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Influence of cultivation practices on yield and post-harvest quality of oyster mushrooms (*Pleurotus ostreatus*)

by

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Declaration

I, Mashudu Ronnie Masevhe, declare that this thesis, which I hereby submit for the degree of Doctor of Philosophy in Agronomy at the University of Pretoria, is my own work and has never been submitted by me for a degree at this or any other tertiary institution.

Mashudu Ronnie Masevhe..  ..Date:…30 April 2023…

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List of symbols and abbreviations

CO_2	Carbon dioxide
O_2	Oxygen
RH	Relative humidity
BE%	Biological efficiency percentage
SAS	Statistical analysis software
LSD	Least significant difference
kg	Kilograms
L	Litre
g	Grams
kPa	Kilopascal
NaOH	Sodium hydroxide
WS	Wheat straw
WC	Wood chips
TG	Thatch grass
NS	Not significant
EC	Electrical conductivity
NaOCI	Sodium hypochlorite (HtH chlorine)
G%	germination percentage
dH_2O	distilled water
P.	*Pleurotus*
IBA	Indole butyric acid
GA	Gibberellic acid
PGRs	Plant growth regulators
IAA	Indoleacetic acid

Influence of cultivation practices on yield and post-harvest quality of oyster mushrooms (*Pleurotus ostreatus*)

By

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Abstract

Previous studies have indicated that oyster mushrooms do not last long under normal storage; the shelf-life can be increased only by storage at cold temperatures and wrapping in polyethylene plastic. Currently, mushrooms regardless of species, can be stored for only a few weeks before they deteriorate rapidly. Studies were conducted in a growth chamber and cold storage facility to determine how the crop responds to agronomic and post-harvest practices such as wheat straw leachate, alternative substrates, extract drainage, pasteurising methods, use of 1-NAA, storage temperatures and drying techniques. The research was performed at the Agricultural Research Council-Tropical and Subtropical Crops (ARC-TSC) at the Mbombela (Nelspruit) experimental farm, Mpumalanga Province, South Africa.

The potential growth inhibition rate of oyster mushrooms by means of wheat straw leachate was determined in growth rooms. Fresh and stored wheat straw was boiled for extraction at five levels of dilution treatments [undiluted extract; 3:1; 1:1; 1:3 ratios and dH_20 control]. The 3:1 ratio encouraged mycelial growth when fresh wheat straw was used, while the 1:1 ratio increased growth with wheat straw that had been stored for 3 to 6 months. However, when using wheat straw as a growing medium in oyster mushroom production, storage of the straw for at least 3 to 6 months prior to spawning is necessary in order to leach out compounds that are capable of inhibiting mycelial growth.

Another follow-up experiment was conducted in a custom made hut-house, where potential growth inhibition of oyster mushrooms by stored wheat straw leachate was determined. Stored wheat straw was drained using five levels of treatment (no drain; 1 h; 2 h; 3 h and 4 h drain)

during spawning. Wheat straw leachate drained for 1 h and 2 h during spawning increased the cumulative number of clusters, caps and fresh mass (g) of the oyster mushrooms.

For effective pasteurisation of mushroom substrates, soaking, boiling and steaming was carried out including the use of NaOCI (household bleach - Jik) and gypsum. Both boiling and steaming expedited the rate of colonisation and prevented attacks by parasitic fungi (*Trichoderma* spp.) which resulted in increased yield and biological efficiency of oyster mushrooms.

Thatch grass (natural pasture) and wood chips were evaluated together with wheat straw (control) as alternative substrates. Both the wheat straw and thatch grass produced a high cumulative number of clusters, caps and fresh mass (g) of oyster mushrooms. Thatch grass can therefore be used as an alternative substrate for inoculating oyster mushrooms, with subsequent increases in clusters, caps or yield and biological efficiency.

Results from different levels of the plant growth hormone auxin (0; 0.1; 0.5; 1; 1.5 and 2 mmol 1-NAA) indicated that 1 mmol 1-NAA significantly increased the growth, yield and biological efficiency of oyster mushrooms during the cooler April – June and the warmer September to November months. There was need to apply 1 mmol 1-NAA during cooler and warmer months, as it did significantly improve the production of oyster mushrooms.

To extend the shelf-life of oyster mushrooms, storage under 2°C (70% RH) and 5°C (70% RH) in covered containers maintained the initial fresh mass (50 g), as well as good quality and colour, without any physiological disorders noticeable during the evaluation periods. The significance of the study is that it suggests that oyster mushrooms can be kept in storage for more than 4 weeks under cold storage and still be usable by consumers without the loss of quality.

Various types of drying techniques, in combination with preservatives [i.e. sun drying; electric dehydration; drying at an ambient (room) temperature (21°C) – no additives; sodium metabisulfite; and lemon juice], were used to investigate the optimum treatment to extend the shelf-life of oyster mushrooms. Oyster mushrooms that dried with or without preservatives under an electric dehydrator or under the sun dry maintained good quality, colour, and lower

moisture content and lasted for six months (still usable by consumers) when stored at ambient (room) temperatures.

Keywords: Biological efficiency, leachate, mycelia, physiological disorders, shelf-life, substrate, wheat straw

CHAPTER ONE

General introduction

1.1. Food security

Food security refers to the state of having reliable access to a sufficient quantity of affordable, nutritious food when need it (Oxford Dictionary). According to Breene (2016), the global food security challenge is straightforward, where by 2050 the world must sufficiently produce food for 9 billion people. The demand for food will escalate to about 60% greater than it is today. The United Nations has set ending hunger, achieving food security and improved nutrition and promoting sustainable agriculture as the second of its 17 Sustainable Development Goals for the year 2030. Peri-urban dwellers are often the poorest of the poor, even though they are situated close to markets where both consumers and producers reside. Even though they may have the will to produce for these markets, they also lack the knowledge to tap into those markets. Finding the right product to penetrate the markets is also a constraint. Mushroom production has the potential to improve food security and alleviate poverty in peri-urban areas. In South Africa, the Agricultural Research Council-Tropical and Subtropical Fruit Crops (ARC-TSC) was established in 1926 and has vast of knowledge and experience of doing research and publishing books on tropical and subtropical fruit crops (Hemming, 2013), whereas mushroom research was only introduced in 2005. Similarly, the Agricultural Research Council-Plant Protection Research Institute (ARC-PPRI) began to work on mushrooms from 2003, where they were able to assist rural, peri-urban and urban dwellers with mushroom production.

From 2014, the demand from the general public for information and training regarding oyster mushroom farming increased dramatically due to a documentary that was regularly broadcasted on the National Television Channel SABC 2, in the programme Living Land (Koch *et al*., 2016). The programme encouraged emerging farmers from rural, peri-urban and urban areas to venture into oyster mushroom cultivation. One advantage of mushroom production is that it does not require soil or large production areas. A mushroom production unit can be set-up easily in any backyard wooden structure, with some basic equipment. This is convenient and easily accessible to the majority of poor black South Africans.

1.2. State of mushroom production worldwide

Total world production of mushrooms (including truffles) was nearly 9 000 kg in 2017, with China being by far the leading producer (7 855 kg). Among over 20 cultivated species, *Agaricus bisporus* (button mushroom, white mushroom, brown mushroom, or portobello) dominates worldwide production, followed by *Lentinula edodes* (shiitake) and *Pleurotus* spp. (particularly *P. ostreatus*, oyster mushroom). The monetary value of processed mushroom in 2018 was estimated at $50,034.12 million (Dulal, 2019). From 2015 to 2018, in terms of mushrooms exports, Poland was number one where most mushrooms were exported to Western Europe, including speciality items like oyster mushrooms and shiitake (FruitLogistica.Com, 2019). In 2018, Polish exports of fresh mushrooms was 228 000 tonnes. Poland was able to grow its production of fresh mushrooms from 315 000 to 330 000 tonnes from 2015 to 2018 compared to Netherlands with a decrease in production from 310 000 to 300 000 tonnes without exportation (FruitLogistica.Com, 2019). The United Kingdom is by far the world's biggest importer of fresh mushrooms, (FreshPlaza.Com, 2017; Ramya and Meenakshi, 2015) with Germany and Russia in second and third place, respectively. South Africa is a net importer of preserved mushrooms and truffles (NAMC, 2013). Over the last 8 years, South Africans are finding more and more interest in exotic mushrooms, as well as medicinal mushrooms (Reynders, 2014). Oyster mushrooms are the second most cultivated mushrooms worldwide following *Agaricus bisporus* (Berne *et al*., 2008) except in South Africa where the production of this mushroom is not yet reaching the recognized status.

1.3. Botanical description of fungi

Botanically, a fungus is neither a plant nor an animal, although it is similar to a plant, but it has no chlorophyll and cannot manufacture its own food like a plant through photosynthesis (Nordqvist, 2014). However, fungi get their food by absorbing nutrients from their surroundings. The kingdom Fungi includes mushrooms, rusts, smuts, puffballs, truffles, morels, molds and yeasts and thousands of other organisms and microorganisms. They range from microscopic single-celled organisms, such as yeast, to gigantic multicellular organisms (Nordqvist, 2014), while some are in the kingdom Protista. They play a crucial role in decomposition and returning nutrients to the soil, while others are used in medicine, such as the antibiotic penicillin, as well as in industry and food preparation. They belong to the family

Pleurotaceae and genus *Pleurotus* (Stamets, 2000) consisting of more than 70 species (Kong, 2004).

1.4. Description of oyster mushrooms

Oyster mushroom refers to the shape of the cap, which resembles a bi-valve of the same name (Phillips, 2006). Oyster mushrooms are saprophytes that are generally found on hardwoods, such as dead and decaying wooden logs or sometimes on dying trunks of hardwoods (deciduous) or soft (coniferous) woods. The caps range between 5 to 25 cm and are convex in shape when young and flatten out and turn up as the mushroom senesces. The colour ranges from white, yellow, brown, tan, blue and even pink. Certain strains form clusters while others grow as individuals (Stamets, 2000).

Oyster mushrooms are said to be extremely delicious and traditionally used to strengthen veins and relax tendons (Hobbs, 2002). They have the potential to inhibit tumours, improve liver and kidney functioning and aid in gastrointestinal disorders (Stamets, 2000). They also have a relatively high folic acid content that is capable of protecting humans against diseases such as anaemia, diabetes and high blood pressure (Vetayasuporn, 2004). In the Czech Republic, extracts from the fruiting bodies, included as the main ingredient in food preparations, have been recommended for prevention of high blood pressure and cholesterol (Rathee *et al*., 2012). The mushrooms are believed to be capable of stimulating the immune system to fight cancer and constitute a rich source of protein, minerals, and vitamins C and B (Caglarirmak, 2007).

1.5. Cultivation techniques and storage of oyster mushrooms

In China, oyster mushrooms are cultivated mainly on sawdust and cotton seed hull (Yang *et al*., 2013), whereas in the agricultural economy of temperate and subtropical countries, cotton plant residue from stem-leaf and gin trash, wheat straw and peanut shells, were of particular interest to local farmers in Greece as they were produced in large quantities (Philippoussis *et al*., 2001). However, for the commercial production of *Pleurotus* mushrooms, the main substrate used was wheat straw (Pant *et al*., 2006). The utilization of rice straw and or wheat straw in oyster mushroom cultivation is not popular in China because of its low yield and low biological efficiency (Wang, 2010), or the need for varying degrees of pre-treatment. The mushroom industry was probably the only significant one which converted wheat straw directly

into a protein source for the human diet (Labuschagne *et al*., 2000). The production of edible or medicinal mushrooms was a successful example of agro-waste recycling (Chiu *et al*., 2000). This is particularly so since oyster mushrooms can be grown on a variety of substrates, at times supplemented with additional nitrogen sources such as wheat bran, rice bran, sorghum or millet (Kwon and Kim, 2004). The ability of a fungal species to colonise different substrates is dependent on the genetic properties of the strain used, as well as the physical, chemical and biological conditions of the substrates (Sindhu *et al*., 2016). Furthermore, colonisation of substrates is dependent on the incubation technique used. It is therefore necessary to ensure that the best conditions for the growth of mushrooms are prevalent all the time (Wasser, 2010). Higher rates of mycelial growth assist in the rate of penetration of the substrate by fungi, thereby simplifying the overall cultivation of mushrooms (Kumar, 2015).

The most accepted method for preserving mushrooms for an extended shelf-life is by means of cooling (Singh *et al*., 2010). Storage temperature is one of main factors that affect post-ripening and processes such as respiration, transpiration, senescence and other physiological activities (Luo and Cai, 2001). Relative humidity (RH) also plays an important role in the quality parameters of mushrooms, such as appearance and texture, which can be adversely affected by low RH, due to water loss (Singh *et al*., 2010).

Drying or dehydration of mushrooms is defined as the reduction of water activity of product-defined levels that guarantee the micro-biological and physicochemical stability (Lewicki and Jakubczyk, 2004). Dried mushrooms can be used in many different types of products, such as pizzas, in soups, in sauces and other dishes (Stamets, 2000). Importantly, elevated temperatures during drying may enhance enzymatic reactions that can result in improved flavour of the dehydrated mushrooms, such as shiitake, oyster, morels, and reishi. Many mushrooms dry readily and can be stored for many months (Stamets, 2000). Oei (2005) remarked that treatments for different mushroom species do differ and that little was known about the preservation practices of mushrooms. Usually fresh markets for mushrooms are considered first and later for processed markets. A number of conservation methods that differ according to the target market, have been developed. For example, canning, air-drying, brining (salting), freeze-drying and freezing and vacuum drying are some of the methods that are employed in mushroom preservation (Oei, 2005).

Over 80% of the total production of *Agaricus* mushrooms, including white and brown strains (crimini and portabello), are sold on the fresh market as whole or sliced mushrooms (Combs, 2004). Currently, pieces of sliced mushrooms that are smaller in size or have an irregular shape are considered to be waste and are usually disposed of. Sliced products are still of good quality and can be used in processing, such as drying. This would provide mushroom growers with a way to profit from the product that they currently dispose of. Dried mushrooms can be used by consumers and animal feed industry (where mushrooms are dried, ground and then incorporated to the feedlot ratio). Consumer benefits include the fact that they can purchase dried mushrooms to use in products that do not need fresh mushrooms and could be stored for longer periods (Combs, 2004).

Product-oriented quality refers to the various attributes used to evaluate the effects of post-harvest treatments, such as handling techniques and storage conditions (Kader and Rolle, 2004). Quality changes can be plotted as a function of time and can be directly associated with the post-harvest treatments under study. To ensure good quality and the safety of fresh oyster mushrooms, washing before consumption might be necessary to reduce the microbial population on the crop (Chikthimmah and Beelman, 2005). The opening of caps in *Agaricus bisporus* mushrooms is the most important morphological quality parameter (Braaksma *et al*., 2001), which has a beneficial effect of earlier harvest times upon subsequent postharvest development. The button mushrooms are graded into Grade A, B and C or Grade M1 and M2 (Government Gazettes, 2018), whereas oyster mushrooms are graded following the system denoted as Extra class, Class 1 and Class 2 (Asean Stan 35: 2014). The cap opening is one of the quality parameters of button mushrooms whereas it does not apply to oyster mushrooms as they develop with open caps. Generally, fresh mushrooms have a very short shelf-life and are therefore traded on the world markets in a processed form (Xu *et al*., 2002).

According to various researchers, the shelf-life of fresh mushrooms was limited to 1-3 days at an ambient temperature (Singh *et al*., 2010) and 4-7 days at 4°C (Mahajan *et al*., 2008). Other studies indicated that oyster mushrooms can last for only 5 to 7 days in storage at a temperature of 1-2°C, before deterioration begins (Hill, 2004). The fact remains that oyster mushrooms cannot last for long periods, whether at room (ambient) or cold room storage temperature, even though the cold chain can extend their shelf-life slightly. South Africa has the potential to engage in commercial oyster mushroom production due to their nutritional and medicinal

benefits to human beings. How cultural or production practices affect post-harvest practices, however, has not been studied previously.

1.6. Problem statements

The problem statement for the study was the lack of oyster mushroom knowledge with reference to: (i) Nutritional value, (ii) Beneficiation, (iii) Growth requirements (iv) Correct harvesting procedures, and (v) Extension of shelf-life. This has led to its under-utilisation and lack of food security reality. South Africa is not adequately tapping into this key product to alleviate malnutrition, including for sustainable economic development and food security.

1.7 Aim, hypothesis, and objectives

Aim of the study: To optimise production practices for the small-scale production of oyster mushrooms using local sourced materials.

The hypothesis

Full understanding of the growth requirements and shelf-life extension of oyster mushrooms will lead to food security and sustainable economic development in South Africa

Objectives of the study

The objectives of the study were:

(i) Full study on growth medium requirements for oyster mushrooms

(ii) To improve postharvest storage practices of oyster mushrooms

(iii) To evaluate the influence of season on the production rates of oyster mushrooms

(iv) Design a training manual of oyster mushrooms for small-scale farmers

1.8. Outline of the thesis

The outline of the thesis starts with **Chapter 1**, which is an introductory chapter giving background information to mushroom production and provides the problem statement, hypothesis, aim and objectives of this study. This is then followed by extensive information gathered through a literature survey in **Chapter 2**. **Chapter 3** introduces the actual

experimental work. **Chapters 3 to 5** investigate wheat straw leachate effect on general oyster mushroom growth. **Chapter 3** deals with growth of oyster mushroom spawn on wheat straw leachate. **Chapter 4** investigates growth and production of oyster mushrooms as affected by drainage of the wheat straw leachate. **Chapter 5** looks into the growth and yield of oyster mushrooms as affected by wheat straw pasteurising methods. **Chapter 6** introduces the possibility of using alternative substrates for cultivating oyster mushrooms. **Chapter 7** also introduces the possibility of using 1-Naphthaleneacetic acid (1-NAA) for improving growth and yield of oyster mushrooms. **Chapter 8** and **Chapter 9** investigate factors affecting the shelf-life. Results of the effect of seasonal and different storage temperatures on the shelf-life of oyster mushrooms are included in **Chapter 8**. Parameters affecting the shelf-life of oyster mushrooms was thoroughly dealt with in **Chapter 9**. The overall concluding remarks substantiated by gathered information is given in **Chapter 10**.

CHAPTER TWO

Literature review

2.1. Introduction

In general, South Africans have a mushroom knowledge, as they are well acquainted with naturally occurring mushrooms that spring up on manure and compost rich soils (Personal views). Although some of these mushrooms are edible, others are poisonous and the communities have experience in distinguishing between edible and non-edible fungi. These mushrooms are very scarce and are not found all the time. Therefore, there is a need to introduce other mushroom types, for example, oyster mushrooms, button mushrooms, shiitake, reshi, etc., to the said people, as mushrooms are highly nutritious (i.e., dry matter, protein, fat, carbohydrates, minerals and vitamins) (Bernas *et al*., 2006). Since poor people cannot afford red meat, mushrooms can be a substitute, as they are rich in proteins. In developing countries, malnutrition is one of the major problems because most of the population remains under the economic bread line (Kandala *et al*., 2011). In South Africa, mushrooms possess the potential to alleviate the protein deficiencies in the human diet. Even those who can afford to buy meat, tend to experience the monotony of buying mostly chicken because it is more affordable and mushrooms can be eaten as a supplement (Personal views). In South Africa, the mushroom growers have formed "The South African Mushroom Farmers' Association" (SAMFA) which was formally established in 1984. They celebrated its 25th year in 2009 by hosting the XVIIth International Congress on the Science and Cultivation of Edible Mushrooms in Cape Town, South Africa during May 2008. The association was able to attract scientists, growers, researchers, suppliers, marketers, educators and partners to the City of Cape Town to attend this prestigious event, which brilliantly showcased the latest in mushroom production and marketing technology from all the very best suppliers and farmers from around the globe. SAMFA is funded by its members who are mostly mushroom growers, industry suppliers and trade alliances (SAMFA, 2017).

Taxonomy of Pleurotus

The classification of species within the genus *Pleurotus* is difficult due to high phenotypic variability across wide geographic ranges, geographic overlap of species and ongoing evolution

and speciation (O'Reilly, 2011). The genus *Pleurotus* is characterised by the absence of a distinct anamorph, except for *P. cystidiosus* (Bao *et al*., 2004). The colour of the basiodiome varies from white, yellow, brown, grey, black to blue or salmon. There has been confusion regarding the position of *Pleurotus* within the Basidiomycota (Zervakis *et al*., 2014), whereas other authors prefer *Tricholomataceae* with order *Agaricales* and others *Polyporaceae* within *Polyporales*. There are some features which show that *Pleurotus* is related to both families, i.e., hyphal system which is mono to dimitic, the cylindrical basidiospores, the haptomorphosis in single growing basidiomes. The occasional occurrence of a peg and the saprophytic to parasitic have been noted (Jibrin *et al*., 2017).

Maftoun *et al*. (2015) found that the most cultivated species of oyster mushrooms belong to the *Pleurotus ostreatus* group, which includes species of subtropical parts of the world like *P. cornucopiae*, *P. cystidiosus*, *P. eryngii*, *P. flabellatus*, *P. fossulatus*, *P. sapidus* and *P. ulmarius* and temperate and tropical strains such as *Agaricus* and *Volvariella*. *V. volvacea* is another important species, which is commonly known as straw mushrooms, paddy straw mushrooms, Chinese mushrooms or warm mushrooms; and belongs to the family *Plauteaceae* of Basidiomycetes (Bao *et al*., 2013). It can grow at relatively high temperatures and its vegetative growth can be active at 32-34°C. It is a fast growing mushroom and the time required from spawning to harvesting is only approximately 5-10 days (Bao *et al*., 2013). Species of *Pleurotus* may be called oyster, abalone, or tree mushrooms and are some of the most commonly cultivated edible mushrooms in the world (Chang and Miles, 2004). *Pleurotus* fungi have been used in mycoremediation of pollutants, such as petroleum and polycyclic aromatic hydrocarbons (Cohen *et al*., 2002; Stamets, 2005). *Pleurotus* means "side ear" from Greek, where '*pleure*', side + '*ous*', ear.

Description of Pleurotus

The caps of *Pleurotus* species may be laterally attached with no stem. If there is a stem, it is normally eccentric and the gills are decurrent along it. The term *Pleurotoid* is used for mushrooms having the same general shape (Kang, 2004). The spores are smooth and elongated and described as "cylindrical". Where hyphae meet, they are joined by clamp connections with a soft consistency. However, remarkably, *P. dryinus* can sometimes be dimitic, meaning that it has additional skeletal hyphae, which gives it a tougher consistency like bracket fungi (Knudsen and Jan, 2008) in Plate 2.1 and Plate 2.2.

Ecology of Pleurotus

Pleurotus fungi are found in both tropical and temperate climates throughout the world (Chang and Miles, 2004). Most species of *Pleurotus* are white-rot fungi on hardwood trees, although some also decay conifer wood (Cohen *et al*., 2002). *P. eryngii* is unusual in its association with herbaceous plants, and *P. tuber-regium* produces underground sclerotia. In addition to being saprotrophic, all species of *Pleurotus* are also nematophagous, trapping nematodes by paralyzing them with a toxin (Thorn *et al*., 2000)

Morphology

P. ostreatus is a wood-destroying fungus which is widespread in temperate zones (Kong, 2004). It forms fruit bodies under cold temperature conditions, in comparison with other *Pleurotus* species. It requires a cold shock temperature treatment from 2°C - 10°C in order to initiate formation of primordia. According to Moonmoon *et al*. (2010), 66 commercial strains were recognised in Korea and different strains have different degrees of heat or cold tolerance, while other strains are less affected by unfavourable temperature conditions at the latter flush stages. *Agaricus* mushrooms are preferred at the immature button stage with the cap (pileus) unopened, the gills (lamellae) not visible and the stem (stipe) plump rather than elongated (Spahr, 2018) (Plate 2.1). In *Agaricus* buttons, lamellae are already formed but covered by a membranous veil which extends from the cap margin to the stipe. Following harvesting, mushrooms do not continue to grow and therefore have a short shelf-life (Harun, 2017). The button and egg stages of the *Volvariella* species are sold in the market at a premium price (Ahlawat and Tewari, 2007).

At the egg stage, the pileus is pushed out of the veil which remains to form the volva and pileus at these two stages, which are similar to that noticed in the mature and elongation stages. Fruit bodies of *Pleurotus* are characterised by an off-centre stalk that may be small or long or even absent, and the annulus and volva are also absent (Stamets, 2000). Fruit bodies appear like petals of a flower in a cluster or individually. They open up like an oyster shell with the widest margin away from the stalk (Stamets, 2000). The size varies from species to species and within the same species when cultivated under different climatic and nutritional conditions. The fruit bodies measure a few to several centimetres in width, with the minimum sizes about 2-3 cm

and the maximum sizes around 15-20 cm (Chang and Miles, 2004). The margins may be smooth, broken or slightly serrated or dented, depending on the species.

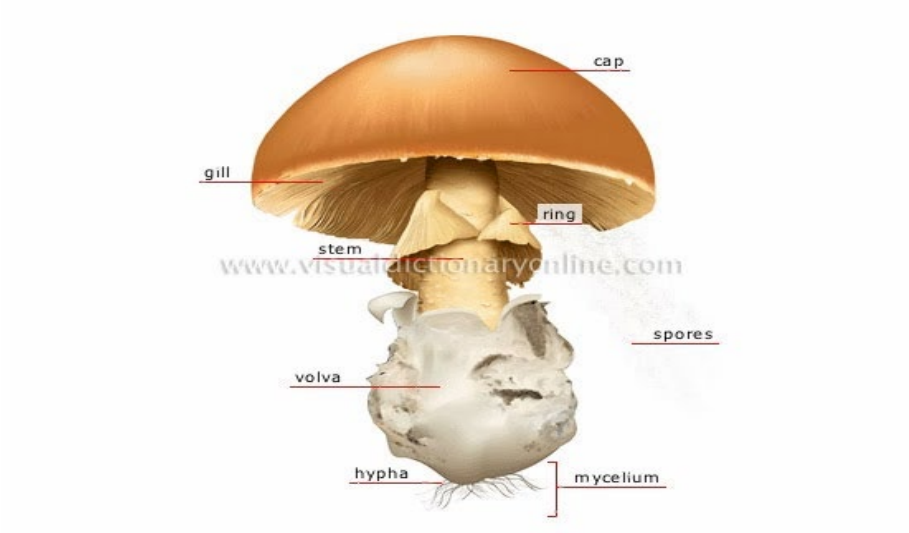


Plate 2.1. General structure of a mushroom (Thian, 2014)

More recently, molecular phylogenetics has been utilized to determine genetic and evolutionary relationships between groups within the genus, delineating discrete clades (Gonzalez and Labarere, 2000). *Pleurotus* along with the closely related genus *Hohenbuehelia* have been shown to be monophyletic (Thorn *et al*., 2000). Tests of cross-breeding viability between groups have been used to further define which groups are deserving of species rank, as opposed to subspecies, variety or synonymy. If two groups of morphologically distinct *Pleurotus* fungi are able to cross-breed and produce fertile offspring, they meet one definition of species. These reproductively discrete groups, referred to as intersterility groups have begun to be defined in *Pleurotus* (Rosnina, 2017). Many binomial names used in literature are now being grouped together as species complexes using this technique and may still change in future.

Phylogeny of species

According to Gonzalez & Labarere (2000) and Peterson *et al*. (2011), there are many oyster mushrooms species organised according to phylogenetic clades, sub-clade and any older binomial names that have been found to be closely related which are reproductively compatible. Sometimes they may no longer be taxonomically valid as displayed in Table 2.1.

Table 2.1. Phylogeny of *Pleurotus* species showing scientific name, common name, subgroup and area found (Gonzalez and Labarere, 2000; Peterson *et al*., 2011)

Scientific name	Common name	Subgroup	Area to be found
***P. ostreatus* clade**			
P. ostreatus	Oyster or pearl oyster mushroom	*P. florida*	North America and northern Eurasia
P. pulmonarius	Phoenix or Indian oyster mushroom	*P. columbinus* and *P. sapidus*	North America, Eurasia and Australasia;
P. populinus			North America
P. eryngii	King oyster mushroom	*P. ferulae*, *P. fossulatus*	Europe and East Afghanistan
P. abieticola			Asia;
P. albidus			Caribbean, Central America, South America
***P. djamor-cornucopiae* clade**			
P. cornucopiae	Golden oyster mushroom Tarragon oyster mushroom	*P. citrinopileatus* *P. euosmus*	Eastern Asia
P. djamor	Pink oyster mushroom	*P. flabellatus*, *P. salmonicolor*	Pantropical
P. opuntiate			North America, New Zealand
P. calyptratus			

***P. cystidiosus* clade**			
P. cystidiosus	Abalone mushroom	*P. abalonus* *P. fuscosquamulosus* *P. smithii*	Global - Taiwan, Africa and Mexico
P. dryinus			North America, Europe and New Zealand;
P. levis			Subtropical to tropical
P. tuber-regium	King tuber mushroom		Africa, Asia, Australasia
P. australis	Brown oyster mushroom		Australia and New Zealand
P. purpureo-olivaceus			Australia and New Zealand
Other species of unclear relationship			
P. gardneri, *P. parsonsii* and *P. velatus*			

Life cycle of oyster mushrooms

The life cycle of oyster mushrooms begins with the germination of a basidiospore in a suitable substrate, which gives rise to a monokaryotic mycelium containing genetically identical nuclei and is capable of indefinite independent growth (Adebayo and Martinez-Carrera, 2015) (Figure 2.1). When two compatible monokaryotic mycelia are in close contact, they are able to establish a fertile dikaryon by hyphal fusion or plasmogamy (Badalyan *et al*., 2004; Kang, 2004). This dikaryon, which has clamp connexions and is binucleate in each hyphal compartment, contains two genetically different nuclei throughout the mycelium (Figure 2.1). Appropriate environmental conditions, such as temperature, light and relative humidity, play a role in the dikaryotic mycelium being able to develop into different fruit bodies with specialised structures called the basidia (Kües and Liu, 2000). In these club-shaped, binucleate cells, which are formed in the lamellae (hymenium) of each fruit body, karyogamy (fusion of the paired nuclei; 2n) and meiosis (recombination and segregation) take place (Kües, 2000). Four resulting haploid nuclei move to the sterigmata on basidium to form four new basidiospores (Figure 2.1). When the fruit bodies are mature, basidiospores are discharged, thus starting the sexual life cycle again.

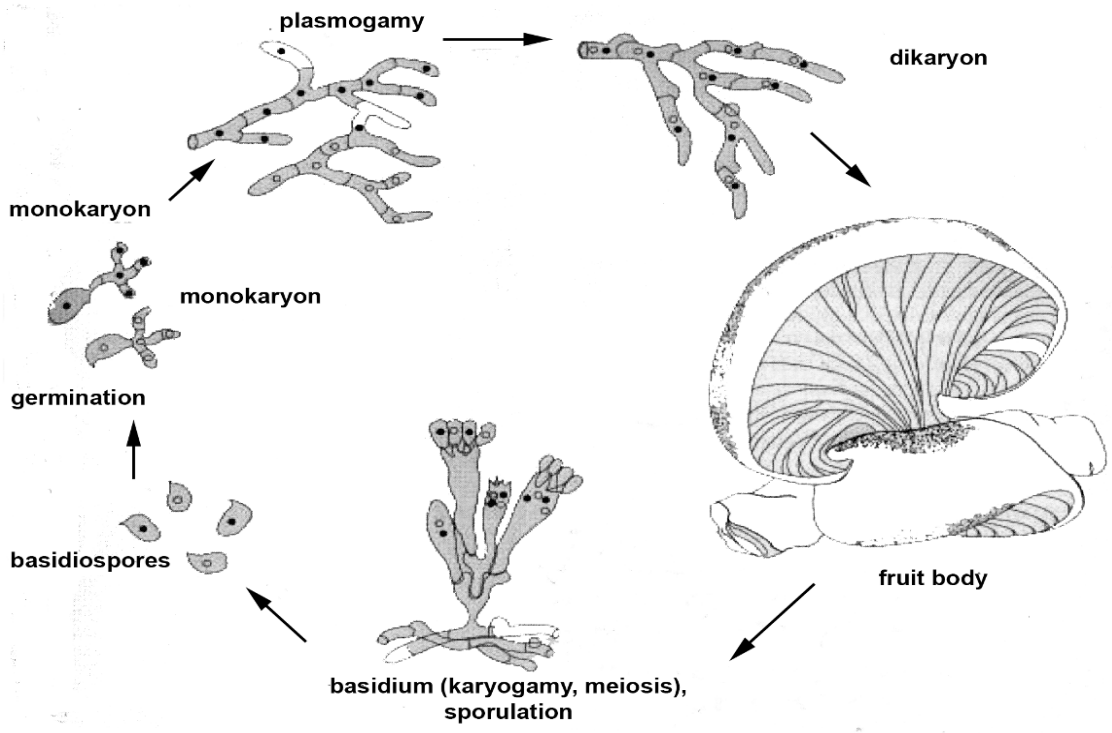


Figure 2.1. Life cycle of oyster mushrooms (Kües and Liu, 2000)

Oyster mushrooms

Oyster mushrooms include some of the more important cultivated edible species in the world (Marshall, 2007). Both the scientific and common names refer to the shape of the fruiting body. The genus *Pleurotus* refers to the sideways-growth of a stem with respect to the cap, while the species *ostreatus* (English common name, oyster) refers to the shape of the cap which resembles a bi-valve of the same name (Phillips, 2006). Oyster mushrooms consist of seven species, namely, golden (*Pleurotus citrinopileatus*), abalone (*Pleurotus cystidiosus*), pink (*Pleurotus djamor*), king (*Pleurotus eryngii*), tarragon (*Pleurotus euosmus*), tree (*Pleurotus ostreatus*) and Phoenix or Indian (*Pleurotus pulmonarius*) oyster mushrooms. The *Pleurotus* group mainly grows on wood. Some are parasitic while others are saprophytes. Most have lateral stems that grow out of the host.

Description of selected Pleurotus species

Tree oyster mushrooms

P. ostreatus has long been a favourite of mushroom hunters in China, especially in spring time in lowland hardwood forests (Stamets, 2000). *P. ostreatus* has a convex cap at first, expanding to being broadly convex, eventually flat and even upturned with age. It is 5 - 20 cm in width, with white to yellow to grayish yellow to tan, rarely with pinkish tones, to lilac gray to gray brown colours during its growth stages, as illustrated in Plate 2.2 (Kuo, 2005). The cap margins are smooth to undulating like an oyster shell (Plate 2.2). The colour varies according to strain, lighting and temperature conditions. Stems are typically eccentrically attached to the cap. The flesh is generally thin. Certain strains form clusters, while others form individual mushrooms. They are believed to possess the potential to inhibit tumours and improve liver and kidney function, as well as alleviate gastrointestinal disorders. While this mushroom is often seen growing on dying hardwood trees, it only appears to be acting saprophytically, rather than parasitically (Hall, 2010). They actually benefit the forest by decomposing the dead wood, returning vital elements and minerals to the ecosystem in a form usable to other plants and organisms. In New Zealand, oyster mushroom importation is banned as they believe that it could damage their forestry industry.

Plate 2.2. Matured tree oyster mushroom clusters ready for consumption (Photo taken at ARC-TSC, Postharvest Laboratory, 2006)

Golden oyster mushrooms

The fruiting bodies of *P. citrinopileatus* grow in clusters of bright yellow to golden brown caps with a velvety, dry surface texture (Liu *et al*., 2012). Caps range from 20-65 mm in diameter. The flesh is thin and white, with a mild taste and without a strong smell (Plate 2.3). Stems are cylindrical, white in colour, often curved or bent and about 20-50 mm long and 2-8 mm in diameter. Its extreme fragility post-harvest limits its distribution to distant markets.

Plate 2.3. Golden oyster mushrooms (Robinson, 2010)

These mushrooms are spicy and bitter at first, and impart a strong nutty flavour when thoroughly cooked. *P. citrinopileatus* grows quickly through pasteurised straw and sterilised sawdust and thrives at high temperatures. It is native to the forested, subtropics of China (Chang and Miles, 2004), southern Japan and adjacent regions. They have the potential to cure pulmonary emphysema.

Abalone mushrooms

They are by far the most interesting of all the oyster mushrooms with a sexual and asexual life cycle (Plate 2.4). Abalone mushrooms are mainly used in stews and stir-fries with very rich taste (Hill, 2008). Due to the large, meaty texture, they are popular in vegetarian dishes. The stems take longer to cook than the caps, so many cooks trim the caps off and add those last with a pleasant smell – no sour odour. It is distributed from eastern to south-eastern United States, Taiwan and South Africa.

Plate 2.4. Abalone oyster mushrooms (Mushroom Source, 2010)

Pink oyster mushrooms

A fresh and glowing flush of bright pink oyster mushrooms is a sight to behold. Pink oyster varieties are the most commonly occurring wild *Pleurotus* in pan-tropical climatic zones of the world. Pink oyster mushrooms are vigorous growers, fast colonizers and heavy yielding (Plate 2.5) (FreshCap Mushrooms, 2017). They have the ability to flourish on a wide variety of base materials and tolerate high temperatures. This species is so aggressive that it is able to colonise unpasteurised bulk substrates before competitors can flourish and is distributed throughout the tropics and subtropics.

Plate 2.5. Pink oyster mushroom (Robinson, 2010)

King oyster mushrooms

P. eryngii is the largest species in the oyster mushroom genus *Pleurotus*. It has a thick, meaty white stem and a small tan cap in young specimens (Zervakis *et al*., 2001). The king oyster mushroom is the best tasting of the oyster mushrooms (Plate 2.6). It is therefore well deserving to be called the king oyster mushroom.

Plate 2.6. King oyster mushrooms (Robinson, 2010)

The king oyster mushrooms prefer hardwoods and are easy to grow. Although the mushroom grows on cereal straws, their yields are not as substantial as those of *P. ostreatus* and *P. pulmonarius* on this same material, at the same rate of spawning, unless supplements are added or a unique spawning method is employed. Unlike other species of *Pleurotus* which are wood-decaying fungi, the *P. eryngii* complex are weak parasites on the roots of herbaceous plants (Alma and Royse, 2008). It is distributed throughout southern Europe, North Africa, central Asia and in Russia.

Tarragon oyster mushrooms

These fungi are closely related to *P. ostreatus*. This mushroom is generally considered to be a form or variety of *P. ostreatus,* but differs (Plate 2.7) in its strong smell of tarragon (Emanuelli, 2013). *P. euosmus* behaves similarly to *P. ostreatus* in growth culture. It is mainly distributed in England and Scotland.

Plate 2.7. Tarragon oyster mushrooms (Gibson, 2009)

Indian or phoenix oyster mushrooms

P. pulmonarius is virtually indistinguishable from *P. ostreatus* and differs largely in its habitat preference for conifer woods (Stamets, 2000) (Plate 2.8). In the western United States, *P. pulmonarius* is usually found at higher altitudes than *P. ostreatus*, which prefers lowland river valleys. *P. pulmonarius* and *P. ostreatus* grow on a variety of hardwoods, with *P. pulmonarius* primarily a spring mushroom and *P. ostreatus* growing most prevalently in summer to autumn (Amao and Oloke, 2013). The entire description is similar to that of *P. ostreatus*, except that the veil is absent. The strains form clusters of more than five to six mushrooms (Plate 2.8). It is widely distributed in North America and Europe. In North America, *P. pulmonarius* also closely resembles *P. populinus* which is restricted to growing on aspen and cottonwood (Trudell and Ammirati, 2009).

Plate 2.8. Indian or Phoenix oyster mushrooms (Stamets, 2000; Beetz and Kustudia, 2004)

Edible mushrooms with particular reference to the oyster mushroom

Edible mushrooms are used extensively in cooking in many cuisines (Phillips, 2006). Though commonly thought to contain little nutritional value, many of these varieties are high in fibre and protein and provide vitamins such as thiamine (B_1), riboflavin (B_2), niacin (B_3), biotin (B_7), cobalamin (B_{12}) and ascorbic acid (C), as well as minerals including iron, selenium, potassium and phosphorus (Phillips, 2006). Nutritional properties and health benefits of the medicinal mushrooms have been recognised in China for over 2000 years (Sadler, 2003). They have been used as part of traditional Chinese medicine, as well as in Japan and Malaysia. The most important medicinal mushrooms include reishi (*Ganoderma lucidum*), shiitake (*L. edodes*), maitake (*Grifola frondosa*), Jew's ear (*Auricularia auricular*) and oyster mushrooms (*Pleurotus* species). Sadler (2003) mentioned two areas of major interest as being: a) the role of fungi as an immune system enhancer and immunomodulator; and b) their cholesterol lowering effects. Other areas of scientific interest include the impact of mushrooms on high blood pressure and diabetes and their anti-viral, anti-bacterial, anti-oxidant and free-radical scavenging effects.

Growth and reproduction

The basic difference between mushrooms which belong to the Kingdom of *Fungi* and plants which belong to the Kingdom of *Plantae* is that plants are able to synthesise their own carbohydrates, while mushrooms are unable to do so (Feeney *et al*., 2014). Mushrooms also differ from most plants which have a full root system, which is formed before they appear above the ground (Stamets, 2000) (Plate 2.1). The mycelium of mushrooms should grow in organic material containing carbohydrates, such as sugar, starch, cellulose or lignin, as well as mineral and nitrogen fertiliser, as also required by plants. The reproductive unit of all fungi is a spore, which may be formed asexually or sexually (Pringle and Taylor, 2002). All fungi, with the exception of yeasts, form the so-called hyphae, tiny threads that originate from spores (Oei, 2003). These hyphae will branch out to form the mycelium. After some time, they will enter a sexual phase and form spores. Larger spore-producing structures (larger than 1 mm) are called mushrooms. In nature, this is the most striking part of the organism, when in fact it is just the fruiting body. The major part of the living organism can be found under the ground or in wood (Oei, 2003).

The role of fungi in nature is determined by the particular mode of nourishment and growth, which may be saprophitic, parasitic or symbiotic (Pringle and Taylor, 2002; Oei, 2003). Saprobic fungi grow on dead organic material, whereas, parasitic fungi attack living plants and animals. Fungi living in association with other organisms, such as plants and insects, in a way that is beneficial to both partners form an association known as symbiosis (Pringle and Taylor, 2002; Oei, 2003). In nature, saprobic organisms will grow on fallen leaves, animal droppings and stumps of dead wood (Oei, 2003), while some are specialised in breaking down hairs of mammals and others may decompose bird feathers. It is their role in nature to decompose complex organic structures left behind by plants and animals.

Poisonous mushrooms

Mushrooms are categorised as edible and non-edible (poisonous). Poisonous mushrooms can cause illness (Boa, 2004; Miles and Chang, 2004; Wieland, 2012). Most poisonous mushrooms are categorised into three groups of dangerous fungi such as *Amanita*, False morels and a catchall category known as little brown mushrooms (Conservation Commission of Missouri, 2015). These poisonous mushrooms have been reported to have caused death in the United

States and other countries. *Amanita* has three dangerous species, namely, *A. phalloides*, *A. verna* and *A. virosa*. A few other poisonous mushrooms are from the *Lepiota* genus. *Lepiota josserandii*, *Lepiota castanea*, and *Lepiota helveola* are known to have the same toxins as certain species of *Amanita* and can be deadly. *Chlorophyllum molybdites* is sometimes known as the "green spored *Lepiota*" due to its spore print and it can cause extreme sickness. Certain species of *Bolete* are known to be poisonous such as *Boletus satanas*. The *Clitocybe* and *Inocybe* genera contain a number of poisonous species which are difficult to identify. The *Cortinarius* genus also contains many dangerous species which are tricky because they are a beautiful purple. The most important precaution against mushroom poisoning is to positively identify the fungi you want to eat (Wieland, 2012). Toxins caused by poisonous mushrooms may be divided into three categories: those which cause the degeneration of cells of internal organs even before symptoms are manifested, those which directly affect the nervous system and those which cause gastro-intestinal upsets (Wieland, 2012).

2.2. Cultivation of mushrooms

Commercial mushroom production requires fungal strains with excellent cultivation properties, disease resistance and optimum yields in a reasonable time and should also provide high-quality products of appealing taste, odour, shape and texture, as well as a good shelf-life (Kües, 2007). Various strains from different fungal species are available for commercial production (Oei, 2003; Singh *et al*., 2004; Sonnenberg *et al*., 2005; Yamanaka, 2005). Isolation of new strains from the wild serves as breeding material of new production lines with combinations of properties adapted to special needs. A new species might be introduced to a market either from other countries or by establishing suitable cultivation conditions for new species and the breeding of suitable production strains by crossing isolates from the wild (Oei, 2003; Royse *et al*., 2005; Yamanaka, 2005).

The taste preferences of consumers influence the choice of mushrooms for cultivation. The most important factors to be considered in successful and economical mushroom cultivation include the substrate and environmental conditions needed for mycelial growth, substrate colonisation and fruiting (Obodai *et al*., 2003; Bonatti *et al*., 2004; Mendez *et al*., 2005; Salmones *et al*., 2005; Pant *et al*., 2006; Tisdale *et al*., 2006). For example, wood from local fast growing trees provided the substrate in Hawaii for the cultivation of *Pleurotus ostreatus*, whereas various types of straw as waste from local agricultural practices were used for the

cultivation of fungi in Europe, Asia, Latin America and Africa (Obodai *et al*., 2003; Bonatti *et al*., 2004; Mendez *et al*., 2005; Salmones *et al*., 2005; Pant *et al*., 2006; Tisdale *et al*., 2006). Possibilities for investment plays another major role in how to cultivate mushrooms. For example, seasonal cultivation of mushrooms should be considered if high installation costs of specific cultivation chambers are not affordable. *Agaricus bitorquis*, *Coprinopsis cinerea*, *Pleurotus abalonus* and *Volvariella volvacea* require temperatures of 25°C and above for fruiting and are ideal species for cultivation in tropical countries during humid seasons (Oei, 2003).

2.2.1. Mushroom spawn

The spawn constitutes the starting material to rapidly colonise growth of a mushroom on the substrate (Rosado *et al*., 2002; Sánchez, 2004; Nwanze *et al*., 2005; Sainos *et al*., 2005). Oei (2003) and Cho (2004) defined spawn as mushroom seeds. Mycelial cultures on grain, wood sticks or liquid substrates are variably used as an inoculum (Plate 2.9). Spawn is regarded as a substrate which has been impregnated by mushroom mycelia and can be used for propagation purposes (Miles and Chang, 1997). Spawn, unlike spores, is already at its mycelial growing stage on its own substrate, such as sorghum, barley or sawdust (Cho, 2004). There are four types of spawn that can be produced, such as grain spawn, sawdust spawn, plug spawn and liquid spawn (Cho, 2004). The success of commercial mushroom production is not only influenced by fungal strains and conditions under which they produce mushrooms, but also by the type and quality of the cultures taken for inoculation (Kües, 2007).

Mushroom growers usually buy spawn from specialized companies which concentrate on strain improvement and spawn production; although this is a large cost factor (Sánchez, 2010), since the knowledge and equipment needed for maintaining production strains and producing spawn is complex and time consuming. Spawn needs to be produced under sterile conditions in order to diminish contamination of the substrate (Sánchez, 2004). It is recommended that mushroom growers produce their own spawn when commercially sourced spawn lacks the required quality or when special mushrooms are cultivated (Oei, 2003).

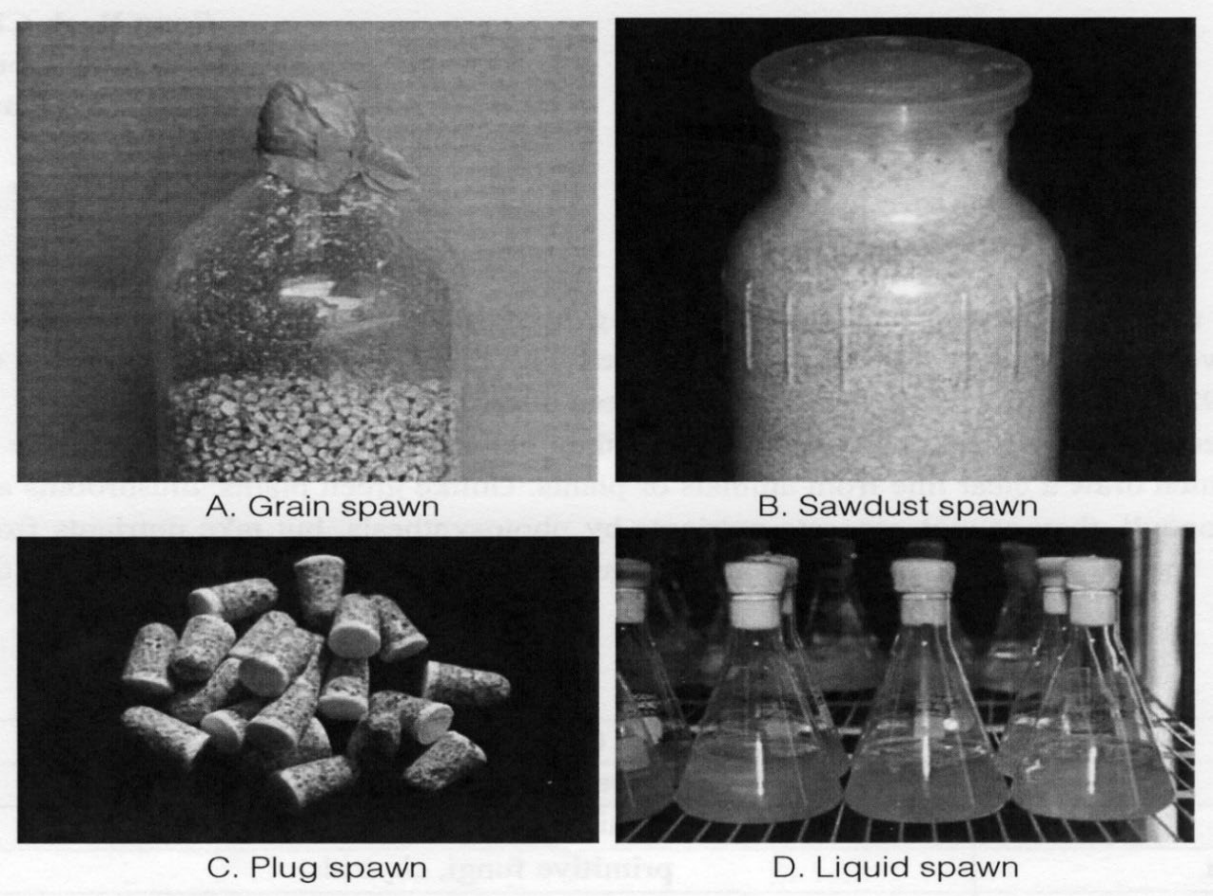


Plate 2.9. Various types of mushroom spawn (Cho, 2004)

2.2.2. Substrates for oyster mushrooms cultivation

The following factors play an important role in determining the suitability of substrate for optimum mycelia growth: relative humidity of air, ventilation, shade or sun, temperature and chemical composition. Oyster mushrooms can grow on a broad range of substrates, while others are very selective (Oei, 2003). For example, *Pleurotus* will grow on the wood of almost any broad-leaf tree, while shiitake requires more specific trees to support its growth. Unless mushrooms are grown on wood logs, the preparation of the growth substrate is one of the most important factors in their cultivation (Kües, 2007).

The role of substrates in oyster mushroom production

Substrate composition has significant impact on growth and nutritional composition of mushrooms (Bellettini *et al*., 2019; El Sebaaly *et al*., 2019). Mushroom potentially can be grown on different lignocellulosic materials like saw dust (Ananbeh, 2003; Hanafi *et al*., 2018), soybean straw, wheat straw (Cao *et al*., 2019) and groundnut shells (Mane *et al*., 2007),

newspaper and tea leaves (Jain, 2005), cotton waste and paddy straw (Ashraf *et al*., 2013), date palm waste (Alananbeh *et al*., 2014). However, for rapid growth of mushrooms an ideal substrate should have nitrogen, carbohydrate, cellulose, and lignin because of its efficient lignin degrading trait (Das and Mukherjee, 2007). Water hyacinth [*Eichhornia crassipes* (Mart.) Solms.] utilization as a low-cost substrate for oyster mushroom cultivation because it is available throughout the year has been reported by Mukhopadhyay (2019). The use of diaper and food waste (comprising of coffee waste, sugarcane bagasse, banana skin, egg shell) via converting them into a unique formulated growth substrate has been reported by Ma *et al*. (2020). This substrate proved to contain high cellulose (27%), hemicellulose (16%), nitrogen (15,779 mg/kg), phosphorus (867 mg/kg) and potassium (7,758 mg/kg). Coffee wastes including coffee crop residues, process waste from cherry processing and spent coffee grounds have been commonly utilized for cultivation of *Pleurotus* in coffee producing countries (Carrasco-Cabrera *et al*., 2019). The agricultural wastes such as maize straw, palm kernel cake, cotton seed hull, spent grain and grass families as they contain cellulose, hemicelluloses and lignin can be used as substrates for cultivating *Pleurotus* species (Dubey *et al*., 2019). Ashrafi *et al.* (2014) reused spent mushroom substrates (wheat straw, sawdust and date palm fiber) for the cultivation of *Pleurotus ostreatus* and *Pleurotus florida* in Malaysia. Waste paper that causes environmental pollution was supplemented with maize stalk and wheat bran as a source of nitrogen for cultivation of *Pleurotus* spp. in Ethiopia (Tesfay *et al*., 2020).

