CHINHOYI UNIVERSITY OF TECHNOLOGY

CARBON SUBSTRATE OPTIMIZATION FOR CULTIVATION OF *PLEUROTUS OSTREATUS* AND A WILD INDIGENOUS MUSHROOM *CANTHERELLUS DENSIFOLIUS*

By

ELYSON NYASHA SILIVANI

C15126201T

A thesis submitted in Partial Fulfilment of the Requirements for the Master of Phiosophy Science degree

Supervisor: Dr N.L Chigu

Co-supervisor: Dr T Chisango

Co-supervisor: Professor K Chitindingu

DECLARATION

I Elyson Nyasha Silivani of student registration number C15126201T confirm that this work submitted for assessment is my own and is expressed in my own words. Any uses made within it of the works of other authors in any form are properly acknowledged at the point of their use. A full list of the references used has been included.

Signed: ..... **Date**: .....

Name of Supervisor: .....

Signed: ..... **Date**: .....

DECLARATION

I Elyson Nyasha Silivani of student registration number C15126201T confirm that this work submitted for assessment is my own and is expressed in my own words. Any uses made within it of the works of other author's in any form are properly acknowledged at the point of their use. A full list of the references used has been included.

Signed: ..... **Date:** 23/8/24

Name of Supervisor: T CHITSANGO T

Signed: ..... **Date:** 23/8/24

APPROVAL

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as University supervisors.

Dr. Nomathembu L Chigu,

Department of Biotechnology,

Chinhoyi University of Technology

Signature..... Date.....

Dr. Tawanda J Chisango,

Department of Biotechnology,

Chinhoyi University of Technology .

Signature..... Date 23/8/24.....

Professor K Chitindingu,

Dean, School of Health Sciences and Technology.

Chinhoyi University of Technology.

Signature..... Date.....

ACKNOWLEDGEMENTS

I would like to express my gratitude to the Almighty God for allowing me to complete my project and giving me the courage to pursue this Mphil degree. My heartfelt thanks go to my academic supervisors Doctor Chigu, Dr Chisango and Professor Chitindingu for direction in order to make this work a success. The valuable advice, corrections, modifications and improvements did enhance the perfection in completing this work. Finally, I would like to thank my family and friends for their unwavering support.

Abstract

The yield and quality of mushrooms are largely dependent on the substrate composition. Many Zimbabweans are venturing into mushroom farming with inadequate knowledge on substrate formulation for maximized yield.The yield and quality of mushrooms are largely dependent on the substrate composition. The main objective of the study was to determine the effect of different individual substrates and substrate combinations on the growth and sustainable development, perfomance during the cultivation of exotic *P.ostreatus* and wild *Cantherellus* in under constant conditions in a mushroom house. This study was carried out during the rainy seoson however these conditions do not have any implications on the results since we controlled the inernal environment of the mushroom.There is low published material related to oyster mushroom cultivation in Zimbabwe. Most of the substrates used in the literature review, highlighted substrates such as rice straw and palm kernek cake are agricutural wastes of crops not cultivated in Zimbabwe.Various substrate combinations in the ratio 1:1 used as treatments were cotton hulls -wheatstraw, cotton hulls-sawdust, cotton hulls-maizecobs, and cotton hulls-banana fronds. These substrates are commonly available in Zimbabwe. The combination producing the highest biological efficiency was used to cultivate wild indigenous *C.densifolius*. Fruition of *C.densifolius* was evaluated on hardwood sawdust and cotton hulls individually and in combination. Each combination weighed 1kg and was replicated 4 times.Compound substrates perfomed much better in terms of biological efficiency and spawn run compared to individual substrates. The highest biological efficiency (76%) and spawn run(17 days) for *P.ostreatus* were obtained from combining cotton hulls and sawdust followed by cotton hulls and maize cobs with biological efficiency of 50% and spawn run of 13 days. Consequently combining cotton hulls with a substrate that has a wide C:N ratio such as sawdust increases the biological efficiency as a result of the boost in the carbon component. Analysis in terms of economic return revealed that mushroom production was most profitable using cotton hulls and sawdust as substrate with a benefit-cost ratio of 5.7, followed by cotton hulls(4.2), cotton hulls-maizecobs(3.7), cotton hulls-banana fronds(2.4) and lastly cottonhulls-wheatstraw(2). The optimised substrate combination was then tested on the indigenous mushroom in an attempt to cultivate that wild and known to be naturally growing in the woods but instead under laboratory conditions. *C. densifolius* mycelia grew best on potato dextrose agar with a pH of 7 cultured in plates incubated in dark conditions, at room temperature (28 C), with no aeration. Mycelia of *C.densifolius* exhibited a higher rate of growth on Potato dextrose agar at a rate of 22.5mm/day obtained after 4 days, compared to Malt Extract agar that produced 12.9mm/day after 7days, to completely cover the petri plates and exhibited a tendency to change colour from white to green after 10 days of incubation. The phenomenon observed on the petri plates was deduced to be a spore print colouration. It took mycelia 6 days to completely cover *Sorghum bicolor* grains at 28^0C. Wheatstraw, banana fronds and maizecobs failed initiate fruiting. Cotton hulls produced green pinheads after 31 days of incubation. Sawdust and cotton hulls combination stimulated pinhead formation and maturation into fruiting bodies. The biological efficieny attained was 9% and the spawn run took 32 days. High yield and economic return can be obtained using sawdust and cotton hulls combination. The same combination can sustain growth and fruit formation of *C.densifolius.* We recommend use of this combination in the cultivation of

P.ostreatus. We also recommend further studies into domestication of *C.densifolius* to increse the biological efficiency of *C.densifolius*.

List of Abbreviations

C.densifolius	*Cantherellus densifolius*
P.Ostreatus	*Pleurotus ostreatus*
BE	Biological efficiency
L.kabansus	*Lactarius kabansus*
B:C ratio	Benefit:cost ratio
C:N ratio	Carbon to nitrogen ratio
ANOVA	Analysis of variance
LSD	Least significant Difference
CRD	Completely randomised design
YEA	Yeast Extract agar
MEA	Malt Extract Agar
PDA	Potato dextrose agar
TSA	Trypticase Soiy Agar
SDA	Saboraud dextrose agar
PGA	Potato Glucose Agar
NA	Nutrient Agar

TABLE OF CONTENTS

List of tables

List of figures

Chapter 3

Chapter 4

CHAPTER 1

1.1General Introduction

Mushrooms are the fleshy, spore-bearing fruiting bodies of fungi, Sitotaw *et al*,. 2020. In the wild it is identified by a fruiting body in the shape of an umbrella attached to logs, twigs and soil.Mushroom cultivation is dated by Kashanguraet al,. 2005to have started in the 18th century by farmers who cultivated mushrooms on composted animal manure beds in caves. Onyeka et al(2017) purports that the natural process of mushroom cultivation occurs on decaying remains of trees. Natural crystallisation into a white tissue of mycelium colony leads to formation of the fruiting part of the mushroom under favourable conditions of temperature, substrate availabilit and moisture.

Mushrooms are unique because they do not fit neatly into the plant or animal kingdoms. They are not capable of making their own food as compared to plants via photosynthesis. They are consumers just as animals but they do not possess structures to move from one place to another. Their main favourite food material are dead plant and animal matter, thereby classifiying them as heterotrophs.

1.1.1Classification of mushrooms based on nutrition

Generally there are two types of mushrooms based on the types of material they decompose. There are primary decomposers and secondary decomposers. Primary decomposers possess lignocellulosic enzymes that can decompose lignin and cellulose in wood and agricultural waste such as straw. (Kashangura et al 2005). In nature primary decomposers degrade logs twigs and leaves. They are characterised by fast rate of colonisation as they release lignocellulosic eenzymes that are produced from mycelium that attach on the substrate. ramify the substrate. Examples of these mushrooms are oyster(*Pleurotus*) and shiitake(*Lentinula endodes*) inncluding all wood inhabiting fungi.(Mshandete and Cuff 2008)

Secondary decomposers do not posses lignocellulosic enzymes. Instead they feed on the by-products of primary decomposers. They normally grow on composted substrate when supplemented with simple sugars.An example of secondary decomposer is the button mushroom whose scientific name is *Agaricus bisporus*.(Kashangura et al,.2005).

1.1.2Nutritional aspects of mushrooms

Mushrooms obtain food from dead plant matter. Mushrooms can be divided into three categories based on how they obtain food from plant matter. Mushroom or fungi can be saprophytic, mycorrhizial or parasitic. Saprophytic fungi include oyster mushrooms which have lignocellulosic enzymes that can degrade lignin and cellulose complexes These fungi are found in nature attached to leaves, twigs, logs and bark of tree. They can reduce dead wood to humus. The saprophytic enzymes are important ecologically for the recycling of plant carbon. (Ntezirayo et al,. 2024)

Mycorrizial mushrooms are mutualistic or symbiotic (*Cantherellus spp*). In nature they are found in close association with specific trees. It is important to note that most of our wild edible mushrooms in Zimbabwe are mycorrizial mushrooms(Kashangura et al,. 2005).Rediet et al,.2020) alludes that there are certain growth of trees can be impeded in the absence of these mushrooms due to their symbiotic circumstances. Furthermore there are some indigenous mushrooms whose existence is bound to certain types of woodland. For example the *Cantherellus* can only grow in *mitombo woodlands(Brachystegia sp).* *Mitombo* woodlands are made of (*Brachystegia spiciformis*)*msasa* and (*Milletia stuhllmannii*)*munhondo* types of trees. The symbiotic association allows the fungi to gain certain nutrients from thtree in return the tree receives certain nutrients from the fungal metabolic processing.

Another mutualistic association exists between mushrooms and animals. There is a relationship that exists between *Termitomyces* and termites. The *Termitomyces* got its name from the fact that such fungi is only found on termite mounds. It forms psedorrhizia or a long root which grows into the comb of the mound as shown in Fig 1. Our indigenous mushrooms are difficult to cultivate because most of them are mycorrhizial. It was observed by Kashanguraet al(2005) that they require a living substrate to survive. Domestication of mycorrhizial mushroom will require formulation of a substrate with similar characterics to a living substrate. In contrast *Pleurotus* species are easier to cultivate because they require a dead substrate instead of a living substrate.

Fig 1Termitomyces growing in symbiosis with termite mounds

The third group of fungi are the parasitic fungi. These ones are found on living plants and may cause the death of the plant they are attached to. They cause cancers on trees and also drying of the tree and eventually its death. Parasitic mushrooms only receive nutrients from the plants they feed on but not necessarily giving anything back to the tree. Some of these mushrooms, after killing the plant can shift to a saprophytic mode and feed on the dead plant material.(Ramsbottom 1954).

Niaz and Ghafoor(2021) state that the enzymes involved in degradation of cellulose produce extracellular enzymes that include endoglucanases, cellubiohydrolases, and glucosidases. The degradation of cellulose produces cellubiose which is later degraded to glucose. Hemicellulose is a complex carbohydrate found in plant cell walls. There are xylan based hemicellulose, mannan based hemicellulose , galactan based hemicellulose and arabinase based hemicellulose. Xylanases, Mannases, Galactanases and Arabinases degrade hemicelluloses. Mushroom fungi that degrade hemicellulose include Shiitake(*Lentinula edode*s) a wood degrading fungi. Lignin is broken down by lignin peroxidase, manganese peroxidase and laccase. Manganese peroxidase being secreted by all wood degrading basidiomycetes.(Kashanura et al 2005) Lignocellullytic fungi also release proteases, pectinases proteases and lipases. *Pleurotus* species of mushrooms contain enzymes that degrage complex biomolecules in the substrate such as proteins, lignin, hemicellulose and cellolose. The enzymes are include proteases for proteins, xylanase for hemicellulose, laccase for lignin and protease for proteins. (Mshandete and Cuff ,.2008)

1.1.3Growth and Reproduction of mushrooms

Fig

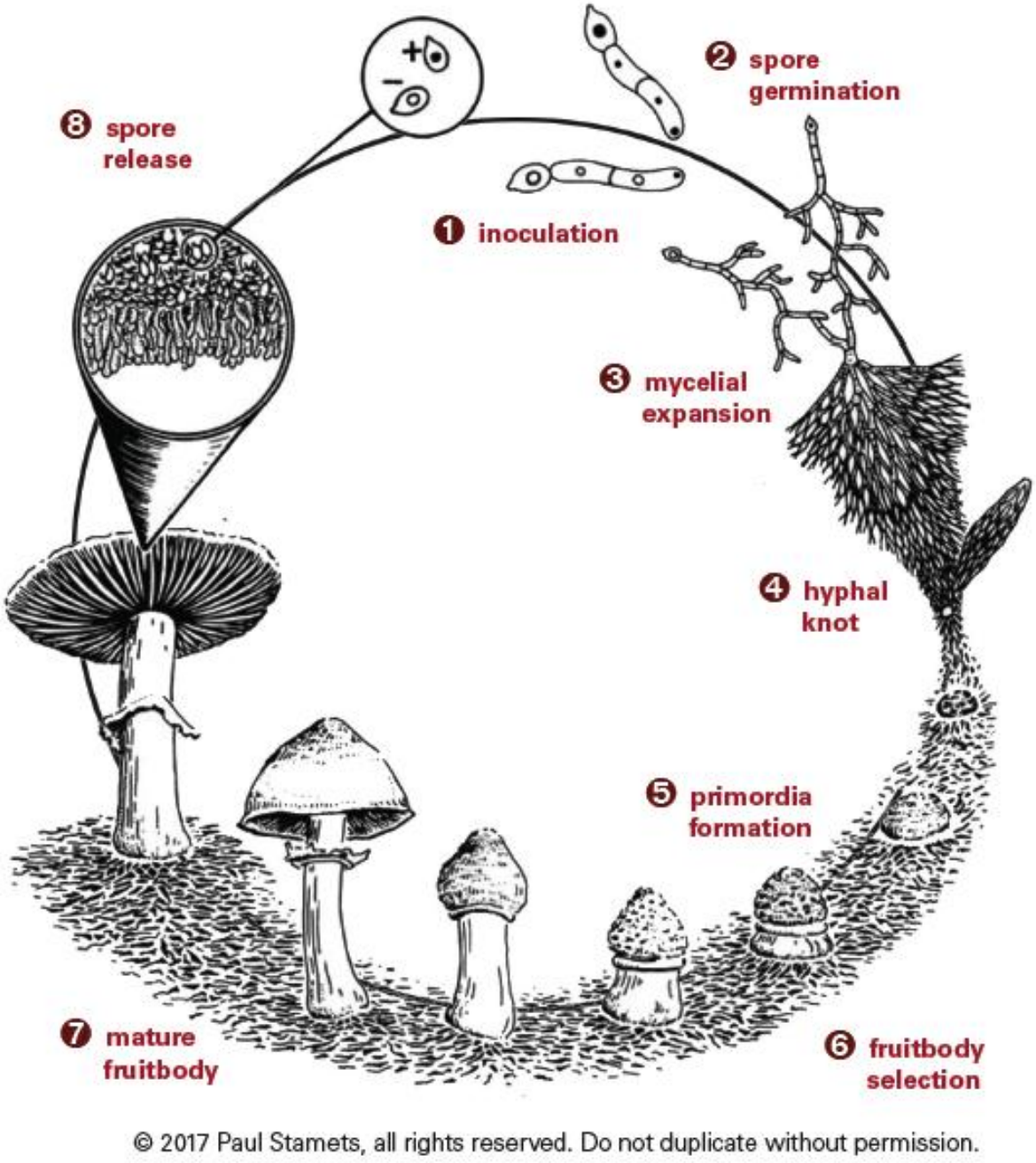

Fig 2,Reproduction cycle of mushrooms

Mushrooms are a group of fungi that obtain organic matter from dead plant matter, forming a fruiting body called the basidiocarp from its mycelium. The stem or the stipe supports the mushroom and has the same colour as the mushroom cap.The two stages in the life cycle of mushroom are the hyphae stage and the fruiting stage. (Deacon 1997)

Mushrooms reproduce from spores as shown in Fig 2. It is the spore which is the reproductive unit of the fungi. The spore can be formed sexually, asexually or vegetatively.

Mature spores are formed on the gills below the basidiocarp. When spores are dispersed they only germinate when they land on a suitable substrate. Under favourable temperature and moisture the spores germinate and send a germ tube that attaches to the substrate. The tube will then elongate and then form the hyphae. The hyphae, being a filamentous tube will divide mitotically to form other cells to form net like threads called mycelium. The mycelium is the vegetative body of the mushroom and is also responsible for its nutritional provision (Alexopoulos & Mims 1979).

Thereafter each nucleus is separated from the others by a septa so that each segment of the mycelium is uninucleate. Mycelium that has multinucleate is homokaryotic and each segment within the mycelium is monokaryotic. Since basidiomycetes species of mushrooms with homokaryotic mycelia do not fruit, two homokaryotic mycelia of different mating types must fuse. When two compatible homokaryotic mycelium meet their cell walls break down followed by nuclear migration (Chang and Hayes 1978).

Following nuclear migration, spores germinate followed by mating between compatible hyphae leading to the formation of dikaryotic mycelia. Mycelia continue to grow up to a time when the growth rate ceases due to decline in heat and carbon dioxide. Formation of fruiting bodies is triggered by a drop in temperature and carbon dioxide together with a rise in humidity. Prevailing moisture conditions cause enlargement of fruiting bodies which then differentiate into the defined structures comprising the cap, stipe and gills. The gills are found underneath the cap, the cap in turn is supported by the stipe. The gills contain the hymenium which is a layer of fertile cells bearing basidium. The basidium develops from arms called sterigmata and the apices to give four basidiospores.From the four basidiospore each basidiospore has a haploid nucleus. Only mature spores become pigmented and are suitable for dispersal by wind,water and animals. If the spore lands on favourable substrate and the climatic conditions are optimum the cycle will repeat again (Kashangura et al 2005).

1.2Challenges in cultivation of mushrooms

1.2.1Physical factors

The conditions required for mycelia growth of commercial mushrooms are known. However, in the case of wild indigenous mushrooms the factors affecting the commencement of the fruiting stage of mushroom are unclear especially. This may be due to the impossibility of producing sporophores in solid cultures.

1.2.2Humidity provision

The enzymes secreted by mushroom fungi work best in an aqueous environment as they are activated by availability of moisture. Optimum humidity during fruiting is 90%. (Kashangura et al 2005). Moisture is important in facilitating enzymatic reactions. The reactions result in the breaking down of cellulose, hemicellulose and lignin into simple sugars, peptides and simple amino acids. These nutrients in the substrate can only be accessed by the fungi when they are in solution. Furthermore, in the absence of water the enzymes cannot function, the mycelia starves and then dessicates and dries out as explained by Siwulski et al ,.2024. In mushroom cultivation the mushroom growth house should be well watered especially at the fruiting stage. This moisture also aids in the regulation of temperature. High humidity above 90% for oyster will limit air exchange and increase the incidence of bacterial blotch

1.2.3Light requirements

Light is essential for fruiting. Verma and Bilgrami(2011) observed and recorded that green light (500 to 600nm) wavelength is essential for the induction of primordial formation in oyster mushroom cultivation. Light illuminating the substrate leads to the formation of reactive oxygen species which being toxic to oyster mycelium inhibits the hyphae stage thereby stimulating the formation of fruiting bodies by changing the activity of catalase. Mushrooms such as oyster exhibit a phototropic response which is the growth towards light. Most mushrooms prefer low lighy intensity.(Moore 1998)

1.2.4Aeration and Carbon dioxide levels

Carbon dioxide is vital for mycelium development. Mycelium branches vegetatively in the presence of carbon dioxide. During the spawn run 5000-10000ppm , fruit body formation 1000-5000 ppm and mushroom maturation 500-2000ppm(Webster & Weber 2007). Different types of mushroom can tolerate different tension levels of carbon dioxide. Oyster can tolerate up to 28 % and button below 2%.(Parmar 2022) For wild indigenous mushroom the tolerance level is unknown. .Excessive CO2 levels will encourage bacterial growth and lead to contamination.

1.2.5Temperature for growth

General temperature for spore production is 25 to 28^{o}C.(Tovell 2021) Initiation of basidiospores is inhibited at 7 to 10^{o}C but this varies with species (Anupam et al 2020). Low

temperatures affect nucleic acid synthesis in mycelia which in turn inhibits reproduction. *Agaricus bisporus* has 23 to 25 ^{o}C as optimum for growth whilst 14 to 18 ^{0}C is optimum for fruiting (Vijay 2022)). However for wild indigenous mushrooms the temperatures are unknown (Dulay et al 2020). On oyster mushrooms temperatures above 35^{0}C will delay fruiting, and if fruiting occurs the caps will be folded. Temperatures below 20^{0}C will delay spawn run.

1.3Mushrooms under investigation

1.3.1 Pleurotus ostreatus

Asmamawet al(2014) alludes that the word Pleuro from *Pleurotus ostreatus*(oyster) means lateral . The stem of mushroom is lateral in relation to the cap. Oyster mushrooms have caps that vary in colour from gray to brown. It is under these fruiting body caps where we find gills that bear fungal spores. The spores are dispersed when the mushroom dries out. They are often dispersed by the wind, however the rain and termites also assist in the dispersal of spores. Once dispersed they act as the mushroom seed.

Pleurotus ostreatus is a member of *Pleurotus* species of mushrooms cultivated globally for food. According to Besufekud et al (2019), oyster mushrooms are the second largest commercially produced mushrooms in the world. They possess a rare capability to convert lignocellulosic waste residues into edible fruiting bodies, rich in nutrients. Oyster mushrooms are reported by Ramsbottom (1954) to contain high amounts of vitamins, particularly vitamin D and high protein contents because they contain enzymatic proteases that can breakdown complex macromolecules in agricultural wastes into individual amino acids. Of much importance is the absence of fatty acids in oyster mushrooms, which makes them ideal for persons willing to maintain a low fat diet. Furthermore, oyster mushrooms contain the appropriate ratios of potassium and sodium, which are essential in reducing heart disease.

Pleurotus ostreatus is a signature mushroom, the easiest mushroom to cultivate by anyone who desires to venture into mushroom cultivation. It has a wide range of substrates that can be used as a source of its nutrition. However *Pleurotus ostreatus* is an exotic mushroom therefore there is need for countries like Zimbabwe to introduce cultivation strategies for indigenous mushrooms inorder to boost our gross domestic product.

Promoting the cultivation of local mushrooms supports sustainable agriculture, enhances food security, preserves cultural heritage, and contributes to the resilience of both ecosystems and communities.

Promoting local mushrooms over cheaper and easier-to-grow foreign varieties may require more initial effort and investment, but it offers significant long-term benefits in terms of sustainability, cultural preservation, and economic resilience.

1.3.2 Wild Indigenous mushrooms

Fig 3Types of wild indigenous mushrooms in Zimbabwe, A-*Cantharellus cinnabarimus*(tsvuke tsvuke),B-*Cantharellus meombionsis*-Chihombiro, C-*Amanita zambiana*(nhedzi),D-*Termitomycetes spp*(huvhe,uzutwe)

Wild indegenous mushrooms of Zimbabwe include *Amanita zambiana, Termitomyces, Cantharellus cinnabarius* and *Cantherellus meombionsis* as shown in Fig 2. In this study we focused on *Cantharellus densifolius*. Most of them remain in the wild occuring as a fruiting body during the short rainy season. These mushrooms face threats of extinction due to deforestation of land for farming and urbanisation of forests (Kavhumbura 2022) Therefore there is need to domesticate them to ensure their preservation.

