affected in terms of mycelial growth rate (MGR), days

to full colonisation of substrate, time taken for pinning (TP), mushroom yield, biological efficiency (BE) and the number of contaminated bags.

(ii) The growth and yield performances of each of the test mushroom species will differ among different supplementation levels of WB and MF.

(iii) For each and every mushroom species there is an optimal amount of WB and MF.

(iv) There is a mushroom species that would perform the best over the other mushroom species in terms of growth and yield performance.

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CHAPTER 2

LITERATURE REVIEW

2.1 Nature of cultivable mushrooms

Mushrooms are fleshy, spore-bearing fruiting bodies of micro-organisms commonly known as fungi (fungus) (Boa, 2004 ; Oei & Nieuwenhuijzen, 2005b; Stevenson & Lentz, 2007). Mushrooms are neither plants nor animals as they belong to the Kingdom *Mycetae* (Miles & Chang, 1997). A mushroom can also be defined as a macrofungus that has a fruiting body that is either epigenous or hypogenous (Anderson, 2008; Chang & Miles, 1992). In contrast to the vegetative parts of the mushrooms, that are not easily seen with the naked eye, the mushroom fruiting bodies are large entities that can be seen with the naked eye (Cho, 2004).

There are two groups of mushrooms consumed by humans; cultivable and non-cultivable mushrooms. In the latter group, the mushrooms are collected from the wild, where they grow in symbiotic association with tree roots (Mycorrhizal fungi) (Pandey *et al.*, 2008) or act as parasites/saprophytes on plants (Yang, 1986). In the latter group, humans have mastered the art of growing them under controlled conditions (Manzi *et al.*, 2001). There are many of the cultivable mushrooms, that includes several button (*Agaricus bisporus*) strains, shiitake (*Lentinula edodes*), oyster (*Pleurotus* spp.), enokitake (*Flammulina velutipes*) and maitake (*Grifola frondosa*) mushrooms. Oyster mushrooms, and to a lesser extent the shiitake mushrooms, have become the mushrooms of choice for production in poverty alleviation programs.

2.2 Oyster mushrooms

The oyster mushrooms are among the most important cultivated mushrooms in the world (Chang & Miles, 1991; Mohamed *et al.*, 2011). In terms of both the production and customer preference, the oyster mushrooms have gained popularity, because of their simplicity and low cost cultivation technology (Baysal *et al.*, 2003; Mswaka & Tagwira, 1997). The oyster

mushroom species grow in a wide range of temperatures ranging between 10 and 21 °C (Stamets, 2000c). This property of oyster mushrooms is advantageous to the mushroom as it allows the mushrooms to be grown and harvested all year round (Alam *et al.*, 2007b). For the vegetative development of oyster mushrooms, high humidity of 80–90% and a high temperature ranging from 25–30 ºC is needed. However, for optimal fruiting, lower temperatures between 18–25 ºC are needed (Ajonina & Tatah, 2012). The oyster mushroom is among the largest fungi (Onuoha, 2007), that is able to grow on a variety of substrates than any other mushroom (Cohen *et al.*, 2002). This is because oyster mushrooms poses a multitude of enzyme systems that can degrade almost all kinds of plant waste material (Poppe, 2004).

2.2.1 Classification, morphological description, and ecology of oyster mushrooms

Oyster mushrooms are a group of fungi in the genus *Pleurotus*. The oyster shell-like appearance of the fruit bodies of these mushrooms is the reason why these mushrooms are named oyster mushrooms (Stanley *et al.*, 2011). The genus *Pleurotus* belongs to the class *Basidiomycetes*, subclass *Hollobasidiomycetidae*, order *Agricales* and is under the family *Pleurotaceae* (Ibekwel *et al.*, 2008). Oyster mushrooms are widely distributed in the Northern Hemisphere, being naturally present in Europe, North Africa, Asia and North America (Singer, 1986). There are 40 known species in the genus *Pleurotus* (Jose & Janardhanan, 2000), which can be distinguished by their temperature requirements as well as by cap shape, size, and colour (Sarker *et al.*, 2008). Table 2.1 contains classifications of some *Pleurotus* mushrooms with the temperature requirements and the colour of their caps.

Within the natural environment, the *Pleurotus* spp. are primary degraders of wood occurring on tree trunks in subtropical and temperate forests. They are commonly called white rot fungi of wood, because of the appearance of the white rot residue after the degradation of lignocellulosic substrate (Ortega *et al.*, 1993). Although, the oyster mushroom fungi are classified as saprophytes, they are also nematophagous, because they are capable of paralyzing

nematodes with their toxins and consuming them through external enzymes (Barron & Thorn, 1987; Thorn *et al.*, 2000).

Table 2.1 Classification of commonly cultivated oyster mushrooms based on cap colour and temperature requirements for vegetative growth and fruiting.

		Temperature requirements	
***Pleurotus* spp.**	**Colour of cap**	**Vegetative growth (ºC)**	**Fruiting (ºC)**
P. ostreatus	White, cream or grayish[^]	5 - 35[#]	10 - 21[*]
P. florida	White[^^^]	25[##]	15 - 25[##]
P. sojar-caju	Pale moose grey[**]	25[##]	18 - 25[##]
P. cystidiosus	Buff, Hazel, White or grey[**]	10 - 35[#]	21 - 27[*]
P. djamor	Pinkish (fresh), Whitish or yellow (dry)[^]	15 - 35[#]	20 - 30[*]
P. eryngii	Buff[**]	10 - 35[#]	15 - 21[*]
P. pulmonarius	Whitish or cream[**]	5 - 35[#]	21 - 29[*]
P. uber-regium	Dark brown [<<]	20 - 40[^^]	30 - 35[*]
P. cornucopiae	Cream[**]	15 - 35[#]	15 - 25[#]

Source: * (Kang, 2004; Stamets, 1993); ** (Da Serra, 1997); # (Oei & Nieuwenhuijzen, 2005a); ^ (Menolli Junior *et al*., 2010); ## (Kong, 2004a); ^^ (Fasidi & Ekuere, 1993); ^^^ (Dhar *et al*., 2011); <<(Afieroho *et al*., 2013).

2.3 Uses of mushrooms

2.3.1 Food uses

Mushrooms are rich in protein, carbohydrates, amino acids, minerals and vitamins (Jiskani, 2001; Khan *et al.*, 1981). Stanley *et al.* (2011), stipulate that the oyster mushroom contains 25-30% protein, 17-44% sugar, 2.5% fat, 7-38% mycocellulose, and 8-12% minerals (such as calcium, sodium, phosphorus and potassium). The nutritional value of mushrooms lie between that of meat and vegetables (Bano, 1976). The quality of mushroom proteins can surpass

proteins from animals (Jonathan *et al.*, 2013). They consist of nine essential amino acids (Rathee *et al.*, 2012) that include lysine, valine, phenylalanine, histidine, isoleucine, tryptophan, threonine, methionine, and leucine. Mushrooms have low levels of fat, sodium and cholesterol (Chang, 1996), but they have high levels of linoleic acids (Chang & Mshigeni, 2001; Sadler, 2003), vitamins, minerals, chitin (carbohydrates), and vegetable proteins (Manzi *et al.*, 1999). Mushrooms are a good source of minerals and vitamins, especially the vitamin B complex group. However, they are relatively poor in fat soluble vitamins (A, D, E and K). Within the B complex vitamins, the mushrooms are relatively rich in thiamine (B1), riboflavin (B2), niacin, biotin, and they are also a good source of ascorbic acid (Smith *et al.*, 2002).

2.3.2 Medicinal uses

Although, mushrooms are recognized for their nutritional value, they also possess medicinal constituents that are active against hypercholesterolemic conditions, diabetes, cancer, hypertension, and other infections (Alam *et al.*, 2007a; Chorvathoba *et al.*, 1993; Manpreet *et al.*, 2004; Nayana & Janardhanan, 2000; Wang *et al.*, 1996; Yoshioka *et al.*, 1985). The medicinal properties of mushrooms are due to the presence of active compounds such as polysaccharides, oligosaccharides, peptides, proteins, triterpenoids, dietary fibres, alcohols, phenols and mineral elements (Pardeshi & Pardeshi, 2009). Among the above compounds, the polysaccharides are well known to have antitumor and immunomodulatory properties (Borchers *et al.*, 1999; Lorenzen & Anke, 1998 ; Mizuno, 1996; Mizuno, 1999a; Mizuno, 1999b; Mizuno, 2002; Ooi & Liu, 1999; Reshetnikov *et al.*, 2001; Tzianabos, 2000; Wasser & Weis, 1999a). The oyster mushrooms have strong anti-inflammatory and immunomodulatory properties (Lavi *et al.*, 2010) as well as anticancer activity (Wasser, 2002). Thus, the consumption of oyster mushrooms has an advantage of preventing as well as reducing diseases such as diabetes, heart disease, high blood cholesterol level, gastric cancer, hepatitis B, liver

illness, kidney problems, hypertension, microbial infection, chronic fatigue syndrome and impaired immune response (Gunde-Cinoerman, 1999; Ooi, 2000; Wasser & Weis, 1999b).

2.3.3 Environmental uses

Although, mushrooms play an important role in providing food and health benefits (medicine) to humans (Meghalatha *et al.*, 2014), they are also valued for other uses. These include tonics, bioremediation (Chang, 1993; Kuforiji & Fasidi, 2009; Mane *et al.*, 2007) and mycoremediation of the polluted environment (Boamponsem *et al.*, 2013). Some mushrooms, such as *Pleurotus* spp., are said to have the potential of producing digestible protein for cattle feed from lignocellusic material (Zadrazil, 1977, 1980).

2.4 Mushroom production steps

There are seven major practical steps in mushroom production (Chang, 1998; Chang & Chiu, 1992). The steps are: (i) selection of acceptable mushroom species, (ii) preparation of selective substrate, (iii) spawn production, (iv) preparation of bulk substrate, (v) growth of mycelia on a bulk substance (spawn run), (vi) fruiting, and (vii) harvesting.

2.4.1 Selection and culture of an acceptable mushroom species

2.4.1.1 Qualities for selection

The first step to be considered in mushroom cultivation is to have a mushroom of interest that consists of organoleptic qualities which are acceptable to the indigenous population. It is also important to examine the availability of the substrate to be used for mushroom cultivation, since the substrate is essentially required in larger quantities. The environmental requirements for the mushroom to grow and fruit without the use of expensive growth systems must also be taken in to consideration (Chang, 1998, 2008; Chang & Chiu, 1992).

2.4.1.2 Production of starting/mother and stock cultures

The mushroom mother, or starter culture, can be produced using both the tissue culture and the multispore culture technique (Sharma & Kumar, 2011; Stamets & Chilton, 1983). The tissue culture technique involves the use of a healthy mushroom which is cleaned thoroughly and thereafter, the mushroom should be cut into halves so that the interior upper tissues of the stipe can be taken out and transferred into the surface of medium aseptically and incubated for certain period and at a specific temperature (Oei & Nieuwenhuijzen, 2005a). The tissue culture is advantageous, since it preserves the genetic trait of the mushroom. However, it has to be taken within one to two days after the mushroom has been harvested (Stamets & Chilton, 1983). The multispore technique makes use of the healthy cleaned mushroom. The spore print should be obtained first by placing gills of the mushroom on white paper or a glass surface. If the specimen is dry, drops of water should be added on the top cap of the mushroom surface in order to allow the release of spores (Stamets, 1983). After obtaining the spore print, the spores should be transferred aseptically into a petri dish with potato-dextrose agar, or test tube, and allowed to germinate at 32 ºC for a period of 48 h (Quimio, 1982). The culture obtained should be stored as a stock culture. There are several methods that have been used for storage of mother cultures. These methods include subcultures, liquid nitrogen, and paraffin sealing (Kong, 2004b). The simplest method of preserving is storing the mother culture in a 3-5 ºC refrigerator.

2.4.2. Spawn production

Mushroom spawn is a medium that serves as the inoculum of the mushroom growth medium. The inoculum is produced by inoculation of sterilised grains (mostly wheat, rye or millet) with high quality stock mycelia cultures (Mata & Savoie, 2005; Stamets, 2000b). After the grains have been fully colonised by mycelia, the mixture is called a spawn. The spawn is grown under axenic conditions in autoclavable polyethylene bags. This ensures gaseous exchange (Philippoussis, 2009). Obtaining a good spawn is essential in mushroom production, because

inadequate spawn and spawn of poor quality can result in unsatisfactory fruiting bodies (harvest), i.e. a poor yield (Miles & Chang, 2004).

2.4.3 Preparation of bulk substrate for mushroom production

The lignocellulose substrate for mushroom production needs to be obtained close to the cultivation site and must be easily available so that production of the mushrooms will be cost effective (Sales-Campos *et al.*, 2011). The lignocellulosic substrate has to be supplemented with cereal grains or cereal bran, especially those substrates with low productivity (Przybylowicz & Donoghue, 1990; Stamets, 2000a; Stamets & Chiiton, 1983). The supplements serve to increase levels of the already available nitrogen and carbohydrates, that help to speed up colonisation and degradation of substrate by the fungus (Sales-Campos *et al.*, 2011).

This activity of supplementation of substrate is used for *Pleurotus* spp. production in order to gain both sufficient yield and development (Carvalho *et al.*, 2010). The pH has to be corrected by additives such as lime stone ($CaCO_3$). Also, gypsum is used as a source of calcium and to improve the physical structure of the substrate as well as to buffer pH changes in the substrate (Przybylowicz & Donoghue, 1990).

The ideal medium for growing edible mushrooms should be free from all competitive microorganisms. Therefore, the substrate needs different degrees of pre-treatment to discourage the growth of the competing microorganisms, and simultaneously promote the growth of mushroom mycelium (Chang, 2008). There are various ways that can be used to pre-treat the substrates, some of which include steam sterilisation, pasteurization, fermentation, and also chemical sterilization (Karnawadi, 2006), but the most commonly used is steam sterilization. The moisture content of the substrate should also be considered since most mushroom fungi require a high moisture (Sales-Campos *et al.*, 2011). Guzman *et al.* (1993) indicated that an optimum growth of fungi is achieved at 70-80% moisture in the substrate.

2.4.4 Inoculation and spawn run

After the substrate has been steam pasteurized, it has to be cooled to around 30 ^{o}C under aseptic conditions, and thereafter, inoculated with an inoculum or spawn previously prepared (Sales-Campos *et al.*, 2011). Inoculation is carried out by spreading the spawn over the surface of the substrate and firmly compacted with the substrate. It can also be inserted deep into substrate (2-2.5 cm) (Chang, 1998; Chang & Chiu, 1992). Following inoculation the mushroom vegetative mycelium grows on the inoculated substrate and this is called the incubation period (Przybylowicz & Donoghue, 1990) or spawn running. The incubation process usually needs a dark place. However, a dark room is not needed for certain fungi that require light for their incubation (Sales-Campos *et al.*, 2011). The temperature during spawn running has to be monitored so that it remains optimum for the mycelium growth (Sales-Campos *et al.*, 2011). Low temperatures, such as 4-5 ^{o}C, may inhibit the mycelial growth of certain species, while temperatures above 35-40 ^{o}C can be deadly to other mushroom species (Bononi *et al.*, 1999). During the incubation process, the entire substrate gets colonised by mycelium, which forms a compact white mass all over the substrate (Sales-Campos *et al.*, 2011). The incubation period varies, since mycelium development depend upon different factors such as the type of inoculum used, quality of compost (substrate), and conditions of inoculation place. For *Pleurotus* spp. it generally takes 20-30 days to reach full growth (Eira & Minhoni, 1997).

2.4.5 Fruiting

When the mycelium has fully colonised the substrate, it can be able to produce fruiting bodies (Okwulehie & Okwujiako, 2008) in response to sudden change to the external physical environment, which will first promote the formation of primordia (initial fruiting bodies) that later develop into fruiting bodies (Bononi *et al.*, 1999). The environmental changes that may trigger fruiting include changes in temperature, gas exchange, relative humidity, light and water availability within the compost. These induction factors also have an impact on the quality of

the mushrooms (ZadraziL & Grabbe, 1983). For *Pleurotus* spp., lowering the temperature from the spawn run temperature (25-27 ^{o}C) to between 12 ^{o}C and 18 ^{o}C is the most common induction method used to stimulate fruiting (Eira & Minhoni, 1997). For shiitake cultivated in sawdust, the cultivation bags are cooled to a temperature of 5-8 ^{o}C for 5-12 days or by placing bags in cold water of 5-16 ^{o}C for 12-24 h, and packed back into the fruiting room (at 16 ^{o}C). The primordia forms at the top of the bags and develops within 3-4 days and is ready to be collected (Eira & Minhoni, 1997).

Mushroom fruiting usually occurs in flushes, also named rhythmic cycles (Chang, 2008). Under proper fruiting conditions, the additional flushes will 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 & Donoghue, 1990).

2.4.6 Mushroom harvesting

The procedure for mushroom harvesting involves grasping each mushroom stalk individually and twisting the mushroom till it is pulled out of the substrate (Khare *et al.*, 2006). The mushroom harvesting period vary between different mushroom strains and usually ranges from 6-12 weeks and they can be harvested on a number of flushes. The determining factor in the amount of flushes to be harvested and production time include, the type of strain, substrate formulation together with the environmental conditions during cultivation (Przybylowicz & Donoghue, 1990). However, a period between the flushing of mushrooms named the resting stage, whereby the mushroom mycelia have to accumulate nutrients. During this stage, contamination must be prevented to allow rapid mycelial growth (Przybylowicz & Donoghue, 1990). Harvesting of mushrooms can be carried out at different maturation stages depending on the mushroom species, market value and consumer preferences (Chang & 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 & Mishra, 2008).

2.5 Production systems

Oyster mushrooms have been grown on different production systems such as tree logs, beds, bags, boxes, and bottle systems (Quimio, 2004). The most commonly used production system for the oyster mushrooms is the bag cultivation system (Kitamoto *et al.*, 1993), but a log system is still in use for shiitake production. However, a bottle system is also becoming more common for oyster mushrooms.

2.5.1 Log system

Oyster mushrooms have traditionally been grown using logs and tree stumps as the substrate (Ivors, 2003), but in recent years, the production has moved largely to synthetic logs (bag system). This is because the synthetic log system is easier to manage and adapt for intensive production akin to a factory system compared to tree logs. Also, the synthetic log system has higher yields within a shorter time compared to using tree logs. Log cultivation is also possible on the shiitake mushroom, although other substrates such as sugar cane bagasse, coffee pulp, vine yard pruning, straw (Gaitán-Hernández *et al.*, 2011; Gaitán-Hernández *et al.*, 2006; Gaitán-Hernández & Mata, 2004; Mata & Gaitán-Hernández, 1992; Mata & Gaitán-Hernández, 1994; Salmones *et al.*, 1999) and wood shavings have also proven to be successful in shiitake cultivation (Mata *et al.*, 1990; Morales & Martínez-Carrera, 1991; Morales *et al.*, 1991; Pire *et al.*, 2001).

The cultivation of oyster mushrooms and shiitake on natural logs have been performed using a variety of tree species (San Antonio, 1981). Traditionally, in the oriental countries, the tree species that was mostly used for cultivation of shiitake mushrooms was the shii tree (Singer, 1961), but the oak tree is also being increasingly used (Harris, 1986; Stamets & Chilton, 1982).

The positive aspect of the log cultivation of mushrooms is that mycelium is fully protected by bark and this process requires less or minimum labour and care, since it is operated under natural conditions (Przybylowicz and Donoghue, 1990). Log cultivation produces mushrooms of high quality (Royse, 2001; Silva *et al.*, 2007) with more polysaccharides available when compared to the mushrooms that are produced using sawdust (Brauer *et al.*, 2002). The mushrooms also have thick caps and a good odour. Besides the positive aspects of log cultivation, there are some disadvantages, e.g., logs take a longer period to be fully colonised. Usually, it takes approximately 6 months and between 1 and 2 years for the first flush of mushrooms to take place (Sabota, 1994), and about 6 years is needed for complete cropping (Campbell & Racjan, 1999). By contrast, it takes 3-4 months for the synthetic log or bag system to complete production (Royse, 2009). The log cultivation of mushrooms poses a major threat to natural forests as it involves cutting down trees (Chiu *et al.*, 2000).