There is a range of more than 200 different wastes available which can be used as substrates for oyster mushroom cultivation (Poppe, 2004), but each grower can make their own choice of the best substrate from among all those genera or species. A few substrates that can be used for cultivating oyster mushrooms are: alang-alang grass, (*Imperata cylindrical*), an abundant herb in Asia, especially in Indonesia, used for *Pleurotus*; artichoke waste is useful after drying for various mushroom substrates; azolla, a fast growing fern in Asia close to tropical rivers, is used for *Agaricus*, *Pleurotus* and *Collybia*; dried banana leaves with 1.45% N, are very productive in bulk for *Pleurotus* or for *Volvariella*; barley straw has a biological efficiency of 96% for *Pleurotus*; bean pods offer a substrate component or used in bulk for *Pleurotus*; bean straw, various genera, is used for *Agaricus* and as a substrate component for *Pleurotus*, it can also be used as a basic substrate; dried citrus fruit peel (*Citrus unshiu*) is a reasonable substrate for *Pleurotus* production; pith and coir coconut fibre can be composted and then used for the cultivation of *Pleurotus* or *Volvariella* in India; coconut husks can be used for *P. cystidiosus* in India and for *Volvariella* in India; coffee sawdust is efficient for *Pleurotus* when mixed with

ipil-ipil powder (Poppe, 2004); maize cobs hammer milled or crushed were tested first for both *Pleurotus* and shiitake and produce variable yields; maize stipes, maize cobs, maize leaves, maize stover, maize stalks (*Zea mays*) were chopped and used as a component of a substrate for *Agaricus*, *Pleurotus* and shiitake; maize waste from the post-shelling dust, the cleaning fiber and the broken pith are useful for *Pleurotus* cultivation with satisfactory yields; wild grasses should be dried for hay before being utilised and lawn grasses, while insufficient research has been carried out, are also cut and dried in India for *P. sapidus* (Poppe, 2004). Mandeel *et al*. (2005) used unsorted office paper, cardboard, plant fibres (*Bromus fasciculatus*, family *Graminae*) and white sawdust as substrates for cultivation of *Pleurotus* spp.

Characteristics of different substrates for oyster mushrooms

Annually, wheat straw is produced in large quantities (11 million tons) by farmers worldwide (Sarwar *et al*., 2002; Solovyev *et al*., 2018). It is regarded as a cheap source for oyster mushroom cultivation. Wastepaper is nearly 100% cellulosic in composition which refers to paper, newspaper and cardboard collected and those discarded from industries, supermarkets and many other shopping places, residential places, and offices. At an international level, about 40-65% of paper is wasted and disposed to the environment (Prognos, 2010) that is increasing pollution. Oyster mushroom cultivation could reduce this pollution at a safe level. Pine needles has more than 50% holocellulose, and hence can be used for production of pulp and paper. It is also rich in lignin, which is a requirement of oyster mushroom cultivation (Ananbeh, 2003; Priti *et al*., 2013). The cellulosic matter in mature green plants is comprised of 40–50% of cell wall, which is a useful source for macrofungi, especially Basidiomycetes due to their ability to release a high amount of exo-enzymes that assist in forming huge biomass (Owaid *et al*., 2017). The lignin has a second level of carbon sources after cellulose, and the soil microbes (fungi in the first level and bacteria in the second level) decompose and use it as an energy and carbon source, significantly (Alam *et al*., 2004).

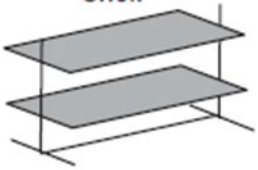
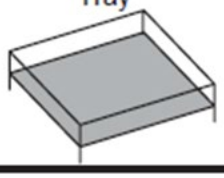
Substrate carbon to nitrogen ratio

For a good growth of *Pleurotus ostreatus*, it is necessary that in the substrate sources of carbon and nitrogen can be found as well as other minerals such as S, Ca, Mg, P, K and some lower concentrations of minerals such as Fe, Zn, Mn, Cu and Mo (Romero *et al*., 2010), with an ash content between 2.5 to 15.7% (Sánchez and Royse, 2002). Mushrooms are known as decomposers of organic matter in general and particularly of cellulose, for which it produces a series of enzymes (Saavedra, 2007; Gutiérrez-Rojas *et al*., 2014). The species of *Pleurotus*

degrades a complex lignin-cellulose-hemicellulose structure and takes nutrients for their development, growing in a wide range of woody materials such as agricultural wastes and forestry, which are mainly composed of polymers present in the walls of the plant cells, such as cellulose (40 to 60%), 15 to 35% hemicellulose and lignin (10 to 30%), being the main source of carbon and nitrogen (Kang, 2004; Grilli *et al.*, 2015). Degradation of caffeine by bacteria to provide a carbon and/or nitrogen source for growth is well characterized, both biochemically (Dash and Gummadi, 2006) and genetically (Summers *et al*., 2015) by N-demethylation steps to yield xanthine, which is further catabolized via the purine degradation pathway.

Individual physiological capabilities of species to attack lignocellulosic waste materials helps to decide upon the choice of substrates (Kües, 2007). Lignocellulosic wastes are usually low in nitrogen; for example, cereal straw has a nitrogen content of only 0.59 – 0.66% (Stamets, 2005). Oyster mushrooms usually perform better on waste that has available nitrogen content ranges from 0.56 – 0.66% (Tisdale *et al*., 2006; Masevhe *et al*., 2016). Addition of nitrogen-rich supplements, such as ammonium sulphate, ammonium nitrate, urea or organic nitrogen sources such as fish meal (10.2% N), soybean meal (77% N), chicken manure and brewer's grain (3.2 – 4.4% N) to improve C:N ratio in a growth substrate can therefore positively influence button mushroom yields (Rajarathnam *et al*., 2001; Noble *et al*., 2002; Demirer *et al*., 2005; Tan *et al*., 2005). Also, growth substrates might be supplemented with micro-nutrient fertilisers (Royse *et al*., 2004; Weil *et al*., 2006). Manganese ions can stimulate the action of certain peroxidases in lignocellulose degradation as the mineral of primary importance. In some instances, for example, for *A. bisporus* and *Caprinus comatus* production, composting of the substrate prior to use, helps to create a better acceptance of the substrate by a fungus (Oei, 2003).

Table 2.2. Substrates for mushroom production, their handling and examples of use (Kües, 2007)

Substrate	Treatment	Type of cultivation	Cultured species
Sawdust (beech, oak, ...)	Sterilisation/ autoclaving	Bag* 	Pleurotus spp., Ganoderma spp., Flammulina velutipes, Tremella fuciformis, Hericium erinaceus, Auricularia spp.
Straw (wheat, rye, rice, oat, barley, cotton, ...)		Bottle* 	
Industrial and agricultural lignocellulose-wastes (coffee pulp, coffee waste, cotton stalks, paper, ...)	Pasteurisation	Shelf 	Pleurotus spp., Stropharia rugoso-annulata, Volvariella volvacea
Manure (horse and chicken dung) **Soil** (as compost or casing layer)	Composting/ fermenting	Tray 	Agaricus spp., Coprinus comatus, Lepista nuda

* Filters in bags and bottles for air sterilisation are indicated by the differential shading

Lime and gypsum are added to substrate in order to stabilise the pH between 6.4-7.8 which encourages the mycelia to colonise the media (Oei, 2003; Mandeel *et al*., 2005). According to Oei (2003), the selection of substrate depends on the availability of nutrients, pH, microbial activity, aeration and water content. The more easily accessible nutrients are available, the higher the yield and the higher the risk of contamination. If infection pressure is high, it might be more profitable to add fewer nutrients, thus obtaining a lower yield, but less infection (Oei, 2003). If the substrate is either too tight or too loose, the mycelium will have difficulty colonising it. If it is too loose, the mycelium will need more energy to reach the next bit of sawdust or straw substrate. If it is too tight, the mycelium cannot respire, as it needs oxygen and will also release carbon dioxide. Both low oxygen and high carbon dioxide concentrations will retard its growth rate; therefore, good aeration is necessary (Oei, 2003).

Pasteurisation methods for substrates

Pasteurisation methods have been fully described by Stamets (2000); they are summarised below:

***Hot water bath*:** The required temperature should be between 65 – 85°C for 1 hour. The best method was also confirmed by Oei (2003).

***Steam pasteurisation*:** The minimum recommended time for pasteurisation is 2 hours above 71°C (Kurtzman, 2010). The temperature is maintained above 71°C with relevant heating when required.

***Hydrated lime bath method*:** Lime of 0.046M can be prepared in water to give a pH value of 9.5 or higher. The optimum ratio used for straw to the prepared lime is 1:22.7 (mass:volume). After 3 or 4 days of initial growth, the pH slowly falls as the mycelia race through straw secreting acids and enzymes. One week after inoculation, the straw should be fully colonised. If colonisation is not complete within 7 to 10 days, competitors usually arise. Optimising the parameters for the species being cultivated greatly influences the success or failure of this simple method.

***Bleach bath method*:** This is similar to the hydrated lime bath method, but household bleach (5.25% sodium hypochlorite) is used as a disinfectant. Due to the presence of caustic soda in sodium hypochlorite, the pH of the water is increased. When sodium hypochlorite dissolves in water, two substances form, which play a role in oxidation and disinfection. These are hypochlorous acid (HOCl) and the less active hypochlorite ion (OCl-).

***Hydroxide peroxide techniques*:** This method was developed by Rush Wayne in 1999 and is more useful to individuals who do not have access to many of the more expensive tools. Hydrogen peroxide works to kill many fungal spores, yeasts and bacteria by producing a reactive form of oxygen, which destroys the cell walls. Although not thorough enough to neutralise most natural fungal contaminants resident in raw sawdust, straw or composts, hydroxide peroxide can help to complete the process initiated with many preheated substrates. When wood is baked in an oven at 149°C for 3 hours, compounds are destroyed in wood that would otherwise neutralise peroxide. This technique is an effective heat pre-treatment where

hardwood fuel pellets used for wood stoves can first be boiled in water, drained and then allowed to cool. Recommended hydrogen peroxide concentration is 0.03% in water (v/v). This water can then be used to drench the substrate to further reduce the likelihood of competitors. When diluted, hydrogen peroxide is added to wood and allowed to sit for 8 hours. The substrate can then be inoculated with a modicum of success.

***High pressure extrusion method*:** This methodology was first developed for the commercial feed industry in the creation of pelletised rabbit, chicken or cattle feed. The effective reduction of substrate causes frictional heat to increase. For example, a 6:1 reduction of straw into a 10 mm sized pellet creates a thermal impaction zone where temperatures exceed 80°C, which is sufficient for pasteurisation. Pellets between 50 and 100 mm in diameter are better suited for the production of the fruit body than smaller pellets.

***Detergent bath method*:** This method simply utilises biodegradable detergents (soap) containing fatty oils to treat bulk substrates. Coupled with surfactants that allow thorough penetration, these detergents kill a majority of contaminants that compete with the mushroom mycelium. The substrate is submerged into a detergent solution, excess water is drained and the substrate ready for inoculation (Aloha Medicinals, 2018).

***Yeast fermentation method*:** Recommended rates vary between 1% to 5% sugar broth. Fermentation proceeds for 2 to 3 days undisturbed in a sealed drum at room temperature (24°C). Another yeast culture can be introduced for a secondary, booster fermentation that lasts for another 24 hours. After this period of fermentation, chopped straw is forcibly submerged into the yeast broth for no more than 48 hours. Not only do these yeasts multiply, thereby absorbing readily available nutrients which can then be consumed by the mushroom mycelium, but metabolites such as alcohol and antibacterial by-products are generated in the process of killing competitors. Upon draining, the straw is inoculated using standard procedures as described by Stamets (2000).

2.2.3. Growth regulators and their role in mushroom production

Fungal growth is controlled by certain chemical substances known as hormones, also known as growth factors, growth substances, growth regulators, and/or phytohormones (Sneha, 2011), which can either promote or inhibit the growth of the mushroom. Baxamusa (2010) and Sneha

(2011) mentioned five major plant hormones, namely auxin, gibberellin, cytokinin, abscisic acid and ethylene. The auxins, gibberellins and cytokinins act as growth stimulators, whereas abscisic acid and ethylene act as growth inhibitors.

These hormones help to regulate the fruit body by responding to the various signals from the mushrooms and the environment (Baxamusa, 2010); they regulate different tissues during the different stages of development. According to Ahlawat (2011), hormones are either plant or microbial products. The microorganisms, which influence the growth characteristics or behaviour of other microorganisms, affect growth through some metabolites like anti-microbial compounds and growth promoting hormones, including indole acetic acid, indole butyric acid, cytokinins and gibberellic acid. There are plenty of reports available on the use of these hormones in horticultural crops either to improve the yield of the fruits or vegetables or their quality, as compared to mushrooms (Ahlawat, 2011). Mushrooms are different from higher plants because of the nature of the growing medium and other aspects like their position in the plant kingdom, but still there are a few reports on the use of hormones in mushroom cultivation. Work done in India have indicated that commercial preparations of indole butyric acid (IBA) stimulate mushroom mycelial growth under in vitro conditions. The spray of such a formulation on mushroom beds stimulates early pinning, as well as higher yield of mushrooms. Most of the yield is harvested during the first 15 days of cropping. The results are equally positive in both *A. bisporus* and *A. bitorquis*. The hormone IBA spray can be done in the following steps: i) The commercial preparation of IBA, i.e. Veradix-2, should be mixed thoroughly in water at 0.1% (w/v), and ii) the spray should be done at 50 ml/10 kg mushroom compost bag each at the pinning initiation stages of 1st, 2nd and 3rd flush of cropping.

Functions of auxin and uses of synthetic auxin: auxin is used for cell elongation, prevention of lodging, apical dominance, photo-tropism and geotropism, promoting root initiation in the callus, stem cutting, delay of abscission of leaves, inducing parthenocarpy, increasing the number of female flowers and activating the cambium (Baxamusa, 2010).

Functions of gibberellins: they stimulate stem elongation, lead to development of seedless fruits, delay senescence in leaves and citrus fruits, and end seed dormancy in plants that require light for induction of germination (Baxamusa, 2010).

Functions of cytokinins: they are responsible for cell division, morphogenesis, apical dominance; they delay senescence, induce flowering, promote phloem transport, accumulation of salts in the cells, and production of female flowers; and increase resistance to low and high temperature and diseases (Baxamusa, 2010).

Functions of abscisic acid: it hastens formation of the abscission layer and senescence of leaves and fruit; stops transpiration; promotes bud dormancy in seed during winters; induces seed dormancy; inhibits cambial activity; induces flowering in strawberry; is active in seed development, maturation; causes the synthesise of carotenoids in green oranges as given by Baxamusa (2010) and Sneha (2011).

Cultivation of Pleurotus species on straw

The cultivation steps were described by Zadrazil *et al*. (2004) for home purposes and on small mushroom farms mostly in Asia. After the pre-treatment of the straw, this substrate is usually inoculated with grain spawn and placed into suitable containers, that is, plastic bags, bottles, blocks covered with plastic, and trays. The spawned substrate containers are transferred to an incubation room for growth at a temperature of around 25°C, followed by the induction of fruiting by lowering the temperature and increasing the humidity (Stamets, 2000; Oei, 2003) (Plate 2.10). During fruiting, CO_2 has to be controlled since it affects the shape of the stipe. High CO_2 levels will increase the length of the stipe. In cultivation of *P. ostreatus,* a low CO_2 concentration is necessary during the formation of the fruiting body in order to suppress the elongation of the stipe (Oei, 2003). In contrast, *P. eryngii*, where a long stipe is expected by consumers, this can be realised by reducing the fresh air supply.

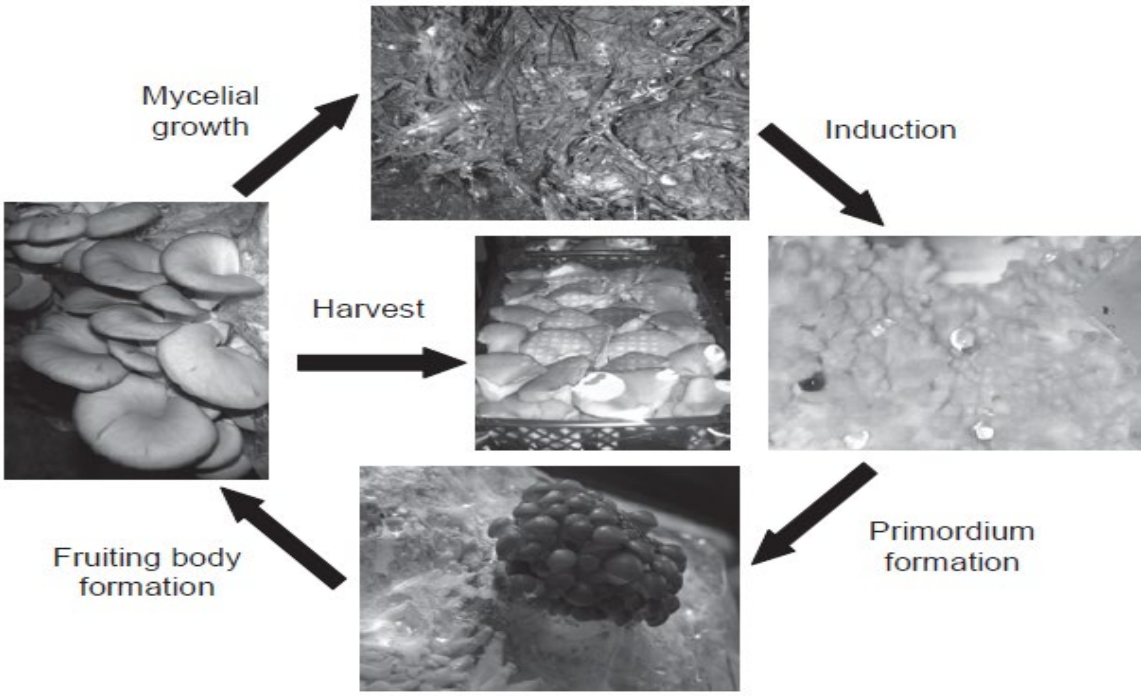


Plate 2.10. Main steps in mushroom cultivation documented for *Pleurotus ostreatus* in pasteurised wheat straw (Kües, 2007)

2.3. Optimal growing conditions for mushrooms

The inoculated substrates are used to fill plastic bags or trays and transferred to an enclosed room or building to incubate for a period of up to 12 weeks, depending on the variety of mushroom (Marshall and Nair, 2009). The plastic bags are normally hung on wires or placed on wooden racks to minimise contamination. Humidity levels are very important for mycelium to colonise over the next two weeks, therefore water needs to be available and the temperature must be controlled from 24 – 27°C by sprinkling water over the plastic bags. The crop should be protected from sunlight and strong winds at all times, which can cause the mushrooms to dry out (Marshall and Nair, 2009). Humidity can be maintained in the growing room by hanging wet rags at several points around the walls or watering the floor using micro-jet sprinklers. The temperature can be regulated by misting with micro-jet irrigation and cooling could be assisted by using a table fan blowing over a container of water, and air circulating between the sacks should help assist with regulation of the temperature, where effective disinfectant such as sodium hypochlorite is used. Fresh Mushrooms (2013) indicate that the correct water content of the substrate is critical, since too much will exclude oxygen by occupying the pore space and too little can limit the growth of fungi. There is a link between water, nutritive value, microbial activity and temperature, because it is a chain, when one

condition is limiting one factor, the whole chain will cease to function. According to Fresh Mushrooms (2013), ventilation is essential for mushroom growing and necessary to control humidity and temperature. It is essential to maintain hygienic conditions by disinfecting the growing room with sodium hypochlorite and replacing the sand over the general cropping cycle in order to protect the crop from contamination from *Trichoderma* species, green mould, bacterial blotch, dry bubble disease, etc (Marshall and Nair, 2009).

2.4. Approximate composition of oyster mushrooms

Apart from being famous for their appetising flavour, mushrooms are a potential source of protein (Hafiz *et al*., 2003). The great advantage is that mushrooms possess the capacity to convert nutritionally valueless substances into high protein food (Tolera and Abera, 2017). The digestibility of mushroom protein was as high as 72-83% in the study conducted by Al-Momany (2011). It is reported that mushrooms are suitable as an addition to all kinds of food, as well as for medical and pharmaceutical purposes. Mushrooms are useful for diabetic and heart patients and constitute an important source of nutritive proteins and minerals for vegetarians (Valverde *et al*., 2015). On an area basis, mushrooms offer a more valuable source of protein than either cattle or fish (Hafiz *et al*., 2003). Mushrooms are a healthy food that is low in calories but rich in protein, dietary fibre, vitamins, and minerals (Farzana and Mohajan, 2015).

***Moisture content*:** Generally, fresh mushrooms contain approximately 90% moisture content and 10% dry weight (Akyuz and Kirbag, 2010). In mushroom consignments, moisture content is affected by environmental factors such as temperature and relative humidity during storage (Table 2.3).

***Fat*:** The fat content in different species of *Pleurotus* ranges from 1.08% to 9.4% in terms of dry weight and on average the *Pleurotus* species contains 2.85% fat (Obodai *et al*., 2017) (see Table 2.3). The crude fat of mushrooms is represented by all classes of lipid compounds, including free fatty acids, mono-, di- and tri-glycerides, sterols, sterol esters and phospholipids. The major neutral lipid of the oidia of *P. ostreatus* is a triglyceride in nature and constitutes 29% of the dry weight (Sharma *et al*., 2008). The main fatty acid is oleic acid (79.4%), with lesser amounts of palmitic (14.3%) and linoleic acids (6.3%).

***Carbohydrate and fibre*:** Carbohydrate is a major constituent of the *Pleurotus* species, ranging from 46.6% to 81.8% (Selvakumar *et al*., 2015). The fibre content ranges from 7.5% to 27.6% in the *Pleurotus* species. The carbohydrate composition of the *Pleurotus* species comprises 4.2% soluble carbohydrates (Table 2.3), 1.66% pentosans and 32.26% hexosans on a dry weight basis (Deepalakshmi and Mirunalini, 2014). The glycogen content in *P. flabellatus* is 8.9% (Kalac, 2016).

Table 2.3. Composition of cultivated mushrooms compared with some common vegetables per 100 g (Kalac, 2009, 2013; Mallikarjuna *et al*., 2012)

Vegetable names	%			
	Moisture	***Fat***	***Carbohydrate***	***Protein***
	Dry weight basis			
Beetroot	87.6	0.1	9.6	12.9
Brinjal	92.7	0.2	5.5	15.1
Cabbage	92.4	0.2	5.3	18.4
Cauliflower	91.7	0.2	4.9	28.8
Green beans	88.9	0.2	7.7	21.6
Green peas	74.3	0.4	17.7	26.1
Potatoes	73.8	0.1	19.1	7.6
Mushrooms	91.1	0.3	4.4	26.9

***Vitamins*:** In *Pleurotus* species, the thiamine content ranges from 1.16 mg to 4.80 mg per 100 g (Yang *et al*., 2001). The niacin content varies from 46 mg to 108.7 mg per 100 g in different species of *Pleurotus* (Caglarirmak, 2007). The ascorbic acid content is higher in the *Pleurotus* species and ranges from 90 mg to 144 mg per 100 g (Caglarirmak, 2007). The vitamin B_{12} content is 1.4 mg per kg dry weight in *P. ostreatus* (Watanabe *et al*., 2014). The ergosterol content, a main sterol in *P. ostreatus,* is 70% (Dupont *et al*., 2012). The ergosterol present in mushrooms can be converted to vitamin D under ultraviolet irradiation (Jasinghe *et al*., 2007).

***Protein*:** Protein content is usually measured by determining the nitrogen content and multiplying this figure by 6.25 (Farzan and Mohajan, 2015). An assumption is that nitrogen is only present in the form of protein. Some of the nitrogen is used in the cell wall (chitin). A

factor of 70% x 6.25 = 4.38, is considered an approximate real protein content for all types of mushrooms (Farzan and Mohajan, 2015). Mushrooms are often referred to as an alternative protein sources in cases where meat products are hard to come by (Farzan and Mohajan, 2015) (Table 2.3) and (Table 2.4).

Table 2.4 Nutritional information of raw oyster mushrooms per 100 g provided by SelfNutritionData (2015) (the nutrition values of 0% were excluded)

Nutritional	***Amounts per serving***	***Percentage Daily Value (%DV)***
Calories	43 g	2%
Carbohydrates	6.5 g	2%
Dietary fibre	2.3 g	9%
Fat	0.4 g	1%
Protein	3.3 g	7%
Vitamin A	48 IU	1%
Thiamin	0.1 mg	8%
Riboflavin	0.3 mg	21%
Niacin	5 mg	25%
Vitamin B6	0.1 mg	6%
Folate	27 mcg	7%
Pantothenic acid	1.3 mg	13%
Iron	1.3 mg	7%
Magnesium	18 mg	5%
Phosphorus	120 mg	12%
Potassium	420 mg	12%
Sodium	18 mg	1%
Zinc	0.8 mg	5%
Copper	0.2 mg	12%
Manganese	0.1 mg	6%
Selenium	2.6 mcg	4%

2.5. Harvesting of mushrooms

Firstly, young mushrooms last much longer after harvest than aged mushrooms since the state of maturity are not the same (Stamets, 2000). Once the spores have developed on the gills, perishability is accelerated. If mushrooms have partial veils, like button mushrooms (*Agaricus brunnescens*) or black popular mushrooms (*Agrocybe aegerita*) do, they are best picked while the partial veils are intact; in other words, when mushrooms are still young. Partial veils protect the gills, limiting moisture loss, preventing spore release and rupture only as the caps expand. Clusters yield (denoted as fresh mushrooms mass per bag) higher quality mushrooms than those grown individually. Bouquets or cluster of mushrooms have obvious advantages both from the point of view of harvesting, as well as marketing (Stamets, 2000).

Mushrooms are harvested based on consumer preferences in the markets (Amin *et al*., 2017). They can be picked with ease needing minimum handling and trimming (Stamets, 2000). Once harvested, they protect one another when bunched together, as they limit damage caused by individual, loose mushrooms jostling against one another. Most importantly, bouquets or clusters at the ideal stage for harvesting are composed of younger mushrooms. Mushroom bouquets or clusters can be sold much like broccoli. All these features combined extend the shelf-life far beyond that of individual mushrooms and make clusters highly desirable (Stamets, 2000). Harvesting standards for oyster mushrooms are dictated upon by what the end product is going to be used for (Amin *et al*., 2017). They are generally harvested before showing slightly curled edges and spore shedding, and with caps measuring 20-25 mm (Miles and Chang, 2004).

2.6. Classification of harvested mushrooms into different grades

There are general acceptable minimum standards for grading of mushrooms which are: free of mechanical and/or physiological damage; sound, produce affected by rotting or deterioration such as to make it unfit for consumption is excluded; cluster or single. Grading of mushrooms is important for marketing. Generally, the grading is done by segregation of mushrooms into various grade standards as per market demand (human consumption). For example, button mushrooms are graded into ***Grade A, B*** and ***C***, whereas oyster mushrooms are graded following the system denoted as ***Extra class, Class 1*** and ***Class 2*** (Asean Stan 35: 2014).

Quality of mushrooms

Visual evaluation: Shewfelt (2014) indicated that visual evaluation of the quality characteristics by an expert panel, despite its limitations, is still a widely used and accepted technique. Numerical scales for specific attributes are available for commodities when no chemical or physical measure that relates to specific purchase characteristics is available (Shewfelt, 2014). Scales of evaluation are treated as objective measures, but they are fraught with problems regarding sensory analysis without having many of the safeguards required for such techniques: Scoring is subject to variability by the expert panels; It is almost impossible to "blind" judge the treatments, particularly when the samples are evaluated over time in a storage study; the full range of scales are rarely used, since studies are usually stopped when the sample drifts into the lower (poor quality) end of the scale and results tend to be analysed assuming linearity of scale, although in many cases no clear evidence exists that the points on the scale occur at equal intervals (Shewfelt, 2014).

***Colour*:** The use of colorimeter generates a composite three parameter L*a*b* number (Kortei *et al*., 2015) and it can be of use to determine colour differential of oyster mushrooms. The CIE 1976 L*a*b* is an approximately uniform colour scale (Hunterlab Technical Manual, 2008). It is used extensively in many industries throughout the world which is sometimes referred to as the CIELAB colour difference metric. The colour indices such as L, a, b denotes the surface colour of oyster mushrooms together with the calculated chroma and hue angle (Mkhathini *et al*., 2017). L* is commonly known as lightness and measures the lightness of mushrooms, ranging from 100 to zero, for perfect white to black, respectively. Colour index a* measures redness when positive and green when negative while b* measures yellowness when positive and blueness when negative. All the indices help to clearly define mushroom colour, including the chroma which indicates pure chromatic colour and hue angle which combines a* and b* coordinates to make up the overall colour (Dar *et al.*, 2019).

***Extra class*,** Oyster mushroom must be of superior quality. They can have slight superficial defects, provided these do not affect the general appearance of the produce, the quality, the keeping quality and presentation in the package. It must also be presented as cluster, only 5% of defects shall be allowed for bloom-cap

Class 1**,** Oyster mushroom must be of good quality. It can have slight defects of colour and shape; slight defects on the cap due to rubbing and other superficial defects such as breakage and blemish not exceeding 5% of the total surface area. The defects must not, in any case, affect the gill. For oyster mushroom presented as cluster, only 15% defects shall be allowed for bloom-cap.

Class 2**,** This class includes oyster mushroom which do not qualify for inclusion in the higher classes, but satisfy the minimum requirements as stated earlier. It can have defects in shape and colour such as slight defects on the cap due to rubbing and other superficial defects such as breakage and blemish not exceeding 10% of the total surface area. The defects must not, in any case, affect the gill. For oyster mushroom presented as cluster, 20% shall be allowed for bloom-cap.

2.7. Maintaining post-harvest quality

Freshly harvested oyster mushrooms are highly perishable because of their high moisture content, metabolism and susceptibility to enzymatic browning (Bhupinder and Ibitwar, 2007).

Packaging and storing the crop for market

Packaging of mushrooms is carried out in a pack-house (Gabriel, 2015). While some growers prefer to trim the stalks prior to packaging, others grade their crop as they pick, carrying them in groups of three to four chips each to receive a particular grade.

Once the mushrooms have been harvested they must be chilled by frequently being ferried to the cold room (Stamets, 2000). Larger farms utilise blast chillers, which precipitously drop the temperature to near freezing. Cardboard boxes when used have the advantage of insulating the mushrooms after harvest. During or after cooling, the mushrooms are sorted and packaged. Once cooled, mushrooms must not be re-warmed until delivery. The ideal temperature for storage is 1-2°C (Stamets, 2000). If mushrooms are covered with clear, anti-condensate, breathable plastic, they can be preserved for extended periods of time. Even with the shelf-life

being extended, mushrooms should be rotated in the stores at least twice weekly to ensure the highest quality product (Stamets, 2000).

Punching of holes in a plastic covering can maintain a high relative humidity around produce but it may be less effective in delaying mushroom spoilage because it does not have a similar effect on the CO_2 and O_2 content of the atmosphere inside the bag. The holes may be very small and, in such cases, they are commonly referred to as micro-perforations. Mushrooms can be stored for a maximum of 7 days in a refrigerator within the plastic as described above (Bhupinder and Ibitwar, 2007). Mangaraj *et al*. (2009) indicated that attempts have been made to maintain the quality of mushrooms by wrapping consumer units with selectively permeable or perforated film.

Paper bags are commonly recommended for storing mushrooms (Tantillo, 2011). They are able to absorb moisture from the mushrooms, and therefore paper bags are also placed in a larger perforated plastic bag. This two-bag system allows mushrooms to breathe but not become dry. Properly stored mushrooms should last several days. Mushrooms should not be frozen unless first blanched, then covered in the blanching water before being placed in a freezer (Tantillo, 2011).

The majority of present-day crop storage methods were known and used thousands of years ago (Prakash, 2001). Some evidence is fragmentary, but there is sufficient present literature on the well-known storage techniques such as drying, sealing, cooling, freezing, fermentation, pickling, salting and smoking. These were all used, a very long time ago, albeit often in a crude manner (Freedman and Freedman, 2007). The main advantage of cold storage over other storage methods is the control of temperature and humidity (Basediya *et al*., 2013).

The optimum temperature for *A. bisporus* mushrooms storage is 0 to 3°C and 95% relative humidity (Aguirre *et al*., 2008). Relative humidity and temperature are the most important factors in soybean seed storage, where the relative humidity of the atmosphere around the seed controls the fungi and insect activity while in storage (Monira *et al*., 2012). Little fungal activity occurs below 75% RH, while all insects and fungi present during storage become inactive at a relative humidity of less than 60% (Mason and McDonough, 2012). Like all fruits and vegetables, mushrooms are perishable, and after harvest, they often change in ways that make them unacceptable for human consumption (Rawat, 2015). The most readily observable

of these changes include wilting, ripening, browning, liquefaction, and loss of moisture, as well as loss of texture, aroma and flavour (Yee, 2001). These changes are usually preceded by an increase in the rate of respiration, which when coupled with other reactions and with the cessation of nutrient supply, will trigger a series of irreversible reactions that damage the mushrooms.

The shelf-life of mushrooms can often be extended by pre-treatments and/or storage at chilling temperatures (0 to 1°C). Chilling storage is not always effective, particularly for some tropical or subtropical mushrooms which may suffer from chilling injury (Kader and Rolle, 2004). For long term storage of mushrooms, canning, pickling and drying processes are employed. The quality of the end product is not often comparable to the quality of fresh mushrooms and furthermore, these processes are not always suitable for all types of mushrooms (Pitt and Hocking, 2009).

An effective method for reducing water loss from fresh mushrooms, is to increase the relative humidity of the air (Wills *et al*., 2000). This reduces the vapour pressure difference between the produce and the air and hence the amount of water required to be evaporated from the produce before the air is saturated with water vapour (Wills and Godling, 2016). Use of a very high relative humidity, however, favours the growth of moulds on certain commodities. A relative humidity of 90% is usually the best compromise of conditions for the storage of fruit; however, a relative humidity of 98 to 100% is better for leafy and certain root vegetables, including mushrooms, which have a higher coefficient of transpiration (Wills *et al*., 2000). Increased air peculation is directly proportional to water loss (Wills and Godling, 2016). Ambient air pressure is inversely proportional to water loss (Wills *et al*., 2000).

2.8. Preserving using sulphur dioxide

Preserving mushrooms in an extract form is much more convenient than to preserve the whole mushroom as this maintains their delicate taste and aroma (Devi *et al*., 2015). Spoilage is controlled by reducing the moisture content to 12% (Fontana, 2000). Treatment of dried mushroom with sulphur dioxide may restrain the growth of certain fungal species. Sulphur dioxide is more effective against moulds and bacteria than yeasts. Sulphur dioxide is widely used as a food preservative because of the following reason: it acts as an antioxidant. Because of this, it maintains the appearance of mushrooms and prevents rotting. During the storage of

dried foods, the presence of sulphur dioxide prevents browning reactions and off-flavour development, as well as loss of vitamin C and carotene (Fontana, 2000). Mostly, the solution is applied as a dip after the blanching but before the drying of vegetables and mushrooms.

2.9. Drying of mushrooms

The aim of preservation is to keep the nutritional value of a crop for a longer period (Oei, 2003). The nutritional value of fresh mushrooms is usually better than that of the non-fresh ones and the taste may become better because of the conservation treatments. Most mushrooms consist of approximately 90% water, while reishi mushrooms, being woody in texture, usually contain between 70 – 80% water. When Shiitake are grown outside, especially in Donko (cracked cap) form, the moisture content is often only 80%. When mushrooms are young, the moisture content is usually higher than when they are mature. Mature mushrooms with their gills exposed, dry faster than young closed mushrooms (Stamets, 2000).

Drying is also based on the principle of limited free water availability (Oei, 2003). Drying has several advantages: it is easy, quick and safe. The market for dried oyster mushrooms is, however, small compared to the market for dried shiitake (Oei, 2003). Shiitake, oyster, morels, reishi and many other mushrooms dry readily and can be stored for many months. Mushrooms can be sold in their natural form or powdered for soups, spice mixtures and teas. For example, during the drying of shiitake mushrooms, they are divided into pieces of a suitable size, which are strung on threads and suspended over a stove until bone-dry (Kantrong *et al*., 2014). Some cultivators actually preserve their dried mushrooms without harming the quality in order to prolong storage. Once dried, mushrooms should be hermetically sealed and ideally frozen until needed (Stamets, 2000).

Table 2.5. Rating scales developed by Shewfelt (2014) for specific mushroom commodities

Commodity	Attribute	Range
Mushrooms	Quality, black to dark gray colour, uniform shape, immature size, undeveloped pilei	1-5

Drying methods

Mushrooms can be dehydrated by various means such as sunlight, a conventional oven, or an electric dehydrator, and a microwave oven (Srilakshmi, 2003; DeLong, 2006; Sharif *et al*., 2017). Drying, like other preservation methods, requires energy. Unless sun drying is possible, the cost of energy to dehydrate foods is higher than that for canning, and in some cases, more expensive than freezing (Constenbader, 2012). For efficient drying techniques, the following tools and facilities are required: trays, sunlight structure, solar structure, electricity, an oven, and drying facility. Dehydrators with thermostatically controlled heat and forced air circulation are available from a number of commercial sources. Dehydrators require: (i) an enclosed cabinet, (ii) a controlled source of heat, and (iii) forced air to carry away moisture. Venting to allow the intake and exhaust of air is necessary (Swanson, 2003).

Sun drying: Sun drying is the cheapest and oldest method among various drying methods (Rai and Arumuganathan, 2008) and the operation thereof is also very simple, because no fuel or mechanical energy is required. However, it is completely dependent on the weather and it is not possible to maintain it around-the-clock and around-the-year. Even though the quality of the product is affected by exposure to sunlight, relative humidity and general hygienic conditions, this method is often used because of the free availability of the heat source. Mushrooms are spread over the trays or sheets and kept in the open under the sun; favourable atmospheric conditions are above 25°C temperature, with less than 50% relative humidity and a high wind velocity (Rai and Arumuganathan, 2008). In order to dry mushrooms using sunlight, one needs to select the right spot that will receive sunlight all day long, prepare the mushrooms, lay them out in the sun to dry sufficiently.

Electric dehydrator: This is an electrical appliance for drying mushrooms indoors and is placed in a dry, well-ventilated area (Boyer, 2009). A food dehydrator has a heating element, a fan and vents for air circulation. It is designed to dry foods quickly (Harrison and Andress, 2000). Mushrooms are cleaned using a mushroom brush and are sliced according to the required sizes (Meredith, 2015). They are arranged on the dehydrator trays, making sure that none of the pieces touch or overlap each other. They are dried at a temperature of 43°C for 4 to 6 hours or up to 8 hours for thicker slices and allowed to cool completely before transferred to suitable containers and glass jars, which must be covered with tightly fitting lids and stored away from

direct light or heat. Dried oyster mushrooms can be stored for at least a year before they may spoil (Meredith, 2015).

***Freeze-drying*:** Freeze-drying is the removal of water from a substance by means of sublimation from the frozen state to the vapour state (Rai and Arumuganathan, 2008). This drying method takes place in three stages, that is, the water present in the product is removed by the formation of ice crystals; the ice crystals are then removed from the outer surface of the material by means of sublimation; after removal of all the ice, the small quantity of water left is removed by sublimation in the freeze dryer. This method retains the original shape and size of the material dried, unlike other methods where shrinkage is a problem (Rai and Arumuganathan, 2008). Mushrooms are freeze-dried at -20°C and the moisture is removed by means of sublimation at a very low vacuum (0.012 mbar) for 12 – 16 h. The freeze-dried mushrooms have superior flavour and appearance, but are brittle (Martinez-Soto and Ocanna-Camacho, 2001). They are packed in sturdy packaging and cushion-packs are flushed with nitrogen in order to preserve the product better (Rai and Arumuganathan, 2008). This product can be stored for up to 6 months, without any change in its quality and appearance.

***Microwave-vacuum driers*:** A microwave oven is used with a modified power output from 0 to 600 Watts by incorporating a 230 Volts AC in the circuit (Sharma and Prasad, 2001; Giri and Prasad, 2006). The microwave-vacuum is carried out at power level of 200 W and a system pressure of 6.5 kPa. A container of mushrooms placed on a perforated Teflon plate can be placed in a microwave oven cavity. A vacuum pump with a pressure-regulating valve is connected to the container in order to maintain the desired level of the vacuum inside it. The extent of the vacuum in the container is monitored with a vacuum gauge and the airtight condenser is used in the vacuum line for condensing the water vapour released from the drying of the mushrooms (Giri and Prasad, 2013).

2.10. Marketing the product

A number of organisations help growers to find markets for their mushrooms (Rai and Arumuganathan, 2008; Jackson, 2014). Some co-operative marketing organisations coordinate production and sales (Stamets, 2000). Co-operative marketing becomes a necessity when multiple growers overwhelm local markets. Like in Asia, marketing gourmet mushrooms has benefited from a long tradition, while in North America and South Africa, gourmet mushrooms

are a relatively new phenomenon having been available for less than twenty years (Stamets, 2000). Cycles of over and under production are typical in any new expanding market place and should be expected. Growers must adapt their production schedules and product lines so that they do not become over extended. As the public becomes increasingly aware of the health properties of mushrooms, the markets will expand. The marketing potential of dried oyster mushrooms is still limited (Oei, 2003) in the world markets, including South Africa. There are ideal marketing strategies for oyster mushrooms in South Africa. Advertising the oyster mushrooms in Public TV stations and Public Radio stations being the first. Others include developing oyster mushroom festivals similar to Marula Festivals in Limpopo and stakeholders where participants can be given freebees like calendars with mushroom logos, T-shirts, caps and key holders. Furthermore, giving out free samples and tasting in supermarkets could also be considered (Personal view).

CHAPTER THREE

The effect of wheat straw leachate on growth of oyster mushroom spawn

3.1. Introduction

"Oyster mushroom" is an exotic edible mushroom with several health benefits such as to strengthen tendons, inhibit tumours, improve liver and kidney functioning and aid in gastrointestinal disorders (Stamets, 2000). It treats anaemia, diabetes, high blood pressure and lowers cholesterol (Vetayasuporn, 2004). It is carcinogenic above certain particular concentrations (Caglarirmak, 2007). Despite all the advantageous, South Africans are not exploiting this crop. One advantage of mushroom production is that it does not require soil or large production areas. In addition, a mushroom production unit can be set-up easily in any backyard wooden structures, with some basic equipment.

This research focuses on the use of wheat straw substrate as a single growing medium for oyster mushroom in S.A. It can be grown from many substrates in other countries. Wheat straw (*Triticum aestivum*) contains chemical compounds that can be used to control weeds, pests and diseases by inhibiting growth of other plant species (Wu *et al*., 2001). So far, the mushroom industry is the only industry that directly converts wheat straw into a protein source for human consumption (Das and Singh, 2004). According to Labuschagne *et al*. (2000), wheat straw consists of the fractions of cereal plants which are above ground after removal of the grains. It consists of structural polysaccharides and lignin as the main components.

Zeng *et al*. (2008) stated that wheat straw contains water soluble toxic substances which inhibit seed germination and growth of maize seedlings. According to Wu *et al*. (2007), roots of wheat, oats and other related crop plants release chemicals that inhibit the growth of their own seedlings. Allelopathy is defined as the direct inhibitory influence of one plant by another through displacement of residues under natural conditions by means of chemical rather than nutritional agents (Ferguson and Rathinasabapathi, 2009; Mubeen *et al*., 2011). Ferguson *et al*. (2016) also defined allelopathy as the beneficial or harmful effects of one plant on another plant, both crop and weed species, from the release of biochemicals, known as allelochemicals. This can be achieved when plant parts leach out through root exudation, volatilization, residue

decomposition, and other processes in both natural and agricultural systems. The effects of allelopathic inhibitory growth vary among different parts of the same plants, with regards to their germination and initial growth (Economou *et al*., 2002; Aziz *et al*., 2008). Allelopathic inhibition can involve a complex interaction of different classes of chemicals such as phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates and amino acids or a mixture of different compounds, sometimes exerting a greater allelopathic effect than individual compounds (Ferguson and Rathinasabapathi, 2009). The phenolic compounds may have inhibitory or stimulatory effects on plant growth depending on their concentration in the soil and the sensitivity of the growing crop (Liebman and Davis, 2000). Previous studies have indicated that plant tissues possess allelopathic potential, but may not be leached or exuded from the plants into the environment to exhibit seedling allelopathy under natural conditions (Wu *et al*., 2000).

Wheat seedlings have allelopathic effects on the growth of a number of agricultural weeds (Wu *et al*., 2000). Grodzinskii (2016) termed phytotoxic substances capable of inhibiting the growth of mushrooms as allelochemicals. The allelopathic effects are due to inhibitory substances that are released directly from plants that are still alive into the environment through root exudation, leaching, volatilisation and the decomposition of plant residue (Weston and Duke, 2003). A potential disadvantage of using leachate extracted as weed control from a crop field is that the inhibitory effects are species specific. Similarly, leachate from wheat straw inhibited cotton seed germination (Liebman and Davis, 2000; Khaliq *et al*., 2013). In addition, crop leachate has been shown to promote the production of phytotoxins by certain bacteria (Martínez-Viveros *et al*. 2010). Pretreatment of leachate to detoxify organic compounds may be necessary before it can be used as a plant nutrient source. Lettuce seeds provide distinct advantages over most other test organisms: they are inexpensive, easy to culture, and require no upkeep between experiments (Gonai *et al*., 2004). They can be used for water and sediments including heavy metals, some pesticides and other organic toxicants.

According to Wu *et al*. (2007) and Alsaadawi (2008), the phytotoxins released from decomposing wheat residue reduce yield. Qasem and Foy (2008) reported an isolated component from wheat glumes that showed strong phytotoxic effects on *Imperata cylindrica*. Farmers around Mpumalanga Province (South Africa) use wheat straw as a growing medium for oyster mushrooms but its non-availability and high cost limit its general use.

The aim of this study was to assess the potential growth inhibition of wheat straw leachate on the growth of oyster mushroom cultures.

The objectives of the study were (i) to assess the potential growth inhibition of wheat straw leachate on lettuce seed germination and oyster mushroom spawn, and (ii) to investigate potassium, calcium and magnesium salt concentrations capable of growth inhibition on stored wheat straw. The following hypotheses were consequently tested:

(i) Null hypothesis (H_0) was that wheat leachate will not have a significant effect on oyster mushroom germination and growth.

(ii) Alternative hypothesis (H_1) was that wheat leachate will have a significant effect on oyster mushroom germination and growth.

3.2. Materials and methods

Location of the study area

The study was carried out at the Agricultural Research Council - Tropical and Subtropical Crops (ARC-TSC), Mbombela Municipality (25°27' S and 30°58' E and 650 m above sea level), Mpumalanga Province, South Africa. The area is a humid subtropical climate area with mild winters and hot summers.

Wheat straw leachate extraction

Prior to the experiments, dry wheat straw was obtained from Marble Hall (Limpopo Province, South Africa). A single bale (10 kg) of wheat straw (*Triticum aestivum* – Duzi) was boiled (for an hour in order to collect the extract) in water in a 225 L steel drum. The other remaining bales of wheat straw were stored in the shed house (average temperature of 22°C and average relative humidity of 55%, Figure 3.1).

Oyster mushroom experiment design and layout

The first experiment was subjected to five dilutions with distilled water starting with, 100% leachate (undiluted leachate); 75% leachate (3:1); 50% leachate (1:1); 25% leachate (1:3) ratios and distilled water (dH_20) was used as a control. This was repeated four times in a plant growth

room. The experimental design was a completely randomised design (CRD) repeated three times (Table 3.1).

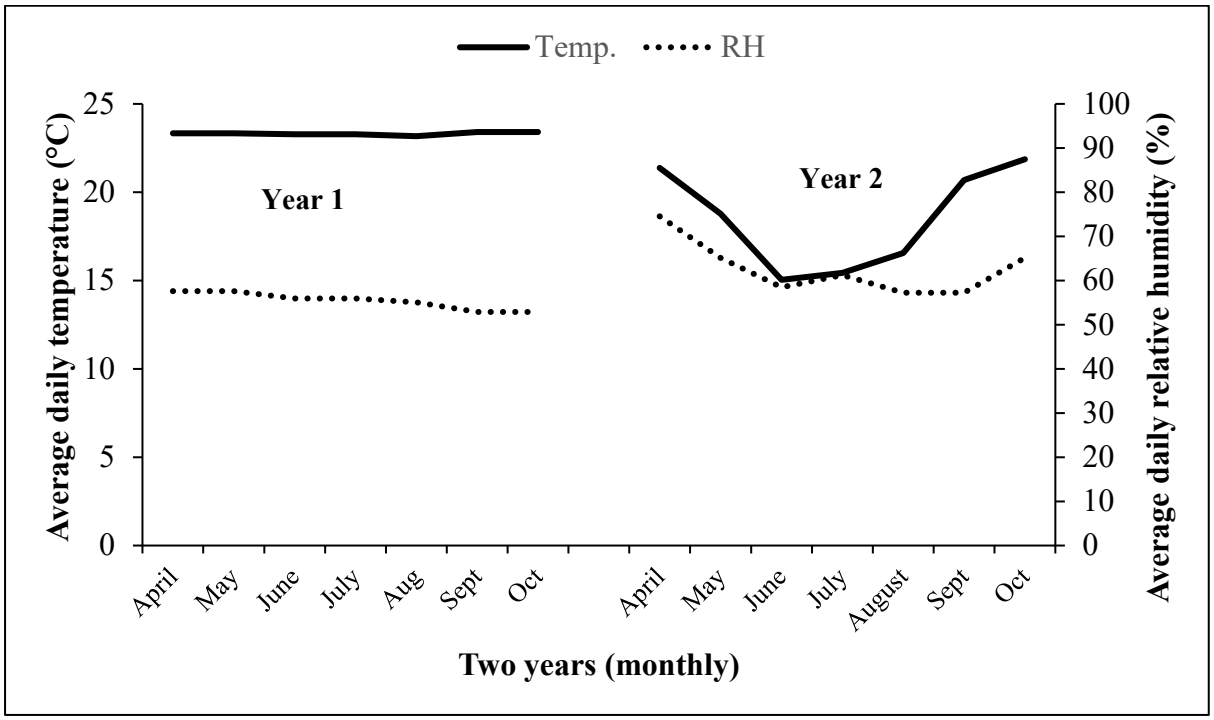


Figure 3.1. Average daily temperature (°C) and relative humidity (%) at the Agricultural Research Council-Tropical and Subtropical Crops (ARC-TSC) experimental farm of Mbombela from 1st year (April to October 2008) and 2nd year (April to October 2010) (ARC-TSC and BASF Friedenheim data, 2008 and 2010)

Table 3.1. Lettuce seed testing and oyster spawn mycelia growth from 0 month to 6 months of wheat straw extract storage period

Cycle	Substrate storage for lettuce	Substrate storage for oyster spawn	Boiling period
1	0 month	0 month	1 h
2	3 months	A*	1 h
3	6 months	6 months	1 h

A* - this come from preliminary studies for mushroom for 3 months did not give any conclusive evidence, hence comparison was between 0 and 6 months.

In the second experiment, for the first phase (0 month), leachate (for oyster mushroom spawn) was collected from the fresh dry wheat straw boiled for an hour, and for the second phase (6 months), leachate was collected from the wheat straw that had been stored for 6 months with separate extractions (Table 3.1). Similar to this experiment, Wu *et al*. (2007) reported a collection of wheat straw in the field from two sites in South Australia of which some used immediately for the experiment while the remaining straw was stored and used as required.

Water and leachate (extracts) analysis

The wheat straw leachates (100%, 75%, 50% and 25% dilutions) as used for this trial were analysed at the ARC-TSC, Soil Science Laboratory. The pH was determined using a Beckman pH meter 61 (Beckman Instrument, USA), which was calibrated at 25°C with a standard buffer solution. The electrical conductivity (EC) was measured using a Cyberscan 300 conductivity meter (Autech Cybernetics, Singapore) using the method outlined by Cavins *et al*. (2000). Potassium (K), calcium (Ca), and magnesium (Mg) were measured using an atomic absorption flame spectrophotometer (Varian Spectr. AA 250 Plus, Australian) as outlined by Masevhe *et al*. (2015).

Initial separation of the allelochemicals

A 20 kg of powder from the plant parts of *Triticum aestivum* (wheat straw), which has the strongest inhibition on wheat germination and growth, was soaked and extracted 3 times with 150 L of the extraction solvent. The filtrate was subsequently concentrated at 35°C in vacuum to give an aqueous residue. A small amount of distilled water was used to dissolve the residues. Petroleum ether, ethyl acetate, and chloroform was chosen as the extraction solvents, and the volume of each extraction solvent was 3 times that of the sample volume. For each extraction, the samples were shaken vigorously for 15 min to ensure complete extraction, and then they were distilled until the full hierarchical analysis. The extracts from the same extraction solvent was combined and concentrated into a paste in a rotary evaporator. A 1.5 g aliquot of extracts was dissolved in 150 mL of methanol, and then, compounds at concentrations of 0.5, 1, and 10 mg/mL were prepared by serial dilution with methanol for the wheat bioassays using the method outlined by Liu *et al*. (2016).

