

EFFECT OF THATCH GRASS (*Hyparrhenia hirta*) AND MAIZE STALK SUBSTRATES ON THE PERFORMANCE AND ECONOMIC VIABILITY OF OYSTER MUSHROOM (*Pleurotus ostreatus*) GROWN INDOORS.

**A dissertation submitted in partial fulfilment of the requirements for the Master of Science
Degree in Food Security and Sustainable Agriculture
(Production)**

Bindura University of Science Education



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The undersigned certified that they have supervised and recommended to Bindura University of Science Education for acceptance of dissertation entitled '**Effect of thatch grass (*Hyparrhenia hirta*) and maize stalk substrates on the performance and economic viability of oyster mushroom (*Pleurotus ostreatus*) grown indoors**' submitted in partial fulfillment of a Master of Science Degree in Food Security and Sustainable Agriculture.

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DECLARATION

I hereby declare that the research project entitled '**Effect of thatch grass (*Hyparrhenia hirta*) and maize stalk substrates on the performance and economic viability of oyster mushroom (*Pleurotus ostreatus*) grown indoors**' submitted to Bindura University of Science Education, Department of Agricultural Economics, Education and Extension is a record of an original work done by me under the guidance and supervision of **Prof. Ronald Mandumbu** and this work is submitted in partial fulfilment of the requirements for the award of a Master of Science Degree in Food Security and Sustainable Agriculture. The results embodied in this thesis have not been submitted to any University or Institute for the award of any degree or diploma.

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DEDICATION

I dedicate this Research project to my nephew Farai Ignitius Goremucheche.

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ABSTRACT

Growing of *Pleurotus Ostreatus* mushroom differ from other horticultural crops which totally depend on the sun as the direct source of energy. Various techniques and substrates are used by farmers to produce mushrooms (Mswaka.,1999). The techniques consist of grow bags layout, tray methods, tier systems and trench beds. Growing media consists of grasses, leguminous by products, cotton seed hulls, saw dust and banana leaves. Mushroom production has proven to be beneficial to many developed countries. It offers good investments, employment opportunities, as well as providing valuable source of protein. Oyster mushroom is second largest commercially produced and important edible mushroom in the world market after (Kang., 2004). It is a labour-intensive crop with excellent potential in the developed countries. A range of organic substances which are readily available within the fields can be utilized. However, there is need to have valuable information on substrate nutritive values and yields that can be obtained in specific measurements for mushroom production. The trial design of completely Randomised Design (CRD) with five replicate and five treatments was done. This experiment was conducted mainly to assess growth rate, yield and economic viability of *Pleurotus ostreatus* grown indoors using *Hyparrhenia hirta* and maize stalk substrates. The mycelium growth rate was measured and recorded on daily basis, whilst stem length, stem diameter, cap size (*basidiocarps*) were weighed and recorded on weekly basis. A combination of *Hyparrhenia hirta* and maize stalk at 1:1 proved to be the highest followed by maize stalk substrate which also gave high levels. Since few farmers are engaged into mushroom farming, there is need for researchers to find the best substrate in terms of productivity levels and time taken from spawning to harvesting. Time frame and productivity curve were used to record difference of peak production per treatment. Significantly high yields were obtained from *Hyparrhenia hirta*: maize stalk resulting in ($P < 0.001$). Maize Stalk had similarly high yields ($P < 0.001$) while other treatments gave the lowest. High yields indicated an economic viability in the utilisation of *Hyparrhenia hirta* and maize stalk substrates. Indoor mushroom cultivation was conducted under controlled environment in terms of light intensity (200-800 lux), temperatures(15-28°C) and humidity (85-95%) and determine optimum yields through use of viable substrate and reliable source of spawn.

Key words: (Mushroom, spawn, basidiocarps, substrates and productivity).

LIST OF ACRONYMS AND ABBREVIATIONS

°C	Degrees Celsius
%	Percent
ANOVA	Analysis of variance
BCC	Bulawayo City Council
BE	Biological Efficiency
CAADP	Comprehensive Africa Agriculture Development Programme
CBR	Cost Benefit Ratio
DART	Developmental and Reproductive Toxicity
DM	Dry Matter
GHG	Green House Gas
Hh	<i>Hyparrhenia hirta</i>
Hrs	Hours
Mg	Magnesium
MDGs	Millenium Development Goals
Ms	Maize stalk
MSD	Millenium Sustainable Development
MRR	Mycelia Running Rate
N	Nitrogen
NGOs	Non-Governmental Organisations
PDC	Provincial Development committee
WK	Week
Pdn	Production
ZAIP	Zimbabwe Agriculture Investment Programme

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1.0 CHAPTER 1

1.1 Introduction

1.1.1 Background to the study

Mushrooms belong to the kingdom of fungi, a group very distinct from plants, animals and bacteria. Fungi depend on other organisms for their nutrition and the common mode is saprophytic. Mushrooms evolved from *mycelium* bodies which are strand like mass of cells that grow on substrate such as dead tree logs, groundnut hulls, cotton seed hulls and banana leaves, sugar cane bagasse and other plant fibres with high cellulose content (Ragunathan *et al.*, 1996). They also grow favourably on wheat straw which contains lignocellulose (Chitamba *et al.*, 2012). The fungus is much sensitive to its food source where it extracts carbohydrates and protein. Well prepared substrates may be appropriate food source to mushroom in its whole life cycle. For this research, mushroom was produced by inoculating spawn into substrates of different proportions. Under conducive conditions, spores germinated into hyphae (collectively mycelia). Germinated hyphae formed primary mycelia and then secondary mycelia through *Plasmogamy* (Hyphal fusion). The accumulated mass of hyphae utilized nutrients from the substrate and colonized to form pin heads which developed into mushroom. Unlike green plants, mushrooms are *Heterotrophs*. They do not have chlorophyll, they cannot generate nutrients by photosynthesis, but take nutrients from other sources. In this research a blend of nutrients was derived from various combinations of *Hyparrhenia hirta* and maize stalk substrates.

Growing of *Pleurotus ostreatus* mushroom indoors, under low temperature conditions and high humidity levels can be more viable economically and ecologically. The term *Pleurotus* originated from the Greek word *Pleura* which means *Side* and *otus* which means *ear*. Mushrooms are also grown on a great variety of substrate and the choice of substrate depends on availability, accessibility, cost and utilization (Ajonina, 2012). This is on the background that variation on the Biological Efficiency (BE), mushroom nutrition content and yield on combination of different substrates have been reported worldwide (Chang and Miles., 1989). Nutrient available within the substrates determines the mushrooms yield potential while differences in Biological Efficiency (BE) have been associated with different levels of substrate combination resulting in specific nutritional composition of the product. It is reasonable to suggest that in order to be able to adequately address issues related to mushroom productivity there is need for thorough assessment of the effects of growing mushroom using different media on growth rate levels; yields using

different treatments under same environment, thus intervention that seek to utilize and improve mushroom production. This has created the need to consider utilization of available and suitable substrates in mushroom cultivation. According to (Rajajapaks *et al.*, 2007), successful mushroom cultivation has three important factors to be considered, these are: reliable spawn, good substrate and a conducive environment. Optimum mushroom grown depends on the existence of specific environmental conditions like temperatures, light intensity as well as relative humidity. Mushroom have nutritional and medicinal value (Bilal., 2016)(Hobbs, 1995). Most mushroom farmers

It is a widely accepted fact that food is a basic necessity of life. Adequate intake of quality food is a key requirement for healthy and productive life (Helen, 2002). The households can benefit from use of tonnes of thatch grass that is destroyed through veld fires every year through converting it to food. Maize stalk destroyed as debris in times of droughts can also be utilized for food production. Oyster mushroom are mostly found in Chinese foods and a range of varieties also grow in Africa. Due to its high amount of nutrients, it is widely popular. They are loaded with various amounts of vitamins, Vitamin B₁ (*Thiamine*), Vitamin B₂ (*Riboflavin*), Vitamin B (*Niacin*) and Pro Vitamin D₂ (*Ergocalciferol*) which makes them a healthy diet. Oyster mushroom consumes significant amount of cellulose, hemicelluloses and nitrogen as their main nourishment source for fruiting body formation and mycelia growth while lignin is rarely used in fruiting bodies (Viziteu, 2000). The iron found in oyster mushroom is high in comparison to meat ((<http://www.healthbenefitstimes.com/oyster-mushroom/>, 2015). World production of mushroom is being dominated increasingly by species which are both edible and have nutritional and medicinal benefits (Zhang, 2004). Use of *Hyparrhenia hirta* grass and maize stalk for growing mushroom have positive impact since both substrates are cheap, available and viable.

1.2 Problem Statement

In Zimbabwe small-scale production of mushrooms has been proposed as a viable option in enhancing livelihoods through reducing vulnerability to poverty by strengthening subsistence as a quick fast yielding source of food and reliable source of income states Assan and Mpofu., (2014). However, despite the fact that mushroom production is becoming popular in Zimbabwe, there is little research done in terms of utilization of local substrates or improving mushroom growing levels and sustainability.

However, little is known about quantities of these substrates and their effect in terms of growth rate and yield. There are so many cheap alternative organic wastes that can be utilized upon for their efficient use for mushroom production in smallholder urban mushroom production sector in Zimbabwe. Despite the fact that the notion of mushroom substrate can be thought of as somewhat comparable to the soil for a plant, the way that mushrooms actually use a substrate is different, and needs to be fully understood in mushroom production in order to achieve the best results. As a result, the utilization of the appropriate mushroom substrate is one of the most crucial aspects of mushroom cultivation (Quimio, 1990). For small urban mushroom growers there is the need for an effective substrate utilization for mushroom production, more especially *Pleurotus ostreatus* which is the most cultivated mushroom in Zimbabwe. Looking forward to optimizing the substrate utilization to oyster mushroom cultivation in smallholder urban production, different substrates were tested on their effect on mushroom growth rate, yield and economic viability.

There is little research done in terms of utilization of local substrates or improving mushroom growing levels and sustainability (Agricuniverse.co.zw, 2015). Mushroom production provides food and medicinal properties, with its culinary, nutritional and health properties. Most material used to produce mushroom is cheap, readily available but most people do not have information about its benefits. (Mswaka, 2000). The cultivation period of mushroom is shorter making it economically viable in such that within that short space of time, one can realise huge profits through selling the produce. Being a science and an art, mushroom production generally requires simple knowledge and use of locally available material as substrate for a sustainable production. As a diverse secondary metabolite, the fungi have a long history in both culinary and medicinal application (Das *et al.*, 2010, Kalaivani *et al.*, 2011; Xu 2012).

A gap noticed in the dietary compliment and lack of knowledge about its benefits, short life cycle clearly shows the need for information dissemination and demonstration as a way of improving dietary needs and employment creation especially in African countries. Since mushroom production can be easily done indoors under controlled environment it can be one major component of poverty alleviation and dietary diversity. Burning of substrates like agriculturally based result in GHG emissions, thus there is need to recycle the material for positive usage like producing food like mushrooms.

Since most edible and medicinal mushroom grow under distinct conditions previous research has evaluated that their safe use for human consumption has been applied under modern analytical approaches with use of recycled material, hay and crop residues (Wang *et al.*, 2014) . While history of useful data should carefully be used, each material used as mushroom substrate need to have foundation for establishing safe use since some toxicological endpoints may clearly need further investigations. Obtaining safe data for developmental and reproductive toxicity (DART), genotoxicity, and chronic end points can particularly be difficult due to limited research funding. Substrate compatibility and spawn run effect requires a conducive environment and timing of humidity and temperature is very critical.

Most bush veld is being burnt every year at the expense of poverty alleviation measure and environment management tool which include mushroom production (Bills *et al.*, 2008)(Bode *et al.*, 2002) (Fiedurek *et al.*,1996) (Miao *et al.*, 2006). However, there is no specific information on substrate combination and yield potential. There has been little research on this note and most donor funding has not shown interest in horticultural crops.

1.3 Objectives of the study

1.3.1 Main objective

To assess performance of *Pleurotus ostreatus* grown indoors using *Hyparrhenia hirta* and maize stalk substrates.

1.3.2 Specific objectives

- To determine growth rate of *Pleurotus ostreatus* on different substrate ratios of *Hyparrhenia hirta* and maize stalk.
- To assess the yield levels obtained under different ratios of *Hyparrhenia hirta* and maize stalk.
- To determine an economical evaluation of the best combination of *Hyparrhenia hirta* and maize stalk substrate.

1.4 Hypothesis

- There is no significant difference in mushroom growth due to *Hyparrhenia hirta* and maize stalk substrates.
- There is significant difference in mushroom yield due to *Hyparrhenia hirta* and maize stalk substrates
- There is positive economic viability in substrate combination.

1.5 Justification

Each substrate needs to be described in such a way that it is possible to predict its yield potential for mushroom production. To achieve a more comprehensive understanding of the ligni-cellulose levels required and mushroom anticipated, there is need to establish substrate combination and yield potential. Various sources of substrates have been used in different localities depending on their availability (Grodzinskaya, 2003) . In Zimbabwe the major substrate used in smallholder urban mushroom production sector is wheat straw and cotton seed hulls. According to (AGRITEX Mat North, 2016) mushroom growers purchase cotton seed hulls from Gokwe and most farmers complain of long distance and huge cost of transport charges. While some people who rear chickens and rabbits collect the *Hyparrhenia hirta* for various uses, it is usually exposed to annual veld fires of which according to Dube and Munsaka., (2018) the tonnes of grass lost could be economically utilised for mushroom production within the city. Urban mushroom farmers can efficiently utilise the grass for lucrative business.

In the past five years' interest in mushrooms has greatly increased in Zimbabwe. This has been due to upcoming agricultural information and technology pertaining mushroom production and lucrative prices (Dzenga, 2017; Khatua *et al.*, 2017) Maize stalks are also another cheapest source of substrate that can be utilised to grow mushroom during normal season and even if the season is bad. Instead of burning the stalks during land preparation farmers can be utilize stalks for mushroom production. Use of *Hyparrhenia hirta* grass and maize stalk for growing mushroom have its own positive impact since both substrates are cheap, available and viable.

High prices of mushroom are mainly due to the current shortage of on the local market. Mushroom cultivation has subsequently become a highly profitable activity in other countries like China,

where a fresh and dried value added mushroom are exported worldwide (Shrestha *et al.*, 2012). Currently the demand is very high but the supply is very low hence the market is lucrative. The use of mushrooms as food crosses all cultural boundaries. However, demand for field picked mushroom has dropped due losses of lives by consumption of poisonous mushrooms. Thus, the need to grow mushroom indoors under regulated and monitored environment. Mushroom consumption in Africa and especially in Zimbabwe, has deep traditional roots, having been gathered since time immemorial and are featured in many local dishes, medicines, and appear in culture and folklore (Mswaka *et al.*, 2001). It is upon this information that this research seeks to find out yield obtained using indoor methods and substrate used during the growing process of the fungi to be economically viable. There is need to have documentation on values of yields obtained from common local substrates. Since mushroom contain huge amount of nutrients it is worth finding out as to how best farmer can grow the crop as way of food security and dietary diversity.