1.3.2.1 *Cantharellus densifolius*

*Cantherellus densifolius*now referred to as *Lactarius kabansus* (Pearce & Sharp,.2000) is a wild edible mushroom commonly found growing on decaying leaves of trees and grass during the rainy season. Prevailing temperatures and moisture conditions during the short rainy season favors the growth of *C.densifolius*. The Zimbabwean rainy season which starts in November and ends in March favors the growth of a wide range of wild mushrooms that naturally grow on forest litter, fallen logs, lawns, gardens and piles of agro-industrial residues. In Zimbabwe *C.densifolius* growth is most prevalent during the onset of the middle and the end of the rainy season when enough moisture is available for spore germination, mycelial growth and fruit body formation. It is commonly referred to in the common language as *nzevedzambuya* meaning ears of an old lady. This name might also have been coined as a reference to the outline of the cap which is rounded in the shape of an ear and the mushroom cap has a brownish appearance which is the natural skin colour of the natives in Zimbabwe. Furthermore the underside of the cap is densely packed with brown gills hence the name *densifoliu*s. In Zambia they refer to the mushroom as kabansa hence the name *Lactarius kabansus*.The inside of the cap is brown and very soft. The outside of the cap measures 2-8cm in diameter , thesurface is blackish brown. The gills are originally white and turn green after picking. The stipe measures from 2 to 4 cm. The smell is weak, pleasant and fruity. When tasted raw they have a mild taste. The inside of the cap is brown and very soft. The mushroom is very soft and the cap is easily broken. This is exhibited in the streets where mushroom gatherers sell the mushrooms. The mushrooms are rarely compact and being fragile the cap easily breaks. This is mainly due to the accumulation of water and failure of the lignin cell wall to support the mushroom (Norrilinia 2023).It is classified in the kingdom fungi, phyllum *Basidiomycota, Agaricomycetes* class, order *Cantharellus* , family *Cantharellaceae* and Genus *Cantherellus.(*Arora 1986)

1.4Cultivation of mushrooms

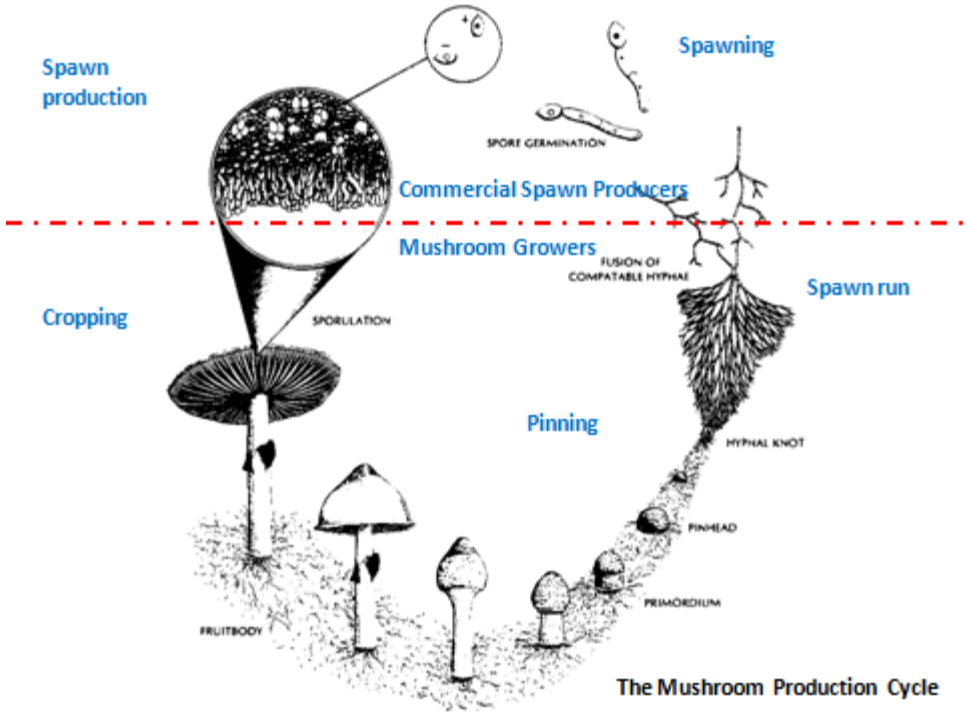

Fig 4:The mushroom production cycle

Mushrooms can be cultivated in closed dark rooms for food. Historically the Chinese were the first to cultivate mushrooms in caves. Europeans then devised methods to artificially produce tissue cultures from fungal spores leading to improved technological methods of cultivating mushrooms. The most important requirement being spawn and substrate, other requirements is a controlled environment. Fig 4 is an outline of mushroom production process from spawn production to nushroom body production.

1.4.1 Spawn Production

This is the seed of the mushroom. The production of spawn begins with aseptic isolation of fungal spores from inner tissues of the mushroom cap followed by placement into mycological media such as PDA or MEA as shown in Fig 4. Once the media is fully covered with fibrous strands of mycelia the mycelia is then aseptically transferred to grains of wheat or sorghum. The grains provide a sustained source o nutrition for the mcelia. They ensure food availability for mycelia during acllimatisation onto substrate. When grain spawn is used as a vehicle to transfer mycelia to substrate the mycelia needs time to acclimatise to the new substrate. During this time, the grains provide a sustained source of nutrition.

Fig 5.Transfer of mycelia from petri plates to grains

1.4.2 Mushroom Substrate types

Mushroom substrate is the material or substance, which provides food for the growing mycelium. Oyster mushrooms can grow on a wide range of substrates compared to other substrates. They can grow on all wood types including sawdust, paper pulp and sludge. They also grow on all types of cereal straw such as maize straw, wheatstraw and rice straw. They also grow well on other types of agricultural substrates such as corncobs, banana fronds, maize stalks and leaves, savanna veld grass, sage grass, cottonseed hulls and coffee grounds.The mechanism by which substrate is broken down is by the extracellular release of enzymes.According to Onyeka **et al** (2017) the extracellular enzymes secreted by fungi contain amorphous homo and heteropolysaccharides often associated with fungal protein. These enzymes bind to cellulose, lignin and hemicellulose, and break them down into simple sugars and peptides. These enzymes have active binding sites or catalytic domains that break down glycodidic bonds between sugar units. The glucose and maltose are used for respiration during the spawn run. At the fruiting stage amino acids are used as buiding blocks for proteins in the fruiting body.

1.5 Problem statement

The key problem with mushroom cultivation is substrate. Substrate is the main factor under analysis in terms of availability, cost, nutrition and yield.

1.5.1Agricultural waste disposal

Agricultural waste disposal represents a critical problem to farmers and environmentalists. Most rural farmes are oblivious to the fact that the waste left from farming can be used to earn extra income. Tesfaw et al(2015) stresses that a vast majority of lignocellulosic agricultural waste is often either left to rot in the field, or fed to livestock, or used as manure or disposed of by burning. Maize ,tobbacco and sugarcane collectively produce 2-3million tons of waste annually. 30-50% of crop residure(600000 to 1.5 million tons) are disposed of by burning in Zimbabwe annually (Zimbabwe Ministry of Agriculture, Mechanisation and Irrigation Development, 2016). The rest is either used as manure or as drought feedstock for livestock.

The intentional burning of agricultural waste after harvesting is a major factor contributing to global warming and the greenhouse effect.Agricultural wastes have high content of hemicellulose, cellulose and lignin which can be fed on fungi producing mushrooms instead of being disposed of by burning.

Agricultural waste has become a source of contamination.The plant process of photosynthesis produces 200 billion tons per year of organic matter, worldwide (Zimmer 2018). The majority of this matter is not directly edible to animals and herbivorous animals. Humans and animals consume the carbon in the form of polysaccharides and simple sugars leaving behind the lignin, cellulose and hemicellulose. This remaining lignin, cellulose and hemicellulose becomes a contamination source of the environment (Wendiro et al 2019).

1.5.2 Substrate

Despite many farmers venturing into mushroom farming there are still lack of enough knowledge for new farmers as to which substrate to use or which combination of substrates works best. Nutritional composition of oyster mushroom is limited when only one substrate is used to grow mushrooms (Hoa et al 2015). A formulation of substrates might contribute to better nutritional souce for mushroom cultivation. Most mushroom farmers in Zimbabwe are using one type of substrate mostly cotton hulls to grow oyster mushroom. As more people are

becoming knowledgeable about cotton hulls a demand for this commodity has been created. In 2023 a kilogram of cotton hulls was going for 14cents per kilogram, this year 2024 the price has more than doubled to 39cents per kilogram (Tapiwa 2021) The end result is an increase in demand for cotton hulls leading to a sharp increase in the price.It then becomes economically expensive to venture into mushroom farming due to expensive substrate.

There is an increase in the price of the mushrooms. In addition to increases in the cost of substrate there are also transport costs. The increase in price can also be attributed to transportation of cotton hulls from Harare to areas further away such as Matebelelend and Masvingo.

1.5.3 Problem Statement regarding Wild indigenous mushrooms

According to Wendiro et al (2019)the amount of indigenous mushrooms being gathered annually is declining due to deforestation. Indigenous mushrooms are disappearing at an alarming rate despite their popularity, culinary, financial appeal and primary dietary role. It is important to conserve indigenous trees in close symbiosis with indigenous mushrooms such as *Cantherellus*, *Amanita* and *Lactarius* species because most of them have an obligate symbiosis with indigenous trees.

Wild mushrooms are only available during the rainy season which is very short. An efficient protocol and substrate formulation needs to be developed for maintaining a consistent availability of wild mushrooms even after the rainy season. Dulayet al(2017) alludes that widespread malnutrition as a result of drought and overpopulation in third world countries has necessitated the search for alternative sources of protein.

Little or no knowledge is available on the growth requirements of wild mushrooms in particular the substrate requirement. Widespread mushroom cultivation of wild mushrooms is hinged on good knowledge of growth requirements and the influence of the substrate on the growth rate and nutrient composition.

The increase in demand for edible mushrooms has resulted in various mushroom training programs and setting up of many mushroom growth houses in urban areas of Zimbabwe. However, provision of quality spawn that is resistant to pests and diseases remains a huge constraint. Furthermore, mushroom spawn is not readily available in agricultural shops and supermarkets. In addition, these exotic varieties require substrates with high nutrition such as cotton husks which are difficult to procure and transport. This reduces the total annual

production of mushrooms in Zimbabwe. Rural communities end up relying on wild edible species that are only available in the rainy season, which unfortunately lasts for only 3 to 4 months.

The seasonality of wild edible mushrooms disqualifies them as a reliable source of nutrition. Furthermore lack of clear cut identification criteria and limited information on their genetic diversity results in unfortunate deaths of the ignorant. This is because there is no true distinction between poisonous and non-poisonous wild mushrooms. As a result this draws back efforts to fully exploit wild edible mushroom for commercial production and breeding purposes.

There is a gap in research regarding to which substrates this fungi prefers. These mushrooms has never been cultivated for the production of fruiting bodies. The nutritional composition of the fruiting body has not yet been investigated. Therefore this study was conducted to establish the optimum conditions for mycelia growth of *C. densifolius* and ultimately evaluate fruiting body on cotton hull and sawdust based substrate formulation.

Apparently there are very few studies in Zimbabwe focusing on the domestication of wild edible mushrooms in Zimbabwe. Inorder to domesticate wild edible mushrooms such as *C.densifolius* there is need to seek knowledge on the physiological events in relation to their growth morphology as a prerequisite to developing large scale economic processes for their production.

Despite the huge potential in wild mushrooms all potentially cultivated mushrooms with potential nutraceuticals, remain in the wild. Wild indigenous mushrooms are direct sources of protein, vitamins and essential mineral elements to support health of human beings (Dulay et al 2012) Assimilation of these mushrooms by innovative production technologies will ensure food security, promote environmental protection and sustain livelihoods. It is therefore necessary to continuously come up with innovative ways of cultivating wild mushrooms to ensure conservation of wild genetic resources.

A prerequisite of domestication is a full understanding of the optimum conditions in terms of nutrition and physical parameters. This will then lead to the establishment of a successful production technology. This research investigates optimum growth conditions of *C.densifolius* and determines the nutritional and physical requirements for mycelia growth and the production of fruiting bodies.

Collection and identification of wild mushrooms is not enough to ensure their conservation. This study seeks to investigate possibility of cultivation and evaluation of growth parameters of a Zimbabwean wild strain of *Cantherellus*. Substrates used to investigate growth including cotton hulls and sawdust.

1.6Substrate and combinations

Oyster mushroom mycelial growth is optimum in the presence of more carbon compared to nitrogen(Besufekud et al 2019). Therefore, substrates comprised primarily of cellulose, hemicellulose and lignin are good candidates for oyster mushroom cultivation(Bhattacharjya 2015). In this study, we will investigate cotton hulls, sawdust, wheatstraw, banana fronds and maize cobs individually and in combination to determine potential in optimisation of *Pleurotus ostreatus* growth and domestication of *Cantherellus densifolius*. A parallel investigation into the potential capability of growing on cotton hulls and sawdust as sole carbon source and as combined was investigated. The characteristic features and C: N ratios of the substrates under investigation below are indicated in the table below

Table 1:Carbon to nitrogen ratios of the substrates selected for investigation in this study

Substrate	Characteristic	C:N ratio	References
Banana fronds	Dried leaves from banana. Brown	70:1	ScienceMate 2022
Maize cobs	Cobs from maize. Cob highly dense.	80:1	Lentz and Lindsey 2016
Cotton seed hulls	Waste product resulting from the pressing and crushing down of sottonseed into cottonseed cooking oil.	100:1	Haziran 2013
Sawdust	Wood shavings from different wood typese.g oak, teak e.t.c	500:1	Rynk 1992

1.7Hypothesis

- Hypothesis 1

- H_0 : Substrate formulations designed for optimum *Pleurotus ostreatu and C.densifolius s* mushrooms have no effect on productivity levels set by individual substrates.

- H_1 : Substrate formulations designed for optimum *Pleurotus ostreatu and C.densifolius s* mushrooms can exceed productivity levels set by individual substrates.

- Hypothesis 2

- H_0 :Systematic cultivation of Cantherellus densifolius cannot be established.

- H_1 : Systematic cultivation of Cantherellus densifolius can be established

1.8Research questions

- Can pure cultures be developed from wild edible *Cantherellus densifolius* mushroom and propagation spawns developed?

- Can local agricultural waste residues support mycelial growth and fruiting body formation of wild *Cantherellus densifolius?*

- Can combinations of substrates increase biological efficiency and shorter spawn for optimum growth of *Pleurotus ostreatus?*

- Is there a significant increase in profitability(B:C ratio) by combining different substrates?

1.9 Objectives

1.9.1 Main objective

- To establish substrate formulations for increased, efficient and profitable *Pleurotus ostreatus* and *Cantharellus densifolius* mushroom production.

1.9.2 Secondary objectives

- To reduce the spawn run of cotton hulls by 5 days by bulking it with other substrates

- To increase the biological efficiency of cotton hulls by 10% by bulking it with other substrates

- To increase the benefit of cultivating P.ostreatus on cotton hulls by 50% through diluting cotton hulls with inexpensive substrates.
- To develop a system for producing indigenous *Cantharellus densifolius* mushroom spawn.
- .To develop a system for cultivating indigenous *Cantharellus densifolius* on locally available agricultural wastes.
- To improve storage and pasteurisation of substrates by 20% though pelletisation..

1.10 Significance of the study

1.10.1 *Pleurotus ostreatus*

Combinations of substrate will decrease pressure on limited substrates on high demand (cotton hulls and wheatstraw) for the cultivation of exotic *Pleurotus ostreatus*. This will ultimately result in the value addition of other agricultural wastes which will either be used as bulking agents or dilution in combinations. The end result is reduced price of the oyster mushrooms making them affordable to the consumers.

Combinations of substrate will ensure increased oyster mushroom yield. Mixing substrate will lay the foundation for a diverse nutrient profile enhancing mycelial growth. Substrates such as wheatstraw and banana fronds have low water retention. Mixing them with cotton hulls will optimise moisture levels.

The increase in demand for oyster mushrooms as an alternative source of protein will be met by mixing substrates. There will be reduction in the cost of expensive substrate brought about by dilution with inexpensive substrates. This will in turn lead to lessened production costs leading to increased productivity. Hence, demand for oyster mushrooms will be met and the mushroom industry will become profitable.

1.10.2 Wild Indigenous mushrooms

This study will ensure achievement of National Development Strategy 1(NDS-1) that proposes utilisation of idle natural resources. Indigenous mushrooms and Agricultural waste are idle natural resources that can be harnessed. The potential can be used to feed the nation reduce poverty and ultimately increase our Gross Domestic Product.(Government of Zimbabwe (2021)

Characterisation will contribute to addition of indigenous edible mushrooms knowledge in Zimbabwe, also provide information on the morphogenetic characteristics which is important in selecting suitable strains for breeding purposes. Spawn propagation techniques will provide data on propagation feasibility.

Domestication of wild mushrooms will allow for value addition of agricultural waste and achievement of food security. (Sharareh and Hamid 2016). Therefore, the bioconversion potential of wild *C.densifolius* will positively impact the mushroom industry. To sustain production of native wild mushrooms at a commercial level there is need to develop a cultivation process considering substrate management, economic and cutural characteristics of the local population. (Shweta et al 2021)

This study will add to the body of knowledge pertaining to the acquisition of new strains of mushrooms from the wild. Wild strains are disease resistant and high yielding. Successful domestication of *C.densifolius* will provide the foundation for domesticating other types of indigenous mushroms such as *Amanita zmbiana* and *Termitomyces*.

Domestication of wild indigenous mushroom will lessen ecosystems degradation. The natural ecosystem in the wild is disturbed every time mushroom gatherers forage for wild mushrooms during the rainy season. This affects the natural reproduction cycle of the mushrooms. Therefore domestication ensures preservation by supplying the mushroooms through cultivation, reducing the need to go into the wild to forage for mushrooms.

Chapter 2

Article 1

Developments in substrate formulation for Pleurotus ostreatus cultivation; a review of current substrates, different combinations, and optimization.

Silivani Elyson[1]*, Chigu Nomathembu Loyce[2], Chisango Tawanda, Chitindingu Kudakwashe

[1]Chinhoyi University of Technology, School of Health Sciences and Technology, Department of Biotechnology, P. Bag 7724, Chinhoyi, Zimbabwe

*Corresponding Author:elysonsilivani@gmail.com

Abstract

Substrate composition is key componet for successful oyster mushroom cultivation. The cost and quality of each substrate varies according to availability, downstream processing and storage. The high cost of substrate per kilogram creates a domino effect leading to a high cost per kilogram of mushroom product therefrom. The methodology for this review focused on published literature information from 2015 up to December 2021 primarily reflecting on substrate for mushroom cultivation that is available in Zimbabwe. The major substrates identified and recorded were, cotton seed hulls, wheat straw, maizecobs, banana fronds and sawdust Research gaps identified were on the spawn run, biological efficiency, number of flushes and the cost-benefit ratio. The best substrate formulation gives shorter rates of spawn run, a higher cost benefit ratio(3.498)(Dubey et al 2019), larger mushroom caps and higher biological efficiency(97.9%)[2].A good substrate combination should not only give the best spawn and biological efficiency but also minimize the cost of production and maximize yield per kilogram of substrate. Most published papers on substrate combination consider the spawn run and the biological efficiency but there is a gap on the cost benefit ratio and the number of flushes. Different types of substrates are compared individually and in combinations to determine which combination gives the best result. This study will assist mushroom farmers in selecting the best substrate combination for process optimization of mushroom cultivation at a very low cost thereby ensuring food security.

Keywords: Substrate, supplementation, formulation, mushrooms, *Pleurotus ostreatus*, biological efficiency.

Introduction

Mushrooms obtain energy from dead plant matter. Mushrooms can be saprophytic, mycorrhizial or parasitic based on how they obtain food (Kashangura et al 2005). Saprophytic fungi include oyster mushrooms, which have lignocellulosic enzymes that can degrade lignin and cellulose complexes (Bertelsen 2013) In nature, we find fungi attached to dead leaves, twigs, logs and bark of trees (Stamets 2018)They can reduce dead wood to humus. They contain saprophytic enzymes that are important ecologically for the recycling of carbon from plants(Dubey et al 2019).

The three key ingredients to sustainable mushroom agriculture are cheap substrate, high yielding substrate and good quality spawn. Mushroom substrate is the material or substance that provides food for the growing mycelium. Oyster mushrooms can grow on a wide range of substrates from wood to cereal crop waste. They can grow on all wood types such as wood shavings, sawdust and paper pulp sludge (Hoa et al 2015). They also grow on all types of cereal straw such as maize straw, wheatstraw and rice straw (Tesfaw et al,.2015)(Girmay et a,.2016). They also grow well on other types of agricultural substrates such as corncobs, banana fronds, maize stalks and leaves, savannah veld grass, sage grass, cottonseed hulls and coffee grounds. Extracellular release of enzymes aids in the breaking down of the substrate (Chukwurah et al 2013).

Agricultural waste disposal represents a critical problem to farmers and environmentalists. Most rural farmers are oblivious to the fact that the waste left from agriculture is an extra source of income.(FAO 2016). Most farmers ignorantly dispose agricultural waste by burning whilst others leave the waste to rot in the field. Despite many farmers venturing into mushroom farming there are still lack of enough knowledge as to substrate selection. Furthermore, the farmers who know mushroom farming do not know which combination of substrates works best(Kashangura et al 2005).

Little or no knowledge is available on the growth requirements of wild mushrooms in particular the substrate (Mwafaida et al (2022). Widespread mushroom cultivation of wild mushrooms is hinged on good knowledge of growth requirements and the influence of the substrate on the growth rate and nutrient composition. In Zimbabwe, wild mushrooms are only available during the short rainy season. An efficient protocol and substrate formulation, for maintaining a consistent availability of wild mushrooms post rainy season, has to be developed(Vieira and Meire 2016).

Different dead plant matter has different composition of nutrition. A number of factors influence the selection of a particular substrate. These are nutrition, cost, availability, accessibility and storage. A single particular substrate may be readily available but costly to the farmer. For example, cotton hulls are highly nutritious and readily available throughout the year but they are costly to buy and transport (Haziran 2013). On the other hand, maize stover and maize cobs are easily accessible, cheap but they lack nutrition (Lentz and Lindsey 2016). Banana fronds are cheap, easy to store and transport because they are lighter but they lack nutrition and require downstream processing to reduce the surface area. Nutritional

composition of oyster mushroom is limited when cultivated on individual substrate (ScienceMate (2022). A formulation of substrates will contribute to better nutrition in mushrooms(Nararian et al 2016)

(Sayner 2019) grew mushrooms on a combination of coffee grounds and wheatstraw. One, two, or three substrates are brought together in different concentrations to produce a compressed pellet. The advantage is that pellets do not need a lot of pasteurization since they are pasteurized in the extrusion process.

There is limited knowledge as to which combination of substrate works best to improve the growth and nutrition of oyster mushrooms. (Onyeka et al 2017) argues that the yield and quality of oyster mushrooms is largely dependent on the chemical and nutritional content of the substrates. This study will add to the current body of knowledge of the best combinations of substrates formulated to achieve optimum mushroom growth. Supplementation provides what is lacking in one substrate by another substrate. (Callow 2020) Cultivated chestnut mushrooms with sawdust supplemented with 35% soy hulls. The sawdust act as a carbon source whilst the soy hulls act as a nitrogen source.Furthermore a suitable nitrogen ratio helps to produce an optimum mushroom yield.*Pleurotus* species of mushrooms show much diversity in yield potential depending on the substrates nutrient quality. The best formulation requires the establishment of a consensus of different substrates.