Treatment of lettuce seeds (Phase 1)

The first phase of the lettuce experiment (0 month – April – Autumn trial; 3 months – June - Winter trial and 6 months – October - Spring trial) was conducted using lettuce 'Great Lake 659' obtained from Hygrotech Pty Limited (Mbombela, South Africa) and stored at room temperature. The lettuce seeds were disinfected using a HtH chlorine (12.4% NaOCI - 6.4 g in 500 ml water for 5 minutes - sodium hypochlorite is the grim reaper of bacteria, algae, fungi, slime, and other swimming pool creeps) solution and rinsed three times with dH_20. The dilutions of wheat leachate were sterilised using an autoclave (Hirayama Mfg. Corp.) for an hour to destroy parasitic fungi. Twenty 90 mm diameter Petri-dishes (disposable plastics), each lined with one layer of filter paper (Whatman No. 1) with 25 seeds each, were moistened with 5 ml of extract. The petri-dishes were sealed using parafilm 'M' to reduce moisture loss. The placement of seeds, germination and evaluation of the seedlings were performed in a laminar flow bench (Fibratron – Halfway House) using heat sterilised forceps to minimise contamination. The trial was repeated three times at three-month intervals. Similar methods and procedures were also used in 0 month, 3 months and 6 months wheat straw storage using the same batch of wheat straw with separate extractions.

Treatment of oyster mushroom spawn (Phase 2)

A second phase (0 month – April - Autumn and 6 months – October - Spring trials) was carried out using oyster mushroom spawn (*Pleurotus ostreatus*). Pure oyster mushroom spawn (HK35 strain) was purchased from Exotic Spawn (Pretoria, Irene) and transported to Nelspruit (Dawn wing courier) in cardboard boxes at ambient temperature. The purchased spawn was stored in a cold room at 5°C before use. The wheat straw was stored in a shed at an average temperature of 17°C and a relative humidity of 62% (Figure 3.1). Similar procedures were followed as those for the first phase except that 1 mm of oyster mushroom spawn was placed in each petri-dish. Each petri-dishes was lined with filter paper and remained sealed with parafilm 'M' which was replaced when necessary. The trial was repeated as per Table 3.1 using the same batch of wheat straw with separate extractions.

Controlled plant growth room

A plant growth room with dimensions of 4.0 m length x 2.8 m breadth x 3.1 m height which allowed 16 hours light, 8 hours darkness and the temperature ranging between 25 – 27°C was used for this experiment. The temperature was controlled at 24°C during the day and 16°C during the night, for wheat or oat seedlings. The light intensity (65 µmol/m^2/sec PAR) was measured using a ceptometer (LP-80 ACCUPAR) in the plant growth room.

Growth parameters

The germination rate (%), length of lettuce seedlings (mm), mycelial length (mm) and colony diameter (mm) were measured every 24 hours for four days.

Germination percentage (%)

The germinated seeds were counted manually. The counted germinated seeds were removed every day without damaging the seedling to other petri-dishes for measurement of their length. The germination percentage (G%) was calculated using the method outlined by Saúpe (2009) from *Equation* 1.

$$G\ (\%) = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ (planted)}\ X\ 100 \qquad Eq.\ [1]$$

Seedling length (mm)

The length of the lettuce seedlings was measured using a Vernier calliper (Mitutoyo Manufacturer, China) with the help of forceps from root to tip and calculated using cumulative growth (%) from *Equation* 2.

$$Cumulative\ Growth\ (\%) = \frac{Day\ 2 - Day\ 1}{24\ h\ (Intervals)}\ X\ 100 \qquad Eq.\ [2]$$

Initial length was taken for day one and final length was for day two, which would then be given the estimated growth within 24 hrs.

Colony diameter (mm)

The evaluation of the mycelia was performed using a marking pen and a ruler to measure the colony diameter across two points of the colony. The Petri-dishes were marked at the bottom with a dot in order to see the growth from day to day, where the mycelia stretched into whitish threads. The colony diameter (mm) were calculated in terms of cumulative growth (%).

Statistical analysis

The collected data were subjected to analysis of variance (ANOVA). Treatment means were separated using Fishers' least significance difference (LSD) at 5% ($p < 0.05$) level of confidence. Statistical analysis was carried out using the SAS package, Version 8.2 (Statistical Analysis System Institute Inc. 1999-2001). To determine the relationship between growth inhibition and growth parameters, a quadratic curve was fitted to the data. The used equation was: $Y = a + bx + cx^2$; where Y is the growth parameters (g.); while a, b and c were constants. The mean of the analysed data for 0 month was : $\overline{x}D = 0.554$ and the sample standard deviation (coefficient of variance) was $S_d = 1.13$. The statistical analysis as evident from computation of LSD = 6.09 there was no significant difference between the values, CV = 15 the results are within the same range, $R^2 = 0.80$ the precision of the experiments, Se = 1.13.

3.3. Results and discussion

3.3.1. Lettuce seed germination (%) rate

With distilled water (dH_20 - control), there was 76% of the seeds germinated after Day 1 compared with the undiluted leachate where none germinated during 0-month wheat straw storage period (Figure 3.2). There was a significant difference ($p < 0.05$) between the dH_20 treatment and all other treatments. These results, to our knowledge, are the first to be reported on both lettuce and mushroom growth inhibitions. In contrast to these findings, Kaur *et al*. (2021) reported that the inhibition of seed germination of ryegrass, redroot pigweed and red raspberry in wheat aqueous extracts varied from 4.2% to 8.6%. The dH_20 treatment improved the germination rate of lettuce seeds without any inhibition by the leachate from Day 0 to Day 4. Vozhdayev *et al*. (2015) reported that the germination of maize seeds and the radicle length were inhibited by the leachate from the soil with wheat straw only on the top 2.5 cm and for soil mixed with straw at 42 days compared with soil only as a control.

In 0-month wheat straw storage period (April trial), the inhibition of growth by the undiluted extract was probably due to the freshness of the wheat straw. This straw had a high electrical conductivity (EC) of 3686 mS/m and a high content of potassium (998.51 ppm), calcium (92.29 ppm) and magnesium (57.34 ppm) (Figure 3.2 and Table 3.4). The undiluted extract significantly inhibited the germination of seeds from Day 0 to Day 4. The high calcium of 92.3 ppm in the undiluted extract could probably be the cause of poor germination as it inhibited the uptake of other nutrients such as potassium and magnesium. Similar to these findings, Osemwota *et al*. (2007) reported that the Ca/Mg ratio is related to plant growth and excessive Ca can restrict Mg uptake. Lee *et al*. (2009) also indicated that moderate and excessive treatments of Ca or Mg strongly retarded the growth of the shoots and roots of lettuce plants, while the K concentration in the expanded leaf was lower at higher Ca or Mg concentrations.

The recently dried wheat straw exhibited a strong inhibition of germination in the 0-month wheat straw storage period which can be associated with allelopathic effects. Saffari *et al*. (2010) reported that mulched wheat straw showed the strongest inhibition of germination of maize seedlings 5 days after spawning and that a significant variation existed among different maize cultivars in tolerating allelopathic effects of wheat straw mulch. In the straw with both the dH_20 and the 1:3 ratio (25% extract to 75% dH_20) treatments, the germination of seeds started after Day 1 compared with the straw mulch that had been treated with the dilutions of both 1:1 (50% extract to 50% dH_20) and 3:1 ratios (75% extract to 25% dH_20) as depicted in Figure 3.2. This indicates that the greater the dilution, the greater and earlier the inhibition of germination is broken in an early season as in 0-month wheat straw storage period (Figure 3.2).

With 3 months wheat straw storage period (June trial), the undiluted extract resulted in germination after Day 1 compared with the use of the undiluted extract in the early season when no germination occurred. It also indicates that the stored wheat straw begins to lose its inhibition strength as shown by a decrease in EC (1858 mS/m) and the lower levels of K (548.4 ppm), Ca (15.9 ppm) and Mg (23.1 ppm) in the second cycle (Table 3.4). The undiluted extract resulted in a 3% germination after Day 2, but surprisingly, the germination improved to 40% after Day 3 and to 80% after Day 4. The dH_20 treatment resulted in the germination reaching more than 80% in Day 1 compared with the sample using the undiluted extract, with more than 80% germination being reached only after Day 4. Therefore, the ageing of wheat straw had a

positive effect on the germination of lettuce seeds. The inhibition of germination of seed breaks down with time as witnessed when using the diluted extracts (1:3 and 1:1 ratios).

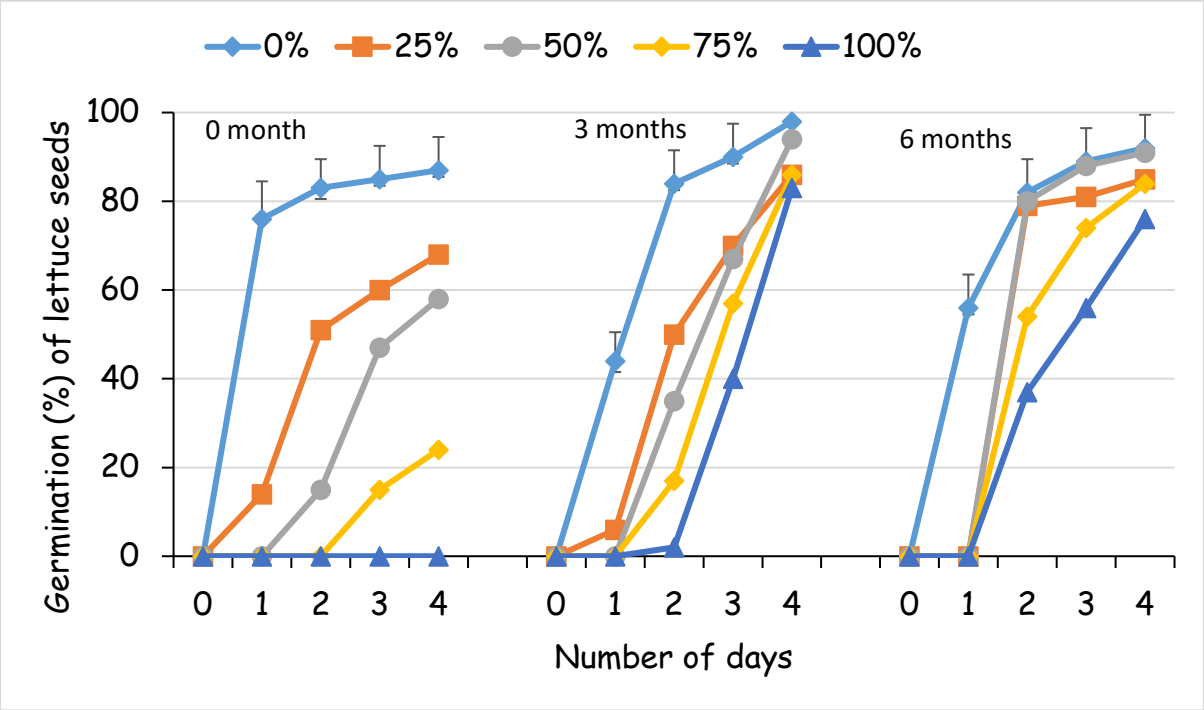


Figure 3.2. Germination (%) of lettuce seeds taken from Day 0 to Day 4 in a 0 month, 3 months and 6 months of wheat straw storage period. [100% leachate (undiluted leachate); 75% leachate with 25% distilled water (3:1); 50% leachate with 50% distilled water (1:1); 25% leachate with 75% distilled water (1:3) ratios and distilled water (dH_20) as a control]. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$). Using the Anova test, all the P-values for Day 1 to Day 4 in 0 month, 3 months and 6 months of wheat straw storage period were less than 5% implying that they were statistically significant difference

Table 3.2. *p*-values and standard error means for Lettuce seed germination (%) from 0-month of wheat straw storage period

Days	0-month		3 months		6 months	
	p-values	Standard error means	*p*-values	Standard error means	*p*-values	Standard error means
Day 1	<.001	5.75	<.001	15.54	<.001	5.27
Day 2	<.001	8.41	<.001	8.42	<.001	16.70
Day 3	<.001	10.46	0.021	14.16	0.014	7.48
Day 4	<.001	14.69	0.224	7.48	0.005	6.07

With 6 months wheat straw storage period (October trial), the undiluted extract resulted in the germination of seeds after Day 1 which is earlier than in the other wheat straw storage (Figure 3.2). With time, the wheat straw leached out the chemical responsible for inhibiting the germination of seeds. Ralph (2007) found that wheat straw that was stored dry for one year produced a higher mushroom yield than the new season straw of the same type. This indicates that with time the strength of the inhibitions which is associated with allelopathic can be reduced. Calcium was lower (28.4 ppm) in the undiluted extract resulted in the undiluted extract with higher germination (of 40%) after Day 1 in 6 months compared to 3 months wheat storage period. The germination rate of the seeds in the straw treated with the extract that had been diluted at a ratio of 1:1 began to exceed that of 1:3 ratio after Day 2 in the 6 months compared with the same ratios in the 3 months wheat straw storage period (Figure 3.2). Both the dH_20 (control) substrate and the substrate treated with a 1:3 ratio extract resulted in similar growth trends from Day 1 to Day 4.

3.3.2. Seedling length (mm)

With 0-month wheat straw storage period (April trial), the recently dried wheat straw exhibited a strong inhibition of seedling growth (Figure 3.3) associated with allelopathic. There were significant differences ($p < 0.05$) between the results using the dH_20 treatment and the other treatments as depicted in Table 3.3. The dH_20 treatment positively affected the growth of

lettuce seedlings compared to all other treatments. According to Wu *et al*. (2006), the growth of cabbage, maize, cucumber and lettuce responded differently to the wheat residue with the larger seeded crops being more tolerant than the smaller seeded species.

With 3 months wheat straw storage period (June trial), the results of the straw with the 1:1 ratio treatment exceeded that of the 1:3 ratio for growth from Day 1 to Day 3, whereas the results of the straw treated with the 1:3 ratio extract were expected to follow those of the control. This indicates that the dilutions were weakened by the ageing of the straw (Figure 3.3). By the end of the 6 months wheat straw storage period, the growth of the seedlings in the straw treated with a 1:1 dilution ratio of the extract fell below that of the 1:3 ratio after Day 4. In contrast, the results for the samples using a 1:3 ratio exceeded both those of the dH_20 and 1:1 ratio treatments after Day 2 to Day 4 (Figure 3.3).

Table 3.3. *p*-values and standard error means for seedling length (mm) from 0-month of wheat straw storage period

Days	**0-month**		**3 months**		**6 months**	
	p-values	Standard error means	*p*-values	Standard error means	*p*-values	Standard error means
Day 1	<.001	0.14	<.001	0.2990	<.001	0.2864
Day 2	<.001	0.94	<.001	0.730	<.001	1.449
Day 3	<.001	1.34	<.001	1.284	<.001	1.680
Day 4	<.001	2.09	<.001	1.951	<.001	2.834

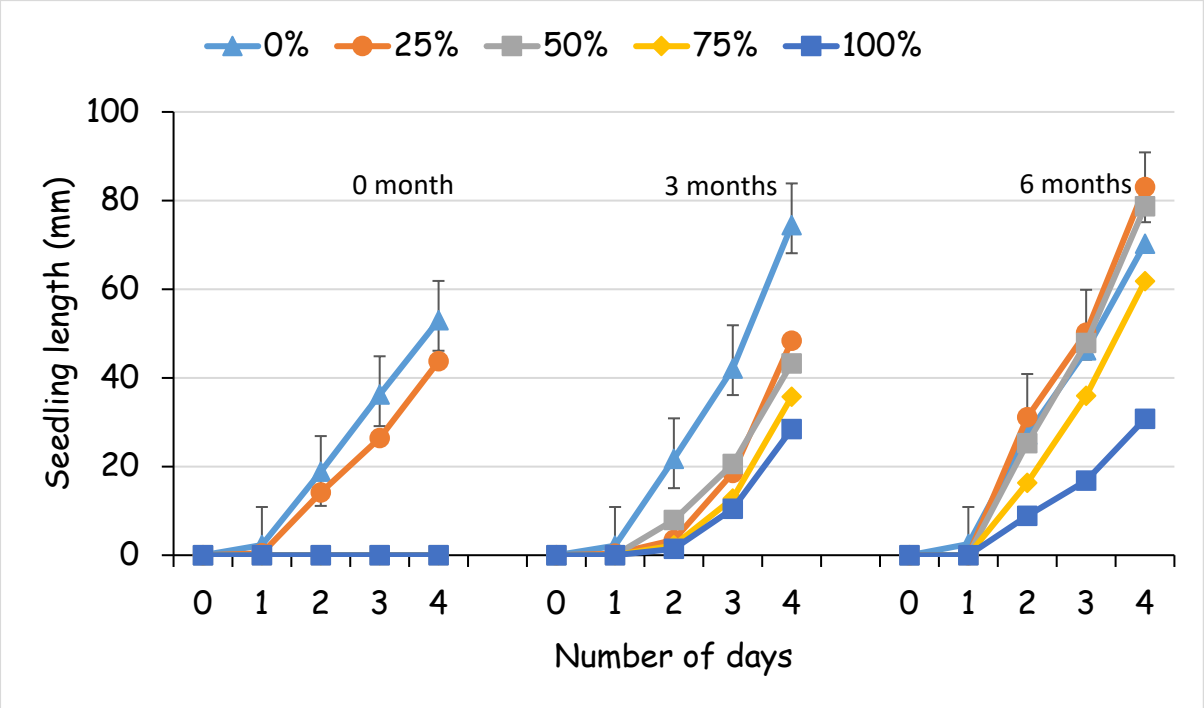


Figure 3.3. Seedling length (mm) of lettuce seeds taken from Day 0 to Day 4 in a 0 month, 3 months and 6 months of wheat straw storage period. [100% leachate (undiluted leachate); 75% leachate with 25% distilled water (3:1); 50% leachate with 50% distilled water (1:1); 25% leachate with 75% distilled water (1:3) ratios and distilled water (dH20) as a control]. Bars on the graph represent Standard Error (SE) means ($p < 0.05$)

Table 3.4. Mineral analysis from the wheat straw leachate collected before the lettuce seed germination in 0; 3 and 6 months of wheat straw storage.

Treatments	Month	pH	EC mS/m	K (ppm)	Ca (ppm)	Mg (ppm)
100%	0	6.23	3686.0	998.51	92.29	57.34
(undiluted	3	6.37	1858	548.38	15.94	23.143
extract)	6	5.84	1934	498.62	28.4	37.73
75%	0	5.13	2916	725.93	67.07	44.0
(3:1)	3	6.53	1422.67	409.36	12.59	17.327
	6	6.11	1454	376.28	20.79	28.2
50%	0	5.15	1755	458.14	43.97	28.59
(1:1)	3	7.02	1042.67	272.95	12.51	11.717
	6	6.17	997	262.48	16.6	19.34
25%	0	5.94	781	176.61	20.78	11.89
(1:3)	3	7.13	548	137.09	7.07	6.036
	6	6.22	542	129.93	6.46	9.38
Control	0	4.52	22	0.75	0.99	0.39
(dH_20)	3	6.05	7.12	0.87	0.79	0.386
	6	6.50	16.07	0.52	1.02	0.23

3.3.3. Mycelial growth (length) (mm)

The mycelial growth of the oyster mushroom in wheat straw treated with a 3:1 dilution of the extract differed significantly from the said growth using the straw leachate with all the other treatments. The results of lettuce seed germination in the first trials using the samples treated with dH_20 differed significantly from those of all the other treatments. The oyster mushroom spawn reacted differently to the wheat straw leachate compared with lettuce seeds. All the treatments had a growth of mycelia spawn between Day 1 to Day 4 compared with no germination of lettuce seeds in the first trial. Muswati *et al*. (2021) reported that the edible oyster mushroom (*Pleurotus ostreatus*) had shown good growth on inedible crop residue such as wheat straw. The wheat straw treated with an extract of a 3:1 dilution resulted in a mycelial

growth length of 15 mm after Day 1 compared to that with the dH_20 treatment, which measured 5 mm in the 0-month wheat straw storage period (Figure 3.4). There was a steady increase in oyster mushroom mycelial growth of 5 mm to 53 mm after Day 2 to Day 4 on the substrate treated with the 3:1 extract. It is noteworthy that the levels of EC, K, Ca and Mg of the straw treated with the 3:1 extract were lower when compared with that of the undiluted extract (Table 3.7), but the growth of the mycelia was better (Figure 3.4). The results using the 1:1 extract followed the same trend as the trial using the 3:1 extract after Day 2 to Day 4.

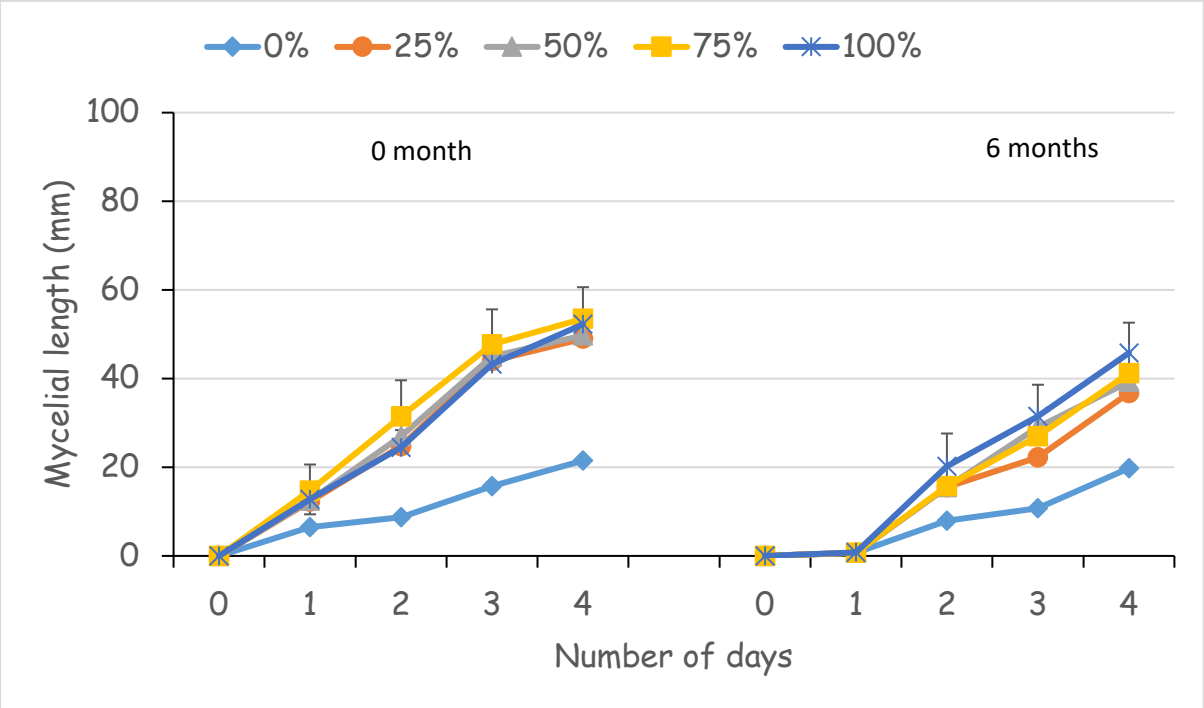


Figure 3.4. Mycelial length (mm) of oyster mushroom spawn taken from Day 0 to Day 4 in a 0 month and 6 months of wheat straw storage period. Bars on the graph represent Standard Error (SE) means ($p < 0.05$). Using the Anova test, all the P-values for seedling length from Day 1 to Day 4 in 0 month and 6 months of wheat straw storage period were less than 5% implying that they were statistically significant difference

The undiluted leachate resulted in lower growth than those of 1:1 and 3:1 dilutions but after Day 4 exceeded the growth using the 1:1 dilution. The dH_20 treatment yielded lower growth of mycelial from Day 1 to Day 4 compared with all the other treatments in the first trial (Figure 3.4). With 0-month wheat straw storage period, the dH_20 had zero amount of Calcium.

However, Ca played a negative role in the growth of the mycelia spawn in the first trial or early season (Table 3.7).

Table 3.5. *p*-values and standard error means for mycelial length (mm) from 0 – 6 months of wheat straw storage period

Storage	**Mycelial length (mm)**	
	p-values	Standard error means
0-month	<.001	0.1025037
6 months	<.001	0.10205177

3.3.5. Colony diameter (mm)

The growth of the mycelia in colony diameter and in length followed similar trends in both the 0-month and 6 months of wheat straw storage period (Figures 3.4 and 3.5). With 0-month, the 3:1 leachate resulted in the mycelium covering (colony diameter) a huge space from Day 0 to Day 4 compared with the wheat straw using all the other treatments during this period (Figure 3.5). This indicate that the wheat straw required few hours of drainage excess extracts. In contrast, in 6 months wheat straw storage period, the use of the 1:1 leachate resulted in the mycelium covering a huge space from Day 1 to Day 4 compared with the straw treated with all the options (Figure 3.5). The wheat straw required a greater number of hours to remove excess leachate to allow for better growth of the mycelia. All the treatments resulted in similar trends in the 0-month and 6 months wheat straw storage period trials.

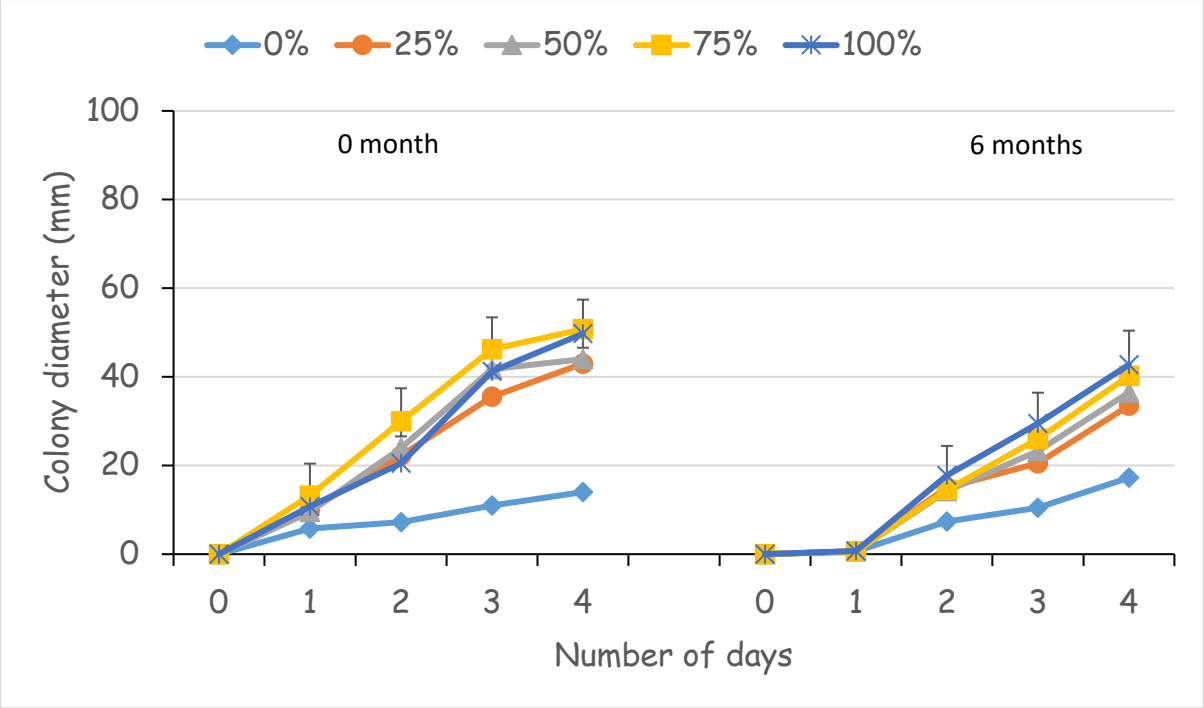


Figure 3.5. Colony diameter (mm) of oyster mushroom spawn taken from Day 0 to Day 4 in a 0 month and 6 months of wheat straw storage period. Bars on the graph represent Standard Error (SE) means ($p < 0.05$). Using the Anova test, all the P-values (0.0001) for seedling length from Day 1 to Day 4 in 0 month and 6 months of wheat straw storage period were less than 5% implying that they were statistically significant difference

Table 3.6. *p*-values and standard error means for colony diameter (mm) from 0 – 6 months of wheat straw storage period

Storage	**Colony diameter (mm)**	
	p-values	Standard error means
0-month	<.001	0.10300269
6 months	<.001	0.10284957

Table 3.7. Mineral analysis from the wheat straw leachate collected before the oyster mushroom spawn growth in 0 and 6 months of wheat straw storage

Treatment	Month	pH	EC mS/m	K (ppm)	Ca (ppm)	Mg (ppm)
100%	**0**	5.66	2180.00	451.90	39.78	30.310
(undiluted	**6**	5.91	3186.67	842.47	22.30	28.347
extract)						
75%	**0**	6.11	1226.00	301.20	27.83	18.710
(3:1)	**6**	5.94	2376.67	620.22	17.47	20.604
50%	**0**	6.06	1140.00	240.10	21.42	14.610
(1:1)	**6**	5.89	1724.67	419.76	10.65	13.889
25%	**0**	6.13	586.00	131.40	12.13	8.090
(1:3)	**6**	5.99	856.67	221.97	5.37	6.961
Control	**0**	6.00	7.88	0.38	0.00	0.000
(dH_20)	**6**	6.42	8.09	0.15	0.15	0.298

3.4. Conclusions and recommendations

Recently dried or new wheat straw and that which is stored for 3 months, need to be leached out the chemical compounds associated with growth inhibition (allelopathic) effects before being used for spawning. The new wheat straw imposed a high inhibition of lettuce seed germination compared with that stored for more than 3 months. On the other hand, wheat straw stored for at least 6 months tends to lose the compounds responsible for inhibiting the growth of lettuce seedlings. Wheat (*T. aestivum*- Duzi) straw possess chemical compounds associated with allelopathic effects that can inhibit germination of lettuce (*L. sativa*) seed and growth of oyster mushroom mycelia (*P. ostreatus*). It might be important for farmers to leach out compounds in wheat straw prior to inoculating it with oyster mushroom spawn. The set null hypothesis (H_0) is rejected and accept the alternative hypothesis as the mycelia length (mm) and colony diameter (mm) of oyster mushroom spawn taken from Day 0 to Day 4 in a 0-month and 6 months wheat straw storage period showed a significance difference in line with the *p*-values.

CHAPTER FOUR

Growth and production of oyster mushrooms as affected by drainage of the wheat straw in real farming practices

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4.1. Introduction

The influence of wheat straw leachate on the growth of lettuce seeds and oyster mushroom mycelium was determined in the experiments discussed in Chapter 3. It was necessary to conduct this trial in the field rather than in the lab in order to investigate whether there were differences or similarities in rate of colonisation and yield. This knowledge will be critical to small-scale farmers.

The wheat cultivar Duzi is very important in South Africa and worldwide as a food grain source for human consumption (Siddiqui *et al*., 2009). Wheat straw has been proven for its growth inhibition capabilities on other crops, as well as its own seed germination and growth (Sarrantonio and Gallandt, 2003), but this has not been proven for oyster mushrooms, particularly under South African conditions.

Water is one of the main factors that influence the success of mushroom production (Bellettini *et al*., 2016). Nutrients are translocated from the mycelium to the fruiting bodies by a steady moisture flow (Oei and Nieuwenhuijzen, 2005). However, high moisture content in the substrate could result in anaerobic conditions for the mycelium, inhibiting transpiration, rendering the development of fruiting body impossible, even with elevated inoculum amounts or number of holes in mushroom cultivation packages, resulting in the development of undesirable organisms such as bacteria and nematodes (Bechara, 2007; Diamantopoulou and Philippoussis 2015). Whereas low moisture content will result in the death of the fruiting body. For this reason, the use of high moisture content limits the growth within the whole substrate, resulting in surface growth (Patel *et al*., 2009). According to Chang and Miles (2004) and Bellettini *et al*. (2019), the appropriate moisture in the substrate should be within the range 50% and 75% in the substrate, enabling the satisfactory growth of *Pleurotus* species.

This need to be studied in order to understand the responses of oyster mushroom to wheat straw as a substrate under field conditions.

Therefore, this study was conducted with the following objectives: (i) to assess the potential growth inhibition of wheat straw extracts on the growth of oyster mushrooms, (ii) to investigate some growth inhibitors in stored wheat straw under field conditions. The following hypotheses were consequently tested:

(i) The null hypothesis (H_0) was that drainage of wheat straw leachate will not inhibit the colonisation and growth of oyster mushroom cultures.

(ii) The alternative hypothesis (H_1) was that drainage of wheat straw leachate will inhibit the colonisation and growth of oyster mushroom cultures.

4.2. Materials and methods

Location of the study area

This particular study was carried out similarly to the one as from Chapter 3, only that this was a scaled up version taking into consideration the real farming protocol and field practices. Chapter 4 then gives the exact real situation and exposure as experienced by farmers. ***NB***: (The actual study followed this well-established methodology to negate the fact of acquiring totally new substrate. The rationale was to take abundantly available resources and knowledge for designing a novel training manual for small-scale farmers).

Experimental design and wheat straw pasteurisation

A trial was laid out in a completely randomised design (CRD) with five levels of drainage [no drain - ND (85 - 95%); for 1 h drain (65 – 75%) moisture content; for 2 h drain (45 - 55%) moisture content; for 3 h drain (25 - 35%) moisture content and for 4 h drain (5-15%)], replicated four times. The moisture content was determined using a RADWAG moisture analyser of balances and scales (Mettler Toledo, Germany) at 65 – 75% (Kwon and Kim, 2004). The wheat straw (substrate) was sourced from Marble Hall (Limpopo Province, South Africa). After harvest, it was stored in a shed for one month before purchased and after collected the straw was kept for four months in a shed house with normal temperature before being utilised. The substrate was pasteurised using a method outlined in the Mushroom Growers Handbook 1 (2004). Three wheat straw bales of approximately ±10 kg each were placed in a 225 L steel drum containing water. The water (1/4) was filled in the drum and then three bales of wheat

straw were placed in the drum and more water was place in the drum to cover the straw. After reaching boiling point (100°C), the drum with the wheat straw was kept above a fire for 1 hour in order to achieve proper pasteurisation. Pasteurisation was carried out to also moisten the substrate in order to render conditions suitable for growing oyster mushrooms and to destroy any competing fungi (allelopathic).

After an hour of boiling, the wheat straw was removed from a drum and placed on a growing table. Industrial polyethylene bags (410 mm x 750 mm x 45 µm, width, length and thickness) were filled with the wheat straw together with spawn (1 kg, which was further split into three equal portions, i.e. ≈0.33 kg as one split each at the bottom, middle and top parts of the polyethylene bag) to make up a total weight of 6.20 kg per treatment for each replicate. During boiling, the temperature was monitored using a pocket thermometer (Checktemp 1, Hanna Instrument - Mauritius). The planted bags were perforated at the bottom in order to drain excess leachate. The top parts of the polyethylene bags were sealed with a rubber band in order to minimise contamination before kept in a growth chamber as depicted in Plate 4.1. The oyster mushroom (*P. ostreatus*, tree species, HK35 strain) spawn (4 kg each bag) were purchased from Exotic spawn (Irene, Rietvlei, Gauteng Province, South Africa). The industrial plastic bags were sourced from Plastilon (Pretoria, Gauteng Province).

Plate 4.1. Custom made growth chamber (hut-house, made of thatch grasses)

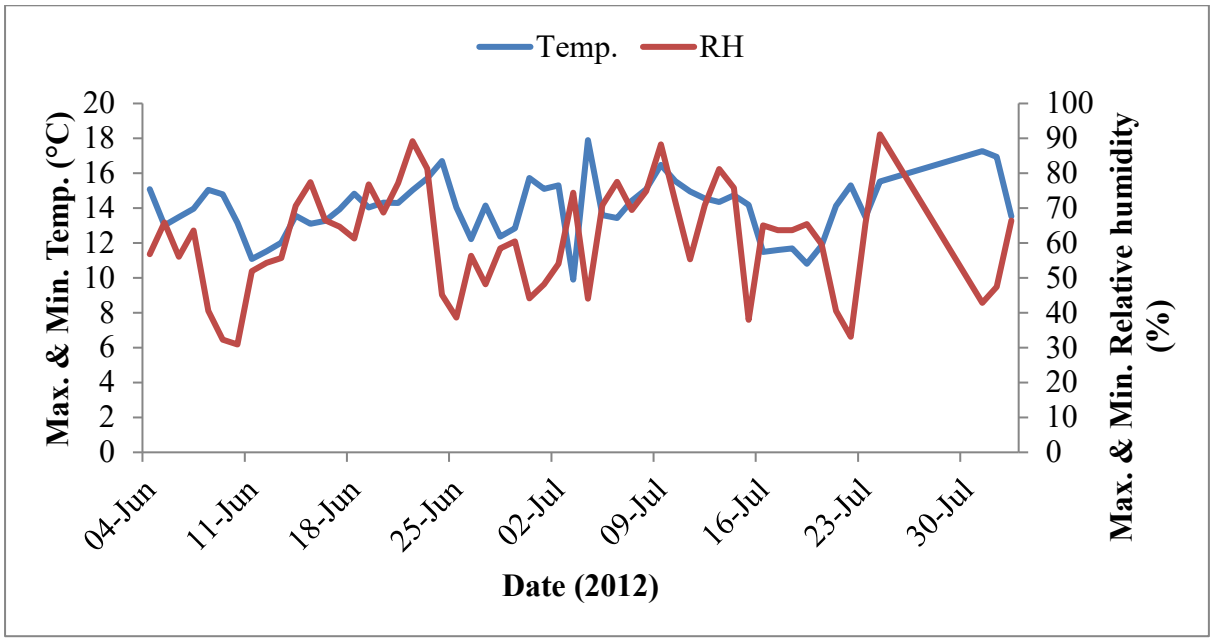


Figure 4.1. Average daily temperature (°C) and relative humidity (%) in a custom made hut-house taken from 04 June 2012 to 02 August 2012

Data collection

Colonisation and contamination

Colonisation of mycelium refers to inoculated spawn that has covered the substrate with mycelia in order to grow. Contamination of mycelium occurs when invader fungi cover a substrate instead of the inoculated spawn. The inoculated plastic bags with substrate were evaluated for both colonisation and contamination. The inoculated bags took at least 12 days for full colonisation to take place, whereas contamination was evaluated on a weekly basis. Both colonisation and contamination were evaluated using a rating scale of 0 to 100%. After full colonisation, the planted plastic bags were made small cross cut from top to bottom using sterile surgical blades to facilitate the growth of mushroom clusters. The temperature (°C) and (RH) relative humidity (%) data in growth chamber were measured using a HOBO Data Logger (U14 – 001 LCD Temperature/RH logger, United Kingdom). The temperature (°C) and relative humidity (%) data that were collected in the growth chamber (Plate 4.1) during the study period are presented in Figure 4.1.

Harvesting

The oyster mushrooms were harvested manually by twisting the clusters off and kept in brown paper bags (SO 12-16.5 cm x 36.5 cm). The harvested oyster mushrooms were taken to a laboratory for post-harvest analysis.

Growth parameters

The growth parameters that were noted were the cumulative number of harvests, the cumulative number of clusters, the cumulative number of caps and the cumulative fresh mass. For each harvest, the oyster mushroom fresh mass was measured using a Scaltec scale 986 (Scaltec Instruments GmbH, Goettingen, Germany) for each treatment and added together to arrive at a cumulative fresh mass at the end. Biological efficiency (BE) was determined according to *Equation 1*.

$$BE\ (\%) = \frac{\text{Fresh mass (g) of oyster mushrooms}}{\text{Total wet substrate used (g)}} X\ 100 \qquad Eq.\ [1]$$

Statistical analysis

As a statistic was carried from Chapter 3, for the data collected from each specific months so was the data from Chapter 4 subjected to same treatments.

4.3. Results and discussion

4.3.1. Rate of colonisation (%) from different drainage periods

There was a steady increase in the colonisation percentage of 1 h, 2 h, 3 h and 4 h treatments from Day 3 to Day 5 (Figure 4.2). There was a significant difference ($p < 0.05$) between 1 h, 2 h, 3 h and 4 h treatments and the control (not drained). The treatments which had been drained for 1 h (65%), 2 h (70%), 3 h (60%) and 4 h (63%) displayed a colonisation rating of more than 60% compared with the control which exhibited a rating of 20% on Day 5. The control had the lowest colonisation rating due to excess water content on substrate compared with the results, from Day 3 to Day 12, of the treatments that had been drained for 1 h, 2 h, 3 h and 4 h. The results for the 4 h (80%) treatment began to exceed those of the samples of all the other

treatments from Day 7. This indicates that the colonisation rate in the wheat straw from which extracts were leached for 4 h begins to recover after a few days. The amendment of substrate with distillery effluent could be beneficial as a nutrient supplement as well as providing moisture to the growing system (Pant *et al*., 2006).

Full colonisation (100%) of the 1 h, 2 h, 3 h, 4 h and control samples was reached on Day 12. This indicates that the number of hours the wheat straw had been drained does not matter because full colonisation was reached by the end of the same period. This could also have been influenced by the water content of substrate, temperature (°C) and relative humidity (%) in the custom made huthouse (Figure 4.2). The 1 h, 2 h, 3 h and 4 h drainage led to an increase in the colonisation rating, displaying similar trends from Day 3 to Day 12, while the control sample increased the colonisation at the same rate as the sample that had been drained for one hour (Figure 4.2). In this study, there was no contamination of oyster mushroom mycelia. This indicates that the substrate was pasteurised properly, which allows the mycelia to cover a substrate more rapidly by reducing the amount of harmful competitor organisms (Mushroom-Appreciation, 2015). The custom made huthouse was sprayed with sodium hypochlorite. The sodium hypochlorite significantly kills the parasitic fungi responsible for causing the infection of green mould.

Table 4.1. *p*-values and standard error means for rate colonisation (%) from drainage periods of wheat straw

Days	**Rate of colonisation (%)**	
	p-values	Standard error means
Day 1	0.0705	0.29975637
Day 2	0.0002	0.27481127
Day 3	<.001	0.28410094
Day 4	0.0032	0.26058869
Day 5	0.0033	0.24428622
Day 6	0.0194	0.24731217
Day 7	0.0297	0.26692124
Day 8	0.1245	0.26537085
Day 9	0.2741	0.27702195

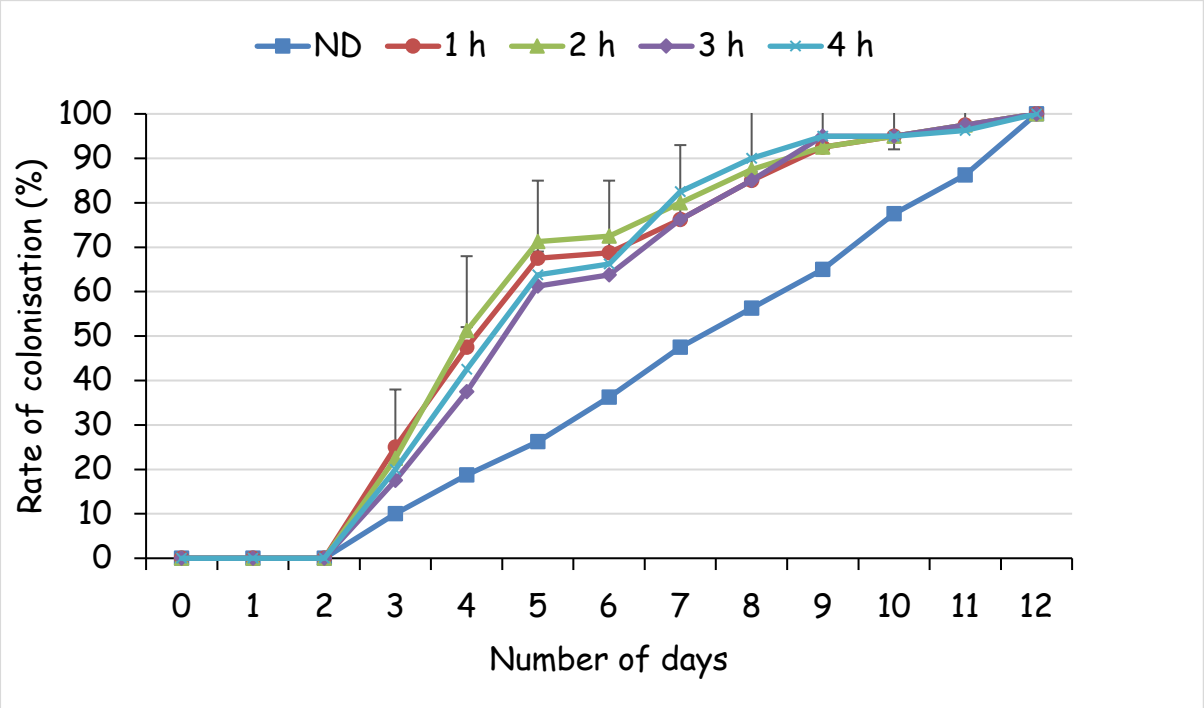


Figure 4.2. Rate of colonisation (%) from different drainage periods. [ND - no drain (85 - 95%); 1 h drain (65 – 75%) moisture content; 2 h drain (45 - 55%) moisture content; for 3 h drain (25 - 35%) moisture content and for 4 h drain (5 - 15%)]. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$). Using the Anova test, all the P-values for from Day 3 to Day 12 were less than 5% implying that they were statistically significant difference

4.3.2. Harvesting of oyster mushrooms from different drainage periods

No significant difference was noticed between the use of the control, and the substrates that had been drained for 1 h, 2 h, 3 h and 4 h regarding the number of clusters (Figure 4.3). The wheat straw that had been drained for 1 h produced the highest number (19) of clusters, followed by the control (17), and the wheat straw that had been drained for 2 h (16), 3 h (14) and 4 h (13). This indicates that the wheat straw that was subjected to 1 h of drainage was suitable for the production of a high number of clusters. The amount of time for which the wheat straw was drained did not make any difference to the number of clusters produced.

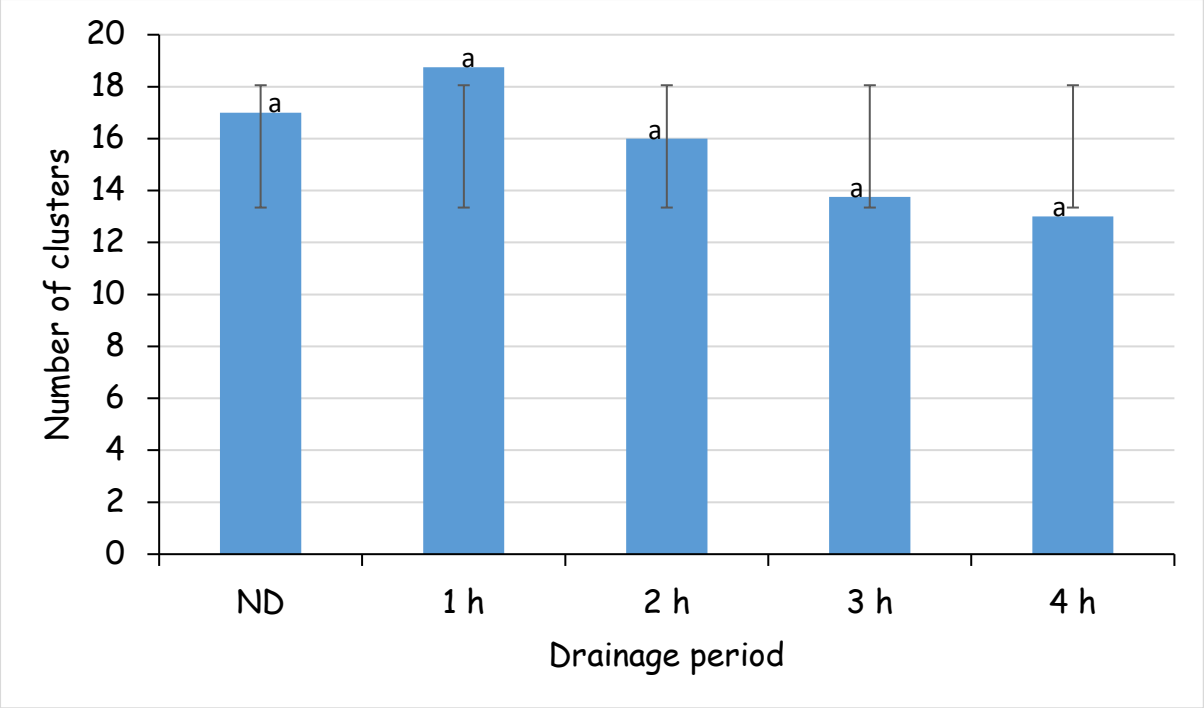


Figure 4.3. Number of oyster mushroom clusters harvested from different drainage periods. T-test Means with the same letter are not significantly different. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

No significant differences were noted between the treatments of the control substrates and those that had been drained for1 h, 2 h, 3 h and 4 h regarding the number of caps produced (Figure 4.4). The treatment that had been drained for 1 h produced the highest number (127) of caps compared to those that had been drained for 2 h (125), 3 h (123), 4 h (110) and the control (107) sample. The control treatment produced the lowest number of caps compared with all other samples. This indicates that the drainage treatments had no effect before spawning the oyster mushroom spawn. Although, the 1 h drainage of the wheat straw can be advisable due to the large number of clusters and caps produced by the oyster mushroom spawn on this substrate.

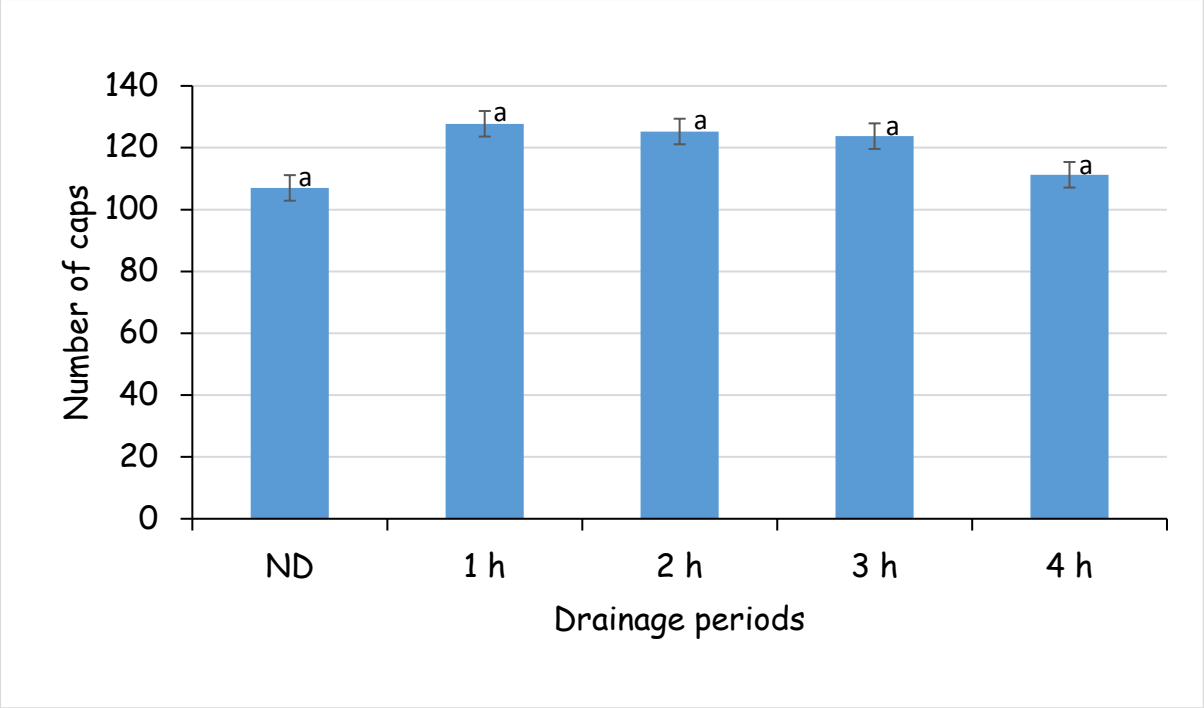


Figure 4.4. Number of oyster mushroom caps harvested from different drainage periods. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

No significant differences were observed for the control, 1 h, 2 h, 3 h and 4 h treated wheat straw samples. However, the samples that were treated for 1 h and 2 h (1400 g) produced the highest cumulative fresh mass (g) compared with those that had undergone all the other treatments (Figure 4.5). The samples that had been drained for 3 h (1300 g) yielded the second largest cumulative fresh mass (g). Both the control and the 4 h samples produced the lowest cumulative fresh mass (g) of them all. This indicates that 1 h and 2 h drainage was necessary in order to produce the largest fresh mass (g). Draining of excess water from the substrates and allowing them to cool for at least 5 – 8 h enables the mushroom spawn to produce mushrooms successfully (MushroomBox, 2015). It was necessary to drain the wheat straw substrate in order to remove the excess leachate that exerts a negative effect on the production of oyster mushrooms.