Researchers have come up with a way to bridge data generated from different raw material, different substrates have been tested and proved but none of the information clarifies specific substrate quantities in terms of ratio combination per location and yield levels. However, discoveries made showed that different performances have been recorded under different environments and levels of toxicity are recorded different due to exposure to certain conditions leading to contamination (Zadril, 1983). According to dietary needs assessment held, humans at different ages have certain nutritional requirements that can be covered by mushroom for sustainability (Feeney *et al.*, 2014). Thus mushroom production is of paramount importance so that it can be inclusive in the diet within meals of different age groups. Availability of raw material like growing media creates opportunity for utilization of the dietary benefits, environmental management and profit marginalisation.

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2.0 CHAPTER TWO

2.1 Literature review

2.2 Introduction

The current state of knowledge, capacity of substrate yield potential and growth rate is of paramount importance in mushroom cultivation. Mushroom forming fungi are poorly collected, sparingly studies relatively underutilized. (Onyango, 2011). Mushroom utilization showed that the collected wild mushroom has drastic sentiments which need useful phenolic feature description and domestication protocols. The benefits of mushroom production are that it prevails to many different growing systems from simple and inexpensive to highly sophisticated and expensive (Mensah, 2015). According to (Grimm and Wösten, 2018) cultivated mushroom and related fungi grow almost everywhere on natural material conceivable termed as substrates. Substrate is one of the critical component of mushroom production, as mushrooms depend on substrates for their nutrition (Miles and Chang, 2004). The growth, development and fruiting of mushroom is based on lignocellulose substrate material (Okhuoya, 2000).

Mushroom can be classified into three groups, namely saprophytes, the parasites and symbiotic (which include mycorrhizal) species. There are a few parasitic species, most of the edible mushroom are saprophytic fungi (Miles and Chang, 1997). These species have a symbiotic relationship with some vegetation particularly trees. Utilization of *Hyperrhenia hirta* and maize stalk as substrate for oyster mushroom under controlled environment enables formulation of ratios that meet mushroom growing requirements possible. The substrate must be rich in essential nutrients, it should also be in forms that are readily available to the mushroom assimilation, and also free from toxic substances that inhibit the growth of the spawn. Knowing the biological efficiency and yield levels is critical. There is need to understand the impact of substrate measurements period so as to establish anticipated yield levels.

In Zimbabwe deaths have been recorded which resulted in consumption of poisonous mushroom (Katongomara and Moyo, 2018). This shows the gap on critical knowledge on edible mushroom identification. The chemical composition of the fresh fruiting bodies of oyster mushroom, *Pleurotus ostreatus* indicates a large quantity of moisture (90.8%), whereas fresh as well as dry oyster mushrooms are rich in proteins (30.4%), fat (2.2%), carbohydrates (57.6%), fiber (8.7%)

and ash (9.8%) with 345 Kcal energy value on 100 g dry weight basis; while vitamins such as *Thiamin* (4.8 mg), riboflavin (4.7 mg) and *Niacin* (108.7 mg), minerals like calcium (98 mg), phosphorus (476 mg), ferrous (8.5 mg) and sodium (61 mg) on 100 g dry weight basis, are also found present (Pandey and Ghosh, 1996). According to Rambelli and Menini, (1985) oyster mushroom have been reported to exhibit anti-tumoral chemical properties. Oyster mushrooms are known to bear therapeutic ingredients such as dietary fibres (*Chitins* and *Citosans*) and phenolic compounds (Kurtzman and Doyma, 2005)(Gregori et al, 2007)(Moharram et al, 2007)(Neyrinck et al, 2009). Various bioactive compounds isolated from *Pleurotus ostreatus* culture extracts of Ethiopian higher fungi showed other biological properties such as antiprotozoal, anthelmintic, phytotoxic and brine shrimp lethality activities (Dagne, 1995). Therefore, mushroom growing is essential for a range of positive benefits.

2.3 Importance of Substrate

Substrate in mushroom cultivation has the same function as soil in plant production (Weon *et al.*, 2005). Many species of *Pleurotus* are commonly grown on a wide range of lignocellulosic materials (Sánchez, 2004). A Study conducted to compare the effects of agro-wastes on the growth, yield and nutritional composition of oyster mushroom indicated that knowledge of substrate yield potential was critical. (Hoa *et al.*, 2015). Different substrates can be recommended per region due to local availability of agricultural wastes (Cohen. *et al.*, 2002). The ideal medium for cultivation of edible fungi must be sterile and rich in essential nutrients (Zadril and Reinige, 1988)(Kwon *et al.*, 2003), such as nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and iron (Fe). Most commonly used substrates include sawdust, cottonseed straw, cereal straw, corncob, sugar cane straw and other plant fibers with high cellulose content (Ragunathan *et al.*, 1996)(Kwon *et al.*, 2003). According to (Labuschagne *et al.*, 2000), wheat straw has been the main substrate used for cultivating *Pleurotus ostreatus*. However, (Buglio, 2001) successfully planted *Pleurotus ostreatus* on a combination of wheat straw, cotton hulls, paddy straw, sugarcane and sorghum leaves.

Although *Pleurotus* species can be produced from various substrate types or residues, productivity and biological efficiency will vary according to strains and substrates used (de Carvalho and andrade, 2010). Cultivation of oyster mushrooms using different substrates has been studied

extensively (Poppe, 2004). However, there is no specific information on the usage of *Hyparrhenia hirta* and maize stalk as alternatives to wheat straw and cotton seed hulls for oyster mushroom cultivation. *Hyparrhenia hirta* can be freely collected from the veld and maize stalks are waste products from cereal fields. These alternative substrates need to be studied in order to determine their suitability for use by both commercial and small-scale farmers in mushroom production.

2.3.1 Substrate Pre-treatment

Different biological characteristics of substrate have effect on nutrient availability for assimilation during initial stages of mushroom growth (Shim, 2001) Due to high nitrogen content of *Hyparrhenia hirta* and other grasses, the harvesting season and weather must be carefully chosen since interaction of nitrogen and carbon also affect mushroom development. Too much carbon can lead to dwarf stems while too much nitrogen result in rank growth of stem and small cap may result (Mikishvili *et al.*, 2006). Substrate harvesting should not be done in rainy season since during wet period drying and processing may be difficult and that leads to mildew formation and that can harbour fungal formation which will compete with the *Pleurotus* type of mushroom. *Hyparrhenia hirta* is best harvested from May to July when it is ripe and is at flowering stage. Maize stalk is best harvested at its dry state after removal of maize cobs. After harvesting the substrates must be placed in the sunlight to dry and retain its nutritive value. Soaking should be done for 24 hours to facilitate dislodging of hidden contaminants (MushWorld.com, 2004).

2.4 Heat Treatment

Substrate need to be treated before spawning. Heat treatment is most frequently used to kill or reduce pests and micro-organisms, and there are two categories: sterilization and pasteurisation. The right pasteurisation time and temperature depend on the possible pathogens in a given substrate material. Heat treatment is done at 75-100⁰C for 2 hours (Bellettini *et al.*, 2019).

2.5 Nutritive Factors

Substrate nutritive values have great impact on mushroom production and it is critical among other variables at all stages of growth. Mushrooms are endowed with metabolic capabilities which makes them grow and reproduce when supplied with carbon source, nitrogen source, various minerals, vitamins and water (Chen *et al.*, 2000)

2.6 Attributes of maize stalk in mushroom production

The main nutritional sources for oyster mushroom are cellulose, hemicellulose and lignin (MushWorld.com, 2004). Proteins and enzymes are provided by minerals that include phosphorus, sulphur, magnesium and potassium. If appropriate ratios of substrates are incorporated in mushroom production various yields can be obtained. Growth rate is mainly determined by mycelia development which is mainly influenced by substrate quality and environmental factors. Environmental conditions should be aseptic so that there is no contamination during colonization period (Koutrostsios *et al.*, 2017).

Table 1: Chemical composition of maize stalks (on DM basis, %)

Total hydrolyzable carbohydrates%	Ether extract%	Crude protein%	Crude fiber %	Holo-cellulose%	Hemi-cellulose%	Cellulose%	Lignin%	Ash%
35.10	0.52	3.60	42.15	68.80	26.01	41.00	11.45	13.57

The obtained data as shown in Table 2.1, showed that maize stalks contain high content of crude fiber, cellulose, lignin and lower in crude protein (Tanaka, Robinson and Moo-Young, 1985) (Lin *et al.*, 1985). High values of lignin and cellulose indicate that maize stalk can be used as a nutritive substrate for mushroom production.

Table 2: Chemical composition of Hyparrhenia hirta grass (on dry matter basis%)

Ether extract %	Nitrogen free extract%	Crude protein%	Crude fiber%	Lignin %	Ash%
1.8	45.6	3.2	38.1	0.7	11.3

Source: <http://usesplantnet.project.org>> *Hyparrhenia hirta* (PROTA), 2011

2.7 Performance Parameters in mushroom grown indoors.

A study undertaken for identifying effect of abiotic factors on oyster mushroom for Trichy district in India indicated that these factors had influence on yields (Chitra *et al.*, 2018). Abiotic factors can determine mycelia growth rate and cap development in a given environment. Furthermore,

mushroom require oxygen and a specific pH in order to develop a normal metabolism for structural and energy requirements. (Miles and Chang, 2004). Factors like temperature and relative humidity are important to the growing of mushroom (Mahajan., 2008). Major ecological factors that affect stalk height, stalk diameter and cap size are; temperature, humidity, fresh air and compact material (AMGA, 2004).

2.7.1 Temperature

Pleurotus ostreatus can be widely cultivated, and it can adapt to different temperatures and grows throughout the year (Qu, Huang and Zhang, 2016). Oyster mushroom can grow at moderate temperatures, ranging from 18 to 30⁰ C (Jaramillo et al., 2013).(Li et al., 2011) related that the substrate containing inoculum was subsequently kept in a darkened spawn running room at 23⁰C. According to (Ahmed *et al.*, 2013a) culture house should be maintained at between 22 and 25⁰C. Similarly, (Kwon *et al.*, 2003) also cultivated *Pleurotus* species where incubation room temperature was maintained at between 22 to 24⁰C. Low temperature induce fruiting body development which accompanies the enhancement of activities such as acid protease and the accumulation of nutriment around a developing fruit body (Tokimoto and Fukuda, 1997).

2.7.2 Relative Humidity

Water is one of the main factors that influence the success in mushroom growth. Nutrients are transported from the mycelium to the fruiting bodies by a steady moisture flow (Oei and Nieuwenhuijzen, 2005). For most *Pleurotus* species humidity range is 20-70% (Pandey, 2001). During spawn running and mycelia formation humidity should encompass a range between 60-75% and 85-97% respectively. High humidity within the growing room is favourable for pinning and fruiting (Pandey *et al.*, 2008).

2.7.3 Luminosity

Photoperiodism is needed for fruiting body production. For *Pleurotus* species 200-640 lux for 8-12hrs a day⁻¹ at a temperature compatible with the mushroom (Ahmed *et al.*, 2013b)(Mejía and Albertó, 2013)

2.7.4 Air Composition

Gaseous environment control in aerobic solid-state fermentation is an important factor in the development of microorganisms, dependant on oxygen flow speed through the substrate and the speed of oxygen consumption by microorganisms (Li *et al.*, 2015). Monitoring air condition and temperatures on daily are critical in mushroom cultivation.

2.8 Economic Evaluation of mushroom production.

Cultivation of mushroom is one of the lucrative agricultural enterprises. Study carried out in Bangladesh indicated that profitability of mushroom cultivation was found comparatively higher than that of rice and wheat (Ahmed *et al.*, 2013). According to (Ahmed *et al.*, 2008) as funding to promote the production and consumption of mushrooms is limited, local government and NGOs can play a vital role to develop mushroom agriculture and create ample employment opportunities both in semi-urban and rural areas. Mushroom cultivation could be a possible route to alleviate poverty and develop the life style of the vulnerable people in developing countries. While in Bulawayo mushroom production has also shown to be a lucrative business mostly being practiced by small holder farmers, most farmers lack technical skills to increase their yields (AGRITEX, 2018).

Although knowledge and production level are still limited in Bulawayo, mushroom has stimulated much interest within Zimbabwe. Fertilizers, machinery and pesticides are not much used, the market price of mushroom is relatively high and profit margins are relatively high.

Unlike other agronomic crops, the set-up, costs-benefits and space for mushroom cultivation are recommendable. Indoor mushroom production can be profitable thus may play an active role to employment generation and thus alleviate poverty. In general mushroom projects take very little space and can produce returns within a short period of time (Ahmed *et al.*, 2008). Mushroom has potential to steer a country to achieve MSDs poverty and hunger eradication, poverty, improved health, improved environment and potential to the overall national economy (Irene, 2019). However, limited research has been undertaken on mushroom to provide clear information and marketing (Odendo, Kirigua and Kimenju, 2010). There is need for participatory innovation by researchers, farmers, educators, extension policy makers and marketing in order to optimise mushroom value chain.

2.9 Summary of Literature review

Substrate composition and combination is important for high yields in mushroom production. (Lin, 1985). According to Ahmed et al., (2013a) grasses have been found to be high yielding if properly monitored and cultivated using right quality in terms of moisture. *Hyparrhenia hirta* was found to have highest yield levels, slightly higher than wheat straw. (Li, 2011). It is therefore imperative to nutritive values of each substrate so that pin heads can have high number of basidiocarps and consequently that have effects on the mushroom yields. Environmental factors play a critical role in mushroom cultivation, thus the importance of indoor cultivation for proper temperature and relative humidity management.

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3.0 CHAPTER 3

3.1 Methodology

Abstract

Mushroom can be grown on some organic substrates, mostly waste materials from farms, plantations, factories and institutions. (Patra and Pani, 1995). From an environmentalist point of view, a wide range of agricultural waste can be used alone or in combination to create mushroom substrate. There is need to evaluate the productivity of growing media in order to ensure usage of highly productive substrates in the mushroom cultivation technology (Tesfaw, 2015). From an economic point of view, the business of mushroom production is to obtain returns, through profit maximization, hence there is need to assess the performance of different substrates so as to come up with highly productive substrates. (Lin, 2009). This study was conducted to investigate the suitability of two types of substrates (*Hyparrhenia hirta* and maize stalk) with regard to the rate of colonization amongst the substrates, nutritional content and yields obtained.

