A COMPARATIVE STUDY OF THE EFFECTIVENESS OF ALKALINE SOAKING, BLEACH AND BOILING FOR PASTEURIZATION OF SUBSTRATE IN THE CULTIVATION OF WHITE OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*)

by

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Declaration

I Tawanda Michael Chipendo declare that all work in this dissertation is my work and has not been submitted for a degree at any University

Signature ……

Date ……

Approval

This dissertation by Tawanda Michael Chipendo is approved as fulfilling part of the requirements for the award of a Bachelor of Science Honours Degree in Biological Sciences by the Midlands State University.

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Abstract

A study was carried out on substrate pasteurisation for *P. ostreatus* cultivation. The major aim of this work was to compare the effectiveness of wood ash, hydrated lime, bleach and boiling for pasteurisation of the substrate for cultivation of white oyster mushroom, *Pleurotus ostreatus*, on maize straw. To achieve this objective, weed occurrence, the number of days to completely colonise the straw (NDCC), the number of days to initiate primordial sprouting (NDIPS), the number of primordial colonies (NPC), the yield of the first fruiting flush (YFF) and the duration of the first fruiting flush (DFF) were measured. Analysis of Variance for RBD was done to compare NDCC and YFF. NDIPS, NPC and DFF were compared using the nonparametric Friedman's test. There was low infestation of straw with *Trichordema* as well as yellow mould (*Aspergillus* spp.) in three bags. The NDCC was significantly different across treatments (ANOVA, p= 0.000) and boiling recorded the fastest rate of mycelial colonisation of straw taking 16.67 days. There was no significant treatment effect on DTIPS ($\chi^2(3) = 4.188$, p= 0.242) with the mean NDIPS ranging from 4.00, on boiled straw, to 4.67, on both bleach and wood ash. NPC also showed no significant treatment effect ($\chi^2(3) = 1.575$, p= 0.665) with treatment means ranging from 1.33, on bleach-treated straw, to 1.83 on boiled straw. YFF showed significant differences (ANOVA, p= 0.000) for at least two treatments. The lowest yield was obtained on straw pasteurised with bleach (BE = 22.30%) while ash-pasteurised straw gave the highest yield (BE= 37.34%). DFF showed no significant treatment effect ($\chi^2(3) = 4.860$, p= 0.182). The mean DFF ranged from 16.67, on bleach-treated straw, to 18.00, on boiled straw. Pasteurisation of the mushroom substrate using sifted wood ash proved best for achieving high yield. Pasteurising using wood ash however requires more time for spawn run. Mushroom farming trainers can be encouraged to incorporate some of the chemical pasteurisation methods like use of bleach, hydrated lime and wood ash into their training programs especially in in urban areas where wood is not readily available. Instead of disposing of wood ash, farmers may consider using it for the purposes of growing mushroom and reduce the amount of fuel demand for the enterprise.

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Acronyms

NDCC	number of days to completely colonise substrate
NDIPS	number of days to initiate primordial sprouting
NPC	number of primordial colonies within the first three days of primordial sprouting
YFF	yield of first flush
DFF	duration of first flush
BE	biological efficiency

CHAPTER 1: INTRODUCTION

1.1 Background

1.1.1 Mushroom consumption in Zimbabwe

The mushroom industry is based on two main sectors; cultivated mushroom and wild indigenous mushroom (www.mdpafrica.org.zw/cffproducts/documents/uamushroom.doc). Wild indigenous mushrooms are generally obtained mainly from the forest, during the wet season. The formation of mushroom fruiting bodies depends very much on the pattern of rains and, in some years, there may be virtually total lack of mushroom fruiting (www.mdpafrica.org.zw/cffproducts/documents/uamushroom.doc). Some edible types of wild mushrooms are very tasteful and are therefore in demand on the local market for consumption as food, yet others, however, lack taste and flavour. On the other hand some mushrooms are poisonous and may lead to death when eaten. There is thus the need to cultivate known and well-studied species of edible mushroom for human consumption (www.mdpafrica.org.zw/cffproducts/documents/uamushroom.doc, http://www.un-csam.org/publication/tm-mushroom.pdf).

Two main types of edible cultivated mushroom on the Zimbabwean market are the white button (*Agaricus bisporus*) and white oyster mushroom (*Pleurotus ostreatus*). The white oyster mushroom has, however, been fast increasing in production and popularity despite the fact that white button was the first to be grown in Zimbabwe. Button mushrooms are usually sold fresh, while oyster type are available in both fresh and dried form on the local (Zimbabwean) market (Mupaso, 2013). Mushrooms are desired by consumers for their culinary, nutritional and/or medicinal properties (https://kurimaagro.wordpress.com/2012/07/18/mushroom-production-training-zimbabwe/).

Mushrooms add flavour to food and are nutritionally valuable as a fair substitute for meat, with at least a comparable nutritional value to many vegetables (Marshall and Nair, 2009). Oyster mushrooms are rich in non-starchy carbohydrates and have high content of dietary fibre. They are moderate in protein content (1.6 to 2.5% of dry mass), including most amino acids, and are rich in vitamins (C and B) and minerals (Croan, 2004). The niacin content in oyster mushrooms is estimated to be ten times higher than any other vegetable (Croan, 2004; Randive, 2012). Some mushroom species, including *Pleurotus* spp. have immune system boosting, lipid-lowering, anti-tumour, anti-microbial and anti-viral properties, and regulation of blood pressure, among other various medicinal properties (Marshall and Nair, 2009; Randive, 2012). The consumption of mushrooms can therefore be valuable especially in developing countries (including Zimbabwe) where the diets are usually unbalanced (Marshall and Nair, 2009).

1.1.2 Mushroom cultivation in Zimbabwe

Mushroom production in rural communities can alleviate poverty and improve the diversification of agricultural production (Godfrey, Siti and Judith, 2010). It can thus be done in two ways; as a core project or as a side farming activity to blend with the conventional crop production (Mupaso, 2013). Extra benefits, apart from nutrition and profitability, can also be realised from the enterprise as it requires minimum land size, a factor that is ideal for urban mushroom growers. The spent mushroom compost can also be used in horticulture (www.mdpafrica.org.zw/cffproducts/documents/uamushroom.doc). In Zimbabwe, mushroom cultivation is mainly promoted by non-governmental organisations (NGO's) especially in rural areas. Despite the lack of government support on policies to govern mushroom cultivation, the industry has bright prospects (Mupaso, 2013).

Agricus bisporus, is difficult and costly to grow in the local climate (it is impossible to commercially grow it without having air conditioners). Many growers have built complex

and expensive facilities, but small growers have been successful with simple units (Chiroro, 2004; Mupaso 2013). It is possible with a little small capital, to produce mushroom for home consumption and/or commercially (Mupaso, 2013). Oyster mushrooms are essentially cultivated in two ways. The first cultivation method is using woody substrates like logs or sawdust. Composted substrates like agro-wastes (wheat, soybean, maize straws and so on) can also be used. These composted substrates can be spawned using different systems, but the cheapest cultivation system is growing them in plastic bags measuring 30cm x 90cm containing substrate, in a simple growing house for controlled growing conditions around 18-25^0 C and 75% humidity (Chiroro, 2004; Marshall and Nair, 2009).

1.2 Justification

Like in any other crop pests and diseases, which include bacteria, fungi (especially *Trichordema*, the green mould), viruses, nematodes, insects and mites cause direct or indirect damage to mushroom crop. This may affect mushroom growers as it lowers productivity of mushrooms (Sodi and Kapoor, 2009). The substrate must therefore be pasteurised to inhibit and/ or control the development of such organisms. To date there are various methods used to pasteurise the substrate; including boiling, hot water immersion, self-pasteurisation and use of chemicals. In Zimbabwe, most farmers boil the straw in water for one to two hours to control these pests and diseases.

Electricity costs in Zimbabwe rank high in southern Africa thus affecting prices of products (Chronicle, 2015). Therefore, some oyster mushroom farmers in Zimbabwe, like any other industry, are facing difficulties in coping with the elevated rates of electricity for pasteurization of straw, as they use domestic power supply for boilers. In addition, power shortages are also another drawback in the process, especially in areas where power cuts are frequent and unpredictable. Other growers, however are using firewood as their source of energy, but still do not have enough access to the wood especially in and around urban areas.

With the rising concerns about degradation of woodlands in Zimbabwe, the conservation agencies are advocating for green energy and taking strong measures against unlicensed wood trade in urban and peri-urban areas (http://www.cifor.org/library/632/deforestation-of-woodlands-in-communal-areas-of-zimbabwe-is-it-due-to-agricultural-policies/). Such action is further reducing the availability of wood to some mushroom growers. Limited access to firewood is inconveniencing farmers and in some cases leading to higher production overheads as producers may have to search for cheaper firewood far from the farming area. Some farmers in urban areas, however may have to buy the expensive firewood from local traders.

Many farmers confirm that heat pasteurization is labour intensive hence increasing production overheads and is time consuming. The goal of any mushroom farmer is to produce high yields at a minimal cost. Time is also a resource of essence for effective production. Farmers must consider reducing waste of raw materials, contamination or expense in equipment. When pasteurising the substrate, growers need to consider methods that merge with the above ideals. Finding cheaper and easier methods of pasteurization can encourage increase in production scale and also make this easier for new growers to realise income benefits. These methods must characteristically be cost effective, time saving, available and non-compromising on crop productivity.