Problems with substrate

Downstream processing of compacted substrate. Some substrates accumulate more dust particles compared to others. Some substrates require chopping down into smaller particles to increase their surface area. Wheatstraw, banana fronds and maize cobs need chopping down first to increase surface area for mycelia to easily access nutrients. Sawdust, cotton hulls, sunflower cake, sugarcane bagasse and soybean meal being highly nutritious are prone to contamination. They readily attract flies, mites and aphids, which in turn pass on bacteria and fungi to the substrate. They require stronger chemicals and heat for complete pasteurisation(Kashangura et al 2005).

Seasonal availability of individual substrates is another problem. In subtropical regions such as Zimbabwe, wheat is cultivated in winter; therefore, dry wheatstraw is available 5 months after the wheat farmers have harvested their wheat. Fresh dry wheatstraw gives the best yield of oyster compared to wheatstraw that has been stored for months. There are also challenges with storing mushroom substrates for long periods. They will be prone to attack by termites. There is need to create a system of supplementation which closes the gaps of waiting for substrate. The period after wheat harvest the prices of wheatstraw increase sharply due to the increase in demand. The same goes for maize cobs, cotton hulls and soybean leaves(Govera 2020).

Cost of individual substrates. Cotton hulls being more expensive than other substrates determine the cost of every kilogram of mushroom in countries like Zimbabwe. Supplementation with another cheaper substrate would reduce the cost of mushrooms per kilogram whilst maintaining productivity and nutritional composition(Tapiwa 2021).

Nutritional differences and longevity of individual substrates also affect mushroom production. Sawdust and cotton hulls afford a sustained nutrition to mushroom mycelia for a longer period compared to other types of substrates. There are more flushes per kilogram of cotton hulls and sawdust compared to wheat straw and sugarcane bagasse. It has been observed that substrates containing a higher percentage of lignin and cellulose afford oyster mushroom a larger number of flushes compared to substrates witha lower proportion of lignin and cellulose. A compromise by combining cheaper wheatstraw and expensive cotton hulls afford mushrooms sustained nutrition and increased benefit to the farmer.(Lentz and Lindsey 2016).

Factors under analysis

Benefit Cost Ratio

B-C ratio is defined as the ratio of benefit from selling output in monetary terms relative to the total cost during production (Dubey et al 2019). As the value of B:C increases so, does the feasibility of mushroom production in economical aspect. It is the then quotient of the yield of mushrooms by the sum total expenditure of raw materials and substrate. As the B-C ratio falls below 1 the less feasible the project.

Carbon to Nitrogen ratio

C/N ratio is the proportion of carbon to nitrogen in a substrate. According to (Kashangura et al 2005) for mycelial growth and fruiting body formation the carbon to nitrogen ratio should be 1:50,1:100 or 1:500`. Most agricultural wastes such as corn cob, grain straws, sawdust and banana fronds have sufficient C/N ratios to support saprophytic mushroom growth. The C/N ratio directly affects the mushroom spawn run, yield and biological efficiency. For example, the carbon to nitrogen ratio for sawdust is 51.71(Kavhitha et al,.2018), for sugarcane bagasse is 45.83 (Patel et al,.2020) and for corncobs is 34.57 (Zhang et al,.2020). Ratios of 50:50 sawdust and sugarcane bargasse give a C/N ratio of 46.67, and 50:50 sawdust and corncobs is 42.55. Ratios of 80:20 sawdust and sugarcane bagasse give a ratio of 46.68 whereas 80:20 sawdust and corncobs give a C/N atio of 49.05. Therefore an increase in corncobs and sugarcane bargasse in the combined substrates decreases the C:N ratio(Hoa et al 2015).

Biological efficiency

Biological efficiency is defined as the percentage measurement of fresh mushrooms relative to the dry weight of substrate(Pant et al 2006).In theory a 100% biological efficiency is achieved when fresh mushrooms having a moisture content of 90% are harvested from a substrate with a moisture content of 75% of which 25% of dry substrate gives us the mushrooms.

$$\text{Biological Efficiency} = \frac{\text{Fresh weight of mushrooms}}{\text{Initial dry weight of substrate/M}}$$

The mathematical relationship between the biological efficiency and the dry mass of substrate is an inverse proportion. Biological efficiency decreases as the initial dry mass of substrate

increases, this means the more substrate required to produce more mushrooms the lower the bological efficiency. Provided the the mass of substrate increases but mass of mushrooms remains constant, the substrate has a low biological efficiency.

The mathetical relationship between the biological efficiency and the fresh weight of mushrooms is a direct proportion. Biological efficiency increases with the fresh weight of mushrooms. Provided the fresh weight of mushrooms increases but mass of substrate remains constant, the substrate has a high biological efficiency.

A 100% biological efficiency is achievable when a mushroom farmer collects 1 kgs of mushrooms from one harvest after cultivating on 1kgs of dry substrate. Other internal factors such as temperature and humidity, affect biological efficiency (Stamets 2018). A biological efficiency above 75% will be economical for the mushroom farmer. This is only achievable by using a high spawn rate (Kashangura et al,.2005). Usually the first, second and third flushes are the best flushes, with each flush decreasing in yield form the first (Bertelsen 2013). For subsistence, farming households can allow the mushroom bags to fruit until the substrate is spent. For commercial farming only three flushes are economical,

Spawn run

Spawn run is the period it taken by a bag of the substrate to be fully colonized by mycelia. For oyster mushrooms, white fibrous mycelia completely cover the whole bag. The most important factor affecting the spawn run is the substrate composition followed by temperature and pH. The nutritional composition and the compactness of the substrate also contribute to a faster spawn run. Substrates with the appropriate C/N ratio and proper aeration allow faster spawn run compared to powdered substrates with limited air exchanges. For example data recorded by (Hoa et al 2015) revealed spawn run on corncobs took 40 days, sugarcane bagasse 32 days and sawdust 30 days. For combinations of substrates 50:50 sawdust and sugarcane bagasse gave a spawning run of 32 days, sawdust and corncobs 35 days. Combinations of 80:20 sawdust and sugarcane bagasse gave a spawn run of 33 days whereas 80:20 sawdust and corncobs took 33 days a for spawn run.

Number of flushes

The number of flushes per bag depends on nutrient composition of a substrate. Economically viable mushroom cultivation hinges on provision of sustained nutrition. (Wang et al,.2018) cultivated oyster mushrooms on corncobs and concluded that the number of flushes substrate quality, strain selection, environmental conditions and cultivation methods. (Sharma et al,.2020) observed oyster mushrooms cultivated on different substrates and concluded that there are 7-14 days inbetween flushes. (Chang et al 2017) observed growth of different strains of oyster mushrooms and concluded that the maximum number of economical flushes is five. If a particular combination of substrate cannot supply sustained supply of nutrients, the number of viable flushes is reduced. (Hoa et al 2015) accounted for the number of flushes per bag of substrate and provided the yield of mushrooms per bag of mushrooms as shown in the table 1:

T**able 1:Substrates combinations, and corresponding flushes (Hoa et al 2015).**

Substrate formula	First flush(g)	Second flush(g)	Third flush(g)	Fourth flush(g)	Fifth flush(g)	Sixth flush(g)	Total yield(g)	BE
Sawdust	70	53	39	30	22	17	232	46
Sugarcane Bargasse	76	61	45	35	23	16	257	65
Corncobs	75	60	45	32	20	17	250	58
50:50 SD:SB	75	50	38	31	25	15	235	52
80:20SD:SB	77	56	45	37	31	23	270	66
50:50SD:SC	76	62	43	36	23	16	258	58
80:20SD:CC	71	54	39	28	22	22	233	48

In Table 1 six flushes for oyster mushrooms were recorded. There is a gradual decrease in the yield per flush. The highest total yield was recorded for a combinaion of sawdust(SD) and sugarcane bargasse(SB) at a ratio of 80:20 respectively. The lowest yield was for sawdust alone with 232g. The biological efficiency is directly affected by the total yield as observed by a low yield giving a low biological efficiency and a higher yield giving a higher biological efficiency.

Literature review

In this review we assessed developments in the substrate formulation using tables 2 and 3 with the dependent variable being spawn run, biological efficiency, carbon to nitrogen ratio(C/N)benefit /cost ratio, and number of flushes. Where no data is supplied(blank spaces) we assumed gaps in that particular research.

Table 2. Analysis of individual substrates.

Substrate	Spawn run	Biological efficiency	C/N ratio	benefit cost ration	# of flushes	References
Wheatstraw	68.16					(Tesfaw et al 2015)
Sugarcane bagasse	32days	65%	45.83	--------	6	(Hoa et al 2015)
Corn cobs	40 days	58%	34.57	--------	6	
Sawdust	30 days	46%	51.71		6	
Sugarcane bargasse	20 days	-------	-------	0.217		(Dubey et al 2019)
Rice straw	25 days	-------	-------	3.498		
wheatstraw		-------	-------	1.108		
banana leaves		-------	-------	1.408		
sawdust						(Itelima 2011)
		30%				(Itelima)
		60%				(Adjapong et al 2015)

cotton hulls		92,9%	(Islam et al 2016)
	18	86.2%	(Muswati et al 2021)
maize cobs		9.5%	(Aadjapong et al 2015)
corn cobs		70%	(Itelima 2011)
maize husk		(32%)	(Adjapong et al 2015)
wheatstr aw	16 days	35.88%	(Girmay et al 2016)
sawdust	20days	9.73%.	(Girrmay et al 2016)
	14 days	74.17%,	(Girmay et al 2019)

Table 2 details investigations carried out by 6 authors from 2011 to 2021 of individual substrates closest to the substrates under investigation. I. The best perfoming substrate is cotton hulls (Islam et al 2016, Muswati et al 2021) whilst the leat perfoming substrate is sawdust (Girrmay et al 2016)

Table 3. Analysis of substrate combinations

Substrate combination	Spawn run	Biological efficiency	C/N ratio	benefit cost ration	References
25 % plam pressed fibre, 25% sugarcane bagasse and 50% rice straw		79.72%			(Zakil et al 2019)
50:50 sawdust and sugarcane bagasse,			46.67	6	[Hoa et al 2015)
50:50 sawdust and corncobs			42.55.	6	
80:20 sawdust and sugarcane bagasse			46.68	6	
80:20 sawdust and corncobs			49.05	6	
sawdust and cassava peel		46.30%			(Islam et al 2016)
sawdust and teff straw					(Besufekud et al 2019)
sawdust (CA) corn cobs and coffee bean husk	0.69cm/day	77.83%			(Gume et al 2013)
.palm kernel cake and maize cobs		97.9%			(Chukwurah et al 2013)
Chebodium album (weed)(16.66%) and cotton waste at 83.34%.					(Islam et al 2016)
sugarcane bagasse and sawdust at 50/50		58.94 %		6	(Hoa et al 2015)
wheatstraw , cotton waste and baobab fruit shells		85.9%.		1.93	(Muswati et al 2021)

Table 3 is a summary of work done by 7 authors on substrate combination of oyster mushrooms.from 2013 to 2021.

Discussion

Comparative analysis of substrates

Individual substrates

Research gaps were identified on the feedstock of mushroom cultivation paying attention to C/N ratio, spawn run, cost benefit ratio and number of flushes. However most research articles used for this review provided enough information on biological efficiency.

Most authors do not consider the importance of profitability in mushroom cultivation. More focus is highlighted on the biological efficiency and the spawn run. A higher benefit cost ratio is an indication of profitability compared to a low benefit cost ratio. Only [12] provided an account of the profitability of sawdust, corncobs and sugarcane bagasse.

Biological efficiency

The most significant biological efficiency for cotton hulls at 92.9 % (Islam & Riaz,.2016), followed by 86.2%(Muswati et al,.2021) and lastly 74.17%(Girmay et al 2019). This means for every 1kg of cotton hulls to grow mushrooms the yield of mushrooms ranges from a lower limit of 740grams to an upper limit of 929 grams. The average weight of mushrooms a farmer can expect to harvest from cultivating oyster mushrooms of cotton hulls is approximately 844g which translates to a biological efficiency of 84.4%. The differences in biological efficiency of cotton hulls indicate a difference in cultivation methods and environmental conditions which resulted in three different values on the same substrate. Substrate quality is not the only limiting factor for a high biological efficiency.

12 recorded a biological efficiency of 58% for corn cobs in 40 days.. 15 recorded a biological efficency of 70% for corncobs but the spawn run is missing. The average biological efficiency for these results is 64%. The average weight of fresh mushrooms a mushroom farmer can expect to harvest from cultivating oyster mushrooms on corncobs is 640 grams for every 1000 grams of dry corncobs. Compared to cotton hulls there is a difference of 20.4% more biological efficiency.

12 recorded a biological efficiency of 46% for sawdust in 30 days.. 19 recorded a biological efficiency of 9.73% in 20 days. 15 recorded a BE of 30% and 16 recorded 60% but detais pertaining to the span run are missing. The average biological efficiency from the four investigated is 36.4%. The average weight of fresh mushrooms a farmer can expect to harvest from cultivationg oyster mushrooms on sawdust is 364 grams for every 1000grams of dry sawdust. Compared to cotton hulls(84.4%) and corncobs(64%) sawdust has the lowest biological efficiency.

12 cultivated oyster mushrooms on sugarcane bagasse finishing spawn run in 32 days and obtaining a biological efficiency of 65%. 1 also cultivated on sugarcane bagasse and recorded a spawn run of 20 days, however data representing the biological efficiency is missing. According to 1 a farmer can expect to harvest oyster mushrooms in 20 days on sugarcane bagasse. According to 12 a farmer can harvest 650 grams of oyster mushrooms in 32 days on sugarcane bagasse. Compared to cotton hulls sugarcane bagasse has a shortfall of 190 grams and compared to corncobs there is a difference of 10grams. Sugarcane bargasse and corncobs have almost the same biological efficiency Compared to sawdust sugarcane bagasse brings about 220 grams more yield per kg of dry substrate.

14 cultivated oyster mushrooms on wheatsraw and harvested in 68 days however theere is agap in biological efficiency. 1 also cultivated oyster mushrooms on wheatstraw however values for the spawn run and the biological efficiency are missing. 19 cultivated on wheatstraw and harvested within 16 days obtaining a biological efficiency of 35.88%. The biological efficiency for wheatstraw according to 19 is 35.88 grams, translated to 358.8 grams of mushrooms per kg of wheatstraw. The difference in biological efficiency between wheatstraw and sawdust is 0.5% which translates to 50 grams of mushrooms meaning whetstraw and sawdust have almost the same biological efficiency. However compared to cotton hulls, corncobs and sugarcane bagasse wheastraw has the lowest biological efficiency.

1 cultivated oyster mushrooms on banana leaves but data representing biological efficiency and spawn run is missing. 1 cultivated oyster mushrooms on rice straw obserrving and recording a spawn run of 25 days. 16 cultivated oyster mushrooms on maize cobobs observing and recording a biological efficiency of 9.5%. Compared to corn cobs maize cobs are 54,5% less biologically efficient even though they are both cereal straws. Compared to wheatstraw maize cobs have a lower biological efficiency. 16 also cultivated oyster mushrooms on maize husk obtaining a biological efficiency of 32% meaning the husk of maize is more nutritious compared to the cob. The husk is 22.5% more efficient compared to the cob. Compared to corncobs maize husk has a lower biological efficiency even though wheatstraw(36.4%) and sawdust(35.9%) maize husk falls within the same range of 30% to 40%.

Spawn run

1 cultivated oyster mushrooms on rice straw observing and recording a spawn run of 25 days. 19 cultivated on wheatstraw and harvested within 16 days obtaining a biological efficiency of 35.88%. 12 cultivated oyster mushrooms on sugarcane bagasse finishing spawn run in 32 days and obtaining a biological efficiency of 65%. 1 also cultivated on sugarcane bagasse and recorded a spawn run of 20 days, however data representing the biological efficiency is missing. 12 recorded a biological efficiency of 46% for sawdust in 30 days.. 19 recorded a biological efficiency of 9.73% in 20 days. 15 recorded a BE of 30% and 16 recorded 60% but detais pertaining to the span run are missing. 12 recorded a biological efficiency of 58% for corn cobs in 40 days.. 15 recorded a biological efficency of 70% for corncobs but the spawn run is missing.

The are two observations for spawn run of oyster mushrooms on cotton hulls. 18 recorded 18 days for oyster mushroom mycelia to completely cover the substrate. The corresponding biological efficiency was 86.2%. 19 observed and recorded a spawn run of 14 days on the same type of substrate. The average cotton hulls spawn run for spawn run for articles in this review is 16 days. The corresponding biological efficiency was 74.17%. 18 recorded a longer spawn run but a higher biological efficiency. In contrast 19 recorded a faster spawn run and a lower biological efficiency. The difference in biological efficiency being 12.03% tanslating to 120 grams of fresh mushrooms. Therefore there must be a mathematical correlation between the spawn run and the biological efficiency. It can be assumed that there is an inverse variation between the spawn run and the biological efficiency. As the spawn run takes longer the biological efficiency increases. As the spawn run takes shorter the biological efficiency decreases. A longer spawn run indicates enough time taken for mycelia to absorb most of the nutrients in the substrate however a longer spawn may also be caused by low incubation temperatures and improber balance of carbon dioxide and oxygen levels.

Comparison of the biological efficiency and the spawn leads to the introduction of a new variable, The Biological Efficiency to Spawn Run Ratio.

The Biological Efficiency to Spawn Run Ratio

Table 4. The Biological Efficiency to Spawn Run Ratio

Substrate	**Theoretical BE**	**Average Biological efficiency**	**Theoretical Spawn run**	**Average Spawn run**	**BE/SR**	**Author**
Cotton Hulls	92,9%,86.2%,74.17%,	84.4		16	5.275	[17], [18], [19]
wheatstraw	35.88%	35.88		42	0.854	[19], [1], [14]
rice straw,	----------	--------	25 days	25	------	[1]
sugarcane bagasse	32days	65	20 days	26	2.5	[1], [12],
sawdust	30%,60%,9.73%.	36.4	----------	25	1.456	[19], [15], [16]
corncobs	70%	64.0	----------	40	1.6	[15]
banana leaves	----------	--------	----------	------	-------	[1]
maize cob	----------	9.5%	----------	------	-------	[16]
maize husk	----------	32%	----------	-------	-------	[16]

Table 4 is an illustration of how we came up with the biological efficiency to the spawn run ratio using the theoretical values of the biological efficiency and the spawn run. It explains the time taken for a certain quantity of fresh mushrooms to be harvested from a kilogram of dry substrate. It can be observed that a high BE/SR ratio results from a combination of a high biological efficiency and a short spawn run.

Cost benefit ratio

Only one article provided information related to the cost benefit ratio [1]. Rice straw had the highest(3.498) , followed by banana leaves(1.408), wheatstraw(1.108) and sugarcane bagasse at 0.217 These values cannot be further analysed because the biological efficiency and the spawn run is missing. The ratio rises with the decrease in the cost of production. Inversely it falls with the rise in the cost of production. From these values it can be deduced that rice straw is more economical and sugarcane is most expensive.

A B:C ratio of 3.498 for rice straw is due to low cost, availability and high nutrition. A B:C ratio of 0.217 for sugarcane bagasse is due to high cost of substrate and the low yield of mushrooms. The low yield being caused by high moisture content and high sugar content resulting in fermentation producing alcoholic substances lowering the pH and suppressing the colonisation of the fungus (Dubey et al 2019).

Number of flushes

Only one author provided data on the number of flushes per substrate [12]. According to [12] sugarcane bagasse, corn cobs and sawdust have the same number of flushes. Provision of the number of flushes would have assisted in extrapolating the total yield for the whole cultivation process.

C/N ratio

Low nitrogen content gives low biological efficiency of sawdust. As the C/N ratio goes down the yield increases. Substrates with high lignin and phenolic content decrease the activity of cellulose, but less lignin enhances enzyme activity and thus ensures higher mushroom yield and biological efficiency (Hoa et al 2015).

Conclusion

An ideal substrate as alluded before, should merit a high biological efficiency and short spawn run. A high biological efficiency ensures high yields. A short spawn run ensures quick returns and minimises operational costs. Only cotton hulls fit this criteria, with an average effiency of 84% and an average spawn run of 16 days. Furthermore dividing the biological efficiency by the spawn run gives the BE/SN ratio which gives a representation of the time taken to achieve the biological efficiency. Cotton hulls, with a ratio greater than 5, gives the highest biological efficiency in a short space of time.

Comparative analysis of substrate combinations

Biological efficiency

Combinations of substrate vary depending on availability and location. For the articles under review the highest biological efficiency(97.9%) is obtained by combining palm kernel cake and maize cobs [2], followed by wheatstraw, cotton waste, sawdust at 85.9%[18]. The lowest biological efficiency was obtained from combining sawdust and cassava peel(46.30%)[7] followed by a combination of sugarcane bagasse and sawdust(58.94%)[12].

Almost all the authors provided a biological efficiency result for their substrate combinations. However, for the spawn run there are research gaps. Only two authors for the publications under review provided data recorded on the spawn run (Gume et al 2013). A figure on spawn run gives a representation of the accessibility of substrates to the mycelia. Aspects of aeration, surface area, nutrition and spawn quality all affect the performance of a particular combination of substrates.

A high biological efficiency (97.9%) exhibited by palm kernel cake and wheatstraw (Chukwurah et al 2013) may be a result of the high C: N ratio in palm kernel combined with the high cellulose content in wheatstraw.

Research gaps

In comparison to individual substrates there were missing data related to the spawn run and the cost benefit ratio. There is low published material related to oyster mushroom cultivation in Zimbabwe. Most of the substrates observed such as rice straw and palm kernek cake are agricutural wastes of crops not cultivated in Zimbabwe.

Conclusion

The ideal substrate should satisfy all the requirements regarding the spawn run, biological efficiency and cost benefit ratio. Research from Zimbabwe concerning possible substrate combinations from agricultural waste products of our most cultivated crops such as maize, tobbacco, cotton, soyabeans, banana, sorghum, sugarcane and potato.for oyster is lacking. There is need to fill reseach gaps related to spawn run, biological efficiency and most importantly the benefit cost ratio.

Chapter 3

Article 2

Optimizing Pleurotus ostreatus Cultivation: Evaluation of Substrate Combinations Including Wheat Straw, Sawdust, Banana Fronds, Maize Cobs, and Cotton Hulls

Silivani Elyson[1*], ChisangoTawanda Jonathan[1], Chitindingu Kudakwashe[1], and ChiguNomathemba Loice[1]

[1]Chinhoyi University of Technology, School of Health Sciences and Technology, Department of Biotechnology, P. Bag 7724, Chinhoyi, Zimbabwe

*Corresponding Author:elysonsilivani@gmail.com

Abstract

The yield and quality of oyster mushrooms (Pleurotus ostreatus) are significantly influenced by the chemical and nutritional content of the substrate. This study aimed to evaluate the effects of different individual substrates and their combinations on the performance of P. ostreatus, with a focus on improving mushroom cultivation in Zimbabwe. Substrates tested included wheat straw, cotton hulls, sawdust, maize cobs, and banana fronds, each at 1 kg and replicated four times. Mushrooms were cultivated at 25^0C during the spawn run and 20 ^{0}C at the fruiting stage.Humidity was below 50% during spawn run and was increased to 90% at the fruiting stage. Substrate combinations were arranged in a factorial design of 1 kg and replicated four times, including cotton hulls with wheat straw, sawdust, maize cobs, and banana fronds in a 1:1 ratio. Results showed that compound substrates outperformed individual substrates in biological efficiency and spawn run. The combination of cotton hulls and sawdust yielded the highest biological efficiency (76%) and a spawn run of 17 days, while the fastest spawn run (12 days) was achieved with cotton hulls and wheat straw. Combining cotton hulls with a substrate that has a wide C:N ratio such as sawdust increases the biological efficiency as a result of the boost in the carbon component Economically, cotton hulls and sawdust provided the highest benefit-cost ratio of 5.7. Therefore, combining cotton hulls and sawdust is highly recommended for farmers to meet growing consumer demand while minimizing environmental impact. Future research should explore the scalability and economic feasibility of these substrate combinations in varying conditions.