3.2 Introduction

Pleurotus species can efficiently degrade agricultural wastes and they grow on wide range of substrates (Sánchez, 2010). Oyster mushroom stem length, stem diameter, cap size and nutrient content is determined by substrate quality (Obodai, Cleland-Okine and Vowotor, 2003). The use of residues in bioprocess may be one of the solutions to bioconversion of inedible biomass residue into nutrition protein rich food in the form of edible mushroom. More than 2000 species of mushroom exist in nature, but only approximately 22 species are intensively cultivated (Manzi, Aguzzi and Pizzoferrato, 2001). However, mushrooms cultivation is not easy, it involves a suitable technique and strain to spawn manufacturing, and growing the crops. Cultivation of mushroom in Zimbabwe is viewed as an effective way to extract bio resources left behind in agricultural residues and as a sound environment protection strategy (Chitamba *et al.*, 2012). According to (Miles and Chang, 1997) yield levels of mushroom are determined by substrate quality. The objectives of the experiment were mainly focusing on yield levels obtained on different substrate combination, time taken for fruit development and an economic evaluation was conducted.

3.3 Materials and Method

3.3.1 Experimental Site

Preparation of growing facilities

Laboratory equipment and assembling was done during the 2nd week of November 2018. Substrate procurement and collection was done within a week. Substrate crushing was done using a shredding machine. Cleaning and fumigation of spawning room, incubation room and growing room was done on the 3rd week of November 2018. A total of 600 grow bags were spawned and placed in the incubation room where they were monitored under a period of darkness which was imposed by covering the using 80micron black polythene.

3.4 Description of Study Site and Design

The experiment was done at Municipal Parks Nursery of Bulawayo City Council (BCC) which is located in Mabutweni (S.200142540E.28.530870) of western part of the city. It was conducted from November 2018 to February 2019. The area was reserved for urban agriculture activities within the community. These included goat farming and cereal crop production, however currently the land has since been abandoned with some of the urban farmers growing mushroom and maize as their main enterprises. Within the fields there is a massive *Hyparrhenia hirta* species of grass dominating in the area, using it for mushroom production could be a competitive advantage. The experiment was conducted under conducive environment where temperature and humidity were constantly monitored. The temperature range was maintained at 22-24°C during incubation period and at 18-30°C during all stages of growth up to harvesting. Air conditioner was used for controlling the temperature. Humidity levels were monitored at 75-95%. Hygrometer was used to measure relative humidity and the humidifier was used for cooling the growing room. A period of darkness was observed during the incubation period by covering the grow bags with black polythene plastic as curtaining. Light was gradually introduced during the second week of incubation and later monitored at (200-800lux) up to harvesting period.

The experiment was carried out on Completely Randomized Design (CRD) with five replicates and five treatments. The five treatments included (T1) maize stalk (100%), (T2) *Hyparrhenia hirta* (100%), (T3) *Hyparrhenia hirta*: maize stalk (3:1), (T4) *Hyparrhenia hirta*: maize stalk (3:2), (T5) *Hyparrhenia hirta*: maize stalk (3:3). Each treatment had a total of 120 grow bags.

3.5 Materials used

Two types of growing media were used; thatch grass (*Hyparrhenia hirta*) and maize stalk. Both substrates were tested indoors at mixed ratios of 3Hh:1Ms, 3Hh:2Ms, 3Hh:3Ms. Tubing plastics were used as grow bags and spawn was used for inoculation at 5% per 2.5ks of wet substrate. Black polythene was used for covering grow bags during the incubation period. Hygrometers and thermometers were used for measuring relative humidity and temperature respectively. Agriculture lime was used to keep pH at neutral levels (6.5-7.0). Digital scale and kitchen scale were used to weigh wet substrate and mushroom at all stages of mushroom growth. Quantities for material used is tabulated in (Appendix 1).

3.5.1 Spawn Source

The wheat grain spawn of *Pleurotus Ostreatus* was obtained from a local spawn producer (Empire Mushrooms) of Magwegwe Township in Bulawayo.

3.6 Preparation of Substrates

Two different types of substrates were used at different treatments during the experiment. The substrates were subjected to same environment to derive growth rate and yield thereafter. Design used consisted of randomization and replication to monitor accuracy. Treatments that was applied was done within the stipulated time frame, objectives that were considered were the growth rate, yields and economic evaluation. *Hyparrhenia hirta* and maize substrates were chopped to same length of 3-5cm using a shredder. They were then soaked for 24hrs to soften them and create optimal surface area for release nutrients and enzyme activity during mycelium development. Substrates were then treated with agriculture lime to stabilize pH and create easy flow of nutrients. The substrates were then mixed using the following ratios; 3:1,3:2 and 3:3 *Hyparrhenia hirta*: maize stalk respectively. The two substrates (Hh and Ms) were independently given same treatments as control of experiment at 100%. The weights used were 25kgs in all treatment ratios given. Constant values that were considered were temperatures and relative humidity that was maintained at 15-28 °C and 75-85%. Temperature and humidity were regulated by mistifying using knapsack sprayer. Time was recorded in days for completion of growth of mycelium on substrates, mycelium growth (%), pin numbers, stem length stem diameter and cap sizes and weights.

3.6.1 The Sterilization Process

High temperature sterilization process was observed at 100⁰C for 2hrs for all treatments. Application of agriculture lime at 5% was also done at this stage to facilitate even distribution of nutrients, temperature and pH. Substrate was then allowed to cool on the cleaned disinfected polythene plastic sheet.

3.6.2 Mixing of substrates

After cooling the substrates, substrates were weighed and mixed according to the following ratios *Hyparrhenia hirta*: maize stalk: 3Hh:1Ms, 3Hh:2Ms, 3Hh:3Ms, 100% Hh:100%Ms substrates were used as control.

3.6.3 Bagging of substrates and Spawning.

Substrates were bagged according to treatments and inoculation of spawn was done at this stage. This was one of the most critical stage since accuracy was to be observed. Inoculation was done at a rate of 75g (Spawn): 2.5kg (wet substrate). Relative Humidity was maintained at 75%. Temperatures were maintained at 15-25⁰C. Continuous cooling was observed through mystifying using knapsack sprayers.

3.6.4 Incubation Period

Spawned bags were covered with black polythene to observe the incubation phase of a maximum of two weeks. During this phase the grow bags started the spawning running process (mycelia formation) at different rates according to given treatments. At this point the mycelium began to secrete extra-cellular enzymes which were then digested to provide some nutrients for the mushroom formation in each treatment. (Onyango., 2011) At this stage the room temperature was kept at a range of 15 to 28⁰C and relative humidity was kept at 70%. This was done for all treatments within the first two weeks of mushroom initiation and mycelia development rate was recorded as shown in (Appendix 2).

3.7 Data Collection

Initial data collection was done during incubation period where mycelia growth rate was recorded for all treatments and that was measured through measuring flush length, diameter and time taken for the percentage of colonization. Pin heads were counted and recorded, stem length, stem

diameter and cap size were measured and recorded also (Appendix 2). Fruiting bodies which accounted for the yield levels were harvested and the number of mushroom and its fresh weight were recorded every 7 days in ten consecutive weeks (Appendix 3). The minimum period (6.13 days) between flushes was recorded. The total number of fruiting bodies, total number of flushes, and yield of each bag was calculated after cropping period.

3.7.1 Data Analysis Procedure

The mushroom weights and numbers were subjected to square root transformation before statistical analysis. Statistical significance was determined by one (one way) analysis (ANOVA) using GenStat 16th edition. Mean separation was done using Least Significant Difference and effects were declared at $P < 0.05$ level (Appendix 7).

After the last harvest, dry weight of the spent substrate was determined by exposing it to direct sunlight heat for 3 consecutive days. The Biological efficiency (BE) of each treatment was calculated using the formula:

$$\text{BE} = \frac{\text{Total weight of mushroom (g) per substrate} \times 100}{\text{Dry weight of spent substrate}}$$

Highest value was recorded in the 3Hh/3Ms treatment ($P < 0.001$) while the lowest value was recorded in Hh as ($P < 0.05$)

3.8 Ethical considerations

All measurements were done using accurate scales and right types of instruments for mushroom production. The decision-making process during treatment and design layout is influenced by the results of assessment for instruments and tools used. According to (Altman, 1991), decision-making should not be undermined by an inappropriate choice of statistical methods of analysis. For this experiment Genstat 16 Edition was used and that can be considered as an ethical way for validity purposes and accuracy.

3.9 Summary

The growth rate of mycelium varied widely and pinhead reduced as the developed into fruiting bodies due to differences in substrate nutritive values. However, *Hyparrhenia hirta* and maize

stalk combination at equal proportions (100%:100%) had the highest yield 108.1c with the value of (P<0.001) while *Hyparrhenia hirta* had the lowest of 78.9c indicated low nutritive values of the substrates used.(Appendix 14)

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4.0 CHAPTER 4

Growth rate of *Pleurotus ostreatus* on *Hyparrhenia hirta* and maize stalk substrates determination.

Abstract

Oyster mushrooms (fungi of genus *Pleurotus*) are the second largest cultivated mushroom species group in the world after agaricus species, constituting approximately 27% of the total global production (Mamiro *et al.* 2014). In search for alternative to cotton seed hulls and wheat straw as growing media in oyster mushroom production, two substrates (*Hyparrhenia hirta* and maize stalk) were used to determine growth rate. Time taken for mycelia formation, pin head formation and fruiting was recorded. The objective of this study was to determine the growth rate of *Pleurotus ostreatus* on *Hyparrhenia hirta* and maize stalk substrates. The Completely Randomised Design (CRD) used included five treatments which indicated high growth rate of mycelia on maize stalk which had average yield 108.1g while treatment *Hyparrhenia hirta* recorded a negative average yield of 78.9g. The main strain used as spawn for the experiment was *Pleurotus* species which is adaptable to a range of temperatures and relative humidity but its growth rate is influenced by the nutrients within the substrate used. According to Hoeksema *et al.*, (2012), mushroom growth rate is influenced by nutrients of substrates. Growth rate trial recorded significant differences in maize stalk treatments ($P < 0.001$) and *Hyparrhenia hirta* treatments recorded negative results ($P < 0.05$). Positive growth rates were observed on maize stalk treatments. No significant differences were noted in stem diameter and stem length.

Key words: Substrate, Mycelia, Spawn, Growth rate, *Pleurotus ostreatus*.

4.1. Introduction

Indoor mushroom cultivation can be done throughout the year, being a primary-saprophyte it grows well in organic material where the substrates are partially decomposed or highly degraded. *Pleurotus ostreatus* has a unique adaptation to a wide range of lignocellulosic substrates (Shah, 2004); hence, it is common to find wild oyster mushroom spouting in clusters on dead trees. According to Ashraf *et al.*, (2013), various crop residues have been used in producing oyster mushrooms and either as main substrates or in combination with supplements. Oyster mushroom is consumed for its taste, medicinal and nutritional properties. (Rajapakse *et al.*, 2007).

The utilization of maize residues as substrates for oyster mushroom under controlled conditions has been reported by Atikpo *et al.*, (2008). Spawn running is an important phase of mushroom cultivation since it has a bearing on how fast the substrate will be colonized. This study

investigated the relative performance of different ratios of *Hyparrhenia hirta* and maize stalk combinations in *Pleurotus ostreatus* grown indoors under same environmental conditions. The potential of these substrates and nutritive values of the substrates. Growth rate parameters included mycelia growth, pin head numbers, fruit body numbers, stem length, stem diameter and cap size. The available literature on substrate chemical composition of maize stalk and *Hyparrhenia hirta* (DM basis %), indicated that the two substrates had the potential to be utilized for mushroom production (Chang et al., 1999). Little work has however, been done to establish relationships among these chemical properties linked to growth rate and yield of mushroom production. Such information is very critical if the best substrate combination is to be identified. Understanding the effects of substrate combination on growth rate of oyster mushroom assist mushroom growers to design appropriate substrates that ensure fast growth rate, optimum yields and cost benefits achieved.

4.2. Materials and Methods

The experiment was conducted in Mabutweni, in Bulawayo under conducive environment where temperature and humidity were constantly monitored. The temperature range was maintained at 22-24°C during incubation period and at 18-30°C during all stages of growth up to harvesting. Air conditioner was used for controlling the temperature. Humidity levels were monitored at 75-95%. Hygrometer was used to measure relative humidity and the humidifier was used for cooling the growing room. A period of darkness was observed during the incubation period by covering the grow bags with black polythene plastic as curtaining. Light was gradually introduced during the second week of incubation and later monitored at (200-800lux) up to harvesting period. Parameters used during mycelia growth stage to fruit initiation were flush growth rate (mycelium development) and colonization rate. These were measured using the Tensiometer, ruler and girth gage. Among the 5 treatments: Ms, Hh, Hh/Ms1, Hh/Ms2 and Hh/Ms3, used ratios and time taken for each treatment were measured and recorded on daily basis. According to Moore et al, (2008), micro-climatic conditions regulate fungal culture homeostasis, affect the duration of fruit body morphogenesis, and define the transition from incubation (vegetative stage of growth) to fruiting (development of basidiocarps). Thus choice of substrate used in mushroom production is of paramount importance at all stages of growth. The study indicated fast colonization rate, fast pin head development and high yield in terms of cap size, stem length and stem diameter. The experiment was conducted under conducive environment

4.2.1. Source of Substrate and Spawn

Hyparrhenia hirta was sourced from local fields around Mabutweni and maize stalk was sourced from farmers within the Parks Nursery. The spawn (Exotic Royale Oyster) was sourced from spawn producer based in Magwegwe.

4.2.2. Description of study Area

Mabutweni parks Nursery is located in the western part of the City of Bulawayo (S.200142540E.28.530870). The area has a hub of agricultural activities including crop and small livestock production. The study was a managed indoor experiment with 5 treatments housed under similar environmental conditions. Relative humidity and temperatures were recorded on daily bases.

4.2.3. Research Design

The experiment was carried out on Completely Randomized Design (CRD) with five replicates and five treatments. The five treatments included (T1) maize stalk (100%), (T2) *Hyparrhenia hirta* (100%), (T3) *Hyparrhenia hirta*: maize stalk (3:1), (T4) *Hyparrhenia hirta*: maize stalk (3:2), (T5) *Hyparrhenia hirta*: maize stalk (3:3). Each treatment had a total of 120 grow bags. The layout consisted of factorial combination of three ratios with each treatment replicated five times. Two types of substrates were used *Hyparrhenia hirta* and maize stalk. Substrates were sterilized using the method out lined by Kang (2004).

4.2.3.1. Spawning and Incubation

Substrates were initially sterilized through boiling for 2 hours. They were then cooled and mixed and bagged into grow bags. The bagging was done concurrently with spawning and 2.5kgs of wet substrate were inoculated with 100g spawn. Spawning was done in the sterile spawning room. Spawned bags were placed into the sterile incubation room where the bags were monitored under darkness to initiate spawn run. They were observed under controlled environment of 15-25⁰C and relative humidity of 75-85%. During the incubation period all treatment were kept under dark environment to facilitate mycelia growth.