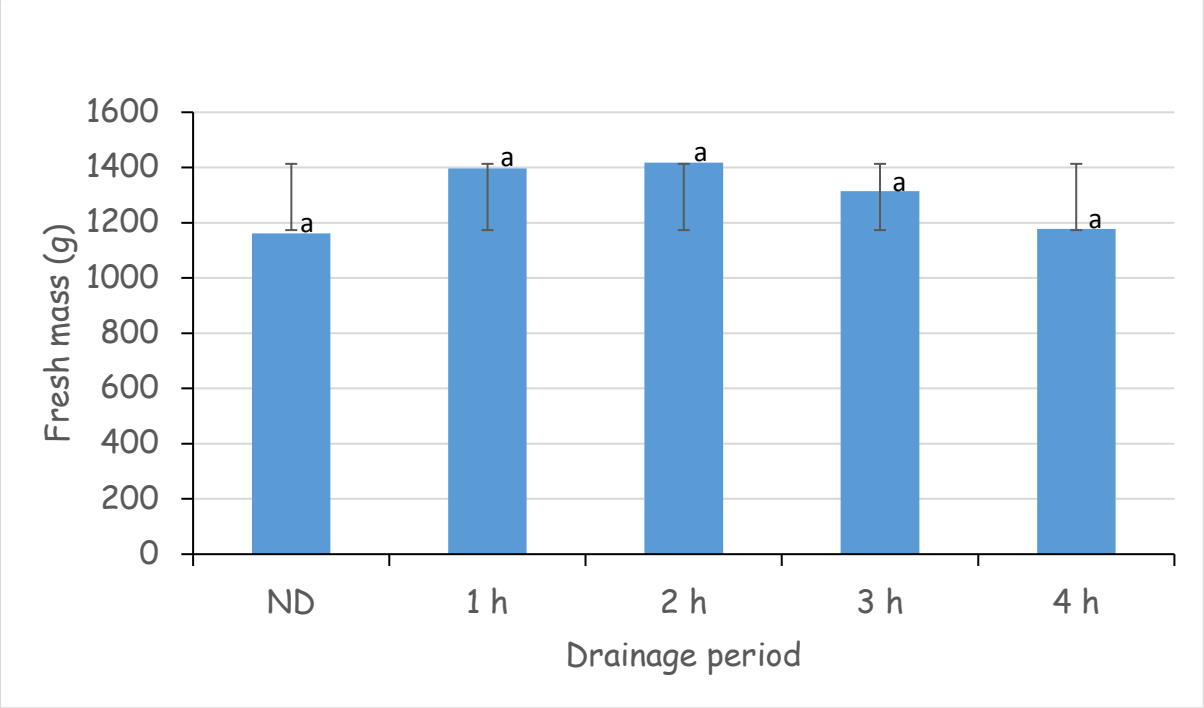


Figure 4.5. Fresh mass (g) of oyster mushrooms harvested from different drainage periods. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

Table 4.2. *p*-values and standard error means for harvesting of oyster mushroom from different drainage periods of wheat straw

Harvesting	***p*-values**	**Standard error means**
Number of clusters	0.5794	0.24959941
Number of caps	0.7159	0.25949098
Fresh mass (g)	0.9043	0.26036067

4.3.3. Biological efficiency (%)

The 2 h drainage had the highest biological efficiency (92%) compared to other treatments (Figure 4.6). Although, the 2 h, 1 h and 3 h drainage did not show any differences, the differences were visible on both 4 h drainage and not drain treatments. Both the not drained and 4 h drainage had the lowest biological efficiency (75% and 76%) compared to 2 h, 1 h and 3 h drainage (92%, 90% and 85%).

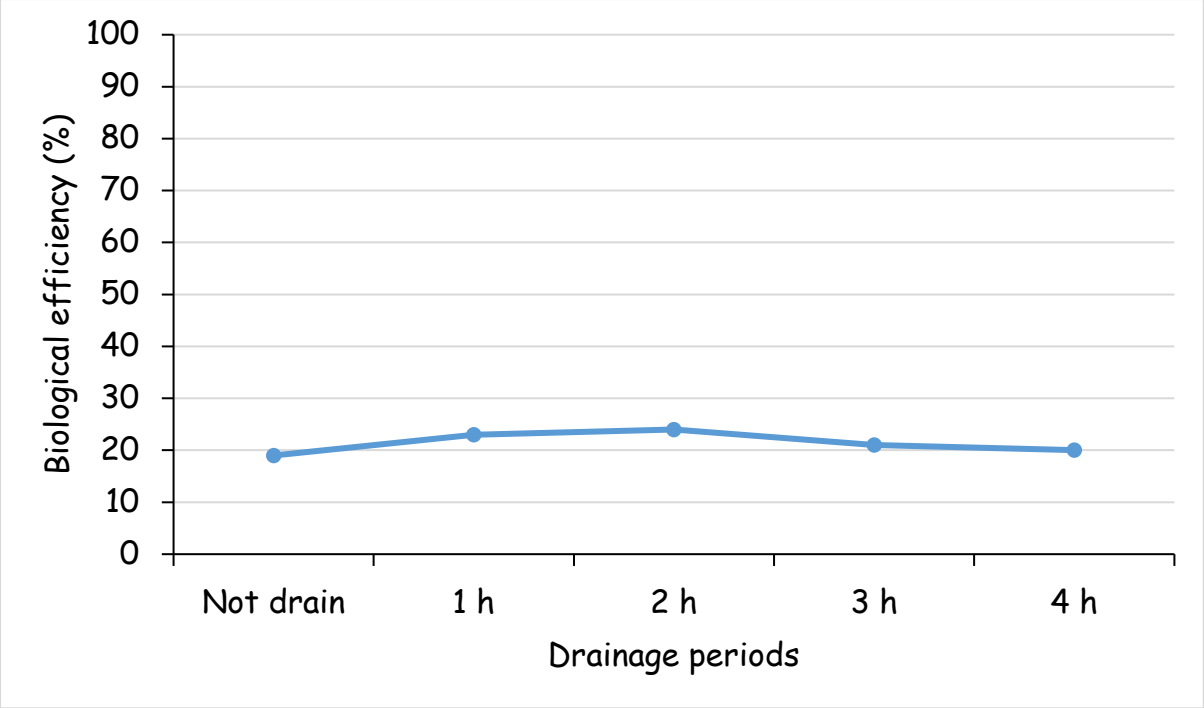


Figure 4.6. Biological efficiency (%) of harvested oyster mushrooms drained for certain periods

4.4. Conclusions and recommendations

This experiment demonstrated that drainage of wheat straw extracts accelerates the colonisation of oyster mushroom mycelia compared to not drained at all. It is always necessary to leach out wheat straw extracts before inoculating oyster mushroom spawn. Accelerated colonisation of mycelia helps to suppress the amount of contamination. Draining wheat straw leachate for 1 h produces a larger number of clusters and caps; similarly, for the cumulative fresh mass with wheat straw that had been subjected to 2 h drainage. It can be advisable that drainage is necessary for enhancing the colonisation of oyster mushroom mycelia and improving the yield of oyster mushrooms under field conditions. The results compare favourably with those obtained from the samples in the growth chamber. The set null hypothesis *(H_0)* is rejected and accept the alternative hypothesis (H_1) as the results extracted from this chapter indicate that colonisation and growth of oyster mushrooms are significantly affected by drainage of the wheat straw leachate.

CHAPTER FIVE

Growth and yield of oyster mushrooms as affected by wheat straw pasteurising methods

5.1. Introduction

Sterilisation is the process in which microbes are killed in food and drinks/fluids (Meyer *et al*., 2000), whereas pasteurisation refers to a process of eliminating only the competitors but keeping useful nutrients (Sánchez, 2010). Pasteurisation is a cultural practice during the cultivation of oyster mushrooms. Each growing medium requires pasteurisation due to various contaminants that invade the materials.

Different pasteurisation methods can be used for the cultivation of oyster mushrooms and to improve the yield (Khan, 2009). In contrast to wood waste which should be sterilised, most agricultural by-products are better pasteurised using steam or hot water baths (Stamets, 2000). Pasteurisation or sterilisation is often the most expensive practice because it requires fuel-consumption for steaming or boiling (Khan, 2004). Pasteurisation selectively kills the majority of competitors, preserving populations of non-competitive susceptible fungi, thus resulting in a substrate with a limited window of opportunity for contamination and in turn favours the dominance of mushroom mycelia. Sterilisation of substrates is a much more appropriate method to destroy a number of micro-organisms for the effective and smooth cultivation of mushrooms (Oei, 2003; Apihidean, 2006). Pasteurisation can propagate thermophilic micro-organisms that can improve the selection of a substrate by immobilising readily available nutrients to competitors and by producing toxic or inhibitory molecules to limit the rapid growth of competitors (Mata and Savoie, 2005). Without sterilisation, high yields cannot be achieved (Khan, 2004). Pasteurisation typically occurs between 60°C and 82°C at atmospheric pressure (Stamets, 2000).

Sterilisation is carried out at above boiling point of water at 100°C and above atmospheric pressure at 121°C kPa. A hybrid treatment, which is atmospheric sterilisation or super-pasteurisation, calls for the exposure of substrates to prolonged elevated temperatures exceeding 88°C for at least 12 hours (Stamets, 2000). In any case, a carefully balanced aerobic environment must prevail throughout the incubation process, or competitors will flourish (Stamets, 2000). Wheat straw was ideal for both the home and commercial cultivator, whilst

straw was a forgiving substrate for the small to midsized cultivator accepting a limited number of contaminants and selectively favouring mushroom mycelia (Stamets, 2000). However, growing on straw was found to be less expensive than growing on sawdust.

Several methods to heat-treat bulk substrates are given by Stamets (2000), namely, the hot water bath; steam; hydrated lime bath; bleach bath; hydrogen peroxide technique; high-pressure extrusion method; detergent bath method and yeast fermentation method. Certain studies have indicated that different species of cultivated mushrooms have different substrate requirements (Philippoussis *et al*., 2001), while certain species grow best in fermented pasteurised substrates, while other species grow best in sterilised, pasteurised or simply moistened substrates (Stamets, 2000). Suitable substrates together with proper sterilisation may lead to fast colonisation and high density growth of mycelia, which in turn enhances primordial formation and fructification, and thus increases mushroom yield (Stamets, 2000). However, no research data is available for pasteurisation or sterilisation methods of substrates in South Africa.

Therefore, the objective of this study was: effect of pasteurisation methods on the rate of colonisation, contamination and the yield of oyster mushrooms. The following hypotheses was consequently tested: (i) The null hypothesis (H_0) was that growth and yield of oyster mushrooms is not affected by wheat straw pasteurising methods. (ii) The alternative hypothesis (H_1) was that growth and yield of oyster mushrooms is affected by wheat straw pasteurising methods.

5.2. Materials and method

Location of the study area

A study was carried out as described in Chapters 3. ***NB***: (The actual study followed this well-established methodology to negate the fact of acquiring totally new substrate. The rationale was to take abundantly available resources and knowledge for designing a novel training manual for small-scale farmers. The study was done only once as the focus has been the finding of pasteurisation methods).

Experimental design and treatments

The experimental layout was a completely randomised design (CRD) with substrates that had been subjected to five different treatments, that is, five disinfection methods, with each treatment replicated four times (20 units). The five disinfection methods used were soaking in water (control), household bleach - Jik (sodium hypochlorite – 3.5%), gypsum (calcium sulfate dehydrate), steaming, and boiling. A single bale of wheat straw (±10 kg) was used for each treatment. The moisture content of 65% to 75% was determined using a RADWAG moisture analyser after pasteurisation or disinfection (Mettler Toledo, Germany). The wheat straw was one month when collected from the source and was utilized immediately. As per the normal academic standards, the experiments were repeated and statistically evaluation were performed and given in the results.

Soaking of wheat straw

A plastic drum of 225 L capacity was filled with 56 L of water and a single bale of wheat straw was added (Plate 5.1A). The drum was then filled to capacity with water to avoid floating over of wheat straw and closed with a lid. The soaked wheat straw was left in the drum overnight. Subsequently, the wheat straw was taken to a table to remove excess leachate before spawning.

Pasteurisation of wheat straw using boiling method

A steel drum (225 L capacity) was placed on a fire and then filled with 56 L of water. A single bale of wheat straw was then placed into the steel drum and water was added to its full capacity (Plate 5.1B). The steel drum containing wheat straw was then closed with a lid, in order to ensure accelerated pasteurisation, and boiled for an hour. During boiling, the temperature was monitored using a pocket thermometer (Checktemp 1, Hanna instrument - Mauritius). After an hour, the wheat straw was removed from the drum using a garden fork and placed on a sterilised growing table to cool down and to remove excess leachate.

Plate 5.1. (A) Wheat straw soaked in 225 L drum of water, (B) Wheat straw pasteurised using boiling water, (C) Jik (Sodium hypochlorite) added to the drum of wheat straw, (D) Gypsum added to the drum of wheat straw

Disinfection of wheat straw using household bleach - Jik (sodium hypochlorite)

Jik was used at a strength of 3.5% (sodium hypochlorite) in 2 L of container (Plate 5.1C). A plastic drum (225 L capacity) was filled with 56 L of water. About 6 x ½ cups (125 ml) of Jik were added to the drum. A single bale of wheat straw was placed into the plastic drum and water was subsequently added to full capacity. The wheat straw was allowed to soak overnight in the drum. The wheat straw was taken out and laid on a growing table to remove excess leachate before inoculating the next day.

Disinfection of wheat straw using gypsum (Gluex – white wash)

A plastic drum (225 L capacity) was filled with 56 L of water (Plate 5.1D). About 6 x ½ cups (125 ml) of gypsum (Gluex – white wash paint) were added to the drum of water and stirred to ensure that it dissolved properly. A single bale of wheat straw was placed into the plastic drum and thereafter water was added to full capacity. The wheat straw was allowed to soak overnight. The substrate was taken out to a growing table to remove excess leachate before spawning.

Pasteurisation of wheat straw using a steam boiler

A single bale of wheat straw was soaked overnight in a 225 L capacity plastic drum of water. The following day, the soaked wheat straw was taken to the nursery unit for steam pasteurisation. The steam boiler was filled with water and set at a temperature of 100°C. A 50 L rubber bin was then filled with the wet wheat straw for steaming (Plate 5.2). As soon as the steam boiler began to release steam, a steam pipe was placed into the bottom of the bin and then the top was closed with a lid. The wheat straw was steamed for 5 minutes similar to the time used for pine bark pasteuration. The wheat straw was steamed using an electrode steam conditioner (Marshall Fowler, model: Esc 60, year: 1994, volts: 380, pressure: 60 kPa, serial no.: 1685) as depicted in Plate 5.2.

Plate 5.2. (A) Electrode steam conditioner machine and (B) a 50 L of rubber plastic dust bin used for pasteurising the wheat straw

Preparation for spawning oyster mushroom

Industrial polyethylene bags (410 mm x 750 mm x 45 µm, width, length and thickness) were filled with wheat straw (5 kg) together with spawn (1 kg, which was further divided into three equal parts, i.e. ≈0.33 kg at the bottom, middle and top parts of the polyethylene bag) to make up a total weight of 6 kg per treatment. During inoculation, the mass of planted bags was measured using a Sasco Africa scale (Plate 5.3). Twenty plastic bags were planted in one day. After spawning, the plastic bags were marked accordingly and placed on shelves randomly. A custom-made hut house was irrigated twice daily for 5 minutes (the choice of 1 kg spawn was made as it was sufficient to cover the whole bag).

Plate 5.3. Floor scale used to measure the required mass of straw and spawn in bags for oyster mushroom production

Data collection

Colonisation and contamination

Colonisation of mycelia occurs when the inoculated spawn covers the substrate with mycelia in order for growth to begin. Contamination of the mycelia occurs when invader fungi cover the substrate instead of the inoculated spawn. The oyster mushrooms planted inside the plastic bags were evaluated for both colonisation and contamination. It took 12 days to reach full colonisation, and contamination was evaluated on a weekly basis. Both the colonisation and contamination were evaluated using a rating scale of 0 to 100%. After full colonisation of the mycelia, the plastic bags were made small cross cut from top to bottom using sterile razor blades to facilitate the growth of mushroom clusters.

Harvesting

Oyster mushrooms were harvested manually by twisting the cluster and kept in brown paper bags (SO 12; 16.5 cm x 36.5 cm). The harvested mushrooms were taken to a laboratory for post-harvest analysis. Both the temperature and relative humidity were recorded using a HOBO data logger (U14 – 001 LCD Temperature/RH logger, Newbury - United Kingdom).

Growth parameters

i) *Cumulative number of harvests*

Harvesting was carried out approximately three times for each of the inoculated plastic bags. The oyster mushrooms were not harvested at the same time due to the variations in the time it took for them to reach maturity.

ii) *Cumulative number of clusters*

A cluster is a set of mushrooms that comes out of the plastic bag through the cross-cut holes. Each treatment was harvested separately and the clusters were counted each time during harvesting from the bags.

iii) *Cumulative number of caps*

A cap is an umbrella or head produced by the mycelia to form a mushroom. After harvesting the oyster mushrooms, the clusters were separated individually according to treatment and the caps were counted per harvest.

iv) *Cumulative fresh mass (g)*

Cumulative fresh mass refers to the total mass of fresh oyster mushrooms harvested at different times. At each harvest, the oyster mushroom mass was measured using a Scaltec scale (Germany) for each treatment and added together to arrive at the cumulative fresh mass of the oyster mushrooms at the end of the experiment. The biological efficiency (%) was determined using *Equation* 1.

$$BE\ (\%) = \frac{\text{Fresh mass (g) of oyster mushrooms}}{\text{Total wet substrate used (g)}} X\ 100 \qquad Eq.\ [1]$$

Statistical analysis

Data from this Chapter was treated for statistical evaluation as for Chapter 3 and 4.

5.3. Results and discussion

5.3.1. Rate of colonisation as affected by pasteurisation

The rural communities of Gert Sibande District Municipality were trained to use a single method for pasteurising the growing medium when cultivating oyster mushrooms. It was necessary to research and document other methods that can be used as alternatives for pasteurising growing media when cultivating oyster mushrooms. The well-known method is boiling of the substrate. Most of the rural communities are familiar with the boiling method as the only method to use, while it is also considered to be the simplest and best method. This method was very simple to relate to in rural communities because they use firewood daily when cooking their food. Boiling of wheat straw is merely a change from using a small pot to using a bigger drum. It is convenient for them as they all have the knowledge and experience necessary to make this a success. The ARC-TSC has researched other methods that can be used for pasteurising wheat straw, namely the use of sodium hypochlorite (household bleach - Jik), gypsum, and steaming. Soaking the growing media in boiling water is also effective.

There was no significant difference between the rate of colonisation by the mycelia of the non-disinfected (control) wheat straw and that which had been treated with boiling and steaming from Day 1 to Day 18 (Figure 5.1), whereas differences ($p < 0.05$) were observed in the rate of mycelial colonisation between the control, gypsum and Jik (bleached) treated wheat straw from Day 1 to Day 18. On Day 1, both the gypsum and Jik samples yielded a lower colonisation percentage (25%) compared with the control (42.5%), steamed (40%) and boiled (37.5%) samples. The mycelia covers the substrate in two to eight weeks depending on the amount of spawn, the type of substrate and the strain employed (Grimm *et al*., 2021). Other differences were identified between the substrates that had been treated with boiling, gypsum and bleach - Jik and the amount of colonisation from Day 1 to Day 18 (Figure 5.1). The rate of mycelial colonisation of the steamed substrate differed significantly from that of both the gypsum and Jik treated substrates from Day 1 to Day 18. The poor colonisation of mycelial from both the gypsum and jik can be imposed by the chemical residues in the wheat straw substrates during disinfection. Steamed (70°C) as well as sterilised (121°C) straw with spawn mixed into the whole bulk of the substrate, steamed straw was completely colonised by *Pleurotus ostreatus* mycelium whereas on sterilised straw, *Trichoderma pleuroti* suppressed the growth of *P.*

ostreatus mycelium (Jablonsky *et al*., 2016). This can be due to the absence of microbial population in the sterilised substrate which normally inhibits the development of *T. pleuroti* where it was consequently prone to contamination. The boiled substrate reached full (100%) mycelial colonisation by Day 11 compared with all the other treatments. These results disagree with the findings by other researchers, for example, Shah *et al*. (2004), who found that the colonisation of the mycelia took 2 weeks for its completion on saw dust, whereas Khan (2009) reported that *Pleurotus ostreatus* took 24 to 25 days for the completion of 100% spawn colonisation of wheat straw. In contrast to this finding, Masarirambi *et al*. (2011) reported that the number of days taken to colonise various substrates ranged from 52 days to 98 days, which was far too long when compared with these findings.

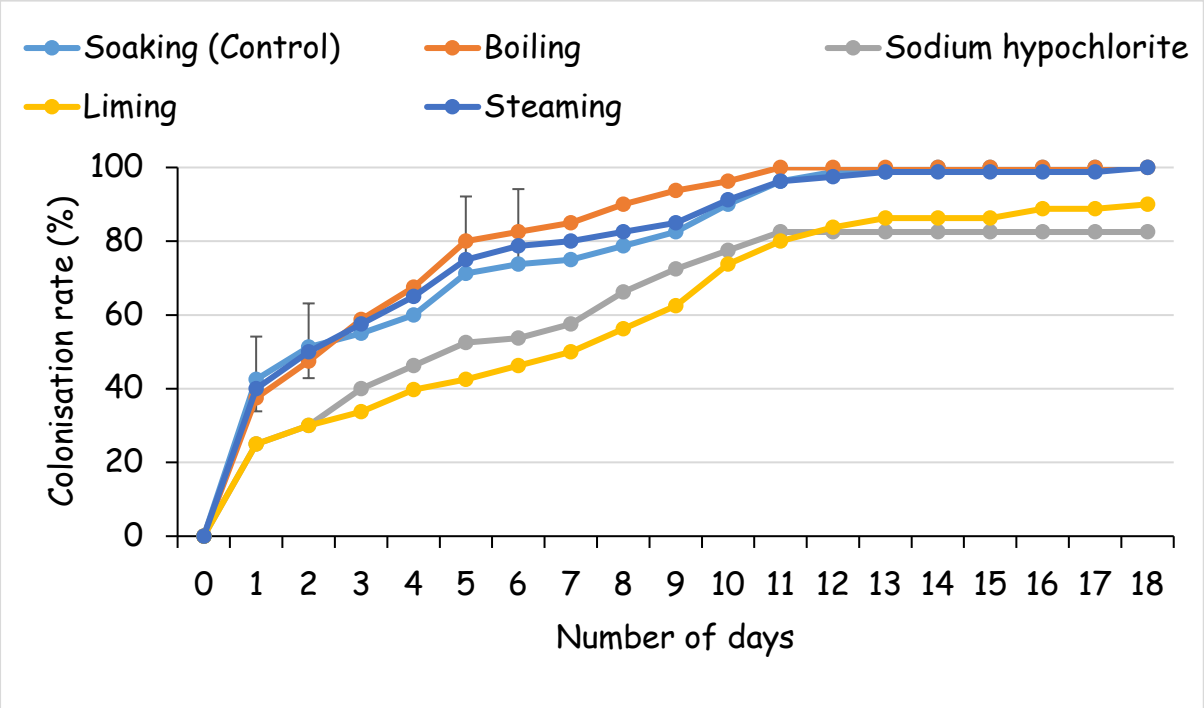


Figure 5.1. Rate of colonisation affected by pasteurisation method from Day 1 to Day 18. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

The mycelial growth in the substrate treated with Jik (bleach) reached carrying capacity with a rate of colonisation (82%) from Day 11 to Day 18, whereas, the gypsum treated substrate began to improve from Day 13 to Day 18 where it reaches a high rate of colonisation (90%). The sterilising methods of the substrate affects the number of days taken for mycelial colonisation (36 to 64 days), while the mycelia failed to colonise the horse manure compost substrate (Oseni *et al*., 2012). Colonisation was lower for the substrate treated with gypsum than that treated

with Jik from Day 2 to Day 12, but the results begin to overlap from Day 12 to Day 18 (Figure 5.1). Boiling was regarded as the best method to improve the rate of colonisation percentages of oyster mushroom mycelia. Boiling was also regarded as the cheapest method for the pasteurisation of wheat straw because most of rural communities are familiar with this technology and they can collect wood in the forest for free. The second best method was steaming, but this method is the most expensive practice due to the required equipment (Figure 5.1). The differences in the rate of colonisation using the various sterilisation methods could be due to climatic conditions, temperature and the relative humidity variations in a custom made huthouse. When the temperature in the growth chamber is higher, the growth of the mycelia accelerates resulting in shorter periods required for the colonisation of mycelia (Sher *et al*., 2011) as depicted in Figure 5.2.

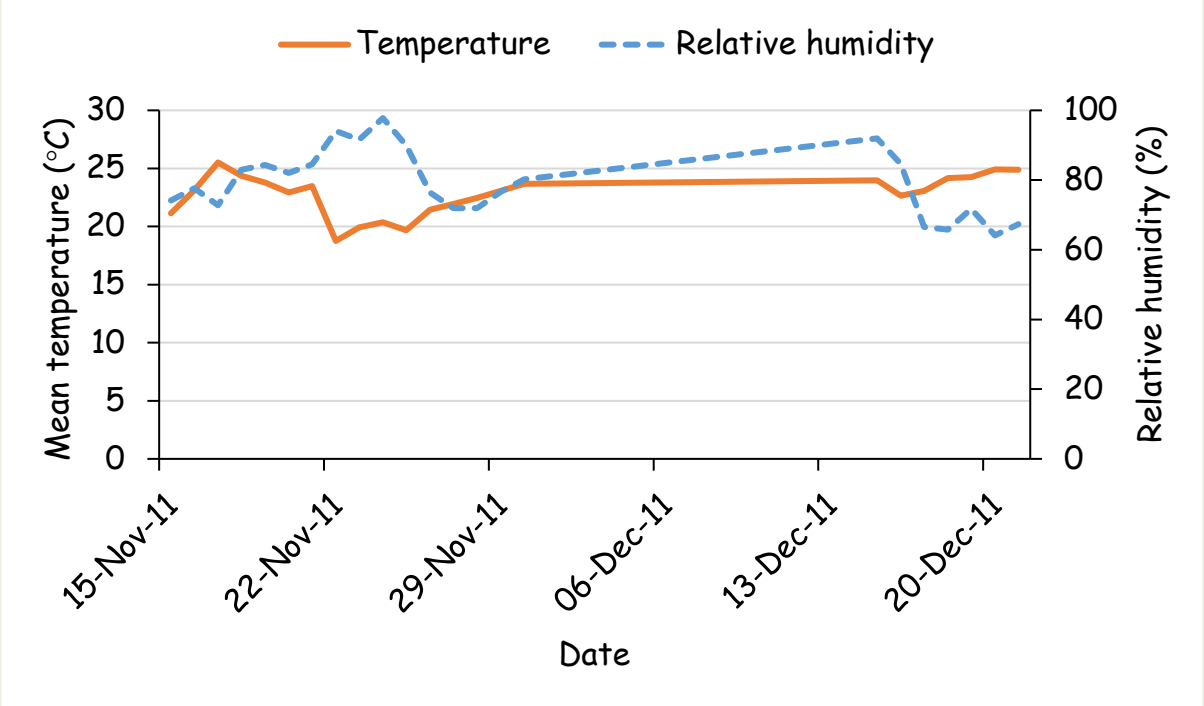


Figure 5.2. Mean daily temperature (°C) and relative humidity (%) in the custom-built hut house recorded from 15 November 2011 to 21 December 2011

Table 5.1. *p*-values and standard error means for rate colonisation (%) from pasteurisation methods of wheat straw

Days	**Rate of colonisation (%)**	
	p-values	Standard error means
Day 1	0.0130	0.2408
Day 2	0.0155	0.2431
Day 3	0.0972	0.2558
Day 4	0.1640	0.2787
Day 5	0.0561	0.3322
Day 6	0.0475	0.3111
Day 7	0.0833	0.2764
Day 8	0.0704	0.2778
Day 9	0.0883	0.2761
Day 10	0.2905	0.2926
Day 11	0.2544	0.2931
Day 12	0.3115	0.2917
Day 13	0.4123	0.2885
Day 14	0.3962	0.2885
Day 15	0.3962	0.2885
Day 16	0.4388	0.2976
Day 17	0.4388	0.2976
Day 18	0.3646	0.3116

5.3.2. Rate of contamination (%)

Bleach (sodium hypochlorite) was sprayed in the custom made hut before placing planted plastic bags in it. The bleach suppressed the amount of contamination from Week 0 to Week 2 (Figure 5.3). However, there were no significant differences in the contamination results among the various treatments of the substrate from Week 0 to Week 2. In contrast to this finding, Oseni *et al*. (2012) observed differences with horse manure which had been pasteurised using an autoclave, with a number of bags being contaminated by green mould and other bacteria

during incubation. Mushroom green mould was first considered a minor problem in the 1980s, which was associated with low quality compost or poor hygiene and was corrected by modifying the composting process, and improving the sanitation and chemical intervention (Ayilara *et al*., 2020). The amount of contamination increased in the wheat straw that had been treated with steam from the beginning of Week 1 (5%) compared with the samples with other treatments which presented with low contamination (3%). This indicates that the steam pasteurisation method was not able to overcome the amount of parasitic fungi available in the substrate at the initial stage.

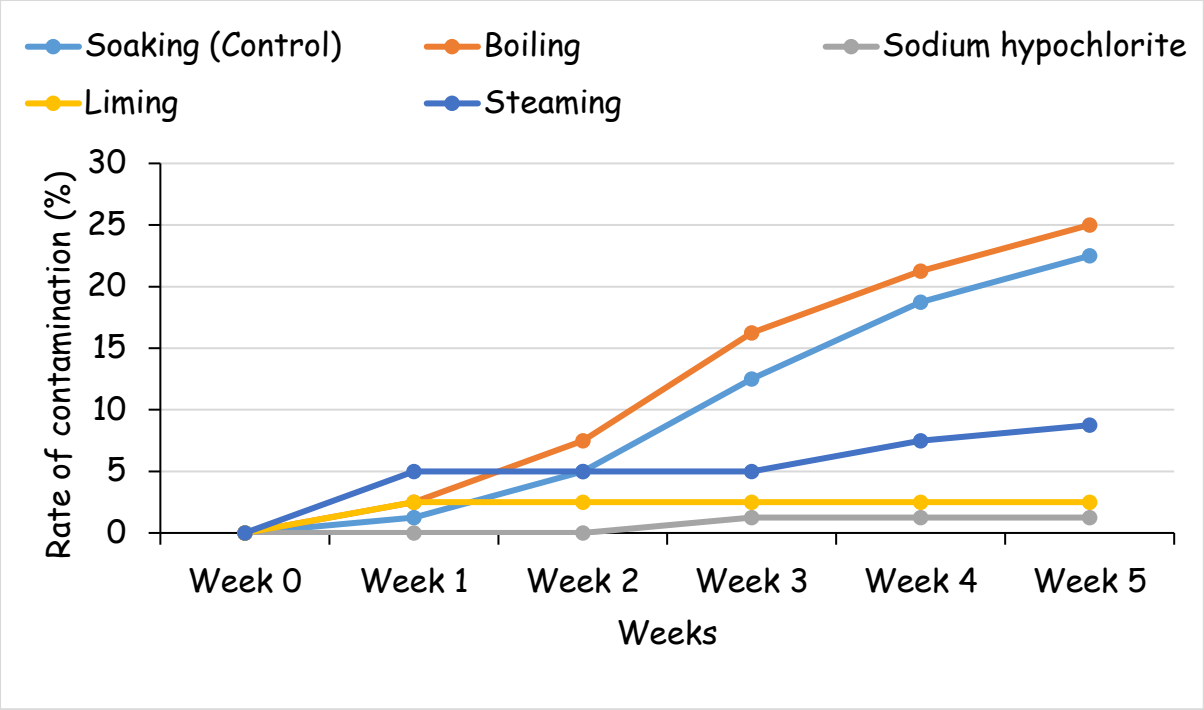


Figure 5.3. Rate of contamination affected by pasteurisation method from Week 0 to Week 5. There were significance difference but the statistical computation displayed no signifance differences as the pool of data used was very low

The boiling, steaming and control pasteurising methods allowed contamination to begin to increase from Week 2 to Week 5 significantly for all other treatment methods except for lime and sodium hypochlorite. The substrates treated with the boiling (24%) and control (22%) methods exhibited the highest amount of contamination in Week 5. This indicates that as the weeks progress, the parasitic fungi increase their colonies in the substrates treated with the boiling and control methods. The ability of the pasteurisationn method to eliminate substrate

contaminants is evident by the presence or absence of contaminants in the substrate after pasteurisation, spawning and incubation (Oseni *et al*., 2012).

Bleaching was regarded as the best pasteurisation methods for suppressing contamination, with the next best methods being the treatments with steaming or the addition of lime (Figure 5.3). In a previous study, the maximum contamination with green mould occurred with pasteurised cattle manure compared with pasteurised thatch grass in oyster mushroom production (Masarirambi *et al*., 2011).

Table 5.2. *p*-values and standard error means for rate of contamination (%) from pasteurisation methods of wheat straw

Weeks	**Rate of contamination (%)**	
	***p*-values**	**Standard error means**
Week 1	0.4802	0.2416
Week 2	0.2218	0.2471
Week 3	0.2507	0.2443
Week 4	0.2548	0.2459
Week 5	0.2295	0.2435

5.3.3. Harvesting of oyster mushrooms from different pasteurisation methods

Cumulative number of clusters

Considering all the treatments of the wheat straw, no significant differences were evident in the cumulative number of clusters among all the samples (Figure 5.4). The maximum cumulative number of clusters (33) was recorded for the substrate pasteurised with the gypsum method compared with those of all the other treatments. The substrate that had been boiled produced 28 clusters (cumulative number), which did not differ from that of the substrate that had been pasteurised with steam which yielded a cumulative 27 clusters. The Jik pasteurisation method led to a production of the lowest cumulative number of clusters (17) compared with the control sample with 20 cumulative clusters (Figure 5.4).

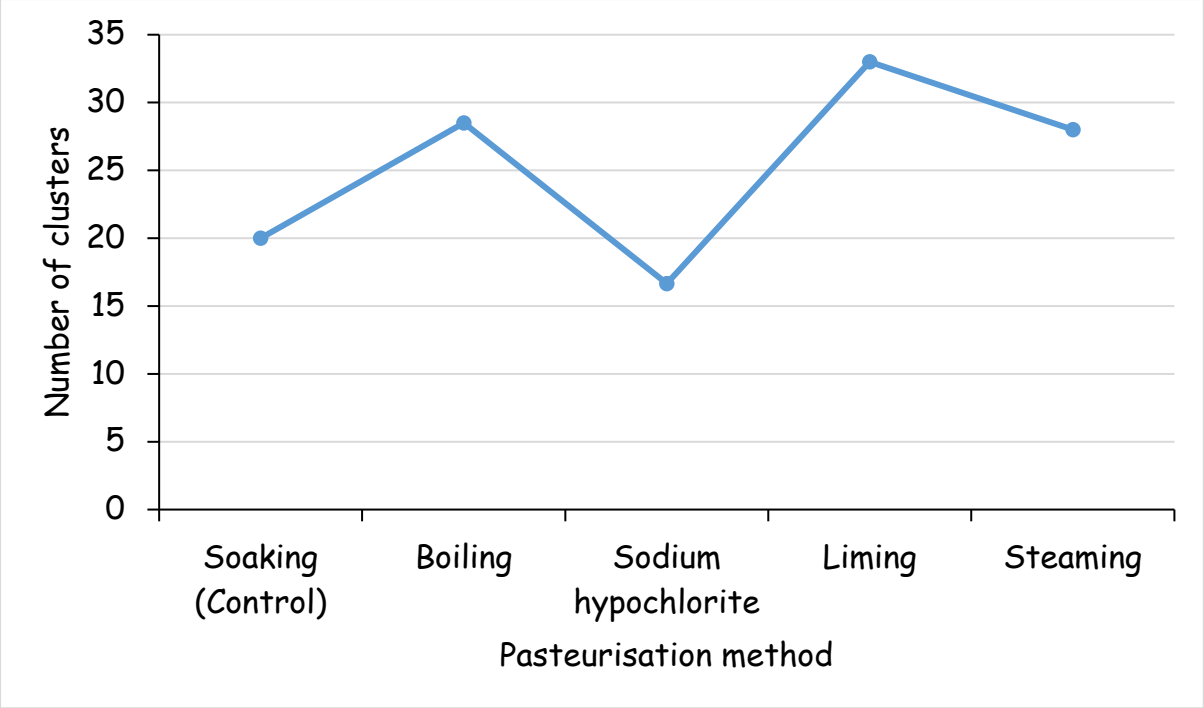


Figure 5.4. Number of clusters as affected by different pasteurisation methods

Oseni *et al*. (2012) harvested oyster mushrooms three times and the maximum yield was obtained in the first flush rather than the second and third clusters. The first clusters showed a significant difference compared with both the second and third clusters. Similar to this experiment, there were three harvests, but with no significant differences at all. Contrary to our findings, Pathan *et al*. (2009) found significant differences in the number of clusters in the substrates treated with soaking and boiling from 15 minutes to 90 minutes; however, the methods of pasteurisation were not similar to those of the present study.

Cumulative number of caps

The cumulative number of caps plays an important role in the overall yield of oyster mushrooms because the greater the cumulative number of caps produced, the higher the yield. There was no significant difference ($p < 0.05$) in the cumulative number of caps among all the treated samples (Figure 5.5). Although no significant difference occured, the steam and gypsum pasteurising methods led to a production of the highest cumulative number of caps (269), respectively, while boiling the substrate led to the production of a lower cumulative number of caps (220).

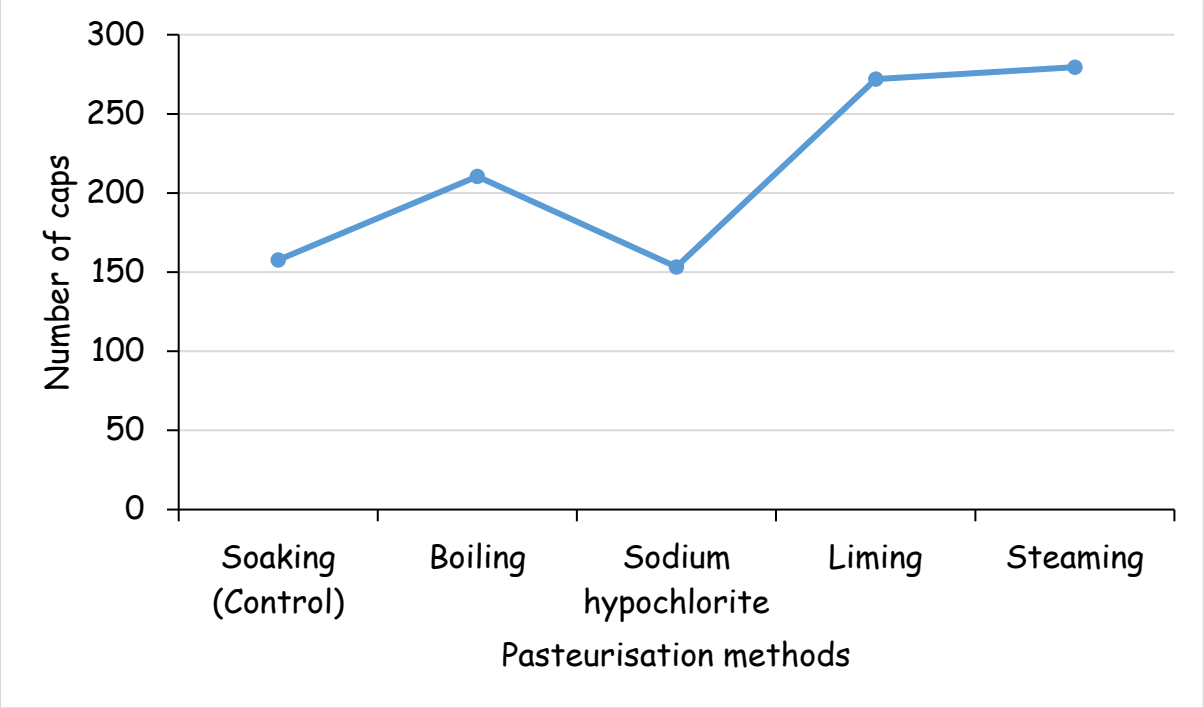


Figure 5.5. Number of caps as affected by different pasteurisation methods

It should be desirable for a substrate to produce a large number of caps, particularly when coupled with favourable additives and environmental conditions for mushroom production (Onyango *et al*., 2011). Both the control and substrates treated with bleach produced a similar cumulative number of caps (160) as depicted in Figure 5.5.

Cumulative mass (g) of oyster mushrooms

There were no significant differences observed in the cumulative fresh mass of the harvested oyster mushrooms with regard to all the treated substrates (Figure 5.6). The steamed substrate produced the highest yield (1 900 g) of the substrates subjected to all the other treatments. The boiled substrate yielded the second highest cumulative fresh mass (1 700 g). These yields were produced from 6 kg of wheat straw planted in plastic bags at the beginning. The wheat straw treated with gypsum produced (1 250 g) the lowest yield, whereas it was expected to produce a high cumulative fresh mass due to the high cumulative number of caps produced. Oseni *et al*. (2012) obtained a maximum average yield of 410.4 g from autoclaved bagasse compared with the lowest yield (118.9 g) from bagasse that had been pasteurised for 2 h in 18 x 14 cm autoclaveable bags from the plastic bags filled with 500 g of bagasse at the beginning.

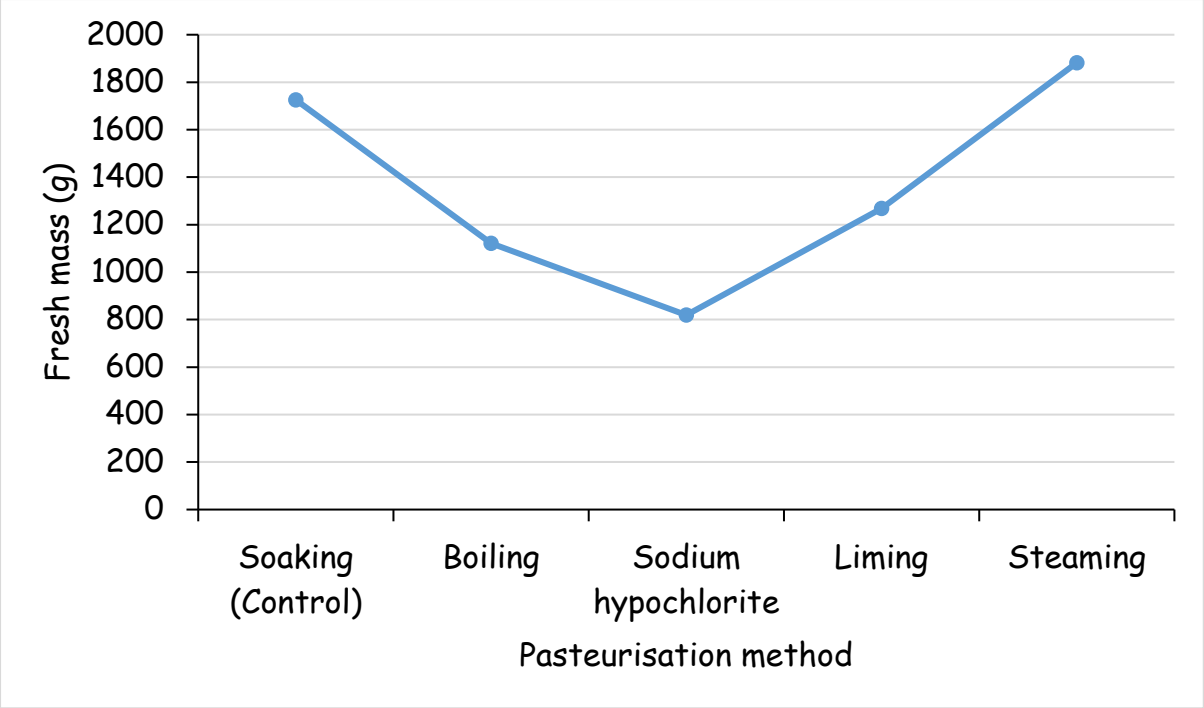


Figure 5.6. Fresh mass (g) of oyster mushrooms as affected by different pasteurisation methods

Similarly, there was no significant difference between the yields from the wheat straw treated with lime or the soaked substrate (Anonymous). Both the control (1 100 g) and jik (800 g) treated wheat straw had a disappointing yield compared with that of the other treatments (Figure 5.6). These results signify that steaming, boiling, gypsum, control (soaking) and jik pasteurisation did not promote yields of oyster mushrooms, but both boiling and steaming of the wheat straw led to higher yields than all the other treatments.

Table 5.3. *p*-values and standard error means for harvesting of oyster mushrooms from pasteurisation methods of wheat straw

Harvesting	***p*-values**	**Standard error means**
Number of clusters	0.8217	0.2997
Number of caps	0.2080	0.3025
Fresh mass (g)	0.2536	0.3078

5.3.4. Biological efficiency (%)

Both the steaming (31%) and boiling (29%) had the highest biological efficiency (%) compared to liming (21%), soaking (19%) and jik (14%) treatments. This was not in agreement with Wilcox (2017) where the best biological efficiency was achieved with calcium hydroxide. The Electrode steam conditioner machine used for steaming can be expensive to small-scale farmers compared to boiling method of pasteurising. Although, the steaming produces the highest biological efficiency (%), it cannot be recommended for small-scale farmers. But boiling method with 2% less compared to steaming, it can still do justice to small-scale farmers due to its affordability. The chemical treatment had the highest biological efficiency (72%), followed by hot water (68%) and the least soaking in water (2%) (Siddhant *et al*., 2014)

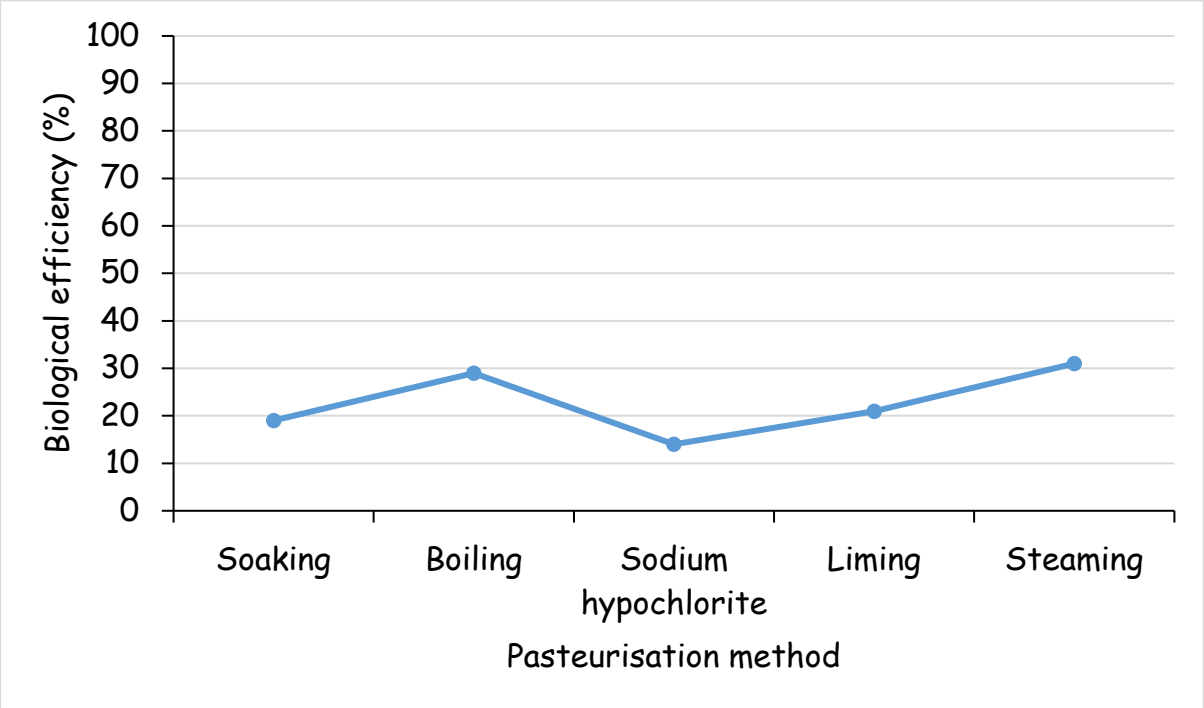


Figure 5.7. Biological efficiency (%) of harvested oyster mushrooms from different pasteurisation methods

5.4. Conclusions and recommendations

Both the boiling and steam pasteurising methods, which help to overcome attacks by parasitic such as fungi (*Trichoderma* species), showed an increasing trend in enhancing the rate of mycelial colonisation. Disinfection with jik was ideal for suppressing the amount of

contamination from Week 0 to Week 2. Both the boiling and steam pasteurising methods led to the production of the highest cumulative fresh mass (yield) of oyster mushrooms. Both the boiling and steam pasteurising methods can be encouraged and recommended to farmers to be used as alternatives (i.e. instead of their current practices) depending on affordability by the individual because farmers need higher yields of oyster mushrooms in order to increase their sales. Pasteurisation by means of soaking, gypsum and jik did not lead to good yields of oyster mushrooms, therefore they cannot be recommended to farmers. Both boiling and steaming methods of pasteurisation did very well in terms of the resultant higher yields of oyster mushrooms. The set null hypothesis *(H_0)* is been rejected and accept the alternative hypothesis (H_1) as pasteurisation methods of wheat straw affect the rate of colonisation (%), growth and yield of oyster mushrooms.

CHAPTER SIX

Alternative substrates for cultivating oyster mushrooms

6.1. Introduction

For successful mushroom cultivation, three factors must be considered, namely good quality and reliable spawn, a good substrate and a conducive environment (Rajapakse *et al*., 2007). Substrates in mushroom cultivation have the same function as soil in plant production (Kwon and Kim, 2004). Many species of *Pleurotus* are commonly grown on a wide range of lignocellulosic materials (Sánchez, 2004). Different substrates can be recommended per region due to local availability of agricultural waste (Cohen *et al*., 2002). Growers typically select the most effective, economical and locally available substrate materials for mushroom production (Li *et al*., 2015). The ideal media for cultivation of edible fungi must be mold free and rich in essential nutrients (Kwon and Kim, 2004) such as nitrogen, phosphorus, potassium, magnesium and iron. The most commonly used substrates include sawdust, cottonseed straw, cereal straw, maize cob, sugarcane bagasse and other plant fibres with a high cellulose content (Kwon and Kim, 2004). According to Labuschagne *et al*. (2000), wheat straw has been the main substrate used for cultivating *P. ostreatus*. However, Bughio (2001) successfully planted *P. ostreatus* on a combination of wheat straw, cotton ball straw, rice straw, sugarcane and sorghum leaves.

The substrate can also be supplemented, if necessary, with additional nitrogen sources, such as wheat bran, oat bran, copra cake (from spent coconut), rice bran, sorghum or millet in order to obtain quality mushrooms. Additives such as gypsum, limestone and chalk can function as pH buffers in a substrate (Kwon and Kim, 2004). Although *Pleurotus* species can be produced from various substrate types or residues, productivity and biological efficiency will vary according to the strains and substrates used (Bernardi *et al*., 2007). Different substrates, therefore, can be recommended per region depending on local availability of agricultural wastes (Cohen *et al*., 2002). The cultivation of oyster mushrooms using different substrates has been studied extensively (Poppe, 2004). According to Bellettini *et al*. (2016), the C:N ratio (28–30% carbon and 1% nitrogen) is an important condition for mushroom production (spawn running). However, there is no specific information on the use of thatch grass (*Hyparrhenia hirta*) and wood chips (Pine or

Eucalyptus) as an alternative to wheat straw for oyster mushroom cultivation. Thatch grass can be freely collected from the veld (wild or open fields) and wood chips are waste products from wood milling companies. These alternative substrates need to be studied in order to determine their suitability for use by small-scale farmers in mushroom production.

Therefore, this study was conducted with the following objectives (i) testing of different substrate for rate of colonisation, amount of contamination and yield of oyster mushrooms. (ii) comparison of mineral content and nutritional content from oyster mushrooms produced from different substrates. The following hypotheses was consequently tested: (i) The null hypothesis (H_0) was that alternative substrates will have no significant effect on rate of colonisation, contamination and yield of oyster mushroom. (ii) The alternative hypothesis (H_1) was that alternative substrates will have a significant effect on rate of colonisation, contamination and yield of oyster mushroom.

6.2. Materials and method

Study site

Details of the study site are described in Chapter 3. ***NB***: (The actual study followed this well-established methodology to negate the fact of acquiring totally new substrate. The rationale was to take abundantly available resources and knowledge for designing a novel training manual for small-scale farmers).