2.5.2 Bed/shelf system

The shelf system uses wooden shelves or multi-tiered metal shelves placed indoors. It has the advantage of being mechanised and utilises nets and winches to fill and empty shelves (Staunton *et al.*, 1998). However, its disadvantage is that it requires a high capital investment. The system also has the risk of rapid disease spread through the compost down the length of the shelves, since it has no barriers (Staunton *et al.*, 1998). The fermentation process for shelf cultivation of oyster mushroom production system that uses a fermented substrate can be performed in three steps (Choi, 2004). The steps are: (i) pre-fermentation of the substrate outdoors for 2-3 days, (ii) pasteurization at 60-65 °C for 8-10 h, and (iii) post-fermentation at 45-55 °C for 3-4 days. As soon as the fermentation is finished, the substrate can be inoculated with spawn, thereafter, the substrate is stored in shelves. The substrate must be covered with a perforated plastic sheet to provide ventilation. After a period of about 17-23 days (for oyster mushrooms), the mycelia will develop. The temperature is maintained at around 20-22 °C

during the first stage and is gradually increased up to 25 °C. For the fruiting process to occur, light is provided (80-120 lux for 3-4 days), and temperatures lowered to provide a cold shock and the relative humidity maintained between 85-95% before the plastic sheet is removed.

2.5.3 Bag (synthetic log) system

The bag cultivation system is in fact a synthetic log system. It offers some important advantages over the natural tree log production system. Some of the advantages are that bag production takes a shorter time and it is also efficient (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. The cultivation of oyster mushrooms using the bag system has been described by Kwon and Kim (2004). Firstly, the substrate of choice is supplemented by nitrogen sources such as WB, rice bran, sorghum or millet bran only if needed. Other additives such as gypsum, limestone, and sugar may be added whereby the gypsum and limestone act as buffers to control the pH of the substrate. The substrate mixture must be moistened to a water content of 65% and packed into bags. The bagged substrate is then steam sterilised/pasteurized to remove contaminants from the substrate. After the heat treatment, the substrate is allowed to cool to room temperature and inoculated with the spawn. The inoculated bags are then incubated at an optimum temperature (20-25 °C or room temperature) in a dark area, since the spawn run does not need light. The incubation period is usually 15-25 days, depending on various factors such as bag size, substrate used, and conditions provided for growth. After the mycelia have fully colonised, the top of each bag is opened and fruit production is initiated by lowering the room temperature to below 15 °C. For large bag systems, the colonised bags have to be punctured in order to expose a fructication surface and the bags may be watered 2-3 times daily in order to prevent fruiting bodies from drying.

2.5.4 Bottle system

The bottle system was originally developed for cultivating enokitake mushrooms. It has, however, been adopted to cultivate many other mushrooms including oyster and shiitake mushrooms. The cultivation procedures for the bottle system are in all respects similar to that of the bag system. The bottle cultivation system has the advantage of being mechanized, and the bottles are re-usable, easily handled and are also autoclavable (Rodriguez & Royse, 2005). The bottle cultivation system has the disadvantage of requiring high humidity for the formation of sporocarp and this frequently results in the production of watery fruit bodies, that is an unwanted character in mushroom production (Mayuzumi & Mizuno, 1997).

2.6 Substrates for artificial logs

Shiitake and oyster mushrooms are fungi that thrive on a substrate high in lignocellulose content. Thus, most agricultural plant materials, are suitable as base substrates for the production of oyster and shiitake (Heltay, 1957; Heltay & Zavodi, 1960). Some of the agricultural waste that have been used in the production of oyster mushrooms include sawdust, rice husk, sorghum, cocoa beans, cotton waste (Belewu, 2001, 2002, 2003; Belewu & Lawal, 2003), sugar cane bagasse, cotton seed husk (Chang, 1989; Fan & Ding, 1990; Royse, 1995 ; Wang, 1995; Yang, 1986), peanut shells, wheat straw, sunflower seed hulls, wood chips, vine prunings, and coffee pulp (Campbell & Racjan, 1999; Philippoussis *et al.*, 2001a; Philippoussis *et al.*, 2000, 2001b; Poppe, 2000; Ragunathan *et al.*, 1996). However, not all of these lignocellulosic materials are suitable. For example, the use of eucalyptus material has been cautioned, since there has been some reports that it produces mushrooms that can cause stomach problems (Stamets, 2000c). For high yields and acceleration of production, various lignocellulosic substrates used in mushroom production require supplementation with additives that provide extra nitrogen and easily degradable carbohydrates (Royse, 2002). The reason for supplementation of a substrate is that the mycelia of mushrooms require specific nutrients for

growth, that are provided by supplements, and therefore, mushroom yield increases (Oei, 1996). The proportions of the supplements required vary with the type of base lignocellulosic material. A downside of supplementation is that higher supplementation may increase contamination (Miller and Jong, 1987; Przybylowicz and Donoghue, 1990), especially with nitrogenous material, since nitrogen supplements cause the competitor bacteria and fungi to grow more competitively on the substrate (Stamets, 2000c).

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CHAPTER 3

Performance of *Pleurotus ostreatus* mushroom grown on maize stalk residues supplemented with various levels of maize flour and wheat bran.

(This chapter has been published in Food Science and Technology)

3.1 Abstract

Improving the performance of mushrooms in terms of high production and fast growth rate is essential in mushroom cultivation. In this present study, the performance of *Pleurotus ostreatus* was evaluated using varying levels of wheat bran (WB) and maize flour (MF). The results indicated that *Pleurotus ostreatus* was highly influenced by different levels of supplementation, with 8% WB, 18% WB and 2% MF having higher contamination rate. The low levels of supplementation gave a significantly better mycelial growth rate (MGR) and shorter colonisation period as observed that the control had highest MGR whereby 20% MF had lowest MGR. The pinning time (TP) was shortest at the first flush with minimum of 3 days (12% MF). The higher levels of supplementation showed maximum biological efficiency (BE) such as 14% MF, 12% WB and 14% WB. The yield was also higher at high levels of supplementation such as 20% MF and 8% MF being the exception in the lower levels. Based on the results, it was observed that for fast production of oyster mushroom, there is no need to supplement the maize stalk substrate. However for improved productivity, supplements may be added up to certain limits such as 14% MF and 12% WB. The results observed can be useful to small scale farmers who are willing to obtain a good yield within minimal time at minimal cost with reduced contamination problems.

Key words: Biological efficiency, *Pleurotus ostreatus*, supplement, maize stalk.

3.2 Introduction

Oyster mushrooms, such as *Pleurotus ostreatus*, are not usually attacked by both diseases and pests and their cultivation does not require sophisticated control of the growing environment, hence, they are cultivated in a cheap and easy way (Kues & Liu, 2000; Sánchez, 2010). The *P. ostreatus* mushroom has gained popularity within the Southern African Development Community (SADC) (Earnshaw *et al.*, 2012), because of its pleasant flavour and taste (Khan *et al.*, 2012). In addition, the mushroom has potential for livestock feed and for extraction of enzymes and medicinal compounds (Patil *et al.*, 2010).

According to Garg and Gupta (2009), plant derived agro-industrial waste cause environmental and health problems. This waste can be managed through mushroom cultivation, since mushrooms have the ability to grow on a variety of raw lignocellulosic substrates and under a wide range of temperatures (Jandaik & Goyal, 1995; Sánchez, 2010). Oyster mushrooms (*Pleurotus* spp.) are able to perform such duties, because they contain a number of non-specific lignocellulosic enzymes (Zhang *et al.*, 2002) that have a major impact in the development and growth of the mushrooms (Kuforiji & Fasidi, 2008). The nature and nutrient constituent of the mushroom substrate also have an effect on the mycelium growth, mushroom quality, and crop yield (Baldrian & Val´aˇskov´a, 2008; Kües & Liu, 2000). One of the limiting factors in the cultivation of mushrooms is that the availability of a good substrate is essential in order to promote satisfactory yield of the mushrooms (Ueitele *et al.*, 2014). A good substrate should consist of nitrogen supplement and carbohydrates in order to promote rapid growth of the mushroom (Ogundele *et al.*, 2014). In most cases, the substrate does not have the nitrogen required by the mushroom to grow optimally, hence, the need for nitrogen supplementation of the substrate in order to gain an improved growth of the mushroom (Choi, 2004; Oei, 2003a). Although, supplementing the substrate material leads to improved growth, there are limitations involved. For instance, high supplementation of substrate may lead to contamination (Yildiz *et*

al., 2002), together with reduced yield of the mushrooms (Fanadzo *et al.*, 2010). A variety of substrate supplements have been recommended for oyster mushrooms. These supplements include, but are not limited to, urea, ammonium sulphate, gram flour, molasses, mustard cake, soy bean meal, and cotton seed cake (Ralph & Kurtzman, 1994). Wheat bran (WB) supplements are effective in improving mushroom productivity and also the inclusion of maize additive shows an increase in both the nutritional value and productivity of mushrooms (Oei, 2005; Stamets, 2000a).

This present study focussed on utilizing maize stubble as a substrate for oyster mushroom *(P. ostreatus*) production in the northern KwaZulu-Natal (KZN; South Africa) areas. The information scarcity on supplements required to improve yields of the *P. ostreatus* mushroom with minimum contamination guided the research in order to determine the performance of *P. ostreatus* on maize stalks with varied levels of wheat bran (WB) and maize flour (MF) supplements. The results of the study will be crucial to poor rural communities which utilizes local maize residue materials for *P. ostreatus* production for both food and income generation.

3.3 Materials and Methods

3.3.1 Supplements and substrates collection

The maize residues (leaves and stalks) were obtained from farms in and around the northern KZN area (University of Zululand and local community farms) and stored at room temperature (25 ºC). The vitamin free MF supplement was obtained from Empangeni millers and the WB was obtained from Coastal Farmers (local market).

3.3.2 The test mushroom strain

The test oyster mushroom (*P. ostreatus*) was obtained from Cedara College of Agriculture at Pietermaritzburg in KZN, South Africa. The mushroom strain was pre-cultured on potato

dextrose agar (PDA) (MERCK) and incubated at 25 ºC. These were maintained as working spawn cultures in a refrigerator at 4 °C in order to preserve the mushroom in its active state.

3.3.3 Preparation of mushroom spawn

A modified method outlined by Fritsche (1978) was used to prepare the spawn, whereby 1 kg of sorghum grain was soaked overnight in 1.5 litres of water and the excess water was thereafter drained from the grains. A quantity of 900 g of the grains was mixed with 12 g of the gypsum and 3 g $CaCO_3$. The mixture was packed halfway into 250 ml Schott bottles. The grain in the 250 ml glass bottles were sterilised at 121 °C in an autoclave (Labtech, LAC5060S) for 15 minutes and allowed to cool to room temperature. The sterilised sorghum grain mixture was inoculated with 10 mm^2 of previously grown pure cultures of *P. ostreatus* strain using a flamed and cooled scalpel in a laminar flow hood. The inoculated bottles were incubated in the dark at room temperature (25 ºC) for two weeks until the mycelium had fully colonised the grain.

3.3.4 Bulk substrate preparation

The substrate was prepared using a modified version of the method proposed by Bano and Srivastava (1962). The maize stalks were milled using the milling tractor machine and thereafter, the dry weight of maize stalks was measured. This assisted in the analysis of the results, especially the biological efficiency (BE). Tap water was added to the substrate to achieve 65% moisture content using the rule of thumb (1-2 droplets of water must be released when the substrate is squeezed). Thereafter, the maize stalk substrates were separately supplemented with 8 levels of MF and WB, viz; 0%, 2%, 4%, 8%, 12%, 14%, 18%, and 20%, respectively. After the supplements were added and thoroughly mixed with the base substrates, 1 kg of the resultant substrate was packed into polypropylene bags (22.5 cm × 30 cm) in four replicates and compressed by hand until compactness was achieved. The bagged substrates were pasteurised at 60-65 ºC for six hours (Jang *et al.*, 2003) and allowed to cool to room temperature.

3.3.5 Substrate inoculation, spawn running and fruiting

After cooling, all the bagged substrates were inoculated with previously prepared pure grain spawn of the *P. ostreatus* at the rate of 2% of wet substrate. *P. ostreatus* was grown in four replicates for every level of supplemented substrate. Inoculation was strictly conducted in a sterile environment (laminar flow hood) using one spoonful of mother culture placed aseptically on the top surfaces of each bag with substrates. The inoculated substrates were tightly closed with cotton wool and rubber bands to hold it in place. The cotton wool was used as plugs that prevented contamination into bags but allowed gaseous exchange. The inoculated bags were incubated in a dark room at 25-27 ºC until they became fully colonised. Thereafter, the bags were transferred to a fruiting room that was constructed from plastic film supported by gum poles and covered by a double layer of 60% shade cloth on the outside. The fruiting room was under the canopy of a Zygium tree, that is an evergreen tree and hence, provided shade throughout the year. The fruiting room was fitted with timed micro-jet sprinklers that watered the mushrooms in a fine spray at least three times a day in order to maintain 80-90% relative humidity. The mushrooms were allowed to fruit under ambient temperatures. The experiment was terminated after 3 fruiting flushes.

3.3.6 Performance/productivity measurements

In order to determine the performance and how the supplements affect the performance of *P. ostreatus* when grown on maize stalk, different parameters were investigated. The parameters that were measured for performance of *P. ostreatus* included the number of contaminated bags, mycelial growth rate (MGR), number of days to full colonisation, time to fruiting (TP; initiation of pin heads), fresh mushroom yield (MSY) and biological efficiency (BE). The number of contaminated bags for each and every treatment was recorded or counted manually. The advancement of mycelium down the substrate bag was ringed at 6-day intervals until the substrate was fully colonised. The rate of colonisation of the substrate by the mushroom

mycelia was then estimated by measuring the distance from the top down to each of the rings at four equally spaced points around the bag using a marked ruler. The number of days it took for the mycelia to fully colonise the substrate was also noted from the time it first colonised the substrate up to the point where the mycelia fully covered the substrate. Subsequently, after the mycelia has fully colonised the substrate, the bags were taken to the fruiting room where the bags were opened on the top part. The time taken for by mushroom to initiate pin head after full colonisation of substrate and after every flush was recorded on each replicate.

The mushroom yield was calculated according to Morais *et al.* (2000), using the equation: MY = [Weight of fresh mushroom harvested (g) per fresh substrate weight]. The BE was calculated according to (Royse *et al.*, 2004; Stamets, 2000b), as follows: BE = [weight of fresh mushroom harvested (g)/ dry substrate weight (g)] × 100.

3.3.7 Experimental design and statistical analysis

The experiment was conducted in a completely randomised design with 4 replications (n = 4). Data was analysed and graphs were constructed using SPSS version 23 and Microsoft Excel. Tests used were Pearson's Correlation, Binomial Test for Proportions, and Repeated Measures ANOVA followed by Duncan's post hoc test for homogeneous groups. A 5% level of significance was used throughout.

3.4 Results

3.4.1 The number of contaminated bags

There were eight (12.5%) contaminated bags from a total of sixty four. This indicated that the *Pleurotus ostreatus* was not highly susceptible to contamination. From the total of eight contaminated bags, high contamination was observed at 18% WB, 8% WB, and 2% MF (with 50% of replicates), and the lowest contamination was observed at 12% MF, and 2% WB (with 25% of replicates) respectively (Table 3.1). The MF supplement contained a total of three

(9.3%) contaminated bags whereas WB supplement contained a total of five (15.6%) contaminated bags. The proportion of contaminated bags were not significantly different between the two supplements (p = 0.4005). Over time the contamination was overcome and only 2% WB yielded two flushes whereas the other seven yielded three flushes.

Table 3.1 The effect of WB and MF supplements on the number of contaminated bags on the maize stalk base substrate.

Treatment	Level (%)	Number of contaminated bags
MF	2%	2 (50%)
	12%	1 (25%)
WB	2%	1 (25%)
	8%	2 (50%)
	18%	2 (50%)

3.4.2 Mycelial growth rate of *Pleurotus ostreatus* on maize stalk supplemented with different levels of wheat bran and maize flour

There was significant negative correlation between supplement level and MGR of oyster mushroom for MF (r = -0.71; p = 0.00001) and WB (r = -0.59; p = 0.00075) (Figure 3.1). There was no significant difference between the average mycelial growth for MF and WB (p = 0.06655). The MGR did differ significantly between supplement levels for both MF and WB (p = 0.0000). The MGR ranged from 0.35 cm/day for 20% MF to 0.60 cm/day for 0% WB (Table 3.3). Supplement levels 14%, 18%, and 20% of both WB and MF showed significantly lower MGR than the control.

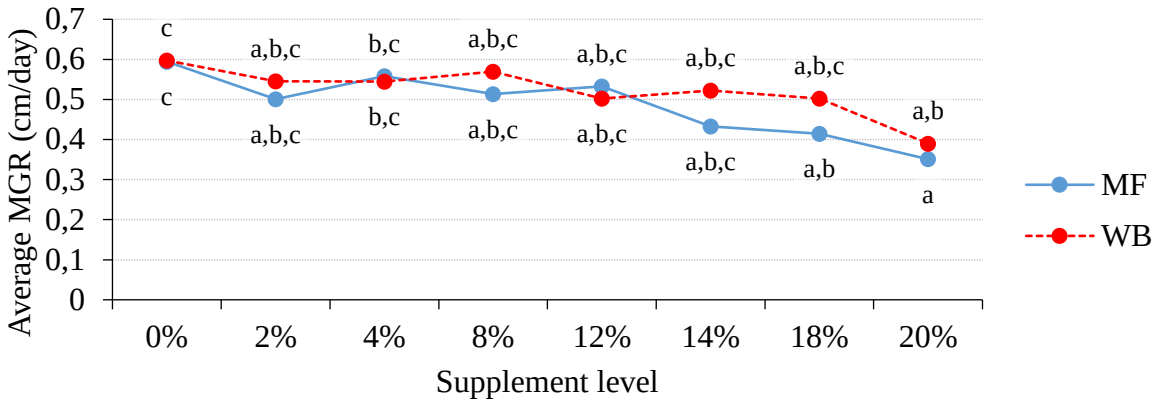

Figure 3. 1 Average MGR of *Pleurotus ostreatus* mushroom per supplement level**.** [a-e]Averages with different letters are significantly different at $p<0.05$.

3.4.3 The number of days to full colonisation of maize stalk substrate by *Pleurotus ostreatus* mushroom

The days to full colonisation displayed a significant positive correlation with the supplement level for both MF (r = 0.58463; p = 0.00055) and WB (r = 0.53651; p = 0.00270) (Figure 3.2)

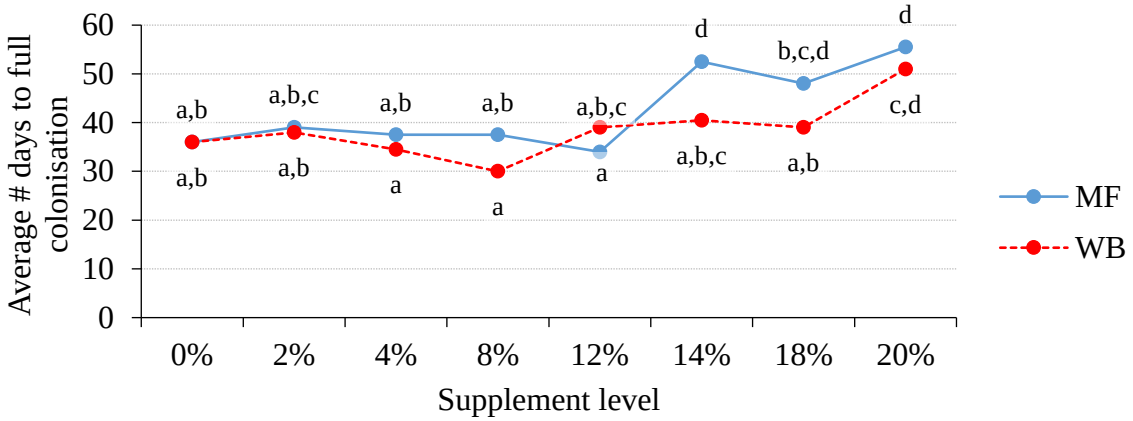

Figure 3. 2 Average days to full colonisation of maize stalk substrate supplement with different levels of MF and WB. [A-d] Averages with different letters are significantly different at $p<0.05$.