4.3 Data collection procedure.

Data were collected on the following parameters; mycelia growth rate, pin head and fruit body development rate and growth rate, stem length, stem diameter and cap size. All parameters were measured using appropriate equipment to ensure accuracy.

4.4 Data Analysis

The mushroom weight, stem length, stem diameter and cap numbers were obtained and the data sets were organised according their respective parameters as indicated in Figure 5.1 ,5.2 and 5.3, the different treatments were subjected to analysis of variance (ANOVA). This procedure was done to separate the different treatments and to determine the magnitude of the difference. Furthermore, according to Biggs, (1999), statistical analysis is the best subsequent economic analysis. The mushroom weights and numbers were subjected to square root transformation before statistical analysis. Statistical significance was determined by one (one way) analysis (ANOVA) using GenStat 16th edition. Mean separation was done using Least Significant Difference and effects were declared at $P < 0.05$ level (Appendix 7).

4.4.1 Challenges encountered during data collection.

No challenged were encountered during data collection, all data was collected on time and appropriate equipment was used for measurements to ensure accuracy.

4.5 Results

4.5.1 Colonization

Initial substrate was recorded and the following results were obtained:

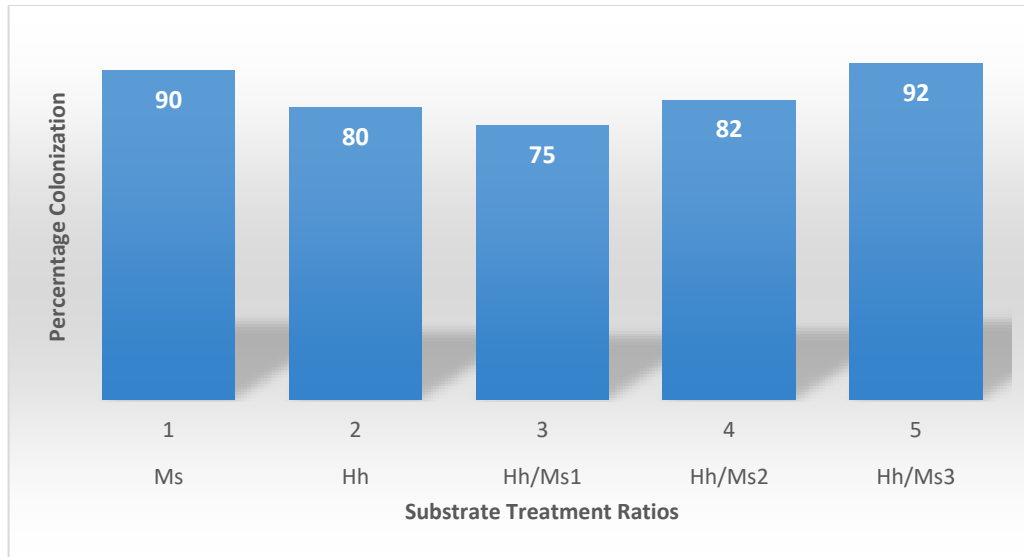


Figure 4.1: Mycelium Colonization % (15th day)-Incubation Period

During the second week all treatment were 75% and above indicating of full colonization within 21 days. Fig. 1.1 above indicates the mycelium growth on the 15th day of incubation. This was the end of incubation period and darkness was withdrawn by removal of black polythene exposing grow bags to light. Treatment 5 (Hh/Ms) indicated a similar percentage with Treatment 1 (Ms). This indicated that there was a significant nutritive value for both substrates. ($P < 0.001$). The lowest percentage of colonization was indicated in Treatment 3 had lowest value ($P < 0.05$). However, other substrates also indicated similar values. The graph also indicated that there was an improvement of mixed substrates at higher percentage and a decrease at lower substrate combination.

4.5.2 Rate of colonisation

Mean daily temperature in the growth room varied between 18-27⁰C during full colonization with mycelia and fruiting, whilst RH was between 80% and 90%. These temperatures appear to be ideal because Shah *et al.* (2004) reported an optimal temperature of 25⁰C for full mycelia colonisation and fruiting. There were high significant differences ($P < 0.05$) in the percentage colonization of *Hyparrhenia hirta* compared with maize stalk substrates (Fig.1.1 above.) on day 10 of incubation, maize stalk indicated 60% colonization while *Hyparrhenia hirta* indicated 45%. Mixed substrate of *Hyparrhenia hirta* and maize stalk at same proportion ratio indicated 68% and other treatments Hh/Ms1 and Hh/Ms2 had lower percentages of colonization, 48% and 56% respectively. This

indicated the nutrient variability and colonization rates were according to values utilized during enzyme process in fruit development.

The colonization result (Figure 1.2) are consistent with the findings of Tan (1981), Shah *et al.* (2004), Mondal *et al.*, (2010) and Khan *et al.* (2009), who reported that spawn took 2-3 weeks (14-21 days) to achieve full colonization. Moreover, Khan (2009) reported that *Pleurotus ostreatus* took 24-25 days for completion of spawn running on wheat straw substrate which is similar to *Hyparrhenia hirta*. From day 1 to 9, no significant difference in percentage was observed between the growing media. This indicated that Hh/Ms3 substrate combination had attained full colonisation in day 16 although significant differences were noted on earlier days. Indoor grown mushroom can cover a designated area (van Nieuwenhuijzen 2007). The variation observed may be due to environment impact and hygiene standard observed during the period.

4.5.3 Pinning

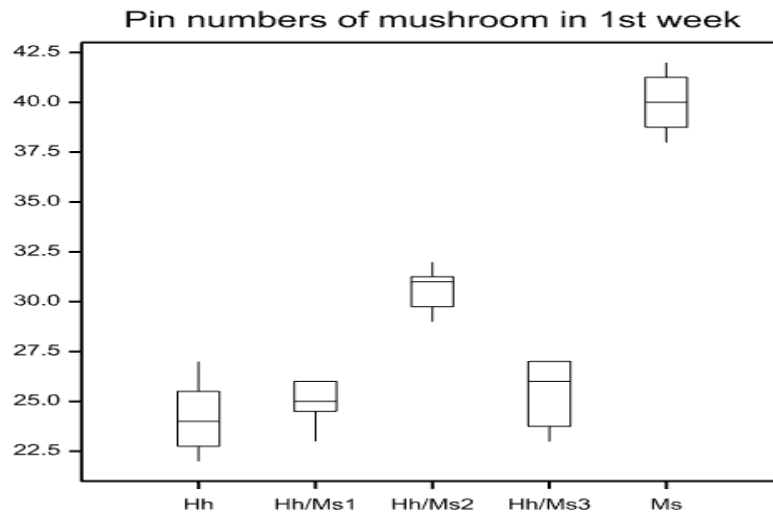


Figure 4.2: Pin numbers recorded for (T1) maize stalk (100%), (T2) Hyparrhenia hirta (100%), (T3) Hyparrhenia hirta: maize stalk (3:1), (T4) Hyparrhenia hirta: maize stalk (3:2), (T5) Hyparrhenia hirta: maize stalk (3:3).

During the 1st week Maize stalk had significant high numbers of pin heads (40.0) and Hyparrhenia hirta had the lowest pin heads of 23. This indicated a high nutritive value of the initiated by the high mycelia recorded of 60% in the 10th Week.

During week 2 there was an increase in mycelia growth rate in T4 (Hh/Ms2) and T2 (Hh) recorded the lowest mycelia growth rate which subsequently recorded pin heads of 31 and 25 respectively. As it is clearly shown in Figure 1.1 above, combined substrates of *Hyparrhenia hirta* and maize stalk at high ratios shows highest percentages in terms of mycelium growth rate. The growth rate had much influence on early fruit development which also resulted in high levels of pin numbers. However, there was no significant difference in stem length for Treatment 1, Treatment 2, Treatment 3, Treatment 4 and Treatment 5 since the average rate was at 1.1cm more all treatments. Similarly, there was no significant difference in stem diameter for all treatment and the average range was between 0.98-1.2cm. There was an indication that among all treatments pin numbers, stem length and stem diameter growth rate had significant relationship with the yields obtained and the time taken for fruit maturity.

4.5.4 Pin Numbers Recorded-week 2

The pin number profile below indicated number of pin heads recorded in 10 bays (average on replicates) of given treatment.

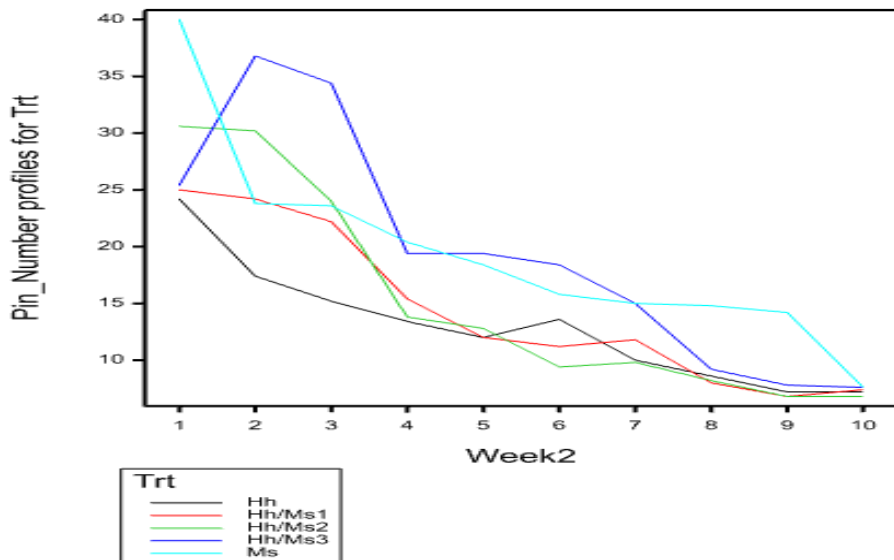


Figure 4.3: Pin numbers recorded during week 2

The pin numbers recorded during week 2 indicated the significant difference between the substrates. Treatment Hh/Ms3 had the highest value ($P < 0.001$) as indicated in Fig 1.3 above. While Hh recorded the lowest value ($P < 0.05$). However, for all treatment week 2 indicated a peak in grow bag bay 2-5 more pin heads were produced during this period showing the abundance of nutrients during the initial stages as indicated by Kang (2004). This also indicated that both Hh and Ms had higher cumulative number of pin if combined (Figure 1.3). Maize stalk also showed potential as an alternative to *Hyparrhenia hirta* when considering pin numbers.

4.5.6 Fruiting

After full colonization the grow bags were removed from the incubation room and were placed in the growing room. Within the 1st week pin heads began to show and they were recorded on weekly bases. A significantly high pin head was recorded in the initial stages of growth and developed into fruiting bodies (basidiocarps). The grow bags were then pierced to facilitate easy flush of mushroom bunches. Observed results indicated that not all pin head developed into oyster mushroom fruit.

Initial stage of growth was the mycelia development where maize stalk substrate recorded greatest value ($P < 0.001$) mycelia growth rate. Combination of 3:3 ratio *Hyparrhenia hirta* and maize stock substrates also gave high mycelia growth rate ($P < 0.001$). While Combination of 3:2 ratio *Hyparrhenia hirta* and Maize stalk similarly recorded high mycelia growth rate. However, combination of 3:1 ratio resulted in the lowest growth rate ($P < 0.05$). These results indicated the importance of substrate combination at different levels.

Each treatment was recorded on daily bases. The growth rate on the second day after inoculation was at initial flush (5%) whilst *Hyparrhenia hirta* recorded the lowest (1%). On the tenth day a marked percentage increase was recorded on the tenth day when treatment Hh/Ms3 recorded 59% level of growth rate. This indicated the link of high growth rate with nutrient availability. The blend of nutrients from both substrates indicated great percentage of growth rate on the tenth day. Subsequently in the fifteenth day full colonization was recorded above 92% and 90% (Hh/Ms3 and Ms) respectively. The lowest percentage was 80% Hh and Hh/Ms1 recorded 75%.

4.5.7 Determination of Biological Efficiency

Biological Efficiency of mushroom on fresh weight basis was calculated by using formula given by Chang and Miles (1989).

$$\text{BE\%} = \frac{\text{Total fresh weight of mushrooms (g) per substrate} \times 100}{\text{Total weight of substrate used (g)}}$$

Biological efficiency indicated that (T1) maize stalk (100%), (T2) *Hyparrhenia hirta* (100%), (T3) *Hyparrhenia hirta*: maize stalk (3:1), (T4) *Hyparrhenia hirta*: maize stalk (3:2) and (T5) *Hyparrhenia hirta*: maize stalk (3:3) combinations respectively indicated the following results: 92%, 48%, 60%, 76% and 98%. Observation indicated that nutrient values within the substrates had a positive impact on growth rate of mushroom since high ligno-cellulose value in maize stalk substrate indicated high growth rate of mycelia and fruiting bodies and recorded significant value ($P < 0.001$).

4.6 Discussion

The study demonstrated that combined substrates of *Hyparrhenia hirta* and maize stalk at 100% values is potentially suitable for mushroom cultivation. Many locally available organic substrates have high potential for *Pleurotus ostreatus* production. In this study although maize stalk had the highest potential and biological efficiency, it was noted that since it is not available through the year alternative means of mixing it with other substrates could increase its availability whilst maintaining the mushroom yields obtained (Onyango., 2011). Observation indicated that number of pin heads recorded had a significant difference between substrate combinations.

4.7 Conclusions

Combination of *Hyparrhenia hirta* and maize stalk substrates accelerated the rate of colonisation as shown in Figure 4.1, this facilitated fast fruit development. However, with *Hyparrhenia hirta* the rate of colonisation recovered at a later stage (day 10) this indicated that both substrates had potential of breaking down the lignin and cellulose, but at different rates Yildiz., (2002).

4.8 Recommendations

Hyparrhenia hirta can be recommended for *Pleurotus ostreatus* mushroom production since it contains N, P and Mg. (Stamets., 2005). Maize stalk also can complement use on *Hyparrhenia hirta* since it contains N and Mg required in mushroom production. Environmental factors are of

paramount importance in indoor mushroom production thus appropriate instruments and equipment should be used to monitor temperature and relative humidity (Khan *et al* 2012).

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5.0 CHAPTER 5

Assessment of yield levels obtained under different ratios of *Hyparrhenia hirta* and maize stalk.