Experimental design and pasteurisation of the substrate

The layout of the experiment was a completely randomised design (CRD) with six treatments consisting of a factorial combination of three types of substrates and two levels of drainage, with each treatment replicated four times. The three types of substrates were wheat straw (control), wood chips and thatch grass (*Hyparrhenia hirta*) (Plate 6.1). The two levels of drainage were no drainage and drainage. The six treatments consisted of control x drain, control x no drain, wood chips x drain, wood chips x no drain, thatch grass x drain and thatch grass x no drain. A single bale each of wheat straw (10 kg), 100% wood chips (20 kg) and thatch grass (15 kg) were boiled separately in 225 L drums. The substrates were pasteurised using a method outlined by Kang (2004). The drained substrates were boiled and excess water was allowed to drain for one hour

before inoculating them with spawn, whereas the undrained substrates were inoculated with spawn immediately after boiling. During boiling, the temperature was monitored using a pocket thermometer (Checktemp 1, Hanna instrument - Mauritius). The culture bags (410 mm x 750 mm x 45 µm, width, length and thickness) were filled with wheat straw only and with wood chips only and thatch grass only (6 kg) together with spawn (1 kg, which was further divided into three equal parts, i.e. ≈0.33 kg at the bottom, middle and top parts of the polyethylene bag) to make up a total wet weight of 7 kg per treatment. The wheat straw substrate was sourced from Marble Hall (Limpopo Province, South Africa), the wood chips were sourced from DENSA, a wood company (Mpumalanga Province, Lydenburg/Sabie Road), whereas the thatch grass was collected from the veld (Mpumalanga Province, Mbombela).

Data collection

The culture bags were evaluated for both colonisation and contamination. The evaluation of colonisation began shortly after inoculation until full colonisation was reached, whereas contamination by mostly *Trichoderma* spp. was evaluated on a weekly basis using a rating scale method of between 0 and 100% as outlined by Tesio (2003). After full colonisation had been obtained, the culture bags were made small cross cut from top to bottom to facilitate the growth of mushroom clusters. The mature oyster mushrooms were harvested three weeks after spawning. These clusters were manually pulled from the culture bags and kept in brown paper bags to avoid moisture loss, and taken to the laboratory for post-harvest analysis. The growth parameters recorded included the cumulative number of harvests, the number of clusters, the number of caps and the fresh mass.

Postharvest analysis

After recording the growth parameters, the samples were kept in two separate plastic bags with caps or stalks and stored in a deep freezer (-21°C) for further analysis. These samples were subsequently sent on ice to the ARC-API Laboratory Services for chemical analysis. Mineral analyses of samples of wheat straw, wood chips and thatch grass substrate were determined at the ARC-TSC Soil Science Laboratory. The wheat straw, wood chips and thatch grass samples were analysed using standard procedures for leaf analysis (P; K; Ca; Mg; Zn; Cu; Mn; Fe and B) as

outlined by Masevhe *et al*. (2015). These nutrients were measured by an Atomic Absorption Flame Spectrophotometer (Varian Spectra AA 250 plus, Australia). The C:N ratios of wheat straw, wood chips and thatch grass were determined by modified Kjeldahl method where nitrogen and loss on ignition for C using method outlined by Greenfield and Southgate (2003).

Analysis of chemical composition of oyster mushrooms

Both caps and stalks (100 g each) were chemically analysed for ash, carbohydrates, dry matter, moisture, fat and protein content. The organic matter was analysed using the method outlined by Chepkwony *et al*. (2001). The carbohydrates, moisture content, crude protein, ash and fat were analysed using appropriate SANAS accredited methods outlined by Greenfield and Southgate (2003). The dry matter content was determined using the method outlined by Mertens (2002). Fats were dissolved in ether and evaporated at 105°C using the method outlined by Greenfield and Southgate (2003).

Cost and economic analysis of the substrates

Wheat straw was bought from a farm in Marble Hall at a cost of R25.00 per 10 kg bale. The transport cost R1 000.00 (480 km return trip) using a 1-ton truck with a trailer. In addition, the wheat straw was stored for 3 months to leach out compounds responsible for inhibiting mushroom growth. Wood chips were collected at no cost from a milling company very close to the experimental site; the transport costs were considered to be negligible. No costs were associated with the thatch grass, as it was collected from the veld (South African wild field) close to the experimental site and used immediately. In South Africa, button mushrooms are sold for R25.00 per kg, whereas oyster mushrooms are sold for R40.00 per kg in local retail markets. The biological efficiency (%) of harvested oyster mushrooms was determined using *Equation 1*.

$$\text{BE (\%)} = \frac{\text{Fresh mass (g) of oyster mushrooms}}{\text{Total wet substrate used (g)}} \text{X } 100 \qquad \text{Eq. [1]}$$

Plate 6.1. (A) Pasteurised wood chips, (B) thatch grass and (C – control) wheat straw on a spawning table before inoculation with oyster mushroom spawn

Statistical analysis

Statistical evaluation of data as done for Chapters 3, 4 and 5,

6.3. Results and discussion

6.3.1. Comparison of rate of colonisation between drained substrates and with excess extracts

The extent of drainage did not affect the rate of mycelial colonisation significantly from Day 1 to Day 4 (Figure 6.1). The rate of mycelial colonisation of the wheat straw with excess extracts did not differ significantly from that of the drained wheat straw from Day 7 to Day 9. Furthermore, rate of mycelial colonisation of the wheat straw with excess extracts reached 100% from Day 9 to Day 11, unlike the drained wheat straw (Figure 6.1). Mane *et al*. (2007) reported a range from 12-14 days for full spawn running in various *Pleurotus* spp. on lignocellulose substrates, although the results of the present study concur with the previous spawn running periods reported by others regarding the *Pleurotus* spp.

The rate of mycelial colonisation of thatch grass with excess extracts from Day 6 to Day 9 increases compared with that of the thatch grass that has been drained. The mycelial colonisation of thatch grass with excess extracts reached 100% from Day 9 to Day 11, which is an indication that it fully colonised the polyethylene bags within two weeks (Figure 6.1). Moreover, there was no significant difference ($p < 0.05$) in the said rate of coverage from Day 0 to Day 11 between the wood chips that had been drained and those with excess extracts. Mycelial growth is a preliminary step that creates suitable internal conditions for fruiting while it is also a vital factor in mushroom cultivation, because ultimately, farmers seek the best yields (Pokhrel *et al*., 2013).

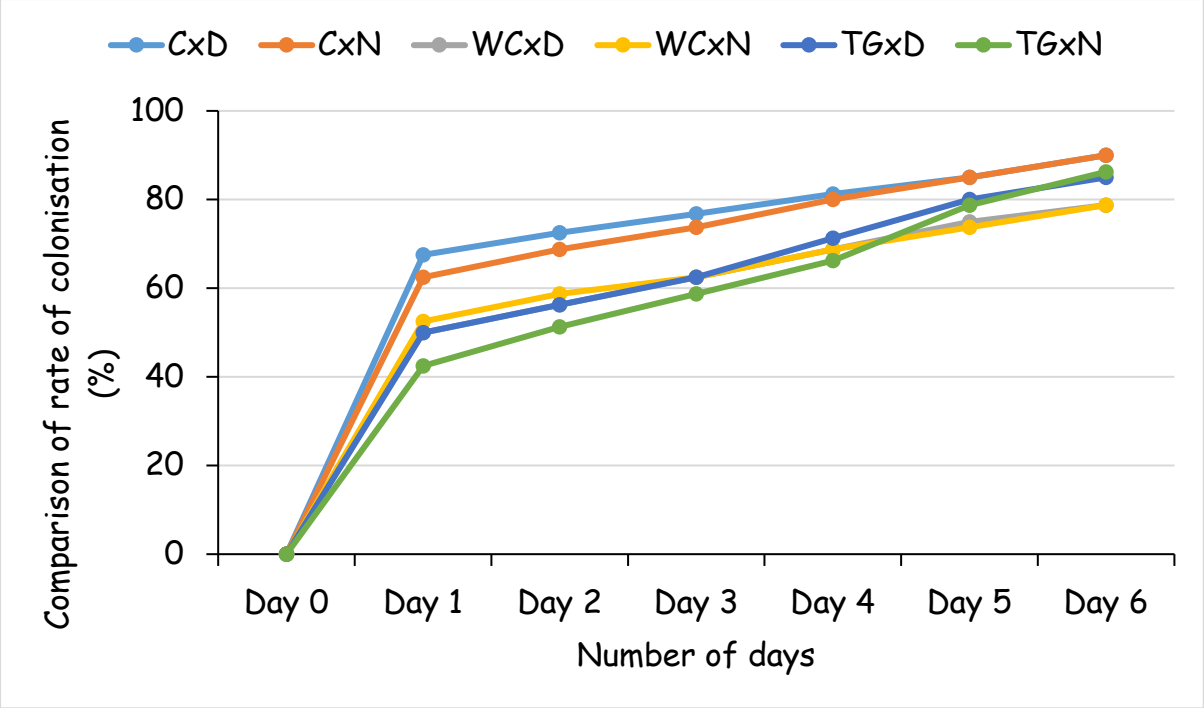


Figure 6.1. Rate of colonisation (%) (CxD-wheat straw x drain, CxN-wheat straw x no drain, WCxD-wood chips x drain, WCxN-wood chips x no drain, TGxD-thatch grass x drain, TGxN-thatch grass x no drain from Day 0 to Day 6. There were no significance differences between the treatments

6.3.2. Rate of colonisation (%) from different substrates

The mean daily temperature in the growth room varied between 19°C and 27°C during the full colonisation of mycelia and fruiting (Figure 6.2) whilst the relative humidity was between 80% and 98% (Figure 6.2). These temperatures appear to be ideal since Shah *et al*. (2004) reported that a temperature of 25°C was required for full mycelial colonisation and fruiting. There were highly significant differences ($p < 0.05$) in the level of colonisation among the substrates (Figure 6.2) with wheat straw compared with either wood chips or thatch grass from Day 1 to Day 8 (Figure 6.2).

From Day 1, the wheat straw had 65% colonisation of the mycelia compared with that of the wood chips and thatch grass with 50% and 45% colonisation, respectively. The wood chips and thatch grass exhibited a low rate of colonisation compared with wheat straw from Day 1 to Day 8. This can be due to the low temperatures and relative humidity in the growth room. In addition, it took 8 days for the mycelia to completely colonise the wheat straw, whereas the

mycelia reached full colonisation of the wood chips and thatch grass from Day 9 to Day 10 (Figure 6.2). The results found in the present study were not consistent with the findings of Khan *et al*. (2012), Mondal *et al*. (2010), Shah *et al*. (2004) and Tan *et al*., (2005), who reported that spawn took two to three weeks (14 - 21 days) to reach full colonisation. Moreover, Khan (2009) reported that *Pleurotus ostreatus* took 24 - 25 days for completion of spawn running on wheat straw substrate. Other researchers (Dehariya and Vyas, 2013) showed that bamboo leaves require spawn run of 17 days, compared to sawdust with unsuitable and poor substrate that require more time for spawn run (32 days). This can be due to environmental conditions and the available minerals in the wheat straw to be able to support the growth of oyster mushrooms. There was no significant difference regarding mycelial colonisation between the various growing media from Day 9 to Day 11. This indicated that wheat straw, wood chips and thatch grass reach full colonisation with time, although differences were evident during the early stages of colonisation. Wheat straw was ideal for improving the colonisation percentage of oyster mushrooms compared with the wood chips and thatch grass.

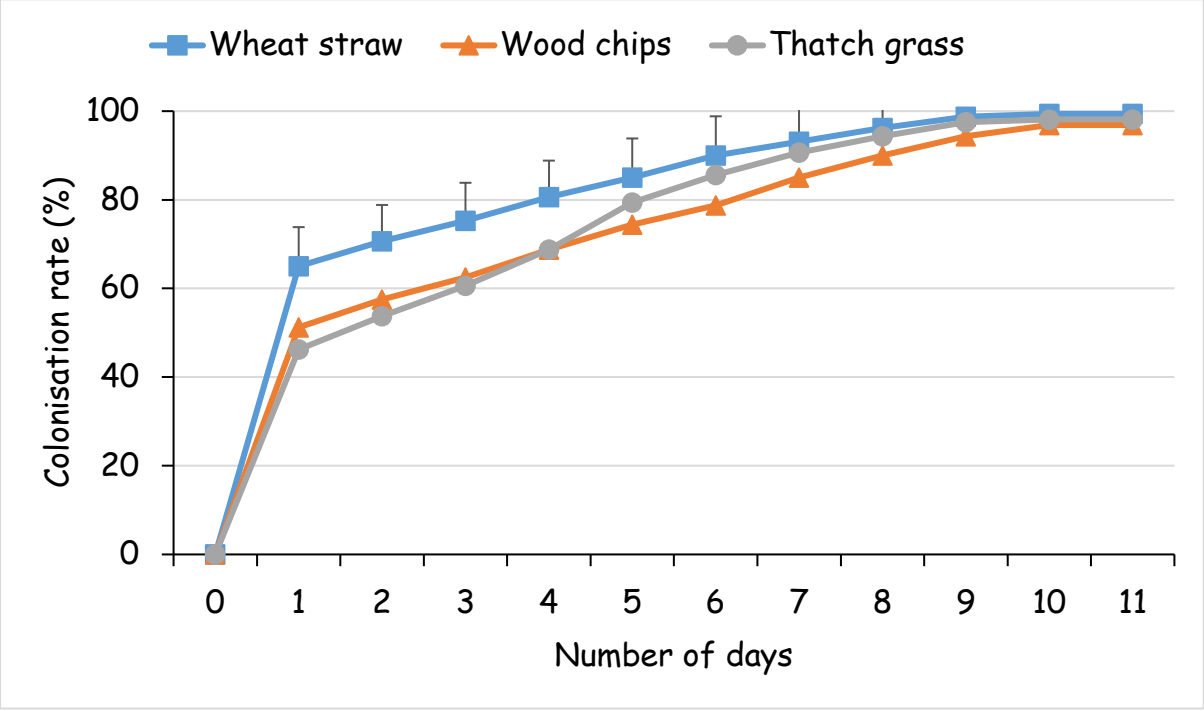


Figure 6.2. Rate of colonisation (%) as influenced by substrate from Day 0 to Day 11. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

Quality and quantity of spawn play an important role in the successful cultivation of any mushroom species (Pani, 2011). The mushroom yield decreased with increase in spawn age and found the quickest substrate colonisation and primordial initiation as well as highest number and weight of sporophores with 21 days old spawn were obtained. Spawning of substrate in 4 to 5 layers induced quicker substrate colonisation (15 days) and fruiting initiation (29-30 days) and sustained higher mushroom yield (68.9-69.0 % BE). Lowest yield (31.0 % BE) and longest fruiting time has been recorded in response to single/middle layer spawning (Pani, 2011).

Girmay *et al*. (2016) recently found that cotton seed followed by paper waste had better mycelia density, time required for mycelia running, pin-head formation and development of fruiting bodies compared with sawdust and wheat straw in Ethiopia. These differences may be due to climatic conditions and other cultivation practices from one country to another or the quality of substrates used. Growing mushrooms should be able to colonise an area quickly before other competative fungi can cover the designated area (Van Nieuwenhuijzen, 2007). The reported variations could be due to environmental differences, hygiene and the viability of the oyster mushroom spawn.

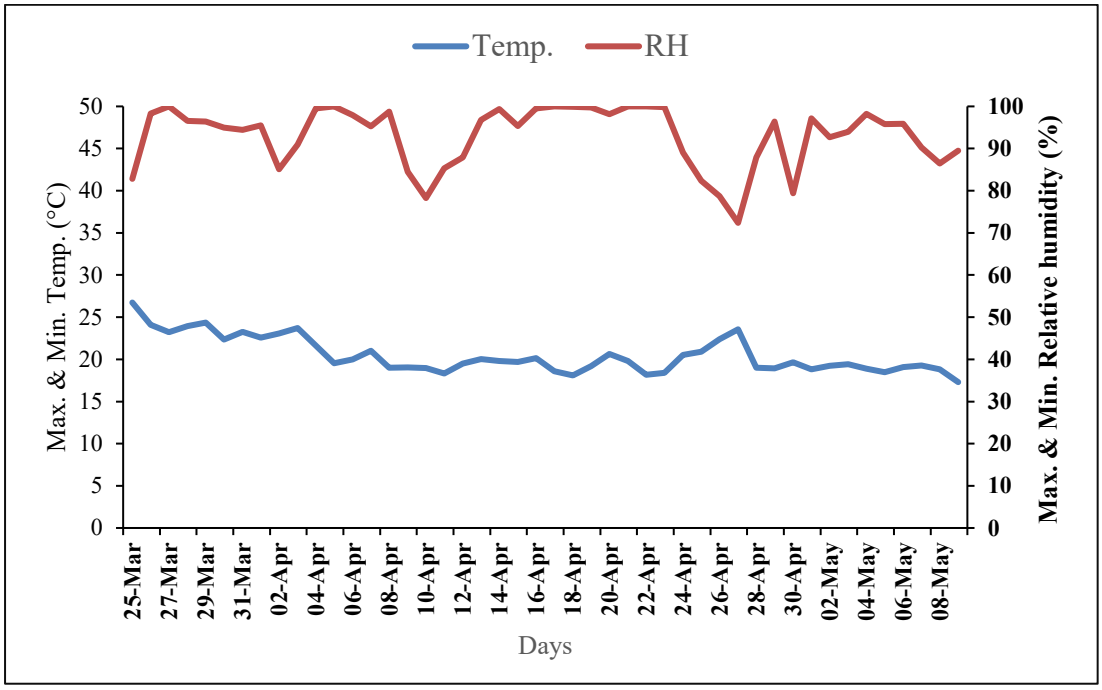


Figure 6.3. Maximun and minimum temperature (°C) and relative humidity (%) data in a custom made hut-house taken from 25 March 2011 to 09 May 2011

Table 6.1. *p*-values and standard error means for rate colonisation (%) from different substrates

Days	**Rate of colonisation (%)**	
	p-values	Standard error means
Day 1	0.0050	0.2197
Day 2	0.0026	0.2168
Day 3	0.0008	0.2153
Day 4	0.0038	0.2254
Day 5	0.0102	0.2253
Day 6	0.0292	0.2210
Day 7	0.0052	0.2242
Day 8	0.0053	0.2269
Day 9	0.0606	0.2216
Day 10	0.1382	0.2284
Day 11	0.1382	0.2284

6.3.3. Rate of contamination (%) from different substrates

The rate of contamination exhibited no significant differences ($p < 0.05$) amongst the treatments during Week 1 (Figure 6.3). In Week 2, the contamination of wood chips did not differ significantly from that of wheat straw. However, in Week 1, the amount of contamination of the wood chips did not differ from that of thatch grass. There was a greater amount of contamination (26%) of the wood chips + drain compared to that of thatch grass + drain (15%) in Week 2, while there was no contamination of the wheat straw + drain or not drain (0%). Van Niewenhuijzen (2007) stated that a fast rate of colonisation of mushrooms within the growing area can suppress the development of other fungi or bacteria. The wood chips were more susceptible to contamination compared with thatch grass. However, the wheat straw did not show any contamination during the experiment (Figure 6.3).

Table 6.2. *p*-values and standard error means for contamination (%) of different substrates

Contamination (%)	
p-value	Standard error mean
<.0001	0.1085

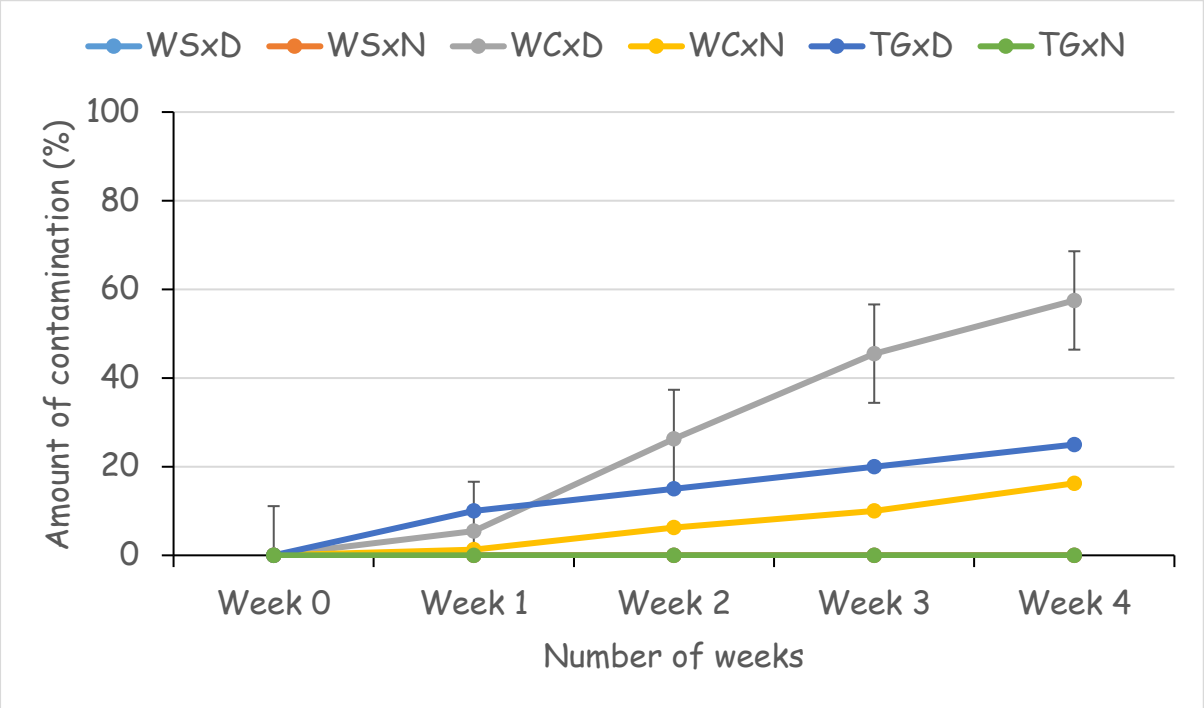


Figure 6.4. Percentage contamination as influenced by substrate and drainage level from Week 0 to Week 4. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

The rate of contamination (45%) in the wood chips + drain was significantly increased compared to that of thatch grass + drain (20%). This could be due to the variation of moisture loss within the different substrates, which could influence the development of other fungi or bacteria. On-farm hygiene can also contribute towards the contamination of both the substrate and the mushrooms (Van Niewenhuijzen, 2007). In addition, in Week 4, the amount of contamination of the wood chips + drain differed significantly from that of wheat straw + drain or not drain and thatch grass + not drain. Moreover, the wood chips + drain displayed the highest contamination (57%) compared with thatch grass + drain and wood chips + not drain (25%) and wheat straw (0%) (Table 6.2). This indicated that wheat straw + drain or not drain and thatch grass + not drain significantly suppressed contamination throughout the production

period. Clearly, both the wood chips + drain or not drain and thatch grass + drain were not effective in suppressing contamination. Bellettini *et al*. (2019) indicated that the substrates with poor nutrition exhibited low mycelial densities thus rendering them prone to contamination, especially by green mold. According to Kumari and Achal (2008), contamination can be caused by improper pasteurisation of straw and available contaminants in a substrate.

6.3.4. Harvesting of oyster mushrooms from different substrates

The cumulative number of clusters that were produced were significantly different ($p < 0.05$) for all the treatments (wheat straw, thatch grass and wood chips). The wheat straw (45) yielded the highest cumulative number of clusters produced followed by the thatch grass (44) and then wood chips (12) (Figure 6.5). Kumari and Achal (2008) cultivated *Pleurotus ostreatus* on different substrates where they reported the highest yield on wheat straw, followed by the combination of paddy straw and wheat straw. Their findings were similar to those of the current study with the higher yield being on wheat straw; however, the other substrates were different from those in this study. The cumulative number of clusters were significantly different between those produced on the wheat straw and the thatch grass.

The cumulative number of caps produced by both the wheat straw and the thatch grass differed significantly ($p < 0.05$) compared to that of the wood chips. Both the wheat straw (395) and the thatch grass (392) yielded the highest cumulative number of caps compared with that of wood chips (91.50) (Figure 6.5).

The yields of both the wheat straw and the thatch grass significantly differed from the wood chips in terms of the cumulative fresh mass of oyster mushrooms. The wheat straw (4 113 g) and the thatch grass (3 944 g) produced the largest cumulative fresh mass of oyster mushrooms compared to that of the wood chips (361 g) (Figure 6.5). This indicates that the yields of both the wheat straw and the thatch grass were greater than that of the wood chips. Thatch grass also showed potential as an alternative to wheat straw considering the cumulative fresh mass produced.

Table 6.3. *p*-values and standard error means for harvesting of oyster mushrooms from different substrates

Harvesting	*p*-values	Standard error means
Number of clusters	0.3868	0.2542
Number of caps	0.3114	0.2358
Fresh mass (g)	0.3017	0.2571

In general, both the thatch grass and the wheat straw yielded a large number of clusters, caps and quantity of fresh mass, which increased the yield. This was not inconsistent with the findings of Bellettini *et al*. (2019), where the wood chips in combination with the wheat straw had the highest yield compared with the single usage of wheat straw and waste paper substrates

6.3.5. Harvesting periods of oyster mushrooms from different substrates

The harvesting intervals of the oyster mushrooms started ±66 days after inoculating. This study was not inconsistent with Bhattacharjya *et al*. (2014) who indicated that the days required for first picking varied from 11.25-12.00 days and the final picking complete from 42.25-50 days depending on different substrates. At the first harvest, the thatch grass produced a high number of clusters compared to both the wheat straw and the wood chips (Figure 6.4). The thatch grass yielded a higher cumulative number of clusters, although each harvest differed regarding the number of clusters, caps and the quantity of fresh mass produced during the entire production period. However, the wheat straw yielded a similar number of clusters compared with that of the thatch grass, while the wheat straw produced a similar number of caps from first to sixth harvests. Surprisingly, the wood chips yielded only three harvests compared with that of both the wheat straw and thatch grass (Figure 6.6). This study was inconsistent with that of Tesfay *et al*. (2020) who harvested only two flushes of mushrooms for each treatment (rice straw, rice straw + wheat straw, rice straw + paper, sugarcane bagasse and sawdust).

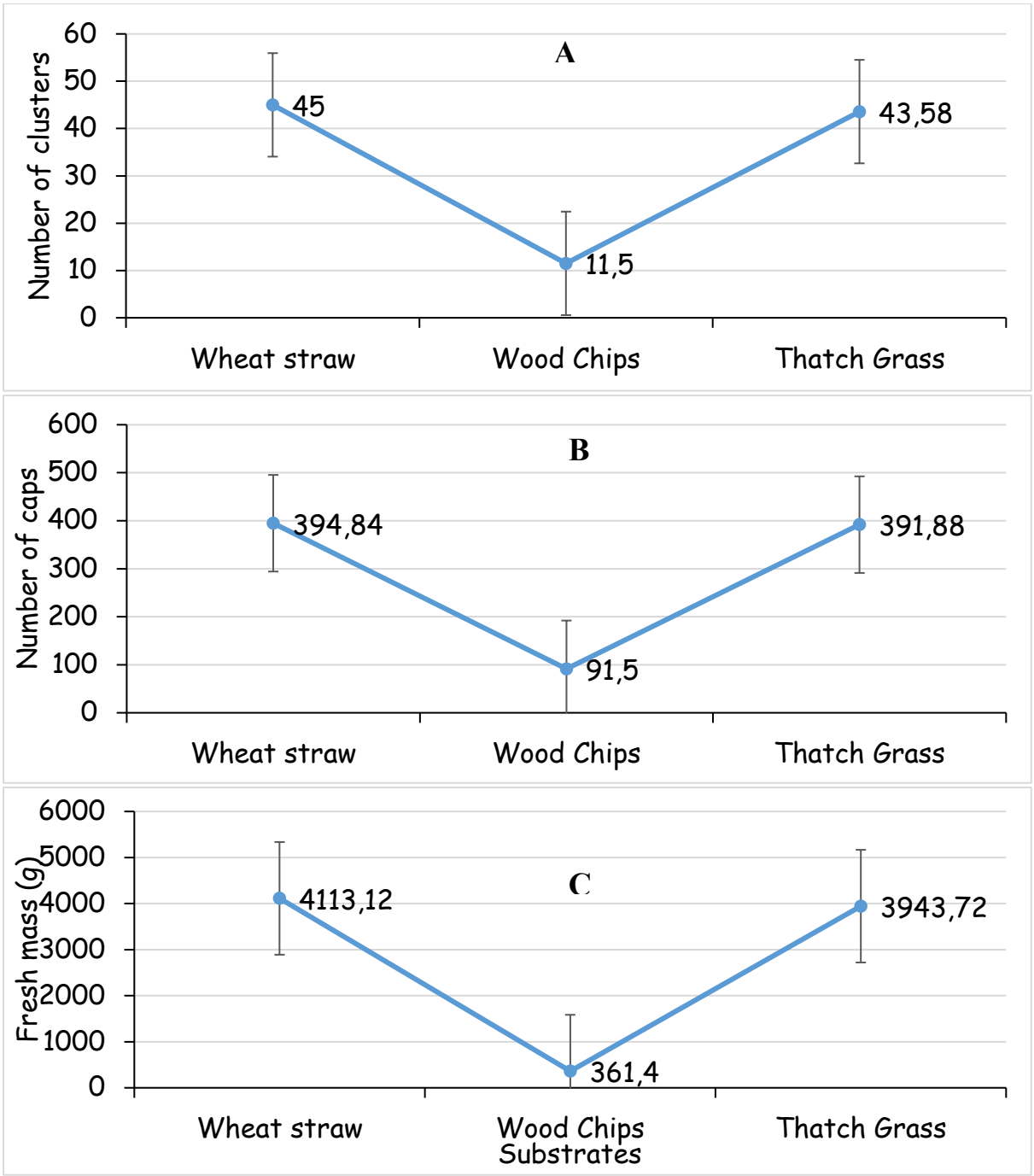


Figure 6.5. Number of clusters, caps and fresh mass (g) as influenced by substrate used. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

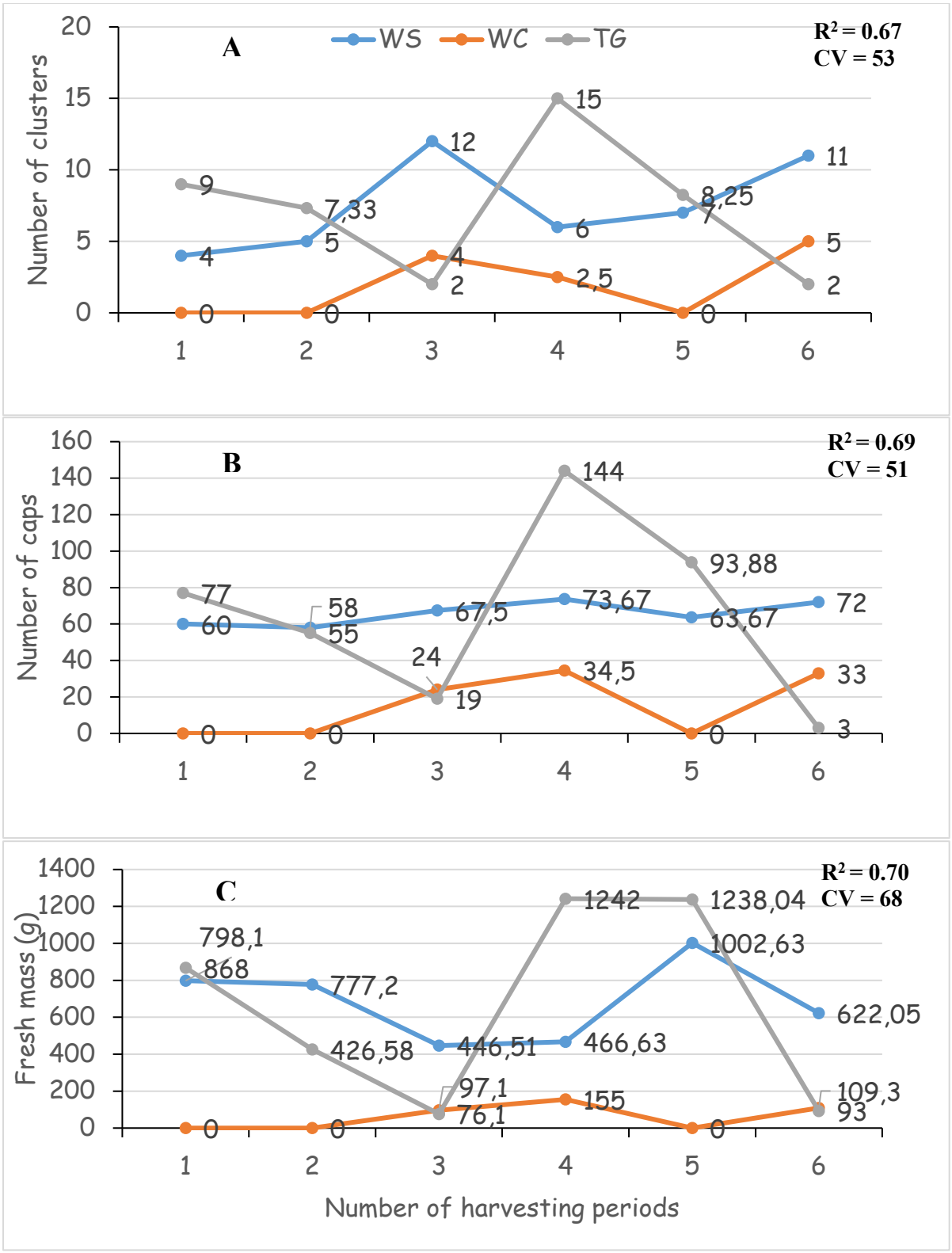


Figure 6.6. Number of clusters, caps and fresh mass (g) per harvest from different substrates. WS = wheat straw; WC = wood chips; TG = thatch grass

6.3.6. Mineral analysis of substrates of fresh oyster mushrooms from different substrates

The wood chips had the highest C/N ratio (400:1), followed with wheat straw (80:1) and the least in thatch grass (40:1). The higher nitrogen content in both wood chips and wheat straw will not immediately be available for uptake as it will need to be converted by microorganisms to ammonium and nitrate (a process called "mineralization"). Thatch grass had a higher N content (1.136%) than both the wheat straw (0.560%) and the wood chips (0.080%) (Table 6.4). Rose *et al*. (2022) indicated that nitrogen plays an important role in building the biomass of *Pleurotus ostreatus*. Thatch grass had a significantly higher available N compared with both the wheat straw and wood chips. This is also supported by Yildiz *et al*. (2002) who reported that while the natural substrates (woods on which *Pleurotus* species grow) are very poor in nitrogen content, they nonetheless produce the fruit bodies. Upadhyay *et al*. (2002) reported that the *Pleurotus* species possess the capability to absorb atmospheric N. The thatch grass had a significantly higher P content (0.161%) compared with both the wheat straw (0.054%) and wood chips (0.001%), whereas the wheat straw contained a high amount of K (0.907%) compared with both the thatch grass (0.537%) and the wood chips (0.037%).

The Ca levels of the thatch grass, wheat straw and wood chips were 0.490%; 0.340% and 0.067%, respectively, which varied significantly. Alananbeh *et al*. (2014) reported that agricultural wastes can be used to supply calcium for the growth and yield of oyster mushrooms. In addition, the application of poultry manure can also be used to supplement and compensate for the lack of Ca, Cu, Mn and Fe in a nutrient deficient substrate for the cultivation of oyster mushrooms (Bandara *et al*., 2006). The available Mg content significantly differed for the thatch grass (0.282%) compared to both the wheat straw (0.108%) and the wood chip (0.024%) substrates. Furthermore, the wheat straw had a higher amount of Fe content (845.00 mg/kg) compared with both the thatch grass (408.33 mg/kg) and wood chips (235.00 mg/kg) (Table 6.4). In general, the wood chips had the lowest mineral (e.g. N, P, K, Ca, Mg and Fe) quantities, which were far below the critical threshold levels required for oyster mushroom production compared with all the other substrates. The lower mineral content of the wood chips was evident by the production of fewer cumulative clusters and caps as well as the lower fresh mass of oyster mushrooms.

Table 6.4. Mineral analysis of wheat straw (WS - control), wood chips (WC) and thatch grass (TG)

Treatment	Mineral analysis											
	%						mg/kg					
	C:N	N	P	K	Ca	Mg	Zn	Cu	Mn	Fe	B	N:K
C (WS)	80:1	0.560	0.054	0.907	0.340	0.108	33.00	24.33	71.33	845.00	7.53	0.63
WC	400:1	0.080	0.001	0.037	0.067	0.024	8.67	3.00	48.33	235.00	3.97	2.07
TG	40:1	1.136	0.161	0.537	0.490	0.282	36.33	11.33	147.00	408.33	10.27	2.13
LSD (α<0.05)	-	0.0405	0.0015	0.0231	0.0211	0.0036	2.9783	0.9418	3.9399	34.01	0.5532	0.4467
p-values	-	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003
Se	-	0.4248	0.5734	0.4102	0.8053	0.4792	0.4109	0.4204	0.4258	0.4662	0.4288	1.0303
CV%	-	3.4236	1.0352	2.3406	3.5267	1.3008	5.7335	3.6574	2.2185	3.4312	3.8162	13.8790
R^2	-	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.93

6.3.7. Nutritional (analysis) composition of fresh oyster mushrooms from different substrates

There were no significant differences ($p < 0.05$) among all the substrates (wheat straw, wood chips and thatch grass) in terms of chemical composition (ash ranged from 0.44%, 0.68% to 0.82%, fat ranged from 0.08%, 0.85% to 0.145%, protein ranged from 1.655%, 1.67% to 2.45% and carbohydrates ranged from 6.735%, 7.09% to 7.22%). Other studies indicate that the protein in rice straw substrates ranged from 22.89% to 25.97%, and the fat and carbohydrate content ranged from 1.03% to 1.50% and 30.24% to 42.26%, respectively, which were far higher than the results obtained from the substrates used in the current study. This also indicates that both carbohydrates and protein depend on the substrate used (Wang *et al*., 2001). However, all the mineral nutrients were available from harvested oyster mushrooms in lower percentages, regardless of the different substrates used (Table 6.5).

All the treatments in this experiment had a moisture content ranging from 89.94% to 90.84%. This finding was similar to those of Dunkwal and Jood (2009), who reported that oyster mushrooms grown on wheat straw and brassica straw contained 89.68% and 88.98% moisture based on fresh weight. Oyster mushrooms are soft in nature due to their high moisture content at a younger stage, but tend to lose moisture with maturity (Prakash *et al*., 2011). Mushroom fungi are regarded as a good source of protein, with high levels of vitamins and fewer calories, and are free from cholesterol (Selvi *et al*., 2007). Generally, no growing media was considered to be outstanding regarding chemical composition when compared with each other.

Table 6.5. Nutritional value of fresh oyster mushrooms harvested in wheat straw (control), wood chips (WC) and thatch grass (TG) substrates

Treatments	**Nutritional value (%)**					
	Dry matter	**Moisture**	**Ash**	**Fat**	**Protein**	**Carbohydrates**
C (WS)	9.84	90.16	0.82	0.145	1.655	7.22
TG	9.16	90.84	0.68	0.085	1.67	6.735
WC	10.06	89.94	0.44	0.08	2.45	7.09
LSD (<0.05)	0.204	0.204	0.916	0.116	0.063	0.014

6.3.8. Biological efficiency (%) of different substrates

The wheat straw + drain had the highest biological efficiency (%) of *P. ostreatus* compared to wheat straw + not drain and other treatments (Figure 6.7). The wheat + drain had the biological efficiency of 80%, followed by wheat straw + not drain (47%), thatch grass + not drain (33%), thatch grass + drain (29%) and wood chips + not drain (4%) and wood chips + drain (3%). This study was not conforming with Pant *et al*. (2006), where the biological efficiency was found in the range of 33.1 to 238.6% for wheat straw and from 33.9 to 46.9% for sugarcane bagasse substrates in *P. sajor-caju* mushrooms.

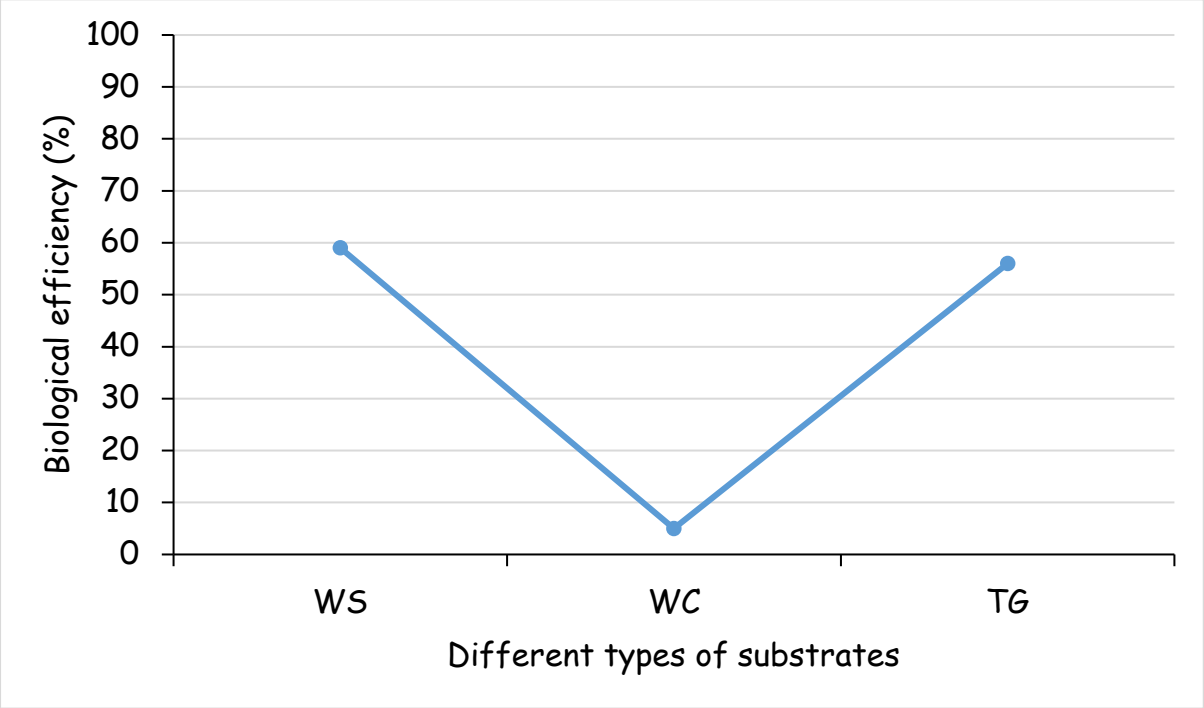


Figure 6.7. Biological efficiency (%) of wheat straw (WS), wood chips (WC) and thatch grass (TG) substrates for oyster mushroom production. The biological efficiency (%) was calculated using the formula from *Equation* 1

Although, the difference of growth and yield of *P. eryngii* and their quality may be due to the genotype of mushroom strains and the biological structure of the substrate (Moonmoon *et al*., 2010), but the production and biological efficiency were comparatively lower than other *Pleurotus* species cultivated in Bangladesh, due to low temperature. Liang *et al*. (2016) reported that the first flush, had the highest yield in a substrate of 45ZMS (Zea Mays), with 54.05% biological efficiency, which showed no significant difference with the yield of the

60PRS (*Panicum repens* stalk) (55.56%) and 45PPS (*Pennisetum purpureum* stalk) (52.53%) substrates.

6.4. Conclusions and recommendations

Wheat straw accelerated the rate of colonisation which helped to prevent contamination with parasitic fungi (*Trichoderma* spp.). However, the thatch grass which had been contaminated in the earlier stages recovered with the colonisation taking place at a later stage. This indicated that both the wheat straw and the thatch grass accelerated the rate of colonisation, but at different stages. Both the wheat straw and the thatch grass suppressed the rate of contamination due to the quick covering of mycelia in a planted bag. The wood chips exhibited a high rate of contamination by the parasitic fungi (*Trichoderma* spp.) due to the late mycelial covering of the planted bags. While the thatch grass had minimal contamination by the late mycelial covering, it is still advisable to be used as an alternative substrate if wheat straw is not available. Thatch grass was recommended because it contains three important nutrients (nitrogen, phosphorus and magnesium) required by oyster mushrooms, whereas wheat straw contains high quantities of only two of the nutrients. Both the wheat straw and thatch grass produced a high cumulative number of clusters, caps and fresh mass of oyster mushrooms. In general, it has been proven that thatch grass can be used as an alternative substrate to wheat straw because it produced a greater yield of oyster mushrooms. The set null hypothesis *(H_0)* could be rejected and accept the alternative hypothesis (H_1) as the alternative substrates have significantly affected the rate of colonisation, contamination and yield of oyster mushroom.

CHAPTER SEVEN

Improving the growth and yield of oyster mushroom (*Pleurotus ostreatus*) using 1-Naphthaleneacetic Acid

7.1. Introduction

Plant growth regulators (PGRs), either produced naturally by the plant or synthetically by a chemist, are small organic molecules that act inside the plant cells and alter the growth and development of plants (Kaur, 2016). PGRs can be broadly divided into two groups: plant growth promoters (auxins, gibberelins and cytokinins) and bio-inhibitors (ABA, methylijasmonate). Growth promoters are involved in cell division, cell enlargement, pattern formation, tropic growth, flowering, fruiting and seed formation (Kaur, 2016). Bio-inhibitors play an important role in plant responses to wounds and stresses of biotic and abiotic origin, and they are involved in various growth inhibiting activities such as dormancy and abscission. The use of plant growth regulators, such as gibberelins, cytokinins, auxins or their synthetic compounds, is becoming popular to ensure efficient production (Verma and Sen, 2008). There are many reports which indicate that application of PGRs enhanced plant growth and crop yield (Mostafa and Abou Al-Hamd, 2011). PGRs modify growth and development in various ways under normal growth conditions (Ud-deen, 2009).

Sprays of GA3 @ 150, 300 and 450 mg/L on the leaves of anthurium promoted an increase in the leaf area. But there was no increase in the number of inflorescences produced or their quality (Lima *et al*., 2014). Effect of auxin and gibberellic acid individually as well as in combination on growth and yield components of linseed (*Linum usitatissimum* L.) was investigated by Rastogi *et al*. (2013). They reported that a combined dose of auxin (1.0 mg/L) and gibberellic acid (200 mg/L) enhanced the seed yield, whereas a 0.5 mg/L dose of auxin enhanced the vegetative growth. GA3 at 10 ppm/packet was the best possible concentration for production of oyster mushroom as it showed a positive effect on number of effective fruiting body, stalk length, pileus diameter, biological yield, economic yield and dry economic yield (Sarkar and Chowdhury, 2013).

Growth regulators are organic substances besides nutrients, synthesized in plants, causing alteration in their cellular metabolism. Synthesis of some plant hormones is adversely affected by environmental factors, which causes restriction on physiological processes of the plant and ultimately, limits their growth potential (Copur *et al*., 2010). The application of these hormones in low concentration regulates growth, differentiation and development, either by promotion or inhibition (Naeem *et al*., 2004), and allows physiological processes to occur at their normal rate (Gulluoglu, 2004). Major plant growth regulators (PGRs) significantly enhanced fibre yield in cotton (Copur *et al*., 2010), protein content in pea (Bora and Sarma, 2006), chemical constituents in croton (Soad *et al*., 2010), fruit size in molina (Vwioko and Longe, 2009), seed germination rate in black gram and horse gram (Chauhan *et al*., 2009), floral buds in jojoba (Prat *et al*., 2008) and other growth parameters in different plants. Thus, to overcome the production constraints, chemical manipulation could be done to improve yield and growth parameters.

Among plant growth regulators, auxin and gibberellin play vital roles in regulating developmental processes within plant bodies (Gou *et al*., 2010). Auxin promotes cell elongation, especially of shoots, and induces apical dominance and rooting, while gibberellin helps in cell growth of stem, leaves and other aerial parts by causing cell elongation, and increase in intermodal length. A higher concentration of gibberellins increases plant growth (Bora and Sarma, 2006) while higher concentration of auxin inhibits it (Hussain *et al*., 2010). Thus, only low doses of auxin are effective in growth promotion (Vwioko and Longue, 2009). The different concentrations of GA had significant effect on growth in mustard (Akter *et al*., 2007). Farooqui *et al*. (2005) reported that indole acetic acid (IAA) application increases oil yield enormously in *Cymbopogan martini* and *Cymbopogan winterianus*. Faizanullah *et al*. (2010) reported that judicious application of growth hormone increases seed yield in linseed. Besides yield, the fibre strength and fineness is also improved by the application of auxin and gibberellin (Ayala-Silva *et al*., 2005). Mckenzie and Deyholos (2011) reported that treatment of GA causes stem elongation, expansion and proliferation and cell wall thickening in bust fibre of linseed. Similarly, growth regulators may also play an important role in the increased biomass production and yield potential of mushrooms. Some growth regulators at different concentrations affect the yield and size of mushrooms too.

Kumar *et al*. (2018) and Da Silva *et al*. (2017) reported that growth regulators such as indoleacetic acid (IAA), 1-naphthaleneacetic acid (1-NAA), gibberellic acid (GA) and kinetin can affect the size and yield of mushroom. In addition, the substrate on which the spawn is grown affects the mushroom production (Royse *et al*., 2004; Onyango *et al*., 2011; Ashraf *et al*., 2013). Plant growth regulators such as IAA, NAA, GA and KIN at different concentrations increased the biomass production of *Pleurotus sajor-caju* by 15-26% and also increased the protein content of the mycelia (Mukhopadhyay *et al*., 2005). This is the first study conducted in South Africa's conditions using 1-NAA to improve the agronomic practices of oyster mushrooms. Therefore, the objective of the study was to investigate the production practices that could encourage growth and yield of oyster mushroom using 1-naphthaleneacetic acid. The following hypotheses was consequently tested: (i) The null hypothesis (H_0) was that 1-NAA cannot improve rate of colonisation, suppress contamination nor increase yield of oyster mushrooms. (ii) The alternative hypothesis (H_1) was that 1-NAA can improve rate of colonisation, suppress contamination and increase yield of oyster mushrooms.

7.2. Materials and Methods

Location of the study area

Details of the study site are described in Chapter 3.

Growth chamber and micro-environmental conditions

The experiment was conducted in a custom-made growth chamber (hut-house, made of thatch grass) as depicted in Plate 4 1, Chapter 4. The floor was covered with river sand, which was sterilized using sodium hypochlorite. Temperature (°C) and relative humidity (%) (Figure 7.1) of the growth chamber was adjusted daily using a micro-jet sprayer with a flow rate of 17 mL every 5 min (at a pressure range of 60 to 70 kPa) and was measured using a HOBO data logger (U14 – 001 LCD Temperature/RH logger, United Kingdom). Vetayasuporn (2006) irrigated each culture medium block using tap water every morning and evening in order to control the temperature (°C) and relative humidity (%). The collected data for temperature (°C) and relative humidity (%) of the growth chamber (Plate 4.1, Chapter 4) during the study period (first and second cycle) are presented in Figures 7.1A and 7.1B.

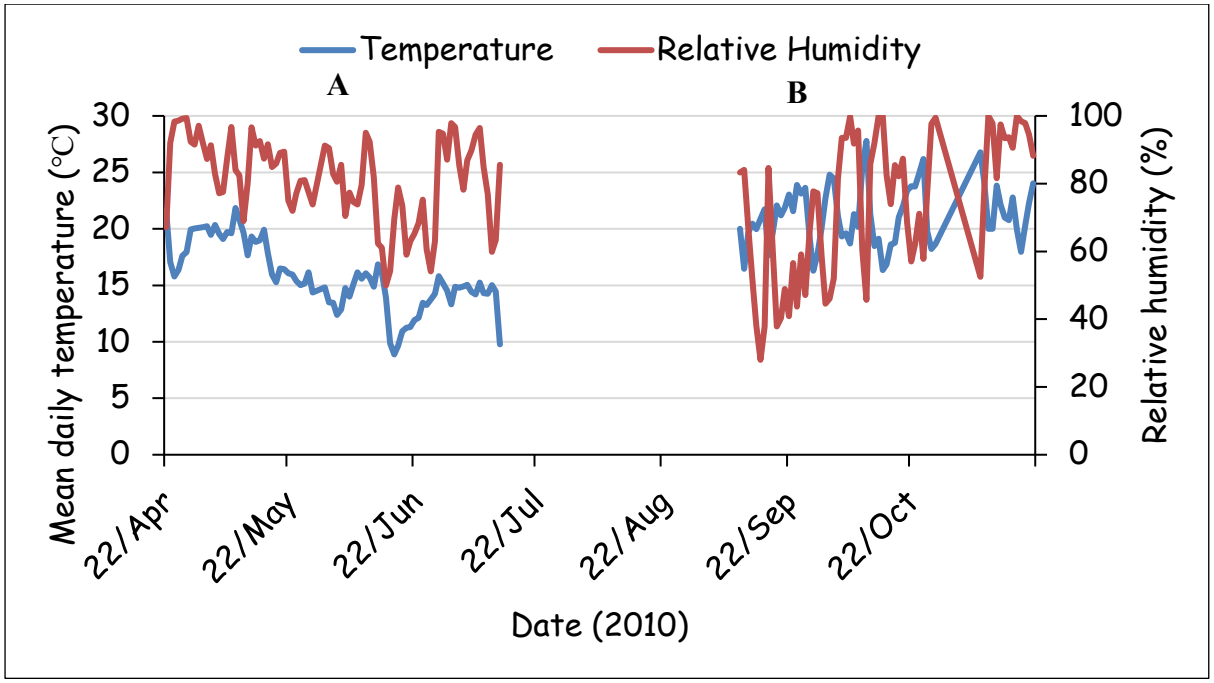


Figure 7.1. Mean temperature (°C) and relative humidity (%) during the (A) first Experiment (April to June 2010) and (B) second Experiment (September to November 2010) of the custom-made growth chamber as shown in Chapter 4 (Plate 4.1)

Experimental design, layout and treatment details

The experiment was laid out in a completely randomized design (CRD) with 6 treatments consisting of 6 levels of 1-naphthalene acetic acid (1-NAA, Lowveld Chemicals, White River, Mpumalanga Province, South Africa) (0; 0.1; 0.5; 1.0; 1.5 and 2.0 mmol), with each treatment replicated four times. For weighing the 1-NAA growth hormone, the measurements were transformed using the *Equation 1*. The solution was then sterilized using an autoclave (Hirayama MFG. CORP., Japan) at a temperature of 130°C and pressure of 0.26 MPa for an hour and allowed to cool down overnight. The following day, solutions of 100 ml in Schott bottles was applied to spawn of 1 kg in a 25 cm x 40 cm vacuum plastic bags (Lowveld Packaging, Nelspruit) and kept overnight.

$$n(mol) = \frac{m\ (g)}{mr(\frac{g}{mol})} \qquad Equation\ 1$$

Where, n(mol) = 1 mol, m(g) = required mass, mr = molar mass

Pasteurisation of the wheat straw substrate

The wheat straw substrate was pasteurised in order to eliminate the few fungi that could potentially compete with the oyster mushroom spawn. In addition, pasteurisation moistens the substrates and makes conditions more favourable for the growth of oyster mushrooms. The wheat straw substrate was sourced from Marble Hall (Limpopo Province, South Africa). In addition, the wheat straw was stored for three months to leach out compounds responsible for inhibiting mushroom growth. The substrate was pasteurised using a method outlined in Masevhe *et al*. (2016). Briefly, three wheat straw bales of about 10 kg each were placed in a 225 L steel drum containing water and boiled on a firewood for 1 hour at 100°C. After an hour of boiling, the wheat straw was removed from the drum and placed on a growing table to cool down and for the leachate to drain. The moisture content of 65% to 75% was determined using a RADWAG moisture analyser of balances and scales after pasteurisation of substrate (Mettler Toledo, Germany). The growing conditions were suitable to harvest mushrooms of good quality. The wheat straw was placed in industrial polyethylene bags (410 mm x 750 mm x 45 µm, width, length and breadth) together with spawn (1 kg, which was divided into equal parts and placed at the bottom, middle and top of the bag) to make up a total weight of 6.20 kg per treatment. During boiling, the temperature was monitored using a pocket thermometer (Checktemp 1, Hanna instrument - Mauritius). The planted bags were perforated at the bottom in order to drain excess leachate and to minimise contamination. The top of the polyethylene bags was sealed with a rubber band to prevent water from micro-jet sprinklers from entering the bags during irrigation. The oyster mushroom (*P. ostreatus*, tree species) spawn (4 kg per bag) was purchased from Exotic Spawn (Irene, Rietvlei, Gauteng Province, South Africa). The two similar trials were conducted during different seasons; the first experiment was conducted from cooler April to June months (2010) and the second experiment was from warmer September to November months (2010).