The average number of days for WB (39 ± 1) was significantly less than for MF (43 ± 1) with $p = 0.04275$. The number of days to full colonisation of maize stalk ranged from 30 ± 5 (8% WB) to 56 ± 4 (20% MF) (Table 3.3). The longest periods to full colonisation were observed for 20% MF (56 ± 4), 14% MF (53 ± 4), and 20% WB (51 ± 4), respectively.

3.4.4 Effect of different levels of wheat bran and maize flour on time required for pin head formation (time for pinning) between flushes

The TP ranged from 3 days to 36 days based on all flushes. There was a positive correlation between the supplement level and TP (averaged over all 3 flushes) for both MF and WB ($r_{MF} = 0.6092$, $r_{WB} = 0.4305$), however, the correlations were not significant ($p_{MF} = 0.0544$, $p_{WB} = 0.1435$). The lowest TP for flush 1 was observed to be 12% MF (3 ± 0 days). This was not significantly different from 0% MF, 2% MF, 0% WB, 2% WB, 18% WB, and 20% WB (Table 3.2). The highest TP for flush 1 was recorded for 8% WB (20 ± 2 days) of which 50% of the replicates initially displayed contamination. The second highest TP for flush 1 was for 12% WB (12 ± 4 days). The latter was significantly different to the aforementioned minimum values. All the first flush values (except for the anomaly at 8% WB which was excluded from the comparison) were significantly different to the second flush and third flush recorded values. The lowest TP for flush 2 was at 14% WB (19 ± 1) and the highest was at 14% MF (30 ± 3), and 20% MF (30 ± 2). The highest and lowest values were significantly different from each other. The only flush 3 TP values that were not comparable with flush 2 values were at 18% WB (36 ± 1). The remaining flush 3 TP values were not significantly different from the flush 2 TP values**.**

Table 3.2 Effect of WB and MF supplementation on TP between flushes.

Supplement	Level (%)	TP flush 1	TP flush 2	TP flush 3
MF	0%	4 ± 0^{a}	$23 \pm 1^{c,d,e,f}$	$25 \pm 1^{c,d,e,f}$
	2%	4 ± 0**	24 ± 1*	31 ± 5*
	4%	$8 \pm 4^{a,b}$	$20 \pm 3^{c,d}$	$24 \pm 1^{c,d,e,f}$
	8%	8 ± 3*	22 ± 2*	25 ± 1*
	12%	3 ± 0^{a}	$23 \pm 3^{c,d,e,f}$	$23 \pm 0^{c,d,e,f}$
	14%	$6 \pm 3^{a,b}$	30 ± 3^{f}	$27 \pm 7^{c,d,e,f}$
	18%	5 ± 1*	26 ± 2*	28 ± 5*
	20%	$6 \pm 1^{a,b}$	30 ± 2^{f}	30 ± 2^{f}
WB	0%	4 ± 0^{a}	$23 \pm 1^{c,d,e,f}$	$25 \pm 1^{c,d,e,f}$
	2%	4 ± 0*	24 ± 1*	30 ± 2*
	4%	$6 \pm 1^{a,b}$	$21 \pm 1^{c,d,e}$	$28 \pm 2^{d,e,f}$
	8%	20 ± 2*	19 ± 12*	26 ± 0***
	12%	12 ± 4^{b}	$23 \pm 3^{c,d,e,f}$	$29 \pm 1^{e,f}$
	14%	$10 \pm 3^{a,b}$	19 ± 1^{c}	$23 \pm 2^{c,d,e,f}$
	18%	4 ± 0**	20 ± 2*	36 ± 1*
	20%	4 ± 1^{a}	29 ± 1^{f}	$26 \pm 3^{c,d,e,f}$

***Based on a single value, **Based on two or more identical values, *Excluded due to ** and ***. Averages with different superscript in a single column are significantly different at $p < 0.05$.

3.4.5 Biological efficiency of *Pleurotus ostreatus* on maize stalk supplemented with different levels of wheat bran and maize flour

The biological efficiency (BE) is used as a measure of substrate conversion into mushroom (Oseni *et al.*, 2012). There was a positive (non-significant) correlation between supplement level and BE for MF ($r_{MF} = 0.4994$, $p_{MF} = 0.1038$) and a negative (non-significant) correlation between supplement level and BE for WB ($r_{WB} = -0.0145$, $p_{WB} = 0.5136$) (Figure 3.3). There

was significant interaction between supplements and the level of supplementation ($p = 0.0005$), since the supplements of both WB and MF caused different reaction in BE of *Pleurotus ostreatus* over different levels of supplementation.

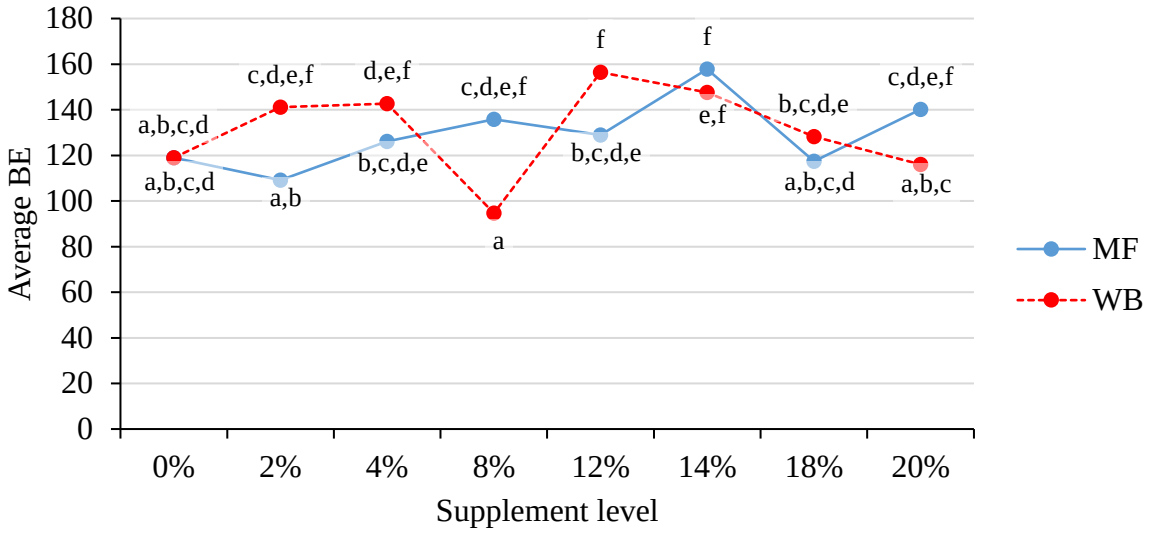

Figure 3.3 Average BE of *Pleurotus ostreatus* mushroom on maize stalk substrate supplemented with different levels of MF and WB. [a-f]Averages with different letters are significantly different at $p < 0.05$.

The BE values ranged from 95 ± 3 (8% WB) to 158 ± 11 (14% MF) (Table3.3). This range indicated that the maize stalk was efficiently profitable for cultivation of *Pleurotus ostreatus*, since it produced BE that was above 50% as stated by Patra and Pani (1995). The highest BE was observed on 14% MF (158 ± 11) and 12% WB (156 ± 6), followed by 14% WB (148 ± 3). The lowest BE was observed on 8% WB (95 ± 30) and 2% MF (109 ± 17) (Table 3.3). When comparing every supplement level from 2% to 20% with the control (0%), the 12% WB (156 ± 6), 14% WB (148 ± 3) and 14% MF (158 ±11) were significantly higher than the non-supplemented maize stalk (control) which had a BE of 119 ± 4.

Table 3.3 Effect of different levels of supplementation of MF and WB on growth of *Pleurotus ostreatus* mushroom.

Supplement	Level	MGR (cm/day)	Days	BE	MSY
MF	0%	0.59 ± 0.05^{e}	$36 \pm 4^{a,b}$	$119 \pm 4^{a,b,c,d}$	$0.12 \pm 0.00^{b,c}$
	2%	$0.50 \pm 0.01^{b,c,d,e}$	$39 \pm 5^{a,b,c}$	$109 \pm 17^{a,b}$	0.11 ± 0.02^{b}
	4%	0.56 ± 0.04^{e}	$38 \pm 4^{a,b}$	$126 \pm 2^{b,c,d,e}$	$0.13 \pm 0.00^{b,c}$
	8%	$0.51 \pm 0.02^{c,d,e}$	$38 \pm 4^{a,b}$	$136 \pm 6^{c,d,e,f}$	0.14 ± 0.01^{c}
	12%	$0.53 \pm 0.02^{c,d,e}$	34 ± 4^{a}	$129 \pm 7^{b,c,d,e}$	$0.13 \pm 0.01^{b,c}$
	14%	$0.43 \pm 0.03^{a,b,c,d}$	53 ± 4^{d}	158 ± 11^{f}	$0.13 \pm 0.01^{b,c}$
	18%	$0.41 \pm 0.02^{a,b,c}$	$48 \pm 4^{b,c,d}$	$117 \pm 6^{a,b,c,d}$	$0.12 \pm 0.01^{b,c}$
	20%	0.35 ± 0.02^{a}	56 ± 4^{d}	$140 \pm 9^{c,d,e,f}$	0.14 ± 0.01^{c}
WB	0%	0.60 ± 0.05^{e}	$36 \pm 4^{a,b}$	$119 \pm 4^{a,b,c,d}$	$0.12 \pm 0.00^{b,c}$
	2%	$0.55 \pm 0.05^{d,e}$	$38 \pm 4^{a,b}$	$141 \pm 6^{c,d,e,f}$	$0.12 \pm 0.01^{b,c}$
	4%	$0.54 \pm 0.02^{d,e}$	35 ± 4^{a}	$143 \pm 4^{d,e,f}$	$0.12 \pm 0.00^{b,c}$
	8%	0.57 ± 0.07^{e}	30 ± 5^{a}	95 ± 30^{a}	0.08 ± 0.03^{a}
	12%	$0.50 \pm 0.01^{b,c,d,e}$	$39 \pm 4^{a,b,c}$	156 ± 6^{f}	$0.13 \pm 0.01^{b,c}$
	14%	$0.52 \pm 0.05^{c,d,e}$	$41 \pm 4^{a,b,c}$	$148 \pm 3^{e,f}$	$0.12 \pm 0.00^{b,c}$
	18%	$0.51 \pm 0.06^{b,c,d,e}$	$39 \pm 5^{a,b}$	$128 \pm 11^{b,c,d,e}$	$0.13 \pm 0.01^{b,c}$
	20%	$0.39 \pm 0.02^{a,b}$	$51 \pm 4^{c,d}$	$116 \pm 5^{a,b,c}$	$0.12 \pm 0.01^{b,c}$

Results are mean ± s.e. MF – maize flour, WB – wheat bran, MGR – mycelia growth rate (a-e), Days – Days until full colonisation (a-d), BE – Biological efficiency (a-f), MSY – Average mushroom yield (a-c) per gram. Means with different superscript in a single column are significantly different at $p < 0.05$.

3.4.6 Yield of *Pleurotus ostreatus* on maize stalk supplemented with different levels of wheat bran and maize flour

There was significant interaction between supplement and supplement levels ($p = 0.0020$) for the yield of *P. ostreatus*. This was due to the behaviour of 8% WB. The minimum yield was recorded at 8% WB (0.08 ± 0.03) and this was significantly less than the average yields at all other MF and WB levels. The maximum yields were recorded at 20% MF (0.14 ± 0.01) and 8% MF (0.14 ± 0.01) (Table 3.3), however these were only significantly different from 2% MF

and 8% WB. The 8% WB showed lowest yield due to the presence of contaminants within this level, since out of four replicates two of them were heavily contaminated.

3.5 Discussion

Supplementation of the substrate is of importance in order to increase production of *P. ostreatus* (Estrada *et al*., 2009), and also to obtain better yield and development (Carvalho *et al*., 2010). The lignocellulosic materials have low protein content and therefore, supplements are useful in the provision of minerals as phosphorus, potassium and nitrogen for production improvement (Mangat *et al*., 2008; Moore-Landecker, 1982). In this present study, maize stalk was supplemented with different levels of WB and MF in order to obtain higher growth and yield of mushroom. Although, supplements enhanced mushroom production, they are required in moderate quantities in order to avoid yield reduction (Fanadzo *et al*., 2010) as well as to decrease contamination risk (Miller & Jong, 1987; Przybylowicz & Donoghue, 1990; Yildiz *et al*., 2002). In this study, the number of contaminated bags ranged from 1 to 2 (25-50%) for different levels of supplementation. This indicated that *P. ostreatus* was not highly affected by contamination, although, 18% WB, 8% WB and 2% MF had slightly higher contamination rates. For 18% WB, this was most likely due to the fact that high supplementation causes possibility of contamination (Yildiz *et al*., 2002), since it becomes too rich in nutrients and therefore run the risk of contamination (Oei, 2003b). As for 8% WB and 2% MF, the slightly higher contamination may most likely be due to partial break down of cellulose and hemicellulose of maize stalk substrate that causes rapid growth of competitor organisms (Balasubramanya & Kathe, 1996). When comparing both supplements, the WB treatment resulted in slightly more contamination of 5 bags (15.6%) than MF treatment which had 3 bags (9.3%). This could be the result of the presence of high nitrogen in WB and also high moisture content (Andrade *et al*., 2007). Overall, this study indicated a low rate of contamination. This implies that *P. ostreatus* was able to colonise maize stalk efficiently and overcome

contamination. One of the important factors in mushroom cultivation is the mycelia with exceptional growth (Pokhrel *et al.*, 2009). In this study, the 0% supplemented maize stalk showed the highest MGR (0.60 cm/day) followed by 8% WB (0.57 cm/day) and 4% MF (0.56 cm/day). These results closely corresponds to the findings of Bhattacharjya *et al*. (2014) who reported closely related MGR on some of the treatments, although their study dealt with sawdust as a substrate. Such high MGR indicated that these level of supplementation consisted of higher carbon to nitrogen ratio (C/N) that favoured high MGR (Yang, 2000). Sarker (2004), established that the mycelium running rate of oyster mushrooms was influenced by different WB levels of supplementation. In this study, by looking at the growth patterns from Figure 3.1, there was a gradual decline in the MGR as the supplement level was increased from 0% to 20%, since the lowest MGR was at 20% MF and 20% WB with respective rates 0.35 cm/day and 0.39 cm/day. This was probably due to high nitrogen content which is known to inhibit mushroom growth if it appears in excessive amounts within the substrate (Yang *et al*., 2013). Therefore, the results of the current study support the findings obtained by Naraian *et al*. (2009) who observed that the increase in the amount of supplements added into the substrate results in the decrease of mycelium growth. The number of days to full colonisation of the maize stalk supplemented with both WB and MF ranged from 30 to 56 days. The shortest periods observed on 8% WB (30 days), 12% MF (34 days) and 4% WB (35 days). This closely corresponds to the 34 days reported for *Pleurotus ostreatus* on sawdust as recorded by Sopit (2006). The longest period to full colonisation was observed at 20% MF (56 days). This was not significantly different to 14% MF (53 days) and 20% WB (51 days). These closely correspond to 58 days reported by Dlamini *et al.* (2012) on maize stover. These results contradict the findings reported by some researchers who stipulated that *Pleurotus ostreatus* took 17-20 days to accomplish full colonisation (Ponmurugan *et al*., 2007; Shah *et al*., 2004; Vetayasuporn *et al*., 2006). Mushroom pinning require conditions that are different from those required for

mycelial growth (Patrick *et al*., 2014). The shortest TP for flush 1 was recorded for 12% MF (3 days). This was not significantly different to 0% supplements (control), 2% MF, 2% WB, 18% WB, and 20% WB with TP of 4 days each. The longest TP for flush 1 was recorded for 8% WB (20 days) and 12% WB (12 days). All flush 1 pinning times were significantly shorter than the pinning times for flushes 2 and 3 (Table 3.2). This may be attributed to the decrease in carbon and nitrogen, since the formation of primordia or pin heads is directly related to availability of carbon and nitrogen (C:N) from lignocellulose substrate (Narain *et al.*, 2008). The lowest TP for flush 2 was for 14% WB (19 ± 1) and the highest was for 14% MF (30 ± 3) and 20% MF (30 ± 2). Flush 3 TP values were not significantly different from the flush 2 TP values, except 18% WB for flush 3 (36 ± 1). The current findings correspond to the results observed by Obodai & Vowotor (2002) who reported that fruiting bodies of *Pleurotus eous* strain Kapak became apparent within 4 days. These also correspond to findings of Rühl *et al.* (2008) who reported 4 days for pinhead formation of *Pleurotus ostreatus*. This corresponds to our lowest TP for the first flush. Obodai and Vowotor (2002) reported that the *Pleurotus quebeca* strain, PQB, took 35 days. This corresponds closely to 18% WB (36 days) for flush 3. Shah *et al.* (2004) reported almost similar results as they reported that the pin head formation ranged from 6-7 days. The highest BE was observed on 14% MF (158 ± 11) and 12% WB (156 ± 6) followed by 14% WB (148 ± 3) which was significantly higher than the control with 119 ± 4. The lowest BE was observed on 8% WB (95 ± 30) and 2% MF (109 ± 17). The reason for lower BE on 8% WB was the presence of contaminants which competed for space and nutrients. The BE tend to increase with increase in the level of supplementation, however, supplementation above 14% MF and 12% WB may cause decrease in BE (Figure 3.3). This may be caused by the overheating of the substrate that affects the mushroom negatively (Oseni *et al.*, 2012). The obtained BE closely corroborates with the findings of Jafarpour *et al.* (2011) who obtained BE of 96.13% on lawn cut complemented with rice bran, 118.20% on barley

straw with rice bran, and 156.40% on chop maize complemented with soybean powder and rice bran. The system of adding supplements to the substrates proved to be useful as research has indicated that addition of certain supplements into the substrates improved yield (Kadiri & Fasidi, 1993 ; Ukoima *et al.*, 2009). The study indicated that there was significant interaction between supplement and supplement level ($p = 0.0020$) with yield ranging from lowest of 0.08 g/g (80 g/kg) to the highest of 0.14 g/g (140 g/kg). The 8% WB recorded the lowest yield, but this is most likely due to the contamination in this level of supplementation that might affected the cellulosic content of the substrates. The results obtained indicated that the yield at 8% MF and 20% MF levels of supplementation are only significantly higher than the yield at 8% WB and 2% MF. The low yield may be attributed to factors such as contamination and carbon and nitrogen balance within the substrate. Zadrazil (1980) states that substrates low in nitrogen content favours the growth of *Pleurotus* species and this might have caused the highest yield that was obtained on 8% MF and 20% MF. The yield for this study corresponds favourably with the findings of Pokhrel *et al.* (2013) who obtained a yield of 153.53 g on banana leaves with rice bran, 131.28 g banana leaves with chicken manure, and a lowest yield of 94.90 g.

3.6 Conclusion

The results of the study stipulate that the performance of *P. ostreatus* was highly influenced by addition of supplements (WB and MF) into the substrate. It was observed that the addition and increase of supplements result in a decrease of the mycelial growth for both WB and MF. In addition, the days to full colonisation of the maize stalk tend to increase significantly with an increased level of supplement for both WB and MF. The TP behave in the same way as the level of supplementation is increased. Therefore, the mushroom production period is said to be delayed by increasing the level supplementation within the maize stalk substrate. In terms of productivity (BE and MSY), it was clear that an increase in level of supplementation lead to an improved performance of *P. ostreatus*.

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CHAPTER 4

Performance of *Pleurotus pulmonarius* mushroom grown on maize stalk residues supplemented with various levels of maize flour and wheat bran

(This chapter has been accepted by Food Science and Technology)

4.1 Abstract

The study evaluated the performance of *Pleurotus pulmonarius* grown on maize stalk supplemented with varying levels of both wheat bran (WB) and maize flour (MF) supplement. The experiments were arranged in a completely random design. The results indicated that *P. pulmonarius* was significantly influenced by different levels of supplementation, with 20% WB supplementation having a higher contamination rate. The low levels of supplementation gave significantly better mycelial growth rate (MGR) and shorter colonisation period. The 2% MF, 2% WB and 4% WB gave significantly higher MGR and faster colonisation. The pinning time (TP) was shortest at the first flush with a minimum of 2 days. The higher levels of supplementation showed maximum biological efficiency (BE) and yield, however, with supplementation levels above 12% WB and 14% MF, the BE and yield declined. The results indicated that low levels of supplementation provides a better growth rate and faster colonisation period, whereas, high levels of supplementation provides better production in terms of yield and BE. Therefore, for the purpose of maximum production, 12% WB and 14% MF could be recommended while for fast production time, 2% MF and 2% WB are recommended.