Abstract

Oyster mushroom is an important crop in Africa, Asia and other parts of the world. However, its production is being greatly affected by environmental factors which include temperature and moisture (Hoa., 2015). Emerging evidence suggest that growing mushroom indoors results in high yields (Mandeel., 2005). It was discovered that several *Pleurotus* species grow well on lignocelluloses (Patra., 1995). This study examined the yield levels obtained under use of combined substrates of *Hyparrhenia hirta* and maize stalk. Ecological requirements of oyster mushroom vary at the various stages of growing period (Tesfaw., 2015). The nutrient utilization determines the yields that can be obtained. (Nadir 2016). The selected substrates were spawned and the study was done indoors under controlled environment where temperatures and humidity were monitored on daily bases. The study laid out a Completely Randomised Design (CRD) with 5 replicates where the grow bags were placed under favourable mushroom growing conditions of temperature range within 15-28°C and Relative humidity of 85-95%. The study aimed at measuring the yield levels of *Pleurotus Ostreatus* obtained from the 5 treatments. The results exhibited that maize stalk yielded positively ($P < 0.001$) while *Hyparrhenia hirta* had negative results ($P < 0.05$).

Key words: *Pleurotus ostreatus*, *Hyparrhenia hirta*, lignocellulose, growing period.

5.1. Introduction

Oyster mushroom possesses the appropriate enzymatic mechanism for transformation of complex organic micro-molecules into simple compounds have been exploited as the means for biodegradation of a wide range of plant litter due to their particular ability for selective delignification. (Osei, 1996). Oyster mushroom is known for its ability to degrade to lignocellulosic residues from agricultural fields and forests and converts them into protein-rich biomass (Rowel *et al.*, 2000). Species of oyster mushroom show good adaptability to a wide range of temperature, making it possible to grow this mushroom almost all year round (Chadha *et al.*, 2001). In Zimbabwe oyster mushroom, particularly *Pleurotus ostreatus*, is the first preference for resource advantaged farmers (Kashangura., 2005).

Substrate nutritive value has effect on the output when growing *Pleurotus ostreatus* species of mushroom. (Onuha, 2007). This is mainly influenced by stage of substrate growth at harvest for

media preparation. In the experiment five treatments were used which were 100% Ms, 100%Hh, 75%:25% (Hh: Ms), 75%:50% (Hh: Ms) and 100%:100% (Hh/Ms) the spawn used was procured from local laboratory of Empire Investments based in Bulawayo. The strain used was an all- season Exotic spawn. In terms of productivity, biological efficiency was considered at which the ability of substrate conversion from dry matter into fresh matter was high (Poppe, 2004).

In Zimbabwe, main substrate used for *Pleurotus ostreatus* cultivation is wheat straw and cottonseed hulls (Kashngura., 2005). However, (Zireva *et al* 2007) highlighted the need to test performance of oyster mushroom under different substrates. Furthermore, the hectarge under wheat is low compared to maize (FAO/WFP,2010) hence, wheat is not readily available in some parts of the country. There is thus need for research on th productivity of alternative substrates that can be used at the same time being easily accessible to oyster mushroom growers in the country.

5.2 Materials and Method

Study was carried out at Mabutweni located within the western area of Bulawayo. Details of this section are given in Section 3.3.

5.2.1. Description of study area.

The study area had all sufficient material for use that was accessible from local farmers and most material was obtained locally. During the incubation period temperatures were maintained within 18 to 25⁰C while humidity was maintained within 75 to 85%. However, both parameters were increased to ranges of 15-28⁰C and 75-95% during the growth stage since there was more requirement for moisture at this stage as recommended by Lin, (2004). Most information is given in section 3.3.

5.2.2. Research Design

The trial was carried out on Completely Randomized Design (CRD) with five replicates and five treatments. The five treatments included (T1) maize stalk (100%), (T2) *Hyparrhenia hirta* (100%), (T3) *Hyparrhenia hirta*: maize stalk (3:1), (T4) *Hyparrhenia hirta*: maize stalk (3:2), (T5) *Hyparrhenia hirta*: maize stalk (3:3). All measurements were proportioned at 25kgs wet weight substrate. Each treatment had a total of 120 grow bags. The grow bags were monitored within the same growing room under same temperatures and same relative humidity conditions.

5.2.3. Data Collection Procedure

The fruiting bodies were harvested, counted, weighed at its fresh state and recorded within 6-7 days for 10 consecutive weeks. The adopted method of harvest break was observed at (6.33) days as reported by (Bahl, 1998). At every stage of harvest, biological efficiency for each treatment was using the formula given in 4.2.3.5. All data was collected at stipulated intervals to maintain accuracy for all treatments. Line chart graphic presentations were used to obtain results for all given parameters.

5.2.4 Data analysis Procedure

The data was subjected to analysis of variance (ANOVA) using GenStat 16th edition. Specific objectives 1 and 2 was analysed using descriptive statistics such as mean, minimum and maximum. Specific objective 3 was analysed using frequency distribution, percentages and cost benefit analysis procedure. The null hypothesis was analysed using Pearson correlation coefficients.

5.3 Results

The results of the summary statistics of growth rate, and yields obtained is presented in Figure 5.1 and Figure 5.2 below:

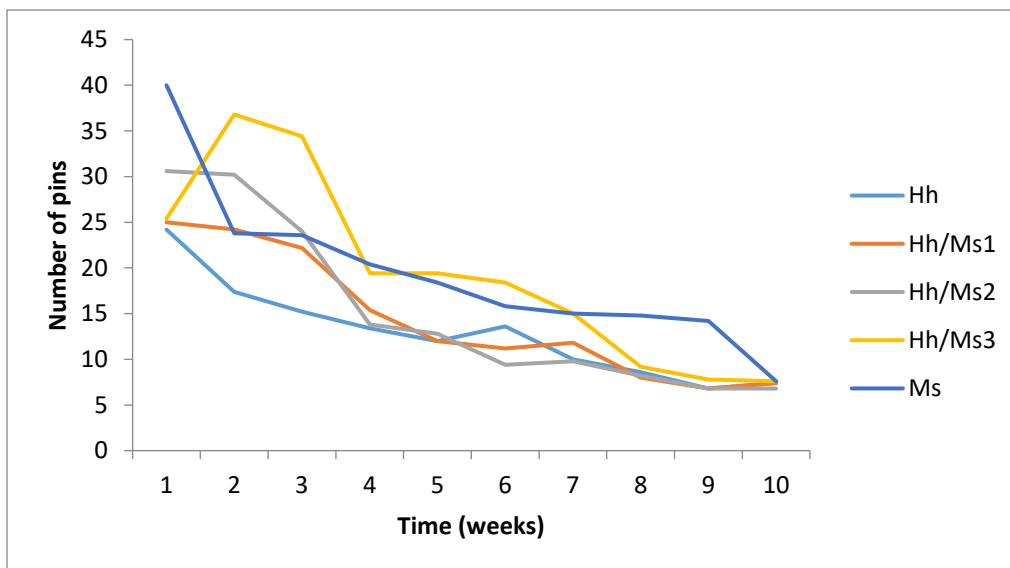


Figure 5.1: Growth rate of pin heads during mycelia phase

Key: T1-Ms
 T2-Hh
 T3-Hh/Ms1
 T4-Hh/Ms2
 T5-Hh/Ms3

The results in figure 5.1 show that the pin number obtained had its peak during week 1-4 recording an average of 30 pin heads per flush, and gradually descended to 10 pin heads during week 8-10. Treatment T1 and treatment T5 gave the highest pin numbers while treatment T2 gave the lowest. There was significant difference between pin numbers recorded on T5 and T2 and the pin numbers recorded in T3 ranged around 14 to 15 pin heads. This Implicated that the nutrients of the initial values gave high yields during the initial stage of growth and decline was due to nutrient utilization during fruit development and growth.

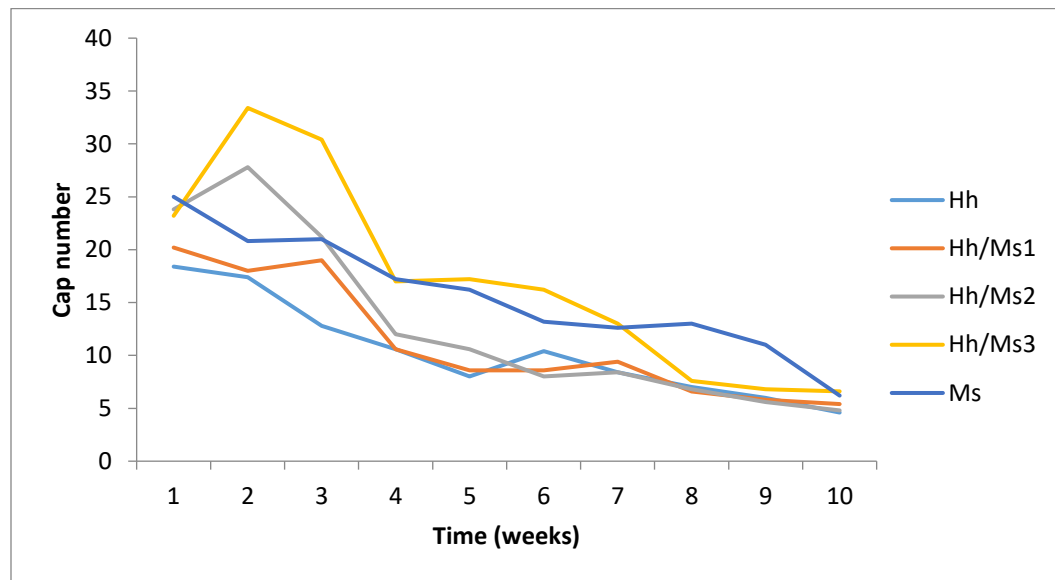


Figure 4.2: Cap number for recorded during growth phase.

Key: T1-Ms
 T2-Hh
 T3-Hh/Ms1
 T4-Hh/Ms2
 T5-Hh/Ms3

Cap numbers recorded in T5 and T1 gave high numbers ranging from 19 to 30 on average between week 1 and 4. While T2 recorded the least cap numbers of an average of 8 within the same period. This indicated the nutrient utilization and values of available nutrients within given substrates. However due to nutrient utilization at productive stage of mushroom growth yields per bag declined.

5.4 Discussion

Several *Pleurotus* species have been successfully cultivated at commercial level world- wide using ligni-cellulose wastes as substrates (Ingale and Ramteke, 2010). An Ideal substrate should contain nitrogen and carbohydrate for rapid mushroom growth (Khare *et al.*, 2010). Various crop residues have been reported to have been used in producing oyster mushroom either as main substrates or in combination with supplements. (Ashraf *et al.*, 2013). In this study, the response of different substrates combination indicated differences in respect of time taken for mycelia growth, pin head formation stem length, stem diameter and cap size.

Maturation of fruiting bodies between flushes and yields obtained had a significant difference and that agrees with report by Lozano (1990). There were 7 flushes obtained within two months for all treatments in this experiment based on 10% spawning rate. As indicated in Figure 5.2 the 2nd flush had the highest yield level and that agrees with what was also reported by Kurtzman., (2005). The flush number with highest yield in this experiment was not definite as each treatment had its peak at different periods.

The results showed that the tested maize stalk substrate had the highest yields as indicated by Mendez *et al* (2005) in mushroom production. The highest yields were recorded on T5 (108.1g) and the lowest T3 (78.9g). Each treatment had different peak mushroom fruiting body numbers as shown in Figure 5.3 the yields varied according to stages of growth the differences. The differences in results reported by other research workers should be expected since it may be due to the variation in the growing requirements for cultivation of oyster mushroom such as temperature, humidity and light regime.

5.5 Conclusion

The study demonstrated that locally available substrates (Hh and Ms) have proven to be suitable for utilization as substrates for oyster mushroom cultivation. Total fresh weight of the first and second flush was significantly found to be the highest for *Hyparrhenia hirta*/Maize stalk combination (T5) 509.6g followed by Maize stalk (T1) 467.8g and the least was *Hyparrhenia hirta* (T2) had the lowest of 368.3g. The low yields might be attributed to low degradation of lignocellulose as reported by Sharma *et al.*, (2013). From the present study it can be concluded that *Hyparrhenia hirta* and maize stalk combination at equal proportions can be the best substrate to grow *Pleurotus ostreatus*.

5.6 Recommendations

Results indicated that high yields can be obtained from a combination of cheap readily available substrates. However, there is need for further trials to confirm the performance of substrate under investigation. Since maize stalk is seasonal is affected by rainfall pattern, there is need to consider further research on combining it with other substrates and do trials on yields obtained.

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6.0 CHAPTER 6

Economic evaluation of best combination of *Hyparrhenia hirta* and maize stalk mushroom substrates.

Abstract

The study which was conducted in Mabutweni, Mzilikazi district of Bulawayo during period year 2018-2019, has analysed the cost and returns of mushroom production. This can be a decision-making tool which accounts for variables such as inflation in mushroom production. According to Ferguson, (2018) this tool contribute towards strategic planning for every business and managers can use it for weighing pros and cons of any new implementation. For mushroom production, this can be an accurate instrument to assist potential mushroom farmers. Understanding of economics of mushroom cultivation is critical depending on availability of manpower, infrastructure, raw materials and market, planning for production volumes. The economics of mushroom vary across regions, and have a bearing on benefit-cost ratio. Mushroom cultivation is one of the efficient ways by which crop residues can be utilized. Therefore, mushroom production can become one of the most profitable enterprises in agribusiness that could produce food from different substrates. The objective of the study was to determine an economical evaluation of the best combination of *Hyparrhenia hirta* and maize stalk mushroom substrate. For this study a total of 600 grow bags of wet substrates were inoculated with 125kgs of *Pleurotus ostreatus* spawn. Each of the treatments were 120 grow bags in a bay and the treatments consisted of T1 (Ms), T2(Hh), T3(Hh/Ms1), T4(Hh/Ms2) and T4(Hh/Ms3). Treatment was subjected to similar growing conditions within the same growing room where temperatures were kept within the range of 15-28⁰C and relative humidity was kept within the range of 75-95%. According to Viziteu, (2000), the growth of *Pleurotus ostreatus* requires high humidity (80-90%) and temperature range of 25-30⁰C. Effect of 2 lignocellulosic substrates were evaluated on mycelial growth, fruit yield level and economic evaluation of *Pleurotus* species were investigated. Production was conducted intensively indoors under controlled and monitored environment. The yields obtained for T1, T2, T3, T4 and T5 are indicated in Table 3.1 to Table 3.5. Strengthening mushroom production sector through availing cost benefits for mushroom cultivation enables urban economy to keep its vibrancy, development, diversification in business and employment creation opportunities.

Key words: Cost benefit, production, planning and economic evaluation

6.1. Introduction

Mushrooms are increasingly being recognised as important food products for their significant role in human health, nutrition and income generation. This study was carried out to assess the most appropriate substrate for *Pleurotus ostreatus* mushroom cultivation. Mushroom classified is heterotrophic organism which get their nutrients from decaying material or other living organisms. There has been significant evidence of high oyster mushroom up-take for health and economic

benefits. (Uddin *et al.*, 2011). Due to the high interest in oyster mushroom, most farmers are cultivating the *Pleurotus* species to meet the market demand (Hearst *et al.*,2009). For a successful mushroom cultivation there is high nutrient requirement and these are mainly obtained from legno-cellulosic plants (Bhugio, 2001). The objective of this research was to determine the economic evaluation of best combination of *Hyparrhenia hirta* and maize stalk substrates. Shrestha *et al.*, (2012), reported that oyster mushrooms are highly profitable in countries like China where fresh and dried mushroom are exported worldwide.