Data collection

Colonisation and contamination

The planted industrial plastic bags were evaluated for both colonisation and contamination in the custom-made growth chamber. The evaluation for colonisation began shortly after

inoculation until full colonisation, whereas contamination, using a rating scale of 0 to 100% depended on infection by mostly *Trichoderma* species. After full colonisation, the plastic bags were made small cross cut from top to bottom using surgical blades to facilitate the growth of mushroom clusters.

Harvesting

The oyster mushrooms were harvested three weeks after inoculation. They were pulled manually from the bags and kept in brown paper bags (SO 12), to avoid moisture loss, and taken to the laboratory for post-harvest analysis. Harvesting of oyster mushrooms was done depending on the maturity of the crop due to flushing differences as outlined in Masevhe *et al*. (2016).

Growth parameters

The growth parameters taken were cumulative number of harvests, number of clusters, number of caps, and fresh mass (g). For each harvesting, the mushroom mass was measured using Scaltec scale for each treatment and added together to make the cumulative fresh mass at the end of the experiment. The biological efficiency (BE%) of the oyster mushrooms was calculated from the cumulative fresh mass (g) using Equation 2.

$$\text{BE (\%)} = \frac{\text{Fresh mass (g) of oyster mushrooms}}{\text{Total wet mass of substrate used (g)}} \text{X } 100 \qquad \text{Eq. [2]}$$

Statistical analysis

Statistical evaluation of data as done for Chapters 3; 4; 5 and 6.

7.3. Results and discussion

7.3.1. Rate of oyster mushroom mycelia colonisation

In the cooler months of April to June (Experiment 1), there was a sharp increase in the colonisation percentage (60%) between Day 0 and Day 1 for all the treatments (Figure 7.2). However, differences among the treatments were not significant. There was a steady increase

in the colonisation percentage between Day 1 and Day 9 in all the treatments. From Day 10 to Day 15, the colonisation percentage reached carrying capacity of 100%. The colonisation of mushrooms was enhanced with the addition of 1-NAA from 3.0 to 10.0 mg/l^{-1} (Guo *et al.*, 2009). The highest mycelial diameter reached was 9.0 ±1.0 mm with the addition of 5 mg/l^{-1} 1-NAA and the growth rate of the mycelia increased constantly from 48 to 96 h at the 1-NAA level of 5 mg/l^{-1} (Guo *et al.*, 2009).

In the warmer months of September to November (Experiment 2), there was a constant increase in the colonisation, reaching 99% in Day 8 with 1 mmol 1-NAA (Figure 7.2). The 1 mmol 1-NAA resulted in the highest colonisation percentage of all the treatments from Day 2 to Day 9 whereas 1.5 mmol 1-NAA yielded the lowest colonisation percentage of all the treatments from Day 4 to Day 9 (Figure 7.2). The production of the mycelial biomass with addition of 5.0 mg/l^{-1} 1-NAA in a shake flask was 6.24 ± 0.18 g/l^{-1} at 168 h and 0.86 ± 0.01 g/l^{-1} at 192 h (Guo *et al.*, 2009), which was enhanced by 15.98% and 56.36% compared with the control.

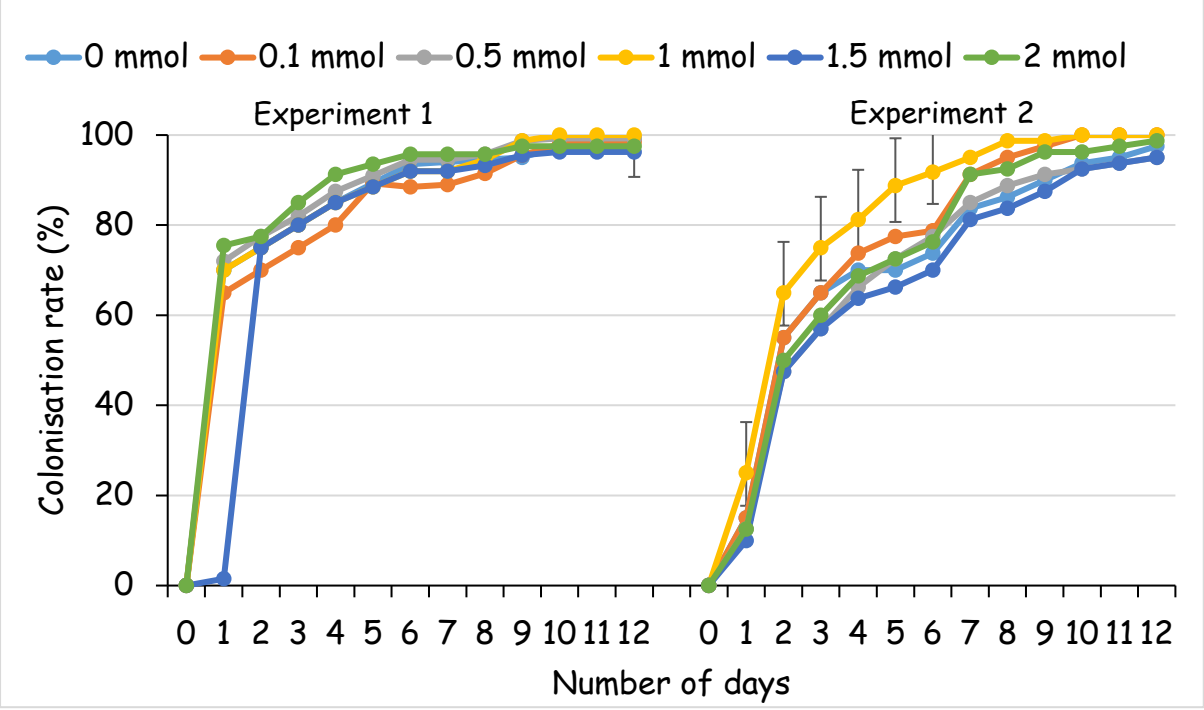


Figure 7.2. Colonisation rate of oyster mushroom spawn from Day 0 to Day 12 conducted in April – June months and September – November months. There were no significant differences between treatments with experiment 1 but significant differences were seen with experiment 2. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

During the cooler months of April to June, the temperatures dropped to lower than optimal for the growth of mushrooms (21°C to 10°C, average 75% RH) and there was no significant difference in the colonisation percentages compared with those of the September to November trial (Figure 7.1 and Figure 7.2). For proper colonisation of oyster mushrooms to occur, spawn running requires temperatures between 20°C – 22°C and a relative humidity between 75 % – 85% in a growing chamber (Kwon and Kim, 2004). The results in Figure 7.2, therefore, suggest that 1-NAA applied at a rate of 1 mmol during the cooler months of April to June increases the rate of colonisation, whereas in warmer September to November months, there was need for application of plant growth hormones as the treatments shows statistical differences on the early days.

Table 7.1. *p*-values and standard error means for rate of colonisation (%) from 1-NAA

Days	**April – June months**		**September – November months**	
	p-values	Standard error means	*p*-values	Standard error means
Day 1	0.3829	0.2173	<.001	0.2182
Day 2	0.3829	0.2173	<.001	0.2329
Day 3	0.3691	0.2169	<.001	0.2329
Day 4	0.3656	0.2163	0.0001	0.2238
Day 5	0.7412	0.2157	0.0020	0.2356
Day 6	0.3278	0.2176	0.0461	0.2356
Day 7	0.2868	0.2184	0.3910	0.2329
Day 8	0.3780	0.2178	0.1739	0.2341
Day 9	0.5589	0.2201	0.1550	0.2399
Day 10	0.7700	0.2396	0.2294	0.2353
Day 11	0.7174	0.2402	0.3469	0.2429
Day 12	0.0585	0.2489	0.3745	0.2461

7.3.2. Contamination of oyster mushroom mycelia

Factors that causes contamination are contaminated water, improper sanitation and growing media, all of which can affect the production of oyster mushrooms. Water has a great impact on fungal contamination as it is used to irrigate oyster mushrooms (Fresh Champignons, 2010). In this trial, there was no significant difference between the treatments in terms of contamination, which indicates that the trials were conducted under sterile conditions (Figure 7.3). Contamination of oyster mushrooms can be controlled if planted in a sterile environment. Cleanliness is one of major requirements in the production or growing of oyster mushrooms (Fresh Champignons, 2010). Hygiene pertaining to the packaging materials can also play a major role the prevention of contamination. This indicates that when growing oyster mushrooms there is a possibility of contamination depending on the materials used and whether they are properly sterilised or not. During the cooler months of April to June, there was a tendency towards increased contamination with both 0 mmol and 2 mmol 1-NAA compared to the warmer months of September to November (Figure 7.3). Competative fungi (*Trichoderma harzianum*) are able to reduce mushroom quality and productivity by 80% (Savoie *et al*., 2001). In cases of high levels of contamination, it may even cause a total inhibition of mushroom growth. In addition, contamination can compete successfully for space and nutrients (Shrestha *et al*., 2021), which can result in discarding the whole batch of oyster mushrooms.

Table 7.2. *p*-values and standard error means for rate of contamination (%) from 1-NAA

Weeks	**April – June months**		**September – November months**	
	p-values	Standard error means	*p*-values	Standard error means
Week 1	0.6345	0.1629	0.6345	0.1629
Week 2	0.0492	0.3764	0.0492	0.3764
Week 3	0.0742	0.3548	0.0742	0.3548
Week 4	0.0635	0.3858	0.0838	0.3821

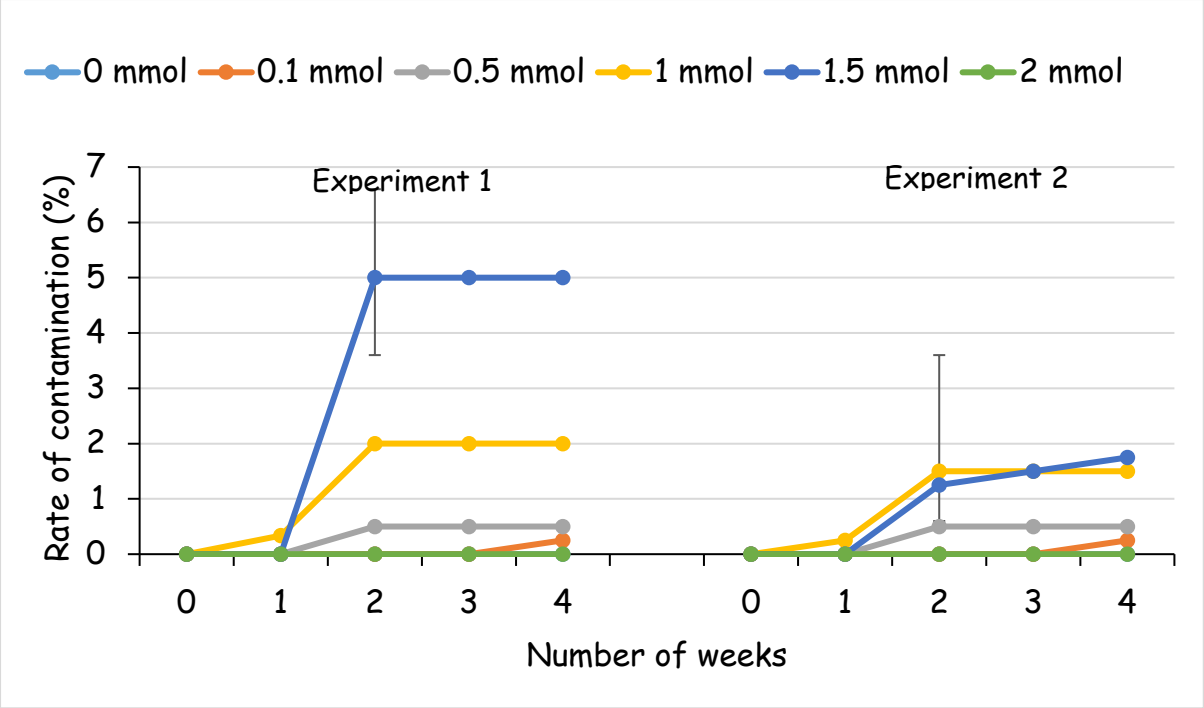


Figure 7.3. Contamination rate of mycelia in the oyster mushroom cultures from Week 1 to Week 4 in April – June and September – November months. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

7.3.3. Harvesting of oyster mushroom crops

The first clusters were evident after 23 days, depending on the season. The results are not consistent with the findings of Vogel and Salmones (2000) who observed pinhead formation before an incubation period of 23 days in commercial cultures of *P. ostreatus* at 19ºC. However, Philippoussis *et al*. (2001) and Obodai *et al*. (2003) observed pinhead formation after incubation periods of 34 days when using wheat and rice straw as substrates. This variation could be due to seasonal differences, temperature and relative humidity, meaning that farmers must be cognisant of the required conditions in their particular area. There was no significant difference regarding the cumulative number of clusters for all treatments in Experiment 1 (Table 7.3). Alam *et al*. (2008) found that the use of higher concentrations of 1-NAA and indole 3-butyric acid (IBA) showed no significant effect on cell division and enlargement, as it failed to increase the number of fruiting bodies, pileus diameter, pileus girth and stalk length in mushrooms. In contrast to Atri *et al*. (2013) had recorded that the 5 ppm concentration of gibberellic acid give maximum mycelial growth of 16.05 mg/ml followed by NAA (15.25 mg/ml) where the least mycelial growth was supported by IBA (11.75 mg/ml). However,

application of gibberellic at 20 ppm had maximum mycelial growth of 17.55 mg/ml, followed by NAA (16.20 mg/ml) where the least mycelial growth was supported by IBA (12.45 mg/ml).

There was a significant difference ($p < 0.05$) regarding the cumulative number of caps for all the treatments (Table 7.3). The treatment with 1 mmol 1-NAA produced the highest cumulative number of caps (196) compared to all the other treatments (0.1 mmol – 112; 1.5 mmol – 114; 0.0 mmol – 115; 2.0 mmol – 144 and 0.5 mmol – 156). There was a significant difference ($p < 0.05$) with fresh mass (g) in all the treatments. The 1 mmol 1-NAA treatment yielded a high fresh mass of 2005 g compared with all the other treatments in Experiment 1, while the 0.1 mmol 1-NAA treatment yielded the lowest fresh mass of 1 181 g compared with all the other treatments. There was no significant difference in the cumulative number of clusters for all the other treatments in Experiment 2, and both the cumulative number of caps and cumulative fresh mass did not differ significantly, but there was a different case in Experiment 1 (Table 7.3). Notable findings were that the data values for the cumulative number of clusters, cumulative number of caps and cumulative fresh mass in Experiment 1 were lower than those of Experiment 2. These could be due to seasonal differences, as the months of April to June during Experiment 1 were cooler compared to Experiment 2 which was run during the warmer months of September to November. Islam *et al*. (2009) suggested that the timing and concentration the application of hormones might be of critical importance regarding the reactions towards mushroom production.

Table 7.3. Mean and total number of clusters, number of caps and final mass (g) for oyster mushrooms treated with different concentrations of 1-NAA for Experiment I (April – June months) and Experiment II (September – November months)

Treatment	**Experiment**						**Total**		
1-NAA (mmol)	**I**			**II**					
	Number of clusters[y]	Number of caps	Fresh mass (g)	Number of clusters	Number of caps	Fresh mass (g)	Number of clusters	Number of caps	Fresh mass (g)
0.0	13.25[a]	112.50[ab]	1181.15[b]	26.75[a]	252.00[a]	2106.08[a]	45.50	371.75	3215.58
0.1	13.50[a]	114.00[b]	1432.58[b]	27.00[a]	259.75[a]	2106.08[a]	45.58	371.75	3287.66
0.5	19.25[a]	156.00[ab]	1440.60[b]	27.33[a]	252.33[a]	2120.60[a]	46.58	408.33	3561.20
1.0	22.50[a]	196.00[a]	2005.00[a]	37.00[a]	307.67[c]	2783.43[a]	50.50	448.00	4314.93
1.5	16.50[a]	114.00[ab]	1251.00[b]	31.00[a]	303.00[c]	2466.23[a]	47.50	417.00	3717.20
2.0	18.25[a]	144.00[ab]	1411.15[b]	32.33[a]	286.00[b]	2335.67[a]	49.25	430.00	4215.58
LSD($\alpha < 0.05$)	11.701	82.46	554.37	28.007	90.686	957.88			
p-value	0.6366	0.5076	0.1563	0.4929	0.0041	0.1648			
Se	0.2198	0.5076	0.1563	0.2925	0.2780	0.2529			
CV%	45.11592	39.23161	25.30481	52.93517	19.18680	23.80061			
R^2	0.290303	0.336005	0.489268	0.418224	0.813254	0.577017			

[y]Within the same column, means followed by the same letters are not statistical different at $p < 0.05$

7.3.4. Harvesting periods of oyster mushrooms

The 1 mmol 1-NAA yielded the highest number of harvests with 13 and 14 for April – June months and September – November months, respectively (Figure 7.4). This is similar to Masevhe *et al*. (2016) who did able to harvest six times with the use of different substrates to 0 mmol in autumn months. The overall results showed that the 1-NAA plays a role in increasing the number of harvests ranging from 8 to 14 times during winter months and autumn months (Figure 7.4). The 0.0 mmol 1-NAA was consistent in giving the lowest number of harvests corresponding to the observations from evaluation of number of clusters, caps and fresh mass (g).

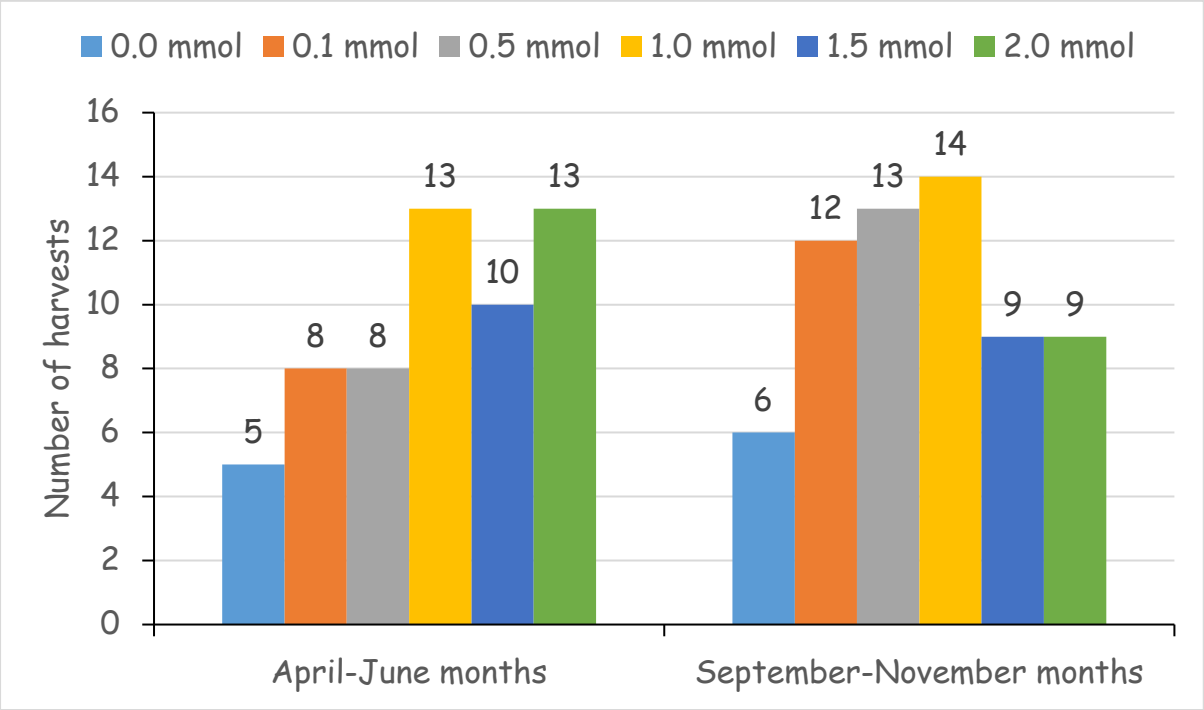


Figure 7.4. Number of harvests from April – June months (Experiment 1) and September – November months (Experiment 2)

7.3.5. Biological efficiency (%) of oyster mushrooms

There were differences in the fresh mass (g) between the cooler months of the April to June trials (Experiment 1) and the warmer months of the September to November trials (Experiment 2); however, the differences were not significant regarding the biological efficiency (%)

(Figure 7.5). Pani (2011) indicated that an increase in the sporophore yield, although statistically insignificant, were recorded with kinetin 10 ppm (76% BE), IAA 25 ppm (73.1% BE) and 1-NAA 25 ppm (71.6% BE). During the cooler months of April to June, the treatment with 1 mmol 1-NAA yielded a high biological efficiency (33%) which differed from all the other treatments. In both the trials during the cooler months of April to June and the warmer months of September to November, the treatment with 1 mmol 1-NAA was not significantly different from all the other treatments. Alam *et al*. (2008) indicate that the use of a high concentration of 1-NAA has no significant effect on economic yield of mushrooms. This study confirms that the use of optimum treatments (2 mmol) did not have any significant effect on the biological efficiency (%) of oyster mushrooms, whereas the use of 1 mmol 1-NAA had a significant impact on the biological efficiency of oyster mushrooms.

A high concentration of 1-NAA (50 ppm) was toxic, while lower concentrations between 10-25 ppm stimulated the biomass production of oyster mushrooms (Pani, 2011). In the other hand, the biological efficiency obtained from application of 20 ppm and 30 ppm gibberellic acid were similar to that of 10 ppm concentration of gibberellic acid (Sarker and Chowdhury, 2013). In the warmer September – November trial, 1-NAA had no impact on the biological efficiency (%) because there were no differences in the cumulative number of clusters, cumulative number of caps and cumulative fresh mass between the treatments. This implies that during the warmer September – November period, the application of 1-NAA will be of no benefit in terms of improving the yield of oyster mushrooms. In the cooler April – June trial, the application of 1 mmol 1-NAA improved the biological efficiency (%) of oyster mushrooms. A higher biological efficiency ranging from 34% to 45% was recorded for all the treatments during the warmer September – November period compared to the cooler April – June period, which could be related to the temperatures and even the freshness of the wheat straw. However, the use of 1 mmol 1-NAA exerted a significant effect only during the cooler months of April to June. This treatment could thus be useful for improving oyster mushroom production under sub-optimal conditions (Figure 7.5).

According to Bellettini *et al*. (2016), in basidiomycete fungi, lignocellulolytic enzymes are affected by many typical fermentation factors such as medium composition, ratio of carbon to nitrogen, pH, temperature, air composition, levels of naa, etc. The survival and multiplication of mushrooms is related to a number of factors which may act separately or have interactive effects among them. Out of that understanding challenges in handling *Pleurotus* spp.

mushroom requires a fundamental understanding of their physical, chemical, biological and enzymatic properties (Bellettini *et al*., 2016).

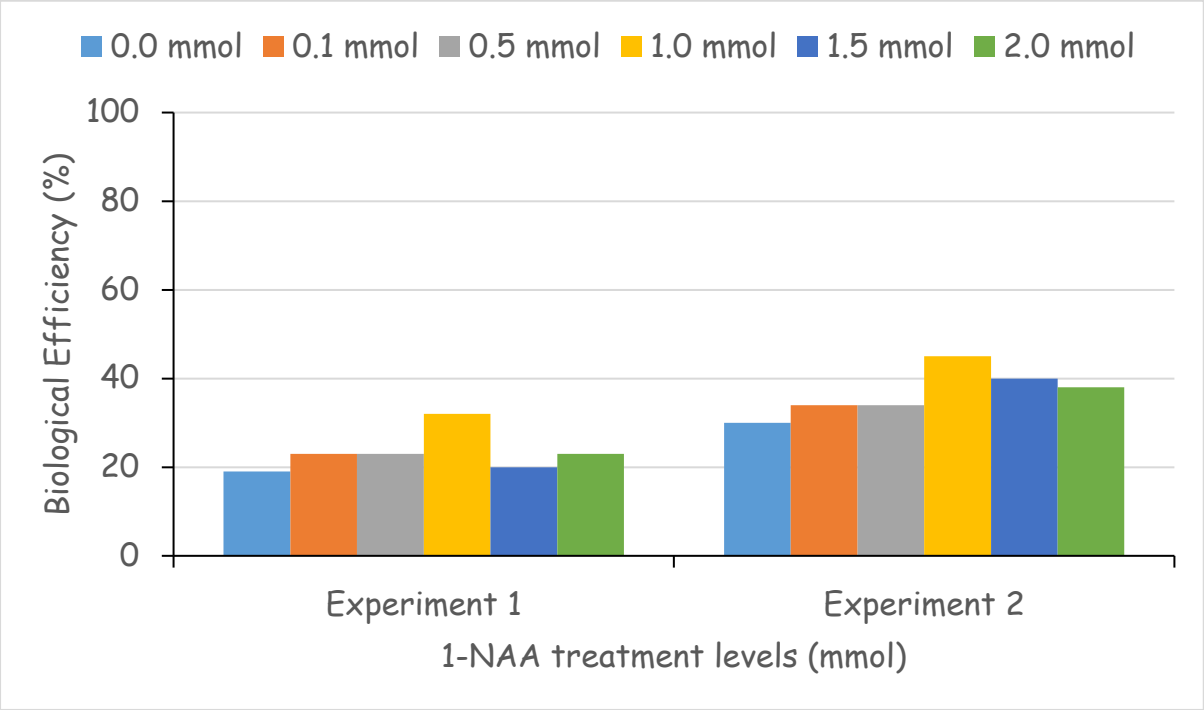


Figure 7.5. Biological efficiency (%) of oyster mushrooms as affected by 1-NAA from Experiment 1 (April – June months) and Experiment 2 (September – November months)

7.4. Conclusions and recommendations

Colonisation differences were not evident during the cooler months of April to June, but there were highly significant differences during the warmer months of September to November. Both the 0 mmol 1-NAA and 2 mmol 1-NAA suppressed the contamination of the oyster mushroom mycelia in both seasons (April – June and September – November). The application of 1 mmol 1-NAA produced the highest fresh mass (g) that influenced the significant biological efficiency (%) of oyster mushrooms. In general, this study concluded that the application of 1 mmol 1-NAA during the cooler months of April to June accelerated the growth and increased the yield and biological efficiency of oyster mushrooms, while during the warmer months of September to November, there was need for the application of this chemical as it would make significant difference.

From the results it is evident that 1-NAA does affect colonisation rate, suppress contamination, and increase yield of oyster mushrooms. This is confirmed by results displayed from Tables 7.1 which is for April –June and September – November months and covering all weeks, where the *p*-value was higher than α which means that the null hypothesis *(H_0)* tested was that 1-NAA cannot improve rate of colonisation, suppress contamination nor increase yield of oyster mushrooms can be rejected. Results for contamination as given in Tables 7.2 for both April-June and September-November covering Week 1 to Week 4, they all had *p*-values greater than α except for Week 2 indicating that the null hypothesis *(H_0)* is rejected and accept the alternative hypothesis (H_1) as number of clusters, caps, and fresh mass (g) were all affected by application of 1-NAA. This is applicable for both April –June and September –November periods. The *p*-values thereof in all cases were higher than α.

CHAPTER EIGHT

Effect of seasonal and different storage temperatures on the shelf-life of oyster mushrooms (*Pleurotus ostreatus*)

8.1. Introduction

FAO 2004 recommended edible mushrooms as a food source to meet protein requirements of developing countries, the majority of which depends mainly on cereals (World Bank, 2004). A mushroom comprises of the visible reproductive structure – in the form of a fruiting body of a living organism which develops seasonally to produce and disperse spores (Moore *et al*., 2008). Their fruiting bodies are shell or spatula shaped with different colours (white, cream, pink, grey, yellow, light brown) (Hasan *et al*., 2008). All mushrooms belong to a group of fungi, a group very distinct from plants, animals and bacteria (Oei, 2003). Most fungi have plant-like cells but lack the most important feature of plants, which is, the ability to use energy from the sun directly through the use of chlorophyll and the process of photosynthesis, to produce their own food. *Pleurotus ostreatus* has the potential to inhibit cancerous tumors, improve liver and kidney function, reduce high blood pressure, and help with gastrointestinal disorders (Stamets, 2000).

An Oyster Mushroom Farming project was established in 2002 by the Agricultural Research Council (Pakela-Jezile, 2006). The initiative was part of the South African Government's national intervention through poverty relief and the establishment of enterprises in the agricultural sector where farmers can trade and contribute to the country's mainstream economy. The initial objectives of the project were to educate the public about mushrooms, cultivate the culture of mushroom consumption and to introduce oyster mushrooms as an alternative, readily available protein in agricultural communities in southern Africa (Pakela-Jezile, 2006). Due to lack of captured information regarding oyster mushroom production in southern Africa as a whole, the Agricultural Research Council was engaged in initiating community projects around the country. Therefore, three provinces were targeted in South Africa, viz. Mpumalanga, Kwa-Zulu Natal and Gauteng where oyster mushroom projects are running with the aid of the Department of Agriculture, Forestry and Fisheries, together with the Provincial Gauteng Department of Agriculture.

The oyster mushroom is scarce in the South African markets compared with the common button mushroom. This makes the oyster mushroom commodity to be expensive to purchase in the market as it is regarded as luxury food item and most perishable. It is known that in South Africa, the button mushrooms can be sold for R25.00 (US $3.1576) per kg, whereas the oyster mushrooms are sold for R40.00 (US $5.0522) per kg in the local retail markets. Mushrooms are rich in protein containing all the nine essential amino acids required by humans and are relatively good sources of nutrients such as phosphorus, iron and vitamins, including, thiamine, riboflavin, ascorbic acid, ergo sterol and niacin (Barros *et al*., 2008).

Storage temperature is one of the main factors that affect post-ripening and qualities such as respiration, transpiration, senescence and other physiological actions (Singh *et al*., 2010) which results in spoilage of the crop. Previous studies indicated that the oyster mushroom can only last for 5 to 7 days in 1 – 2°C storage temperature before the quality deteriorates (Hill, 2004). It is also regarded as a highly perishable mushroom with a normal shelf-life of 1 to 3 days at ambient temperature during marketing (Xiao and Zhang, 2003; Tolera and Abera, 2017). Thus, oyster mushrooms are known not to last long in storage whether in ambient temperatures or in controlled cold room temperatures. This is the first study conducted in South African's conditions in order to maintain good quality of oyster mushrooms and extend their shelf-life using covered and uncovered containers (FT11 – polyethylene container + Econ – plastic wrapper). Therefore, the objective of the study was to examine suitable storage temperatures with plastic film that can improve the shelf-life and maintain good quality of harvested oyster mushrooms. The following hypotheses was consequently tested: (i) The null hypothesis (H_0): Mushrooms can be stored for longer if they are not covered by a plastic film and not kept at 2 - 5°C. (ii) The alternative hypothesis (H_1): Mushrooms can be stored for longer if they are covered by a plastic film and kept at 2 - 5°C.

8.2. Materials and methods

Location and climate of the study area

Details of the study site are described in Chapter 3.

Experimental design and layout

The study was laid out in a randomised complete block design (RCBD) with 6 treatments consisting of a factorial experiment with 2 types of containers and 3 different types of storage temperature, replicated seven times. The two types of containers were foamlite (FT11) container with plastic cover (Econo) and foamlite (FT11) container without plastic cover, while the three storage temperatures were ambient temperature (control), 2ºC and 5ºC with relative humidity maintained at 70%. Two similar trials were conducted during different seasons, with the first experiment conducted from cooler May to July months (2010) and the second experiment from warmer October to November (2010).

Treatments

In order to determine post-harvest storability, 50 g of oyster mushrooms from each treatment were packed in mushroom packaging (FT11) containers, with or without polyethylene covers, with more than 65 caps of oyster mushrooms.

Data collection and analysis

During storage, the quality, fresh mass (g), physiological disorders and colour of the oyster mushrooms were assessed and recorded using the method outlined by Shewfelt (2014). The stored oyster mushrooms were evaluated every 4 days whilst in storage (ambient temperature – evaluated twice; 2°C or 5°C – evaluated four times over a period of 16 days). During each evaluation, photographs were taken per storage date in order to compare the treatments (Plate 8.1). According to Singh *et al*. (2010), indicators for determining the quality of mushrooms are: whiteness, cap development, weight loss and microbial deterioration; therefore, these quality parameters were measured. Statistical evaluation of data as done for Chapters 3; 4; 5, 6 and 7.

Table 8.1. Evaluation method for stored oyster mushroom (After Shewfelt, 2014)

Quality rating	***Physiological disorder***	***Colour rating***
1 – good (without blemishes)	0 – no disorder	1 – light cream
2 – moderate	1 – whitish growth	2 - cream
3 – poor		3 – brown
4 – severe		4 – dark brown
5 – worse (spoiled)		

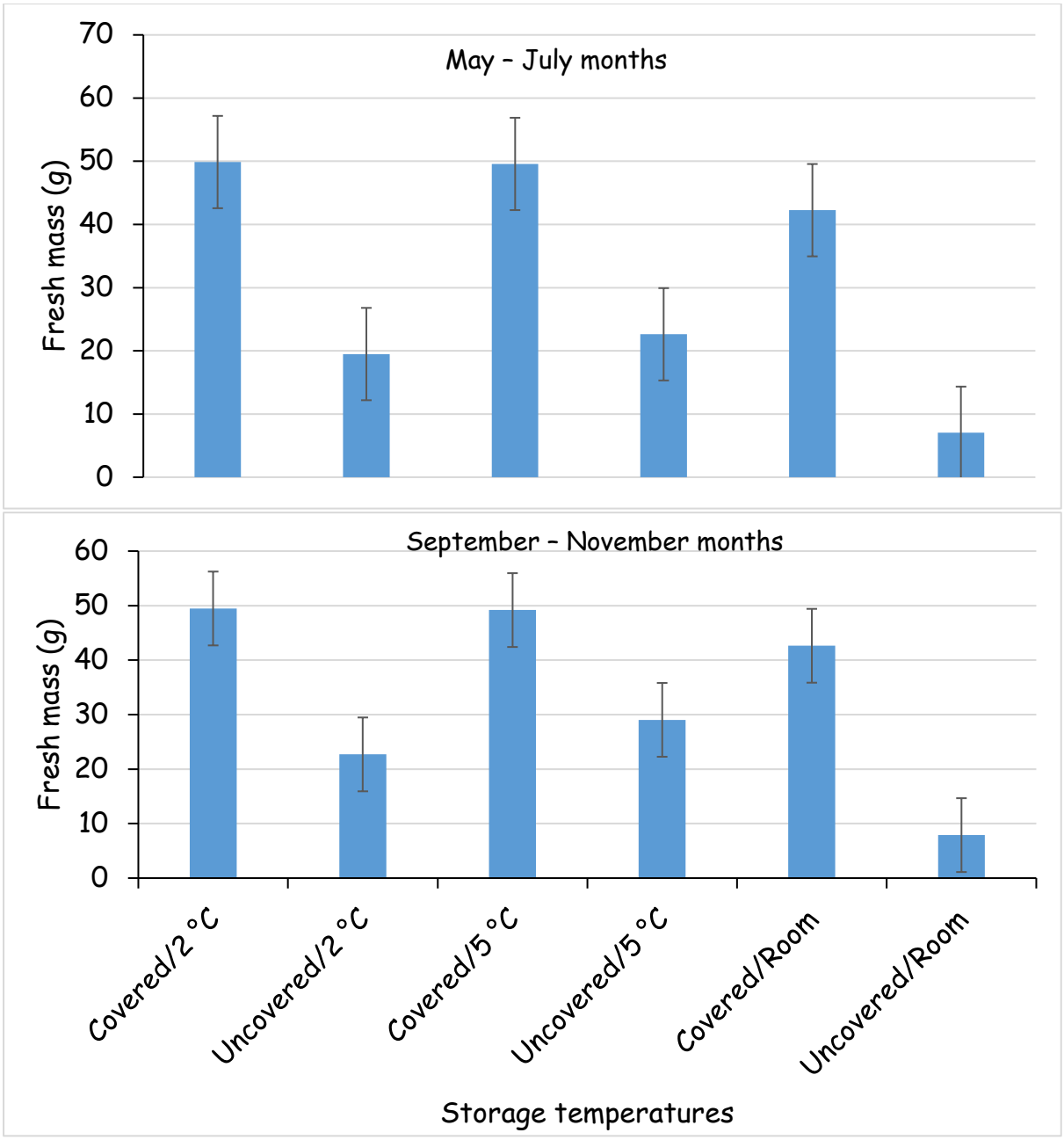
8.3. Results and discussion

8.3.1. Fresh mass (g) of oyster mushrooms during storage for the May – July and October – November trial

During the cooler months of May to July (Experiment 1) and the warmer months of October to November (Experiment 2), there was a significant difference ($p < 0.05$) in the fresh mass of oyster mushrooms stored at ambient temperatures with low relative humidity (RH%) in covered (42 g) and uncovered containers (7 g), which could mainly be attributed to high moisture loss in the uncovered containers (Figure 8.1). When the harvested product looses 5% – 10% of its fresh mass, it begins to shrink and soon becomes no good for human consumption. Singh *et al*. (2010) indicated that the mushroom is one of the most perishable products and tends to loose quality immediately after harvest (1 – 3 days) at ambient or room temperature. Mushroom is extremely perishable as it contains about 90% (wb) moisture content (Kumar *et al*., 2013), some have a life span of less than a day and others may survive one week, while a group of tougher mushrooms may last for months but those with tough wood texture. This study implies that mushrooms in an ambient temperature cannot last for a long period because they tend to lose their quality quickly and farmers are advised to use refrigeration in all the seasons for their produce in order to increase the shelf-life. During the cooler months of May to July and the warmer months of October to November, there was also a significant difference in the mean fresh mass (g) between covered or uncovered containers of oyster mushrooms at both 2°C [70% relative humidity (RH)] and 5°C [70% relative humidity (RH)] (Figure 8.1).

Oyster mushrooms in covered containers maintained a mean mass of 50 g compared to those in uncovered containers with a mean fresh mass of 20 g (Figure 8.1). Castellanos-Reyes *et al*. (2021) found that mass loss from mushrooms stored in open punnets at either 5°C [73% relative humidity (RH)] or 18°C [90% relative humidity (RH)] were linear averaging 4% per day at 5°C and 6% per day at 18°C. Either 2°C or 5°C (70% RH) storage temperatures were able to maintain the good quality of oyster mushrooms during 4 evaluation periods with a short space of four days between each period. In both 2°C and 5°C (70% RH), the good quality of oyster mushrooms was maintained for approximately a month before they were found to be rotten. A notable finding is that oyster mushrooms stored at 2°C displayed chilling injury compared with oyster mushrooms stored at 5°C which did not show any signs of chilling injury (Figure 8.1).

This finding is in contrast to that of Zhu *et al*. (2006) who showed that the freezing point of *Agaricus* mushrooms was -0.9°C because of the delicate structure lacking a protective coat. A storage temperature below 0°C will cause chilling injury. Deterioration in quality of mushroom leads to reduction in consumer acceptability and market price (Kumar *et al*., 2013). The basic findings of this study indicate that storing oyster mushrooms in uncovered conditions results in excessive moisture loss, which in turn reduces the quality of the crop.



Figures 8.1. Fresh mass (g) of oyster mushrooms with storage during May to July and September to November months. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

Table 8.2. *p*-values and standard error means for fresh mass (g) of oyster mushrooms from different storage temperatures in May – July months

	May – July trial					
Evaluations	2°C		5°C		Room Temp.	
	p-values	Standard error means	*p*-values	Standard error means	*p*-values	Standard error means
Fresh mass (g)	<.001	0.0492	<.001	0.0505	<.001	0.0648

Table 8.3. *p*-values and standard error means for fresh mass (g) of oyster mushrooms from different storage temperatures in October – November months

	October - November trial					
Evaluations	2°C		5°C		Room Temp.	
	p-values	Standard error means	*p*-values	Standard error means	*p*-values	Standard error means
Fresh mass (g)	<.001	0.04623	<.001	0.04629	<.001	0.06639

8.3.2. Evaluation of oyster mushrooms during storage for the May – July and October – November trial

The quality of the oyster mushrooms looked good at the first evaluation and after being stored at ambient temperature, had deteriorated at the time of the second evaluation (Plate 8.1). Villaescusa and Gil (2003) found that the shelf-life of the fresh mushrooms is limited to 1-3 days at ambient temperature and to 4-7 days at 4°C. The rate of postharvest deterioration of fresh mushrooms has been directly related to initial microbial load. According to López-Gómez *et al*. (2021), when storing mushrooms at 13 °C, bacterial from initial load of 7 log cfu g^{-1} increased to almost 11 log cfu g^{-1} over a 10 day period.

During the cooler months of May to July, the quality oyster mushrooms in covered containers was significantly different ($p < 0.05$) from those packaged during the warmer months of October to November. During the cooler months of May to July, there were highly significant differences ($p < 0.05$) between the oyster mushrooms in covered containers and the oyster mushrooms in uncovered containers for all storage temperatures. The oyster mushrooms stored at ambient temperatures behave differently, as expected, where the oyster mushrooms stored in covered containers or in uncovered containers displayed the lowest number of good quality (23% or 0%) mushrooms and the lowest number with a light cream colour (27% or 1%) with no physiological disorders (Tables 8.5, Table 8.7 & Table 8.8) because the stored materials dry out. This simply means that the stored ambient (room temp.) oyster mushrooms in covered or uncovered containers had the highest number of poor quality oyster mushrooms as well as the highest number with a dark brown colour. Producers should be advised not to store oyster mushrooms in ambient temperatures because they are highly perishable.

Table 8.4. *p*-values and standard error means for evaluation of oyster mushrooms from different storage temperatures in May – July months

May - July trial						
Evaluations	2°C		5°C		Room Temp. (±25°C)	
	p-values	Standard error means	*p*-values	Standard error means	*p*-values	Standard error means
Quality rating	<.001	-	<.001	-	0.0032	-
Disorder rating	0.0887	-	0.7372	-	0.0097	-
Colour rating	<.001	-	<.001	-	0.0001	-

Table 8.5. Quality class rating in room temperature (±25°C), 2°C and 5°C for Experiment 1 (19 May to 30 July 2010) and Experiment 2 (06 October to 30 November 2010) ($p < 0.001$)

Treatment (°C)	**Rating[a]**	**Experiment**			
		1		2	
		Quality class rankings (%)			
		Covered	Uncovered	Covered	Uncovered
Room	1	23.68	0.69	0^{ns}	0^{ns}
2°C		91.61	39.35	64.9	26.13
5°C		89.07	24.3	50.33^{ns}	28.33^{ns}
Room	2	12.57	1.34	0^{ns}	10.75^{ns}
2°C		6.50	32.22	21.27	27.03
5°C		9.55	39.73	25.67^{ns}	24.9^{ns}
Room	3	28.93	24.6	26.88^{ns}	9.08^{ns}
2°C		0.89	41.9	11.40	35.45
5°C		1.42	32.94	18.79^{ns}	31.15^{ns}
Room	4	22.3	41.4	14.49^{ns}	32.17^{ns}
2°C		0	3.2	2.17	10.91
5°C		0	3.02	5.23^{ns}	13.86^{ns}
Room	5	12.54	32.3	47.92^{ns}	58.43^{ns}
2°C		0	0.38	0.27	0.52
5°C		0	0	0^{ns}	1.68^{ns}
($p < 0.005$)		***	***	***	***

[a]Rating as described in Table 8.1

NS = not significant difference

*** ($p < 0.001$)

Table 8.6. *p*-values and standard error means for evaluation of oyster mushrooms from different storage temperatures in October – November months

October - November trial						
Evaluations	2°C		5°C		Room Temp. (±25°C)	
	p-values	Standard error means	*p*-values	Standard error means	*p*-values	Standard error means
Quality rating	<.001	-	0.0027	-	0.4740	-
Disorder rating	0.0615	-	0.2698	-	0.4244	-
Colour rating	<.001	-	0.0011	-	0.0803	-

Table 8.7. Physiological disorders class rating in room temperature (±25°C), 2°C and 5°C for Experiment 1 (19 May to 30 July 2010) and Experiment 2 (06 October to 30 November 2010) ($p < 0.001$)

Treatment (°C)	**Rating[a]**	**Experiment**			
		1		2	
		Physiological disorder (%)			
		Covered	Uncovered	Covered	Uncovered
Room	0	98.03	100	0.52	18.88
2°C		84.14	93.29	72.53	80.75
5°C		94.13	81.19	52.32	74.72
Room	1	1.96	0	99.48	81.12
2°C		15.86	6.72	27.47	19.25
5°C		17.86	18.82	47.69	38.12
($p < 0.005$)		NS	NS	NS	NS

[a]Rating as described in Table 8.1

NS (not significant difference)

Table 8.8. Colour class rating in room temperature (±25°C), 2°C and 5°C for Experiment 1 (19 May to 30 July 2010) - and Experiment 2 (06 October to 30 November 2010) ($p < 0.001$)

Treatment (°C)	**Rating[a]**	**Experiment**			
		1		2	
		Colour class rankings (%)			
		Covered	Uncovered	Covered	Uncovered
Room	1	27.07	1.39	0^{ns}	0^{ns}
2°C		92.6	22.82	58.43	58.43
5°C		89.25	23.73	2.17	10.91
Room	2	13.88	0.69	0^{ns}	10.75^{ns}
2°C		6.12	33.13	26.13	26.13
5°C		9.33	40.32	0.27	0.52
Room	3	28.07	25.13	26.88^{ns}	9.08^{ns}
2°C		0.88	37.85	27.03	27.03
5°C		1.42	31.43	5.33	28.33
Room	4	31.00	72.78	14.49^{ns}	32.17^{ns}
2°C		0.42	5.64	35.45	35.45
5°C		0	4.53	25.67	24.9
($p < 0.005$)		***	***	***	***

[a]Rating as described in Table 8.1

NS = not significant difference

*** ($p < 0.001$)

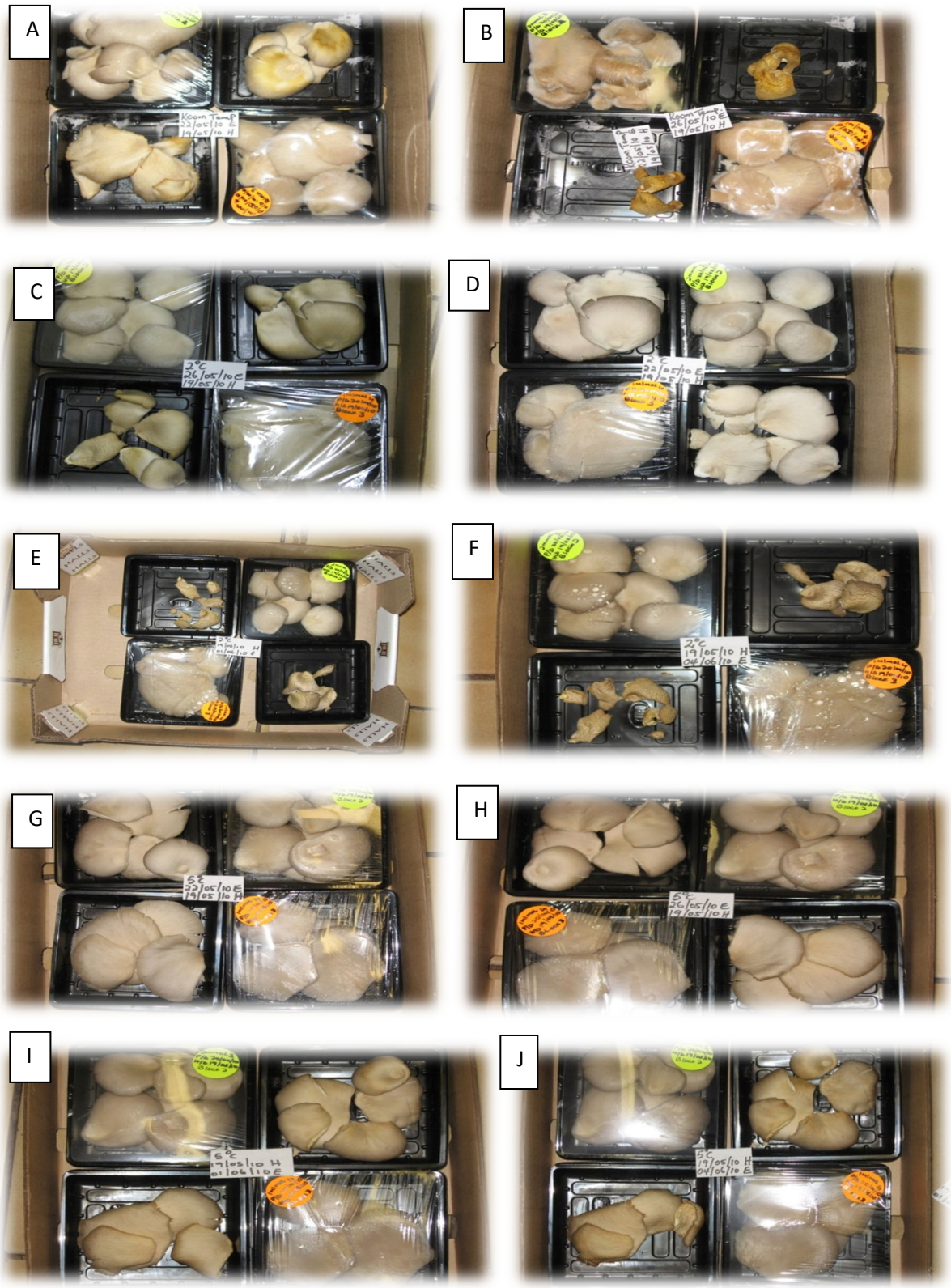


Plate 8.1. Oyster mushrooms harvested and stored in ambient (room) temperatures A and B; 2°C - C, D, E and F; and 5°C - G, H, I and J

The type of packaging materials also play an important role in the quality and colour of the oyster mushrooms, for example, the use of Vitafilm prevents caps from drying and darkening and further controls the gaseous composition of the atmosphere and relative humidity (Czapski, 2000, 2001; Zuchowicz *et al*., 2004ab). Previous studies have shown that both quality and the freshness of the oyster mushrooms can be maintained by refrigerating mushrooms in corrugated cardboard or paper bags immediately after harvest (Hill, 2004).