Key words: Production; biological efficiency; yield; supplement levels.

4.2 Introduction

Mushrooms are recognized as fungi with fruiting bodies that decompose their growth media using biochemical processes in order to obtain energy and growth material (Chang, 1991). Previous studies have reported that there are more than 2000 species of edible mushrooms, however, only about 22 species of these are intensively cultivated (Manzi *et al*., 2001). Among the 22 species of mushroom, oyster mushroom ranks third in the world (Obodai *et al*., 2003). Mushroom farming has many advantages, such as utilising little land for production. This advantage renders mushroom farming economically beneficial (Dzomeku, 2009). Mushroom farming is an alternative way of alleviating poverty, because of relatively quick returns and inexpensive production (Masarirambi *et al*., 2011). Besides these benefits of mushroom farming, oyster mushrooms contain medicinal properties (Gunde-Cimerman & Cimerman, 1995; Pramanik *et al*., 2005; Alarcón & Águila, 2006; Jedinak & Sliva, 2008). For example, *P. pulmonarius* contains anti-inflammatory, anti-nociceptive, and anti-proliferative actions (Smiderle *et al*., 2008; Lavi *et al*., 2010). In common with other *Pleurotus* species, *P. pulmonarius* has characteristics that facilitate intensification of its production. For example, it is an aggressive growing fungi with simple cultivation that gives higher yield with good nutritional value (Wahab *et al*., 2014). Furthermore, this edible and medicinal mushroom is able to break down lignin and other aromatic compounds, since it produces enzymes such as laccase (Lac), manganese oxidizing peroxidase (Mn-dependent peroxidase (MnP) and versatile peroxidase (VP) and aryl-alcohol oxidase (AAO) (Munoz *et al*., 1997). These enzymes allow *P. pulmonarius* to be easily cultivated on various lignocellulosic materials that includes sawdust, paper products, and most agricultural wastes (Croan, 2000). Substrates for mushroom growth may be divided into those regarded as major materials (lignocellulose source) and those regarded as supplementary materials (nitrogen and minerals sources) (Zhang, 2005). Both the major and the supplementary materials are useful in mushroom growth, since the substrate

composition has a huge impact on the biological efficiency (BE) of the mushroom (Chang-Ho & Yee, 1977; Chang & Miles, 1982) and the addition of supplements results in an increased yield (Royse, 2002).

Other researchers have emphasised that the ideal substrate for *Pleurotus* species production should consist of an adequate source of nitrogen and a lignocellulose substrate that provides enough carbohydrate (Oei, 1996; Ayodele & Okhuoya, 2007). To determine whether the substrate had enough nitrogen and carbohydrates, the efficiency of this bioconversion process and also the productivity of mushroom is monitored by the BE of the mushroom (Chang *et al*., 1981).

In northern KwaZulu-Natal (KZN) in the Republic of South Africa (RSA), there is a huge amount of agricultural waste produced by local farms. The local farms produce large maize residues that adds to the environmental pollution, since maize residues are usually burnt leading to negative environmental impact. The current study focuses on converting this waste found within the northern KZN area into value added food in a form of mushroom production. However, there is lack of information on how to convert this local agricultural waste into nutrients for *P. pulmonarius* mushrooms with improved yield, short production time at minimal contamination. Therefore, this study aimed to determine the performance of *P. pulmonarius* on maize stalks with varying levels of wheat bran (WB) and maize flour (MF) supplements.

4.3 Materials and Methods

4.3.1 The collection of substrates and supplements

Maize stalk residues were collected from local farmers in Northern KwaZulu Natal (KZN), Republic of South Africa (RSA) and were kept at room temperature. The maize flour supplement which was free of vitamins was collected form Empangeni millers in KZN. The wheat bran supplement was collected from a local market in KZN named the Coastal Farmers.

4.3.2 The oyster mushroom strain

P. pulmonarius (*M2204*) mushroom was received from the company called Mycelia in Belgium. Potato dextrose agar (PDA) (MERCK) was used to pre-culture the *P. pulmonarius* mushroom and thereafter the mushroom cultures were incubated at room temperature (25 ºC) for 7 days. The mushroom cultures were maintained as working spawn cultures at 4 ºC for further use.

4.3.3 The oyster mushroom spawn preparation

The method described by Fritsche (1978) was modified to prepare the mushroom spawn. 1kg of white sorghum grains were soaked in 1.5 litres of water overnight. The excess water was drained off from the soaked grains. 900 g of the soaked grains were mixed with 12 g of gypsum together with 3 g of CaCO3 and packed half way into 250 ml Schott bottles. The packed grains within the 250 ml Schott bottles were then sterilised in an autoclave (Labtech, LAC5060S) at 121 ºC (103,4kPa) for 15 min and thereafter allowed to cool down to room temperature. After sterilisation the grain mixture were inoculated with ±10 mm^2 of a previously prepared pure culture of *P. pulmonarius* mushroom in a laminar flow hood. The inoculated grain mixtures were incubated in a dark room at 25 ºC for 14 days until the grain mixture were fully colonised by the mycelia.

4.3.4 The preparation of maize stalk substrate

The maize stalk substrate was prepared using a modified procedure by Bano and Srivastava (1962). The dry weight of the milled maize stalk was measured, which assisted in the calculation of other parameters. A measured amount of tap water was added into the maize stalk substrate in order to obtain a standard moisture of 65%. The 65% moisture was obtained using the method called the rule of thumb whereby 1-2 drops of water were released when the substrate was squeezed into the hand. The substrates were supplemented separately with varying levels of both wheat bran and maize flour supplements, wiz; 0%, 2%, 4%, 8%, 12%,

14%, 18% & 20%. After a complete substrate supplementation, 1kg of the supplemented substrate was packed into polypropylene bags of 22.5 cm × 30 cm. The substrate was manually packed into bags and compressed by hand to achieve compactness. The packed substrates within the bags were allowed to be pasteurised at 100 ºC for the period of 12 h (Jang *et al*., 2003). This was done using the Marshall Fowler electrode steamer. After pasteurisation the bagged substrates were allowed to cool at room temperature.

4.3.5 Inoculation of substrate, spawn running and fruiting of mushroom

The sterilised substrate was then inoculated at a rate of 2% of wet substrate with the previously prepared *P. pulmonarius* mushroom spawn. The *Pleurotus pulmonarius* mushroom was cultivated into four replicates for each selected level of the supplemented substrate. The substrates within the bags were inoculated strictly under aseptic conditions (laminar flow hood). The bags were inoculated by placing one spoonful of mother culture into the top surfaces of every bagged substrate. After inoculation, the substrates were closed tightly using rubber bands and cotton wool to prevent contamination but allows free gaseous exchange within the inoculated substrate. After inoculation the bags were incubated in a dark room at 25–27ºC till the bags were fully colonised by the mushroom mycelia. The fully colonised bags were then transferred from the incubation room to the fruiting room that was constructed from plastic film supported by gum poles and covered by a double layer of 60% shade cloth. The inside of the fruiting room was fitted with micro-jet sprinklers that watered the mushrooms three times a day. After three fruiting flushes the experiment was terminated.

4.3.6 Performance and productivity measurements

The performance and productivity of the mushroom were measured using the method outlined by Mkhize *et al*. (2016). The following parameters were measured in order to evaluate the performance and productivity of *P. pulmonarius* mushroom. These included the biological efficiency, mycelial growth rate, number of contaminated bags, time to fruiting, yield of the

mushroom and the number of days to full colonisation of substrate. The rate of mycelial advancement through the substrate was monitored in a six day interval by marking the areas where the mycelia growth ended. The markings were made at four equally spaced points around the bag using the measuring ruler. The days from the first colonisation of the substrate by the mycelia to the time when the mycelia fully covered the whole maize stalk substrate was noted and recorded. The bags which were fully colonised by mushroom mycelia were opened in the fruiting room, therefore the time taken by the mushroom to pin after full colonisation of the substrate was recorded and also the pin head formation after each and every flush was recorded for all the replicates. The yield of *P. pulmonarius* mushroom was calculated using the equation suggested by Morais *et al*. (2000), as follows:

$MY = [\text{Weight of fresh mushroom harvested} / \text{ fresh substrate weight }]$.

The last parameter which was the biological efficiency was calculated according to Royse *et al*. (2004) and Stamets (2000), as follows:

BE = [weight of fresh mushroom harvested (g)/dry substrate weight (g)] × 100.

4.3.7 The analysis of data and experimental arrangement

The experiment was carried out in a completely randomised design with 4 replicates (n = 4). Data was analysed and graphs were constructed using SPSS version 23 and Microsoft Excel. Tests used were Repeated Measures of ANOVA followed by Duncan's post hoc test for homogeneous groups. A 5% level of significance was used throughout.

4.4 Results

4.4.1 Contamination

From the observed results, there were no differences in the number of contaminated bags between the two supplements (WB and MF). For both supplements, there were only three (9.4%) contaminated bags out of 32 replicates (Table 4.1). Although, there were no differences

between WB and MF in the number of contaminated bags, there were differences in the number of contaminated bags within different levels of supplementation. It was observed that for WB, the only level that experienced contamination was 20% (Table 4.1). This level of supplementation was heavily contaminated at 75% (3 out of 4 replicates). The levels of MF that experienced contamination were 8%, 12% and 20%, that only experienced one contaminated bag (25%) out of 4 replicates. This was an indication that an increase in MF did not increase the risk of contamination on *P. pulmonarius* when cultivated on maize stalk substrates. The current results indicated that *P. pulmonarius* was not highly affected by contamination except for the 20% WB supplement that experienced a high rate of contamination.

Table 4.1 Effect of WB and MF supplements on the number of contaminated bags on the maize stalk base substrate.

Treatment	Level (%)	Number of contaminated bags
MF	8%	1 (25%)
	12%	1 (25%)
	20%	1 (25%)
WB	20%	3 (75%)

4.4.2 The mycelial growth rate of *P. pulmonarius* on maize stalk substrates supplemented with different levels of wheat bran and maize flour

The average MGR for the MF supplement treatment levels was not significantly different to the average MGR for WB supplement treatment levels (p = 0.96276) (Figure 4.1). However, the MGR of *P. pulmonarius* was significantly (p = 0.00000) influenced by different levels of both supplements (WB and MF), that ranged from 0.28 cm/day to 0.74 cm/day (Table 4.3). The highest MGR was observed at 2% MF (0.74 cm/day) followed by 4% WB (0.66 cm/day) and 2% WB (0.64 cm/day), respectively (Table 4.3). The 2% MF, 4% WB and 2% WB were

significantly higher in MGR than the other levels of supplementation including the control (0% supplement). The MGR gradually declined immediately after peaking at 2% and 4% for WB supplement (Figure 4.1). For the MF supplement the same pattern of mycelium growth was observed after peaking at 2%.

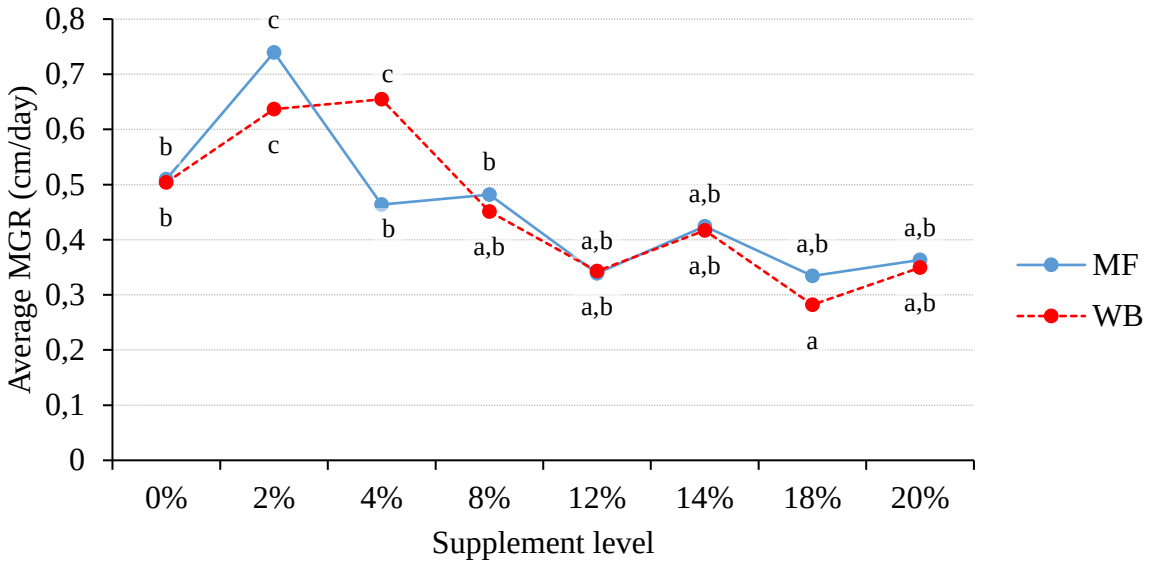

Figure 4.1 Average MGR of *P. pulmonarius* mushroom per supplement level. [a-c]Averages with different letters are significantly different at $p < 0.05$.

4.4.3 The number of days to full colonisation of maize stalk substrate by *P. pulmonarius* mushroom

There was significant interaction between the supplement material and the level of supplementation ($p = 0.00913$) on the number of days taken by *P. pulmonarius* to fully colonise the maize stalk substrate. The results obtained indicate that the time taken by *P. pulmonarius* to fully colonise the maize stalk substrate ranged from 30 to 69 days (Table 4.3). The shortest period to full substrate colonisation of substrate was observed at 2% for both WB and MF (30 days) followed by 4% WB (33 days) and 0% (control) with 39 days. These minimum days to full colonisation that were observed at low supplementation for both WB and MF supplements

were significantly shorter than the rest of the supplement levels. After reaching the minimum days at 2% for both WB and MF, the days it took for *P. pulmonarius* to fully colonise the maize stalk increased as the supplement levels were increased further. Thus, the pattern of days to full colonisation of maize stalk showed that there was a negative relationship between an increase in supplement level and duration to full colonisation of the maize stalk for both WB and MF at levels >2% (Figure 4.2).

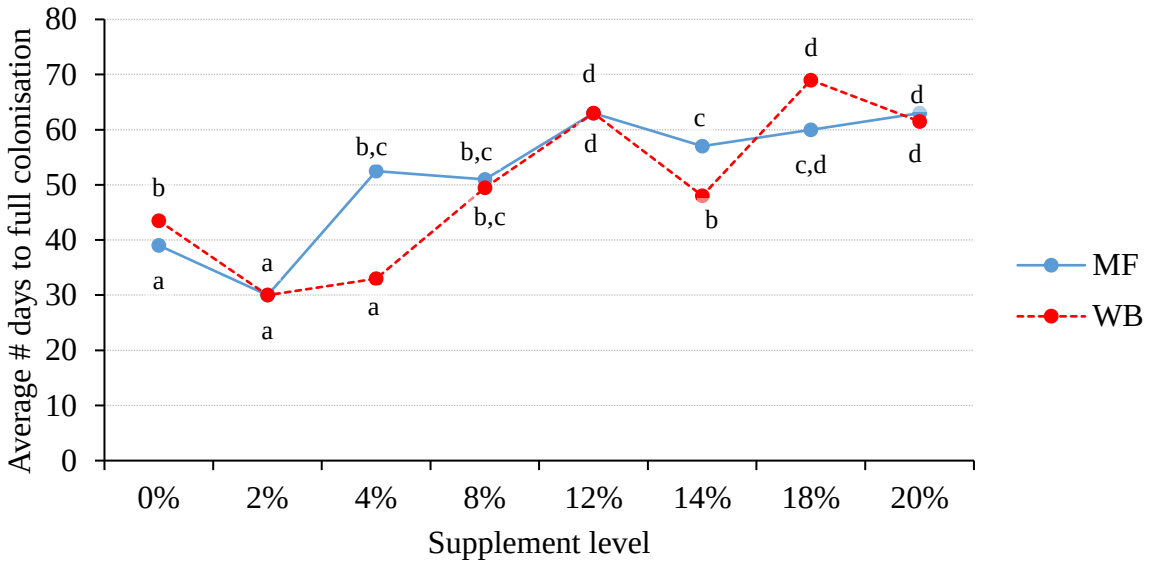

Figure 4.2 Average days to full colonisation of maize stalk substrate supplemented with different levels of MF and WB. [a-d]Averages with different letters are significantly different at $p < 0.05$.

4.4.4 Effect of different levels of wheat bran and maize flour on time required for pin head formation (time for pinning) between flushes

There was no significant interaction in the average TP between the WB and MF supplements. The average TP at different levels of supplementation also showed no significant differences, but the TP of *P. pulmonarius* differed significantly between different flushes with the only overlaps occurring between flush 2 and flush 3 at 2% MF, 14% MF and 20% MF. The TP

ranged from 2 to 16 days (Table 4.2). The shortest TP was observed on the first flush, from 4% to 20% (2 days), and was also statistically similar to other levels for the MF supplement.

Table 4.2 Effect of WB and MF supplementation on TP between flushes.

Supplement	Level	TP flush 1	TP flush 2	TP flush 3
MF	0%	4 ± 0.3^{a}	10 ± 0.5^{b}	14 ± 2.6^{c}
	2%	3 ± 0.4^{a}	9 ± 0.9^{b}	12 ± 1.5^{b}
	4%	2 ± 0.3^{a}	10 ± 0.9^{b}	13 ± 0.8^{c}
	8%	2 ± 0.3^{a}	10 ± 1.1^{b}	12 ± 1.6^{c}
	12%	2 ± 0.3^{a}	8 ± 1.8^{b}	14 ± 1^{c}
	14%	2 ± 0.3^{a}	10 ± 1.8^{b}	11 ± 1.5^{b}
	18%	2 ± 0.3^{a}	8 ± 1.3^{b}	12 ± 0.9^{c}
	20%	2 ± 0^{a}	13 ± 0^{c}	13 ± 1^{c}
WB	0%	4 ± 0.3^{a}	10 ± 0.5^{b}	14 ± 2.6^{c}
	2%	4 ± 0^{a}	8 ± 0.4^{b}	15 ± 3.2^{c}
	4%	3 ± 0.5^{a}	9 ± 0.4^{b}	12 ± 3.4^{c}
	8%	3 ± 0.4^{a}	9 ± 1.1^{b}	15 ± 2.5^{c}
	12%	2 ± 0.3^{a}	9 ± 1.5^{b}	14 ± 2.9^{c}
	14%	3 ± 0.5^{a}	11 ± 1^{b}	13 ± 0.4^{c}
	18%	2 ± 0^{a}	7 ± 1.8^{b}	12 ± 1.1^{c}
	20%	3 ± 0.5^{a}	7 ± 3^{b}	16 ± 3^{c}

Averages with different superscript in a single column are significantly different at $p < 0.05$.

For the WB supplement, 12% and 18% (2 days) showed the shortest TP and was also statistically similar to other supplement levels. The longest TP was recorded on the third flush at 20% WB (16 days) and was statistically similar to other levels of supplementation on flush 3 with the only exception at 2% and 14% MFs. The results in Table 4.2 shows that the *P.*

pulmonarius was quite quicker in initiation of pin heads. Overall, the results observed indicated that there was no significant differences between supplement levels for both WB and MF, however, there were differences between the flushes, since the first flush had the shortest TP.

4.4.5 Biological efficiency of *Pleurotus pulmonarius* on maize stalk supplemented with different levels of wheat bran and maize flour

The results for BE indicated that there was significant interaction between the type of supplement and supplement level (p = 0.01411). The differences between the two supplement materials in BE were small at low supplement levels, but increased at higher supplement levels ≥14% (Figure 4.3).