Keywords: ***Substrate, combination, mushrooms, Pleurotus ostreatus, biological efficiency, spawn run, cost-benefit ratio.***

Introduction

*Pleurotus ostreatus(*oyster mushroom) is a member of *Pleurotus* species of mushrooms cultivated globally for food. According to Besufekud et al (2019), oyster mushrooms are the second largest commercially produced mushrooms in the world. They possess a rare capability to convert lignocellulosic waste residues into edible fruiting bodies, rich in nutrients. Oyster mushrooms are reported by Ramsbottom (1954) to contain high amounts of vitamins, particularly vitamin D and high protein contents because they contain enzymatic proteases that can breakdown complex macromolecules in agricultural wastesinto individual amino acids. Of much importance is the absence of fatty acids in oyster mushrooms, which makes them ideal for persons willing to maintain a low fat diet. Furthermore, oyster mushrooms contain the appropriate ratios of potassium and sodium, which are essential in reducing heart disease.

In Zimbabwe, mushroom cultivation is still at the grassroots level with most small-scale farmers producing enough for subsistence. Much knowledge is still lacking pertaining to the utilisation of substrate individually and in combination to optimise production and achieve commercial production leading to exportation Govera (2020).

Mushroom substrate is the material or substance, which provides food for the growing mycelium. Oyster mushrooms can grow on a wide range of substrates compared to other mushrooms. They can grow on all wood types including sawdust and paper pulp sludge. They also grow on all types of cereal straw such as maize straw, wheatstraw and rice straw. They also grow well on other types of agricultural substrates such as corncobs, banana fronds, maize stalks and leaves, savanna veld grass, sage grass, cottonseed hulls and coffee grounds.The mechanism by which substrate is broken down, is by the extracellular release of enzymes.According to Onyeka et al (2017) the extracellular enzymes secreted by fungi contain amorphous homo and heteropolysaccharides often associated with fungal protein. These enzymes bind to cellulose, lignin and hemicellulose, and break them down into simple sugars and peptides.

Sayner et al (2019) alludes that the yield and quality of oyster mushroom is largely dependent on the chemical and nutritional content of the substrate.In instances where, a nutrient lacking in one substrate it is then provided by another substrate.There is need to establish a consensus by, pulling high yielding substrates together and then come up with the best formulation.

Mushroom yield is closely associated with the chemical and biological composition of the substrate (Bhattacharjya et al 2015) Most mushroom farmers in Zimbabwe today are using cotton hulls to grow oyster mushroom. As more people are becoming knowledgeable about cotton hulls as substrate, it creates a demand for this commodity(Tapiwa 2021). As a result of the increase in demand for cotton hulls there is increase in price of cotton hulls. It becomes economically non-profitable to venture into mushroom farming due to expensive substrate. The price of wheat straw and cotton hulls is increasing every year, as many more people become mushroom farmers. This in turn affects the pricing of the mushrooms themselves.

Dead plant matter has different composition of nutrition. There are many factors, which determine selection of a particular substrate. These are nutrition, cost, availability, accessibility and storage. A single particular substrate may be readily available but costly to the farmer. The length of the mushroom crop or the number of flushes depends on the substrate used for the cultivation of oyster mushrooms. For example, wheatstraw has good nutrition but it has a limited cropping life. Banana fronds also have good nutrition but they has low water retention. Sawdust has high nutrition but it takes longer to fruit. Cotton hulls are the best for fruiting, spawn run, have good nutrition but they are very expensive and seasonal (Pant et al 2006). There are limitations in nutritional composition when only one substrate is used to grow mushrooms. A formulation of substrates might contribute to better nutrition in oyster mushrooms. The best composition of substrates should have a short spawn run, a high biological efficiency and a high benefit cost ratio. C/N ratio directly affects the mushroom spawn run, yield and biological efficiency.

Spawn run

Spawn run is the time it takes for the substrate to be fully covered by the mushroom mycelia. It is affected by qualities of the substrate and qualities of the spawn. For a shorter spawn run a substrate should have a high surface area and good nutrition. Banana leaves require 19 days for spawn run (Asmamaw et al 2014).Sawdust takes 30 days for spawn run.(Hoa et al 2015).Cotton hulls takes 18 days for spawn run.(Muswati et al 2021). Wheatstraw takes 16 days for spawn run.(Girmay et al 2016). Corn cobs takes 40 days for spawn run.(Itelima 2011)

Biological efficiency

Biological efficiency is defined as the percentage measurement of harvested fresh mushrooms relative to the dry weight of substrate.(Chukwurah **et al** 2012). In theory a 100% biological efficiency is achieved when fresh mushrooms having a moisture content of 90% are havested from a substrate with a moisture content of 75% of which 25% of dry substrate gives us the mushrooms.

$$\text{Biological efficiency} = \frac{\text{Fresh weight of mushrooms X 100\%}}{\text{Initial dry mass of substrate}}$$

The mathematical relationship between the biological efficiency and the dry mass of substrate is an inverse proportion. Biological efficiency decreases as the initial dry mass of substrate increases, this means the more substrate required to produce more mushrooms the lower the biological efficiency. Provided the the mass of substrate increases but mass of mushrooms remains constant, the substrate has a low biological efficiency.

The mathetical relationship between the biological efficiency and the fresh weight of mushrooms is a direct proportion. Biological efficiency increases with the fresh weight of mushrooms. Provided the fresh weight of mushrooms increases but mass of substrate remains constant, the substrate has a high biological efficiency.

In practice a 100% biological efficiency is achieved when a mushroom farmer collects 10 kgs of mushrooms from three harvests after cultivating on 10kgs of dry substrate. Outside factors such as temperature and humidity, affect biological efficiency (Girmay 2016).

A biological efficiency above 75% will be economical for the mushroom farmer. This is only achievable by using a high spawn rate. Usually the first, second and third flushes are the best flushes, with each flush decreasing in yield form the first. For subsistence, small scale farmers can allow the mushroom bags to fruit until the substrate is spent. For commercial farming only three flushes are economical (Zakil et al 2019).

B-C Ratio

B-C (Benefit-Cost) ratio is the ratio of benefit from selling output monetary terms relative to the total cost during production. However, as the value of B:C increases so does the feasibility of mushroom production in economical aspect. To find the ratio the yield of mushrooms by is divided bythe sum total expenditure of raw materials and substrate. Rice straw gives a B-C ratio of 3.498, wheat straw 1.108, sugarcane bagasse 0.217 and banana

leaves 1.408. Therefore, as the B-C ratio becomes greater then 1, the more profitable the project and as the B-C ratio falls below 1, the less profitable the project. (Dubey et al 2019).

Carbon to Nitrogen ratio

According to Kashangura et al (2005) for mycelia growth and fruiting body formation the carbon to nitrogen ratio should be 50: 1, 100: 1 or 500: 1. Most agricultural wastes such as corncob, grain straws, wood, sawdust and banana fronds have sufficient C; N ratios to support saprophytic mushroom growth. C: Nratio is the proportion of carbon to nitrogen in a substrate. (Hoa et al2015)

Oyster mushroom mycelial growth is optimum in the presence of more carbon compared to nitrogen. Therefore, substrates comprised primarily of cellulose, hemicellulose and lignin are good candidates for oyster mushroom cultivation. In this study, we will investigate cotton hulls, sawdust, wheatstraw, banana fronds and maize cobs individually and in combination to determine potential in optimisation of *Pleurotus ostreatus*. We discuss the characteristic features and C: N ratios of the substrates under investigation in table 1.

In thisstudy substrates were selected based on availability, cost and literature review in Chapter 2.

In thisstudy substrates were selected based on availability, cost and literature review in Chapter 2.

Table 1:Carbon to nitrogen ratios of the substrates selected for investigation in this study

Substrate	**Characteristic**	**C:N ratio**	**References**
Banana fronds	Dried leaves from banana. Brown	70:1	ScienceMate 2022
Maize cobs	Cobs from maize. Cob highly dense.	80:1	Lentz and Lindsey 2016
Cotton seed hulls	Waste product resulting from the pressing and crushing	100:1	Haziran 2013

	down of sottonseed into cottonseed cooking oil.		
Sawdust	Wood shavings from different hardwood typese.g oak, teak e.t.c	500:1	Rynk 1992

This study will provide sufficient data to make decisions on the utilisation of different types of agricultural and forest waste into nutritional food and to analyse, evaluate and ultimately identify the most appropriate composition of substrate for cultivating *Pleurotus ostreatus*.

Materials and methods

Site of the study

The research was conducted in a steel Mushroom House, at the Department of Biotechnology, School of Health Sciences and Technology, Chinhoyi University of Technology, Zimbabwe from January to March 2022 for evaluating the effect of different substrates on the yield of *Pleurotus ostreatus*.

Mathodology used was used based on mushroom cultivation strategies proposed by Kashangura **et al** 2005

Treatments

Table 2:Experimental design for the allocation of tretments to the mushrooms

EXPERIMENTAL DESIGN

SUBSTRATE	CH	SD	WS	MC	BF	CHSD	CHWS	CHMC	CHBF

1st phase
Treatments-9
Levels-1
Temperature-ambient
pH-constant
Airation-minimum(first three weeks)
Light-none(first 3 weeks)

2nd phase
Temperature <25
Humidity>80
Airation-average
Light-500-700lux

The substrates used in this investigation were wheatstraw, cottonseed hulls, sawdust, banana fronds and maize cobs. Each substrate combination weighing 1kg (dry weight) at a ratio of 1:1 was also distributed into a single factor experiment laid out in a Factorial Design. Nine treatments were replicated four times thus making 36 bags and one control for each treatment as shown in Table 2.

Analysis of variance to test for differences among means was evaluated using computer software R Statistical Package. Line graphs to show spawn run of Pleurotus ostreatus on different substrates and combinations was designed using Microsoft excel 2010. Bar graphs illustrating the differences in biological efficiency were drawn using Microsoft Excel 2010.

Substrate collection and bag filling:

Different substrates were collected from different locations. Cotton hulls purchased from a retailer in Harare. Maize cobs purchased from Citrus farm in Chinhoyi. Banana fronds collected from Bhagudha farm Chinhoyi. Sawdust procured from Chinhoyi University Works and Estates Department. Wheatstraw was collected from Chinhoyi University farm Chinhoyi.

Maize cobs, wheatstraw and banana fronds were ground using a stockfeed grinder at the Chinhoyi University feed factory. Maize cobs were ground into powder, wheatstraw into chunks 2-4cm and banana fronds into chunks 2-4 cm long. Combinations formulated with respect to cotton hulls(Factorial design) at a ratio of 1:1 dry weight. They were soaked with

clean tap water to remove all dust particles. Then the substrate combinations filled into separate 200litre drums and a mixture of 4% sodium hypochlorite and 20% (w/v) cacium hydroxide solution added to cover the substrate. The drums were covered and left overnight. The following day, substrates were drained of excess water separately on steel fence tables. Each substrate was mixed with an equal proportion of cotton hulls.

Inoculation/spawning:

We made our own *Pleurotus ostreatus* grain spawn to avoid bias. Spawning was carried out in transparent polythene plastic bags in layers subsequently with the first layer consisting of substrate and the top layer covered with spawn. Each bag comprised of 3 layers of substrate and 3 layers of spawn. After inoculation each bag approximately measured 2kg of substrate combinations by wet weight. Then the mouth of each bag was secured tightly with string and small holes were punched on the top and lateral sides of each bag for aeration.

Incubation:

The packed bags were incubated in a dark steel mushroom house until the mycelium penetrated to the bottom of the bag. Ambient teperatures were applied during the spawn run. At the fruiting stage moisture was used to maintain temperature and humidity at 20^0C and 90% respectively.The bags were hung from trusses welded below the roof of the mushroom house to allow excess water to drip from the bottom of the bag, to prevent direct contact of the bags with the floor of the mushroom house and to prevent contact with insects such as ants and termites.

Growing:

From the moment bags were introduced into the mushroom house the spawn run was observed and measured until the whole bags were covered with white mycelium. Thereafter the plastics were removed from the bags and the bags were placed on a wooden platform 30cm from the floor with a space of 20cm in-between bags. When pinheads started appearing a light source was introduced into the mushroom house at night and watering commenced 3 times a day at 6 hour intervals using a knapsack sprayer.

Harvesting:

After 5 days of watering mushrooms were harvested and appropriate observations and measurements were taken. Harvesting commenced when the cap attained the maximum

diameter, just before the edges flatten out. Firstly the whole bag and mushroom weight was recorded, then picking was done by gently twisting and pulling out the stalk. Mushrooms were then weighed and packaged into 200g punnets, wrapped with transparent plastic wrapper and sold for $1/200g. Mushrooms were stored in an upright fridge at 15^0C. Then the weight of the empty bag was recorded. The benefit from selling the mushrooms was also recorded.

Data collection and analysis:

Data was recorded periodically during the incubation from colonisation to final substrate weight. Parameters under consideration were number of days to full colonisation, weight of bag and mushroom, total mushroom yield, final substrate weight, total mushrooms sold and benefit cost ratio. Collected data were analysed using R statistical software and Microsoft Excel 2007.

Analysis of variance (ANOVA) was used to test among treatments and means were separated using Least Significance Difference (LSD) at the 5% level of significance.

Results and Discussion

The yield and yield attributing observations of mushrooms obtained from different combinations of substrates were compared. There were notable and considerable variations with respect to physical appearance of mushrooms, spawn run , quantity of first yield and final substrate weight.

Effect of substrate combinations on spawn run and 1st yield weight:

Highly significant results were observed among treatments in terms of spawn run of *Pleurotus ostreatus* as shown in fig3 and Table 3. Of all the combinations under investigation wheatstraw and cotton hulls combination took the shortest time (12 days) followed by cotton hulls and banana fronds(13 days) , cotton hulls and maizecobs(13 days) and lastly cotton hulls and sawdust(17 days).

Table 3: Mean values of first yield, spawn run and final substrate

Substrate	First yield(g)	Spawn run(days)	Substrate final weight(g)

Cotton hulls & wheatstraw	376	12	1576
Cotton hulls & banana fronds	449	13	1716
Cotton hulls & sawdust	428	17	1099
Cotton hulls & maize cobs	420	13	1508
Cotton hulls	400	19	1213
Wheatstraw	212	16	980
Sawdust	155	20	1417
Banana fronds	250	17	1118
Maizecobs	263	18	1842

Table 4:ANOVA table

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
tm	**3**	**11217444**	**3739148**	**146.8**	**<2e-16 *****
Residuals	**32**	**815253**	**25477**		
Signif. codes	**0 '***' **	**0.001**	**' 0.01**	**0.05**	**0.1 ' ' 1**

Using computer Software R Statistical Package ,at the 5% level of significance a P value of 1 X 10^{-16} less than 0.05 which means there are significant differences among treatment means.

Effect of different substrates on total yield,spawn run and final substrate weight of oyster mushroom.

The weight of substrate combination after first harvest was lowest in case of cotton hulls and sawdust(1099g) followed by cottonhulls maizecobs, cottonhulls-wheatstraw and cottonhulls-banana fronds at 1508g, 1576g and 1716g respectively. This is evidence that oyster mushroom absorbs nutrients more efficiently in substrates composed of cotton hulls and sawdust compared to other substrate combinations of cotton hulls. This might also give an indication of particle size and absorption efficiency given the small particle size of sawdust rendering nutrient accessibility easier. Thus, it also indicates that the high biological efficiency obtained from cottonhulls-sawdust combination (76%) is attributed to the small surface area. Statistical analysis of data generated showed that there are significant

differences in the total yield of mushrooms obtained from different substrate combinations. The p-value(1×10^{-16}) in Table 4 , indicates that there are significant differences in the average yield of combinations and individual substrates. We can therefore reject the null hypothesis that all substrates give the same yield of *Pleurotus ostreatus*, Yields from individual substrates pale in comparison to substrate combinations with the exception of cotton hulls(400g) alone which exceeded combinations of cotton hulls and wheatstraw(376g). Combinations of substrates contribute to nutrient compensation as what is lacking in one substrate is supplemented from the other. The high biological efficiency exhibited by cotton hulls sawdust combination may be attributed to high lignolytic and cellulonitic activity of the substrates. Muswati et al (2021)cultivated *Pleurotus ostreatus* on cotton hulls and baobab fruit shells and observed similar results.

Economic analysis

Cost of cultivation:

It is analysed based on the cost of different materials and combinations of substrate. However, in this research the cost of required materials was at a constant for each treatment, except for cost of substrates, which are shown in table2:

Table 5:Mean values of B:C ratio of individual substrates and combinations

Substrate	**Biological efficiency/kg**	**Cost/kg(US$)**	**Benefit(US$)/kg**	**B:C ratio**
Cotton hulls and wheatstraw	34	0.84	1.7	2.02
Cotton hulls and banana fronds	45.6	0.94	2.28	2.43
Cotton hulls and sawdust	76.3	0.67	3.82	5.7
Cotton hulls and maize cobs	50	0.67	2.5	3.73
Cotton hulls	60.3	0.71	3.015	4.24
Wheatstraw	25.2	0.68	1.26	1.85
Sawdust	18.5	0.51	0.925	1.81
Banana fronds	20	0.78	1	1.28
Maizecobs	26.3	0.57	1.315	2.31

B-C ratio:

The B: C ratio relates the benefit from selling mushrooms in monetary terms to the total cost during production. In this case it was calculated in United States Dollars by dividing the cost

of production per kilogram of substrate by the total benefit of selling fresh mushrooms per kilogram of substrate.. Cost of production entails total expenses of raw materials. Benefit of production is calculated by dividing the biological efficiency by the price of selling mushrooms per kilogram of substrate. Substrate combinations were considered profitable if the B: C ratio was greater than one.

Costing

The cost price of cotton hulls is 0.14cents/kg, wheatstraw 20cents/kg, sawdust 5cents per kg, banana fronds 10cents/kg and maize cobs 10cents per kg. Packaging, spawning, punnets, thread and chemicals for pasteurisation were constant at 30cents per kg for all substrates. Transportation for cotton hulls was 0.27cents/kg, wheatstraw 0.1cents per kg, sawdust 0.16cents/kg, bananafronds 0.18cents/kg and maizecobs 0.07cents per kg. Maizecobs ,banana fronds and wheatstraw required grinding to reduce particle size. The cost of milling maizecobs was 0.1cents/kg, banana fronds 0.2cents/kg and wheatstraw 0.08cents/kg.

The differences in B:C ratio are shown in the table 5. The highest most significant ratio was produced by combination of cotton hulls and sawdust(5.7) whereas the lowest least significant ratio was produced by combination of cotton hulls and wheatstraw. For individual substrates cotton hulls produced a significant ratio of 4.24 which was more profitable compared to combinations of cotton hulls with banana fronds (2.43), cotton hulls with wheatstraw (2.02) and cotton hulls with maizecobs(3.73). For individual substrates cotton hulls(4.24) were more profitable compared to all the other substrates with the least profitable being banana fronds with a B;C ratio of 1.28. The data generated in Table 5 is empirical basis for encouraging mushroom farmers to consider combining cotton hulls and sawdust for achieving high economic return with low investment. We suggest the low yied from wheatstraw, maizecobs, sawdust and banana fronds emanating from their high water retention tendencies in contrast to cotton hulls. Furthermore wheatstraw and banana fronds lack compactness due to the low surface area which impedes complete ramification of substrate. Combinations of cotton hulls with wheatstraw and banana fronds did not improve yield due to reasons previously stated. Sawdust and cotton hulls proved to be the ideal combination firstly as a result of the large surface area then secondly due to the rich ratios of lignin and cellulose from sawdust combined with nitrogen and xylanases provided by cotton hulls (Vhargese and Amritkumar 2020).

Table 6: Impact of carbon source and nitrogen source on Biological efficiency

Substrate	Carbon component	Nitrogen component	C;N ratio	BE	Spawn run	References
Cotton hulls	45-55 Cellulose 40-50 Hemicellulose 20-30 Lignin 15-25	1.5-2.5 Protein 50-60 Amino acids 20-30	40:1	60.3	19	Ali ,2019
Sawdust	45-55 Cellulose 30-40 Hemicellulose 20-30 Lignin 5-15	0.5-1.5 Protein 40-60 Amino acids 20-40	400:1	18.5	20	Wiley Blackwell 2019
Maizecobs	45-50 Cellulose 35-40 Hemicellulose 20-30 Lignin 10-20	0.8-1.2 Protein 8-9 Amino acids 39-45	60:1	26.3	18	Singh 2020
Wheatstraw	40-50 Cellulose 30-40 Hemicellulose 20-30 Lignin 5-15	0.5-1.5 Protein 40-60 Amino acids 20-40	85:1	25.2	16	Kumar et al 2020
Banana fronds	40-50 Cellulose 20-30 Hemicellulose 10-20 Lignin 5-10	1.1-1.8 Protein 6-15 Amino acids 27-45 20	40:1	20	17	Abreu,(2020),
CHSD	90-110	2.0-4.0		76.3	17	
CHWS	85-105	2.0-3.7		34	12	
CHMC	90-105	2.3-3.7		50	13	

CHBF	95-105	2.6-3.3	45.6	13

Among the individual substrates cotton hulls and sawdust have the highest carbon component however cotton hulls have the highest nitrogen component.which contributes to the high biological efficiency. Combining cotton hulls and sawdust triples the biological efficiency of sawdust because of the boost in nitrogen contributed by the cotton hulls.

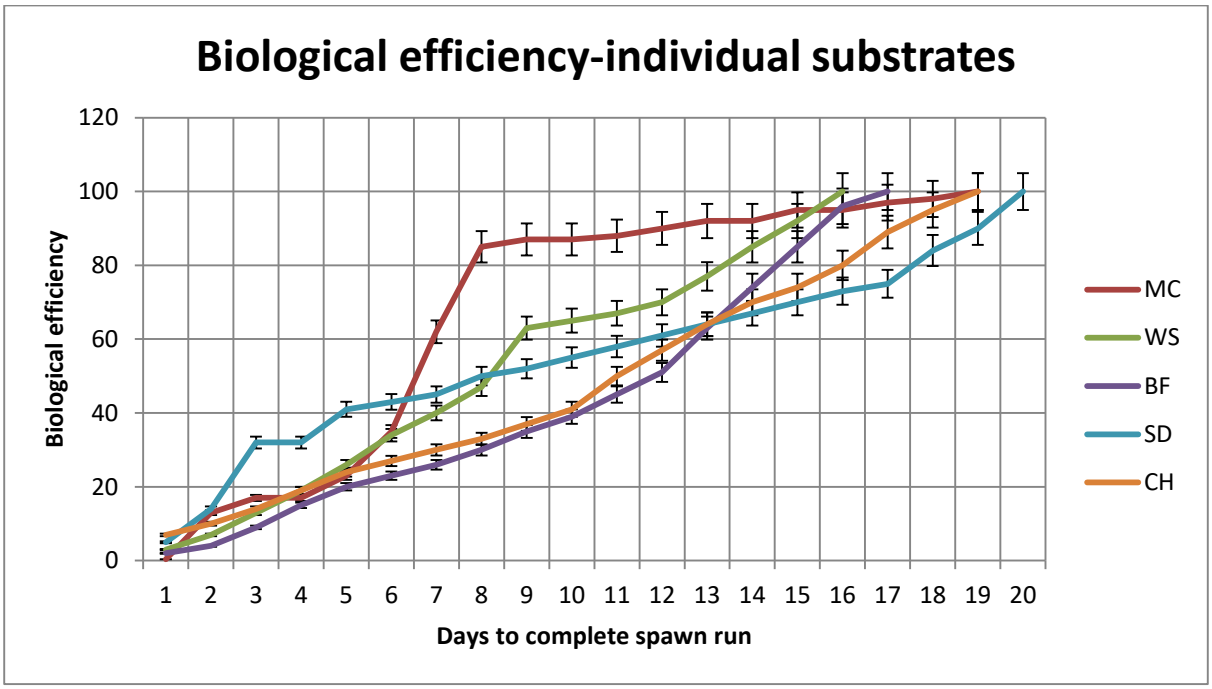

Fig 1: Mean values of *Pleurotus ostreatus* spawn run expressed over 1 day intervals observed on individual substrates

Spawn run was fastest for wheatstraw(16days) due to high nutrient availability and aeration. As the degreee of aeration decreases as a result of compactnaess of the substrate there is

increased longevity of the spawn run as observed for sawdust(20 days) as shown in Fig 1..