In both 2°C or 5°C storage temperatures, oyster mushrooms in covered containers maintained the highest number of good quality (91% or 89%) specimens and also displayed the highest number with a light cream colour (92% or 89%) with only a few with physiological disorders (15% or 17%). The oyster mushrooms in covered containers were able to withstand 16 days of storage at both 2°C or 5°C and were still of good quality, suitable for human consumption. It was also emphasised that fresh mushrooms should be stored at cool temperatures (0 – 2°C and 90% RH) in order to extend their shelf-life quality (Subramaniam *et al*., 2021). However, different to this finding, Hill (2004) indicated that covered oyster mushrooms can last for only 5 – 7 days on the shelf at 1°C – 2°C storage temperatures. Oyster mushrooms in uncovered containers behave differently from those in covered containers where those stored in uncovered containers at temperatures of both 2°C or 5°C maintained the lowest number of good quality (39% or 24%) specimens, the lowest number of light cream colour (22% or 23%) mushrooms, and the lowest number with physiological disorders (6% or 18%). These findings were emphasised by the results after storage at temperatures of both 2°C or 5°C, where the quality rankings were better of compared with ambient temperatures.

During the warmer months of October to November, the differences were significant ($p < 0.05$) for oyster mushrooms stored at 2°C in covered containers and in uncovered containers compared to those stored at both ambient temperature and at 5°C (Table 8.5, Table 8.7 & Table 8.8). The oyster mushrooms stored in covered containers at 2°C, maintained a greater number of good quality (64%) mushrooms with a moderate number of light cream (58%) mushrooms with a lower number of physiological disorders (27%). The mushrooms stored in ambient temperatures, irrespective of whether they were packed in covered or uncovered containers had a moderate number of mushrooms that were of worse quality (47% or 58%) than those stored in colder temperatures with few that were dark brown (14% or 32%) and the highest number of mushrooms with physiological disorders (99% or 81%). This indicates that in ambient

temperatures oyster mushrooms in covered or in uncovered containers were of poor quality and were not attractive to consumers; thus they were not fit for human consumption.

The quality of fresh mushrooms was normally diminished by the extent of the bacterial population (Schill *et al*., 2021). This was also observed in ambient temperatures with the highest number of physiological disorders occurring during the warmer months of October to November. The mushrooms in covered containers lasted for 4 days, whereas oyster mushrooms in uncovered containers were dry after only a few days. When *Agaricus bisporus* is stored in ambient temperatures, it lasts for only 1 – 3 days before deterioration occurs (Mahajan *et al*., 2007). The results of these findings indicate that, regardless of the season, in order to retain better quality and colour without any physiological disorders, oyster mushrooms should be stored in covered containers at either 2°C or 5°C (70% RH). In rural communities, they can store oyster mushrooms in covered containers using their refrigerators to extend the shelf-life of the crop. Commercial farmers can store oyster mushrooms in covered containers using cold rooms or controlled rooms to obtain long shelf-life.

8.4. Conclusions and recommendations

Regardless of seasons and storage temperatures, storing oyster mushrooms in uncovered containers results in excessive moisture loss, which lowers the quality of the crop. Oyster mushrooms in covered containers stored at both 2°C or 5°C maintained the highest mean mass (g), the highest number of mushrooms of good quality and the highest number of light cream mushrooms without physiological disorders. This study recommends that storing oyster mushrooms in covered containers at either 2°C (70%RH) or 5°C (70%RH) during the cooler months of May to July, but preferably at 2°C (70%RH) during the warmer months of October to November, extends the quality of oyster mushrooms.

From the tables 8.2 and 8.3 all have *p*-values less than α (0.05) indicating that the set null hypothesis *(H_0)* is rejected and accept the alternative hypothesis (H_1) as oyster mushrooms stored for longer period with plastic film cover at 2 - 5℃. Effect of seasonal and different storage temperatures on the shelf-life of oyster mushrooms has been clearly demonstrated and quantified from the results. Season and temperature play a major role on fresh mass (g), quality rating and colour rating except for disorder rating production by oyster mushrooms. This was favoured by temperatures between 2 - 5℃.

CHAPTER NINE

Influence of drying methods and selected preservatives on shelf-life of oyster mushrooms

9.1. Introduction

People in South Africa are not familiar with both fresh and dried oyster mushroom (*Pleurotus ostreatus*) and it is a scarce commodity both at the market and retailers (Pakela-Jezile, 2006). For this reason alone, there was a need to introduce oyster mushrooms to people and to train them on how to process them in order to extend their shelf-life. Oyster mushrooms are one of most perishable products and tend to lose their quality soon after harvest (1 – 3 days) at ambient temperature (Singh *et al*., 2010). The shelf-life of oyster mushrooms at 2°C was 9 days and only 3 days when stored at 18°C (Lukasse and Polderdijk, 2003). Processing mushrooms is very expensive since it requires specific equipment such as an electric dehydrator, refrigerator and cold room. However, there are also inexpensive and simple techniques that can be used to process them. One such technique includes using natural sunlight or oven drying so that they can be cured for later use. Since the majority of people in South Africa are poor and cannot afford to buy meat and other sources of protein, dried oyster mushrooms can serve as a substitute. Mushrooms contain high quantities of protein and carbohydrates, minerals and vitamins and are also low in fat content (Mshandete and Cuff, 2007). The energy value of oyster mushrooms (*Pleurotus ostreatus*) is between 13.94 and 21.31 kcal/g of mushrooms (Silveira *et al*., 2006) compared to meat with the energy values from 105.88 and 129.54 kcal/g ().

Of lately, there has been a growing interest in the production of oyster mushrooms in South Africa, probably due to their high nutritional value, as well as the high prices that they fetch in the market. However, only 10% of the mushrooms produced are channelled to canneries and drying and processing entities, therefore, most growers tend to utilise this part of the market only as a last resort should they have surplus stock that could not be sold to the retail industry, greengrocers and restaurants (Country Foods, 2007). Most farmers send their mushrooms to canneries and drying facilities only if they have produced more than the market expectations. Weight loss is one of the most common signs of deterioration of quality in mushrooms (Rai and Arumuganathan, 2008), because they are extremely perishable with a short shelf-life. Processing of oyster mushrooms by drying is important because it extends the shelf-life of the

crop. Currently, regardless of species, mushrooms can be stored for only a few weeks before their quality deteriorates greatly (Combs, 2004). Extending the shelf-life of oyster mushrooms is important to both mushroom producers and consumers. To achieve a longer shelf-life, the traditional method used for preserving *Pleurotus* genus mushrooms was by means of convective drying at 45°C to 65°C (Arora *et al*., 2003). Drying has been regarded as the oldest and cheapest technique known to mankind for preserving food commodities in order to extend their shelf-life (Rama and Jacob-John, 2000; Rai and Arumugarathan, 2008).

Dehydration can be defined as a reduction of water activity of the product until levels that guarantee micro-biological and physicochemical stability are reached (Cao *et al*., 2003; Krokida *et al*., 2003; Lewicki and Jakubczyk, 2004). Therefore, in order to maintain the quality of the produce, the mushrooms are washed in a solution of sodium metabisulfite which has the beneficial effect of whitening the pilei by inhibiting undesirable changes in colour (Bernas *et al*., 2006). Mushrooms treated with boiling water for two minutes also resulted in a better colour of freeze-dried mushrooms (Kotwaliwale *et al*., 2007).

Previous studies have indicated that mushrooms are commodities that decay rapidly and they can begin to decay immediately within a day after harvest (Walde *et al*., 2006). To prevent decay, fresh oyster mushrooms must be processed to extend their shelf-life for off-season use. Other researchers have reported that the shelf-life of mushrooms that are processed using the drying method (Rama and Jacob-John, 2000), and packed in an air-tight container, can be extended for more than a year (Bernas *et al*., 2006; Rai and Arumuganathan, 2008). However, while no research data is available on the processing of oyster mushrooms using drying under South African conditions, the drying of tropical, subtropical and temperate fruit crops is well documented. The objectives of this study were, therefore, (i) to determine the suitable drying techniques for extending the shelf-life of oyster mushrooms and (ii) comparing different preservation chemicals used during drying of oyster mushrooms. The following hypotheses was consequently tested: (i) The null hypothesis (H_0): Varying drying methods and preservatives have no effect on the shelf-life of oyster mushrooms. (ii) The alternative hypothesis (H_1): Varying drying methods and preservatives have an effect on the shelf-life of oyster mushrooms.

9.2. Materials and methods

Location and characteristics of the study area

As described from Chapters 3 to 8.

Experimental layout and treatments

The experiment was laid out in a completely randomised design (CRD) with 9 treatments consisting of three drying methods and three types of preservatives, replicated 3 times. Three methods of drying were sun drying, the use of an electric dehydrator and utilising ambient temperature, whereas the three types of preservatives that were used were: without preservation (control), sodium metabisulfite and preservation with lemon juice. Sodium metabisulfite is normally used for preserving tropical and subtropical fruits and raisins in most of the industry (Komarnisky *et al*., 2003). Sodium metabisulfite was used at a quantity of 25 g in 2 L of cold water and stirred for a few minutes until it dissolved. The lemon juice (100% lemon juice – Brookes True Lem) was mixed with a quantity of 2 cups of 80 ml in 2 L of cold water. Lemon juice concentration and freezing are the best long-term preservation options for its efficacy. The concentrate thus serves as a semi-processed product that can be further processed into various beverages such as lemonade, juice blends, etc. or purely an acidulant (Agricultural Research Council – Institute for Agricultural Engineering; undated). The control sample (without preservatives) was not treated with chemicals or wetted with water. The oyster mushrooms were harvested and dried on the same day. Approximately 200 g of oyster mushrooms was used for each treatment, whereas Apati *et al*. (2010) used 50 g of mushrooms for drying in an oven. Harvested mushrooms were dipped in a sodium metabisulfite solution for at least 5 minutes and the excess water drained before being placed on trays.

Methods for drying oyster mushrooms

Electric dehydrator

The electric food dehydrator is a device that removes moisture from food to aid in its preservation. The preserved oyster mushrooms were laid on trays and placed on a trolley made of plastic nets (Plate 9.1). The mushrooms were spread evenly on trays for proper drying. These

trays allow the heat to easily flow upwards from the bottom. The electric dehydrator was set for 9 h with three steps in between, each lasting for 3 h and set for the first three hours: (60°C, 40% RH), the second three hours (60°C, 27% RH), and the last three hours (55°C, 18% RH). However, Apati *et al*. (2010) used temperature ranges from 40°C, 50°C, and 60°C with a relative humidity of 75% during the drying of mushrooms. The oyster mushrooms were dried properly by the next morning and kept on a tray to cool down before being placed in plastic bags (Plate 9.1). The oyster mushrooms were weighed before and after drying. The cost of the electric dehydrator is +/- R100 000.00. The dehydrator runs for 12 hours in order to dry the commodity to its right state. If you have a 1400Watt appliance, it can cost 13 cents per hour, 700Watt dehydrator, it costs 6.5 cents per hour.

Plate 9.1. Fresh oyster mushrooms in an electric dehydrator

Sun drying

Sun drying is a traditional method used for reducing the moisture content of food; in this case, the fresh oyster mushrooms are spread out in the sun. The oyster mushrooms were placed on trays with the gills facing upwards in order to hasten the drying. The trays (Plate 9.2), which were placed in the sun for about 4½ hours and were covered with veils to protect the mushrooms from flies and dust. The mushrooms were kept in the sun for two days in order to

dry properly. Sundried mushrooms were left on the trays for few days to prevent breakage of the crop before being packaged and sealed in plastic bags.

Plate 9.2. Trays containing fresh (A) and sun-dried (B) oyster mushrooms on paper towels

Ambient drying (temperature)

Ambient drying is a method used to remove moisture content from foods while the product is kept at room temperature. The harvested oyster mushrooms were treated with different preservatives and placed on three trays (Plate 9.3), namely, the control (without preservatives), one with lemon juice, and one with sodium metabisulfite, with the gills facing upwards in order to hasten the drying process. The trays were covered with veils. The oyster mushrooms were kept at ambient temperature for at least 7 days to ensure proper drying.

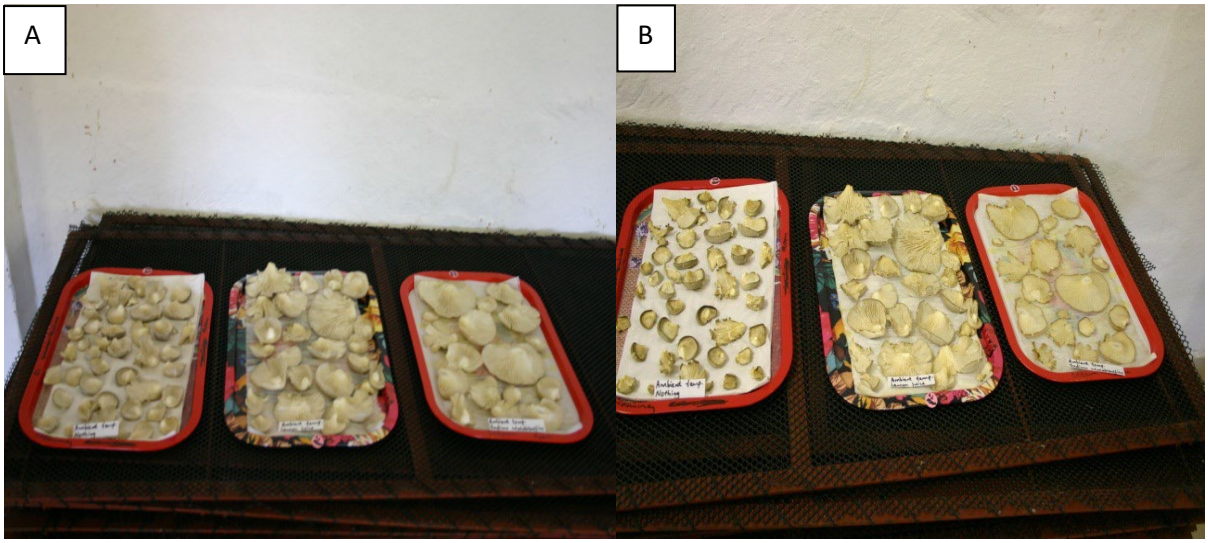


Plate 9.3. Fresh (A) and dried (B) oyster mushrooms dried in ambient temperature

Postharvest analysis

The following postharvest parameters were analysed: storage period of dried oyster mushrooms, moisture content, quality, colour and physiological disorders and the chemical composition of dried oyster mushrooms.

Storage period of dried oyster mushrooms

The mass of dried oyster mushrooms was measured using a Scaltec scale (Germany), packaged in a transparent plastic bag (10 cm x 20 cm) and sealed using a hand impulse sealer (KS 400 - Hongzhan).

Moisture content

The moisture content refers to a quantity of water contained in a material. The moisture content of both the fresh and dried oyster mushrooms was analysed using a RADWAG (Model: MAX 50/NH) moisture analyser machine (Plate 9.4). The moisture content of fresh and dried oyster mushrooms was analysed using 5 g of both fresh and dried oyster mushrooms. On a 0 storage, 5 g of dried oyster mushrooms were analysed for moisture content, whereas at both 6 months and 12 months, 2 g samples were used. Dried oyster mushrooms had less weight compared to fresh mushrooms and it required more pieces of mushroom crops to attain a certain weight. For analysis, the machine was set at a temperature of 100°C and ran for not less than 10 minutes,

depending on the moisture content. Subsequently, the samples of oyster mushrooms were drawn and cut into small pieces, placed into a small pan and placed in a machine to analyse the moisture content thereof. The RADWAG moisture analyser machine determines the moisture content (%), initial mass (g) and final mass (g) of a product.

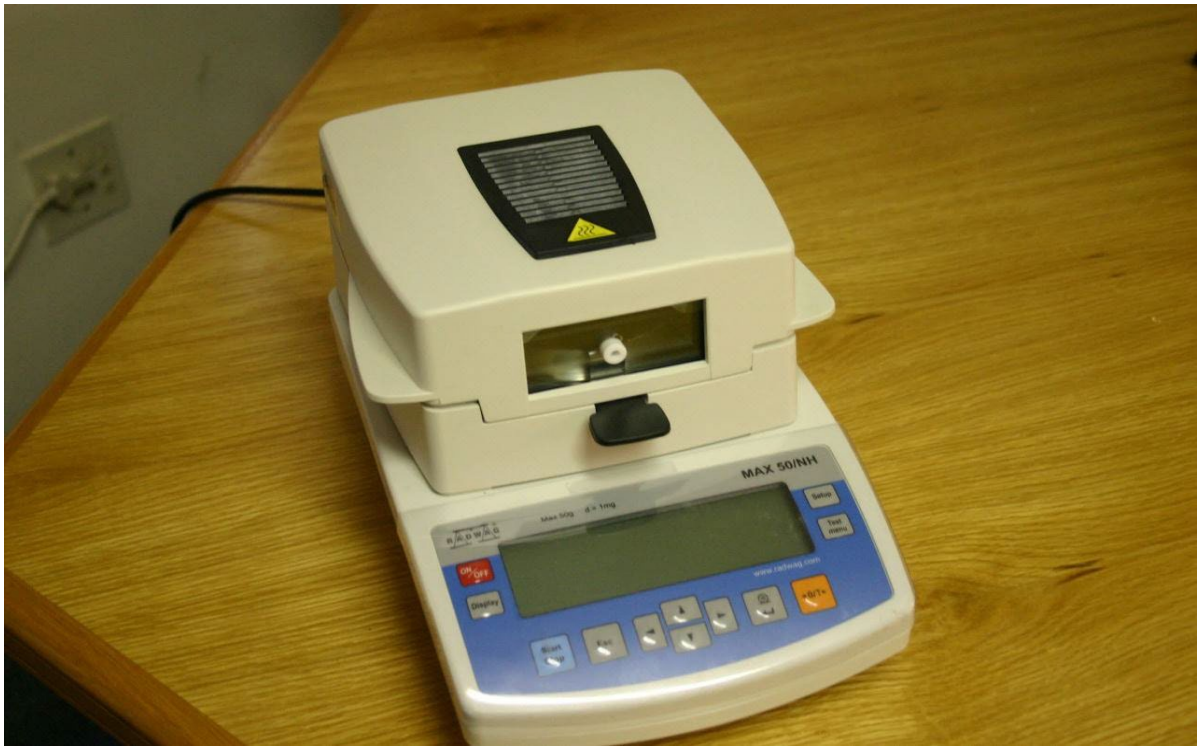


Plate 9.4. RADWAG moisture analyser (Mettler Toledo, Germany)

Quality ratings of oyster mushroom

The quality of the oyster mushrooms is considered to be a degree of excellence of a product or its suitability for a particular use. The dried oyster mushrooms were evaluated thrice during a 6-month period. First, the quality evaluation was performed immediately after drying the oyster mushrooms. The quality was evaluated using five scale ratings (Table 9.1). The dried oyster mushrooms were stored at ambient temperature after each evaluation in order to simulate the storage temperature that could be affordable to small-scale farmers.

Colour

Colour is one of the most important quality parameters as it plays a role in the preferences of the consumers. The dried oyster mushrooms were evaluated using a four-colour rating scale

(Table 9.1). The colour rating was evaluated thrice after 6 months of storage of the oyster mushrooms in ambient temperature.

Physiological disorders

Physiological disorders refer to the damage or problems resulting from the influence of environmental and cultural factors. Physiological disorders were evaluated using two rating scales (Table 9.1).

Table 9.1. Evaluation method for stored dried oyster mushrooms (After Shewfelt, 2014)

Quality rating	Physiological disorder	Colour rating
1 – good	0 – no disorder	1 – light cream
2 – moderate	1 – whitish growth	2 – cream
3 – poor		3 – brown
4 – severe		4 – dark brown
5 – worst		

Statistical analysis

Statistical analysis was done exactly as with Chapters 3, 4, 5, 6, 7 and 8.

9.3. Results and discussion

9.3.1. Dry mass (g) of dried oyster mushrooms

There was a significant difference ($p < 0.05$) between the mass of dried oyster mushrooms over the different months. The mass of the dried oyster mushrooms differed, depending on the storage periods (0, 6 and 12 months) (Figure 8.1). The drying of oyster mushrooms increases their shelf-life by at least 6 months before spoilage sets in (Hill, 2004). At 0 months, the mass

of the dried oyster mushrooms was 16 g, whereas after 6 months of storage, the mass of the dried oyster mushrooms had dropped down to 14 g and later gained mass to 17 g after 12 months of storage (Figure 9.1). Fei *et al*. (2005), who conducted a study under different cold room storage conditions, found similar results, where the different storage conditions exerted a significant effect on the weight loss of the mushrooms. This indicates that the longer the dried oyster mushrooms are stored, the more they tend to accumulate more moisture that builds up mass again.

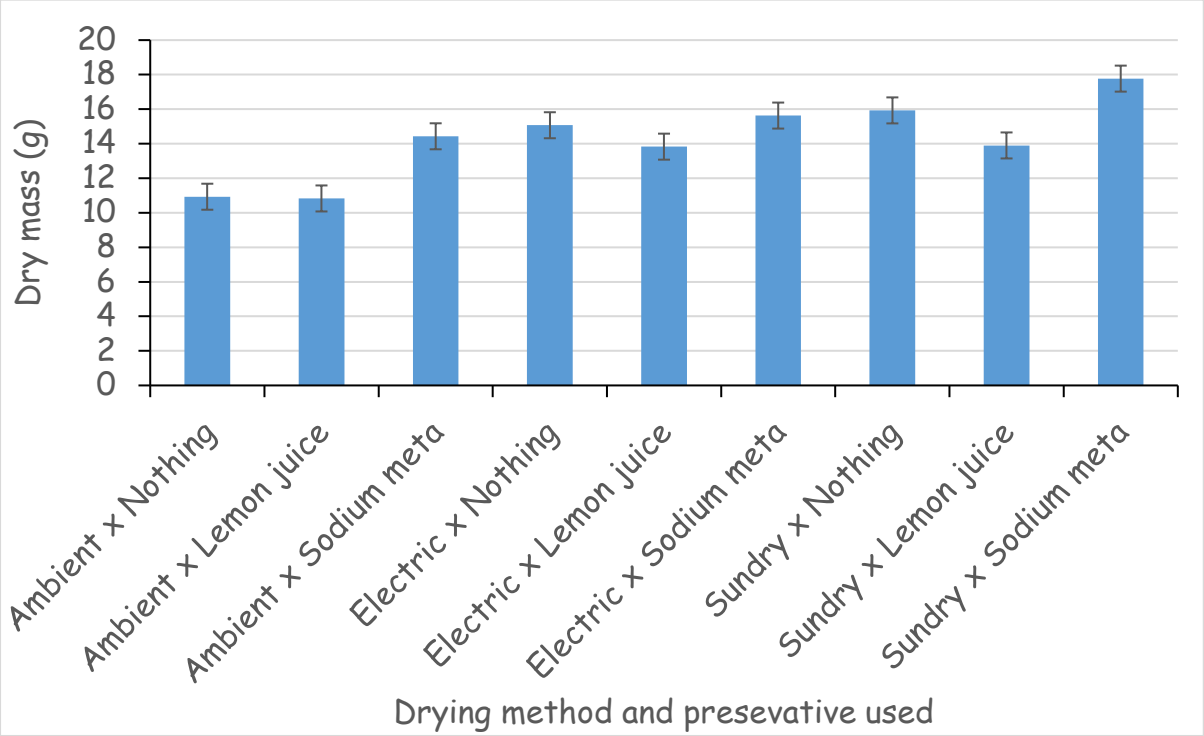


Figure 9.1. Dry mass (g) of oyster mushrooms as influenced by drying method and preservative used. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

9.3.2. Quality rating of dried oyster mushrooms

There was a significant difference ($p < 0.05$) between the quality rankings after the different storage periods (0 month, 6 months and 12 months). In 0 month, the quality was ranked at 11%, which represents good quality dried oyster mushrooms (Figure 9.2). This was also supported by a good quality appearance of the dried oyster mushrooms for all the treatments, except those stored in ambient temperatures and treated with lemon juice, which has shown a poor quality of dried oyster mushrooms (Plate 9.5) represented by photo H. After 6 months of storage, the quality rankings increased to 13%, which indicate moderate quality of dried oyster mushrooms.

The dried oyster mushrooms subjected to both the electric dehydrator and sun drying with different preservatives exhibited good quality after 6 months of storage, except the dried oyster mushrooms using ambient temperature, which had begun to spoil (Plate 9.5). After 12 months of storage, the quality ranking increased to 17%, which indicates poor quality of the dried oyster mushrooms and would be unusable by consumers. The storage conditions of the mushrooms exert an influence on the enzyme reaction with oxygen and form brown pigments; such a disorder reduces the quality of the oyster mushrooms (Kim, 2004).

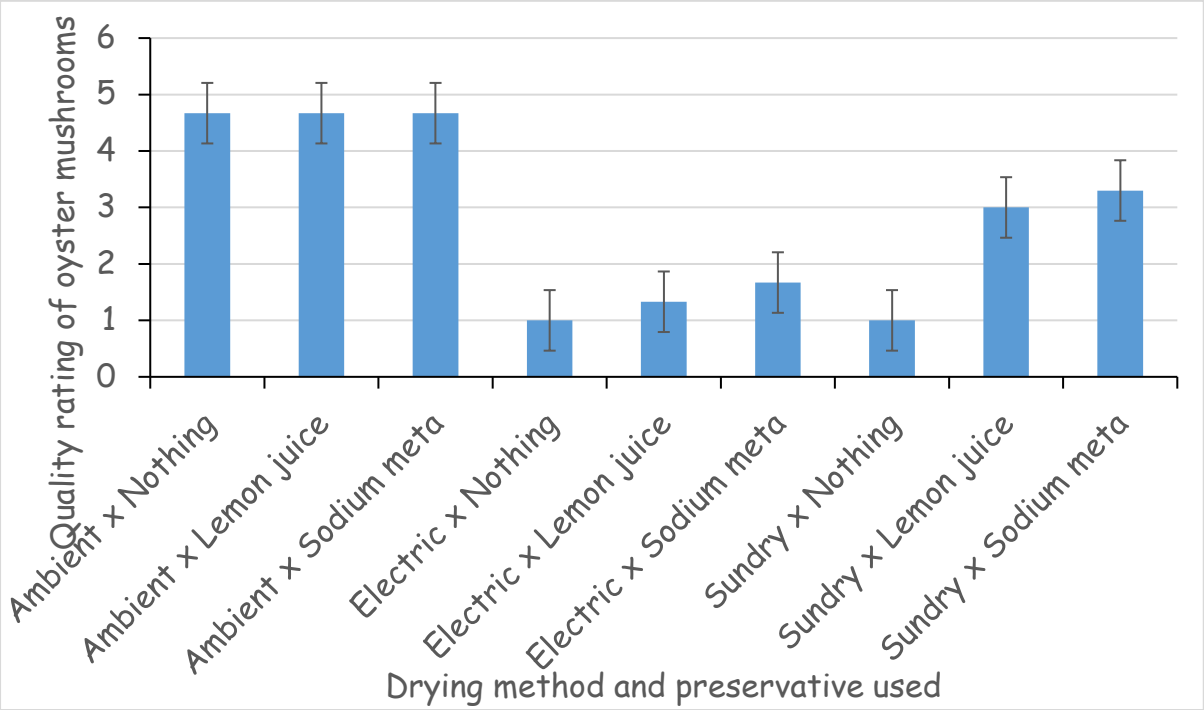


Figure 9.2. Quality of oyster mushrooms as influenced by drying method and preservative used. Bars on the graph represent Standard Error (SE) Means (P < 0.05)

9.3.3. Colour rating of dried oyster mushrooms

There was a significant difference ($p < 0.05$) between the colour rankings for each period (after 0, 6 and 12 months) (Figure 9.3). There was a good colour rating (10%) of dried oyster mushrooms during the first period (0 month) of the storage, whereas within 6 months of storage, the colour ranking increased to 14%, which indicates that the dried oyster mushrooms began to deteriorate as time progressed (Figure 9.3). The appearance of the oyster mushrooms that were dried using either an electric dehydrator or sun drying with different preservatives was of good colour while those dried at ambient temperatures exhibited a poor colour (Plate

9.5). The dried mushrooms that were preserved with sodium metabisulfite produced the lightest colour products, followed those treated with sodium hypochlorite and cys-HCI pretreatment (Hassan and Medany, 2014).

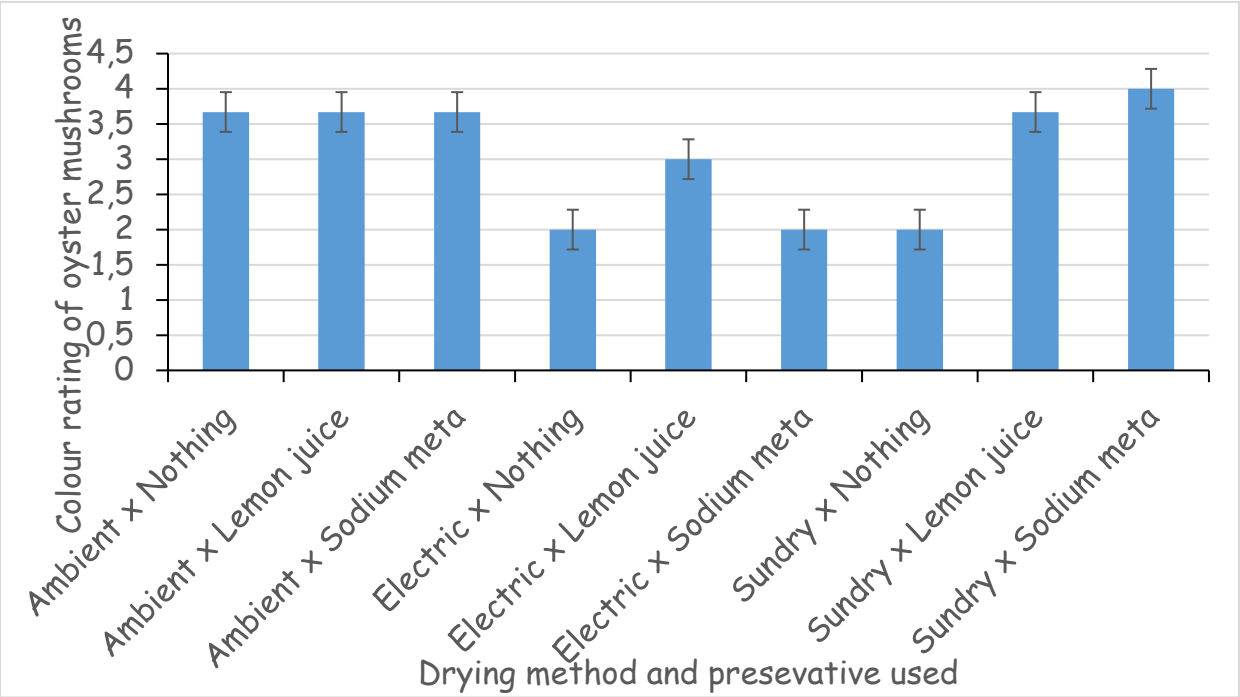


Figure 9.3. Colour of oyster mushrooms as influenced by drying method and preservative used. Bars on the graph represent Standard Error (SE) Means (P < 0.05)

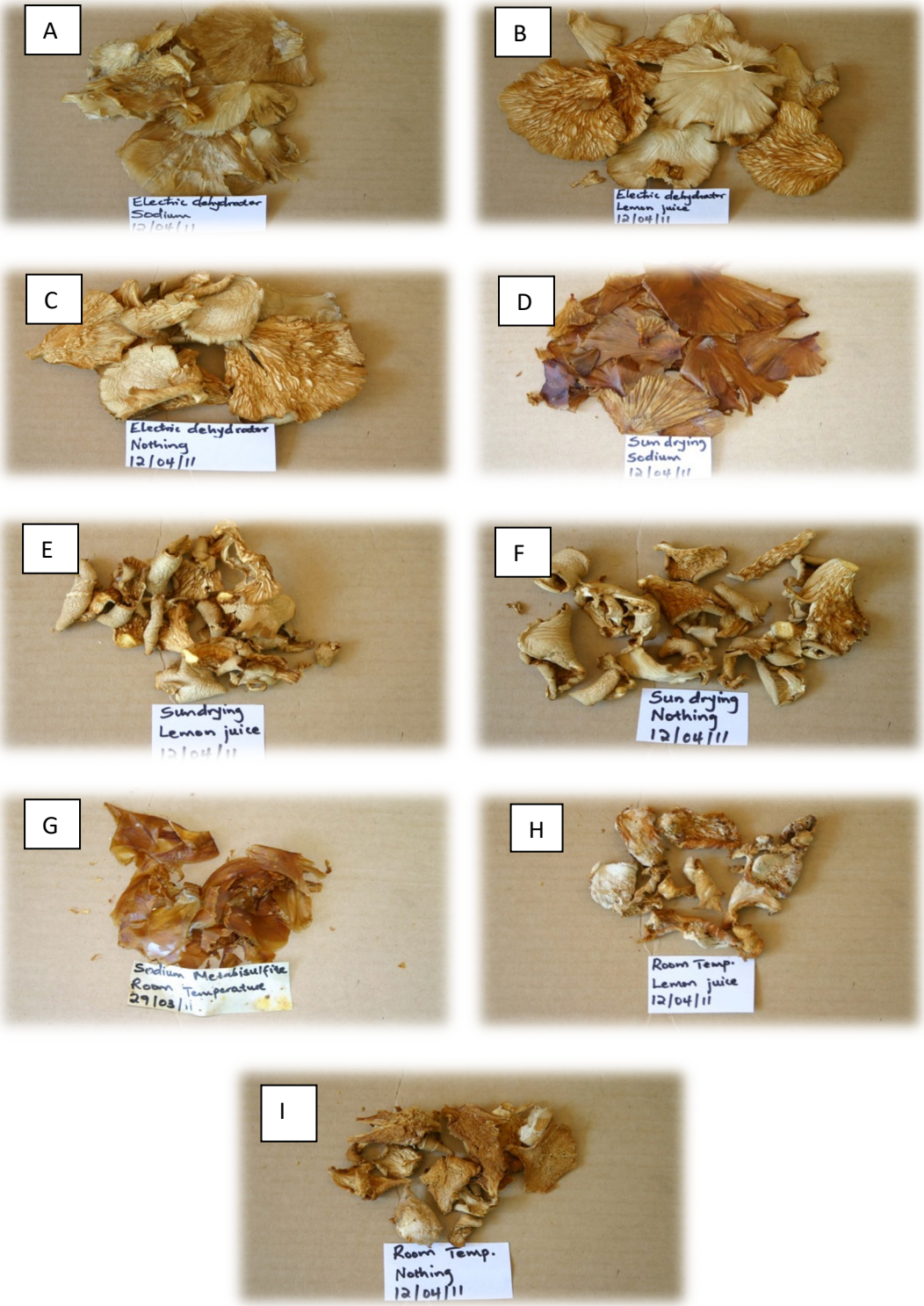


Plate 9.5. Dried oyster mushrooms at 0 month of storage in ambient temperature (A – electric x sulfate, B – electric x lemon, C – electric x nothing, D – sun dry x sulfate, E – sun dry x lemon, F – sun dry x nothing, G – ambient x sulfate, H – ambient x lemon, I – ambient x nothing).

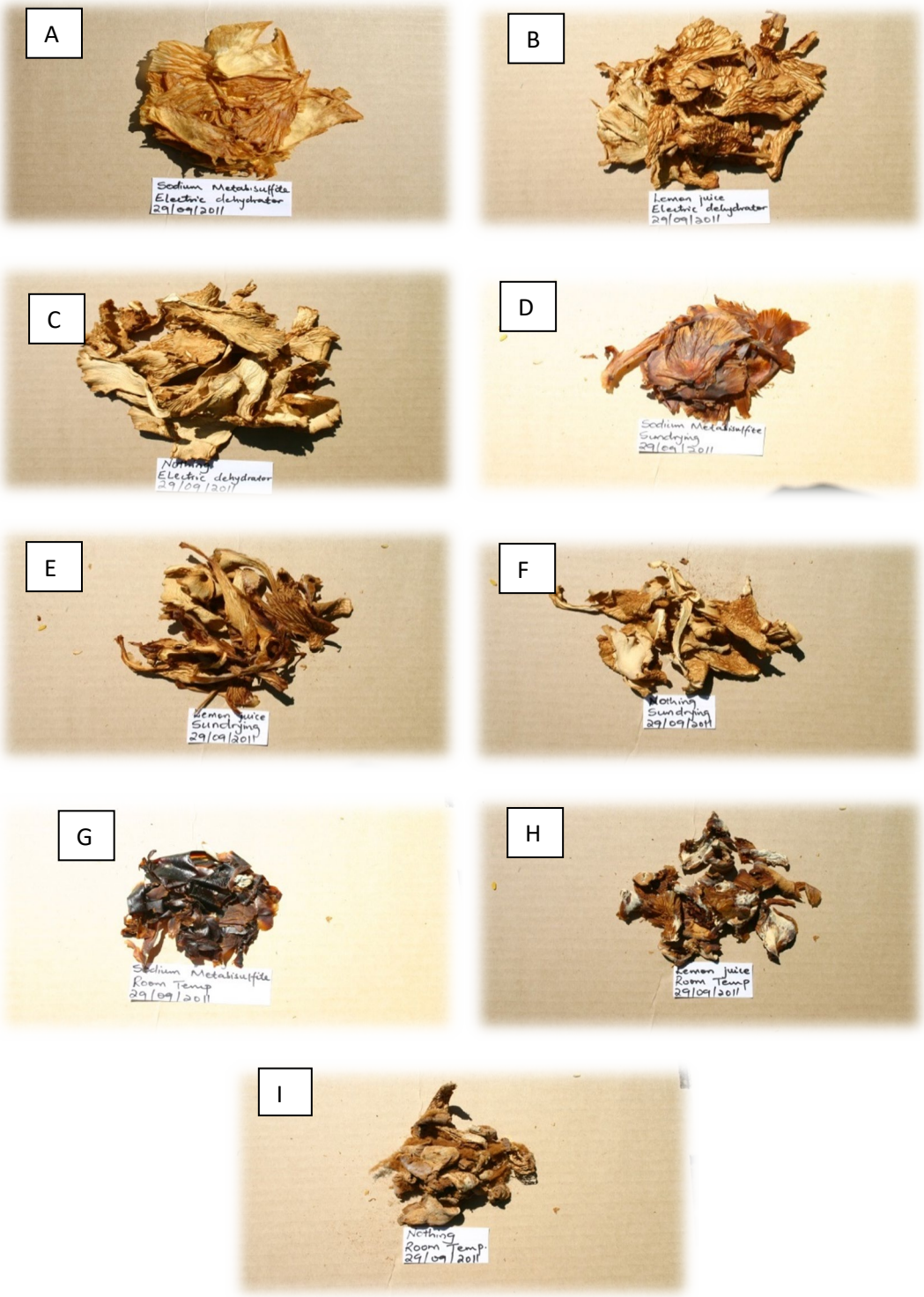


Plate 9.6. Dried oyster mushrooms after 6 months of storage in ambient temperature (A – electric x sulfate, B – electric x lemon, C – electric x nothing, D – sun dry x sulfate, E – sun dry x lemon, F – sun dry x nothing, G – ambient x sulfate, H – ambient x lemon, I – ambient x nothing).

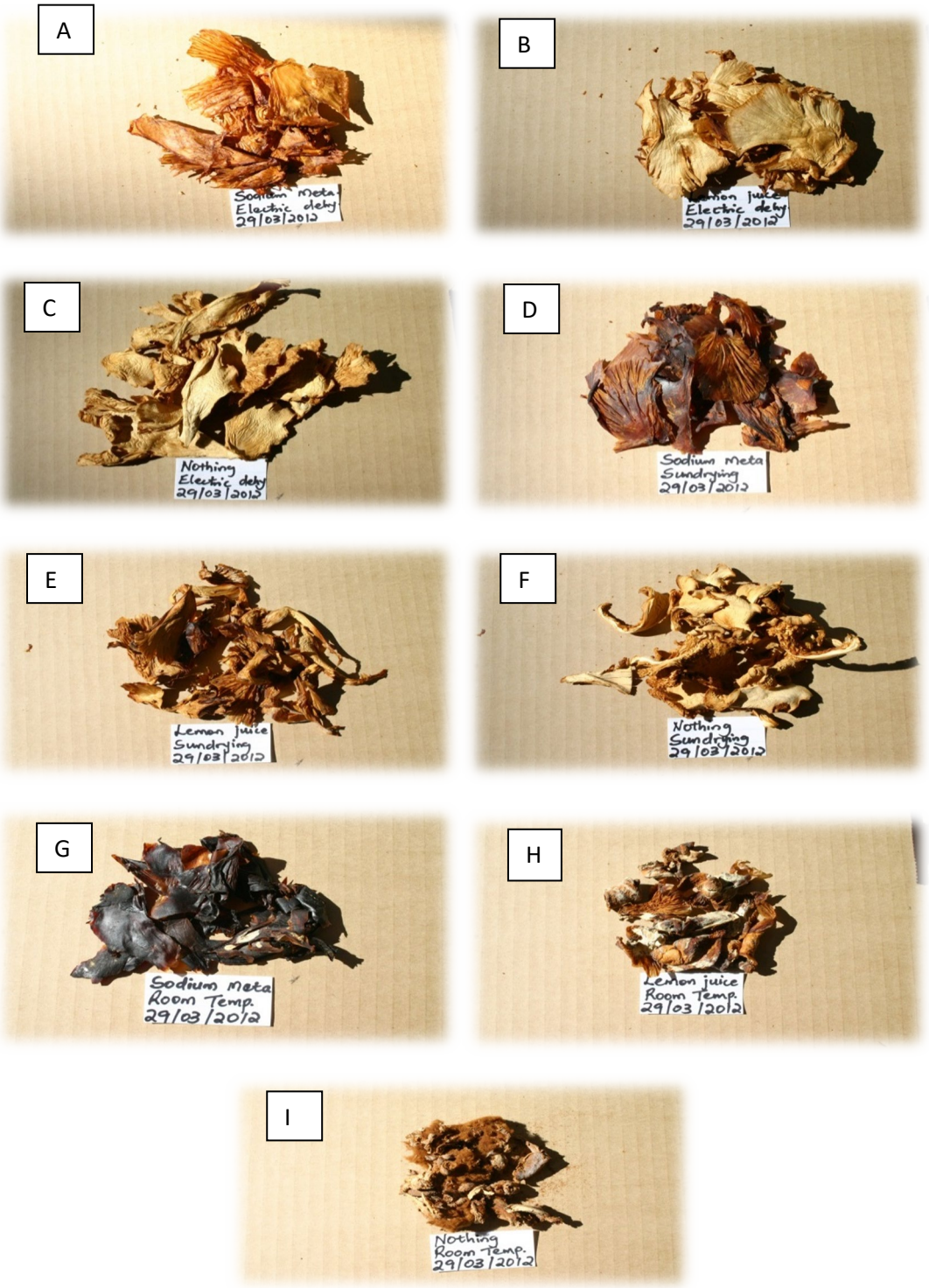


Plate 9.7. Dried oyster mushrooms after 12 months of storage in ambient temperature (A – electric x sulfate, B – electric x lemon, C – electric x nothing, D – sun dry x sulfate, E – sun dry x lemon, F – sun dry x nothing, G – ambient x sulfate, H – ambient x lemon, I – ambient x nothing).

After 12 months of storage, the colour rank increased to 17% which indicates that the quality of the dried oyster mushrooms was deteriorating and they could be unfit for human consumption (Figure 9.3). This can also be confirmed by dried oyster mushrooms using sun drying treated with sodium metabisulfite of all preservatives in ambient temperature storage (Plate 9.7), because their colour was poor. Combs (2004) also confirmed that colour of mushrooms can darken due to drying process and storage periods.

9.3.4. Moisture content (%) of dried oyster mushrooms

There was a significant difference ($p < 0.05$) between the moisture content of the dried oyster mushrooms stored for different periods (0, 6 and 12 months). It is also known that the average moisture content of fresh oyster mushrooms ranges from 85% to 90%. At 0 month of storage, there was a high moisture content (18%) which indicates that the dried oyster mushrooms were of good quality (Figure 9.4). According to Kim (2004), when the moisture content of dried mushrooms reaches about 20%, mushrooms will easily be infested by insects and mould if not packaged, but the opposite was the case in this trial because of the good storage facility. However, after 6 months of storage, moisture content dropped to 9% which indicates a deterioration of the quality. In contrast to this finding, Kim (2004) indicated that the moisture content of dried mushrooms was supposed to be near 10% in order to be of good quality. Whereas, after 12 months of storage, the moisture content depreciated to 7% which indicates that the dried oyster mushrooms were spoiled and unusable by consumers.

Table 9.2. *p*-values and standard error means for evaluation of dried oyster mushrooms from drying methods

Evaluations	***p*-values**	**Standard error means**
Dry mass	<.0001	0.21041
Quality rating	<.0001	-
Colour rating	0.0015	-
Moisture content (%)	0.0011	-

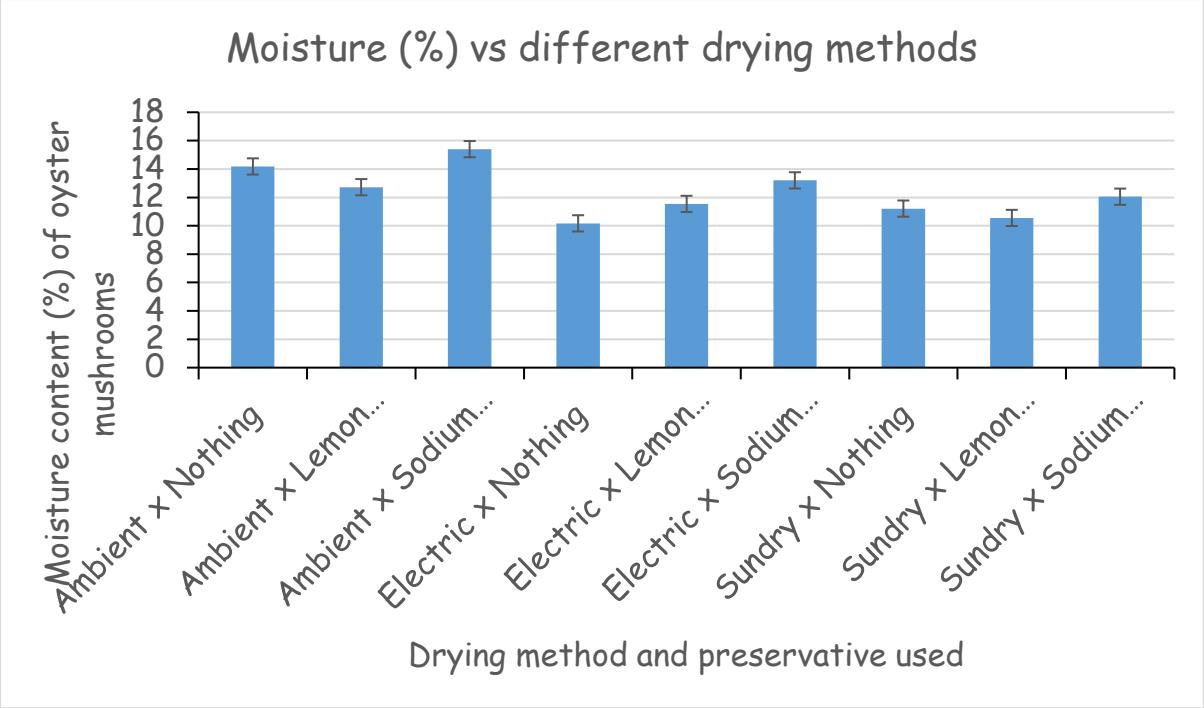


Figure 9.4. Moisture content (%) of oyster mushrooms as influenced by drying method and preservative used. Bars on the graph represent Standard Error (SE) Means ($p < 0.05$)

9.4. Conclusions and recommendations

The dry mass, quality, colour and moisture content of dried oyster mushrooms decreased with the increasing length of the storage period, from 0, 6 and 12 months, respectively. Dried oyster mushrooms should not be stored for more than 6 months to maintain good quality and to stay usable by consumers. Experiments using an electric dehydrator compared with drying with nothing, lemon juice or sodium metabisulfite and sun drying compared with nothing indicated that it was possible to maintain the good quality, good colour and lower moisture content of dried oyster mushrooms. This indicates that there was need to preserve oyster mushrooms while using electric with different to sun drying, but the drying methods played an important role in maintaining their quality. Based on these findings, using both the electric dehydrator and sun drying methods with or without using any preservatives is recommended, depending on the affordability. In other words, the electric dehydrator method (with or without preservatives) is recommended to commercial farmers because it requires more resources, while the sun-drying method (with or without preservatives) is recommended to small-scale farmers since it does not require any resource.

From table 9.2 all have *p*-values less than α (0.05), confirming that the set null hypothesis *(H_0)* should be rejected and accept the alternative hypothesis (H_1) as varying drying methods and preservatives have effect on oyster mushrooms shelf-life. The results as collected from the whole of Chapter 9 confirms that electric x nothing, electric x lemon juice, electric x sodium metabisulfite and sundry x nothing yields the best results as it comes to dried mass (g), quality of dried oyster mushroom, colour ratings and moisture content (%). The acceptance of the alternative hypothesis is in agreement with these results.

CHAPTER TEN

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Food security and sustainable economic development is key to South African development plan. Oyster mushroom has been identified to provide food security and its beneficiation to create employment and allow small-scale farmers to actively participate in economic develpment. In order to promote oyster mushroom as the main driving force, there will be a need to fully understand growth requirements, shelf-life and nutritional value.

Previous studies have reported that plant growth regulators such as IAA, 1-NAA, GA and kinetin can affect the size and yield of oyster mushrooms (Kumar *et al*., 2018; Da Silva *et al*., 2017), while, substrates can affect mushroom production, depending on which spawn is grown (Royse *et al*., 2004; Onyango *et al*., 2011; Ashraf *et al*., 2013). Oyster mushrooms can be stored for only 18 hours under ambient temperature (Jayathunge and Illeperuma, 2001; Singh *et al*., 2010), stored for 2 - 3 days under 15°C, and stored for 7 - 9 days under 0 - 1°C temperature (Suhaili *et al*., 2021). The mushroom industry is probably the only industry that directly converts straw into a protein source for human consumption (Grimm and Wösten, 2018). However, wheat straw contains chemical compounds that can inhibit the growth of other crops, weeds and its own (Wu *et al*., 2006). To extend the shelf-life of oyster mushrooms, an electric dehydrator could be used for drying at 45 – 65°C for good results (Arora *et al*., 2003).

In an effort to extend the shelf-life of mushrooms, a study was carried out on oyster mushrooms to study the effects of agronomic practices on growth, yield and shelf-life determined by the quality of oyster mushrooms produced. This study was conducted in a custom made hut-house and plant growth room at the Agricultural Research Council – Tropical and Subtropical Crops, Mbombela (Nelspruit) Farm. The following features were investigated: the effect of leachate on lettuce and oyster mushroom spawn (undiluted extract; 3:1; 1:1 and 1:3 ratios) and a control (dH_2O) (refer Chapter Three); drainage of wheat straw leachate (1 h; 2 h; 3 h and 4 h drainage) and a control (no drain) (refer Chapter Four); pasteurisation of substrate (Jik; gypsum; steaming and boiling) and a control (soaking) (refer Chapter Five); alternative substrates (wood chips and wild grass) and a control (wheat straw) (refer Chapter Six), uses of 1-NAA at five levels (0; 0.1;

0.5; 1; 1.5 and 2 mmol 1-NAA) and a control (0 mmol 1-NAA) (refer Chapter Seven); storage temperatures of oyster mushrooms (2°C and 5°C with covered or uncovered containers) and a control (room temperature with covered or uncovered containers) (refer Chapter Eight), as well as drying techniques (room; sun drying and electric dehydrator) and a control (no preservation) (refer Chapter Nine).

In a study conducted to determine the potential growth inhibitors using lettuce seeds and oyster mushroom spawns, the dH_20 control did not inhibit the germination and growth of lettuce seeds in all the 3 consecutive three months. However, a 1:3 ratios (25 ml extract with 75 ml distilled water) encouraged germination and growth of lettuce seeds with fresh and stored dry wheat straw for 3 months (experiment 1 and experiment 2), respectively. Dry wheat straw that was fresh as well as that stored for 3 months, needed the extracts to be leached out before spawning. For dry wheat straw stored for 6 months, both samples treated with 1:1 (50 ml extract with 50 ml distilled water) and 1:3 (25 ml extract with 75 ml distilled water) ratios of leachate did not significantly inhibit germination of the lettuce seeds. The growth of lettuce seedlings treated with dH_20, 1:3 and 1:1 dilutions of leachate did not differ significantly in experiment 3. All of these treatments can be used for growing lettuce seedlings as dry wheat straw stored for 6 months tends to lose compounds that are responsible for inhibiting the growth of lettuce seedlings. The dry wheat straw stored for 0-month period imposes a higher level of inhibition of seed germination than that for stored wheat straw (Wu *et al*., 2007). Oyster mushroom (*Pleurotus ostreatus*) spawn reacts quite differently from lettuce (*Lactuca sativa*) seeds in terms of germination and growth because it is a fungus. During experiment 1, the opposite was the case with a 3:1 ratio as it exhibited better growth of mycelia at the early stages when compared with lettuce seeds. Samples treated with undiluted extract yielded lower growth of mycelia than the samples treated with 3:1 and 1:1 dilutions, which indicates that there was a need to leach out excess extracts in order to reduce the amount of growth inhibitors in experiment 1. The dH_20 control sample exhibited poor mycelial growth in both experiment 1 and experiment 2. In experiment 2, the substrate treated with a 1:1 ratio of water and leachate yielded the highest mycelial growth compared with the substrates that had been subjected to all the other treatments. This indicated that it was necessary to leach out the compounds responsible for inhibiting growth before spawning oyster mushroom spawn with wheat straw stored for 6 months. Wheat (*Triticum aestivum* – var. Duzi) straw possesses compounds that can inhibit lettuce (*Lactuca sativa* – Great lakes) seed germination and oyster

mushroom mycelial (*Pleurotus ostreatus*) growth. It might be important to farmers to leach out these compounds prior to inoculating the straw with the oyster mushroom spawn. The use of stored dry wheat straw should be encouraged when farming oyster mushrooms due to the negative effect when using fresh dry wheat straw. Storing wheat straw in a normal outside temperature under a shed for at least 6 months should be encouraged before being utilised so as to break down the compounds responsible for the inhibition of growth.