Cultivation of oyster mushroom in Zimbabwe is an income generating activity becoming popular but there is little research pertaining production costs involved. (Kashangura,2005). According to Munish, (2008) lignin would enhance enzyme activity and thus ensure higher mushroom yield. However, there is need for research on the substrate degradation process and quantities of mushroom produced for cost benefit analysis. (Boddy,2008). The initial stage of measurement was the mycelia running rate, followed by measurement of all parameter on vegetative and productive growth. The inputs used were then costed and the output benefits were recorded too, the data was then subjected to Cost benefit analysis.

6.2 Materials and Method

The study was conducted in Bulawayo. Laboratory equipment and assembling was done in November 2018. Substrate procurement and collection was done within a week. Substrate crushing was done using a shredding machine. Fumigation of spawning room, incubation room and growing room was done on the 3rd week of November 2018. A total of 600 grow bags were spawned and placed in the incubation room where they were monitored under a period of darkness which was imposed by covering the using 80micron black polythene.



Figure 5.1: Mpofu Treggie (January 2019)

6.2.1 Description of Study Site and Design

The experiment was done at Municipal Parks Nursery of Bulawayo City Council (BCC) which is located in Mabutweni (S.200142540E.28.530870) of western part of the city. It was conducted from November 2018 to February 2019. The area was reserved for urban agriculture activities within the community. The experiment was conducted under conducive environment where temperature and humidity were constantly monitored. The temperature range was maintained at 22-24°C during incubation period and at 18-30°C during all stages of growth up to harvesting. Air conditioner was used for controlling the temperature. Humidity levels were monitored at 75-95%. Hygrometer was used to measure relative humidity and the humidifier was used for cooling the growing room. A period of darkness was observed during the incubation period by covering the grow bags with black polythene plastic as curtaining. Light was gradually introduced during the second week of incubation and later monitored at (200-800lux) up to harvesting period. After harvesting, economic data on inputs used was done and output was also recorded and analysed.

The experiment was carried out on Completely Randomized Design (CRD) with five replicates and five treatments. The five treatments included (T1) maize stalk (100%), (T2) *Hyparrhenia hirta*

(100%), (T3) *Hyparrhenia hirta*: maize stalk (3:1), (T4) *Hyparrhenia hirta*: maize stalk (3:2), (T5) *Hyparrhenia hirta*: maize stalk (3:3). Each treatment had a total of 120 grow bags.

6.2.3. Data Collection procedure

Cost of material used was done and recorded at the initial stages during procurement in November 2018. Some of the inputs used had cumulative charges and they were recorded from November 2018 to February 2019. Water charges and electricity charges for cash power was costed. Use of electricity was high during the initial stage when shedding of substrate was done during the 2nd week in November 2018. Costing was done on the following; spawn, Jik, Agriculture Lime, labour days, tubing plastic, transport, black polythene, baling twine and equipment used. Mycelia running rate was done to assess initial growth rate.

Mycelia Running Rate %(MRR)

Mycelia growth rate was measured by taking full grow bag diameter at incubation stage and recording was done on daily basis to confirm the efficiency of the substrate. MRR was in spawned bags for each treatment was measured after mycelium colony cross the shoulder of the grow bag. The linear length was measured at different areas of bag as reported by Dey., (2006) to assure accuracy.

$$MRR = \frac{L}{N} \text{ cm/day}$$

Where L=Average length of mycelium running(cm)

Days required for complete mycelia running were recorded as indicated in appendix 3

After harvesting, the collected mushroom was also costed using the current price which was \$18rtgs/200g punnet (\$1USD) during January-February 2019. Prices of inputs were recorded at all stages of growth. The weighed mushrooms were recorded and a cost benefit analysis was done for all treatments.

Table 3: Summary of mushroom yields and income generated.

Appendix	Treatment	Total number of grow bags	Output (kgs)	Income(\$rtgs)	Income(\$USD)

5	T1(Maize stalk 100%)	120	467.8	42 102.00	2 339.00
6	T2(<i>Hyparrhenia hirta</i> /maize stalk-3:1)	120	396.0	35 640.00	1 980.00
7	T3(<i>Hyparrhenia hirta</i> /maize stalk-3:2)	120	398.3	35 847.00	1 991.50
8	T4(<i>Hyparrhenia hirta</i> /maize stalk-3:3)	120	417.3	37 557.00	2 086.50
9	T5(<i>Hyparrhenia hirta</i> /maize stalk)	120	509.6	45 964.00	2 548.00

Source (Mabutweni trial 2018-2019)

6.2.4. Data Analysis Procedure

The calculation of profitability was based on the conversion of expected costs and revenues on the present value discount rate in amount of 2% (discount rate used I forestry projects) Hlavackova *et al*, (2003). The cost benefit analysis for different substrates and inputs were computed based on present market price of mushroom and prices of output were taken from current going prices on the market. Cost benefit analysis was done in order to determine whether the business for producing mushroom is profitable or not. Statistical significance was determined by one-way analysis (ANOVA) using GenStat 16th edition. The Least Significant Difference (LSD) was used to determine significant differences among treatments at the 0.05 probability level. (Appendix 7).

6.3. Results

Effect of substrate on yield

The time taken from mycelia stimulation to primordial initiation ranged from 5-8 days. The shortest time was observed in Treatment 5 and Treatment 1 (5days). The longest time was observed

on Treatment 2, Treatment 3 and Treatment 4. Numerically the highest average number of pin heads was recorded on Treatment 5 and the average yield on weight was 108.1(g) and the lowest was Treatment 3; 78.9(g). Dey et al (2010) found that the number of primordia and the average yield significantly varied with the substrate used in production of *Pleurotus ostreatus* mushroom (Dey., 2010). Bao, (2012) found that similar findings on growing *Pleurotus ostreatus* on different substrates have different results in terms of yields (Bhuyan.,2008). The costs of inputs were lower compared to the income generated using the current prices source: (Greens Bulawayo February 2019)

The substrates combination used had reasonable lower prices in comparison with the output results shown on Table 6.1 to Table 6.3. The CBR for Treatment 1 was 1:2 and Treatment 5 had the highest of 1:3 while Treatment 2 had the lowest 1.1 closer to break even. These results indicated the levels of nutrients, yields obtained and income generated. Mushroom production can be a valuable enterprise if properly conducted using reliable and nutritive substrates.

6.4. Discussion

The inputs used and the yields obtained revealed the economic viability of mushroom production. (Chitamba *et al.*, 2012) The yield potential was within the reported range by Lozano, (2001) that seven harvestings were carried during 60 days, whereas Jiskani., (1999) reported 6 days between flushes and Bughio., (2001) recorded 9-15 days between flushes. There were 8 flushes recorded within 60 days for all the treatments tested in this experiment based on 10% spawning rate. The results showed that the tested maize stalk and *Hyparrhenia hirta* substrate combination gave high yields and had a high CBR. Maize stalk had almost similar higher yields. Result indicated that the tested maize stalk for the cultivation of *Pleurotus ostreatus* influence the weight of mushroom produced as reported by Mendez *et al.*, 2005.

6.5. Conclusion

The statistical analysis showed significant effect in combination of *Hyparrhenia hirta* and maize stalk substrate combination for mushroom production grown indoors($P < 0.001$). Furthermore, the

CBR showed a high ratio (1:3) of *Hyparrhenia hirta* and maize stalk combinations use. Lower ratios were recorded on *Hyparrhenia hirta* indicating need for substrate combination as reported by Campbell., (2003).

6.6 Recommendations

There is need to conduct further research on substrate combination and through comparative analysis of locally available resources. For obtaining optimal profits, more research is required on identifying substrates that are compatible with oyster mushroom production. Further research can be conducted on assessing substrate impact on the costs and benefits of mushroom experiment for various objectives. There is need to conduct research on *Pleurotus ostreatus* production costs for locally available resources and establishing availability time frames for the substrates.

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7.0 CHAPTER 7

7.1. Introduction

Sufficient evidence exist that nutritive value of substrate has influence on the weight of mushroom harvested Chang et al., (1996). *Pleurotus ostreatus* mushroom have ability to utilize biomolecules for their growth. The suitability of the substrate also confirmed their comparable biological efficiencies as reported by Onyango., (2011). This study was conducted to find solution to economically viable substrates for sustainable *Pleurotus ostreatus* cultivation. Oyster mushroom is the most important cultivated species, favourable to eat, and grow economically on different kind of organic waste raw materials. (Kang, 2004). According to Miles, (1997), *Pleurotus* species grows on a wide range of plant wastes and does not require costly processing method and enrichment nutrition. However, this experiment was conducted on *Hyparrhenia hirta* and maize stalk which are equally cheap and nutritional for oyster cultivation.

The utilization of maize residues as substrate for growing oyster mushroom indoors have been reported by Atikpo., *et al* (2008). Ecological requirements of mushrooms and nutritive requirements vary at various stages of growth. After incubation, pinning stage follows and the harvesting is done. The time for selling the mushroom was after full fruit formation and in this experiment, prices were recorded and cost benefit analysis was conducted.

7.2. Research Summary

The research findings encourage mushroom growers to utilise and maize stalk substrates for mushroom cultivation. The growing media has proved to ensure profit optimization and this can be achieved on small scale and commercial levels. Global mushroom production has increased tremendously, from about 0.3 million tons in 1961 to about 3.41 million tons in 2010. (Zireva, 2003) (Wakchaure.,2011). China is leading producer of mushrooms worldwide, producing about 65% of global mushroom and 83% of oyster mushrooms worldwide (Royse, 2017). According to Onyango *et al.*, (2011) Africa produces only 1% of the total world output of oyster mushroom.

African countries have great potential to produce huge amount of oyster mushroom (Meiying, 1998).

Mushroom growth rate has impact on the yields obtained. Understanding the factors influencing growth rate in mushroom cultivation creates a room for researchers to improve on time taken from spawning to harvesting.

7.3 Conclusions

The study demonstrated that *Hyparrhenia hirta* and maize stalk combination at 100% (1:1) ratio is potentially suitable for use *Pleurotus ostreatus* mushroom production. Maize stalk substrate also proved to be high yielding in terms of mushroom production. However, with lower yields obtained using *Hyparrhenia hirta* alone. Improved yields may be obtained through adding other more nutritious substrates like maize stalk or leguminous by-products. The differences in results reported by other research workers could be due to differences in substrate nutritive composition and other environmental and management factors when growing *Pleurotus ostreatus* mushroom. In terms of income generation, mushroom has high potential. Resources that are cheap and locally available promote mushroom cultivation if good spawn is used. Many locally available organic substrates have high potential for *Pleurotus ostreatus* production indoors. In this study even if maize stalk had the highest potential and biological efficiency, it was noted that since it is not available through the year alternative means of mixing it with other substrates could increase its availability whilst maintaining the mushroom yields harvested.

Observation indicated that number of pin heads recorded had a significant difference between substrate combinations ($P < 0.005$) the influenced the high fruit body number in Hh and Ms combinations. Growth rate was determined in chapter 4 where initial flushes were higher during the first 2 weeks and diminished from week 6-10. The results in figure 5.1 show that the pin number obtained had its peak during week 1-4 recording an average of 30 pin heads per flush, and gradually descended to 10 pin heads during week 8-10. Treatment T1 and treatment T5 gave the highest pin numbers while treatment T2 gave the lowest. High cost benefit ratio of 3;1 was obtained in T5 followed by T1 which showed that maize stalk inclusion in substrate for indoor mushroom cultivation was critical for profit-optimization.

The objective of chapter 5 was determined on yield levels for Maize stalk and *Hyparrhenia hirta* combinations (Ms, Hh, Hh/Ms, Hh/Ms2 and Hh/Ms3). There was significant difference between pin numbers recorded on T5 and T2 and the pin numbers recorded in T3 ranged around 14 to 15 pin heads. Grow bags gave high yields during the initial stage of growth and decline was due to nutrient utilization during fruit development and fruit formation. The time taken from mycelia stimulation to primordial initiation ranged from 5.0-8.0 days was recorded under objective 4. The shortest time was observed in T5 and T1 (5.0 days). Longest time was observed on T2, T3 and T4. (Appendix 4). All treatment was statistically similar but numerically different as shown in Figure 5.1 and Figure 5.2. Numerically the highest average number of pin heads was recorded on T5 and the average yield on weight was 108.1(g) and the lowest was T3 78.9(g). Dey., *et al* (2006) found that the number of primordia and the average yield significantly varied with the substrate used in production of *Pleurotus ostreatus* mushroom (Dey, 2006). Biswas, 2006 found that similar findings on growing *Pleurotus ostreatus* on different substrates have different results in terms of yields. (Afiukwa, 2015). The costs of inputs were lower compared to the income generated using the current prices source: (Greens Bulawayo February 2019).

The objective of chapter 6 was determined by costing the inputs and assessing the output. The substrates combination used had reasonable lower prices in comparison with the output results shown on Table 6.1 to Table 6.3. The CBR for Treatment 1 was 1:2 and T5 had the highest of 1:3 while T2 had the lowest 1.1 closer to break even. These results indicated the levels of nutrients, yields obtained and income generated. Mushroom production can be a valuable enterprise if properly conducted using reliable and nutritive substrates.

7.4. Policy Implications

Since mushroom cultivation is capital intensive and increase with increase in substrate, the financial assistance through institutional agencies at cheaper interest rates would help increase mushroom cultivation. Singh *et al.*, (2007) reported that proper institutional arrangements required to supply a good quality of spawn at reasonable prices and in desired quantities to the mushroom growers. According to FAO, (2009) public policy fundamental prerequisite for small-scale producers to improve their livelihoods through agricultural, horticultural and infrastructural activities.

Policies are instituted to avoid a negative effect that has been noticed in most developing countries. However, there has been challenges as the country tried to implement policies. Other international and Regional Frameworks constitute the Comprehensive Agriculture Policy Framework, Comprehensive Africa Agriculture Development Programme (CAADP), Zimbabwe Agriculture Investment Programme (ZAIP) have seen commitments by some countries to improve on the sector but no specifications are clear on mushroom in particular. According to Malabo Declaration (2003), African countries have been committed in spending 10% of total budgets on agriculture little or nothing is being mentioned about mushroom in terms of sponsor. (Olivier et al.,2019).

For successful mushroom production, proper arrangements are required to supply good quality spawn of reasonable prices and in desired quantities to the mushroom growers (Pathmashini *et al.*, 2009). Mushroom growers can be promoted to initiate positive options about mushroom cultivation through financial support. Small scale mushroom farmers can also be promoted to intensively grow mushroom in small areas they own.