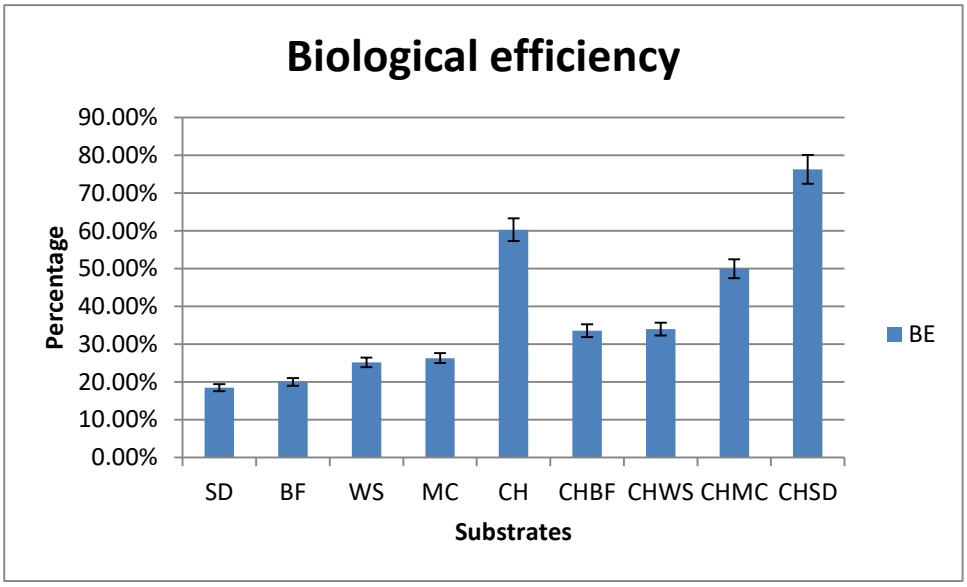

Fig 2: Mean values of *Pleurotus ostreatus* Biological efficiency observed on individual substrates and on combinations with cotton hulls.

In literature the BE of cotton hulls ranges from 74% (Girmay et al 2019) , 86.5% (Muswati et al 2021) and 92.9%(Islam and Riaz 2016). In this study we obtained a BE of 60%..

Hoa et al (2015) cultivated oyster mushrooms on corncobs and obtained a BE of 58%, Itelima (2011) also cultiveted oyster mushroom on corn cobs and obtained a biological efficiency of 70%. **.** Adjapong et al (20150 cultivated oyster mushrooms on maize cobobs observing and recording a biological efficiency of 9.5%. In this study we cultivated oyster mushrooms on maizecobs and obtained a biological efficiency of 25%.

Hoa **et al** 2016 cultivated oyster mushrooms on sawdust and obtained a biological efficiency of 46%. Girmay et al 2016 cultivated oyster mushroom on sawdust and obtained a biological efficiency of 9.73%. Itelima (2011) obtained a biological efficiency of 30% and Adjapong et al (2015) rcorded a BE of 60%. In this study we obtained a BE of 19%.

(Girmay **et al** 2016)cultivated oyster mushrooms on wheatstraw and obtained a BE of 35.9%. We obtained a BE of 23% for wheatstraw.

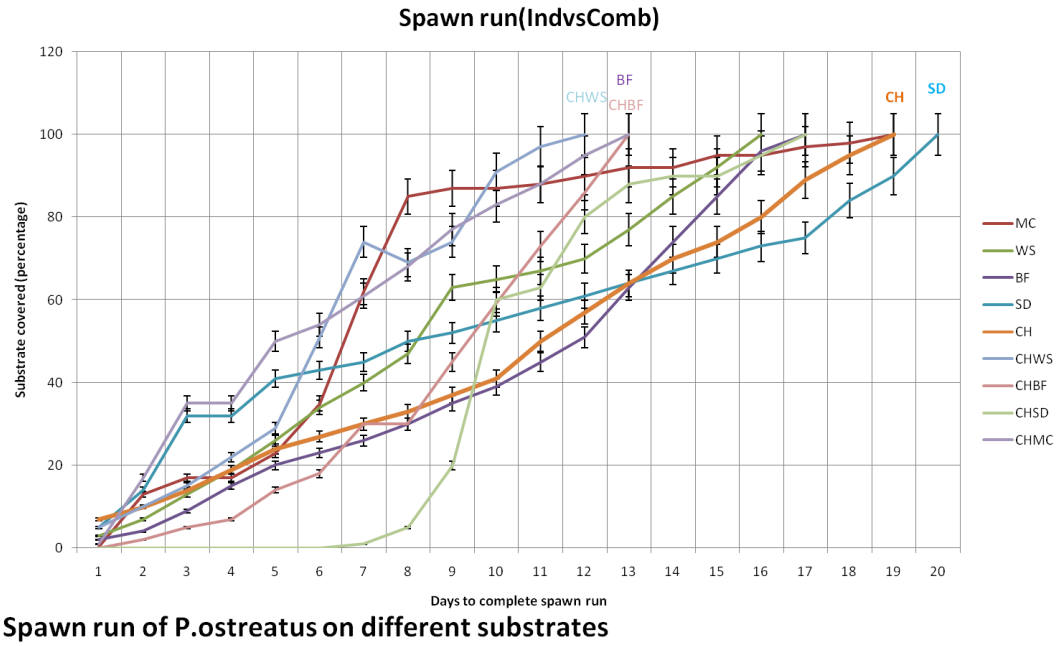

Fig 3: Mean values of *Pleurotus ostreatus* spawn run expressed over 1 day intervals observed on individual substrates and combinations of substrates

Spawn run is fastest for cotton husk and wheatstaw combinations. Spawn run is slowest for cotton husks and sawdust. The improvement in spawn run for cotton hulls from 19 days to a shorter time of 12 days is attributed to increased aeration as shown in Fig 3. Furthermore there is a notable decrease in the spawn ru for sawdust from 20 days to 17 days attributed by increased carbon to nitrogen ratio and aeration. Overall combinations of substrate performed better compared to individual substrate across the board.

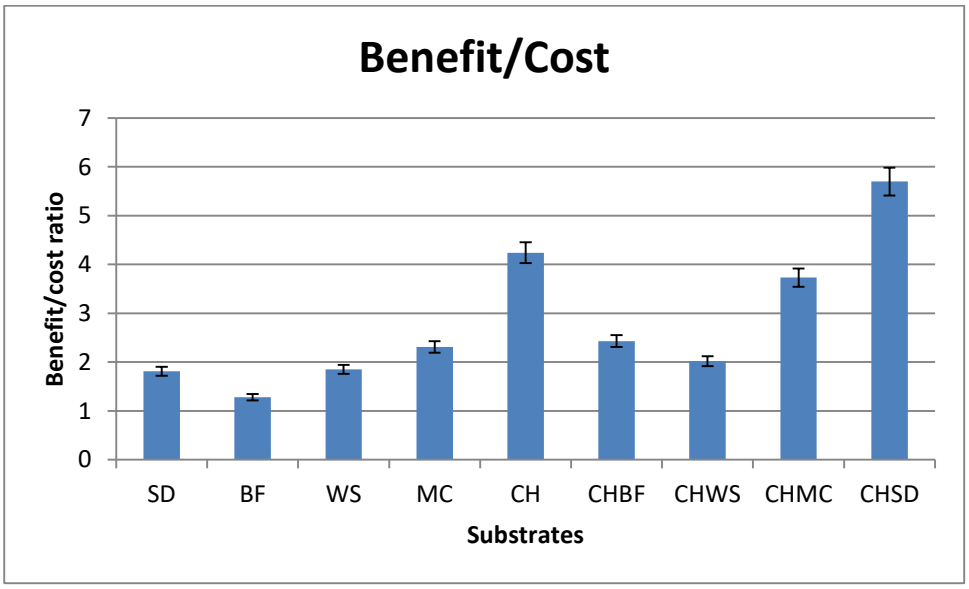

Fig 4: Cost/benefit ratios *Pleurotus ostreatus* mushroom cultivated on different combinations of substrates.

Fig 4 is data translated from Table 2 giving a graphical representation of the benefit cost ratio of using combinations of substrated compared to individual substrates.

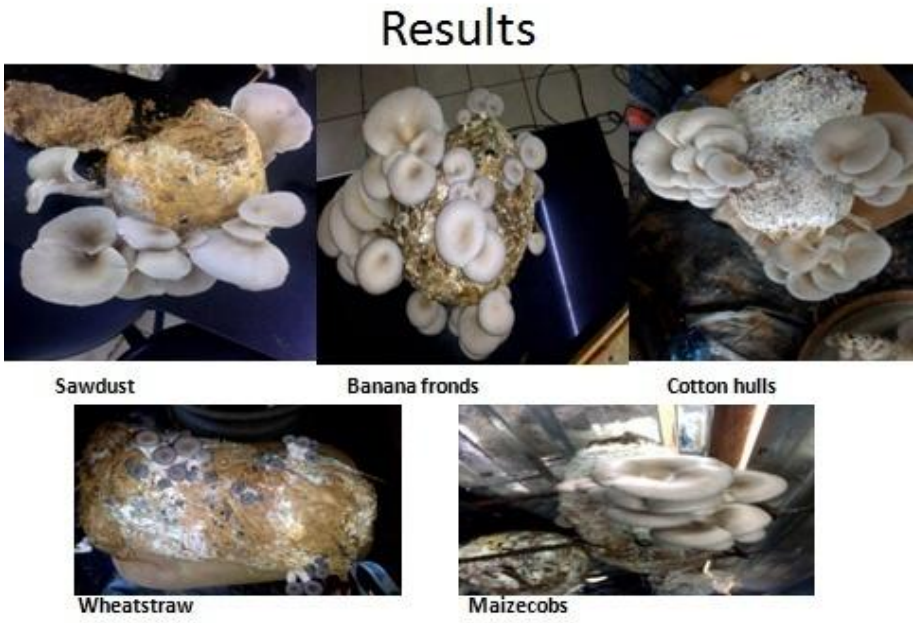

Fig 5 Physiological appearance of *Pleurotus ostreatus* mushrooms cultivated on individual substrates

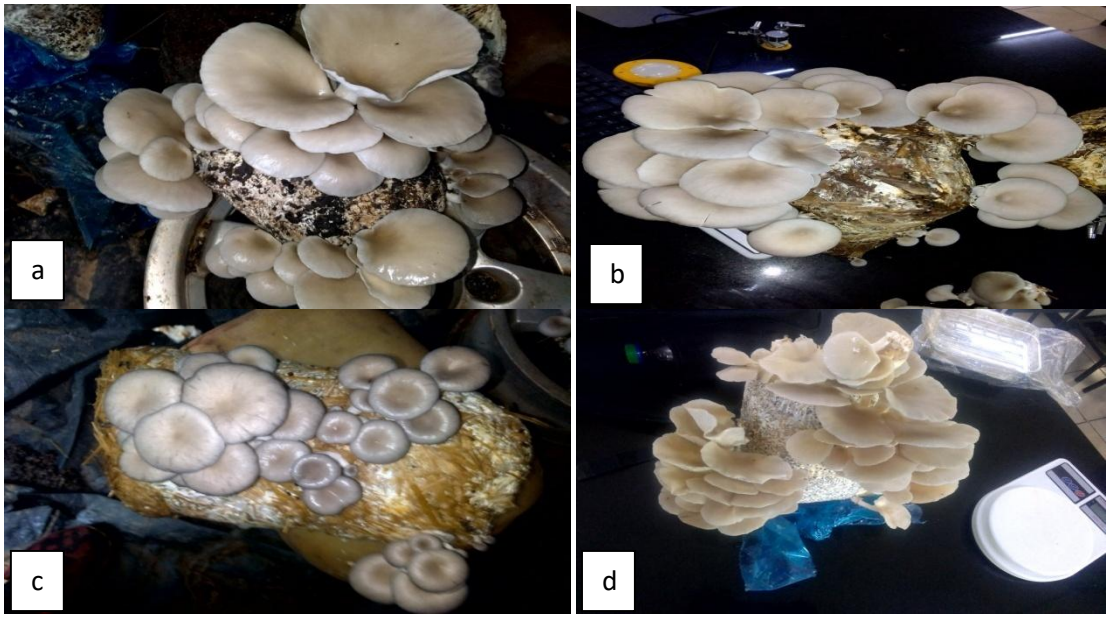

Fig 6: Images of physiological appearance of *Pleurotus ostreatus* on different substrate combinations

A=cotton hulls and sawdust,b=cotton hulls and banana fronds,c=cotton hulls and wheat straw, d=cotton hulls and maizecobs

Fig 5 shows appearance of oyster mushrooms cultivated on different combinations of substrate. On cotton hulls and sawdust, oyster mushrooms are bigger and there is uniform distribution of bunches right round the bag, there is fruiting on all sides of the bag. On cotton hulls and banana fronds there are lesser bunches of mushrooms even though there is also an even distribution of mushrooms right round the bag. On cotton hulls and wheatstraw the mushrooms have a much thicker skin and appear more grey than other mushrooms grown on other combinations. However, there are fewer mushrooms only appearing on one side of the bag. On cotton hulls and maizecobs there is a uniform distribution of mushrooms right round the bag however, the mushrooms appear smaller than the ones on cotton hulls and sawdust. The mushrooms on cotton hulls and maizecobs combination are whiter than mushrooms grown on other substrates.

Discussion

Individual substrate took longer to complete spawn run compared to combinations i.e wheatstraw(16 days), banana fronds(17 days) , maize cobs (19 days), cotton hulls(19 days) and finally sawdust(20 days). This coincides with the findings of Muswati et al 2021 that combinations of cotton hulls perform much better during spawn run compared to individual substrates. Spawn run was also faster for banana fronds (17 days) also due to availability of aeration. Maizecobs and cotton husks managed to achieve the same rate of spawn runner due to compactness. It was observed that, irregardless of nutrient availability particle size of substrate directly affects availability of air pockets within the substrate thereby limiting aiflow and affecting the rate of fermentation. The more compact a substrate is the lesser the airation resulting in a slow spawn run.

The highest benefit being afforded by combining cotton hulls and sawdust and the lowest being cotton hulls and wheatstraw. The only exception being cotton husk individually outperforming combinations with maizecobs, wheatstraw and banana fronds.

Biological efficiency is directly affected by the carbon to nitrogen ratio. The more nitrogen there is in a substrate the higher the rate of protein synthesis leading to a higher yield of mushrooms(cotton husks). In contrast the more carbon there is in a substrate the more the more energy available for cellular replication(mitosis) but less energy available for protein

synthesis,as in the case of wheatstraw and sawdust. However complementation comes into play when the shortfalls of one substrate is covered by suplementation with another substrate resulting in a high biological efficiency as in the case of cotton hulls and sawdust as shown in Fig 2. According to Kashangura et al (2005) a wider C;N ratio indicates slower decomposition whereas a narrow C;N ratio indicates rapid decomposition and release of nitrates. The high nitrogen component of cotton hulls as shown in Table 2 leads to high nutrient decomposition, release of nitrates, faster spawn run and high biological efficiency in Fig 2. Consequently combining cotton hulls with a substrate that has a wide C:N ratio such as sawdust increases the biological efficiency as a result of the boost in the carbon component. It can be seen from Table 2 that biological efficiency falls as the gap betwwen the carbon component and the nitrogen component becomes wider.Cotton hulls also have the highest portion of proteins compared to all the other substrates under investigation, hence the high biological efficiency. The mushroom cap is20-40% protein and the protein efficiency ratio of oyster mushrooms is 2.5-3.5(Melkamu, et al 2019) indicating that a substrate rich in nitrogen will directly produce a higher yield of mushrooms.

Conclusion

This study was conducted by growing *Pleurotus ostreatus* on 5 different agricultural waste substrates namely cotton hulls, maizecobs, wheatstraw, banana fronds, sawdust and on combinations of 4 substrates with respect to cotton hulls. Among the individual substrates wheatstraw colonised the fastest whereas among the combinations wheat straw and cotton hulls were colonised fastest. In terms of biological efficiency, individually cotton hulls performed exceedingly well compared to other substrates. However, combinations of substrates revealed a combination of sawdust and cotton hulls to be superior to other combinations. Combinations of substrates proved to be highly efficient both in spawn run and biological efficiency compared individual substrates. In terms of economic return, combinations of sawdust and cotton hulls (5.7) proved to be the most profitable.

The higher proportion of proteins and amino acids in cotton husk contributed to its high biological efficiency. According to Ali(2019) cotton hulls are high in glycine(10-15%) and alanine(8-12%) essential amino acids contributing to the narrow C:N ratio.Sawdust, being rich in cellulose, hemicellulose and lignin (Table 2) have a wide C;N ratio. When combined with sawdust both extremes in carbon and nitrogen result in a synergistic effect boosting

molecular mitosis during spawn run which requires more carbon and protein synthesis which requires nitrogen to stimulate basidiospore formation.

Hence, we conclude that in terms of spawn run, yield and economic return combination of cotton hulls and sawdust is highly recommended for farmers involved in the cultivation of *Pleurotus ostreatus*.

Chapter 4

Article 3

Domestication of wild indigenous *Cantharellus densifolius* mushroom mycelia and development of a system for cultivation on ligncellulosic agricultural waste.

Silivani Elyson[1*], ChisangoTawanda Jonathan[1], Chitindingu Kudakwashe[1], and ChiguNomathemba Loice[1]

[1]Chinhoyi University of Technology, School of Health Sciences and Technology, Department of Biotechnology, P. Bag 7724, Chinhoyi, Zimbabwe

*Corresponding Author:elysonsilivani@gmail.com

Abstract

Cantherellus densifolius(Lactarius kabansus) commonly referred and known with a Shona name of *Nzevedzambuya* is a tropical brown shaped mushroom, belonging to the family *Basidiomycetes,* indigenous to Zimbabwean forests. In order to domesticate this wild mushroom, the influence of culture media, temperature and grain spawn were evaluated. Furthermore, performance of fruiting body was evaluated on sawdust and cotton hulls individually and in combination. Mycelia of most cultivated mushroooms such as Pleurotus ostreatus is known to grow best on potato dextrose agar with a pH of 7 cultured in plates incubated in dark conditions, at room temperature (28^0C), with no aeration. Mycelia exhibited a higher rate of growth on Potato dextrose agar at a rate of 22.5mm/day(4days) compared to Malt Extract agar(12.9mm/day)(7days) to completely cover the petri plates and exhibited a tendency to change colour from white to green after 10 days of incubation. It took 6 days for mycelia to completely cover *Sorghum bicolor* grains at 28^0C. All other substrates under investigation failed to produce fruiting bodies except for cotton hulls, producing green pinheads after 31 days of incubation. The phenomenon observed on the petri plates is a spore print colouration. Combination of sawdust and cotton hulls (50:50) produced *Cantherellus densifolius* with similar appearance to wild *C.densifolius* mushrooms with 21 fruiting bodies, with a mean yield of 90g/bag and biological efficiecy of 9%. *Cantherellus densifolius* exhibited a tendency to fruit only at the top of the bag and mushrooms appeared individually. This paper is the first report on the isolation, culture and cultivation of *Cantherellus densifolius* on agricultural wastes.

Keywords:, *Cantherellus densifolius*, *Nzevedzambuya*, mushrooms, mycelia, indigenous, wild, substrate, *Lactarius kabansus*

Introduction

Cantherellus densifolius now known as *Lactarius kabansus(*Pearce and Sharp,2000) is a wild edible mushroom commonly found growing on decaying leaves of trees and grass during the rainy season. Prevailing temperatures and moisture conditions during the short rainy season favors the growth of *C.densifolius*. The rainy season which starts in December and ends in March favors the growth of a wide range of wild mushrooms that naturally grow on forest litter, fallen logs, lawns, gardens and piles of agro-industrial residues. In Zimbabwe *C.densifolius* growth is most prevalent during the, onset , the middle and the end of the rainy season when enough moisture is available for spore germination, mycelial growth and fruit body formation. It is commonly referred to in the common language as *Nzevedzambuya* meaning ears of an old lady. This name might also have been coined as a reference to the outline of the cap which is rounded in the shape of an ear and the mushroom cap has a brownish appearance which is the natural skin colour of the natives in Zimbabwe. Furthermore the underside of the cap is densely packed with brown gills hence the name *densifoliu*s. In Zambia they refer to the mushroom as *kabansa* hence the name *Lactarius kabansus* (Hoffmann 2013).The inside of the cap is brown and very soft. The outside of the cap measures 2-8cm in diameter, the surface is blackish brown (Buyck et al 2011) The gills are originally white and turn green after picking. The stipe measures from 2 to 4 cm. The smell is weak,pleasant and fruity. When tasted raw they have a mild taste. The mushroom is very soft and the cap is easily broken. The mushrooms are rarely compact and being fragile the cap easily breaks. This is mainly due to the accumulation of water and failure of the lignin cell wall to support the basidium (Norrilinia 2023).

There are many gaps in research with respect to *C.densifolius*.*C.densifolius* belongs to the family *Basidiomycota* and class *Agaricomycetes*.Its culture conditions are unknown and production technologies are not yet established(De Crop 2017). In the wild *C.densifolius* mushrooms do not grow in clusters. This mushroom remains in the wild existing as spore in the dry season and as fruiting body in the rainy season. Fundamental information on the growth conditions and cultivation are still unknown, thus the need to conduct this study (Pegler and Piearce,1980).

Mushrooms in the wild are tended to or farmed by leaf cutting ants and termites. They have the capability to create weed free chambers underground where they store leaves and wood chips and accidentally drop fungal spores on them (Watling et al 2010)This is followed by crystallisation into a white tissue of mycelium colony. From this colony the flowering part of the mushroom appears under favourable conditions of temperature and humidity. In return the ants and termites gain food from the fungi in the form of mushrooms (Chang and Hayes 1978).

Cantherellus densifolius is under threat of extinction. The rapid growth in population in Zimbabwe is posing a great threat to natural biodiversity of mushrooms. Land cleared for

farming using fire inadvertently destroys fungal spores such that when the rain comes there are no mushrooms (Mwafaida et al 2022). The energy crisis has led to people seeking for other sources of energy such as firewood. As more and more trees are cut down food required for mushroom growth is limited and therefore there is a reduction in wild edible mushrooms. There is high prevalence of extinction of natural habitat of the wild species of edible fungi (Mbaluto 2015).

The growth of the mushroom industry requires acquisition of new strains either from the wild or developed through breeding techniques. This is necessary to achieve fast maturity period, increased resistance to pests and diseases and ultimately high yield (Ngokeu 2015). To improve productivity in the mushroom industry in Zimbabwe we need to introduce new mushroom species with improved characteristics such as high yields and increased resistance to pests and diseases (De Leon et al 2017).