Many mushroom growers pasteurise the substrate by soaking in hot water. Although the system works well, it is the least favourite to achieve high productivity at low cost. The technique is labour intensive, costly and often leads to yields less than 100% biological efficiency (BE). Cold pasteurisation using lime, bleach and wood ash works well as cheaper alternatives treatments for pasteurisation (http://www.alohaculturebank.com/mushroom-growing.pdf). Cold pasteurization methods are more convenient, cheap and always yield higher (http://www.alohaculturebank.com/low-tech-growing.html#.V22gW3mIodU. This

was supported by some local farmers in Harare who, responding to interviews by SIRDC personnel, indicated that they obtained more fruit when using hydrated lime for pasteurisation compared to hot water.

Hydrated lime is also considered to be cheaper (< US $ 1/kg), time saving and easier to use. Hydrated lime is said to be far the best method to use (http://www.alohaculturebank.com/low-tech-growing.html#.V22gW3mIodU). It also suggests wood ash works equally well, but gives higher yields. If farmers are to opt for wood ash, it may be convenient and readily available especially to growers in communal areas, where wood is used daily as a fuel for cooking. In urban areas, it can also be obtained for free from people who dump it as waste. It is therefore reasonable to consider it as it reduces costs of substrate pasteurisation. Sodium hypochlorite solution (bleach) can also effectively kill pests at low concentrations making it a cheaper alternative of hot water.

This study will focus on the use of the three cold pasteurisation methods (hydrated lime, ash and bleach) and heat pasteurisation by boiling. It will, in turn help farmers with information and help them to make decisions on what materials they may use in their mushroom ventures to realise minimum production costs, convenience and better returns. The study will, however not only dwell on productivity, but also on quality of product (whether, or not, it is compromised).

1.3 Objectives

1.3.1 Main objective:

- to compare the effectiveness of wood ash, hydrated lime, bleach and boiling as substrate pre-treatments on the growth and development of white oyster mushroom, *Pleurotus ostreatus*, cultivated on maize straw.

1.3.2 Specific objectives:

- to compare occurrence of weeds on substrate pre-treated with wood ash, hydrated lime, bleach and boiling,
- to compare the time taken for mushroom mycelia to completely colonise the substrate pre-treated with wood ash, hydrated lime, bleach and boiling,
- to compare the time taken to initiate primordial sprouting of mushroom on substrates pre-treated with wood ash, hydrated lime, bleach and boiling,
- to compare the number of mushroom primordia during the first seven days after first observed primordial colony on the substrates pre-treated with wood ash, hydrated lime, bleach and boiling,
- to compare the yields and biological efficiencies of mushrooms grown on substrates pre-treated with wood ash, hydrated lime, bleach and boiling during the first fruiting flush, and
- to compare the durations of the first flush of mushroom fruiting on substrates pre-treated with wood ash, hydrated lime, bleach and boiling.

CHAPTER 2: LITERATURE REVIEW

2.1 What is mushroom?

A mushroom is a macrofungus with a distinctive fruiting body large enough to be seen with the naked eye and be picked up by hand (Zoberi, 1972). The fruiting bodies can be either epigeous or hypogeous. The term mushroom is commonly used in reference to the fruit body of the fungus. Oyster mushroom is the ordinary name used in reference to edible mushrooms belonging to the genus *Pleurotus*; *P. ostreatus* being the most widely known and widely studied species (Hussain, 2001; Zoberi, 1972).

Mushrooms exhibit heterotrophic feeding, relying entirely on outer sources for their nutrition. They mostly exhibit the saprobic mode of feeding, utilising dead plant and animal tissue. *Pleurotus* spp. feed on the lignocellulose components of dead plants, hence reference to them as white rot fungi. Mushroom cultivation is therefore a very efficient and economically viable biotechnology for the conversion of lignocellulose waste materials into high quality protein food (Hussain, 2001).

2.2 History and development of mushroom cultivation

Mushroom has been part of the human diet in history (Chang and Bushwell, 1993). Gathering of edible mushrooms has been practiced since time immemorial (Addey, 1992). Mushrooms were consumed for their flavour, nutritional value, medicinal and tonic properties (Quimio, 2004). Mushrooms were also a delicacy in early civilised Egypt and Rome (Chang and Bushwell, 1993). In Rome, they were perceived as the food for the Gods (Chang and Bushwell, 1993).

The first reported attempts at mushroom domestication were in 600 AD, China (Poppe, 1978). The development of a cultivation technique for *Agaricus bisporus* (the white button

mushroom) was, however, discovered in Paris, France, and first reported in 1707 (Federal University of Lavras, 2010). Since then, white button mushroom cultivation has had major developments (Federal University of Lavras, 2010). Mushroom cultivation is highly technical in developed countries, but however, in developing countries, low production scales due relatively less technology occur (Federal University of Lavras, 2010). In these countries, including Brazil, with an increase in demand, an increase in the number of companies that use cultivation houses with controlled parameters like temperature, humidity and aeration has also been observed (Federal University of Lavras, 2010).

2.3 Mushroom cultivation today

Mushroom cultivation is practiced in most regions worldwide (Chang and Bushwell, 1993). China is the world leader in mushroom production, contributing as much as 40% as of 2002 (USDA, 2003). The USA followed in production of the fungi with an estimated 13% (USDA, 2003). Many types of edible mushrooms suitable for cultivation include species of genera *Auricularia, Agricus, Shiitake, Ganodema, Lentinus, Volvariella, Tremella, Hericium* and *Pleurotus*. *Shiitake* and *Pleurotus* mushrooms are the best choice mushrooms for growers considering their production requirements (Atkins, 1972).

India is recognised as an important potential producer of mushroom, white button mushroom having the highest potential for production (USDA, 2003). However, oyster mushroom cultivation has been common since the end of the 20th century in the country (Pakale, 2004). Most oyster mushroom growers in India are self-employed, operating small-scale farms and most have low or no knowledge of running such projects (Pakale, 2004). Some grow the mushrooms on purchased straws, while others rely on their own raw materials (Pakale, 2004).

In Brazil, two cultivated strains of *P. ostreatus* are preferred; the grey and white strain (Federal University of Lavras, 2010). The white strain is referred to as Hiratake when

harvested with well-developed pileus, or white shimeji when harvested in the primordial stage. Cultivation is done during cool seasons or under controlled environments since *Pleurotus* spp. normally need a cold shock (usually under 20º C) to begin fruiting (Federal University of Lavras, 2010).

In most of Southern Africa, including Zimbabwe, *P. ostreatus* is cultivated during winter season, while the more heat tolerant *P. sajor- caju* is grown in summer. Up to 6 fruiting flushes may be obtained during the harvest period in the production of oyster mushroom. However, only the first three flushes have significant economic importance in commercial cultivation. More than 80 % of the total yield of mushroom can be harvested from the first 3 flushes (Chiroro, 2004).

2.4 *Pleurotus* (oyster) mushrooms

Peurotus mushrooms come in various colours; white, pink, brown, yellow, and grey being the most popular (Mkhatshwa, 2002). The characteristic feature of oyster mushrooms (*Pleurotus*) is a stipe that attaches laterally to the cap at some point. The name *Pleurotus* comes from the Greek word that means side, referring to the stem's position. The stipe may occur at the margin or may be wanting altogether (Marshall, 1923). The taxonomy of the white oyster mushroom *Pleurotus ostreatus* is as follows:

Kingdom: Fungi

Phylum: Basidiomycota

Order: Agaricales

Family: Pleurotaceae

Genus: *Pleurotus*

Species: *Pleurotus ostreatus* (http://www.ncbi.nlm.nih.gov/genome/909)

2.5 Some commercially grown *Pleurotus* species.

Pleurotus olearius and *P. nidiformis* are the only reported poisonous oyster mushrooms (Upadhyay, 2011). The rest of the 36 species recorded are edible and 25 commercially cultivated throughout the world (Upadhyay, 2011). These include *P. ostreatus, P. flabellatus, P. sajor caju, P. florida, P. sapidus, P. cystidiosus, P. eryngii, P. fussolatus, P. opintiae, P. djamor, P. yuccae, P. australis, P. tuber-regium, P. platypus, P. cornucopiae, P. poulinus, P. purpureo-olivaceus, P. levis, P. columbinus* and *P. membranaceus* (Upadhyay, 2011).

Pleurotus ostreatus is the most frequently cultivated species among the genus *Pleurotus* (Chiroro, 2004). It is white in colour and is delicate. As of December 2003, 66 commercial strains of *P. ostreatus* were available in Korea (Kong, 2004). The growing temperatures for fruiting of *P. ostreatus* range from 20 to 30℃, but the mushroom requires a cold shock of about 10 to 20 ^{0}C for primordial sprouting (Cho, 2004). Different strains have different degrees of heat or cold tolerance (Godfrey *et al*., 2010).