The need for long term investment in mushroom cultivation is supported by the potential impact to both personal and group farmer level. At the individual level the farmer intensively makes money. The short growth periods of the mushrooms, the capacity for high volume production and consistence market demand for mushrooms makes selling them profitable for farmers in both foods and medicinal markets. Furthermore, increasing the prevalence of mushroom creates jobs, in particular for women, and provides protein rich nutrient dense produce that can help combat micronutrient deficiencies and food insecurity.

Mushroom being highly perishable enterprise and prone to high temperatures and marketing infrastructure such as cold storage facilities is immense importance. Similarly, suitable arrangements are needed by the canning and processing industry for the management of surplus mushroom especially in Zimbabwe where power cuts are prevalent. There is need to establish drying facilities to make soups for export.

7.5 Recommendations

There is need for effective awareness on *Pleurotus ostreatus* cultivation using maize stalk and *Hyparrhenia hirta* under intensive indoor production. This has potential to improve growing conditions and cascading relevant information to mushroom farmers on the growth rate, yields that can be obtained when combinations of *Hyparrhenia hirta* and maize stalk are used as substrates for *Pleurotus ostreatus* grown indoors. The other critical information is the cost benefits of using *Hyparrhenia hirta* and maize stalk as cheap resources that can be obtained local within the farming area. Research institutions and extension personnel at large should avail information on substrate use and its benefits including specifications on nutrient components that add value to the substrates for mushroom cultivation practised indoors. Costing of the substrates and other inputs is also of paramount importance since it provides farmers with expenditure then that information can be used in relation to the yields obtained linking that to selling prices of mushroom business viability. The most effective way to impact skills to the potential mushroom growers is to teach the fundamental aspects of the mushroom farming system to provide hands-on-training on site using maize stalk and *Hyparrhenia hirta*. Mushroom cultivation also requires good planning and coordination a very positive and practical way of providing this training is through Farmer Field Schools (FFS). These provide an opportunity by-doing based on principles of non-formal education. There is need also to support extension officers or trained farmers to discover key agro-ecological concepts and develop management skills through self-discovery skills practiced in the field. However, there should be awareness programmes on mushroom identification so that farmers can be able to differentiate between edible and none edible mushroom particularly those who pick in the forests. Indoor mushroom should be encouraged since it is highly profitable and can be practised even by land less farmers on small scale. Participation at National and International Trade Fairs can provide an opportunity to exchange information about overcoming processing techniques.

7.6. Area for further Research

There is need therefore to do further research on Hh and Ms substrate combination for at least 2 or more trials so as to ascertain that the yields obtained in indoor mushroom cultivation using these

substrates is standard. The properties within the substrates should be known and documented so that mushroom growers can have access for the quantities required for growing of *Pleurotus ostreatus*, research can be conducted on that area as well. Furthermore, the potential of the spawn should be known as a guideline to oyster mushroom growers to capacitate compatibility of substrate and spawn thus have a standard spawning rate. Research should be done along that area of nutrient values of mushroom growth using *Hyparrhenia hirta* and maize stalk as locally available substrates. Further studies can be conducted *Pleurotus ostreatus* mushroom yields obtained in other Provinces or regions using *Hyparrhenia hirta* and maize substrates grown indoors so as to assess whether geographical differences of these areas have similar results. Mushroom pricing in relation to substrate type for mushroom grown indoors can be another area of research to assess indoor mushroom cultivation viability.

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Appendices

Appendix 1: Materials and Quantity used

ITEM	QUANTITY
Spawn	48kgs
Maize Stalk	500kgs
<i>Hyparrhenia Hirta</i>	1000kgs
Tubing plastics (grow bags)	600(30x15cm) x50micron
Water	80 000L
Jik	5L
Sunlight liquid	2litres
Baling twine	5kgs
Hygrometer	5
Thermometer	5
Digital scale	(200kg max) x 1
Kitchen scale	(2kg max) x 1
Scissors	1
Surgical blade	4
3” nail	10
Black polythene	1 roll (80micron)
Firewood	1.5t
Knapsack sprayer	16Lx4L
Tensiometer	1
pH meter	1
Ruler	(30cm) x 1
Methylated spirit	500ml
Mutton cloth	1kg

Source: Mabutweni mushroom Experiment 2018-2019

Appendix 2: Mycelium Growth Rate per day

Mycelia growth rate

Mycelia growth rate (%) during Incubation Period					
Days of mycelia growth/development	Treatments of Substrate Ratios				
(Days)	Ms (%)	Hh(%)	Hh/Ms1(%)	Hh/Ms2(%)	Hh/Ms3(%)
1 (Incubation)	0	0	0	0	0
2(Incubation)	5	1	2	3	4
3 (Production)	8	3	4	5	7
4(Production)	10	5	5	5	10
5(Production)	20	13	12	15	25
6(Production)	35	20	25	22	40
7(Production)	36	25	35	30	45
8(Production)	37	30	40	38	50
9(Production)	50	35	45	45	59
10(Production)	60	45	48	56	68
11(Production)	65	45	48	60	75
12(Production)	70	65	60	65	80
13(Production)	75	70	65	75	85
14(Production)	85	75	70	80	90
15(Production)	90	80	75	82	92

Source: Mabutweni mushroom experiment 2018-2019

Key:

Treatments

Ms Maize Stalk

Hh- *Hyparrhenia Hirta* (Thatch grass)

Hh/Ms1- Ratio 3:1

Hh/Ms2-Ratio 3:2

Hh/Ms3-Ratio 3:3

Appendix 3: Analysis of variance table for mushroom weight.

Chi-square 308.67 on 53 degrees of freedom: probability <0.001

F-test 5.73 on 53 and 3816 degrees of freedom: probability <0.001

Analysis of variance

Variate: Weight

Source of variation	d.f. pr.	s.s.	m.s.	v.r.	F
Subject stratum					
Trt	4	30571.32 <.001	7642.83		201.05
Residual	20	760.28	38.01		0.86
Subject. stratum					
d.f. correction factor 0.1981					
Trt	9	230053.44 <.001	25561.49		575.47
Week2.Trt	36	73442.04 <.001	2040.06		45.93
Residual	180	7995.32	44.42		
Total	249	342822.40			

*Trt=Treatment, *df=degrees of freedom; s.s.=sum of squares; m.s.= mean square; v.r.=variance ratio

Appendix 4: Experiment residuals.

Subject 23	-7.02	s.e. 1.74
Subject 6 Week2 1	-28.04	s.e. 5.66
Subject 10 Week2 1	20.56	s.e. 5.66
Subject 21 Week2 5	16.82	s.e. 5.66
Subject 23 Week2 5	-63.38	s.e. 5.66
Subject 25 Week2 5	18.12	s.e. 5.66

Appendix 5: Standard errors of differences of means for mushroom weight.

Production(wk)	Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms
1		61.20	101.20	121.80	193.20	160.00
2		109.00	101.40	123.40	193.20	119.60

3	119.80	100.40	120.80	174.60	128.80
4	89.20	89.20	84.20	96.40	97.20
5	84.80	95.00	84.20	77.40	96.00
6	82.60	88.80	58.40	86.20	87.60
7	81.80	62.80	79.80	83.60	80.20
8	78.20	63.60	76.40	86.20	69.00
9	56.40	48.00	48.80	51.40	64.60
10	29.80	39.40	36.80	39.00	32.60

Appendix 6: Standard errors of means for mushroom weight.

Table	Trt	Trt	Trt
rep.	25	50	5
e.s.e.	1.333	0.872	2.959
d.f.	35.65	20	42.08
Trt			2.981
d.f.			35.65
s.e.d.	1.885	1.233	4.185
d.f.	35.65	20	42
Trt			4.215
d.f.			35.65

*rep=replication; e.se=standard errors of means; d.f.=degrees of freedom; Trt=Treatment

Appendix 7: Least significant differences of means (5% level) for mushroom weight.

Table	Trt	Trt	Trt
rep.	25	50	5
l.s.d.	4.639	2.572	10.265
d.f.	35.65	20	42.08
Trt			10.373
d.f.			35.65

*l.sd= least significant difference; df=degrees of freedom; rep=replication; Trt=treatment

Appendix 8: Stratum standard errors and coefficients of variation for mushroom weight.

Variate: Weight

Stratum	d.f.	s.e.	cv%
Subject	20	1.950	2.2
Subject	180	6.665	7.5

*df=degrees of freedom; s.e.=standard errors; cv%=coefficients of variation

Analysis of variance

Variate: Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	4	30571.	7643.	6.00	<.001
Residual	245	312251.	1274.		
Total	249	342822.			

*Trt=Treatment, *df=degrees of freedom; s.s.=sum of squares; m.s.= mean square; v.r.=variance ratio

Appendix 9: Summary of means for mushroom weight.

Variate: Weight

Grand mean 88.7

Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms
	79.3	79.0	83.5	108.1	93.6

Appendix 10: Summary of standard errors of means for mushroom weight.

Table	Trt
rep.	50
d.f.	245
e.s.e.	5.05

*rep=replication; d.f.=degrees of freedom; e.se.=standard errors of means

Appendix 11: Standard errors of differences of means for mushroom weight.

Table	Trt
rep.	50
d.f.	245
s.e.d.	7.14

*rep=replication; d.f.=degrees of freedom; s.e.d.=standard errors of differences of means

Appendix 12: Least significant differences of means (5% level) for mushroom weight.

Table	Trt
rep.	50
d.f.	245
l.s.d.	14.06

*rep=replication; d.f.=degrees of freedom; l.s.d.=least significant difference of means.

Appendix 13: Estimated stratum variance for mushroom weight.

variance	effective d.f.	variance component
1274.49	245.000	1274.

Appendix 14: Fisher's protected least significant difference test for mushroom weight.

Trt	Mean	
Hh/Ms1	78.98	a
Hh	79.28	a
Hh/Ms2	83.46	ab
Ms	93.56	b
Hh/Ms3	108.12	c

Chi-square 144.20 on 53 degrees of freedom: probability <0.001

F-test 2.67 on 53 and 3816 degrees of freedom: probability <0.001

Appendix 15: Analysis of variance for mushroom Stem Length.

Variate: Stem Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Trt	4	1139.416	284.854	64.13	<.001
Residual	20	88.840	4.442	0.84	
Subject stratum					
d.f. correction factor 0.3739					
Trt	9	51911.056	5767.895	1094.29	<.001

Trt	36	5218.184	144.950	27.50	<.001
Residual	180	948.760	5.271		
Total	249	59306.256			

*Trt=Treatment, *df=degrees of freedom; s.s.=sum of squares; m.s.= mean square; v.r.=variance ratio

Appendix 16: Tables of means for mushroom stem length.

Variate: Stem Length

Grand mean 23.82

Week	1	2	3	4	5	6	7
	47.88	46.60	41.88	19.76	13.84	12.60	16.04
Week	8	9	10				
	16.08	12.04	11.52				
Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms		
	23.88	27.46	24.04	20.90	22.84		

Week	Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms
1		35.00	54.20	55.20	39.60	55.40
2		39.40	62.20	55.00	35.60	40.80
3		44.00	46.20	40.00	39.60	39.60
4		26.40	26.40	14.60	15.80	15.60
5		20.00	12.00	11.80	12.80	12.60
6		12.00	12.20	13.20	13.40	12.20
7		21.80	21.80	11.80	10.80	14.00
8		15.60	18.20	15.20	17.40	14.00
9		13.20	10.60	11.80	12.20	12.40
10		11.40	10.80	11.80	11.80	11.80

Appendix 17: Standard errors of differences of means for mushroom stem length.

	Trt	Trt	Trt
rep.	25	50	5
s.e.d.	0.649	0.422	1.441
d.f.	67.30	20	78.18
Trt			1.452
d.f.			67.30

*rep=standard errors of differences of means; d.f.=degrees of freedom; Trt=Treatment

Appendix 18: Least significant differences of means (5% level) for mushroom stem length.

Source of variation	Trt	Trt	Trt	
rep.	25	50	5	
l.s.d.	1.507	0.879	3.336	
d.f.	67.30	20	78.18	
Trt			3.369	
d.f.			67.30	

*rep=replication; l.s.d.=least significant differences of means; d.f.=degrees of freedom; Trt=Treatment

Appendix 19: Analysis of variance for mushroom stem diameter.

Variate: Stem_Diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Trt	4	0.014960	0.003740	0.52	0.723
Residual	20	0.144400	0.007220	1.23	
Subject. stratum					
d.f. correction factor 0.6974					
Trt	9	0.058760	0.006529	1.11	0.361
Trt	36	0.186640	0.005184	0.88	0.631
Residual	180	1.059600	0.005887		
Total	249	1.464360			

*Trt=Treatment, *df=degrees of freedom; s.s.=sum of squares; m.s.= mean square; v.r.=variance ratio

Appendix 20: Summary of mushroom stem diameter

Variate: Stem Diameter

Grand mean 1.0484

Week	1	2	3	4	5	6	7
	1.0280	1.0400	1.0440	1.0440	1.0280	1.0760	1.0680
Week	8	9	10				
	1.0440	1.0480	1.0640				
Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms		
	1.0560	1.0580	1.0420	1.0380	1.0480		

Week	Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms
1		0.9400	1.0600	1.0400	1.0600	1.0400

2	1.0400	1.0400	1.0400	1.0400	1.0400
3	1.0600	1.0200	1.0600	1.0600	1.0200
4	1.0600	1.0800	1.0200	1.0000	1.0600
5	1.0200	1.0200	1.0200	1.0600	1.0200
6	1.0800	1.1400	1.0200	1.0600	1.0800
7	1.1200	1.0600	1.0400	1.0200	1.1000
8	1.0800	1.0600	1.0200	1.0200	1.0400
9	1.0800	1.0400	1.0800	1.0000	1.0400
10	1.0800	1.0600	1.0800	1.0600	1.0400

Appendix 21: Standard errors of means for mushroom stem diameter.

Sources of variation	Trt	Trt	Trt
rep.	25	50	5
e.s.e.	0.01534	0.01202	0.03470
d.f.	125.53	20	145.16
Trt			0.03431
d.f.			125.53

*rep=replication; Trt=Treatment; d.f.=degrees of freedom

Appendix 22: Least significant differences of means (5% level) for mushroom stem diameter.

Table	Trt	Trt	Trt
rep.	25	50	5
l.s.d.	0.04568	0.03545	0.10316
d.f.	125.53	20	145.16
Trt			0.10213
d.f.			125.53

*rep=replication; l.s.d.=least significant differences; d.f. degrees of freedom

Chi-square 108.02 on 53 degrees of freedom: probability <0.001

F-test 2.00 on 53 and 3816 degrees of freedom: probability <0.001

Appendix 23: Analysis of variance for mushroom pin numbers.