Commercial production of fresh edible mushrooms is a rapid growing industrial activity. It is an easy way of producing food from lignocellulosic waste utilising the degrading capabilities of mushrooms.(Shweta et al 2021)

Problem statement and justification

The increase in demand for edible mushrooms has resulted in various mushroom training programs and setting up of many mushroom growth houses in urban areas of Zimbabwe. However, provision of quality spawn that is resistant to pests and diseases remains a huge constraint. Furthermore, mushroom spawn is not readily available in agricultural shops and supermarkets. In addition these exotic varieties require substrates with high nutrition such as cotton husks which are difficult to procure and transport. This reduces the total annual production of mushrooms in Zimbabwe. Rural communities end up relying on wild edible species that are only available in the rainy season, which unfortunately lasts for only 3 to 4 months.

The seasonality of wild edible mushrooms disqualifies them as a reliable source of nutrition. Furthermore lack of clear cut identification criteria and limited information on their genetic diversity results in unfortunate deaths of the ignorant because there is no true distinction between poisonous and non-poisonous wild mushrooms. As a result this draws back efforts to fully exploit wild edible mushroom for commercial production and breeding purposes.

There is a gap in research regarding to which substrates this fungi prefers. This mushroom has never been cultivated for the production of fruiting bodies. The nutritional composition of the fruiting body has not yet been investigated. Therefore this study was conducted to establish the optimum conditions for mycelia growth of *C.densifolius* and ultimately evaluate fruiting body performance on cotton hull and sawdust based substrate formulation.

Wild edible mushrooms have to be domesticated because of their potential use in the culinary and nutraceutical industry. Through domestication *C.densifolius* can be used to sustain livelihoods among local communities through production, sale of spawn and selling of mushrooms.

Apparently there are very few studies in Zimbabwe focusing on the domestication of wild edible mushrooms in Zimbabwe. Inorder to domesticate wild edible mushrooms such as *C.densifolius* there is need to seek knowledge on the physiological events in relation to their growth morphology as a prerequisite to developing large scale economic processes for their production.

The foundation for domesticating any wild edible mushroom is to establish optimal nutritional and physical parameters for basidiospore germination, mycelial growth and *basidiomata* formation.(Magday et al 2014). The present study seeks to clarify the steps in spawn production of *C.densifolius* with special attention on fruiting body formation as a foundation towards successful production technology for its cultivation in the country.

Zimbabwe is blessed with rich mycological resources that remain in the wild waiting to be rescued, domesticated and exported. At present of all wild edible mushroooms such as *Amanita, Termitomycetes* and *Cantherellus* species none has yet to be domesticated using different agro-industrial based substrates. The requirements for setting up of a production technology for wild mushrooms are culture media, medium composition, and optimum physical conditions such as temperature, illumination, moisture and aeration.(Dulay et al2017). Zimbabwe's native trains are faced by threats of extinction due to over harvesting during the rainy season. Furthermore, clearing of forests for agricultural purposes destroys habitats of wild mushrooms. The only solution to conserve natural wild edible mushrooms is by development of cultivation and production technologies to encourage propagation and conservation from extinction. Onyanga et al (2011) proposes that the most important factor in the domestication of wild mushrooms is spawn production. A clarification of nutritional and physiological needs of mushroom mycelia is essential to its domestication.

Wild indigenous mushrooms are direct sources of protein, vitamins and essential mineral elements to support health of human beings.(Dulay et al 2012) Despite the huge potential in wild mushrooms all potentially cultivated mushrooms with nutraceuticals potential, remain in the wild. Assimilation of these mushrooms by innovative production technologies will ensure food security, promote environmental protection and sustain livelihoods. It is therefore necessary to continuously come up with innovative ways of cultivating wild mushrooms to ensure conservation of wild genetic resources for health.

This research investigates optimum growth conditions of *C.densifolius*, determines the nutritional and physical requirements for mycelia growth and production of fruiting bodies. A prerequisite of domestication is a full understanding of the optimum conditions in terms of nutrition and physical parameters. This will then lead to the establishment of a successful production technology.

This study will also investigates possibility of cultivation and an evaluation of growth parameters of a Zimbabwean wild strain of *Cantherellus*. Collection and identification of wild mushrooms is not enough to ensure their conservation. Domestication of wild mushrooms will allow for value addition of agricultural waste and achievement of food

security. (Sharareh and Hamid 2016). Hence the bioconversion potential of wild *C.densifolius* will positively impact the mushroom industry.

This study will also characterise indigenous wild edible *C.densifolius* and develop a feasible and viable technology for its commercial production whilst utilising the abundant agricultural residues as substrate. To sustain production of native wild mushrooms at a commercial level there is need to develop a cultivation process considering substrate management, economic and cultural characteristics of the local population. (Shweta et al 2021) Therefore the aim is to identify, develop propagation spawn and domesticate wild edible *C.densifolius* in Zimbabwe.

Finally, it is necessary to generate a practical production technology that closely resembles the natural habitat of the mushroom *C.densifolius*, being a dead leaf /grass soil inhabiting fungi. In this study cotton hull and sawdust based formulations are evaluated for fruit body formation of *C.densifolius*. The objective being to demonstrate cultivation of rescued mycelia of wild *C.densifolius* on these agricultural residues.

Materials and methods

Methodology used is a modifiication of oyster mushroom cultivation based on mushroom cultivation strategies proposed by Kashangura **et al** (2005).

Source of mushroom strain

Wild fruiting bodies of *C.densifolius* were collected form forest area of Chinhoyi caves, Chinhoyi, Zimbabwe and brought to the Biotechnology laboratory at Chinhoyi University of Technology to rescue the mycelia. Internal tissues of the mushroom cap were aseptically isolated and inoculated onto different types of solid media namely PDA,MEA, TSA, NA, PGA,YEA and SDA. The cultures were covered with parafilm and incubated at 3 different temperatures in the dark until the plates were fully covered with mycelia. Pure cultures were then used as sources of inoculum for spawn production and substrate inoculation.

Study 1:Nutritional requirements for the mycelia growth of *Cantherellus densifolius*

Mycelial growth evaluation on different mycological culture media

The mycelia growth performance was evaluated using different commercial culture media, Potato Glucose Agar, Malt Extract Agar, Potato Dextrose Agar, Nutrient Agar, Trypticase soy agar, Saboraud Dextrose Agar and Yeast Extract Agar. Each culture media was prepared according to the specifications of the manufacturer and were filled up to 1 litre in Duran Schott botles. The media was then sterilised at 121^0C for 15 minutes in an autoclave. After sterilisation media were poured into petri plates. Then using a sterile blade the inside portion of the basidiocarp was aseptically transferred from the mushroom to the centre of the petri plate. The inoculated plates were divided into three groups with each representative media allocated into 3 separate incubators at 15^0C, 28^0C and 33^0C. A flamed sterile surgical blade was used to extract small internal tissues of *C.densifolius* and subsequently inoculated onto different types of media. Inoculated plates were sealed with parafilm and distributed into

different incubators. Successful cultures were used as sources of mycelia disks inoculants in the grain spawn. The diameter of mycelia growth was measured each day in millimetres using a ruler. Each setup was replicated 4 times.

Study 2.Physical requirements for Mycelial growth

Factors under investigation

Temperature

Light

pH

Aeration

Experimental design –Factorial Design

Table 1 Factorial design allocation of treatments to *C.densifolius*

MEDIA	PDA			MEA			TSA			NA			PGA			YEA			SDA		
temperature	15	28	32	15	28	32	15	28	32	15	28	32	15	28	32	15	28	32	15	28	32
Airation																					
No aeration																					
Light																					
No light																					

Treatments-10

Treatment levels:Temperature-3

Aeration-2

lighting-2

pH-commercial media pH

Study 3:Evaluation of mother spawn production at different temperatures on *Sorghum bicolor*

The grain spawn is a reservoir of nutrients for the mycelia and acts as a vehicle for transfer of mycelia from the grain spawn to the substrate. The grain has enough nutrients stored up to support mycelial growth. The grain spawn is a sustainable source of nutrients for the mycelia such that it facilitates rapid colonisation of the substrate thus reducing the spawn run.

Sorghum bicolor is reported by Jiskani et al(2007) to have the shortest incubation time of 6 days. Therefore the efficient growth of most commercial varieties of mushroom and newly introduced wild edible mushrooms is attributed to the nutrient composition of *Sorghum bicolor*. 100g of *Sorghum bicolor* contains 10.9 g protein, 3.2 g fat, 2.3 g crude fiber, 1 g ash, 329 kcal of energy, 27 mg calcium, 4.3 mg iron, 0.3 mg thiamin, 3.83 mg niacin and 0.138 mg rivoflavin. (De Leon 2017). Dulay et al (2020) transferred mycelia of wild *P.sanguineus* onto sorghum grains and it took 10 days for complete ramification of the grains. Furthermore sorghum grains are used extensively for spawn production of *Cantherellus sp* and *Pleurotus sp* .Since *Sorghum bicolor* has proven to be the best grain for spawn development we used it in this study.

The grains were sieved to remove dust grit and insects. They were then washed three times with clean water in a drum. The grains were soaked in clean water overnight then drained the following morning. The grains were boiled until tender but firm. Then the grains were dried until a moisture content of 60% was achieved. Then hydrated lime($Ca(OH)_2$) 2% was sprinkled on the grains to regulate pH and also to further sterilise them. The grains were then dispensed into clean jam jars, the jars were sealed tight and autoclaved at 121^0 C and 15 psi for 45 minutes. After autoclaving the bottles were immediately transferred to the laminar air flow cabinet for cooling. Once cooled the bottles were inoculated with 10mm diameter mycelia disc of *C. densifolius*. Bottles were incubated at three different temperatures 15, 28 and 32^0C. The spawn which is fully ramified would be characterised by a thick mycelia density.

Study 4:Evaluation of substrate formulation on fruiting body performance on cotton hull-sawdust based formulation

Individual substrates were weighed individually and soaked in water treated with 4% sodium hypochlorite and 2% hydrated lime in separate drums for 24 hours. Then the drums were overturned upside down with the lid loosened to permit water to drain. The substrates were then placed on drying racks to allow further water drainage until water capacity was 60%. Following drainage the substrates were mixed at ratio of 1:1 .

Spawning was carried out in transparent polyethene bags. 650 grams of substrate was the first layer then a handful of *C.densifolius* spawn was sprinkled on top of the layer until all the substrate was covered. Subsequently two more layers of substrate and two more layers of spawn were added to make a 2kg bag made up of 3 layers of substrate and 3 layers of spawn. Combinations were made separately and individual substrates of cotton hulls and sawdust were also made separately. Finally the mouth of each bag was secured tightly with string then small holes were punched on the top and the sides of each bag for aeration. The bags were then transferred to a steel mushroom house to allow for the spread of mycelia. On the onset of fruiting plastics were removed from the bags and watering commenced three times a day with a knapsack sprayer. Mature fruiting bodies were harvested and weighed to determine the biological efficiency.

Statistical analysis

All the treatments were laid out in a factorial design under laboratory conditions. One way analysis (ANOVA) was used to ascertain significant differences using least significant differences(LSD) at 5% level of significance. R software and Microsoft Escel 2007 were used for data visualisation and statistical analysis.

Results

Table 2:Mean values of yield and spawn run

Substrate	Spawn run(days)	First yield(g)
Cotton hulls	25	0
Sawdust	40	0
Cotton hulls and sawdust	33	90

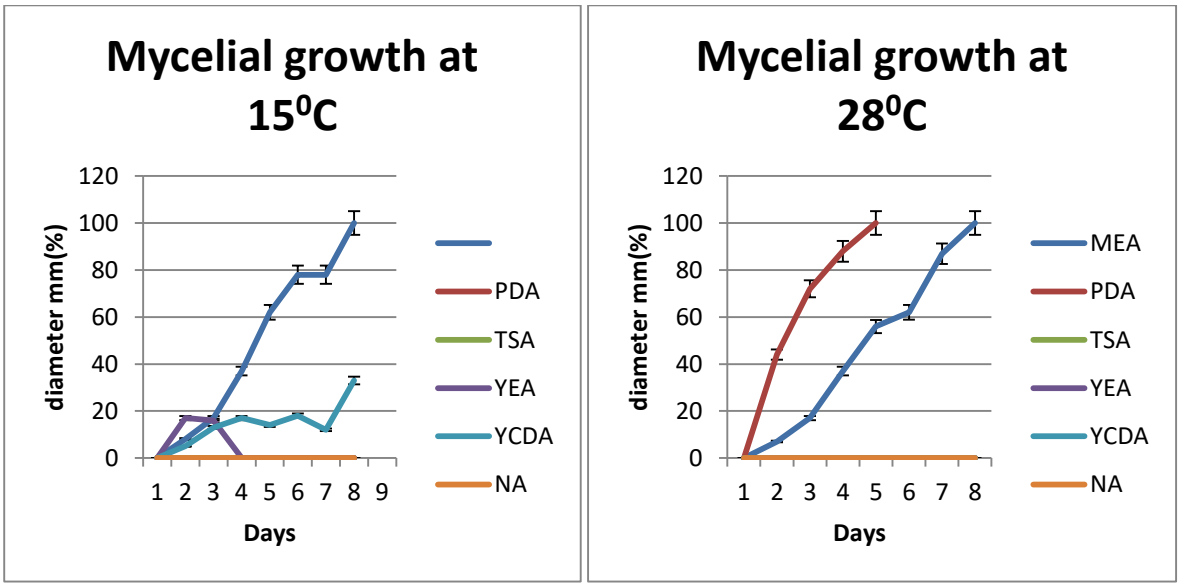

Fig 1.Spread of *C.densifolius* mycelia on different types of solid media over time

Observations of the green phenomenon occuring from pure culture production , to grain spawn production and on cotton hulls might be evidence of natural state of mycelia of C.densifolius in the wild.. It may also be related to photosynthesis, sequestration of carbon from the air. Ancestors of fungi were green coloured algae that had the ability to convert light energy from the sun into chemical energy using a green pigment called chlorophyll and enzymes.

The higher proportion of proteins and amino acids in cotton husk contributed to its high biological efficiency. According to Ali(2019) cotton hulls are high in glycine(10-15%) and alanine(8-12%) essential amino acids contributing to the narrow C:N ratio.Sawdust, being rich in cellulose, hemicellulose and lignin (Table 2) have a wide C;N ratio. When combined

with sawdust both extremes in carbon and nitrogen result in a synergistic effect boosting molecular mitosis during spawn run which requires more carbon . The availability of nitrogen stimulates basidiospore formation through protein synthesis

Malt extract agar contains malt extract which is rich in carbohydrates(maltose, gluose), amino acids(glutamic acid arginine) vitamins(vitamin B1 (thiamine), vitamin B2(riboflavin) and minerals(potassium magnesium, iron and zinc) . Maltose and glucose are reducing sugars that provide energy for fungal growth and vegetative reproduction during spawn run. Amino acids glutamic acid and arginine provide musch need buiding blocks for protein synthesis during basidiospore formation. Vitamins riboflavin and thiamine are essential micronutrients for fungal metabolism. Minerals act as enzyme cofactors for ezymatic reactions. Furthemore the hihg sugar content and slightly acidic pH in malt extract inhibits bacterial growth.

Potato dextrose agar contains potato extract(2%) and dextrose(2%). Pottato extract is rich in starch and dextrose providing energy for the growing mycelia. Amino acids asparagine, glutamic acid and arginine in potato dextrose agr provide assembly materials for protein synthesis. Vitamins B1(thiamine), niacin and B6(pyridoxine) are essential for fungal metabolism. Minerals potassium, magnesium iron and zinc are exxential cofactors for ezymes.

The similarities in amino acid, vitamin, high sugar content and slightly acidic pH make PDA and MEA suitable for isolating fungal spores from *C.densifolius*. However the differences in the type of sugar make PDA more effective compared to MEA. The carbohydrate composition of mat is maltose and glucose but the carbohydrate composition of dextrose is starch and gucose. Malt extract being rich in glucose and maltose forms a slightly acidic pH whereas dextrose containing starch has a close to neutral to slightly acidic pH. The neutral to slightly acidic pH of PDA provides a more suitable environment fror fungi to proliferate spores and form hyphae. Hence PDA was able to support growth of *C.densifolius* in 5 days compared to 8 days for MEA.

At 15^0C only MEA was able to support growth. According to solubility of dextrose decreases with temperature. Hence PDA fails to dissolve at 15^0C thereby limiting nutrient access. Malt extract already contains dissolved maltose and glucose that remain in solution even at low temperatures. Hence at low tempratures of 15^0C *C.densifolius* spores can be isolated using Malt extract agar.

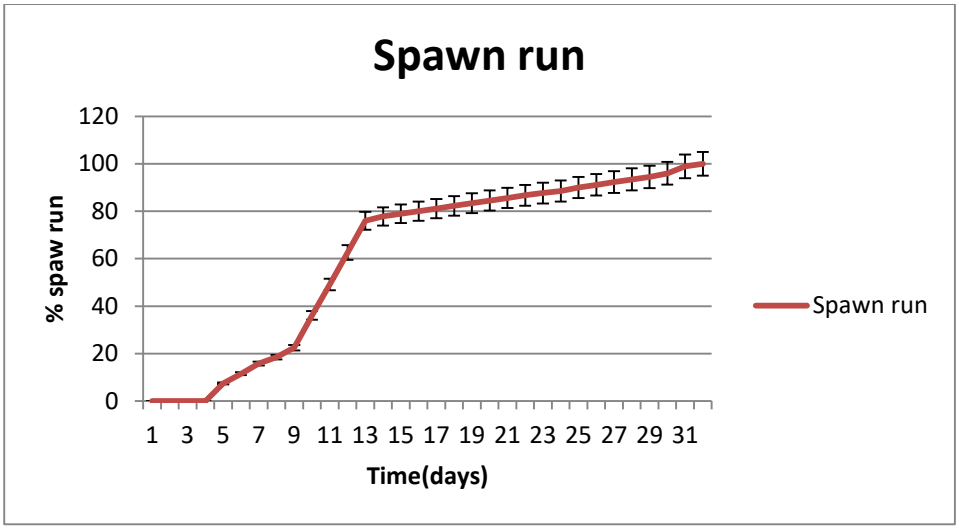

Fig2. Spread of *C.densifolius* mycelia on cotton husk and sawdust combination over time

Spawn run took 21 days on cotton hulls and sawdust combination. The first 4 days there was no visible growth. From day 4 to day 9 the rate of groth was 4%/day. Gradiebnt was steepest from day 9 to day 14 at a rate of 12%/day. The gradient from day 14 to day 31 is 1.4%/day which was the slowest.

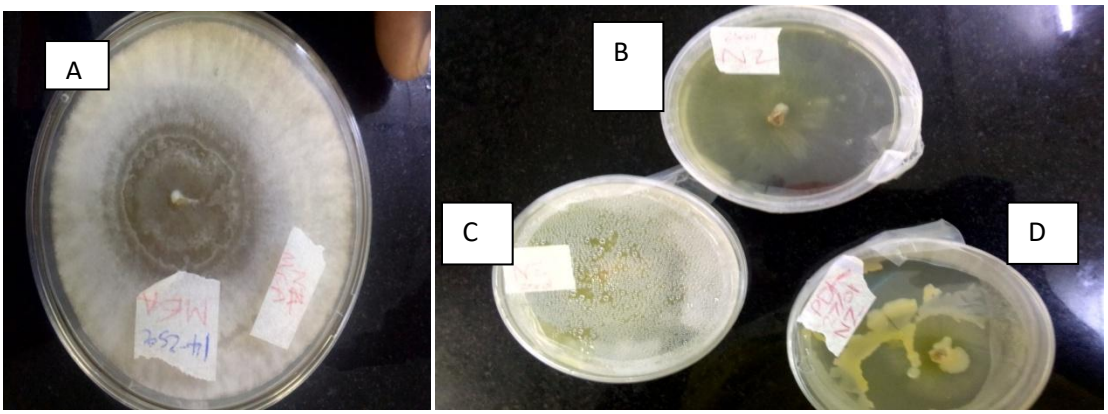

Fig3.Appearance of *C.densifolius* mycelia on PDA, MEA and *Sorghum bicolor*

Fig 4 are images of petri plates cultivated with *C.densifolius* mycelia on PDA nad MEA. Plate A is PDA with white fibrous mycelia of C.densifolius. Plate C is another PDA plate showing a fully covered plate culture with *C.densifolius*. Plate B is PDA plates with mycelia strands beginning to turn brown./ Plate D is an MEA plate also showing the brown green characteristic of *C.densifolius*. Plate Eshows MEA plate with *C.densifolius* mycelia beginning to turn green from white.

Mushrooms undergo heterotrophic nutrition. The phenomenon observed on the petri plates is a spore print colouration. It is a form of basidiospore pigmentation resulting from the introduction of fungi into a new environment. When fungi are introduced to a new environment they undergo evolution.(Kuo 2007) They switch from asexual reproduction to sexual reproduction. *Basidiomycota* changes colour through a process termed basidiospore colouration(Kuo 2014). They switch modes of reproduction to ensure spore dispersal. Sexual reproduction results in the dilution of genes leading to creation of new genetic variants. The green coloured spore is a much stronger variant than the white coloured spore. Spore colour varies from species to species for example white colour is dominant for *Agaricus bisporus* and *Pleurotus ostreatus*, yellow spore print for *Cantherellus cibarius* and *Boletus edulis* and *Chlorophyllum* species exhibit the green colour(Kuo 2009)

Chlorophyllum species of mushrooms exhibit green spore sprints. Mushroom members of this species include *Conocybe filaris*, *Pholotiona rugosa*, *Macropiota procera*, *Leococoprinus procera* and *Agaricus arvensis*. (Arora D, 1979). However *C.densifoliius* is not a member of the *Chlorophyllum* species of mushrooms. Therefore the theory of evolution through spore print colouration better explains observations captures in Fig 4.

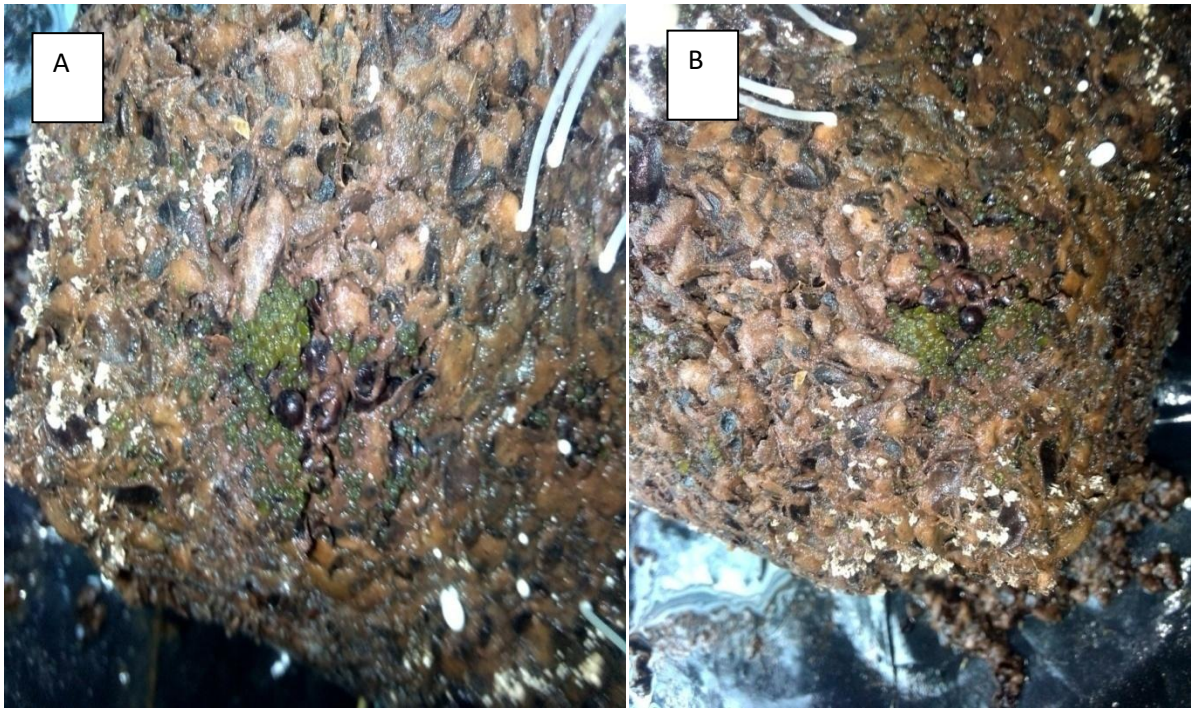

Fig4.Growth of *C. densifolius* on cotton husk

A-Bunches of green pinheads of *C.densifolius* observed on cotton husks after 39 days of incubation. White stems are inkcaps.