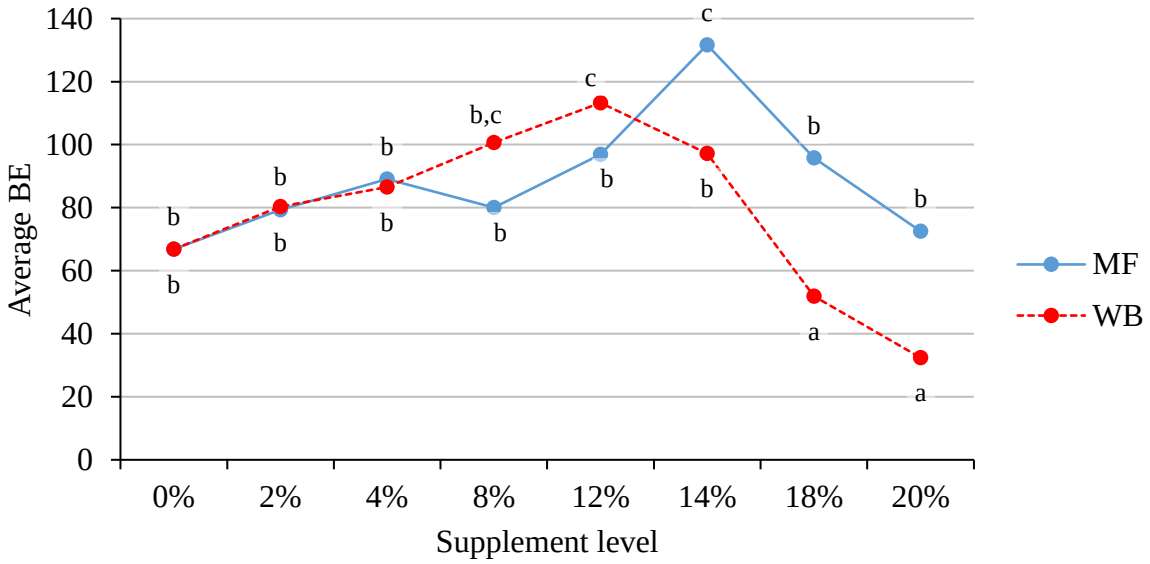

Figure 4.3 Average BE of *Pleurotus pulmonarius* on maize stalk substrate supplemented with different levels of MF and WB supplement. [a-c]Averages with different letters are significantly different at $p < 0.05$.

It was noted that for the MF supplement, BE increased from 66.8 ± 5 at 0% to 131.7 ± 9 at 14% MF and thereafter decreased to 72.5 ± 24 at 20% MF (Figure 4.3). For WB the BE increased

from 66.8 ± 5 at 0% to 113.3 ± 9 at 12% after which it decreased to 32.4 ± 24 at 20% (Figure 4.3). The BE for WB decreased more than that for MF at supplement treatment levels ≥14%.

4.4.6 Yield of *P. pulmonarius* on maize stalk supplemented with different levels of wheat bran and maize flour

There was no significant interaction between type of supplement and the level of supplement (p = 0.16642). However, the average yield of MF supplement was significantly higher than the average yield of WB supplement (p = 0.00124). The yield response pattern to increasing levels of supplementation indicated a gradual yield increase with increasing supplement levels peaking at 14% for MF and 12% for WB and declining afterwards. The decrease was greater for WB than it was for MF (Figure 4.4)

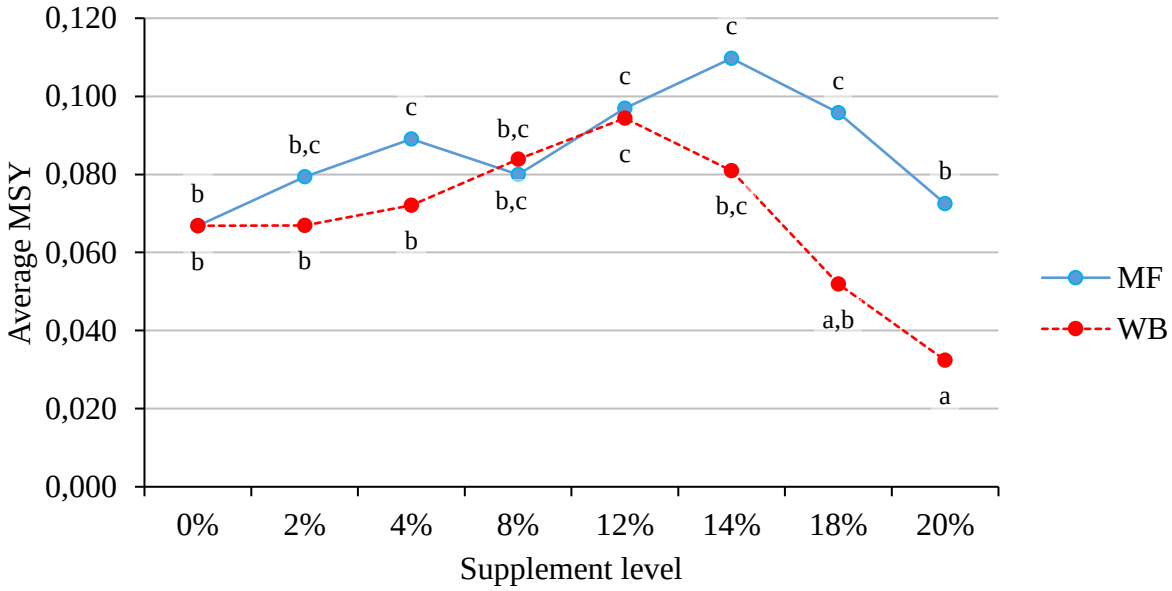

Figure 4.4 Average yield of *Pleurotus pulmonarius* mushroom on maize stalk substrate supplemented with different levels of MF and WB. [a-c]Averages with different letters are significantly different at $p < 0.05$.

Table 4.3 Effect of different levels of supplementation of MF and WB on growth of *P. pulmonarius.*

Supplement	Level (%)	MGR (cm/day)	Days	BE	MSY
MF	0%	0.51 ± 0.09^{b}	39 ± 3^{a}	66.8 ± 5^{b}	0.07 ± 0.00^{b}
	2%	0.74 ± 0.04^{c}	30 ± 0^{a}	79.4 ± 3^{b}	0.08 ± 0.00^{bc}
	4%	0.47 ± 0.04^{b}	53 ± 4^{bc}	89.1 ± 5^{b}	0.09 ± 0.01^{c}
	8%	0.48 ± 0.10^{b}	51 ± 3^{bc}	80.0 ± 6^{b}	0.08 ± 0.01^{bc}
	12%	0.34 ± 0.02^{ab}	63 ± 4^{d}	96.9 ± 4^{b}	0.10 ± 0.00^{c}
	14%	0.42 ± 0.02^{ab}	57 ± 4^{c}	131.7 ± 9^{c}	0.11 ± 0.01^{c}
	18%	0.33 ± 0.01^{ab}	60 ± 2^{cd}	95.8 ± 6^{b}	0.10 ± 0.01^{c}
	20%	0.36 ± 0.07^{ab}	63 ± 3^{d}	72.5 ± 24^{b}	0.07 ± 0.02^{b}
WB	0%	0.50 ± 0.09^{b}	39 ± 5^{a}	66.8 ± 5^{b}	0.07 ± 0.00^{b}
	2%	0.64 ± 0.01^{c}	30 ± 0^{a}	80.3 ± 2^{b}	0.07 ± 0.00^{b}
	4%	0.66 ± 0.02^{c}	33 ± 2^{a}	86.5 ± 12^{b}	0.07 ± 0.01^{b}
	8%	0.45 ± 0.06^{ab}	50 ± 6^{bc}	100.7 ± 9^{bc}	0.08 ± 0.01^{bc}
	12%	0.34 ± 0.02^{ab}	63 ± 4^{d}	$113.3 \pm 9c$	0.09 ± 0.01^{c}
	14%	0.42 ± 0.04^{ab}	48 ± 2^{b}	$97.2 \pm 8b$	0.08 ± 0.01^{bc}
	18%	0.28 ± 0.01^{a}	69 ± 2^{d}	$51.9 \pm 6a$	0.05 ± 0.01^{ab}
	20%	0.35 ± 0.06^{ab}	62 ± 4^{d}	$32.4 \pm 24a$	0.03 ± 0.02^{a}

Results are mean ± SE. MF–maize flour, WB–wheat bran, MGR–mycelia growth rate (a-c), Days– Days until full colonisation (a-d), BE–Biological efficiency (a-c), MSY–Average mushroom yield (a-c). Column results with different superscripts indicate significant differences at 5%.

4.5 Discussion

The cultivation of mushrooms requires a good substrate that consists of sufficient nitrogen and carbohydrates to promote rapid growth of the mushroom (Ayodele & Okhuoya, 2007; Oei 1996). However, a major obstacle in mushroom cultivation is that the likelihood of contamination may also increase with an increase in nutrient concentration of the substrate (Wabali & Wocha, 2013). The incidence of contamination, such as fungal contamination within the substrate, has a hugely negative impact on the growth and yield of mushrooms, since contaminants compete with the cultivated mushrooms (Tisdale, 2004). Therefore, finding ways of improving yield and production of mushroom has become the major objective of mushroom research (Soko *et al*., 2008).

In this current study, the rate of substrate contamination was not highly affected by the increase in the concentration of the supplements in both the WB and MF. The 20% WB was the level that showed exception in the number of contaminated bags, since it experienced a relatively high rate of contamination of 3 bags (75%) out of 4 replicates. This indicated that the *P. pulmonarius* was highly susceptible to contamination when the 20% WB was mixed with maize stalk. Low levels of contamination was observed at 8%, 12%, and 20% MF and was probably due to partial breakdown of the substrate (Balasubramanya & Kathe, 1996). The high level of contamination on the 20% WB may be due to the fact that the substrate at this level of WB supplementation became enriched with nitrogen, which promoted the growth of competitor organisms (Oei, 2003). This supported the findings by Wabali and Wocha (2013) who stated that higher nutrient concentration promoted the growth of competitor fungi. Furthermore, it is known that the addition of supplements into the mushroom growing substrate causes some changes in the decomposition rate of the substrate by the mushroom (Ayodele & Okhuoya, 2007). Similar phenomenon was observed in this current study, since different levels of supplements had remarkably different rates of mycelium growth that was significantly different

to the control and other levels. It was observed that the lowest levels of supplementation, such as 2% MF, 4% WB and 2% WB, had significantly higher MGR that showed similarities with the findings of Gume *et al.* (2013) who reported the mycelial extension of 0.69 cm/day on sdZcCh (combination of *Cordia africana* and *Pouteria adolfi-friederici* sawdust, corncobs and coffee bean husks), 0.64 cm/day on ZcCh (corncobs and coffee bean husks), 0.60 cm/day on sd^1ch (combination of *C. africana* sawdust and coffee bean husks) and sd^2ch (*P. adolfi-friederici* sawdust and coffee bean husks). The higher levels of supplementation showed significant lower MGR, especially from 8% to 20% for both WB and MF. There was a gradual decline in MGR as the level of supplementation was increased (Figure 4.1). This was probably caused by high levels of nitrogen that the substrate experienced as the levels of supplementation increased. Therefore, these results are in agreement with Maziero (1990) who stated that high nitrogen concentration results in reduced degradation of lignin by mushrooms. High MGR and short duration of substrate colonisation could be useful in mushroom cultivation, since the shorter colonisation period minimises the growth of flies and contaminants within substrate, resulting in quicker return of investment (Kopytowski Filho & Minhoni, 2004).

The results from this current study indicated that higher concentrations of supplements causes a delay in the period of mushroom growth, whereas lower concentrations of supplements displayed shorter colonisation periods (Table 4.3). The delay caused by higher supplementation was probably due to the presence of high nitrogen levels which is known to inhibit growth of mushroom if it is abundant (Yang *et al*., 2013). The delayed growth of *P. pulmonarius* resulted in the maize stalk being more prone to fungal and bacterial infections (Diamantopoulou *et al*., 2006; Philippoussis *et al*., 2001), hence, there is a need to balance the supplement with nitrogen to avoid long durations before full colonisation and contaminations without compromising on yield. The observed results of the shortest time to full colonisation of substrate corresponded with the findings by Liang *et al.*, (2011) who reported the period of 30.9 ± 0.9 days for *P.*

pulmonarius. The TP was significantly different between different flushes. The time it took for *P. pulmonarius* to initiate pin heads was significantly faster in the first flush than in second flush and the third flush, respectively (Table 4.2). This indicated that the TP of *P. pulmonarius* is affected by an increase in the number of flushes and this might be due to the availability of free circulating carbohydrates within the substrate (Chang, 2006). The earliness in pinning on the first flush clearly showed that the first flush contained high levels of nutrients that are believed to influence the mycelia to be vigorous in growth, and therefore calls for early pinning (Onyango *et al*., 2011). The early or fast TP observed in the current study concur with Dahmardeh *et al*. (2010) who reported TP of 2-3 days.

Parameters such as yield and BE are important parameters to be taken into consideration for success in mushroom cultivation, since the BE parameter indicates the ability of the mushroom to fruit on the substrate (Fan *et al*., 2000). The results recorded showed that the addition of both WB and MF supplement onto the maize stalk substrate greatly influenced the production of *P. pulmonarius* as the values of BE ranged from 66.8% (control) to 131.7%. The BE gradually increased with increasing levels of supplements for both WB and MF, however, a turning point was reached after which the BE decreased rapidly. The decrease in BE after achieving peak in both WB and MF supplement could be due to heat generated by an increase in supplementation. This is because the overheating of substrate is known to influences the growth of the mushroom leading to an unsatisfactory or poor yield (Assan & Mpofu, 2014). Over all, the results of BE indicated that *P. pulmonarius* was successfully cultivated on the maize stalk substrate with exception on the 20% WB that showed low BE of 32.4% due to contamination. The BE of the control (0% supplement) corresponds to the findings of Assan and Mpofu (2014) who reported the BE of 66.88 ± 4.59 on wheat straw. The yield is another important parameter in mushroom production that is influenced by different factors, such as environmental conditions and substrate supplementation with different additives (Mane *et al*., 2007). The recorded results of

the *P. pulmonarius* mushroom indicated that the yield increased with an increase in level of supplementation. These findings support the findings of Moonmoon *et al.* (2011), Patil *et al.* (2011) and Raymond *et al*. (2013) who reported that the yield showed a trend of increasing with an increase in level of supplementation and at some point it decreases. The lower yield or decrease in yield of *P. pulmonarius* on higher levels of WB and MF was probably due to heat generated by higher supplement level (Assan & Mpofu, 2014), and also excess nitrogen within substrate with high levels of supplements that is known to have an influence on yield and growth of mushroom (Déo & Faustin, 2015). The 14% MF supplement and 12% WB gave the highest mushroom yield that shows that at this level, there was appropriate levels of nutrients.

4.6 Conclusion

The outcome of the study indicates that the addition of supplements (WB and MF) has great influence in the yield and growth performance of *P. pulmonarius* on the maize stalk substrates. The overall contamination was low with the exception of 20% WB that appeared to be highly affected by contamination. The different levels of MF and WB influenced the production period and production rate of *P. pulmonarius*. This is because high levels of supplementation tend to slow the growth rate and increase the period to full colonisation of maize stalk and vice versa. Although, high levels of supplementation delays the production period, they are however, advantageous, since they improve the quantity and quality of the production (yield and BE). It should be note that a turning point is reached after which production decreases with an increase in supplement concentration. Based on the outcome of the current work, it may be recommended that for fast production low levels of supplements should be used. For higher yield or production the 14% MF and 12% WB is recommended but beyond these values the production decreases.

4.7 References

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CHAPTER 5

Performance of *Pleurotus salmoneostramineus* mushroom grown on maize stalk residues supplemented with various levels of maize flour and wheat bran

(This chapter was submitted to Scientia Horticulturae)

5.1 Abstract

Promoting the use of local agricultural waste must be one of the environmental friendly strategies in poverty alleviation. The objective of the present study was to monitor the growth and yield performance of *Pleurotus salmoneostramineus* mushroom grown on maize stalk supplemented with varying levels of wheat bran (WB) and maize flour (MF). The experiments were arranged in a randomized complete block design with four replications per level of treatment. The results indicated that the performance of *P. salmoneostramineus* was affected by different levels of supplementation, with 20% MF having highest contamination rate (75%) followed by 8% MF, 12% MF and 20% WB at 50%. The low supplementation levels (especially the control at 0%, and 4% WB) gave significantly better mycelial growth and a quicker colonisation period. The time for pinning (TP) of *P. salmoneostramineus* was significantly faster within the first flush, with the minimum of 1 day observed at 14% WB and 20% WB. Higher levels of supplementation had a better mushroom yield and biological efficiency (BE). These included 18% WB, 12% MF, 12% WB and 14% MF supplementation levels. From these results, it may be concluded that for fast mushroom production, lower or no supplementation is essential, while for improved productivity, higher supplementation may be recommended. However, production limits are reached at 18% WB, 14% MF and 12% MF.

Key words: Production, supplements, maize stalk, yield, biological efficiency.

5.2 Introduction

Oyster mushrooms are usually classified as fungi that fall under the phylum *Basidomycota*, class of *Agaricomycetes*, under *Pleurotaceae* family (Vilgalys & Sun, 1994). The morphological characteristics of oyster mushrooms that distinguish them from other mushrooms include their cap size (5-20 cm in diameter) and their shell-like appearances (Figure A.3). They are also fleshy with stipe that is lateral or eccentric and may have diverse colour, ranging from white, cream, yellow, pink, brownish or dark grey (Martínez-Carrera, 1999). The pink colour observed in *Pleurotus salmoneostramineus* is usually due to the presence of the chromoprotein that has a photosynthetic function (Shibata *et al.*, 1997).

Finding methods to improve the yield and production of mushrooms is one of the major aims for mushroom researchers (Soko *et al.*, 2008). Therefore, various techniques, substrates, cultivation conditions, and various strains have been examined in order to gain improved productivity and nutritional value (Nunes *et al.*, 2012). Some studies have shown that quality mushrooms with improved yield and growth is achieved through a combination of proper environmental conditions as well as introducing various additives (that include nitrogen sources) into mushroom substrates (Royes, 2002; Panjabrao *et al.*, 2007; Onyango *et al.*, 2011). Another factor that should not be ignored is the that mushrooms that have the potential to produce large number of enzymes are said to better colonise the substrates and at same time, produce healthier and larger fruiting bodies (Ruiz-Rodríguez *et al.*, 2011). However, this is influenced by the composition of the mushroom substrates for the white rot fungi (*Pleurotus* mushroom) (Papinutti *et al.*, 2003; Lechner & Papinutti, 2006). Nevertheless, the substrate material used for mushroom production should retain water tightly so that aeration does occur, otherwise, if substrates retain water poorly, then growth may be limited, since air spaces would be blocked (Patel & Trivedi, 2015). Cultivation of mushrooms in different places is dependent on the availability of local agricultural substrates (Cohen *et al.*, 2002; Liang *et al.*, 2005), also

factors such as the cost and abundance of substrates plays a role in substrate usage (Thomas *et al.*, 1998; Obodai *et al.*, 2003; Mukherjee & Nandi, 2004; Mandeel *et al.*, 2005).

The coastal areas of KwaZulu-Natal (KZN) in South Africa contains a high proportion of maize stalk biomass (on farms). The maize stalk biomass is wasted by burning, and this also results in environmental pollution. All these plant wastes may be usefully converted into nutritious food through cultivation of *P. salmoneostramineus* (pink oyster mushrooms). Although, this could be a useful process, there is however an important concern, since there is a lack of knowledge on how to use the locally available agro-industrial waste in order to obtain improved yields of *P. salmoneostramineus* mushrooms with minimum contamination. Thus, this study aimed to determine the performance of *P. salmoneostramineus* on maize stalks with increasing levels of wheat bran (WB) and maize flour (MF) supplements.

5.3 Methods and Materials

5.3.1 Collection of substrates and supplements

The maize stalk substrate was collected from the local community farms around University of Zululand in the northern Kwa-Zulu Natal (KZN) area, Republic of South Africa (RSA). The collected maize stalk substrate was stored at room temperature. Supplements such as maize flour (vitamin free) and wheat bran were received from the local market named Empangeni millers in KZN.

5.3.2 *Pleurotus salmoneostramineus* mushroom strain

The mushroom of interest, *Pleurotus salmoneostramineus* (*M2708*), was imported from the company named Mycelia in Belgium. The *Pleurotus salmoneostramineus* mushroom was cultured into a potato dextrose agar (PDA) (MERCK) and thereafter incubated at 25 ºC for 7 days. The pre-cultured mushroom was stored in the refrigerator for future use working spawn culture.