Pleurotus florida is widespread in temperate, subtropical and tropical areas (Kong, 2004). It is similar in appearance to and was in the past considered subspecies of *P. ostreatus* (Kong, 2004). Mycologists are, however inclined to regard it as another species with different colour and different characteristics (Kong, 2004). There are two main subspecies of *P. florida*, one sexually compatible with *P. ostreatus* and the other with *P. pulmonarius*. At low temperatures, the colour of the caps is light brown, but turns pale with increasing temperature. It shows the highest yield among the *Pleurotus* species (Kong, 2004).

Pleurotus cystidiosus is also widely distributed in subtropical and tropical regions (Kong, 2004). Although it is mainly grown in subtropical region, its productivity is relatively low (Kong, 2004). Its unique characteristic among the *Pleurotus* spp. is the presence of conidia

(asexual spores composed of white coremia surmounted by black heads of arthroconidia, which occur on the mycelia) (Kong, 2004).

Wild *P. eryngii* are usually collected in Southern Europe, North Africa and Central Asia (Kong, 2004). The species possesses a cream to grey-brown coloured cap, with a whitish stipe 10-14cm long. This "King Oyster" mushroom is increasing in popularity due to its unique flavour. The mushroom is more prone to diseases and more sensitive to growing conditions but grows slower than *P. ostreatus*. It, as in *P. ostreatus*, requires cold shock for primordia formation and forms fruiting bodies, however at 13-18℃ (Kong, 2004).

Pleurotus sajor-caju (the grey oyster mushroom) grows wild in subtropical and tropical regions like India. It is known to be compatible with *P. sapidus*. With its optimal temperature range for fruit-body development relatively high, it is suitable for growing in subtropical and tropical areas (Kong, 2004).

P. cornucopiae is widely distributed in Asia and throughout Europe. It occurs on the stumps of broad-leaf trees from summer through fall. The mushroom cap is yellowish, 4- 12cm with a white stipe. It has a wheat flour odour, and because it tastes good and has a pretty colour, its cultivation is expected to increase (Kong, 2004).

2.6 Benefits of cultivating oyster mushrooms in Zimbabwe

Oyster mushroom cultivation is economically efficient for the farmers of other crops. There is no need to buy the raw materials for the substrate. Low cost structures can also be an advantage for mushroom cultivation (Pakale, 2004). Living in an agro-based country, Zimbabweans can utilise the vast crop residues that are rendered as waste by the farmers (Chiroro, 2004). Maximum profits are realised if substrate materials are agro-wastes from the grower's own fields (Pakale, 2004).

Central and Southern Africa face high costs in conventional agriculture arising from the frequency of drought. With the coupled effect of droughts and livestock diseases in the country, Zimbabweans need to increase their range of alternative sources of protein. When cultivated commercially, these alternative foods must also have the potential to generate income to deal with the issue of poverty. The solution lies, partly, in mushroom cultivation for some native communities. When compared to other crops, oyster mushroom growers incur lower setup costs benefiting from relatively high market prices; giving large profit margins (Chiroro, 2004).

2.7 Selection and pre-treatment of substrates

2.7.1 Choice of Substrate for Cultivation

Pleurotus mushrooms occur naturally on dead wood as saprophytes. They decompose cellulose, hemicellulose and lignin in wood to acquire their essential nutrients. However, straws of other crops are useful as substrates (Poppe, 2000). Agro-wastes rich in cellulose and lignin can be used for their cultivation (Oei, 1991). About 200 different types of wastes are available and can be used as substrates for the cultivation of oyster mushrooms (Poppe, 2000). Wheat straw, maize stalks and cobs, water hyacinth, coffee husks, and cotton among other similar agro-wastes can be successfully exploited in the industry if available and abundant (Chiroro, 2004).

An oyster mushrooms grower can therefore make their own substrate choice from the broad range of possible residues and effectively utilize these wastes, thus reducing the environmental and health hazards of indiscriminate dumping (Poppe, 2000). Training organisations in Zimbabwe put much emphasis on using those that are readily available within the grower's locality. The most common substrate used in Zimbabwe is wheat straw and thatch-grass (Chiroro, 2004). Many studies have been conducted to investigate the

effectiveness of using the wastes and it has been observed that mushroom crop performance differs from one substrate to another (Reed, 2000).

Cultivation of oyster mushrooms on wheat straw yielded more (B/E= 110% for *P. cornucopiae* and 90% for *P. sajor-caju*) than those grown on maize stalks (B/E= 95% for *P. cornucopiae* and 57% for *P. sajor-caju*) after harvesting four fruiting flushes (Reed, 2000). Rice straw was also compared with banana leaves and maize cobs for cultivation of *P. ostreatus* in hanging plastic bags. Rice straw exceeded maize cobs with respect to mushroom yields with rice scoring a B/E of 84.3% compared to maize cobs that had 33.5% (Mamiro, Mamiro and Mwatawala, 2014). Studies by Sharma, Yadav and Pokhrel (2013) on five substrates showed highest yields on rice straw (B/E= 95.46%), followed by rice straw + wheat straw, rice straw + paper and sugarcane bagasse. Sawdust gave the least yield (B/E= 61.96 %).

Water hyacinth can also be a good substrate for oyster mushroom cultivation. The species, although not an agro-waste, is a highly abundant problem weed on some dams and lakes, including some of the most important lakes in Zimbabwe, for example, Lake Chivero. The lake is important as a domestic and industrial water source for Harare. The weed has been used in cultivation by Margaret Tagwira in Mutare (Chiroro, 2004).

2.6.2 Substrate Pasteurisation

Raw substrate does not occur clean enough to be used for cultivation of white oyster mushroom. *Trichoderma* spp. (green mould), the most common contaminant of mushroom substrates, causes great losses in mushroom cultivation. The fungus is prevalent in the early stages of cultivation, especially during spawn run, but also occurs during cropping period (Jandaik and Guleria, 1999). Oyster mushrooms grow very well on properly prepared substrate (Sánchez, Moreno and Gallegos, 2011). To minimise contamination, substrates for

P. ostreatus cultivation require pre-treatment in order to exclude other microorganisms and consequently promote growth of the mushroom mycelium (Earnshaw, Dlamini and Masarirambi, 2012). The substrate is cleaned and treated by pasteurisation to supress or inhibit such weed fungi.

Different treatments were studied by Colavolpe, Mejía and Albertó, (2014); immersion in hot water, steam sterilisation and immersion in alkaline water. An auto-regulated thermal bath was used to pasteurise for 30 minutes at 60 or 80 °C, steam sterilization was done at 120 °C and 1.2 psi for two hours, and alkaline immersion was done with 0, 5% of calcium oxide for 0, 5 min, 12 h, 24 h or 36 h. From the study, two possible treatments were recommended; immersion in hot water at 60 °C for 30 min and immersion in 5% alkaline water for 36 h, for the control of *Trichoderma* (Colavolpe *et al.*, 2014).

Pre-treatment of substrates by self-heating and steam pasteurization were compared on cultivation of strain ECS-1123 of *P. ostreatus*. The biological efficiencies recorded for two flushes were 69.8 ±17.5%, for the self-heating treatment and 75.6 ± 10.2% for the steam pasteurization (Sanchez *et al*., 2011). There was no statistically significant difference between these two treatments. These findings were in agreement with those obtained by Barrios-Espinoza, Ruiz, and Sánchez (2009) for the same strain.

However, these results differ from those obtained by Contreras, Sokolov, Mejia and Sanchez', (2004). Their findings were that steam pasteurization and alkaline soaking were statistically better than the self-heating method. In their research, however, a different strain was used; ECS-0152 of *Pleurotus ostreatus*. The contrast may have resulted from use of a smaller crate (50 x 50 x 50cm) for composting used in the study by Conteras *et al*. (2004)

Treatment by self-heating and alkaline immersion were compared. Two trials were done; using a regular wood crate and the second had the same crate covered with polyurethane. For

the first trial, there was no statistically significant difference (p= 0.591), B/E= 62.8% for alkaline soaking and 68.2% for self- heating. However, the second trial showed significant differences between the two methods, B/E= 57.6% and 78.6% for alkaline immersion and self- heating, respectively (Avendaño-Hernandez and Sánchez, 2013). These results suggested greater yields could be obtained when using self-heating than using alkaline solution.

Caral, Vinay, Manasa, Vinothkumar and Ramesh Babu (2013) compared autoclaving and the use of formaldehyde for pre-treatment of the substrate in the cultivation of *P. ostrearus*. Autoclaving was more efficient than formaldehyde as it gave a better yield on both sawdust and straw. The average yields for autoclaving method were 416g and 360g of mushroom on straw and sawdust, respectively, whereas in chemical sterilization straw yielded 371g while sawdust yielded 310g.

2.7 Spawning and Spawn Run

2.7.1 Spawning methods and techniques

Various cultivation methods have been developed, including log, shelf, box, bag and bottle. The substrate commonly used as good sources of mushroom nutrition are sawdust, logs and agro-waste, including cereal and soybean straw, and cotton waste (Chiroro, 2004). Proper cultivation methods vary from one variety to another. Shelf and box cultivation methods are commonly used in cultivation of *P. florida, P. sajor-caju* and *P. cornucopiae*. Bag and bottle methods are used in the cultivation of *P. eryngii* and *P. cystidiosus*. Mushroom farmers select cultivation methods basing on the mushroom variety, market requirements and demands, and sometines merely subjective farmers' preferences (Kong, 2004).