Variate: Pin_Number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
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Subject stratum					
Trt	4	1750.976	437.744	257.19	<.001
Residual	20	34.040	1.702	1.21	
Subject. stratum					
d.f. correction factor 0.5362					
Subject	9	13283.156	1475.906	1046.08	<.001
Trt	36	2379.984	66.111	46.86	<.001
Residual	180	253.960	1.411		
Total					
	249	17702.116			

*Trt=Treatment, *df=degrees of freedom; s.s.=sum of squares; m.s.= mean square; v.r.=variance ratio

Appendix 24: Tables of means for mushroom pin numbers

Variate: Pin Number

Grand mean 16.244

Week	1	2	3	4	5	6	7
	29.040	26.480	23.880	16.480	14.920	13.680	12.320
Week	8	9	10				
	9.760	8.560	7.320				
Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms		
	12.880	14.400	15.240	19.340	19.360		

Week	Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms
1		24.200	25.000	30.600	25.400	40.000
2		17.400	24.200	30.200	36.800	23.800
3		15.200	22.200	24.000	34.400	23.600
4		13.400	15.400	13.800	19.400	20.400
5		12.000	12.000	12.800	19.400	18.400
6		13.600	11.200	9.400	18.400	15.800
7		10.000	11.800	9.800	15.000	15.000
8		8.600	8.000	8.200	9.200	14.800
9		7.200	6.800	6.800	7.800	14.200
10		7.200	7.400	6.800	7.600	7.600

Appendix 25: Standard errors of means for mushroom pin numbers.

Sources of variation	Trt	Trt	Trt
rep.	25	50	5

e.s.e.	0.2376	0.1845	0.5367
d.f.	96.52	20	114.22
Trt			0.5312
d.f.			96.52

* rep=replication; Trt=Treatment; d.f.=degrees of freedom

Appendix 26: Standard errors of differences of means for mushroom pin numbers.

Sources of variation	Week	Trt	Week
rep.	25	50	5
s.e.d.	0.3360	0.2609	0.7589
d.f.	96.52	20	114.22
Trt			0.7512
d.f.			96.52

* rep=replication; s.e.d.=standard errors of differences; Trt=Treatment; d.f.=degrees of freedom

Appendix 27: Least significant differences of means (5% level) for mushroom pin numbers.

Sources of variation	Trt	Trt	Trt
rep.	25	50	5
l.s.d.	0.7390	0.5443	1.6668
d.f.	96.52	20	114.22
Trt			1.6525
d.f.			96.52

*rep=replication; l.s.d.=least significant differences; Trt=Treatment; d.f.=degrees of freedom

Appendix 28: Analysis of variance for mushroom pin numbers.

Variate: Pin_Number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	4	1750.98	437.74	6.72	<.001
Residual	245	15951.14	65.11		
Total	249	17702.12			

*Trt=Treatment, *df=degrees of freedom; s.s.=sum of squares; m.s.= mean square; v.r.=variance ratio

Chi-square 80.01 on 53 degrees of freedom: probability 0.010

F-test 1.48 on 53 and 3816 degrees of freedom: probability 0.013

Appendix 29: Analysis of variance for mushroom cap numbers.

Variate: Cap_Number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Trt	4	1857.104	464.276	236.88	<.001
Residual	20	39.200	1.960	1.29	
Subject stratum					
d.f. correction factor 0.4885					
Trt	9	8893.904	988.212	648.24	<.001
Trt	36	1750.496	48.625	31.90	<.001
Residual	180	274.400	1.524		
Total	249	12815.104			

*Trt=Treatment, *df=degrees of freedom; s.s.=sum of squares; m.s.= mean square; v.r.=variance ratio

Appendix 30: Genstat Generated Tables of means for mushroom cap numbers.

Variate: Cap Number

Grand mean 13.328

Week	1	2	3	4	5	6	7	8	9	10
	22.120	22.280	20.880	13.480	12.120	11.280	10.360	8.200	7.040	5.520

Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms
	9.760	11.220	12.900	17.140	15.620

Week	Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms
1		18.400	20.200	23.800	23.200	25.000
2		11.400	18.000	27.800	33.400	20.800
3		12.800	19.000	21.200	30.400	21.000
4		10.600	10.600	12.000	17.000	17.200
5		8.000	8.600	10.600	17.200	16.200
6		10.400	8.600	8.000	16.200	13.200
7		8.400	9.400	8.400	13.000	12.600
8		7.000	6.600	6.800	7.600	13.000
9		6.000	5.800	5.600	6.800	11.000
10		4.600	5.400	4.800	6.600	6.200

Appendix 31: Standard errors of means for mushroom cap numbers.

Source of variation	Trt	Trt	Trt
rep.	25	50	5
e.s.e.	0.2469	0.1980	0.5600
d.f.	87.92	20	105.38
Trt			0.5522
d.f.			87.92

*rep=replication; e.s.e.=standard errors of means; Trt=treatment; df=degrees of freedom

Appendix 32: Standard errors of differences of means for mushroom cap numbers.

Source of variation	Trt	Trt	Trt
rep.	25	50	5
s.e.d.	0.3492	0.2800	0.7920
d.f.	87.92	20	105.38
Trt			0.7809
d.f.			87.92

*rep=replication; s.e.d.=standard errors of differences of means; Trt=treatment; df=degrees of freedom

Appendix 33: Least significant differences of means (5% level) for mushroom cap numbers.

Source of variation	Trt	Trt	Trt
rep.	25	50	5
l.s.d.	0.7796	0.5841	1.7647
d.f.	87.92	20	105.38
Trt			1.7433
d.f.			87.92

*rep= replication; l.s.d=least significant differences; Trt=Treatment; df=degrees of freedom

Appendix 34: Analysis of variance for mushroom cap numbers.

Variate: Cap_Number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	4	1857.10	464.28	10.38	<.001

Residual	245	10958.00	44.73		
Total	249	12815.10			

*Trt=Treatment, *df=degrees of freedom; s.s.=sum of squares; m.s.= mean square; v.r.=variance ratio

Appendix 35: Fisher's protected least significant difference test for mushroom cap numbers

Trt	Mean	
Hh	9.76	a
Hh/Ms1	11.22	ab
Hh/Ms2	12.90	b
Ms	15.62	c
Hh/Ms3	17.14	c

*Trt=Treatment

Chi-square 160.98 on 53 degrees of freedom: probability <0.001

F-test 2.99 on 53 and 3816 degrees of freedom: probability <0.001

Appendix 36: Analysis of variance for mushroom cap diameter.

Variate: Cap_Diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Trt	4	890.920	222.730	75.66	<.001
Residual	20	58.880	2.944	2.79	

Subject stratum

d.f. correction factor 0.3179

Source of variance	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	9	223790.160	24865.573	23566.78	<.001
Trt	36	3935.720	109.326	103.62	<.001
Residual	180	189.920	1.055		
Total	249	228865.600			

*Trt=Treatment, *df=degrees of freedom; s.s.=sum of squares; m.s.= mean square; v.r.=variance ratio

Appendix 37: Genstat Generated tables of means for mushroom cap diameter.

Variate: Cap_Diameter

Grand mean 27.440

Week	1	2	3	4	5	6	7	8	9	10
Means	75.440	74.560	69.200	8.720	8.400	8.600	8.680	8.080	6.240	6.480

Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms
	25.180	29.840	28.100	28.800	25.280

Week2	Trt	Hh	Hh/Ms1	Hh/Ms2	Hh/Ms3	Ms
1		64.600	89.600	77.200	72.800	73.000
2		67.600	89.600	78.400	73.400	63.800
3		66.800	64.200	70.600	81.200	63.200
4		8.200	8.200	9.000	9.800	8.400
5		8.600	7.800	7.800	9.800	8.000
6		8.200	9.000	9.000	9.400	7.400
7		8.600	8.800	8.800	8.800	8.400
8		7.600	8.200	8.000	8.400	8.200
9		5.600	6.400	6.800	6.800	5.600
10		6.000	6.600	5.400	7.600	6.800

Appendix 38: Standard errors of means for mushroom cap diameter.

Source of variation	Trt	Trt	Trt
rep.	25	50	5
e.s.e.	0.2054	0.2427	0.4988
d.f.	57.22	20	77.02
Trt			0.4594
d.f.			57.22

*rep. replication; e.s.e.=standard errors for means; Trt=Treatment; df=degrees of freedom;

Appendix 39: Standard errors of differences of means for mushroom cap diameter.

Source of variation	Trt	Trt	Trt
rep.	25	50	5
s.e.d.	0.2905	0.3432	0.7054
d.f.	57.22	20	77.02
Trt			0.6496
d.f.			57.22

*rep. replication; s.e.d.= standard errors of differences of means; Trt=Treatment; df=degrees of freedom;

Appendix 40: Least significant differences of means (5% level) for mushroom cap diameter.

Source of variation	Trt	Trt	Trt
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rep.	25	50	5
l.s.d.	0.6879	0.7158	1.6637
d.f.	57.22	20	77.02
Trt			1.5382
d.f.			57.22

*rep=replication; l.s.d.=least significant difference; d.f.= degrees of freedom; Trt=Treatment

Appendix 41: Analysis of variance for mushroom cap diameter.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	4	890.9	222.7	0.24	0.916
Residual	245	227974.7	930.5		
Total	249	228865.6			

*df=degrees of freedom; s.s.=sum of squares; m.s.= mean square; v.r.=variance ratio

Appendix 42: Input used and yields for Treatment 1 (Maize stalk substrate).

Period of growth stage	Week	Water	Spawn (kgs)	Agric Lime(Kgs)	Labour days	Jik (L)	Output Ms (Kgs)	Wet Substrate(kgs)
Incubation	1	4 500	25	2	8	1	0	300
Incubation	2	2 200	0	0	8	0.25	0	0
Production	3	1 800	0	0	6	0.5	80	0
Production	4	1 600	0	0	6	0.5	59.8	0
Production	5	1 800	0	0	6	0.5	64.4	0
Production	6	1 850	0	0	6	0.5	48.6	0
Production	7	1 850	0	0	6	0.5	48	0
Production	8	1 800	0	0	8	0.5	43.8	0
Production	9	1 850	0	0	8	0.5	40.1	0
Production	10	1 840	0	0	4	0.5	34.5	0
Production	11	1 810	0	0	4	0.5	32.3	0
Production	12	1 800	0	0	4	0.25	16.3	0

Total		24700	25	2	74	6	467.8	300
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Source (Growing house data 2018-2019)

Appendix 43: Input used and yields for Treatment 2 (*Hyparrhenia hirta*).

Period of growth stage	Week	Water(L)	Spawn (kgs)	Agric Lime(Kgs)	Labour days	Jik (L)	Output Hh (Kgs)	Wet Substrate (Kgs)
Incubation	1	4 200	25	2	8	0.1	0	300
Incubation	2	2 200	0	0	8	0.3	0	0
Production	3	1 800	0	0	6	0.5	30.6	0
Production	4	1 600	0	0	6	0.5	54.5	0
Production	5	1 800	0	0	6	0.5	59.9	0
Production	6	1 850	0	0	6	0.5	44.4	0
Production	7	1 850	0	0	6	0.5	42.2	0
Production	8	1 800	0	0	8	0.5	41.3	0
Production	9	1 850	0	0	8	0.5	40.9	0
Production	10	1 840	0	0	4	0.5	39.1	0
Production	11	1 810	0	0	4	0.5	28.2	0
Production	12	1 800	0	0	4	0.3	14.9	0
Total		24 000	25	2	74	5.2	396	300

Source (Growing house data 2018-2019)

Appendix 44: Inputs used and yields for Treatment 3 (3 parts *Hyparrhenia hirta*/2 parts Maize stalk).

Period of growth stage	Week	Water	Spawn (kgs)	Agric Lime(Kgs)	Labour days	Jik (L)	Output Hh/Ms1	Wet Substrate (Kgs)
Incubation	1	4 500	25	2	8	1	0	300
Incubation	2	2 200	0	0	8	0.25	0	0

Production	3	1 800	0	0	6	0.5	50.6	0
Production	4	1 600	0	0	6	0.5	50.7	0
Production	5	1 800	0	0	6	0.5	50	0
Production	6	1 850	0	0	6	0.5	48.6	0
Production	7	1 850	0	0	6	0.5	47.5	0
Production	8	1 800	0	0	8	0.5	44.4	0
Production	9	1 850	0	0	8	0.5	31.4	0
Production	10	1 840	0	0	4	0.5	31.4	0
Production	11	1 810	0	0	4	0.5	24	0
Production	12	1 800	0	0	4	0.25	19.7	0
Total		24700	25	2	74	6	398.3	300

Source (Growing house data 2018-2019)

Appendix 45: Input used and yields for Treatment 4 (3 parts *Hyparrhenia hirta* /2parts maize stalk).

Period of growth stage	Week	Water	Spawn (kgs)	Agric Lime(Kgs)	Labour days	Jik (L)	Output Hh/Ms2	Wet Substrate (Kgs)
Incubation	1	4 500	25	2	8	1	0	300
Incubation	2	2 200	0	0	8	0.25	0	0
Production	3	1 800	0	0	6	0.5	60.9	0
Production	4	1 600	0	0	6	0.5	61.7	0
Production	5	1 800	0	0	6	0.5	60.4	0
Production	6	1 850	0	0	6	0.5	42.1	0
Production	7	1 850	0	0	6	0.5	42.1	0
Production	8	1 800	0	0	8	0.5	29.2	0
Production	9	1 850	0	0	8	0.5	39.9	0
Production	10	1 840	0	0	4	0.5	38.2	0
Production	11	1 810	0	0	4	0.5	24.4	0

Production	12	1 800	0	0	4	0.25	18.4	0
Total		24700	25	2	74	6	417.3	300

Source (Growing house data 2018-2019)

Appendix 46: Input used and yields for Treatment 5 (3 parts Hyparrhenia hirta /3 parts maize stalk).

Period of growth stage	Week	Water	Spawn (kgs)	Agric Lime(Kgs)	Labour days	Jik (L)	Output Hh/Ms3	Wet SubStrate (Kgs)
Incubation	1	4 500	25	2	8	1	0	300
Incubation	2	2 200	0	0	8	0.25	0	0
Production	3	1 800	0	0	6	0.5	96.6	0
Production	4	1 600	0	0	6	0.5	96.6	0
Production	5	1 800	0	0	6	0.5	47.3	0
Production	6	1 850	0	0	6	0.5	48.2	0
Production	7	1 850	0	0	6	0.5	47.7	0
Production	8	1 800	0	0	8	0.5	43.1	0
Production	9	1 850	0	0	8	0.5	41.8	0
Production	10	1 840	0	0	4	0.5	43.1	0
Production	11	1 810	0	0	4	0.5	25.7	0
Production	12	1 800	0	0	4	0.25	19.5	0
Total		24700	25	2	74	6	509.6	300

Source (Growing house data 2018-2019)