5.3.3 *Pleurotus salmoneostramineus* spawn preparation

Method stipulated by Fritsche (1978) was modified in order to prepare mushroom spawn. The white sorghum grain of 1 kg was overnight soaked in ±1.5 litres of water. The water which was excess was drained off from the white sorghum grains. 12 g gypsum and 3 g of CaCO3 was mixed together with sorghum grains and thereafter packed half-way into the 250 ml Schott bottles. The grains within Schott bottles were sterilised in an autoclave for 15 min at 121 ºC. The sterilised sorghum grains were cooled down at 25 ºC and thereafter inoculated with at least one square of 10 mm^2 of mycelium of a previously grown *Pleurotus salmoneostramineus* culture. The inoculated sorghum grains were incubated in a dark room at 25 ºC for a period of two weeks whereby the mycelia had fully colonised the sorghum grains.

5.3.4 Preparation of the base substrates (maize stalk)

Method proposed by Bano and Srivastava (1962) was slightly modified to prepare maize substrate. Tap water was added to the milled maize stalk substrate in order to achieve a standard moisture of 65%. The moist maize stalk substrate was supplemented separately with different concentrations of wheat bran and maize flour supplements, wiz; 0%, 2%, 4%, 8%, 12%, 14%, 18% &20%. After supplementation of maize stalk substrate with wheat bran and maize flour, 1 kg of supplemented substrate was packed into 22.5 cm × 30 cm polypropylene bags and manually compressed with hands to achieve compact bag. The packed substrate within the polypropylene bags was pasteurised at 60-65 ºC for 6 h (Jang *et al.*, 2003) using Marshall Fowler electrode steamer and thereafter cooled down at 25 ºC.

5.3.5 Inoculation of substrate, spawning and fruiting

Method outlined by Mkhize *et al*., 2016 was slightly modified for inoculation, spawn running and fruiting of substrate. The previously prepared maize substrate was inoculated with 20g *P. salmoneostramineus* mushroom spawn. The maize stalk substrate was inoculated in a sterile environment in a laminar flow hood. The spawn was aseptically inoculated into top surfaces of

each bag substrate. After inoculation, bags were sealed at the top with the rubber bands together with cotton wool to allow gaseous exchange and prevent contamination. The inoculated substrate was incubated at 25-27 ºC in a dark room until the mushroom mycelia fully colonised the substrate. The fully colonised substrate was then transferred to a fruiting room. The mushrooms within the fruiting room were watered with sprinklers at least 3 times a day to achieve ±85% standard humidity. After 3 fruiting flushes the experiments were terminated.

5.3.6 *Pleurotus salmoneostramineus* growth and yield performance evaluation

The parameters such as rate of mycelial growth, days to full colonisation, contaminated bags, time for pinning, biological efficiency and yield were used to evaluate growth and productivity of *P. salmoneostramineus* mushroom on supplemented substrate. The bags which experienced contamination were noted and recorded. The mycelial growth within the substrate was measured every 6 days until maize stalk substrate was fully impregnated with mycelia. The bags were measured on each and every 6 day interval using the measuring ruler in order to estimate the mycelial growth rate within the substrate. The days taken by the mycelia to colonise maize stalk fully was monitored as from the point of inoculation till the entire substrate was colonised. The fully colonised bags were transferred from incubation room into fruiting room where they were opened and the time taken by the mycelia to initiate pin head after full colonisation of substrate bag and after every flush was recorded for every replicate. The yield of *P. salmoneostramineus* mushroom was calculated according to Morais *et al.* (2000), using the equation:

MY = [Weight of fresh mushroom harvested (g)/fresh substrate weight (g)].

The BE was calculated (Stamets, 2000; Royse *et al.*, 2004) as follows:

BE = [weight of fresh mushroom harvested (g)/dry substrate weight (g)] × 100.

5.3.7 Statistical analysis

All experiments conducted were in 4 replicates (n = 4). The experimental data was analysed using SPSS 23 and the Microsoft Excel was used to construct the graphs. The Pearson's Correlation test, Binomial test for Proportions and also Repeated Measures ANOVA were used which was followed by post hoc Duncan's homogeneous groups. A 5% level of significance was used throughout.

5.4 Results

5.4.1 The number of contaminated bags

There were no significant differences in the number of contaminated bags between WB and MF supplements. The results in Table 5.1 indicate that MF supplement had a total of 7 contaminated bags out of 32 replicates, whereas WB had 6 contaminated bags out of 32 replicates. The 20% MF experienced the highest contamination rate with 3 (75%) out of 4 contaminated bags followed by 8% MF, 12% MF and 20% WB which have 2 (50%) contaminated bags, while lower levels of contamination were recorded for 12% MF, 14% MF, 4% WB and 18% WB with only one contaminated bag (25%). The observed results indicate that *P. salmoneostramineus* mushroom was not highly susceptible to contamination when maize stalk was supplemented with different levels of supplements with the only exception being for 20% MF that showed relatively high levels of contamination.

Table 5.1 The effect of WB and MF supplements on the number of contaminated bags on the maize stalk base substrate.

Treatment	Level (%)	Number of contaminated bags
MF	8%	2 (50%)
	12%	1 (25%)
	14%	1 (25%)
	20%	3 (75%)
WB	4%	1 (25%)
	12%	2 (50%)
	18%	1 (25%)
	20%	2 (50%)

5.4.2 Mycelial growth rate of *Pleurotus salmoneotramineus* on maize stalk supplemented with different levels of wheat bran and maize flour

The MGR of *P. salmoneostramineus* was significantly influenced by the different levels of supplementation for both WB and MF, since the results observed indicated that there was significant interaction between the supplement and the level of supplement (p = 0.005). The levels that were highly contaminated (and the corresponding levels for the other supplement) were excluded from the analysis (20% MF and 20% WB). Table 5.3 show that the MGR ranged from 0.598 ± 0.02 to 0. 350 ± 0.03. The un-supplemented (0%) substrate had a significantly higher MGR of 0.598 ± 0.02 followed by 4% WB (0.538 ± 0.02) and 14% WB (0.490 ± 0.02) as shown on Table 5.3. The rest of the supplementation levels had significantly lower MGR compared to the control (0%). Based on the growth pattern in Figure 5.1, it is clearly observed that for the WB supplement the MGR was highest at the 0% level where after the MGR decreased as the level of supplement increased to 18%. For MF supplement, the maximum

MGR was reached at the control where after it decreased and remained constant from 2% to 18% and then finally decreasing at 20%.

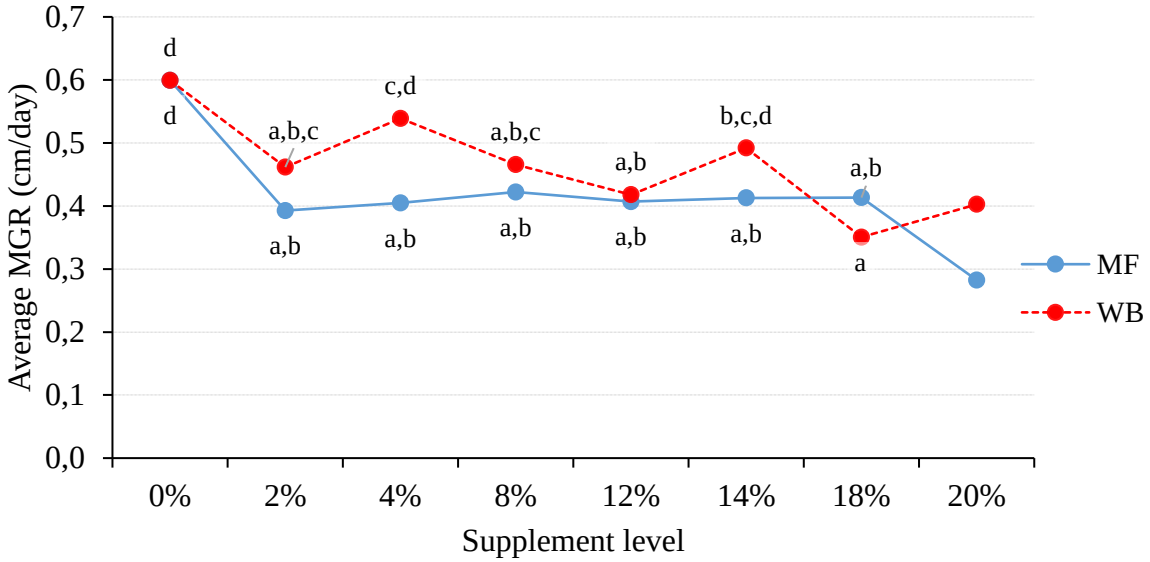

Figure 5.1 Average MGR of *Pleurotus salmoneostramineus* mushroom per supplement level.

[a-d]Averages with different letters are significantly different at $p < 0.05$.

5.4.3 The number of days to full colonisation of maize stalk substrate by *Pleurotus salmoneo-stramineus* mushroom

The duration (days) to full colonisation of maize stalk when supplemented with different levels of supplements indicated that there was significant interaction between the supplement and the level of supplementation ($p = 0.048$). Results represented in Table 5.3 indicated that the days it took for *P. salmoneostramineus* to fully colonise the maize substrate ranged from 34.5 to 56 days. The shortest time to full colonisation of substrate was observed in the control while an extended waiting time was experienced for 18% WB. The colonisation pattern in Figure 5.2 indicate that the addition of supplements had an effect on the time it took for *P. salmoneostramineus* mushroom to fully colonise the substrate. Subsequently after addition of both supplements the days to full colonisation gradually increased.

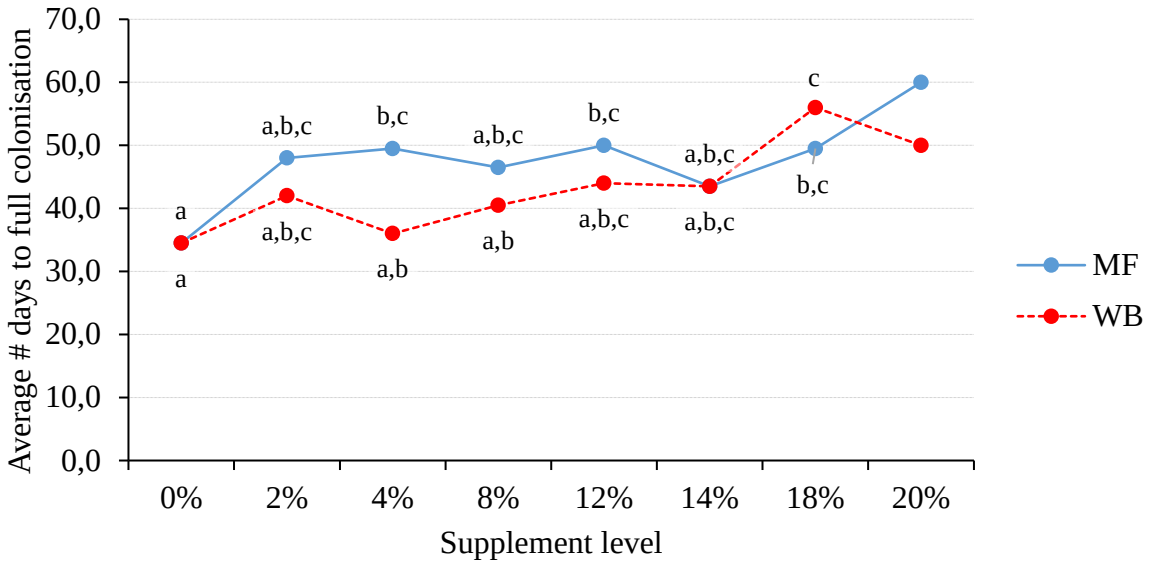

Figure 5.2 Average days to full colonisation of maize stalk substrate supplemented with different levels of MF and WB. [a-c]Averages with different letters are significantly different at $p < 0.05$.

5.4.4 Effect of different levels of wheat bran and maize flour on time required for pin head formation between flushes

The TP of *P. salmoneostramineus* showed no significant interaction between supplement and the level of supplement ($p = 0.148$). However, there were significant differences between the flushes, with the first flush being completely different to the second and third flushes. The first flush TP was significantly shorter than the second and third flushes, respectively. The shortest TP of 1 day was observed at 20% WB and 14% WB. These were not significantly different to the rest of the levels of supplementation for both WB and MF. The second and third flushes had slightly longer TPs, as observed in Table 5.2. The longest TPs were 14 and 15 days.

Table 5.2 Effect of WB and MF supplementation on TP between flushes.

Supplement	Level	TP Flush 1	TP Flush 2	TP flush 3
MF	0%	2 ± 0.3^{a}	15 ± 1.4^{f}	$12 \pm 1.3^{e,f}$
	2%	3 ± 0.3^{a}	$15 \pm 0.6^{e,f}$	$11 \pm 0.7^{d,e,f}$
	4%	2 ± 0.3^{a}	$11 \pm 0.6^{d,e,f}$	$12 \pm 1.2^{e,f}$
	8%	2 ± 0.3^{a}	$14 \pm 1.0^{e,f}$	$10 \pm 0.8^{c,d,e,f}$
	12%	2 ± 0.3^{a}	$12 \pm 0.6^{e,f}$	$12 \pm 0.9^{e,f}$
	14%	3 ± 0.3^{a}	$10 \pm 2.3^{c,d,e,f}$	$14 \pm 2.4^{e,f}$
	18%	2 ± 0.3^{a}	$13 \pm 1.4^{e,f}$	$14 \pm 1.1^{e,f}$
	20%	$5 \pm 3.3^{a,b,c,d}$	$11 \pm 1.2^{d,e,f}$	$9 \pm 0.3^{b,c,d,e,}$
WB	0%	2 ± 0.3^{a}	15 ± 1.4^{f}	$12 \pm 1.3^{e,f}$
	2%	$4 \pm 0.6^{a,b,c}$	$12 \pm 1.0^{e,f}$	$11 \pm 0.9^{d,e,f}$
	4%	$3 \pm 0.3^{a,b}$	$13 \pm 0.8^{e,f}$	$11 \pm 0.7^{d,e,f}$
	8%	2 ± 0.4^{a}	$10 \pm 1.5^{c,d,e,f}$	$14 \pm 0.7^{e,f}$
	12%	$3 \pm 0.0^{a,b}$	$12 \pm 0.3^{e,f}$	$12 \pm 0.3^{e,f}$
	14%	1 ± 0.0^{a}	$15 \pm 0.5^{e,f}$	$11 \pm 1.7^{d,e,f}$
	18%	$3 \pm 0.0^{a,b}$	$10 \pm 0.3^{c,d,e,f}$	$10 \pm 0.7^{c,d,e,f}$
	20%	1 ± 0.3^{a}	$11 \pm 0.3^{d,e,f}$	$11 \pm 0.6^{d,e,f}$

Averages with different superscript in a single column are significantly different at $p < 0.05$.

5.4.5 Biological efficiency of *Pleurotus salmoneostramineus* on maize stalk supplemented with different levels of wheat bran and maize flour

The BE results in Table 5.3 indicate that there was no significant interaction between supplement and the level of supplementation ($p = 0.063$), and the pattern observed in Figure 5.3 shows that the lower levels of supplementation (including the control), resulted in lower BE compared to the higher levels of supplementation. The BE values of *P.*

salmoneostramineus mushroom ranged from 79.69 ± 7.52 (2% MF) to 130. 32 ± 8.69 (18% WB). The highest BE was observed on 18% WB (130. 32 ± 8.69), followed by 12% WB (127.32 ± 8.69), 20% WB (125. 73 ± 8.69), and 14% MF (124.11 ± 7.52). The lowest BE were within the lower levels of supplementation that included 2% MF (79.69 ± 7.52), 8% WB (80.95 ± 7.52), 0% (85.26 ± 7.52), 2% WB (86.42 ± 7.52), and 20% MF (88.55 ± 8.69). Higher levels of supplementation had greater impact on the BE as they gave significantly higher BE values whereas, lower levels of both WB and MF supplementation gave lower BE values.

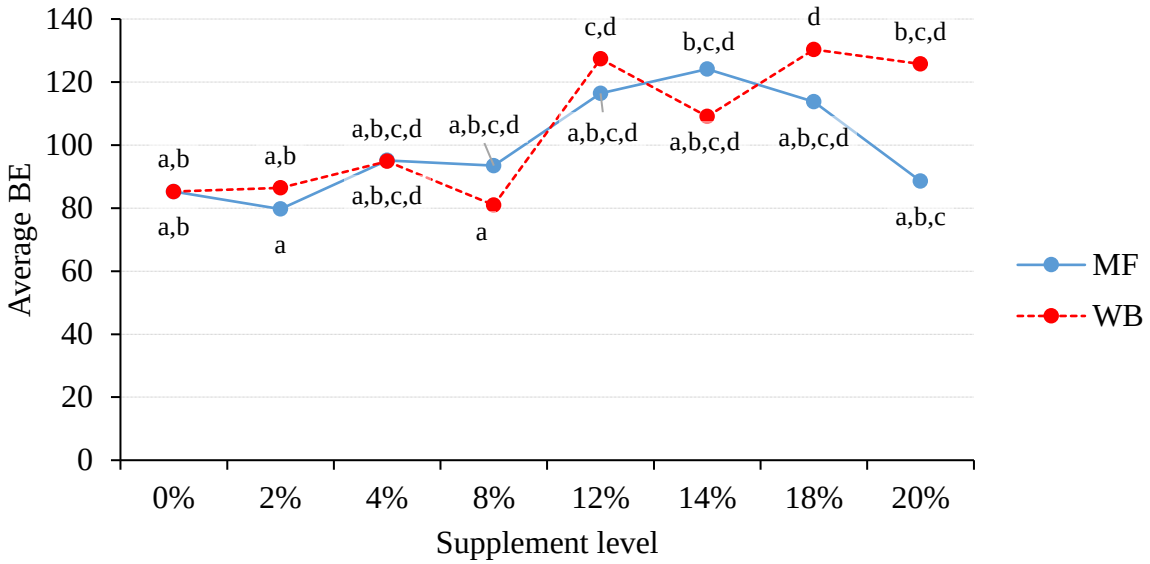

Figure 5.3 Average BE of *Pleurotus salmoneostramineus* on maize stalk substrate supplemented with different levels of MF and WB. [a-d]Averages with different letters are significantly different at $p < 0.05$.

5.4.6 Yield of *Pleurotus salmoneostramineus* on maize stalk supplemented with different levels of wheat bran and maize flour

The yield displayed significant interaction between type of supplement and levels of supplementation ($p = 0.003$). The yield ranged from 0.072 g/g (72 g/kg) to 0.130 g/g (130 g/kg) (Table 5.3) with the maximum yield observed at 18% WB (0.130 g/g) followed by 20% WB (0.126 g/g). These where not significantly different from 12% MF, 18% MF, 12% WB, and

14% MF. The addition of supplements resulted in higher yield as observed on Figure 5.4. The yield gradually increased with the addition of supplements, however, for 20% MF a sharp decrease was observed. For the WB supplement the yield was increasing steadily up to 18% WB and then slightly dropped at 20%.

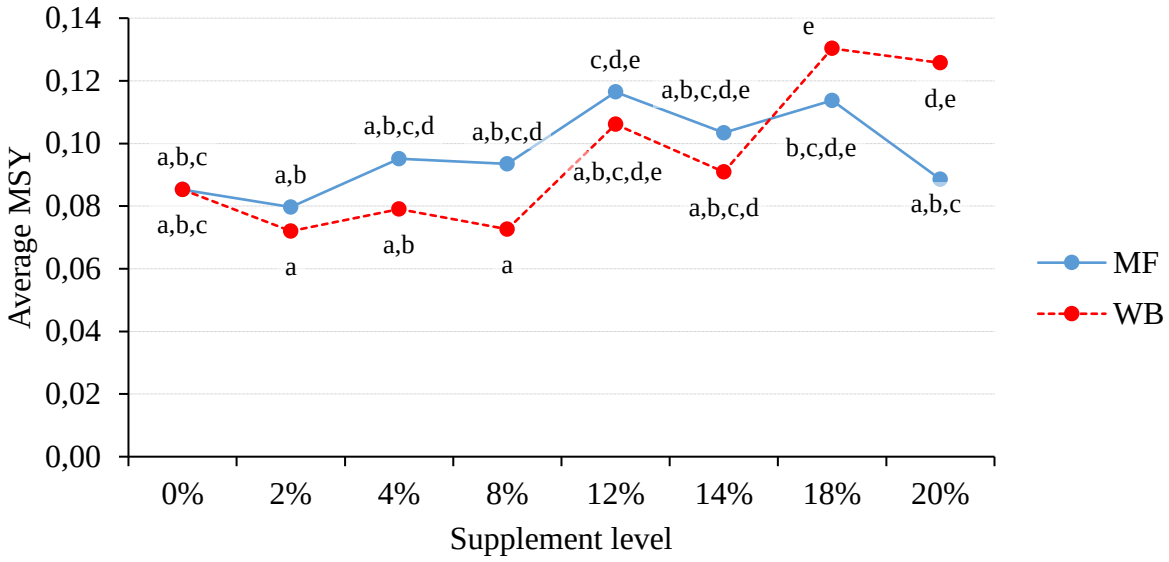

Figure 5.4 Average yield of *Pleurotus salmoneostramineus* on maize stalk substrate supplemented with different levels of MF and WB. [a-e]Averages with different letters are significantly different at $p < 0.05$.