During spawning using the bag method, the spawn can be inoculated using three techniques; alternate layer, top layer and substrate mixed spawning. These techniques were investigated

by Subramanian, Shanmugasundaram and Muthu, 2014) for colonising rate, primordial sprouting days for first, second and third harvest, and yields of *Pleurotus eous* (pink oyster) grown on paddy straw. The alternate layer method proved the best as it had fastest mushroom development and gave the highest yields. Days to colonise were 13-14 for top layer, 7-8 for alternate layer and 10-12 for substrate mixed. First primordia emerged after 17-18 days for top layer, 9-10 for alternate layer and 12-13 for substrate mixed. Pink oyster first harvest was on day 20-22 for top layer, 11-13 for alternate layer and 13-14 for substrate mixed method. The second harvest on day 25-27 for top layer, 15-16 for alternate layer and 19-21 for substrate mixed method. The third harvest day was 30 for top layer, 17-18 for alternate layer and 22-25 for substrate mixed. Finally, the biological efficiencies were 70.42 for top layer, 122.16 for alternate layer and 92.8% for substrate mixed method.

2.7.2 Spawn run

Spawn run refers to the period during which mycelia spread and colonise the substrate so that it covers the whole substratum. It is a vegetative stage in the development of the fungi which requires specific conditions to be successful (Oei, 1991). It is important to ensure that the environment is conducive for the process as it is a vital stage of the development of the fungi being cultivated. For *Pleurotus* mushrooms, spawn run duration differs depending on such factors as species type or variety and substrate used.

Mycelial growth is the foundation of the mushroom development. Good growth of mycelium is essential in mushroom cultivation (Pokhrel, Yadav and Ohga, 2009). Five different types of substrates were investigated by Sharma *et al*. (2013) to determine the growth and development of *P. ostreatus*. Mycelia extension was studied and observations showed fastest rates on rice straw followed by mixture of rice-wheat straw, sugarcane bagasse, mixture of rice straw plus paper and sawdust, respectively. Colonization of the substrate was also determined. It was fastest in rice straw completing in 22.40 days. Primordial formation (a

stage that comes soon after spawn run) averaged at 26.40 days and first harvest period 32.40 days from the same.

Differences in spawn run durations among species were evident in the studies conducted by (Ashraf, Ali, Ahmad, Ayyub, and Shafi, 2013) comparing three *Pleurotus* species (*P. sajor-caju, P. ostreatus* and *P. djmor*) on three wastes (cotton waste, wheat straw and paddy straw). *P. ostreatus* averaged the least number of days 16.27 ± 0.63, while *P. sajor-caju* and *P. djmor* showed no significant differences taking averages of 18.07 ± 0.69 and 18.67 ± 0.61 days respectively to complete mycelial. However their (*P. sajor-caju* and *P. djmor*) scores differ significantly from *P. ostreatus*. Results from the study also suggest consequent higher yields associated with less time to complete spawn run. *P. ostreatus* recorded highest yield 41.60 ± 1.49 g, while *P. sajor-caju* and *P. djmor* yielded less, 34.60 ± 1.70 g and 33.80 ± 1.30 g, respectively.

Temperature and pH are important parameters to be controlled. In a study by Nwokoye, Kuforiji, and Oni, (2010) the mycelium of *P. ostreatus* grew optimally at a temperature of 28^0 C and pH 9. However, the ability of the mycelia to tolerate this temperature and pH range of 3-10 enabled them to grow well on agro wastes. The temperature was, however not in agreement with the standard 10 -20^0 C, inferring that it may have been a high temperature tolerant strain. There are three strains of *P. ostreatus*, the high temperature, medium and low temperature strains in the range of 25-30^0 C, 16-22^0 C and 12-15^0 C, respectively. High pH tends to suppress the growth of weed fungi in substrate thus reducing competition for the mushroom.

2.8 Oyster mushroom fruiting

2.8.1 Environmental conditions suitable for fruiting

Creating and maintaining the optimal environment for fruiting is important for the success of mushroom cultivation (Cho, 2004). The four most important environmental factors in mushroom cultivation are temperature, light, humidity and ventilation (Cho, 2004). The correct temperature and humidity level enables the mushrooms to grow well in the growing house. Light and ventilation have influence on the colour, size and texture of the mushroom fruit (Kwon, 2004).

2.8.1.1 Temperature

High quality oyster mushrooms are obtained when grown between 10 and 18℃. *Pleurotus eryngii* falls into the temperature range 13 to 18℃, while *P. florida* and *P. sajor-caju* are best produced at a wider temperature range of 15-25℃. However, some oyster mushrooms, like *P. cornucopiae* and *P. cystidiosus*, can be grown at such high temperatures as 30℃. Mycelia of white oyster mushroom grow optimally at temperatures ranging from 20 to 25℃. There is need to lower the room temperature below 15℃ to induce fruiting after full colonization. Temperature can also affect the colour of caps; dark coloured mushrooms are produced when grown at lower temperatures within the species' recommended range (Kong, 2004; Kwon, 2004).

2.8.1.2 Light

Mycelia propagation of mushroom needs darkness. However, though mycelia can grow without light, some species require light for fruit-body formation (Cho, 2004). Mushroom formation and growth stages require 80-210 lux of light. Without light, fruiting bodies of *P. ostreatus* would abort or become malformed (Kwon, 2004). Light intensity influences the colour of the fruitbody and stipe length. Under poor light, mushrooms develop an elongated

stipe and light-coloured cap. On the other hand, when grown under excessive light, the stipes are short and dark-coloured (Kwon, 2004).

2.8.1.3 Humidity

Over 80% of the fruit-body is water (Kong, 2004). Substrate moisture content should be 60-75% and log moisture content, 35-45%. Excessive substrate moisture causes lack of oxygen in the substrate, and this could keep mycelia growing vigorously (Kong, 2004). The growth of fruitbodies is thus delayed and stunted. Little moisture, on the other hand, stunt mushroom due to the lack of water. Young mushroom caps may consequently upturn earlier and release more spores. During fruiting, different relative humidity levels, ranging from 80-95%, are needed throughout the fruiting period (Kong, 2004). It is essential, therefore for growers to water the growing room frequently to raise the relative humidity during fruiting. Most growers water the floor of the growing house and misting around the bags, and some growers use humidifiers (Cho, 2004; Kwon, 2004).

2.8.1.4 Ventilation

Proper ventilation is essential in the growing room during fruiting to control ambient CO_2 concentration (Kong, 2004). Although anaerobic conditions support mycelial development, young mushrooms may suffocate in such anoxic conditions. Mushrooms produce long stipes with tiny caps under high CO_2 levels that may result from less frequent ventilation (Figure 2.1) (Kong, 2004). *Pleurotus ostreatus* produces stipes that are too long and low quality mushrooms at a CO_2 concentration higher than 1 000 ppm. Excessive ventilation, however, causes heavy loss of water from the mushroom house, resulting in lower humidity in the room and substrate (Kong, 2004; Kwon, 2004).

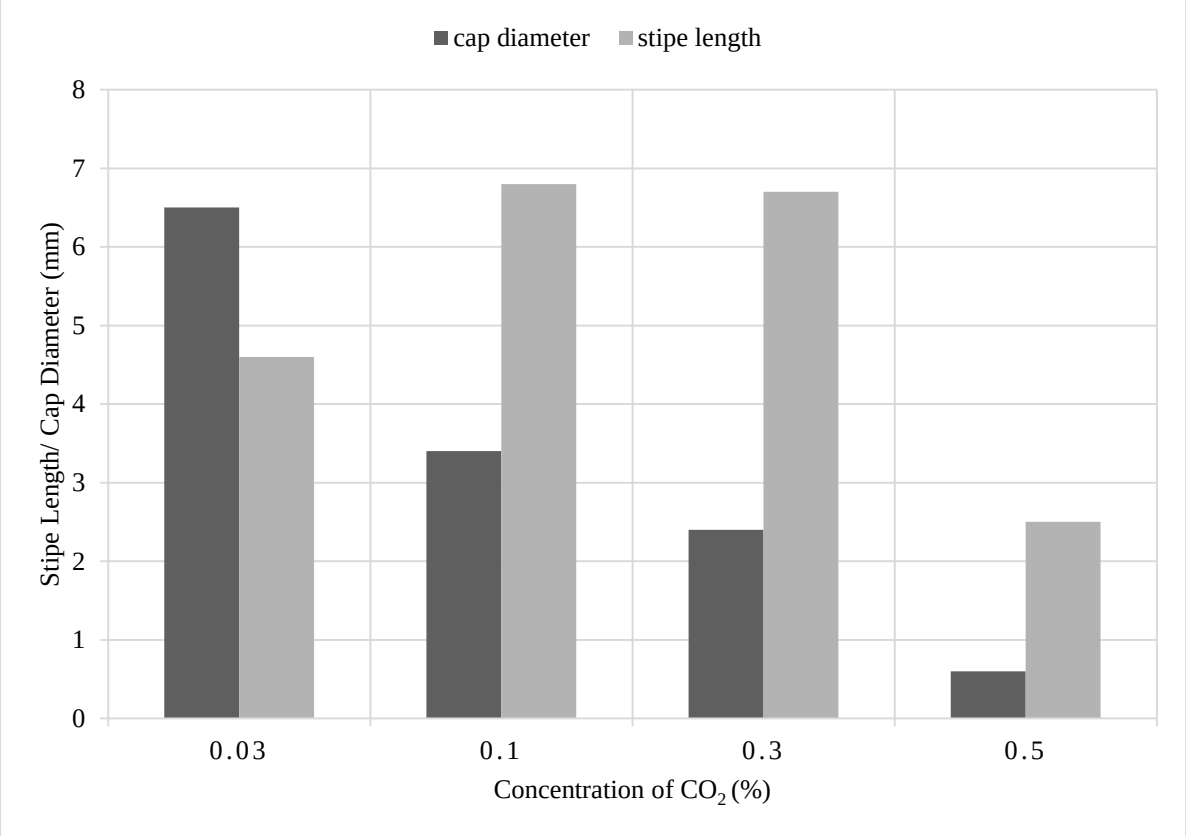

Figure 2.1: The effect of carbon dioxide concentration on the stipe length and cap diameter of *P. ostreatus*. The graph was plotted using data obtained from (http//www.aloamedicinals.com/book1/chapter-8-4.pdf). Good fruiting occurs between 0.1 and 0.3% carbon dioxide.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study site