B-Bunches of small green pinheads observed on another bag of cotton husks. White spots of inkcaps also growing and stems of inkcaps growing as well.

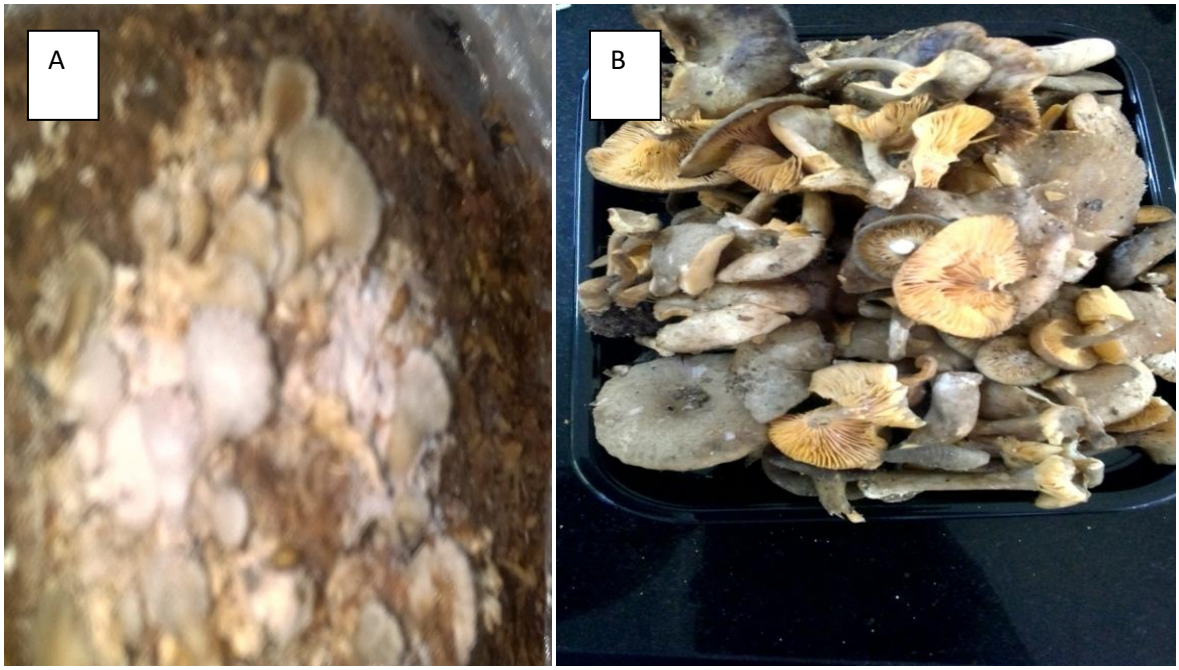

Fig 5.Brown mushrooms growing on cotton husk and sawdust

A-*C.densifolius* mushrooms growing on substrate composed of 50% cotton husk and 50% sawdust. The mushrooms obsereved exhibit similarities in colour and structure to the mushrooms on B.

B-*C.densifolius* collected from the wild. It has a brown circular cap, orange gills and very short stipes.There is a clear cut similarity between the wild collected *C.densifolius* and the ones cultivated on cotton hulls and sawdust combination.

Discussion

Mycelia performance on different mycological culture media

The mycelia growth performance of *C.densifolius* fumgi on the seven commercial culture media was evaluated. Potato dextose agar managed to support quickest rate of mycelia growth which resulted in complete coverage of the plates in 4 days. This was followed by Malt extract agar which was fully coveredwith *C.densifolius* fungi in 8 days.

Mycelia of wild *C.densifolius* was successfully rescued through tissue culture on Potato dextrose agar and Malt extract agar, this is evidence of presence of lignocellulosic enzymes. Furthermore spores of C.densifolius prefer starch sources of carbohydrate(dextrose) compared to malt. The mycelia was cottony with very long fine threads of hyphae which ran parallel to the culture media due to the presence of chitin in their cell walls. Most basidiodpore producing fungi of the phyllum basidiomycota exhibit cottony mycelia (Deacon 2006).

Pure cultures were used to produce spawn while the rest was stored as stock cultures in the fridge. On PDA the mycelia changed colour from white to brown then green after seven days of incubation. The change in colour might be a signal indicating a physiological shift from a vegetative stage to a reproductive stage. Three theories may explain this phenomenon. The first theory is physiological relationship to ancestry, the second is evolution, and the third is carbon sequestration.

The ancestors of basidiospore producing fungi were algae. Green algae evolvved into Chlorophyllum species of fungi that exhibit green coloured hyphae. In Fig 4we observed green coloured mycelia on PDA plates, green coloured mycelia on grains of *Sorghum bicolor*, and green pinheads on cotton husk substrate (Fig 5). The consistency of the green colour may be an indication that *C.densifolius* is a *Chlorophyllum* species. However this theory is discredited by Kuo (2009) who found that most *Chlorophyllum* species are poisonous and inedible.

The second theory is evolution. We may explain the green phenomenon as a survival mechanism employed by fungi when they are introduced intoa new environment. Kuo (2014) states that fungi employ sexual reproduction instead of vegetative reproduction so as to disperse spores with new genetic combinations that can qualify to survive in a new environment. The change from white coloured mycelia observed on Fig 4 changes to a green colour after 7 days of incubation. The green colour may be a more powerful genetic variant

capable of surviving more extreme changes in environmental factors. There is more merit to this theory

The third theory proposes the presence of chlorophyll pigment within *C.densifolius* as a result of a possible ancestral relationship between fungi and plants. The green phenomenon observed was only observed in Fig 5 at the fruiting stage when *C.densifolius* was cultivated on cotton hulls. *C.densifolius* might have employed carbon sequeastration to supplement limited quantities of carbon in cotton husk. It has been alluded before that cotton husk has the highest prortion of nitrogen compared to other substrates. This may also explain why there was succesful fruiting body formation on combination of cotton husk and sawdust.

Grain colonisation in 10 at 28^0C days is evidence of rapid mycelial growth. Compared to other species of basidiomycota such as *Pleurotus ostreatus* (7 days) Hoa et al (2015). Itelima et al (2011) alos states that a rapid mycelial growth is an indication of capability to outgrow competitors such as bacteria , fungi such as *Trichorderma* and pests such as scarid fly. In addition to vigorous mycelial growth C.densifolis exhibits tendency to form thick and dense mycelia. Dense mycelia can rapidly colonise Sorghum bicolour grains and can easily be transferred from gain spawn to substrate. *C.densifolius* can be captured as spawn.

Effect of Temperature on the rate mycelial growth

Temperature affects the rate of enzyme reactions of enzymes released during mycelial growth. Room temperature of 28^0C recorded the fastest rate of mycelial growth on PDA at 25mm/day and thick mycelial density. Growth was slowed at 15^0C and no growth was observed at 32^0C.

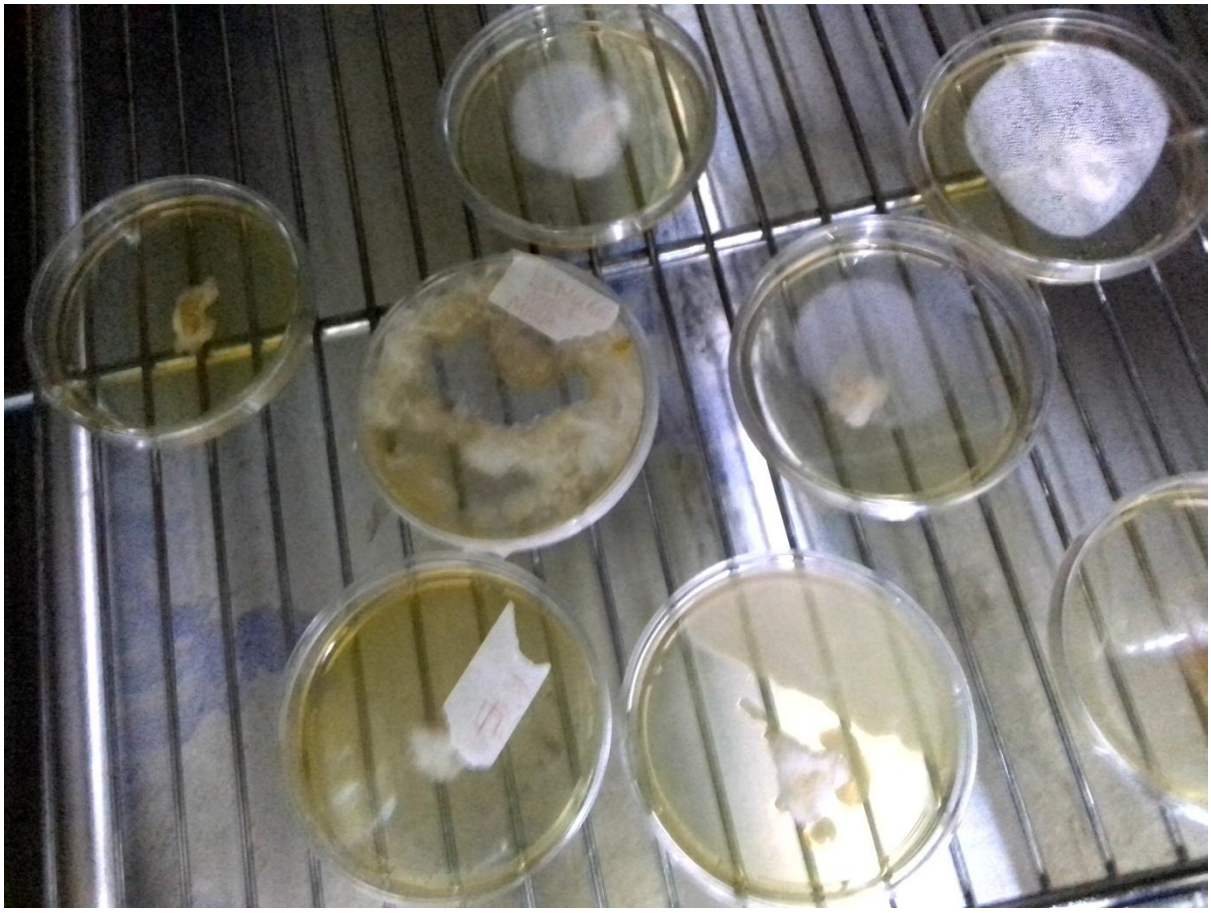

Fig 6 plates showing no growth in an incubator at 32^0C

These results are supported by findings by Verma and Bilgrami(2011)who purports that optimal range for mycelial growth is between 25^0C and 28^0C. Furthermore moist and warm conditions in the rainy season promote mycelial growth. In this investigation we used 3 different temperatures 15^0C, 28^0C and 32^0C. Enzymatic processes require optimum temperatures to maintain optimum growth. According to Kashangura et al 2005 there primary decomposing and secondary mushrooms. Primary decomposers break down dead plant matter and secondary decomposers break down animal waste. Primary decomposers such as *Pleurotus ostreatus* is an example of a primary decomposer with lignocellulosic enzymes working at an optimum of 28-32^0C. Secondary decomposers such as *Agaricus bisporus* require lower temperatures ranging from 15 to 25^0C. Hence our choice of temperatures ranging from 15 to 20 , 25-28 and 30 -32^0C.

Fruiting Development and Production

Given the biological efficiency of the first flush was very low, which means subsequent flushes will produce even lower yields.

The growth and development of *C.densifolius* on cotton hulls was observed as greed pinheads occurring on the lateral sides of the bag. The pinheads were green with a cap and a stipe measuring 0.5mm in diameter and 0.5mm in length. The green colour observed might be evidence of a physiological shift to a photosynthetic stage whereby the fruiting body utilises

chlorophyll related pigments to sequester carbon from the air. The pinheads also appeared singularly and crowded in certain regions of the bag. Upon further watering the pinheads turned brown and dried out without increase in size.

The growth and development of *C.densifolius* was exhibited limited to the top of the mushroom bag. The top of the bag had a notable increase in myelia density compared to the lateral sides of the bag. Furthermore, there appeared whitish pinheads with white pileus upon further watering the pinheads increased in size and became distinct mushroom caps turning brown and taking on a similar appearance to the natural indigenous wild edible *C.densifolius*. With a diameter of 10mm and a pileal length of 5mm there were smaller than the naturally occurring *C.densifolius*. The mushrooms quickly dried up in 24 hours and their weight was 90g on average for each bag.It took 31 days from the time of planting to the time the mushrooms were harvested. Only sawdust and cotton hulls combination managed to produce mushrooms with similar appearance to *C.densifolius*.

The results of this study reflect an interaction of substrates which increased the biological efficiency compared to sawdust and cotton hulls alone. Thus we recommend the addition of enriching supplements to further improve the yield and biological efficiency of *C.densifolius*.

In general any initial attempt at domestication of wild indigenous mushrooms often produces very low biological efficiencies. Magday et al (2014) cultivated wild indigenous *Ganoderma lucidum* on 70% rice straw and 30% sawdust and obtained a biological efficiency of 6.2%. Shweta et al (2021) cultivated wild indigenous *Schizophyllum commune* on paddy straw supplemented with wheat bran and obtained a biological efficiency of 18.33%. Dulay et al (2012) cultivated wild indigenous *Lentinus tigrinus* on rice and sawdust combination and obtained a biological efficiency of 15.93%. Enrichment of the best perfoming substrate in this study with commonly used supplements such as wheat bran, rice bran, soyabean cake and sunflower cake might significantly improve the biological efficiency of *C.densifolius*. Even though a very low biological efficiency was obtained in this study this is the first work to successfully demonstrate successful cultivation of fruiting body of *C.densifolius*.

Conclusion

This study introduces cultivation of an indigenous species of *C densifolius*. Malt extract agar and Potato dextrose agar are the best solid culture media for rescuing mycelia of *C.densifolius*. *Sorghum bicolor* supports the growth of *C.densifolius*.The results of this study provide proof that a naturally occurring strain of *C.densifolius* can be cultured in locally available agro-industrial waste and has great potential for large scale production. We managed to successfully rescue myelia from the basidiocarp of wild indigenous *C.densifolius*, demonstrate mycelial growth on different types of commercial solid culture media, demonstrate growth on grains of red sorghum (*Sorghum bicolor*) and successfully cultivated *C.densiflius* on sawdust and cotton hulls formulation obtaining a significant biological efficiency of 9%. A production system for cultivating *C. densifolius* has been established.

Recommendations

We recommend further studies into cultivation of other wild mushrooms such as *Amanita zambiana* and *Termitomycetes.* We suggest evaluations into secondary decomposition capabilities of *C.densifolius* through investigating its ability to decompose manure based substrate. We recommend supplementation as a strategy to increase the low biological efficiency. The physical conditions for fruiting body formation and nutritional supplements to increase biological efficiency need to be optimised.To minimise the spawn run, increase the biological efficiency and shorten the lag phase in the mushroom cultivation cycle there is need for a proper balance of pH, a high rate of spawning and appropriate nutrient composition (Magday et al 2014).

The biological efficiency obtained from this study of 9% pales in comparison to other commercially cultivated exotic mushrooms with very high biological efficiences of 76.3% for oyster and other native wild mushrooms in other countries on the same substrate composition. Therefore there is need for further optimisation studies into substrate enrichment to improve fruiting body formation and biological efficiency.

Chapter 5

General Discussion

5.1.1 Substrate evaluation for *Pleurotus ostreatus*

Among the individual substrates cotton hulls and sawdust have the highest carbon component however cotton hulls have the highest nitrogen component.which contributes to the high biological efficiency. Combining cotton hulls and sawdust triples the biological efficiency of sawdust because of the boost in nitrogen contributed by the cotton hulls.

Spawn run was fastest for wheatstraw(16days) due to high nutrient availability and aeration. As the degreee of aeration decreases as a result of compactnaess of the substrate there is increased longevity of the spawn run as observed for sawdust(20 days) as shown in Fig 1 chapter 3. Spawn run was also faster for banana fronds (17 days) also due to availability of aeration. Maizecobs and cotton husks managed to achieve the same rate of spawn runner due to compactness. It was observed that, irregardless of nutrient availability particle size of substrate directly affects availability of air pockets within the substrate thereby limiting aiflow and affecting rate of fermentation. The more compact a substrate is the lesser the airation results in a slow spawn run.

Aspects of aeration, surface area, nutrition and spawn quality all affect the performance of a particular combination of substrates.An ideal substrate should merit a high biological efficiency and short spawn run. Moreover, a ratio of cost over benefit ratio would be very accurate in providing information about profitability to a farmer.

In terms of biological efficiency, individually cotton hulls performed exceedingly well compared to other substrates. However, combinations of substrates revealed a combination of sawdust and cotton hulls to be superior to other combinations. Apparently there is an exponential increase in the cost of individual substrate such as cotton husk. Our results will lessen the cost of buying cotton husk by diluting it with sawdust and other less biologically efficient substrates.

We came up with the biological efficiency to the spawn run ratio using the theoretical values of the biological efficiency and the spawn run. It explains the time taken for a certain quantity of fresh mushrooms to be harvested from a kilogram of dry substrate. It can be observed that

a high BE/SR ratio results from a combination of a high biological efficiency and a short spawn run.

The decrease in the cost of cotton hulls will reduce the cost of oyster mushrooms . Currently a punnet of oyster mushrooms has increased from $1USD per punnet to $1.50 per punnet in our major supermarkets. Using these combinationns we can reduce the price of a punnet of mushrooms by 33%. The main goal is to solve malnutrition provide an alternative source of protein at a very low price. The market is ready for the introduction of this new substrate, however there is need for training and workshops to equip farmers with this knowledge.

5.1.2Domestication of *C.densifolius*

Only sawdust and cotton hulls combination managed to produce mushrooms with similar appearance to *C.densifolius*. The growth and development of *C.densifolius* on cotton hulls was observed as greed pinheads occurring on the lateral sides of the bag. The pinheads were green with a cap and a stipe measuring 0.5mm in diameter and 0.5mm in length. The phenomenon observed is spore print colouration whereby mushroom species undergo evolution as a strategy to adapt to a new environment characterised by the new substrate.

Most of our indigenous mushrooms are only available during the rainy season. Our results have revealed that it is possible to cultivate an indigenous mushroom C.densifolius on locally available combinations of substrate throughout the year. There is potential for exportation of this mushroom inreasing our gross domestic product. However the biological efficiencyy has to be improved before farmers can be trained on how to cultivate this mushroom.

5.2 Conclusion

5.2.1Substrate evaluation for *Pleutotus ostreatus*

In terms of biological efficiency, individually cotton hulls performed exceedingly well compared to other substrates. However, combinations of substrates revealed a combination of sawdust and cotton hulls to be superior to other combinations.

The higher proportion of proteins and amino acids in cotton husk contributed to its high biological efficiency. According to Ali(2019) cotton hulls are high in glycine(10-15%) and alanine(8-12%) essential amino acids contributing to the narrow C:N ratio.Sawdust, being rich in cellulose, hemicellulose and lignin (Table 2) have a wide C;N ratio. When combined with sawdust both extremes in carbon and nitrogen result in a synergistic effect boosting

molecular mitosis during spawn run which requires more carbon and protein synthesis which requires nitrogen to stimulate basidiospore formation.

Aspects of nutrition and spawn quality all affect the performance of a particular combination of substrates.An ideal substrate should merit a high biological efficiency and short spawn run. Economically, a high benefit over cost ratio is profitable to a farmer.

A substrate formulation for increased, effiecient, profitable and sustainable *Pleurotus ostreatus* cultivation in Zimbabwe has been established. Assessments on growth of *C.densifolius* and *P.ostreatus* using spawn run and biological efficiency revealed that combinations of sawdust and cotton hulls is most profitable.

5.2.3Domestication of *Cantherellus densifolius*

This study introduces cultivation of an indigenous species of *C densifolius*. Malt extract agar and Potato dextrose agar are the best solid culture media for rescuing mycelia of *C.densifolius*. Wild indegenous *Cantherellus densifolius*spores can be successfully extricated from the caps, cultured on solid agar media and transferred to *Sorghum bicolor* grains. *The Cantherellus densifolius* spawn can be cultivated on combination of sawdust and cotton hulls to reproduce *Cantherellus densifolius* mushroom caps. The ability of *C.densifolius* to grow on cotton hulls nd sawdust qualifies it as a primary decomposer.

In literature most initial attempts at domestication of wild indigenous mushrooms often produces very low biological efficiencies. Magday et al (2014) cultivated wild indigenous *Ganoderma lucidum* on 70% rice straw and 30% sawdust and obtained a biological efficiency of 6.2%. Shweta et al (2021) cultivated wild indigenous *Schizophyllum commune* on paddy straw supplemented with wheat bran and obtained a biological efficiency of 18.33%. Dulay et al (2012) cultivated wild indigenous *Lentinus tigrinus* on rice and sawdust combination and obtained a biological efficiency of 15.93%. Enrichment of the best perfoming substrate in this study with commonly used supplements such as wheat bran, rice bran, soyabean cake and sunflower cake might significantly improve the biological efficiency of *C.densifolius*. Even though a very low biological efficiency was obtained in this study this is the first work to successfully demonstrate successful cultivation of fruiting body of *C.densifolius*.

The results of this study provide proof that a naturally occurring strain of *C.densifolius* can be cultured in locally available agro-industrial waste and has great potential for large scale production.

5.3Recommendations

5.2.1Substrate evaluation for Pleutotus ostreatus

Further dowstream processing of pelleted substrate into pellets will help in storage, pasteurisation and marketing of substrate. Financial constraints hindered us from fulfilling this objective.

Further studies into other types of agricultural wastes such as soybean meal, sunflower cake and sugarcane bagasse as substrates combinations for cultivating *Pleurotus ostreatus*.

5.3.2Domestication of Cantherellus densifolius

We recommend further studies into cultivation of other wild mushrooms such as *Amanita zambiana* and *Termitomycetes.* We suggest evaluations into secondary decomposition capabilities of *C.densifolius* through investigating its ability to decompose manure based substrate. We recommend supplementation as a strategy to increase the low biological efficiency. The physical conditions for fruiting body formation and nutritional supplements to increase biological efficiency need to be optimised.To minimise the spawn run, increase the biological efficiency and shorten the lag phase in the mushroom cultivation cycle there is need for a proper balance of pH, a high rate of spawning and appropriate nutrient composition. (Magday et al 2014)

The biological efficiency obtained from this study of 9% pales in comparison to other commercially cultivated exotic mushrooms with very high biological efficiencies of 76.3% for oyster and other native wild mushrooms in other countries on the same substrate composition. Therefore there is need for further optimisation studies into substrate enrichment to improve fruiting body formation and biological efficiency.

Research into special mushroom growth house designs, with hardware installed to control physical factors such as light, temperature, moisture and aeration will further improve results collected in this study.