Table 5.3 Effect of different levels of supplementation of MF and WB on growth of *Pleurotus salmoneostramineus* mushroom.

Supplement	Level	MGR (cm/day)	Days	B.E	MSY
MF	0%	0.598 ± 0.02^{d}	34.5 ± 2.76^{a}	$85.26 \pm 7.52^{a,b}$	$0.085 \pm 0.01^{a,b,c}$
	2%	$0.395 \pm 0.02^{a,b}$	$48.0 \pm 2.76^{a,b,c}$	79.69 ± 7.52^{a}	$0.080 \pm 0.01^{a,b}$
	4%	$0.408 \pm 0.02^{a,b}$	$49.5 \pm 2.76^{b,c}$	$95.12 \pm 7.52^{a,b,c,d}$	$0.095 \pm 0.01^{a,b,c,d}$
	8%	$0.420 \pm 0.02^{a,b}$	$46.5 \pm 2.76^{a,b,c}$	$93.48 \pm 7.52^{a,b,c,d}$	$0.093 \pm 0.01^{a,b,c,d}$
	12%	$0.407 \pm 0.03^{a,b}$	$50.0 \pm 3.19^{b,c}$	$116.42 \pm 8.69^{a,b,c,d}$	$0.116 \pm 0.01^{c,d,e}$
	14%	$0.413 \pm 0.02^{a,b}$	$43.5 \pm 2.76^{a,b,c}$	$124.11 \pm 7.52^{b,c,d}$	$0.103 \pm 0.01^{a,b,c,d,e}$
	18%	$0.413 \pm 0.02^{a,b}$	49.5 ± 2.76^{c}	$113.79 \pm 7.52^{a,b,c,d}$	$0.114 \pm 0.01^{b,c,d,e}$
	20%	0.280 ± 0.04*	60.0 ± 5.52*	$88.55 \pm 8.69^{a,b,c}$	$0.089 \pm 0.01^{a,b,c}$
WB	0%	0.598 ± 0.02^{d}	34.5 ± 2.76^{a}	$85.26 \pm 7.52^{a,b}$	$0.085 \pm 0.01^{a,b,c}$
	2%	$0.460 \pm 0.02^{a,b,c}$	$42.0 \pm 2.76^{a,b,c}$	$86.42 \pm 7.52^{a,b}$	0.072 ± 0.01^{a}
	4%	$0.538 \pm 0.02^{c,d}$	$36.0 \pm 2.76^{a,b}$	$94.84 \pm 7.52^{a,b,c,d}$	$0.079 \pm 0.01^{a,b}$
	8%	$0.465 \pm 0.02^{a,b,c}$	$40.5 \pm 2.76^{a,b}$	80.95 ± 7.52^{a}	0.073 ± 0.01^{a}
	12%	$0.417 \pm 0.03^{a,b}$	$44.0 \pm 3.19^{a,b,c}$	$127.32 \pm 8.69^{c,d}$	$0.106 \pm 0.01^{a,b,c,d,e}$
	14%	$0.490 \pm 0.02^{b,c,d}$	$43.5 \pm 2.76^{a,b,c}$	$109.08 \pm 7.52^{a,b,c,d}$	$0.091 \pm 0.01^{a,b,c,d}$
	18%	$0.350 \pm 0.03^{a,b}$	$56.0 \pm 3.19^{b,c}$	130.32 ± 8.69^{d}	0.130 ± 0.01^{e}
	20%	0.403 ± 0.03*	50.0 ± 3.19*	$125.73 \pm 8.69^{b,c,d}$	$0.126 \pm 0.01^{d,e}$

Results are mean ± s.e. MF – maize flour, WB – wheat bran, MGR – mycelia growth rate (a-d), Days – Days until full colonisation (a-c), B.E. – Biological efficiency (a-d), MSY – Average mushroom yield (a-e. Column results with different superscripts indicate significant differences at 5%.

5.5 Discussion

The *Pleurotus salmoneostramineus* is one of the mushrooms that are known to have good properties, such as rapid growth on substrate and has mycelium with high saprophytic activity (Chang & Hayes, 2013). Such good properties are important in mushroom growth, because the media that is non-thoroughly impregnated or slowly impregnated by hyphae are sensitive to fungal and bacterial infections which results in yield reduction (Zharare *et al.*, 2010). Although, *P. salmoneostramineus* has good attributes, there are some factors that can alter or disturb these good properties. These may include diseases and contamination, both of which are known to negatively affect yield and production rate (Royse, 1989), since they compete for space and nutrients with the mushroom (López-Arevalo *et al.*, 1996).

The study indicated that the *P. salmoneostramineus* was successfully cultivated in maize stalk supplemented with varying levels of WB and MF, since it can be seen in Table 5.1 that very few levels of supplementation were affected by contamination. The 20% MF was the only level that had a relatively high rate (75%) of contamination. The higher rate of contamination observed at 20% MF for *P. salmoneostramineus* corresponds with the findings of Oseni *et al.* (2012) who reported that 20% WB resulted in higher contamination rates in *P. ostreatus* when supplemented with sawdust. The high contamination rate obtained at 20% MF supports the fact that when substrate become enriched with nitrogen it results in increased contamination (Rinker & Wuest, 1995; Oei, 2003). The mycelium may have also been damaged due to an increase in substrate temperature (Upadhyay *et al.*, 2002) that is known to be caused by rapid metabolic activities triggered by extra nitrogen within the substrate (Fanadzo *et al.*, 2010). During mushroom growth the mycelial growth is the first important step that provides suitable internal conditions for fruiting of mushroom (Zairul-Fazwan *et al.*, 2015). Thus, finding the method that may increase the mycelial growth is of interest in mushroom cultivation (Menolli Junior *et al.*, 2010). However, it is known that the enzymes plays a significant role in growth

and development of mushroom (Kuforiji & Fasidi, 2008), since they are useful in substrate colonisation and are decisive in fruiting bodies production (Leonowicz *et al.*, 2001). The results of this current study indicated that supplementation of the maize stalk substrate caused a decreased MGR as observed in Figure 5.1 that the control gave maximum MGR compared to the rest of supplementation levels. The decrease in MGR due to addition of supplements, especially at 18% WB, may have been caused by the destruction in enzyme activity, since excessive nitrogen is known to cause inhibition of the synthesis of lignin degrading enzyme (Bisaria *et al.*, 1997). The lowest MGR observed on 18% WB (0.35 cm/day) corresponds to the findings by Amin *et al.* (2010) who reported the MGR of 0.37 cm/day for the *Calocybe Indica* on cotton substrate. Wondimu (2016) reported the mycelial growth of 0.45 cm/day on maize stem and cotton seed waste with ratio 60:40 and 20:80 as the fastest mycelial extension. This does not correspond to the observed results of the current study, since for the current study the fastest MGR was 0.60 cm/day on the un-supplemented maize stalk. This indicated that the un-supplemented maize stalk gave vigorous mycelial growth which is a prerequisite for good growth and product yield of *Pleurotus* mushrooms (Chang, 2009).

The results obtained in the study showed that the MGR was negatively affected by an increase in supplement concentration. The slower MGR implies extended waiting times to full colonisation. The shortest times to full colonisation of the substrate that were recorded in the study closely relate to the findings of de Siqueira *et al.* (2011) who observed 35 days to full colonisation of stalk (90% stalk + 10% WB). The findings by Noonsong *et al.* (2016) also indicated that the control (rubber sawdust) had the least days (36 days) to full colonisation of substrate by *Agrocybe cylindracea*. The observed results, however, contradicts with the range of 12-14 days that was reported by other authors for various *Pleurotus* species (Mane *et al.*, 2007). The days to full colonisation of maize stalk gradually increased with an increase in the level of supplements. This was contrary to the findings of Oseni *et al*. (2012) who observed

that the number of days to full colonisation of sawdust decreased with an increase in WB supplementation. The shortest time for days to full colonisation of maize stalk that was observed at low levels of supplementations (including the control) is said to be advantageous for successful cultivation of mushroom, because the substrate that is not fully impregnated with mycelia is usually prone to fungal and bacterial infection which could disturb the yield of the mushroom (Diamantopoulou *et al.*, 2006; Philippoussis *et al.*, 2001). After the mycelium has fully colonised the substrate, it matures from primordia (pin heads) that are classified as small outgrowth of mycelium that become fruiting bodies once fully matured (Khan *et al.*, 2013). The TP of the *P. salmoneostramineus* on maize stalk substrate ranged from 1-15 days. The results obtained in Table 5.2 indicated that the *P. salmoneostramineus* took a significantly shorter time to start pinning during the first ranging from 1-4 days, whereas the second and third flushes gave significantly longer TPs ranging from 9-15 days. The results obtained disagreed with the findings of Kimenju *et al.* (2009) who observed that the duration taken by mycelia to start pinning depends on the supplement level used on the substrates.

On the other hand, the current observed results indicated that the TP of *P. salmoneostramineus* is quite fast for the first flush only and by increasing flushes the *P. salmoneostramineus* tends to take longer to start pinning. This was probably caused by the unavailability of nutrients as flushes increase because the availability of free simple carbohydrates within the substrate has an influence on rapid pinning of mushrooms (Chang, 2006). Previous studies have proven that supplementing the substrate is of importance in order to enhance mushroom production (Estrada *et al.*, 2009). The results of the current study correlate with these findings as observed in Table 5.3 that both the yield and BE were influenced by the addition of supplements into the substrate. These results indicated that adding more supplement into the substrate enhanced both the yield and the BE. Figure 5.3 and Figure 5.4 show that increasing the rate of supplementation resulted in gradual increase in both yield and BE. The higher levels of supplementation (18%

WB, 12% MF, 12% WB, 14% MF) gave better yield and BE that were significantly different to the control and other lower levels of supplementation. Such findings are contrary to the results obtained of days to full colonisation and MGR whereby increasing the supplementation resulted in decreased MGR and days to full colonisation. This supports the report of Menolli Junior *et al.* (2010) where it was argued that colonisation speed does not always guarantee good productivity, as observed in this current study that the control together with lower levels of supplementation proved to work best on fast colonisation and MGR but did not produce best yield and biological efficiency. This denotes that mycelial growth and yield of mushrooms poses different requirements (Oei, 1991). The results obtained, especially for yield, corresponds with the findings of Noonsong *et al.* (2016) who reported that the control (sawdust) gave faster mycelial growth and time to first crop, however, yield obtained was reserved or lower.

The overall results of BE indicated that *P. salmoneostramineus* was successfully cultivated on maize stalk with varying levels of supplements, since the BE values obtained were within the range of 75-125% which is believed to be the normal range for the good mushroom grower (Stamets, 1993). The highest BE (130%) that was obtained in this current study exceeded 125%, although, the exceptional range of 250% was not reached. This shows that the *P. salmoneostramineus* grows successfully on supplemented maize stalk substrate. The best BE of 130% obtained closely relate with the BE obtained by Navathe *et al.* (2014) who reported BE of 130% on *Calocybe indica* cultivated on dried biogas spent slurry. On the other hand, the BE value of 113% recorded on 18% MF corresponds to the BE obtained by Siddhant and Singh (2009) on *P. florida* cultivated on mixed fresh wheat straw with 25% spent mushroom compost. The observed results have shown that higher levels of supplementation gave better yields and BE. However, it was observed that both the yield and BE increased up to a certain point then decreased after reaching the maximum point. This is in agreement with Moonmoon *et al.*

(2011) who stated that the yield of mushroom increases with the level of each supplementation but up to a certain level and then decreases. Also, Shen and Royse (2002) reported that increasing the percentage of WB had a negative effect on the quality and BE of mushroom.

5.6 Conclusion

It may be concluded from this study that *P. salmoneostramineus* can be successfully cultivated on maize stalk with different supplement ratios. The addition of both WB and MF was both advantageous in obtaining significantly higher yield and BE, but had negative effect on MGR and period to full colonisation of substrate. Thus, a quicker growth rate and colonisation period do not necessary guarantee better yield and BE. Therefore, it is the choice of the mushroom grower to decide on whether to use supplement rates that gives fast production with low productivity or choose supplement levels with slow growth but with enhanced productivity. It can thus be recommended, based on the results obtained from this present study, that levels such as 18% WB, 14% MF and 12% MF may be used in order to obtain improved yield and BE, but for quick production, low supplementation or no supplementation may be required, which may save production costs.

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CHAPTER 6

GENERAL DISCUSSION

6.1 Review of the overall results

Previous Chapters, 3, 4 and 5 have reported that the performance of the three oyster mushrooms (*P. ostreatus, P. pulmonarius and P. salmoneostramineus*) was affected by different supplements and different levels of supplements. However, there is a need to determine which supplement and at what level will optimize the performance of one oyster mushroom species over the performance of other mushroom species in terms of growth, BE and yield, without sacrificing significant time in the form of TP.

To determine such behaviour, Figure 6.1, Figure 6.2 and Figure 6.3 were constructed. Table A.1 in Appendix A contains all the results for all 48 combinations of mushroom type, supplement, and supplement level. Table A.1 is placed in the Appendix, since it contains all the numerical results already tabled in Chapters 3 to 5. All combinations of mushroom type, supplement, and supplement level were compared using descriptive statistics. Only descriptive methods were used. The reason for this is two-fold. Some supplement levels had to be excluded from the previous analyses due to contamination that reduced the number of replicates down to one (the single replicate measurements were, however, included on the graphs). Also, the large number of comparisons required (with 48 combinations of mushroom, supplement, and supplement levels) and the small number of replicates available would yield unreliable results for a formal statistical test. Contaminated replicates were omitted, since their performance was erratic. Figure 6.1 displays the number of contaminated replicates (bags) expressed as percentage of contamination. The weighted averages were calculated as follows: 0.1 × % Average MGR + 0.4 × % BE + 0.4 × % Average MSY + 0.1 × (100 – % Average TP). The average number of days were excluded from the calculation, since it is negatively correlated with the MGR. Firstly, the four performance parameters were converted to percentages, since

they are measured on different scales. Secondly, weights were assigned based on personal judgement whereby productivity was given higher weights compared to time (period), therefore the focus remained on the BE and yield. The 48 measurements for the four measured/weighted parameters (MGR, TP, BE and MSY) were ranked, respectively, from 1 to 48, where 1 is the best performer and 48 the worst performer. This allowed the identification of the parameters that contributed most to the weighted average. The 48 weighted averages were arranged from highest to lowest on Figure 6.1 and the respective ranks were indicated alongside each average. The top 5 rankings were considered the best performers of the particular mushroom on the certain supplement level as observed on Figure 6.1. The weighted average indicate the overall performance of the mushroom in a particular supplement level. The mushrooms with a high weighted average are considered the best performers at a certain supplement level and those with lower weighted average are considered as the poorer performers at the particular level.

Figure 6.1 is a graphical summary of Table A.1. Finally, Figures 6.2 and 6.3 depict the MGR and MSY, respectively, for the different mushroom and supplement combination over all eight supplement levels. In previous chapters it was observed that different mushrooms have their own optimum point at which growth performance and productivity were optimized. However, determining the mushroom species that would perform better over the other mushrooms was considered within our study in order to provide knowledge on which mushroom is more advantageous to be used over the other mushroom species. Figure 6.1 and Table A.1 indicated that there was no mushroom species which can be perceived as having the best performance over other mushroom as it was observed from Figure 6.1 that the performance of different oyster mushrooms was affected by different supplement levels at different rate. There were levels of supplementation that optimized the performance of one oyster mushroom, but limited the performance of another. For example, 18% WB, 2% WB and 0% WB/MF for *P.*

pulmonarius, but for *P. salmoneostramineus* 2% MF, 2% WB and 8% WB were the levels that showed to limit the combined performance of these oyster mushrooms which was contrary to 20% WB and 18% WB for *P. salmoneostramineus* and for *P. ostreatus* 12% WB, 14% MF 14% WB, 8% MF and 20% MF, were all the levels of supplementation which optimized the combined performance of these oyster mushrooms. Based on the results in Figure 6.1 it can be stipulated that out of the three oyster mushrooms, the *P. ostreatus* experienced the highest mushroom yield (MSY) and BE. For example, 14% MF and 12% WB had high BE whereas 20% MF together with 8% MF had the highest yield for *P. ostreatus*. The *P. pulmonarius* experienced lower yield and BE when compared to the *P. ostreatus*, however, it had the advantage of experiencing fast production time in terms of TP and MGR. This was observed at levels of 2% MF, 4% WB (fast MGR, moderate TP but lower BE and MSY) and 18% WB together with 18% MF (fast TP but lower MGR, BE and MSY) for the *P. pulmonarius* mushroom. Such differences in the performance of oyster mushroom in different supplement levels may be due to the fact that mushroom species within the same genus can have varied nutritional requirements (Choi, 2003) and they require different substrate mixes to perform optimally (Chitamba, 2007).

In general it was observed from Figure 6.1 that the three oyster mushroom species were cultivated successfully on maize stalk substrate, since the mushroom species were not heavily prone to contamination. However, it was observed that out of the three oyster mushroom species the *P. salmoneostramineus* was more prone to contamination with 8 out of 16 supplement level combinations experiencing contamination (i.e., 13 out of 64 replicates). The *P. pulmonarius* and *P. ostreatus* mushrooms experienced less contamination with 4 and 5 out of 16 supplement level combinations (i.e., 6 and 8 out of 64 replicates), respectively. In terms of the individual supplement combinations, high contamination was experienced only at 20% MF for *P. salmoneostramineus* and for *P. pulmonarius* at 20% WB with 75% rate of

contamination. The most likely reason is that these levels of supplement combinations resulted in substrates that are overly rich in nutrients and therefore increases the risk of contamination (Oei, 2003), since excess supplements also give nutrients to competitor microorganisms within the substrate (Tesfaw *et al.*, 2015). Performance parameters, such as growth and yield, are of concern for mushroom growers, hence, the substrate should be supplemented in order to achieve maximum growth and yield (Josphine, 2014). In this current study, when the three oyster mushrooms were compared to each other in terms of yield, it was observed on Figure 6.2 that out of the three oyster mushrooms, the *P. ostreatus* appeared to have better yield for both supplements (WB and MF) compared to other mushroom species. However, 8% WB for *P. ostreatus* showed to cause lower mushroom yield. This is most likely due to contamination that was experienced at this level of supplementation. Based on Figure 6.2, it was observed that the mushroom species which showed to have least yielding capacity when compared to other mushrooms was the *P. pulmonarius* mushroom, especially the WB supplementation resulted in a lower yield of *P. pumonarius* compared to other oyster mushrooms.