The study was conducted at Lingfield suburb in Gweru, Zimbabwe from July to September 2016. The mushroom house conditions were partially controlled, hence the local weather conditions may have had some effects on the experiment. The climate in Gweru is classified as warm and temperate with a temperature average of 18.1 °C (https://en.climate-data.org/location/3057/). Gweru is found at latitude -19.4513 and longitude of 29.8177. The altitude for Gweru city is 1424m above sea level.

3.2 Preparation and pasteurisation of straw

3.2.1 Preparation and weighing of straw

Maize straw was cleaned and excess soil was removed by dipping the straw in clean tape water in a 200*l* drum. The straw was then chopped into small pieces of five to seven centimetres using a matchet and a wooden block. Four 5kg portions of maize straw were weighed using a digital balance (Ohaus Scout SPX2201). Each of the portions was placed into nets for pasteurisation.

3.2.2 Pasteurization by boiling

The straw was soaked in a covered 200*l* steel drum, completely submerged in 70*l* of cold tap water for 13 hours. The drum was heated using firewood and allowed to boil for one hour. The straw was drained of excess water and then unloaded from the drum onto a sterilised plastic sheet. The straw was left to cool to 25–28 ^{0}C.

3.2.3 Hydrated lime pasteurisation

Powdered hydrated lime (415g) was weighed using a digital balance (Ohaus Scout SPX2201). The hydrated lime powder was dissolved in 50*l* tap water in a 100*l* plastic drum.

The straw was added immediately, completely submerged and the drum covered with a lid. The straw was left to soak for 12 hours, unloaded onto a sterilised plastic sheet and allowed to drain excess water.

3.2.3 Sodium hypochlorite solution pasteurisation

Three hundred and thirty millilitres of 5% sodium hypochlorite solution were mixed with 50*l* water in a 100*l* plastic drum. The straw was added immediately, completely submerged and the drum covered with a lid. The straw was soaked for 12 hours, unloaded onto a sterilised plastic sheet and allowed to drain excess water.

3.2.4 Wood ash treatment

Sifted wood ash (1415g) was weighed using a digital balance (Ohaus Scout SPX2201) and mixed with 50*l* water in a 100*l* plastic drum. The straw was added immediately, and the drum was covered with a lid. The straw was left to soak for 12 hours, unloaded onto a sterilised plastic sheet and allowed to drain excess water.

3.3 Sterilisation of spawning equipment

A sodium hypochlorite solution for sterilisation of apparatus was made by mixing 50m*l* of 5% sodium hypochlorite with 20*l* of water in a 20*l* plastic bucket. All equipment and working surfaces were sterilised by soaking in or washing with the sodium hypochlorite solution. The spawn sleeves were also dipped in the solution for five minutes and placed on a sterilised surface prior to spawning.

3.4 Spawning and experimental setup

The substrate was drained of excess water and unloaded from respective drums onto sterilised black polythene plastic surface. The pH of each pasteurisation solution was measured using a digital pH meter (model: Hanna YSI pH 100). The substrate was bagged into sterilised plastic sleeves mixing with spawn at rates of 1kg spawn per 8kg substrate and the bags closed and

tied with thatching twine. Substrate temperature was measured using a mercury thermometer for each spawned bag. The spawned bags were cleaned of any dirt and placed in a dark mushroom growing house. The experiment consisted of four treatments with six replicates per treatment arranged in randomized blocks. Each of the three blocks had a total of eight units (Table 3.1).

Table 3.1: Experimental arrangement of bags in the mushroom growing house.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24

KEY

BLEACH
HYDARTED LIME
BOILING
WOOD ASH

3.5 Maintenance during spawn run

The room was kept clean by sweeping and removing any dirt regularly, when necessary. The room was kept dark and depleted of oxygen circulation by leaving little ventilation. Air humidity was maintained between 80-90% (with the use of a hygrometer to monitor the moisture). Watering of the floor was done at least once daily using a horse pipe. The mushroom house temperature was measured using a mercury thermometer at 6am, 12pm and 6pm daily.

3.6 Maintenance during fruiting and harvesting

After three days of primordial sprouting, five slits were cut on each of the sleeves using a bleach-sterilised razor blade. Mature mushroom fruit was harvested every two days from day seven of primordial sprouting by twisting the stipe at the base and gently plucking it out. Air humidity was maintained between 80-90%. Watering of the floor using a horse pipe and misting of bags with a knapsack sprayer was done at least once daily. The mushroom house temperature was measured using a mercury thermometer at 6am, 12pm and 6pm daily

3.7 Data collection

3.7.1 Determination of weed occurrence

Weed occurrence data was collected every day from the day of spawning to the end of the 60 days period. Bags were turned and fully observed for development of any other form of fungal contaminants during the 60 day period. A scoring system of 0-10 (0= no observable weed, 1= low weed infestation, 10= high weed infestation) was used to determine severity of weed.

3.7.2 Determination of number the of days to complete colonisation (NDCC)

The date of spawning was marked as day one for the mycelia to begin colonising the straw. Mycelial growth was observed daily and the number of days taken to completely cover the substrate were recorded for each bag. The NDCC were determined by counting from day one to the day when all substrate was completely covered by mycelia.

3.7.3 Determination of the number of days to initiate primordial sprouting (NDIPS)

The day of completion of colonisation of substrate was marked as day one. The number of days taken to produce first visible primordia were determined by counting the number of days from completion of colonisation to the day that the first primordium/ primordia were observed, for each bag.

3.7.4 Determination of the number of primordial colonies (NPC)

Primordial colonies at least 10mm in length were counted and recorded for each bag from the day of primordial initiation for each bag up to day three of initiation.

3.7.5 Determination of the yield of first flush (YFF) and biological efficiency (BE)

The mass of mushroom was weighed using a digital balance (Ohaus Scout SPX2201) and recorded for each bag on each harvest day. Total yields and biological efficiencies were calculated, for each treatment, at the end of the first fruiting flush (Appendix 1, Appendix 2).

3.7.6 Determination of the duration of first flush (DFF)

The day of primordial emergence was marked as day one. The number of days for the first flush were determined for each bag by counting from day one to the last day that fruit was harvested on the bag.

3.8 Data analyses

Data on the weed occurrence and severity were presented as line graphs for comparison. Analysis of variance (ANOVA for RBD) was performed on days to NDCC and YFF as they conformed to normality. The Dunnet's multiple comparison was used in the comparison of alkaline solution and bleach to boiling treatment. The variables NDIPS, NPC and DFF were analysed using the Friedman nonparametric test as they failed to conform to normality (Appendix 3).

CHAPTER 4: RESULTS

4.1 Measured parameters

The substrate temperature means at spawning were 25^0C in boiled straw, followed by hydrated lime (21^0C), then 20^0C in both bleach and wood ash-treated straw. The temperature ranged from 18 to 20^0C throughout the 60 day growing period (table 4.1). The pH for pasteurisation solutions at 20^0C were 11.34, 9.88, 6.75 and 6.19 for hydrated lime, ash, boiled water and bleach solution, respectively.

Table 4.1: Temperature of the substrate (^{0}C) during the growing period.

Pasteurisation treatment	At spawning	Day 1 to day 20	Day 21 to day 40	Day 41 to day 60
Boiled	25	19	18	20
Bleach	20	18	18	20
Hydrated lime	21	18	18	20
Wood ash	20	18	18	20

4.2 Weed occurrence

During the first 18 days of spawn run, all the bags had no visible weed of any form. On day 19, the first observed weed; yellow mould was observed in BA 1.1, followed by BB 1.1 on day 21 where some *Trichoderma* spp. (green mould) was present. BB 2.1 developed observable green mould on day 31. The severity of the yellow mould in BA 1.1 increased from a score of one to a score of two on day 57, while that of green mould observed in BB1.1

showed no observable change. The rest of the bags had no weed observed throughout the 60 day period (Figure 4.1).