More funding neds to be injected into mushroom research to circumvent changes in the climate. El-nino induced drought and erratic rainfall patterns have affected the natural growth patterns of wild edible indigenous mushrooms.

REFERENCES

Abreu,(2020), *Banana (Musa spp) Leaf and stem, A review on chemical composition and utilisation*, Journal of food science and technology, 57(4), 1220-1233

Adjapong A, Abena O. Kwame D. Ansah, Faustina A, and Henry O. Sintim, (2015) *Maize Residue as a Viable Substrate for Farm Scale Cultivationmof Oyster Mushroom(Pleurotus ostreatus)*, Hindawi Publishing Corporation Advances in Agriculture Volume 2015, Article ID213251, http://dx.doi.org/10.1155/2015/213251

Alexopoulus C.J, Mims C.W ., (1979), Introductory Mycology, Wiley , ISBN:0471022144 available at https://books.google.co.zw/books/about/Introductory_Mycology.html?id=EHrwAAAAMAAJ&redir_esc=y

Ali (2019) *Cotton production and processing* CRC Press)

Amara (2019), Chemical composition of maizecobs, Journal of Agricultural Science, 17(2), 257-265. DOI:10.5539/jas.v17n2p257

Arora David, (1979), Muushrooms Demystified, 1st edition, Ten Speed Press

Barh A, Sharma V.D , Sudheer K. A, (2020), Growing mushrooms-A technical guide,,ICAR-Directorate of Mushroom Research, India Available at https://www.reseachgate.net/publication/36962586

Bertelsen Cynthia,(2013), "Mushrooms": A Global History

Besufekad Yohannes, Mekonnen Abraham, Girma Bikila, Daniel Robel, Getahun Tassema, Melkamu Jale, Asefa, Malesu Fikiru Tsehaynesh, Denboba Lalise,(2019) *Selection of appropriate substrate for production of oyster mushroom* (*Pleurotus ostreatus*)..... College of Natural and Computational Science, Department of Biotechnology, Wolkite University, Ethiopia.

Bhattacharjya, D. K., Paul, R. K., Miah, M. N. and Ahmed, K. U. (2015) *Comparative Study on Nutritional Composition of Oyster Mushroom (Pleurotus ostreatus Fr.)* Cultivated on Different Sawdust Substrates. *Biores Comm.* **1**(2), 93-98.

Buyck,(2011), *Lactarius kabansus, a new species from tropical Africa*, Fungal Biology, Vol. 111, Issue 3.

Callow Brian,(2020), *WHAT THE FUNGI, Avaible online at:*https://m.youtube.com/watch?v=GXxAtUV38b4 Accessed 19/11/2020

Chang S.t, Cheung P.C.K, Wong Y.s, Wong K.h, Ng T.B, Liu J.J,(2017),Strain selection for improved yield and quality in oyster mushroom cultivation. Journal of Fungi, 3(3), 39

Chang S.T and Hayes W.A ,(1978), The Biology and Cultivation of Edible Mushrooms, ISBN;9780121680503, Academic Press

Chukwurah, N. F.1, Eze, S. C, Chiejina, N. V., Onyeonagu, C. C.2, Ugwuoke, K. I., Ugwu, F.
S. O.1, Nkwonta, C. G.1, Akobueze, E. U, Aruah, C. B.and Onwuelughasi,(2012) C. U.*Performance of oyster mushroom (Pleurotus ostreatus) in different local agricultural waste materials*, African Journal of Biotechnology Vol. 11(37), pp. 8979-8985, DOI: 10.5897/AJB11.2525 ISSN 1684–5315 Available online at http://www.academicjournals.org/AJB

De Crop E,(2017), *Taxonomy and Ecology of Lactarius Species in Tropical Africa*, University of Ghent

De Leon AM, Guinto LJZG, De Ramos PDV, Kalaw SP (2017)*Enriched cultivation of Lentinus*
squarrosulus (Mont.) Singer: A newly domesticated wild edible mushroom in the Philippines. Mycosphere 8(3), 615–629, Doi 10.5943/mycosphere/8/3/9:

Deacon, J.W.(1997), Modern Mycology, 3rd edition Blackwell Science

Dubey D, Dhakal B, Dhami K, Sapkota P, Rana M, Poudel NS, Aryal L (2019*) Comparative study on effect of different substrates on yield performance of oyster mushroom*, GJBAHS 7: 7.

Dulay Rich Milton R. Arthur Grisha C. Rivera, Erlyn Jane B. Garcia,(2017) *Mycelial growth and basidiocarp production of wild hairy sawgill Lentinus strigosus, a new record of naturally occurring mushroom in the Philippines*, Center for Tropical Mushroom Research and Development, Department of Biological Sciences, College of Arts and Sciences, Central

Luzon State University, Science City of Munoz, Nueva Ecija 3120, Philippines, Biocatalysis and Agricultural Biotechnology http://dx.doi.org/10.1016/j.bcab.2017.03.017

Food and Agriculture Organisation (FAO) OF THE United Nations,(2011), Global food losses and Food waste.

Girmay, Z., Gorems, W., Birhanu, G.(2016).. *Growth and yield performance of Pleurotus ostreatus (Jacq. Fr.) Kumm (oyster mushroom) on different substrates*. *AMB Expr* **6**, 87 https://doi.org/10.1186/s13568-016-0265-1

Govera C.,EcoAfrica,(2020), Chido Govera teaches young Zimbabwean women the art of Mushroom farming, **Availableonlineat https://m.youtube.com/watch?v=nDud2g1RVY accessed 15/06/2020**

Government of Zimbabwe,(2021), National Development Strategy(2021-2025)

Piearce G, Sharp Cathy (2000,) *Vernacular Names Of Zimbabwean Fungi: A Preliminary Checklist* Kirkia , Vol. 17, No. 2 (2000), pp. 219-228 Published by: National Herbarium & Botanic Garden Stable Available online at URL: https://www.jstor.org/stable/2350231

Gume Beje, Muleta Diriba, Abate Dawit, *(2013)Evaluation of locally available substrates for cultivation of oyster mushroom (Pleurotus ostreatus) in Jimma, Ethiopia,*African Journal of Microbiology Research Vol. 7(20), pp. 2228-2237, 14 May, 2013 DOI: 10.5897/AJMR12.895 ISSN 1996-0808

Haziran,(2019),*Cottonseed hulls:Oyster mushroom cultivation,* available online at https://www.kulturkia.com/2019/06/cottonseed-hulls-mushroom-compost.html Accessed 16/01/2022

Hoa Ha Thi, Li Wang Chun-,Chong-Ho Wang (2015*) The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (Pleurotusostreatus and Pleurotuscystidiosus),* Mycobiology, 43:4, 423-434, DOI:10.5941/MYCO.2015.43.4.423
Available online at : https://doi.org/10.5941/MYCO.2015.43.4.423

Hofmann V,(2013), *Mushrooms of Africa.*

Islam Waqar, Riaz Asif, Zaynab, Zujian Madiha Wu,(2016) *Efficacy of different weeds and agricultural substrates for cultivation of pleurotus ostreatus (JACQ. FR.),* Asian J Agri Biol, 2016, 4(4): 108-113.

Itelima J. U.,(2011), Cultivation of mushroom (Pleurotus ostreatus) using corncobs and saw dust as the major substrates , Global journal of agricultural sciences vol 11, no. 1, 2012: 51-56 DOI: http://dx.doi.org/10.4314/gjass.v11i1.9

Jiskani M.M., M. I. Bhatti, K. H. Wagan, M. A. Pathan, A. G. Bhatti,(2007) *Determination of sorghum grains for spawn growth of oyster mushroom, pleurotus ostreatus (jacq. Ex. Fr) kummer*, Department of Plant Pathology, Sindh Agriculture University, Tandojam, Pakistan.

Kashangura C, Kunjeku , E.C, Mabveni, A.R.S, Chirara T, Mswaka, A, Manjolo-Dalu, V, (2005), *Manual for mushroom cultivation*, Biotechnology Trust of Zimbabwe, Harare, Zimbabwe.

Kavitha R, Sivakumar N, Velan M, Sivakumar K, (2018), Optimisation of C:N ratio for co-composting of sawdust and sewage sludge. Journal of Environmental Science and Health, Part B, 53(6), 399-406

Kavhumbura Gamuchirai,(2022), Development of protocols for the domestication wild edible mushrooms and the proximate nutriitional analysis of the wild edible mushrooms, CUT REPOSITORY.

Kumar A, Singh J, Sharma S, (2020).*Chemical composition and carbon content of wheat straw*, Journal of agriculture and food chemistry,

Kuo Michael,(2007-2014), Mushroom identification trilogy,

100 edible mushrooms,(2007),ISBN 978-0-472-03126-9

100 poisonous mushrooms,(2009), isbn 978-0-472-03127-6

Mushrooms of the Midwest and the Northeast,(2014) ISBN 978-0-472-11829-6

Lentz Ed, Lindsey L,(2016), *Nutrient value of wheatstraw*,Agronomic Crops Network, Ohio State University, College of food agricultural and environmental sciences, available online at : https://agcrops.osu.edu/newsletter/corn- newsletter/nutrient-value-wheat-straw Accessed 16/01/2023

Kennedy , Tapfumaneyi Linda , Mutetwa Moses, Ngezimana Wonder,(2021) *The Effects of Different Substrate Combinations on Growth and Yield of Oyster Mushroom (Pleurotus ostreatus)* Hindawi International Journal of Agronomy Volume 2021, Article ID 9962285, Available online at https://doi.org/10.1155/2021/9962285

Magday Jr. JC, Bungihan ME, Dulay RMR 2014 – *Optimization of mycelial growth and cultivation of fruiting body of Philippine wild strain of Ganoderma lucidum*. Current Research in Environmental & Applied Mycology 4(2), 162–172, Doi 10.5943/cream/4/2/4

Marek Siwulski, Przemyslaw Niedzielski, Malgorzata Szostek, Anna budka, (2024), Mineral composition variation in Boletales mushrooms-indication of soil properties and taxonomic influence, Environmental Science and Pollutioon Research 31(28), DOI:10.1007/S11356-024-33916-4 available at https://www.researchgate.net/publication/381258516_Mineral_composition_variation_in_Bolates_mushrooms-indication_of_soil_properties_and_taxonomic_influenceMuswati Charles, Simango

Melkamu Jale, Asefa, Malesu Fikiru Tsehaynesh, Denboba Lalise,(2019) *Selection of appropriate substrate for production of oyster mushroom* (*Pleurotus ostreatus*)..... College of Natural and Computational Science, Department of Biotechnology, Wolkite University, Ethiopia.

Mbaluto Crispus M,(2015),*characterisation and development of propagation spawns for selected wild edible mushrooms from aberdare national forest*, Kenya

Milton D.R, Damaso E.J,(2020), *The successful cultivation of Philippine wild mushroom Pycnoporus sanguineus (BIL7137) using rice straw and sawdust-based substrate*, Bioassay Laboratory, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. Journal of Applied Biology & Biotechnology Vol. 8(05), pp. 72-77, Sep-Oct, 2020 Available online at : http://www.jabonline.in
DOI: 10.7324/JABB.2020.80509

Milton D.R., Sofronio P. Kalaw, Renato G. Reyes, Esperanza C. Cabrera, Noel F. Alfonso (2012), *Optimization of Culture Conditions for Mycelial Growth and Basidiocarp Production of Lentinus tigrinus (Bull.) Fr., A New Record of Domesticated Wild*

Edible Mushroom in the Philippines, PHILIPP AGRIC SCIENTIST ISSN 0031-7454 Vol. 95 No. 3, 278–285

Moore D Fungal photobiology, A review, Mycological research, 102(6),655-655

Mshandete AM, Cuff J,(2008) *Cultivation of three types of indigenous wild edible mushroomsCoprinus cinerius, Pleurotus flabellatus and Volvariella volvoceaon composted sisal decortications residue in Tanzania*. Afr J Biotechnol. 2008; 7(240:4551-62

Mudzikitiri, (2021), *personal communication, identification of edible wild mushrooms*, Treelawny, Banket, Zimbabwe.

Muswati Charles, Simango Kennedy , Tapfumaneyi Linda , Mutetwa Moses, Ngezimana Wonder,(2021) *The Effects of Different Substrate Combinations on Growth and Yield of Oyster Mushroom (Pleurotus ostreatus)* Hindawi International Journal of Agronomy Volume 2021, Article ID 9962285, Available online at https://doi.org/10.1155/2021/9962285

Mwafaida Joseph, Okoko Miguel, Kabugu Emma, Kahindi James H.P, (2022), Domestication of indigenous edible mushrooms using thhe open nursery systemm, Pwani University, Kilifi Kenya, Afr. J. Hort. Sci j.mwafaida@pu.ac.ke

Naraian R, Singh M. P2, Ram S, (2016), *Supplementation of Basal Substrate to Boost up Substrate Strength andOyster Mushroom Yield: An overview of Substrates and Supplements, International Journal of Current Microbiology and Applied Sciences*ISSN: 2319-7706 Volume 5 Number 5 (2016) pp. 543-553, Available at: http://dx.doi.org/10.20456/ijcmas.2016.505.056Ramsbottom J,(1954),*Mushrooms and Toadstools:A study of the activities of fungi*.Collins,London.

Ngokeu A.M, (2015), *Mycorrhizial Fungi Associated with Ebony Trees in Cameroon*, University of Yaunde

Niazi A, R and Ghafoor A, (2021), Different ways to exploit mushrooms: A review, All Life https://doi.org/10.1080/26895293.2021.1919570

Norrilinia (2023),*Zambian mushrooms*, Available online at https://www.luomus.fi/sites/default/files/files/zambianmushrooms.pdf

Onyango B.O.1, Palapala V.A.2, Arama P.F.1, Wagai S.O.1 and Gichimu B.M.3,(2011), *Morphological characterization of Kenyan native wood ear mushroom [Auricularia auricula*

(L. ex Hook.) Underw.] and the effect of supplemented millet and sorghum grains in spawn production, Department of Botany and Horticulture, Maseno University, Kenya, Department of Biotechnology, Masinde Muliro University, AGRICULTURE AND BIOLOGY JOURNAL OF NORTH AMERICAK doi:10.5251/abjna.2011.2.3.407.414

Ntezirayo Vincent, Anthony M Mshandete, Donatha D Tibuhwa, (2024), Successful domestication pf Neonothopanus Hygrophanus (Mont) De Kesel and Degreef and Lentinus Squarrolus Mont., indigenous saprophytic edible mushrooms from Kibira National Park in Burundi, CABI Agriculture and Bioscience (2024) 5:7 https://doi.org/10.1186/s4170-023-00212-z

Onyeka, E. U., Udeogu, E, Umelo, C.Okehie, M. A(2017) *Effect of substrate media on growth, yield and nutritional composition of domestically grown oyster mushroom (Pleurotus ostreatus)*Department of Food Science and Technology, Federal University of Technology, Owerri, Imo State, Nigeria. 2 Department of Food Science and Technology, Abia State University Uturu, Nigeria.**http://www.academicjournals.org/AJPS**

Pant D, Reddy UG, Adholeya A, (2006), *Cultivation of oyster mushroom on wheat straw and bagasse substrate amended with distillery effluent*. World Journal of Microbiology and Biotechnology.

Patel H, Shah R.N, Dave, S.R, (2020), Optimisation of C/N ratio for cultivation of Pleurotus ostreatus on sugarcane bargasse,Bioresource Technology, 306, 123011

Ramsbottom J, (1954),*Mushrooms and Toadstools:A study of the activities of fungi*.Collins,London.

Rediet Sitotaw, Ermias Lukekai, Dawit Abate, (2020), Ethnomycological study of edible and medicinal mushrooms in Menge District, Assossa Zone, Benshangul Gumuz Region, Ethiopia, J Ethnobiol Ethnomed DOI:10.1186/s13002-020-00361-9 Available at : https://doi.org/10.1186/s13002-020-00361-9

Rynk, R., ed. 1992. *On-Farm Composting Handbook*. Northeast Regional Agricultural Engineering Service.. *Available on-line at:* http://www.cals.cornell.edu/dept/compost/OnFarmHandbook/apa.taba1.html , *or in print from:* Northeast Regional Agricultural Engineering Service, 152 Riley-Robb Hall, Cornell University, Ithaca NY 14853.

Sayner, A, Grocycle(2019),*5 things I wish I knew before I started growing mushrooms - youtube*,**Availableonlineat https://m.youtube.com/watch?v=nDud2g1RVY accessed 15/06/2020**

ScienceMate,(2022),*Biogeology and Heat Generation*,NGSS available online at https://www.msnucleus.org/membership/ngss/fourth_ngss/04greens_browns.html

Accessed 16/01/2023

Sharareh Rezaeian, Hamid R. Pourianfar,(2016), *A Comparative Study on Bioconversion of Different Agro Wastes by Wild and Cultivated Strains of Flammulina velutipes*, Waste Biomass Valor DOI 10.1007/s12649-016-9698-7

Sharma S, Singh V.K, Kumar. A, Thakur M.P, Singh R, (2020), Nutritional analysis of oyster mushrooms grown on different substrates, Journal of Food Science and Technology, 57(5), 1510-1518

Shweta Singh a, Chandramani Raj a, Harvinder Kumar Singh b, Ravi Kant Avasthe a, Prashant Said c, Arumugam Balusamy d, Susheel Kumar Sharma e, Sangay Chuzem Lepcha a.

Stamets Paul,(2018), "Fungi", A Very Short Introduction Tapiwa,Grozim Mushrooms.,(2021), *personal communication, productivity of oyster mushrooms*, Marlborough, Harare, Zimbabwe.

Tesfaw A., Tadesse A, Kiros G., (2015),*Optimization of oyster (Pleurotus ostreatus) mushroom cultivation using locally available substrates and materials in Debre Berhan, Ethiopia*, Available online at http://www.jabonline.in

Tovell Jonathan,(2021),*Growing mushrooms indoors: Incubation and grow rooms*,JCB Gourmet mushrooms.

Varghese Bably Pearl, Amritkumar Pavithra,(2020)Comparative Study on Cultivation of Oyster Mushrooms using Nutrition Enhancing Substrates, International Journal of Scientific Research. in Biological Sciences Vol.7, Issue.2, pp.105-111, April (2020) **E-ISSN:** 2347-7520 DOI: https://doi.org/10.26438/ijsrbs/v7i2.105111

Varsha Kerketta,(2021*), Characterization and development of cultivation technology of wild split gill Schizophyllum commune mushroom in India*, Department of Processing and Food Engineering, College of Agricultural Engineering and Post Harvest Technology (CAU, Imphal), Ranipool, Gangtok, Sikkim 737135, India, https://doi.org/10.1016/j.scienta.2021.110399

Verma R.N. and K.S.Bilgrami,(2011),Physiology of fungi,,3rd edition,Scientific Publishers India, www.scientificpub.com (450-465)

Vijay R,(2022), Button mushroom(Agaricus bisporus, Crop management, Directorate of mushroom research,India, Available at https://dmrsolan.icar.gov.in/mushroompprofile/button_Mushroom/button_mushroom.html

Vieira F. R , Meire C N, (2016), *Optimization of substrate preparation for oyster mushroom (Pleurotus ostreatus) cultivation by studying different raw materials and substrate preparation conditions (composting: phases I and II),* World J Microbiol Biotechnol (2016) 32:190, DOI 10.1007/s11274-016-2152-y

Wang Q, Liu X, Zhang J, Li H, Chen M, (2018), Effects of C/N ratio on growth and yield of oyster mushrooms on corncob substrate, Journal of Environmental Science and Health, Part B, 53(6), 407-416

Watling R,(2010),*Fungi of Tropical Africa*

Webster, J & Weber RWS(2007), *Introduction to Fungi*.3rd edition. Cambridge University Press

Wendiro Deborah, Alex Paul Wacoo, Graham *Wise Identifying indigenous practices for cultivation of wild saprophytic mushrooms: responding to the need for sustainable utilization of natural resources* Journal of Ethnobiology and Ethnomedicine (2019) 15:64 https://doi.org/10.1186/s13002-019-0342-z

Wiley Blackwell (2019), *Biomass to biofuels*, Strategies for global industries.

Yashwant Singh Parmar,(2022), *Oyster mushroom*, University of Horticulture and Forestry, Solan, Nauni,India

Zakil Fathie Ahmad, Sueb Mohd Shafiq Mohd,Isha Ruzinah (2019)*Growth and yield performance of Pleurotus ostreatus on various agro-industrial wastes in Malaysia*, AIP Conference Proceedings 2155, 020055 https://doi.org/10.1063/1.5125559

Zimbabwe Ministry of Agriculture, Mechanisation and Irrigation Development, (2016), Agricultural Waste Management Policy

Zimmer C,(2018), 'Global Greening' Sounds Good. In the Long Run It's Terrible,New York Times, Science Available online at https://www.nytimes.com/2018/07/30/science/climate-change-plants-global-greening.html

APPENDICES

Table 3:Substrate evaluation of *Pleurotus ostreatus* growth on different substrates

substrate	ch	sd	ws	mc	bf	chsd	chws	chmc	Chbf
				Yield(g)					
	380	145	202	250	240	400	350	400	420
	420	165	222	262	260	456	380	450	470
	410	160	190	300	300	500	400	380	480
	390	150	234	240	200	356	374	450	426

				Spawn run					
	19	21	16	18	17	18	12	13	14
	19	19	16	19	17	16	13	13	14
	20	22	16	19	18	19	13	13	13
	18	18	16	18	16	15	12	13	13
				Substrate weight					
	1556	1407	970	1829	1108	1071	1550	1488	1687
	1596	1427	990	1855	1128	1127	1580	1538	1700
	1586	1422	958	1865	1168	1171	1600	1468	1745
	1566	1412	1002	1819	1068	1027	1574	1538	1732

Data for *Lactariuskabansus(Cantherellus densifolius*) evaluation on different commercial media and physical factors

Nzevedzambuya mycelial growth evaluation in 24 hour intervals(a petri plate is considered fully covered when the diameter of mycelia reaches 90mm)

Table 4:performance of C.densifolius on different nutrient media

Media	**15^0C(mm)**	**28^0C(mm)**	**32^0C(mm)**
TSA	0	0	0
YEA	15	0	0
YCDA	5	0	0
PDA	0	40	0
MEA	7	0	0
NA	0	0	0
	48 hours		
TSA	0	0	0
YEA	14	0	0
YCDA	12	0	0
PDA	0	75	0
MEA	15,30	0	0
NA	0	0	0
	72 hours		

TSA	0	0	0
YEA	0	0	0
YCDA	15	0	0
PDA	0	90,90,90	0
MEA	33,37	0	0
NA	0	0	0
	96hours		
TSA	0	0	0
YEA	0	0	0
YCDA	13	0	0
PDA	0	90,90,90	0
MEA	56,41	0	55
NA	0	0	0
	120 hours		
TSA	0	0	0
YEA	0	0	0
YCDA	16	0	0
PDA	0	0	0
MEA	10,58	90,90,90	63
NA	0	0	0
	144 hours		
TSA	0	0	0
YEA	0	0	0
YCDA	11	0	0
PDA	0	80,90,90	0
MEA	68,71	0	70
NA	0	0	0
	168 hours		
TSA	0	0	0
YEA	0	0	0
YCDA	30	0	0
PDA	0	90,90,90	0
MEA	90,70	0	50
NA	0	0	0
	``		

Table 5:Anova table for analysis of variance on substrate means

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
tm	**3**	**11217444**	**3739148**	**146.8**	**<2e-16 *****
Residuals	**32**	**815253**	**25477**		
Signif. codes	**0 '***' **	**0.001**	**' 0.01**	**0.05**	**0.1 ' ' 1**

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
tm	3	11217444	3739148	146.8	<2e-16 ***
Residuals	32	815253	25477		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1