The difference in yield capacity that was observed for these three oyster mushrooms was due to the fact that the different strains of mushroom fungi vary in their production of ligninolytic enzyme (Elisashvili & Kachlishvili, 2009; Moldes *et al.*, 2004) that affect colonisation and mushroom yield, because the mushroom strains that are able produce these enzymes in large numbers are said to be suited to colonise the substrate and give higher yields (Ruiz-Rodríguez *et al.*, 2011). The general overview of the mushroom yield for all the mushroom species in this study indicated that the mushroom yield was positively correlated with the increase in the level of supplementation, but only up to a certain point after which the correlation became negative. This is an indication that adding supplements into mushroom substrates impacts on mushroom yield, since it promotes or optimizes mushroom yield to a certain extent. Such patterns of mushroom yield concur with the findings by Moonmoon *et al.*, (2011) who observed similar

effects when increasing the supplement levels. In terms of the MGR, it was observed form Figure 6.3 that the *P. pulmonarius* mushroom behaved differently in comparison to both *P. ostreatus* and *P. salmoneostramineus*. This was because the *P. pulmonarius* mushroom at 0% (non-supplemented) had a slower MGR, whereas the *P. salmoneostramineus* and *P. ostreatus* mushroom had similar MGR that was greater than that of *P. pulmonarius*. However, for the *P. pulmonarius* the MGR increased immediately after addition of supplements (especially at 2% MF), where after it declined to a rate that was lower than those of *P. salmoneostramineus* and *P. ostreatus*. The *P. salmoneostramineus* and *P. ostreatus* were also affected by the addition of supplements in a different way compared to *P. pulmonarius*, since immediately after introducing the supplements, the MGR started to decline gradually as the supplement level was increased. For *P. salmoneostramineus*, with MF supplements, the MGR remained constant through addition of MF supplements, but a decline was recorded at the 20% MF level. This was most likely due to severe contamination observed at this level. Comparing the MGR of the three oyster mushrooms, it can be stipulated that the *P. ostreatus* mushroom had better MGR compared to all other mushroom species. Also, the growth rate of all mushrooms was negatively correlated with the level of supplementation.

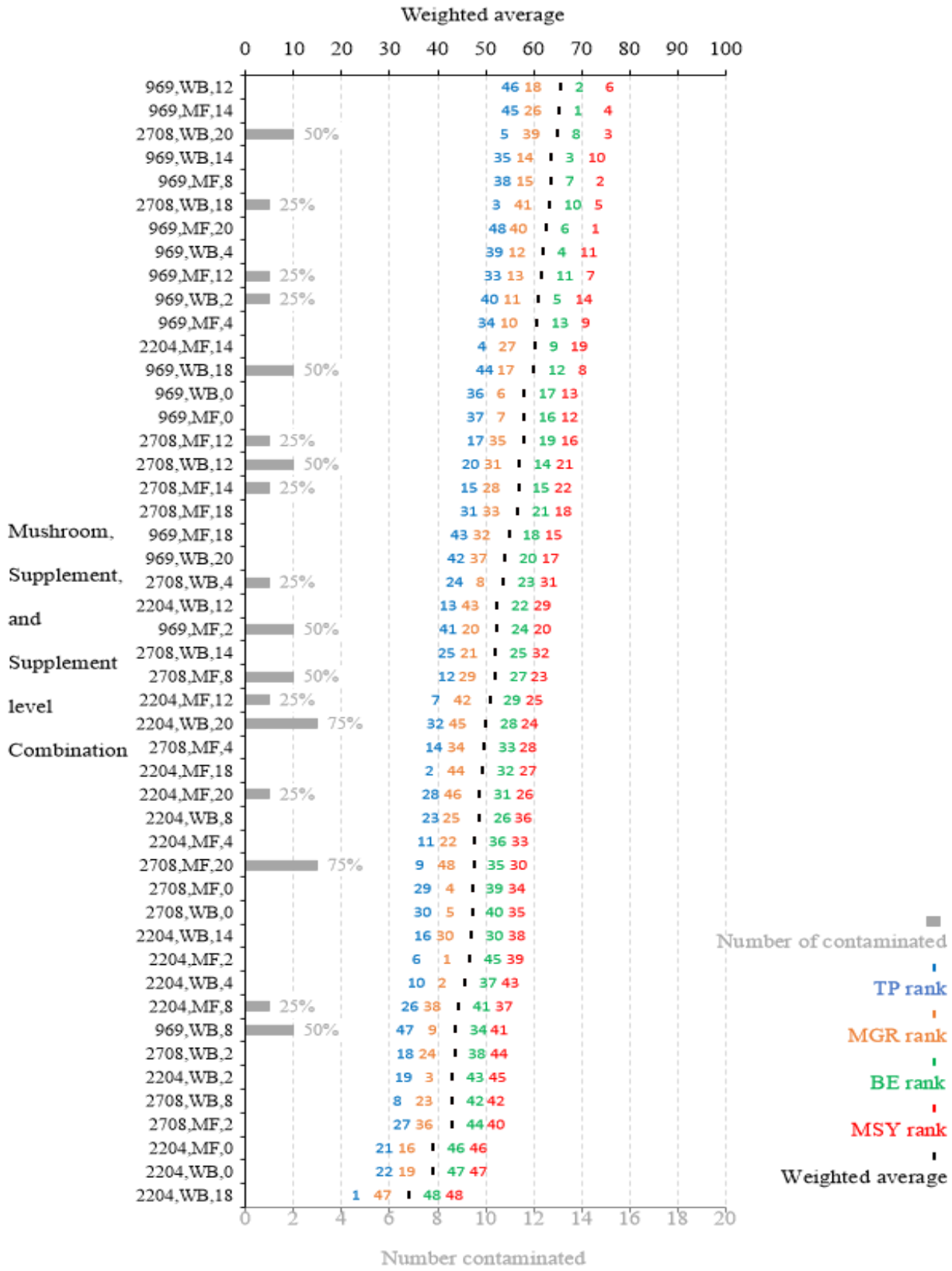

Figure 6.1 Weighted averages, ranks, and number (%) of contaminated bags for the variables for every mushroom, supplement, and supplement level.

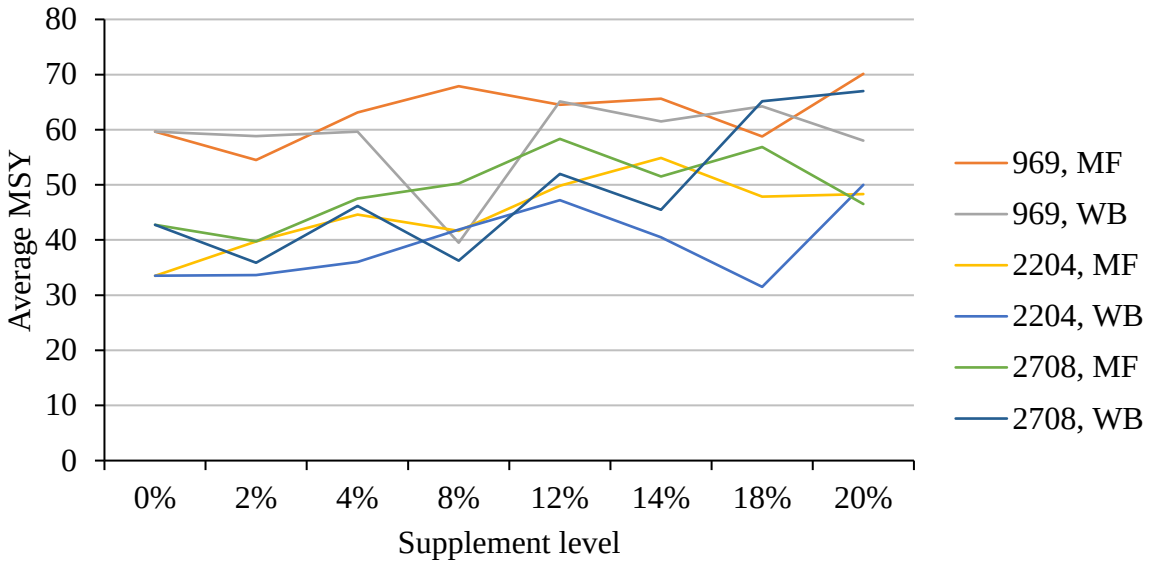

Figure 6.2 Averages of mushroom yield of three oyster mushroom species over different levels of supplementation.

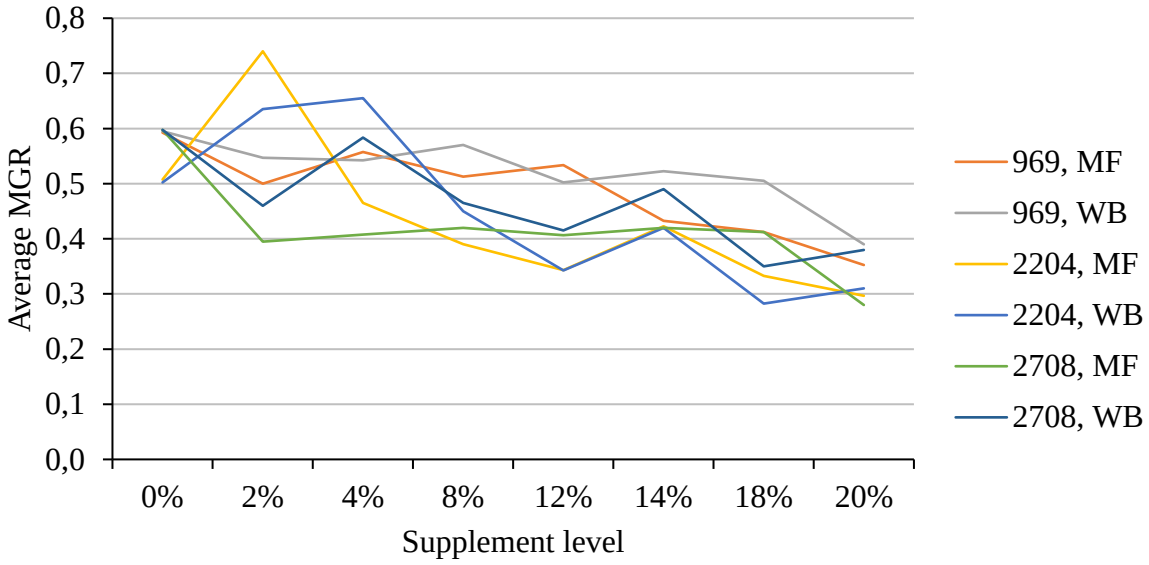

Figure 6.3 Average MGR of the three oyster mushrooms over different levels of supplementation.

6.2 General Conclusion

The present study demonstrated that the maize stalk can be successfully used to cultivate the oyster mushrooms (*P. ostreatus*, *P. salmoneostramineus* and *P. pulmonarius*). It was noted that the addition of different supplements at different levels influenced the performance of all the three oyster mushrooms. Comparing the behaviour of the three oyster mushrooms, it was observed that each mushroom species has its own supplement level combination that optimise the performance of a particular mushroom. However, each mushroom species also had supplement level combinations that disadvantaged their performance, especially those which resulted in higher rate of contamination since they tend to limits mushroom productivity. The increase in supplementation of both WB and MF had negatively affected production time (MGR and Days to full colonisation), however, it had a positive effect on the productivity (BE and MSY) of the oyster mushrooms. For the purpose of utilizing the maize stalk as the base substrate in cultivation of oyster mushrooms, it is therefore the choice of the mushroom grower to decide on whether to add supplements or use un-supplemented maize stalk. This would depend on whether the mushroom grower is concerned with fast and quick production but not considering high productivity, however, if high productivity is a concern over production time therefore the supplements may be added up to a certain extent.

6.3 Recommendations

- Further studies must be carried out using the combination of both WB and MF whereby the best levels of both supplements should be mixed in order to determine if and how it would impact the performance of each mushroom.
- Further studies must be done using the higher supplementation levels of both WB and MF whereby the increase in contamination would be minimised using the hydrogen peroxide. This could be a potential source of limiting the contamination problem that affects the overall productivity.

- Although, the maize stalk is a good substrate to grow oyster mushroom, further studies must be carried out to check the edibility of the mushrooms grown on maize, since most of the farmers use fertilisers and pesticides or the farms are nearby industrial areas that may cause heavy metal pollution. Maize stalk could assimilate the pollutants into the mushroom and that might render the mushroom toxic if the toxicity levels within the fruiting body exceeds safety limits.

6.4 References

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APPENDIX A

Table A.1 Weighted averages, measurements, ranks, and number (%) of contaminated bags for the variables for every mushroom, supplement, and supplement level.

Mushroom, Supplement, and Level			Weighted Average	MSY	BE	MGR	Average TP	Average Days	Number of Contaminated Bags (%)
969	MF	0	57.8	0.119[12]	119.0[16]	0.593[7]	17.3[37]	36.0	
		2	52.2	0.109[20]	109.1[24]	0.500[20]	19.3[41]	39.0	2 (50%)
		4	60.3	0.126[9]	126.1[13]	0.558[10]	17.2[34]	37.5	
		8	63.4	0.136[2]	135.8[7]	0.513[15]	18.1[38]	37.5	
		12	61.4	0.129[7]	128.9[11]	0.533[13]	16.4[33]	34.0	1 (25%)
		14	65.2	0.131[4]	157.8[1]	0.433[26]	20.8[45]	52.5	
		18	54.6	0.118[15]	117.4[18]	0.413[32]	19.4[43]	48.0	
		20	62.4	0.140[1]	140.2[6]	0.353[40]	21.7[48]	55.5	
	WB	0	57.8	0.119[13]	119.0[17]	0.595[6]	17.3[36]	36.0	
		2	60.9	0.118[14]	141.2[5]	0.547[11]	19.0[40]	38.0	1 (25%)
		4	61.7	0.119[11]	142.8[4]	0.543[12]	18.3[39]	34.5	
		8	43.3	0.079[41]	94.7[34]	0.570[9]	21.3[47]	30.0	2 (50%)
		12	65.3	0.130[6]	156.4[2]	0.503[18]	21.2[46]	39.0	
		14	63.6	0.123[10]	147.6[3]	0.523[14]	17.3[35]	40.5	
		18	59.7	0.129[8]	128.2[12]	0.505[17]	20.0[44]	39.0	2 (50%)
		20	53.8	0.116[17]	116.0[20]	0.390[37]	19.3[42]	51.0	
2204	MF	0	38.9	0.067[46]	66.8[46]	0.508[16]	8.9[21]	39.0	
		2	46.6	0.080[39]	79.4[45]	0.740[1]	7.7[6]	30.0	
		4	47.6	0.089[33]	89.1[36]	0.465[22]	8.1[11]	52.5	
		8	44.2	0.083[37]	83.1[41]	0.390[38]	9.0[26]	48.0	1 (25%)
		12	50.6	0.100[25]	99.6[29]	0.343[42]	7.9[7]	64.0	1 (25%)
		14	60.0	0.110[19]	131.7[9]	0.423[27]	7.5[4]	57.0	
		18	49.2	0.096[27]	95.8[32]	0.333[44]	7.2[2]	60.0	
		20	48.5	0.097[26]	96.7[31]	0.297[46]	9.3[28]	64.0	1 (25%)
	WB	0	38.8	0.067[47]	66.8[47]	0.503[19]	8.9[22]	43.5	
		2	42.9	0.067[45]	80.3[43]	0.635[3]	8.8[19]	30.0	
		4	45.6	0.072[43]	86.5[37]	0.655[2]	8.1[10]	33.0	
		8	48.4	0.084[36]	100.7[26]	0.450[25]	9.0[23]	49.5	

		12	52.2	0.095^{29}	113.3^{22}	0.343^{43}	8.3^{13}	63.0	
		14	46.9	0.081^{38}	97.2^{30}	0.420^{30}	8.7^{16}	48.0	
		18	33.9	0.063^{48}	51.9^{48}	0.283^{47}	5.7^{1}	69.0	
		20	49.7	0.100^{24}	100.1^{28}	0.310^{45}	10.3^{32}	54.0	3 (75%)
2708	MF	0	47.0	0.086^{34}	85.3^{39}	0.598^{4}	9.4^{29}	34.5	
		2	42.7	0.080^{40}	79.7^{44}	0.395^{36}	9.3^{27}	48.0	
		4	49.3	0.095^{28}	95.1^{33}	0.408^{34}	8.3^{14}	49.5	
		8	51.7	0.101^{23}	100.6^{27}	0.420^{29}	8.2^{12}	45.0	2 (50%)
		12	57.8	0.117^{16}	116.4^{19}	0.407^{35}	8.7^{17}	50.0	1 (25%)
		14	56.6	0.103^{22}	123.4^{15}	0.420^{28}	8.6^{15}	44.0	1 (25%)
		18	56.5	0.114^{18}	113.7^{21}	0.413^{33}	9.5^{31}	49.5	
		20	47.3	0.093^{30}	93.0^{35}	0.280^{48}	8.0^{9}	60.0	3 (75%)
	WB	0	47.0	0.086^{35}	85.3^{40}	0.598^{5}	9.4^{30}	34.5	
		2	43.3	0.072^{44}	86.4^{38}	0.460^{24}	8.7^{18}	42.0	
		4	53.5	0.092^{31}	110.8^{23}	0.583^{8}	9.0^{24}	34.0	1 (25%)
		8	42.7	0.073^{42}	80.9^{42}	0.465^{23}	8.0^{8}	40.5	
		12	56.9	0.104^{21}	124.2^{14}	0.415^{31}	8.8^{20}	45.0	2 (50%)
		14	51.9	0.091^{32}	109.1^{25}	0.490^{21}	9.0^{25}	43.5	
		18	63.1	0.130^{5}	130.3^{10}	0.350^{41}	7.4^{3}	56.0	1 (25%)
		20	64.9	0.134^{3}	134.2^{8}	0.380^{39}	7.7^{5}	54.0	2 (50%)

Superscripts denote the rank (1 – best; 48 – worst) of the value within the column.

Figure A.1 Lamina flow hood used for inoculation of both spawn and substrate

Figure A.2 The outdoor shed cloth fruiting room under the ever green Zygium tree.

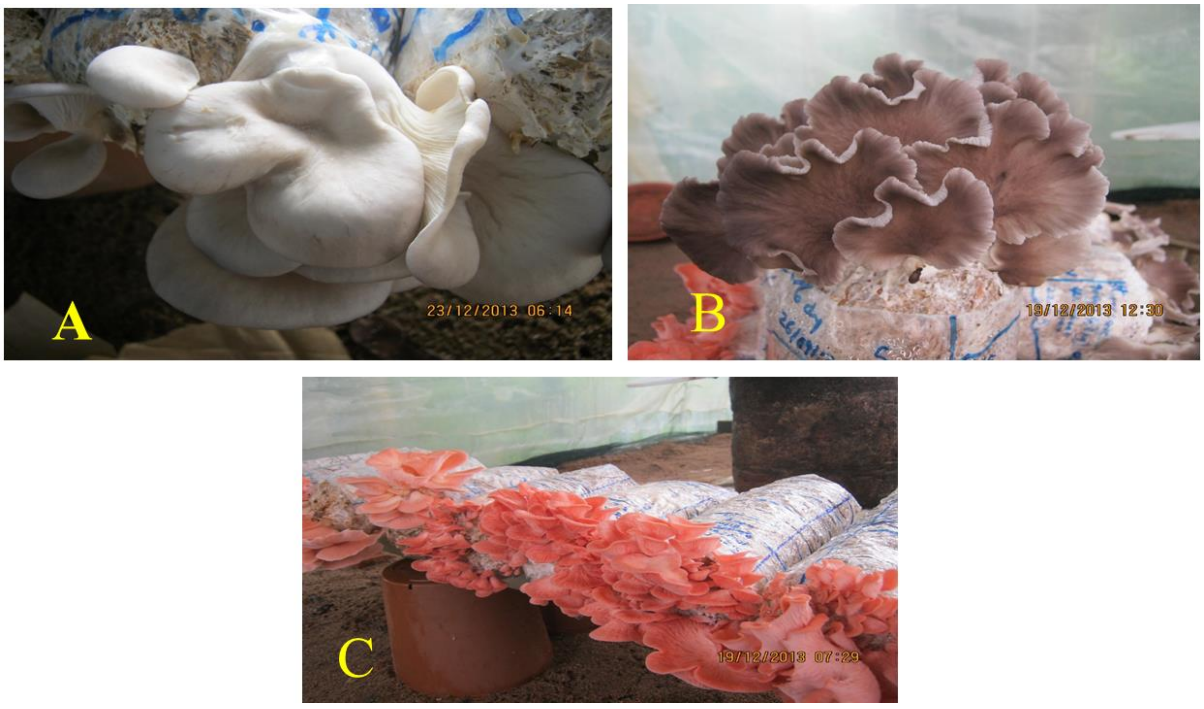

Figure A.3 The three oyster mushroom cultivated on maize stalk: A) *P. ostreatus*, B) *P. pulmonarius*, C) *P. salmoneostramineus.*