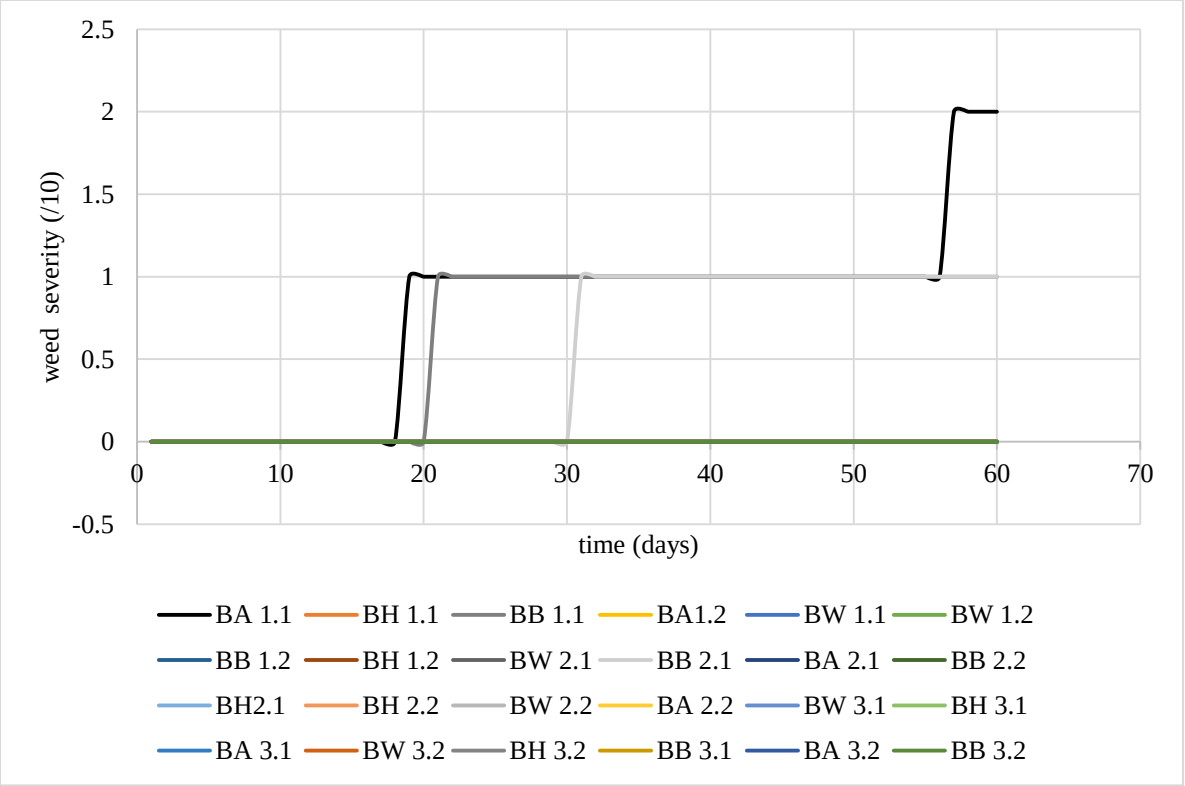

Figure 4.1: Occurrence and severity of weed fungi on pasteurised substrate.

4.3 Colonising period

All mushroom bags showed mycelial growth and successful complete colonisation of the substrate. The differences in time taken to complete colonisation of the substrate was significant at 0.05 significance level (ANOVA, p= 0.000) for at least two treatments (Appendix 4.2). Colonisation was fastest on boiled straw, followed by bleach-pasteurised, then hydrated lime and slowest on ash-treated straw (Figure 4.2).

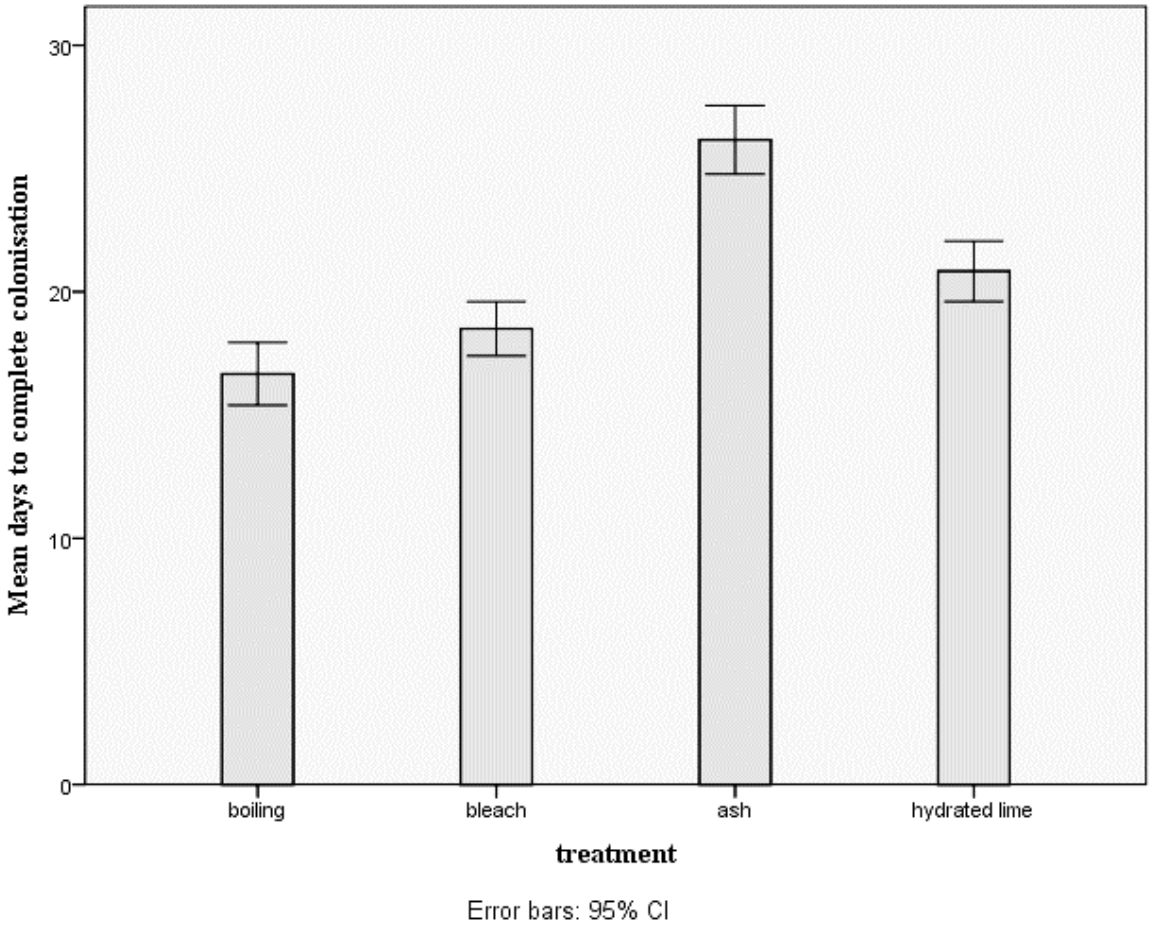

Figure 4.2: Number of days taken by mushroom mycelia to completely colonise straw.

Colonisation was completed in 16.67 days, 18.50, 20.83 and 26.17 days for boiled, bleach-treated, hydrated lime-treated and ash-treated straw respectively (Appendix 4.1). Dunnet's statistic showed that there was significant difference in the pairwise comparisons; boiling and bleach treatment (p= 0.049), boiling and ash treatment (p= 0.000), and boiling and hydrated lime treatment (p= 0.000) on NDCC (Appendix 5.1).

4.4 Primordial sprouting

Mushroom primordia successfully sprouted on all bags. The least number of days to initiate primordia was three, recorded for boiled straw on BW 3.1. The highest number of days; 6 days, was recorded on bleach-treated substrate (BB 1.2) (Appendix 6). However, most bags

showed initiation of primordia four to five days after completion of substrate colonisation for all treatments. The mean number of days to initiate primordia was least in boiled straw (4.00), followed by hydrated lime-treated straw (4.33), and scored highest for both wood ash and bleach-treated straw, 4.77 (Table 4.2).

Table 4.2: Number of days taken to initiate primordial sprouting.

Treatment	Minimum	Maximum	Mean
Boiling	3	5	4.00
Bleach	4	6	4.67
Sifted wood ash	4	5	4.67
Hydrated lime	4	5	4.33

There was no significant treatment effect, at 0.05 significance level, on DTIPS ($\chi^2(3) = 4.188$, $p= 0.242$), (Appendix 7.1).

4.5 Number of primordial colonies

Most of the bags scored one to two primordial colonies within the first three days of sprouting. However, a high of 3 was recorded for boiled straw (BW 2.1) and ash-treated straw (BA 3.3) (Appendix 6). The least mean number of primordial colonies within the first three days of emergence was recorded on bleach-treated straw (1.33), followed by hydrated lime-treated straw (1.50), then sifted wood ash-treated straw (1.67) and highest in boiled straw (1.83) (Table 4.3).

Table 4.3: Number of mushroom primordia within the first three days of primordial emergence.

Treatment	Minimum	Maximum	Mean
Boiling	1.00	3.00	1.83
Bleach	1.00	2.00	1.33
Sifted wood ash	1.00	3.00	1.67
Hydrated lime	1.00	2.00	1.50

There was no significant treatment effect, at 0.05 significance level, on NPC ($\chi^2(3) = 1.575$, p= 0.665), (Appendix 7.2).

4.6 Yield of first flush (YFF) and biological efficiency (BE)

The harvest yields for each bag during the first flush ranged from 173.5g (BB 3.1) to 321.9g (BA 2.1) (Appendix 6). There was a significant difference in the yields of the first flushes at 0.05 significance level (ANOVA, p= 0.000) for at least two treatments (Appendix 4.2). The average yields of mushroom fruit were highest on ash-treated substrate, followed by hydrated lime, boiling and least on bleach-treated substrate (Figure 4.3).

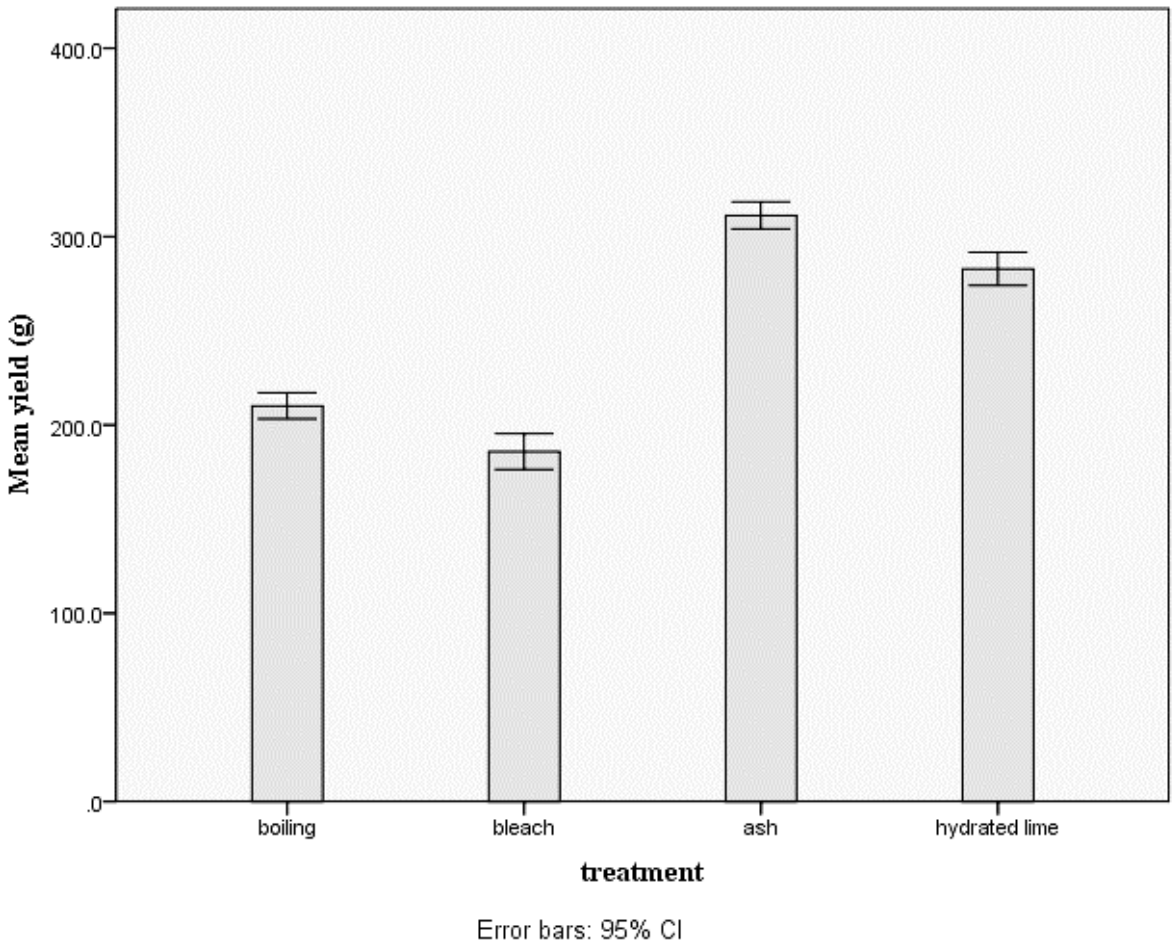

Figure 4.3: Mushroom yields for the first fruiting flush.

The Dunnet's test showed significant differences on pairwise comparison between boiling, and bleach (p= 0.000), ash (p= 0.000) and hydrated lime (p= 0.000) on YFF (Appendix 5.2).

The biological efficiency (BE) followed the same trend as yields, with 37.34% for wood ash treatment, followed by hydrated lime (33.94%) and boiling (25.04%). Bleach treatment gave the least BE, 22.30% (Table 4.4).

Table 4.4: Total yields and biological efficiencies of mushroom for the first flush.

Treatment	Total yields	BE (%)
Boiling	1252.10	25.04
Bleach	1114.90	22.30
Sifted wood ash	1866.80	37.34
Hydrated lime	1696.90	33.94

4.7 Duration of first flush (DFF)

Overall, the first flush ranged from 16 to 19 days in length. Bleach had the shortest DFF (16.67 days), followed by sifted wood ash (17.33 days), hydrated lime (17.83 days) and boiled straw (18.00 days) (Table 4.5).

Table 4.5: Duration of the first fruiting flush.

Treatment	Minimum (days)	Maximum (days)	Mean (days)
Boiling	16.00	19.00	18.00
Bleach	16.00	18.00	16.67
Sifted wood ash	17.00	19.00	17.33
Hydrated lime	17.00	19.00	17.83

There was no significant treatment effect, at 0.05 significance level, on DFF ($\chi^2(3) = 4.860$, $p= 0.182$), (Appendix 7.3).

CHAPTER 5: DISCUSSION

5.1 Weed occurrence

In all the bags where weed was observed, the severity was negligible. The results suggest that the boiling, wood ash, bleach and hydrated lime treatments were all similarly effective in the control of fungal weeds (due to their antimicrobial effect) thus allowing good growth of *P. ostreatus* mycelia. For a good yield and high quality of *P. ostreatus* crop, the mushroom needs to be grown on weed-free substrate, hence the need to pasteurise the straw on which the mushroom is grown. *Trichoderma* is the leading weed in the mushroom cultivation industry. The weed has a faster colonising ability compared to that of *P. ostreatus* so it outcompetes the mushroom in resource utilisation. The presence of *Trichoderma* may slow or inhibit the process of mushroom colonisation of the substrate and consequently results in poor yields, hence much emphasis is on its control compared to other moulds, bacteria and viruses. *Trichoderma*, however may not be able to grow well and cover the substrate if the mushroom mycelia colonises first. The results are thus confirmed because *Trichoderma* developed after the mycelia had grown so its mycelia could not spread. *Trichoderma* control is thus of great importance for high productivity in the oyster mushroom enterprise (Sodi and Kapoor, 2009).

5.2 Colonising period

All bags showed successful completion of substrate colonisation, confirming that the substrate was successfully pasteurised to suppress weed fungi (which are a major cause of mushroom failure). There was a significant difference in time taken to complete colonisation of substrate, showing that there was treatment effect on the growth of the mycelia. Mushroom mycelia need to colonise the substrate in order to achieve high primordial counts and high yields. Mushroom mycelia is able to grow well and completely colonise the substrate in the

absence of weed moulds. It is therefore expected that when the substrate is well pasteurised, the mushroom mycelia grow well on the substrate. Mushroom mycelial colonisation on the maize stover was fastest on boiled straw suggesting pasteurisation by boiling was the best for fast colonisation of substrate. This may be an effect of fast growth of mycelia as spawning on boiled straw was done while substrate was warmer (25^0C) compared to the rest which were spawned at 20^0C. Heat may have taken long to escape for the boiled substrate to reach the room temperature of 18^0C so mycelia had a good start as the mycelia grow well at 25 to 27^0C (Pakale, 2004). The results are, however in disagreement with Contreras *et al.,* (2004) who determined that substrate fully colonises in 14 to 15 days. Such a deviation may be an effect of low temperatures (especially at night and early morning) which may have caused slow mycelia extension.

5.3 Primordial sprouting

Mushroom primordia successfully sprouted on all bags and most bags showed initiation of primordia four to five days after completion of substrate colonisation for all treatments. There was no significant difference across all treatments, suggesting that pasteurisation regime had no effect on the number of days to initiate primordia. Primordial sprouting occurs when the substrate is completely colonised (Pokhrel *et al,*. 2009). Mushroom primordia sprout well under a conducive moisture, temperature, pH, light and ventilation.

Spawn run refers to the period from spawning to primordial sprouting spawn run (Oei, 1991. The spawn run is strain-dependant, but usually takes 20-25 days on boiled substrate (Kwon 2004). The duration of spawn run (NDCC+NDIPS) in the study was consistent with Kwon, with a mean of 20.67 days.

5.4 Number of primordial colonies

Most of the bags had one or two primordial colonies at least 10mm in length in the first three days of initiation of primordial sprouting, except in two bags where a score of three was observed on boiled straw and ash-treated straw. The pasteurisation regimes showed no significant effect on the number of primordia that developed within the first three days of initiation of sprouting. The number of primordial colonies on each bag can be used as a measure of mushroom vigour. *P. ostreatus* also requires a cold shock for induction of good primordial sprouting.

The low primordial counts across all treatments may be due to the lack of a mechanism to induce the initiation of primordia by cold shock.

5.5 Yield and duration of first flush

All mushroom bags fruited well and none showed low yield. Good yields of mushroom fruitbody are obtained when substrate is well pasteurised and good mycelial colonisation occurs. Most mushroom farmers are primarily concerned with yields of the crop, especially when grown at a commercial scale. The highest total yield for the first fruiting flush was obtained on straw treated with wood ash treatment, followed by hydrated lime. Therefore pasteurisation by alkaline soaking proved to be the best treatment for realising high yields. Soaking the substrate in alkaline solution kills weed fungi but not some species of bacteria as boiling and bleach do. The first flush ranged from 16 to 19 days in length. However, there was no significant treatment effect on the duration of the first flush.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Oyster mushroom cultivation is an enterprise that can be economically viable and sustainable for both commercial and small holder farmers. Cultivation of *P. ostreatus* utilises cheaply available resources, including space, substrate and other necessary equipment. One important factor to the realisation of a good *P.* ostreatus crop is pasteurisation. In Zimbabwe, pasteurisation of the straw is commonly done by boiling it for one hour. However, pasteurisation can also be achieved by use of some chemicals that may inhibit or supress growth of weeds. In the present study, the effectiveness of wood ash, hydrated lime and bleach were the chemical pasteurisation agents. These are, in most instances, cheaper, easier to transport and readily available especially to smallholder farmers in urban areas. It was found that soaking maize straw in alkaline solution and bleach can also be as effective as boiling in the pasteurisation of the straw. Of much importance to a mushroom farmer, this study showed that these chemicals are as equally effective as boiling in the control of weeds and development of mushroom on maize straw. However, alkaline soaking, although it takes more time to produce fruit, proved to be superior to leach and boiling in the mushroom yields obtained. There is an increased need to consider using such pasteurisation technique as it is cheap and gives higher mushroom produce.

6.2 Recommendations

The current work may serve as a baseline study for further studies during different seasons and on specified strains of *P. ostreatus*. Most mushroom farmers in Zimbabwe are non-adventurous in terms of pasteurisation of substrate. Since mushroom farming trainers have influence on the decisions that farmers may take in their ventures, trainers can be encouraged to incorporate some of the chemical pasteurisation methods like use of bleach, hydrated lime

and wood ash into their training programs so that farmers can be equipped with knowledge on how the substrate is handled when using such methods. This may also help farmers to use easily available materials for pasteurising their substrate, especially in in urban areas where wood is not readily available.

There is rising concern about the degradation of forests as trees are being harvested for many purposes, including fuel. Instead of disposing of wood ash, farmers may consider using it for the purposes of growing mushroom and reduce the amount of fuel demand for the enterprise. Cheap or free wood ash may be obtained from other firewood users, who may not need it, and utilised for pasteurisation.

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APPENDICES

Appendix 1: Calculation of total yield

Total yield (TYFF) = $mass_1 + mass_2 + \ldots mass_n$

Appendix 2: Calculation of BE

$$BE = \frac{\text{Mean Total Yield} \times 100}{\text{Dry mass of straw}}$$

Appendix 3: Tests for Normality

	Treatment	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	df	Sig.
days to complete colomisation	Ash	.209	6	.200*	.907	6	.415
	Bleach	.183	6	.200*	.960	6	.820
	Boiling	.283	6	.143	.921	6	.514
	hydrated lime	.223	6	.200*	.908	6	.421
days to initiate primordial sprouting	Ash	.333	6	.036	.827	6	.101
	Bleach	.293	6	.117	.822	6	.091
	Boiling	.407	6	.002	.640	6	.001
	hydrated lime	.407	6	.002	.640	6	.001
number of primordial colonies	Ash	.254	6	.200*	.866	6	.212
	Bleach	.407	6	.002	.640	6	.001
	Boiling	.293	6	.117	.822	6	.091
	hydrated lime	.319	6	.056	.683	6	.004
yield of first flush	Ash	.172	6	.200*	.962	6	.833

	Bleach	.135	6	.200*	.992	6	.993
	Boiling	.183	6	.200*	.959	6	.814
	hydrated lime	.188	6	.200*	.916	6	.480
duration of first flush	Ash	.285	6	.138	.831	6	.110
	Bleach	.293	6	.117	.822	6	.091
	Boiling	.492	6	.000	.496	6	.000
	hydrated lime	.302	6	.094	.775	6	.035

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Appendix 4.1: Descriptive statistics for NDCC and YFF

Descriptive Statistics				
	treatment	Mean	Std. Deviation	N
days to complete colonisation	boiling	16.67	1.211	6
	bleach	18.50	1.049	6
	Ash	26.17	1.329	6
	hydrated lime	20.83	1.169	6
	Total	20.54	3.811	24
yield of first flush	boiling	210.183	6.5998	6
	bleach	185.817	9.0380	6
	Ash	311.283	6.8744	6
	hydrated lime	282.817	8.4203	6
	Total	247.525	52.8717	24

Appendix 4.2: ANOVA table for NDCC and YFF

Tests of Between-Subjects Effects						
Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	Days to complete colonisation	305.458[a]	3	101.819	71.452	.000
	Yield of first flush	63077.672[b]	3	21025.891	345.533	.000
Intercept	Days to complete colonisation	10127.042	1	10127.042	7106.696	.000
	Yield of first flush	1470447.015	1	1470447.015	24164.846	.000
Treatment	Days to complete colonisation	305.458	3	101.819	71.452	.000
	Yield of first flush	63077.672	3	21025.891	345.533	.000
Error	Days to complete colonisation	28.500	20	1.425		
	Yield of first flush	1217.013	20	60.851		
Total	Days to complete colonisation	10461.000	24			

	Yield of first flush	1534741.700	24			
Corrected Total	Days to complete colonisation	333.958	23			
	Yield of first flush	64294.685	23			
a. R Squared = .915 (Adjusted R Squared = .902)						
b. R Squared = .981 (Adjusted R Squared = .978)						

Appendix 5.1: Multiple comparisons for NDCC

Multiple Comparisons						
Dependent Variable: days to complete colonisation						
Dunnett t (2-sided)[a]						
(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bleach	Boiling	1.83*	.713	.049	.01	3.66
Ash	Boiling	9.50*	.713	.000	7.67	11.33
hydrated lime	Boiling	4.17*	.713	.000	2.34	5.99
Based on observed means. The error term is Mean Square(Error) = 1.523.						
*. The mean difference is significant at the .05 level.						
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.						

Appendix 5.2: Multiple comparisons for total yield

Multiple Comparisons						
Dependent Variable: yield of first flush						
Dunnett t (2-sided)[a]						
(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bleach	boiling	-24.367*	4.2629	.000	-35.294	-13.440
Ash	boiling	101.100*	4.2629	.000	90.173	112.027
hydrated lime	boiling	72.633*	4.2629	.000	61.706	83.560
Based on observed means. The error term is Mean Square(Error) = 54.518.						
*. The mean difference is significant at the .05 level.						
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.						

Appendix 6: Data on measured variables for each bag.

Pasteurization method	Bag code	NDCC	NDIPS	NPC	YFF	DFF
Ash	BA 1.1	24	5	2	302.9	17
	BA 1.2	26	4	1	311	17
	BA 2.1	26	5	2	321.9	19
	BA 2.2	28	5	1	316	17
	BA 3.1	27	5	1	307.3	17
	BA 3.2	26	4	3	308.1	17
Bleach	BB 1.1	18	4	1	188.5	16
	BB 1.2	19	6	2	199.2	18
	BB 2.1	19	4	1	182.7	17
	BB 2.2	18	5	1	191	16
	BB 3.1	17	4	2	173.5	17
	BB 3.2	20	5	1	180	16
Boiling	BW1.1	17	4	2	210.1	18
	BW 1.2	15	5	1	221.2	16
	BW 2.1	16	4	3	207	19
	BW 2.2	18	4	1	213.2	19

	BW 3.1	18	3	2	201.7	17
	BW 3.2	16	4	2	207.9	19
Hydrated lime	BH 1.1	22	4	1	291.7	17
	BH 1.2	21	4	2	286	18
	BH 2.1	19	5	2	277.2	17
	BH 2.2	22	4	1	271.3	19
	BH 3.1	21	4	2	292	19
	BH 3.2	20	5	1	278.7	17

Appendix 7.1: Friedman tests for NDIPS

N	6
Chi-Square	4.188
Df	3
Asymp. Sig.	.242

a. Friedman Test

Appendix 7.2: Friedman test for NPC

N	6
Chi-Square	1.575
Df	3
Asymp. Sig.	.665

a. Friedman Test

Appendix 7.3: Friedman test for DFF

N	6
Chi-Square	4.860
Df	3
Asymp. Sig.	.182

a. Friedman Test