In the follow-up experiment conducted to demonstrate practices in the oyster mushroom industry, it was found that the drainage of wheat straw extracts accelerates rate of mycelial colonisation. Accelerated colonisation of mycelia helped to suppress the contamination by the proliferation of parasitic fungi. Draining wheat straw leachate for 1 h produced a greater cumulative number of clusters, the number of caps and fresh mass. Similarly, no difference was evident in the cumulative fresh mass produced with 2 h and 1 h drainage. However, it can be concluded that draining of wheat straw leachate for 1 h was necessary to enhance the rate of mycelial colonisation which improves the growth and yield of oyster mushrooms. It has always been necessary to leach out wheat straw extracts before spawning oyster mushroom spawn as this was confirmed by Pant *et al*., 2006.

Evaluation of pasteurisation methods yielded the following; both boiling and steaming accelerated the rate of colonisation, but it was attacked by parasitic fungi, yet both jik and gypsum were able to suppress the amount of contamination from Week 0 to Week 2. Surprisingly, gypsum, followed by both boiling and steaming methods, produced the greatest cumulative number of clusters. Wheat straw treated with both the boiling and steaming methods produced a greater cumulative fresh mass of oyster mushrooms, which represents the yield. Therefore, it can be concluded that boiling and steaming encouraged the rate of colonisation, the cumulative number of clusters, the number of caps and the fresh mass of oyster mushrooms, as these were also findings by Khan (2009) and Stamets (2000).

Substrates have significant influence on rate of colonisation (%), contamination (%) and yield of oyster mushroom (Rajapakse *et al*., 2007). From the results, the following were observed; wheat straw (control) significantly increased the rate of colonisation (65%) compared with both wood chips (50%) and thatch grass (45%) from Day 1 to Day 8. This indicated that the substrates

accelerated the rate of mycelial colonisation to overcome attacks by parasitic fungi. However, thatch grass improved the rate of colonisation at a later stage from Day 6 to Day 9. This indicated that thatch grass can also be considered to accelerate the rate of mycelial colonisation. Wood chips were completely exposed to massive contamination by *Trichoderma* fungi due to the late colonisation of mycelia, whereas thatch grass had minimal contamination due to the early recovery of the mycelia. During harvesting, the thatch grass and wheat straw (control) increased the cumulative number of clusters, the number of caps and the fresh mass of oyster mushrooms. In addition, thatch grass had a high nitrogen content (1.136%) compared with both the wheat straw (control) (0.560%) and wood chips (0.080%), mostly utilised for building protein in oyster mushrooms. The thatch grass and the wheat straw (control) substrates had high quantities of nutrients (phosphorus and magnesium; potassium and iron) required by oyster mushrooms for growth. Thatch grass contained all three important nutrients in high quantities, whereas the wheat straw substrate contained high quantities of only two nutrients. Therefore, it can be concluded that the thatch grass substrate can be used as an alternative to wheat straw in oyster mushroom production. The idea is to have a low-cost substrate that would, however, yield an appreciable quality of harvested oyster mushrooms. All these support the findings by Li *et al*., 2015.

Mycelia were slower to reach their full colonisation potential during the cooler April to June months owing to cold of winter. It took 11 days for the mycelia to reach their full colonisation potential to fully cover the plastic bags, whereas, during the warmer September to November months, the mycelia reached full colonisation cover in 8 to 9 days. It was necessary to apply 1-NAA during the warmer September to November months to accelerate the colonisation of mycelia in order to avoid attacks by parasitic fungi (*Trichoderma* species) (Hussain *et al*., 2010). Both 0 mmol and 2 mmol 1-NAA suppressed the contamination of oyster mushroom mycelia during the cooler April to June and the warmer September to November months. During harvesting, 1 mmol 1-NAA increased the cumulative number of clusters, the number of caps and the fresh mass (g). This indicated that during the cooler April to June months, it was necessary to apply 1 mmol 1-NAA to improve growth and yield; however, the use of either lower amounts of 0.1 mmol or optimum level of 2 mmol did not make any significant difference. In the warmer months (September to November), it was necessary to apply plant growth hormone (1-NAA), although it was lower than in cooler months with growth and yield of oyster mushrooms. The results in Figure 7.2, suggest that 1-NAA applied at a rate of 1 mmol during the cooler months of April to June

increases the rate of colonisation, suppressed the amount of contamination, and improved the yield by increasing the cumulative number of clusters, the number of caps and the fresh mass. In warmer September to November months, there was need for application of plant growth hormones as the treatments shows statistical differences on the early days.

Experiments designed to determine shelf-life yielded the following important information. In the cooler months (May to July), using covered containers at 2°C the initial mass of 50 g (still usable after four weeks) was maintained with good quality (92%), and good colour (92%) without physiological disorders. Similarly, at 5°C with covered containers, it was possible to maintain the initial mass of 50 g (still usable after four weeks) with good quality (89%), and good colour (89%) without physiological disorders. In the warmer months (October – November), 2°C with covered containers the initial mass was slightly changed to 49 g with moderate quality (64%), moderate colour (58%) without physiological disorders. During both seasons (cooler May - July and warmer October - November months), a temperature of 2°C or 5°C was ideal for maintaining the initial mass, good quality, and good colour without physiological disorders.

In the shelf-life of oyster mushrooms using drying methods, the dry mass, quality, colour and moisture content of the dried oyster mushrooms decreased during storage for periods of 0, 6 and 12 months, respectively. Dried oyster mushrooms should not be stored for more than six months to maintain good quality and stay usable for consumption. Electrical dehydration with or without preservatives and sun drying with or without preservatives were able to maintain a good quality and good colour of dried oyster mushrooms with a low moisture content. This indicated that there was no need to preserve oyster mushrooms during drying, but rather, the drying methods play an important role in preserving the quality of a crop. The various treatments result in significant differences in terms of the chemical composition of the mushrooms, except for the content of ash, protein and fat, regardless of whether the mushrooms are of good or poor quality, their colour, and their moisture content, whether high or low (Arora *et al*., 2003). Based on this finding, drying with both the electrical dehydrator with or without preservatives and sun drying with or without preservatives can be used as alternatives, depending on the affordability by farmers. The use of an electric dehydrator with or without preservatives can be more useful to commercial farmers as it does require more resources, whereas sun drying with or without preservatives can be utilised by small-scale farmers, as this method does not need any resources.

The results of this study revealed a general trend that thatch grass increased the cumulative number of clusters and caps as well as fresh mass (g) similarly to wheat straw. This effect qualifies thatch grass to be used as an alternative substrate of wheat straw. On the other hand, wheat straw substrates needed storage for at least 3 to 6 months prior to spawning in order to leach out compounds responsible for growth inhibitions. In addition, boiling and steaming of substrates could overcome attacks by parasitic fungi. Use of 1 mmol 1-NAA during cooler and warmer months could improve growth and yield of oyster mushrooms. Oyster mushrooms in covered containers stored better for four weeks and were still usable for consumption at 2°C or 5°C. This indicates the shelf-life of oyster mushrooms could be extended for longer periods under certain storage temperatures. Electric dehydrator with and without preservatives and sun drying with or without preservatives maintained good quality and colour with less moisture content during storage under ambient temperatures.

Therefore, it can be concluded that substrates, pasteurisation methods, plant growth regulators, storage temperatures and preservation methods should be considered to ensure optimal oyster mushroom production. This is in line with the very first hypothesis and aim of the research project. All these point to the importance of, substrates, pasteurisation, drainage, growth hormone, storage and shelf-life as optimum choice. One of the critical achievements that came out of this research, was the successive development of training manual for small scale farmers using low cost materials.

Based on the obove-mentioned findings, the following recommendations can be made to small-scale farmers of oyster mushrooms:

OPTIMUM PARAMETERS	
Parameters	***Optimum***
Substrate used	Wheat straw
Drainage period	1 hour
Pasteurisation (Temp.)	(60 – 82°C) for 1 hour
Alternative substrate	Thatch grass
Growth hormone	1-NAA (1 mmol)
Storage temperatures	2 – 5°C
Type of drying methods	Electric dehydration and sun dry with or without preservatives

Future work:

- Breeding of new variety of oyster mushroom spawn that will able to colonise substrate quickly, resist contamination and produce higher yield.
- Correctly formulate growth medium which contains planter bags, growth hormone and moisture retention enhancement for cultivating oyster mushrooms.
- Developing low cost packaging materials for oyster mushrooms that can be used by small-scale farmers

RESEARCH OUTPUTS RESULTING FROM THIS PROJECT

This has been presented or published in part as follows:

MASEVHE, M.R., TAYLOR, N.J. & SOUNDY, P. 2021. Training manual for oyster mushroom production by small-scale farmers. Department of Plant and Soil Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria.

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APPENDIX A

Table A1: Mean daily temperature (°C) and relative humidity (%) during first cycle (April to July 2010) in the custom built hut growth chamber

Date	Temp. (°C)	RH (%)	Date	Temp. (°C)	RH (%)	Date	Temp. (°C)	RH (%)	Date	Temp. (°C)	RH (%)
04/22/2010	22	67	05/13/2010	19	97	06/03/2010	12	81	06/24/2010	13	75
04/23/2010	17	92	05/14/2010	19	91	06/04/2010	13	86	06/25/2010	13	61
04/24/2010	16	98	05/15/2010	19	93	06/05/2010	15	70	06/26/2010	14	54
04/25/2010	16	99	05/16/2010	20	87	06/06/2010	14	77	06/27/2010	14	63
04/26/2010	18	99	05/17/2010	18	92	06/07/2010	15	75	06/28/2010	16	95
04/27/2010	18	99	05/18/2010	16	85	06/08/2010	16	74	06/29/2010	15	95
04/28/2010	20	92	05/19/2010	15	86	06/09/2010	16	80	06/30/2010	15	87
04/29/2010	20	92	05/20/2010	16	89	06/10/2010	16	95	07/01/2010	13	98
04/30/2010	20	97	05/21/2010	16	89	06/11/2010	16	92	07/02/2010	15	97
05/01/2010	20	92	05/22/2010	16	75	06/12/2010	15	82	07/03/2010	15	85
05/02/2010	20	87	05/23/2010	16	72	06/13/2010	17	62	07/04/2010	15	78
05/03/2010	19	91	05/24/2010	15	77	06/14/2010	16	61	07/05/2010	15	87
05/04/2010	20	83	05/25/2010	15	81	06/15/2010	14	50	07/06/2010	14	90
05/05/2010	20	77	05/26/2010	15	81	06/16/2010	10	54	07/07/2010	14	94
05/06/2010	19	78	05/27/2010	16	78	06/17/2010	9	70	07/08/2010	15	96
05/07/2010	20	88	05/28/2010	14	74	06/18/2010	10	79	07/09/2010	14	85
05/08/2010	20	97	05/29/2010	15	79	06/19/2010	11	74	07/10/2010	14	77
05/09/2010	22	84	05/30/2010	15	84	06/20/2010	11	59	07/11/2010	15	60
05/10/2010	21	83	05/31/2010	15	91	06/21/2010	11	63	07/12/2010	14	63
05/11/2010	20	69	06/01/2010	13	90	06/22/2010	12	65	07/13/2010	10	86
05/12/2010	18	80	06/02/2010	13	83	06/23/2010	12	68	07/14/2010	11	76

Table A2: Mean daily temperature (°C) and relative humidity (%) during first cycle (September to November 2010) in the custom built hut growth chamber

Date	Temp. (°C)	RH (%)	Date	Temp. (°C)	RH (%)	Date	Temp. (°C)	RH (%)
09/10/2010	20	83	10/01/2010	23	45	10/22/2010	24	57
09/11/2010	16	84	10/02/2010	25	46	10/23/2010	24	62
09/12/2010	20	67	10/03/2010	24	52	10/24/2010	25	71
09/13/2010	20	51	10/04/2010	21	82	10/25/2010	26	58
09/14/2010	20	38	10/05/2010	19	94	10/26/2010	20	78
09/15/2010	21	28	10/06/2010	20	94	10/27/2010	18	98
09/16/2010	22	38	10/07/2010	19	100	10/28/2010	19	99
09/17/2010	18	85	10/08/2010	21	92	11/08/2010	27	53
09/18/2010	20	61	10/09/2010	20	96	11/09/2010	24	78
09/19/2010	22	38	10/10/2010	25	60	11/10/2010	20	100
09/20/2010	21	40	10/11/2010	28	46	11/11/2010	20	98
09/21/2010	22	49	10/12/2010	21	86	11/12/2010	24	82
09/22/2010	23	41	10/13/2010	18	92	11/13/2010	22	97
09/23/2010	22	57	10/14/2010	19	100	11/14/2010	21	93
09/24/2010	24	44	10/15/2010	16	100	11/15/2010	21	94
09/25/2010	23	59	10/16/2010	17	83	11/16/2010	23	91
09/26/2010	24	47	10/17/2010	19	74	11/17/2010	20	100
09/27/2010	19	65	10/18/2010	19	86	11/18/2010	19	86
09/28/2010	16	78	10/19/2010	21	82	11/19/2010	20	98
09/29/2010	18	77	10/20/2010	22	87	11/20/2010	22	95
09/30/2010	20	59	10/21/2010	23	69	11/21/2010	24	88

APPENDIX B

Table B1: Mean temperature (°C) and relative humidity (%) of ARC-TSC experimental farm in Nelspruit (April to October 2008)

Date	Temp. (°C)	RH (%)
April	19	75
May	19	70
June	16	68
July	16	61
August	18	63
September	20	63
October	22	65

Table B2: Mean temperature (°C) and relative humidity (%) of ARC-TSC experimental farm in Nelspruit (April to October 2010)

Date	Temp. (°C)	RH (%)
April	21	75
May	19	65
June	15	58
July	15	61
August	17	57
September	21	57
October	22	65

APPENDIX C

Table C1: Mean daily temperature (°C) and relative humidity (%) in custom built hut taken from June to August 2012

Date	Temp. (°C)	RH (%)	Date	Temp. (°C)	RH (%)
06/04/2012	15	57	07/04/2012	15	56
06/05/2012	13	66	07/05/2012	14	71
06/06/2012	13	56	07/06/2012	13	78
06/07/2012	14	64	07/07/2012	14	70
06/08/2012	15	41	07/08/2012	15	75
06/09/2012	15	32	07/09/2012	16	88
06/10/2012	13	31	07/10/2012	16	72
06/11/2012	11	52	07/11/2012	15	55
06/12/2012	12	54	07/12/2012	15	71
06/13/2012	12	56	07/13/2012	14	81
06/14/2012	14	71	07/14/2012	15	76
06/15/2012	13	77	07/15/2012	14	38
06/16/2012	13	67	07/16/2012	11	65
06/17/2012	14	65	07/17/2012	12	64
06/18/2012	15	61	07/18/2012	12	64
06/19/2012	14	77	07/19/2012	11	65
06/20/2012	14	69	07/20/2012	12	60
06/21/2012	14	77	07/21/2012	14	41
06/22/2012	15	89	07/22/2012	15	33
06/23/2012	16	81	07/23/2012	13	66
06/24/2012	17	45	07/24/2012	16	91
06/25/2012	14	39	07/25/2012	16	75
06/26/2012	12	56	07/26/2012	16	65
06/27/2012	14	48	07/27/2012	16	60
06/28/2012	12	58	07/28/2012	16	58
06/29/2012	13	60	07/29/2012	17	55
06/30/2012	16	44	07/30/2012	17	55
07/01/2012	15	48	07/31/2012	17	43
07/02/2012	15	54	08/01/2012	17	47
07/03/2012	10	74	08/02/2012	14	66

APPENDIX D

Table 3.8. Analysis of variance for germination (%) and seedling length (mm) of lettuce seeds from 0 month of wheat straw storage period

		Mean Square [Z]							
Source of variation	DF	Germination (%)				Seedling length (mm)			
		Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
Treatment	4	4352.00**	946.80**	661.2**	713.2*	3.9552**	34.7075**	81.162**	150.640**
Error	15	33.07**	70.67**	109.3**	215.7*	0.01901**	0.8867**	1.807**	4.400**
p-value		<.001	<.001	0.004	0.039	<.001	<.001	<.001	<.001
Se		5.75	8.41	10.46	14.69	0.1379	0.942	1.344	2.098
LSD		8.67	12.67	15.76	22.14	0.2078	1.419	2.026	3.161
CV%		31.9	71.2	90.1	59.7	25.6	40.4	30.3	30.3

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 3.9. Analysis of variance for germination (%) and seedling length (mm) of lettuce seeds from 3 months of wheat straw storage period

		Mean Square [Z]							
Source of variation	DF	Germination (%)				Seedling length (mm)			
		Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
Treatment	4	1464.80**	1245.20**	804.8*	90.00^{NS}	3.46092**	32.5169**	61.221**	108.072**
Error	15	30.67**	70.93**	200.5*	56.00^{NS}	0.08938**	0.5331**	1.648**	3.806**
p-values		<.001	<.001	0.021	0.224 n/s	<.001	<.001	<.001	<.001
Se		5.54	8.42	14.16	7.48	0.2990	0.730	1.284	1.951
LSD		8.35	12.69	21.34		0.4506	1.100	1.935	2.940
CV%		54.3	30.5	52.1	124.7	60.6	27.9	21.9	16.4

[Z]F-values significant (*), highly significant (**) or not significant (NS) at 5% level of probability

Table 3.10. Analysis of variance for germination (%) and seedling length (mm) of lettuce seeds from 6 months of wheat straw storage period

		Mean Square [Z]							
Source of variation	DF	Germination (%)				Seedling length (mm)			
		Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
Treatment	4	2508.80**	2366.8**	250.00**	214.00**	5.11060**	30.615**	57.041**	110.866**
Error	15	27.73**	278.9**	56.00**	36.00**	0.08201**	2.101**	2.823**	8.034**
p-values		<.001	<.001	0.014	0.005	<.001	<.001	<.001	<.001
Se		5.27	16.70	7.48	6.07	0.2864	1.449	1.680	2.834
LSD		7.94	25.17	11.28	9.14	0.4316	2.185	2.532	4.272
CV%		47.0	30.3	66.8	75.8	56.6	25.3	16.9	17.6

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 3.11. Analysis of variance for mycelia growth (mm) and colony diameter (mm) of oyster mushroom spawn from 0 month of wheat straw storage period

		Mean Square [Z]	
Source of variation	DF	Mycelia growth (mm)	Colony diameter (mm)
Treatment	39	837.87846**	781.50513**
Error	60	16.05500**	9.63833**
p-values		<.0001	<.0001
Se		0.1025037	0.10300269
LSD		6.13	5.7079
CV%		11.82665	10.31417

[Z]F-values highly significant (**) at 5% level of probability

Table 3.12. Analysis of variance for mycelia growth (mm) and colony diameter (mm) of oyster mushroom spawn from 6 months of wheat straw storage period

		Mean Square [Z]	
Source of variation	DF	Mycelia growth (mm)	Colony diameter (mm)
Treatment	39	587.95756**	507.84757**
Error	60	11.18508**	9.79197**
p-values		<.0001	<.0001
Se		0.10205177	1.10284957
LSD		3.6461	3.2334
CV%		21.90184	22.21347

[Z]F-values highly significant (**) at 5% level of probability

Table 4.3. Analysis of variance for rate of colonisation (%) for oyster mushroom from different drainage periods of wheat straw

		Mean Square [Z]							
Source of variation	DF	Rate of colonisation (%)							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Treatment	7	84.2857143^{ns}	389.642857**	800.000000**	514.285714**	516.071429**	461.964286**	411.428571*	153.571
Error	12	32.5000000^{ns}	34.791667**	60.000000**	83.750000**	84.375000**	119.166667**	120.000000*	73.3333
p-values		0.0705	0.0002	<.0001	0.0032	0.0033	0.0194	0.0297	0.1245
Se		0.29975637	0.27481127	0.28410094	0.26058869	0.24428622	0.24731217	0.26692124	0.26537
LSD		8.7831	9.0875	11.934	14.099	14.152	16.818	16.877	13.193
CV%		30.00462	14.93277	13.35511	14.88049	12.66977	13.51870	12.44824	9.35900
R^2		0.602041	0.867250	0.886076	0.781759	0.781081	0.693380	0.666667	0.54987

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 4.4. Analysis of variance for number of clusters, caps and fresh mass (g) for oyster mushroom from different drainage periods of wheat straw

		Mean Square [Z]		
Source of variation	DF	Number of clusters	Number of caps	Fresh mass (g)
Treatment	7	24.7000000^{ns}	909.34286^{ns}	71875.245^{ns}
Error	12	29.6083333^{ns}	1420.05000^{ns}	195598.665^{ns}
p-values		0.5794	0.7159	0.9043
Se		0.24959941	0.25949098	0.26036067
LSD		8.3832	58.057	681.38
CV%		34.65831	31.66685	34.19639
R^2		0.327338	0.271956	0.176516

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 5.4. Analysis of variance for rate of colonisation (%) for oyster mushroom from different pasteurisation methods

		Mean Square[Z]													
Source of variation	DF	Rate of colonisation (%)													
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Treatment	7	212.857**	289.107**	313.929^{ns}	375.357^{ns}	604.107*	617.143*	557.857^{ns}	475.179^{ns}	354.821^{ns}	248.036^{ns}	252.500^{ns}	227.500^{ns}	203.750^{ns}	209.643^{ns}
Error	12	49.167**	70.000**	136.042^{ns}	201.458^{ns}	215.417*	208.333*	228.333^{ns}	183.125^{ns}	148.333^{ns}	177.292^{ns}	167.708^{ns}	169.375^{ns}	182.292^{ns}	182.292^{ns}
p-values		0.0130	0.0155	0.0972	0.1640	0.0561	0.0475	0.0833	0.0704	0.0883	0.2905	0.2544	0.3115	0.4123	0.3962
Se		0.2408	0.2431	0.2558	0.2787	0.3322	0.3111	0.2764	0.2778	0.2761	0.2926	0.2931	0.2917	0.2885	0.2885
LSD		10.803	12.89	17.97	21.867	22.612	22.237	23.28	20.849	18.764	20.514	19.952	20.051	20.801	20.801
CV%		20.623	20.039	23.803	25.574	22.844	21.543	21.742	18.104	15.368	15.528	14.231	14.069	14.479	14.440
R^2		0.72	0.71	0.57	0.52	0.62	0.63	0.59	0.60	0.58	0.45	0.47	0.44	0.39	0.40

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 5.4. Analysis of variance for rate of contamination from pasteurisation methods

		Mean Square [Z]				
Sources of variation	DF	Rate of contamination				
		Week 1	Week 2	Week 3	Week 4	Week 5
Treatment	7	16.964^{ns}	47.143^{ns}	176.071^{ns}	328.750^{ns}	478.929^{ns}
Error	12	17.083^{ns}	29.167^{ns}	116.042^{ns}	218.542^{ns}	301.458^{ns}
p-values		0.4802	0.2218	0.2507	0.2548	0.2295
Se		0.2416	0.2471	0.2443	0.2459	0.2435
LSD		6.3678	8.3205	16.596	22.776	26.75
CV%		183.6977	135.0154	143.6302	144.2259	144.6880
R^2		0.37	0.49	0.47	0.47	0.48

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 5.5. Analysis of variance for number of flushes, caps and fresh mass (g) from different pasteurisation methods

		Mean Square[Z]		
Sources of variation	DF	Number of clusters	Number of caps	Fresh mass (g)
Treatment	7	129.800^{ns}	16386.929^{ns}	717917.604^{ns}
Error	7	269.286^{ns}	8620.500^{ns}	425942.651^{ns}
p-values		0.8217	0.2080	0.2536
Se		0.2996	0.3025	0.3078
LSD		36.124	204.39	1436.5
CV%		66.7070	44.0032	45.4941
R^2		0.33	0.66	0.63

[Z]F-values not significant (ns) at 5% level of probability

Table 6.6. Analysis of variance for rate of colonisation (%) from different substrates

		Mean Square [Z]										
Sources of variation	DF	Rate of colonisation (%)										
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
Treatment	8	337.500**	307.292**	276.729**	258.333**	203.646**	160.938^{ns}	137.500**	117.708**	63.542^{ns}	27.604^{ns}	27.605^{ns}
Error	15	72.222**	57.153**	40.986**	52.153**	51.111**	52.431^{ns}	29.722**	25.486**	25.486^{ns}	14.652^{ns}	14.653^{ns}
p-values		0.0050	0.0026	0.0008	0.0038	0.0102	0.0292	0.0052	0.0053	0.0606	0.1382	0.1382
Se		0.21969	0.21679	0.21526	0.22539	0.22528	0.22103	0.22417	0.22691	0.22163	0.22837	0.22837
LSD		9.0569	8.0568	6.8228	7.6963	6.221	7.717	5.8101	5.3802	5.3802	4.0795	4.0795
CV%		15.689	12.740	9.682	9.932	8.983	8.539	6.086	5.396	5.211	3.901	3.901
R^2		0.71	0.74	0.78	0.73	0.68	0.62	0.71	0.71	0.57	0.50	0.50

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 6.7. Analysis of variance number of clusters, caps and fresh mass (g) from different substrates

		Mean Square [Z]		
Source of variation	DF	Number of clusters	Number of caps	Fresh mass (g)
Treatment	17	16.608^{ns}	1451.564^{ns}	262796.020^{ns}
Error	10	13.662^{ns}	1058.709^{ns}	188553.768^{ns}
p-values		0.3868	0.3114	0.3017
Se		0.2542	0.2358	0.2571
LSD		4.3467	38.264	510.64
CV%		52.5358	51.2839	67.8299
R^2		0.67	0.70	0.70

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 7.4. Analysis of variance for rate of colonisation as influenced by 1-NAA within the April – June experiment

		Mean Square [Z]											
Sources of variation	DF	Rate of colonisation (%)											
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Treatment	8	114.583^{ns}	114.583^{ns}	120.833^{ns}	127.083^{ns}	49.979^{ns}	73.667^{ns}	74.000^{ns}	38.479^{ns}	16.979^{ns}	9.896^{ns}	10.521^{ns}	11.729*
Error	15	98.888^{ns}	98.889^{ns}	101.944^{ns}	106.597^{ns}	79.344^{ns}	57.975^{ns}	54.175^{ns}	32.942^{ns}	19.433^{ns}	16.711^{ns}	15.919^{ns}	14.008*
p-values		0.3829	0.3829	0.3691	0.3656	0.7412	0.3278	0.2868	0.3780	0.5589	0.7700	0.7174	0.0585
Se		0.2173	0.2173	0.2169	0.2163	0.2157	0.2176	0.2184	0.2178	0.2201	0.2396	0.2402	0.2489
LSD		14.988	14.988	15.217	15.561	13.425	11.476	11.093	8.650	6.644	6.161	6.013	5.641
CV		14.206	13.259	12.555	12.057	9.897	8.213	7.925	6.092	4.552	4.168	4.059	3.801
R^2		0.38	0.38	0.38	0.38	0.25	0.40	0.42	0.38	0.31	0.24	0.26	0.30

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 7.5. Analysis of variance for rate of colonisation (%) as influenced by 1-NAA within the September – November experiment

		Mean Square [Z]											
Sources of variation	DF	Rate of colonisation (%)											
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Treatment	8	106.250**	206.250**	206.250**	210.417**	239.063**	129.167*	96.875^{ns}	115.625^{ns}	99.479^{ns}	85.938^{ns}	53.646^{ns}	31.250^{ns}
Error	15	10.000**	18.889**	18.889**	22.153**	42.500**	47.778*	84.722^{ns}	67.222^{ns}	55.208^{ns}	56.389^{ns}	43.611^{ns}	26.597^{ns}
p-values		<.0001	<.0001	<.0001	0.0001	0.0020	0.0461	0.3910	0.1739	0.1550	0.2294	0.3469	0.3745
Se		0.2182	0.2329	0.2329	0.2238	0.2356	0.2536	0.2329	02341	0.2399	0.2353	0.2429	0.2461
LSD		4.7661	6.5503	6.5503	7.0937	9.8255	10.418	13.873	12.357	11.199	11.318	9.953	7.773
CV		21.081	8.149	6.862	6.664	8.692	8.919	10.469	9.026	7.943	7.836	6.832	5.278
R^2		0.85	0.85	0.85	0.83	0.75	0.59	0.38	0.48	0.49	0.44	0.40	0.39

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 7.6. Analysis of variance for rate of contamination (%) as influenced by 1-NAA within the April – June and September – November experiments

		Mean Square [Z]							
Sources of variation	DF	Rate of contamination (%)							
		April – June months				September – November months			
		Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4
Treatments	11	0.037^{ns}	3.115*	2.987^{ns}	3.079^{ns}	0.037^{ns}	3.115*	2.987^{ns}	2.919^{ns}
Error	12	0.046^{ns}	1.141*	1.248^{ns}	1.181^{ns}	0.046^{ns}	1.141*	1.248^{ns}	1.268^{ns}
p-values		0.6345	0.0492	0.0745	0.0635	0.6345	0.0492	0.0745	0.0838
Se		0.1629	0.3764	0.3548	0.3858	0.1629	0.3764	0.3548	0.3821
LSD		0.4795	2.3909	2.5006	2.5004	0.4795	2.3909	2.5006	2.521
CV%		514.072	197.181	191.495	156.234	514.072	197.181	191.495	168.926
R^2		0.42	0.71	0.69	0.72	0.43	0.71	0.69	0.68

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 7.7. Analysis of variance for number of clusters, caps and fresh mass (g) of oyster mushroom produced from wheat straw treated with different levels of 1-NAA within April – June and Septermber - November months

		Mean Square [Z]					
Sources of variation	DF	April – June months			September – November months		
		Number of clusters	Number of caps	Fresh mass (g)	Number of clusters	Number of caps	Fresh mass (g)
Treatment	8	46.229^{ns}	2840.167^{ns}	243015.104^{ns}	261.899^{ns}	16634.111**	581353.372^{ns}
Error	15	60.275^{ns}	2993.375^{ns}	135293.939^{ns}	264.959^{ns}	2777.937**	309935.790^{ns}
p-values		0.6366	0.5076	0.1563	0.4929	0.0041	0.1648
Se		0.2198	0.2203	0.2352	0.2925	0.2780	0.2529
LSD		11.701	82.459	554.37	28.007	90.686	957.88
CV%		45.1159	39.2316	25.3048	52.935	19.187	23.801
R^2		0.29	0.34	0.49	0.42	0.81	0.58

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 8.7. Analysis of variance for fresh mass (g), qualityRank, disorderRank and colourRank at 2°C for the May to July trial

		Mean Square [Z]			
Sources of variation	DF	Fresh mass (g)	QualityRank	DisorderRank	ColourRank
Treatment	46	2153.0560**	285209.77**	34471.92^{ns}	285595.90**
Error	367	62.3282**	30047.51**	26154.19^{ns}	33135.90**
p-values		<.0001	<.0001	0.0887	<.0001
Se		0.0492	-	-	-
LSD		2.899	58.976	60.123	58.394
CV%		23.019	39.115	37.776	40.579
R^2		0.81	0.54	0.14	0.52

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 8.8. Analysis of variance for fresh mass (g), qualityRank, disorderRank and colourRank at 5°C for the May to July trial

		Mean Square			
Sources of variation	DF	Fresh mass (g)	QualityRank	DisorderRank	ColourRank
Treatment	42	1710.4693**	229749.85**	35556.14^{ns}	242847.48**
Error	353	63.3296**	31277.19**	41907.54^{ns}	31486.52**
p-values		<.0001	<.0001	0.7372	<.0001
Se		0.0505	-	-	-
LSD		3.965	49.495	64.610	53.144
CV%		21.687	40.722	43.891	40.319
R^2		0.76	0.47	0.09	0.48

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 8.9. Analysis of variance for fresh mass (g), qualityRank, disorderRank and colourRank at room temperature for the May to July trial

		Mean Square [Z]			
Sources of variation	DF	Fresh mass (g)	QualityRank	DisorderRank	ColourRank
Treatment	47	1585.9477**	89335.05**	65818.24**	109840.35**
Error	192	32.0938**	49818.94**	39767.14**	50015.99**
p-values		<.0001	0.0032	0.0097	0.0001
Se		0.0648	-	-	-
LSD		1.731	79.901	103.48	80.018
CV%		23.880	27.286	25.211	27.900
R^2		0.92	0.31	0.29	0.35

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 8.10. Analysis of variance for fresh mass (g), qualityRank, disorderRank and colourRank at 2°C for the October to November trial

		Mean Square [Z]			
Sources of variation	DF	Fresh mass (g)	QualityRank	DisorderRank	ColourRank
Treatment	40	2084.8362**	250147.30**	86557.07^{ns}	257289.83**
Error	430	63.1386**	60170.70**	62194.91^{ns}	64820.21**
p-values		<.0001	<.0001	0.0615	<.0001
Se		0.0462	-	-	-
LSD		2.4293	114.34	106.42	110.05
CV%		21.932	51.880	51.642	16.752
R^2		0.75	0.28	0.11	0.89

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 8.11. Analysis of variance for fresh mass (g), qualityRank, disorderRank and colourRank at 5°C for the October to November trial

		Mean Square [Z]			
Sources of variation	DF	Fresh mass (g)	QualityRank	DisorderRank	ColourRank
Treatment	42	1171.8292**	134532.39**	90770.53^{ns}	148967.11**
Error	428	44.6186**	75618.83**	80178.44^{ns}	79174.70**
p-values		<.0001	0.0027	0.2698	0.0011
Se		0.04629	-	-	-
LSD		2.4661	80.462	76.812	84.024
CV%		17.0811	54.0481	50.8566	54.738
R^2		0.72	0.15	0.09	0.16

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 8.12. Analysis of variance for fresh mass (g), qualityRank, disorderRank and colourRank at room temperature for the October to November trial

		Mean Square [Z]			
Sources of variation	DF	Fresh mass (g)	QualityRank	DisorderRank	ColourRank
Treatment	43	1674.4569**	27816.502^{ns}	30430.790^{ns}	43480.532^{ns}
Error	191	26.3904**	27717.806^{ns}	29429.206^{ns}	31786.000^{ns}
p-values		<.0001	0.4740	0.4244	0.0803
Se		0.06639	-	-	-
LSD		1.6974	66.661	62.969	77.821
CV%		19.8865	16.8003	19.6435	18.5308
R^2		0.93	0.18	0.19	0.23

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

Table 9.3. Analysis of variance for dry mass (g), quality, colour rating and moisture content of oyster mushrooms as influenced by drying method and preservative used

		Mean Square [Z]			
Sources of variation	DF	Dry mass (g)	Quality	Colour rating	Moisture content
Treatment	10	19.1206**	6.8815**	1.9926**	70.2774**
Error	16	0.2475**	0.3287**	0.3704**	12.3344**
p-values		<.0001	<.0001	0.0015	0.0011
Se		0.21041	-	-	0.23298
LSD		0.4971	0.5729	0.6082	3.5097
CV%		3.4886	20.3682	19.7972	28.4721
R^2		0.98	0.93	0.77	0.78

[Z]F-values significant (*), highly significant (**) or not significant (ns) at 5% level of probability

APPENDIX E

TRAINING MANUAL ON OYSTER MUSHROOM PRODUCTION FOR SMALL-SCALE FARMERS

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1. Introducing oyster mushrooms

Oyster mushrooms belong to the genus *Pleurotus* which is cultivated worldwide and include *Pleurotus ostreatus*, *Pleurotus citrinopileatus, Pleurotus eryngii, Pleurotus pulmonarius*, *Pleurotus populinus,* and *Pleurotus euosmus*. In this training manual, the focus will be on *Pleurotus ostreatus* or oyster mushroom, which is also known as the tree species. Oyster mushrooms refer to the shape of the cap, which resembles oysters or bivalves found in the sea (Phillips, 2006) as depicted in Figure 1.

Figure 1: Harvested oyster mushrooms showing the shape of the caps

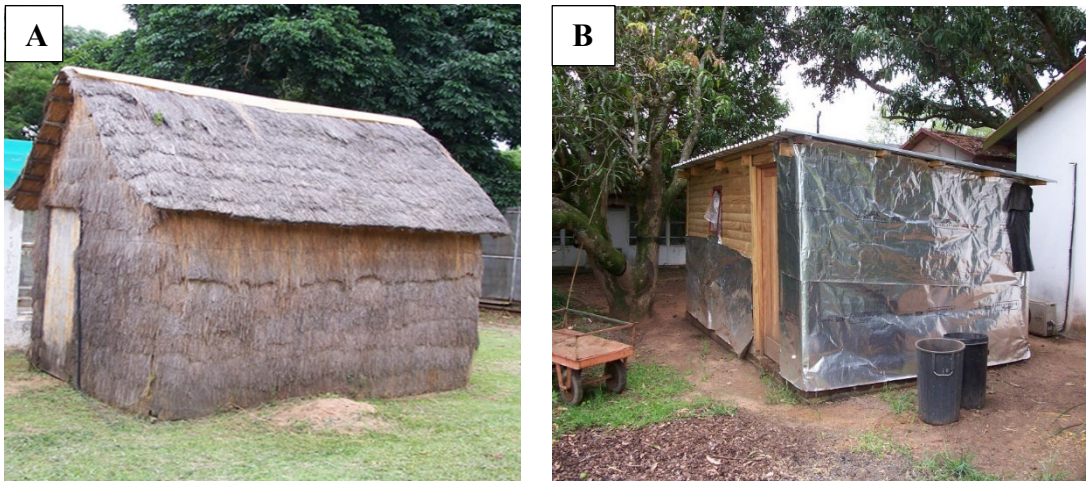
Oyster mushrooms are saprophytes (a plant, fungus, or microorganism that lives on dead or decaying organic matter) that are generally found on hardwoods such as dead and decaying wooden logs or sometimes on dying trunks of hardwoods (deciduous) or soft (coniferous) woods. The caps range from 5 to 25 cm in size and are convex (curved outwards) in shape when young and flatten out further and turn up as the mushroom senesces. Their colour ranges from white, yellow, brown, tan, blue, and even pink. Certain strains form clusters, while others grow as individuals (Stamets, 2000). These mushrooms are said to be extremely delicious and traditionally used to strengthen veins and relax tendons (Hobbs, 2002). They have the potential to inhibit tumours, improve liver and kidney functioning and aid in gastrointestinal disorders (Stamets, 2000). Oyster mushrooms have a relatively high folic acid content, which is capable of protecting humans against diseases such as anaemia, diabetes and high blood pressure (Vetayasuporn, 2004).

It is relatively easy and economically sustainable to farm with oyster mushrooms. The turnaround time for harvesting is very short, only just over a month. Harvesting can continue for two to three months in matured oyster mushrooms (Figure 2). The need for fresh oyster mushrooms and its beneficiation has increased quickly leading to expansion of market demand. They are highly tradable and require low start-up fund. Small-scale farmers can utilize any old buildings which are not currently being used for mushroom production. The setting up of structures such as hut-houses (sheds) are not costly as the grass can be collected from the veld, while the main poles can be chopped from old trees in the bush and the roof can be made out of grass with thin poles and ropes for strengthening the structure. In short, material from old structures can be recycled for this purpose.

Figure 2: Tree oyster mushroom growing in industrial polyethylene bags (410 mm x 750 mm x 45 µm, width, length and thickness)

To start producing oyster mushrooms, you will need:

1) A growth room such as a shed which may be a hut-house (Figure 3A) or a wooden house (Figure 3B),



Figures 3A: A hut-house or **3B:** wooden house

2) A growth medium such as wheat straw (Figure 4A), woodchips (Figure 4B), thatch grass (Figure 4C), rye straw, maize straw, millet straw, etc.

Figures 4A: Wheat straw, **4B:** Thatch grass and **4C:** Wood chips

3) Quality spawn or healthy spawn (Figure 5),

Figure 5: Oyster mushroom spawn inoculated in sorghum seeds

4) Available and sustainable water supply with micro-jet irrigation system (Figure 6) where possible, or alternatively keeping the floor bedding moist at all times,

Figure 6: Planter bags with micro-jet irrigation system clearly indicated in wooden house

5) Drums for pasteurisation (Figure 7),

Figure 7: Steel drum for pasteurisation

6) Wood for making a fire as shown in Figure 7,

7) Planting tables and industrial plastic bags been filled with wheat straw substrate (Figure 8),

Figure 8: Planting table with industrial plastic bags

8) Implements for cutting the plastic bags as shown in Figure 9,

Figure 9: Razor blades and scissor

9) Sodium hypochlorite (Figure 10),

Figure 10: Sodium hypochlorite (bleach), 3.5% m/v

10) Brown bags for harvesting as depicted in Figure 8,

Figure 8: Brown bag (SO12) for packing harvested oyster mushrooms

2. Ideal Infrastructure for mushroom cultivation

There are plenty of structures such as hut house, wooden house and unused old houses that could be ideal for oyster mushroom cultivation (refer to Figure 3A and 3B).

The general requirements for these structures are sufficient aeration and with the inside environment being able to be controlled. These will be ideal or optimum conditions suited for maximum oyster mushroom growth and cluster production.

Thatch grass can be collected from the veld with ease, although treated poles for the supporting structure may need to be purchased. Whereas for old buildings, one could just clean and make them ready for propagation of oyster mushrooms.

Oyster mushroom planter bags inside a *hut-house* on shelves with a micro-jet irrigation system above, adjusted to a flow rate of 17 mL·5 min^{-1} (at a pressure range of 60 to 70 kPa), are displayed in Figure 6. The shelves are made in such a way that a person can move freely within the structure without bumping into the growth bags which could cause damage to the growing mushrooms. This will allow one to monitor the planter bags and perform harvesting with ease. The floor is normally covered with river sand at ± 2 cm thickness for proper drainage with perforations on the wall to control the amount of water in the sand (damp not wet) to avoid structural damage and oyster mushroom spoilage. If the small-scale grower does not have access to running water, they can opt for making the floor wet with water in containers on a daily basis to lower the temperature relative to the outside and to maintain high relative humidity in the growing chamber. The optimum growth conditions for oyster mushroom are 20 – 22°C temperature and relative humidity between 75 – 85% in the growing chamber.

A wooden house structure with a corrugated iron roof and silver foil covering on the outside, in order to regulate temperature for growing oyster mushrooms, is shown in Figure 3B. This structure is not so suitable as aeration is restricted and it can be costly to construct. Treated wooden planks for protecting from termites or regular wetting from irrigation water must be purchased from reputable retailers. For this wooden house, there must be a foundation constructed using bricks with cement (Figure 9) and corrugated iron as shown in Figure 3B.

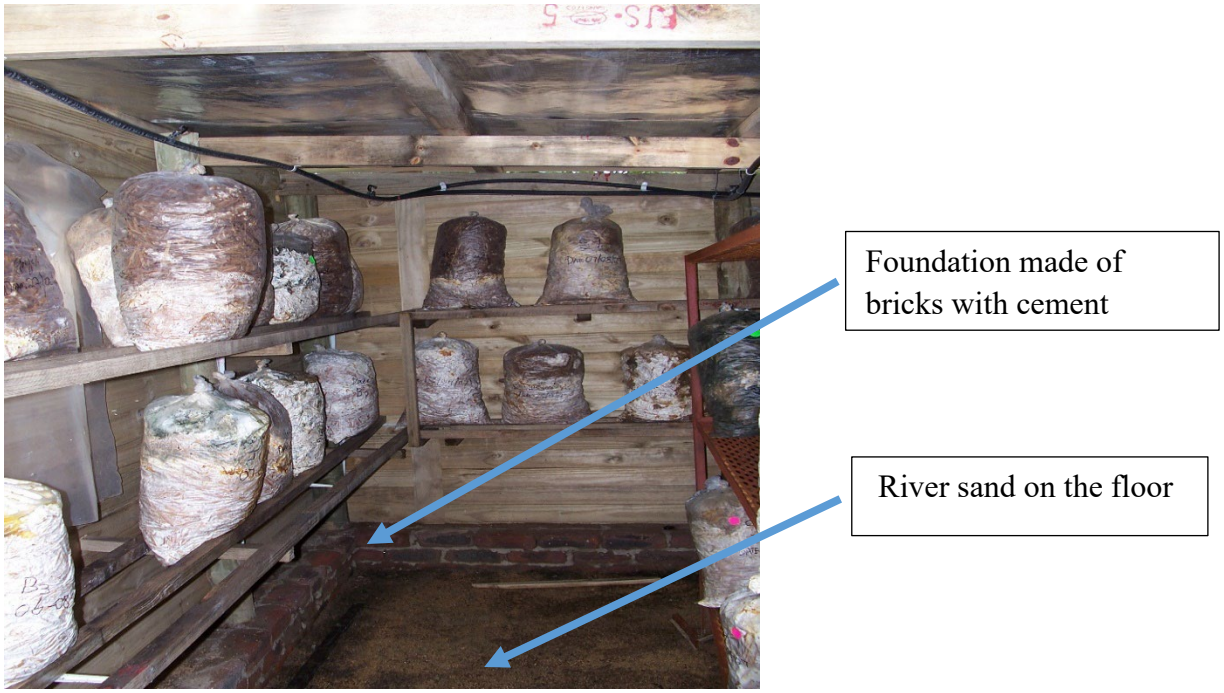


Figure 9. Oyster mushrooms planter bags inside a wooden house on the shelves with a micro-jet irrigation system, adjusted with a flow rate of 17mL·5min^{-1} (at a pressure range of 60 to 70 kPa) in the morning and late afternoon on a daily basis. The floor is normally covered with river sand for proper drainage and to make it easy to disinfect for getting rid of parasitic fungi or spores. The growth chamber is disinfected by spraying the floor and the shelves with pure solution of sodium hypochlorite [3.5% (m/v) – JIK brand]. If the small-scale grower does not have running water, they can opt for making the floor wet by pouring water to the floor on a daily basis in the morning and late afternoon to keep the temperature lower and increase the relative humidity in the growing chamber.

Description of the hut-house

The hut-house was 6 x 3 m (18 m^2) in dimension as an appropriate structure for maximising the yield, although for production it can be bigger than this dimension. This growth chamber can be able to accommodate ±25 planter bags (410 mm x 750 mm x 45 µm) as shown outside the hut-house in Figure 3A and inside the hut-house in Figure 2. The floor must be covered with river sand for keeping proper moisture in the house (quicker drainage and still wet for 3-5 days). Regular changing of river sand is needed after bringing in new planted bags to the growth house (after each planting cycle of oyster mushroom) to avoid build-up of unwanted spores in the growth chamber. Cleaning removes contaminants while promoting growth of quality

mushroom. The over mature oyster mushrooms which are not yet harvested normally release spores to the floor and if the same river sand is used for the next batch of planter bags, these spores can contaminate the other crop. The hut-house itself can be built from grass with supporting treated poles for protecting the structure from collapsing and avoiding being eaten by termites. Inside the structure, there must be sufficient light during the day to be able to work but avoiding direct sunlight as this will cause spoilage of the mushrooms.

Description of the wooden-house

The wooden-house was 10 x 6 m^2 in dimension for commercial purposes. It is advisable to have separate houses in case where there is a breakout of contamination in the planted bags. This growth chamber can be able to accommodate ±50 planter bags (410 mm x 750 mm x 45 µm). The aluminium foil used on the outer wall is for sunlight reflection to insulate the structure, thereby maintaining a suitable temperature and relative humidity in the house. A single window frame without windowpane is recommended for proper aeration by loosely covering with clear plastic and orientated for ease of gaining access to the crops.

Compared to the wooden-house, the hut-house is the most ideal structure for cultivation of oyster mushrooms because of the ease of controlling and maintaining the essential environmental parameters.

3. Preparation or procurement of spawn

The ideal and correct procedure is to prepare or procure a pure culture of *Pleurotus ostreatus*, to provide quality yield of identified mushroom species. Poor quality spawn will result in a poor quality crop and poor harvest stemming from contamination. There are two important methods for spawn acquisition. Spawn can either be bought from a reputable company with an excellent track record such as ***Exotic Spawn Cc*** **(**http://www.zibb.co.za/food/profile/exotic+spawn**);** ***Funguys Gourmet*** **(**https://funguys.co.za/**);** ***Mushrush*** (https://mushrush.co.za/) etc., or alternatively, spawn can be produced by following the correct agronomic procedures. If you are going to purchase spawn, then you need to consider the following from the company supplying the spawn: Spawn bags must be sealed properly on top and on the inoculation side; the spawn must show growth of mycelia already with white threads and the bags must be properly sealed with no holes in the plastic bags.

The correct agronomic procedure to produce spawn is as follows:

1) Using a sterile blade, and wearing rubber gloves, a hair net and face mask to keep the environment sterile, cut out an inside section of the mushroom stem or cap and place it in an empty sterile Petri dish with agar. Agar can be sourced or prepared from a Potato Dextrose Agar (PDA) using the following method: Wash two medium sized potatoes (200 g) and cut them into 2.5 cm cubes and place in a clean pot and add 1.25 litres of clean water. Bring to boil and keep the simmering temperature for 10 – 15 minutes. At this time, remove the pot from the stove and allow to cool down for 5 minutes. Strain the water away from the potatoes (use a fine sieve) and use this water to prepare your potato infusion. Fill this potato infusion into empty petri-dishes (Figure 10).

Figure 10: Petri-dish

2) White spores will start to form around the mushroom stem in approximately 2 - 3 days.
3) When the dish is well populated, transfer the spores to a bag with well-cooked sorghum (Figure 11) with drained water, that will continue to facilitate spore development. One can also use wheat, rye, brown rice, white rice, wild bird seed and even popcorn. The recommended quantities are 1 spoon of spores into 1 litre of sorghum.

Figure 11: Cooked sorghum

4) When the bag is fully colonised, between 40 and 60 days from the start of the process, the spores can then be planted in straw-filled grow bags for mushroom production. Good quality mycelium and sorghum are critical for an excellent spawn (Figure 12) that will produce good mushroom yields. This can be described as viable (higher percentage of colonisation of the substrate, fresh and rigid, good mycelia strain and appear white with no spots for contamination). During spawning, it is vitally important to weigh spawn during planting of oyster mushroom in order to determine input-output as shown in Figure 13.

Figure 12: Oyster mushroom spawn (*Pleurotus ostreatus* - tree species – HK35 strain) which refers to spores of mushroom inoculated into pasteurised sorghum seeds in order to grow. This spawn was purchased from Exotic Spawn (Irene, Rietvlei, Gauteng Province, South Africa) and it is how good quality spawn should look like.

Figure 13: Weighing of oyster mushroom spawn into a plastic bag (1000 g) in order to determine the input-output and for uniformity

4. Substrate choice and preparation

Many species of *Pleurotus* are commonly grown on a wide range of plant based materials such as *Pleurotus ostreatus* which is a type of fungus known to aid in the degradation of wood. Different substrates can be recommended depending on the local availability of agricultural waste as shown in Figures 16; 17 & 18. The ideal media for cultivation of edible fungi must be mould-free and rich in essential nutrients such as nitrogen, phosphorus, potassium, magnesium and iron. The most commonly used substrates include wheat straw, sawdust, cottonseed straw, cereal straw, maize cob, sugarcane bagasse (the dry pulpy residue left after the extraction of juice from sugar cane) and other plant fibres such as wood and agricultural residues. Substrates are normally kept dry at all times.

Quick steps for cultivating oyster mushrooms

Make firewood and place a steel drum on a supporting structure (3 legged stand) (Figure 15)

⬇

Boiling of the substrate (wheat straw, thatch grass or wood chips (Figure 15), etc.)

⬇

Boil (100°C) for 1 hour

⬇

Place the substrate on a planting table, allow to cool and drain the leachate (Figures 16; 17 & 18)

⬇

1 kg of spawn, must be split into three equal portions in an industrial polyethylene bag (410 mm x 750 mm x 45 µm, width, length and thickness) together with substrate and tied at the end with a rubber band (for ease of manipulation in each bag)

⬇

All planted bags should have their edges cut for controlling drainage (Figure 14)

Figure 14: Weighing planter bags with edges cut

⬇

Place the planted bags on shelves in the growing chamber for incubation

Figure 15: Wood chips of eucalyptus and pine trees from a milling company (**A**) and (**B**) a drum during boiling for pasteurising (refers to a process of eliminating competitors and it is done between 60°C and 82°C and at atmospheric pressure but keeping useful nutrients) the substrate

Figure 16: Pasteurised wood chips on a spawning table before inoculation with oyster mushroom spawn

Figure 17: Pasteurised thatch grass on a spawning table before inoculation with oyster mushroom spawn

Figure 18: Pasteurised wheat straw on a spawning table before inoculation with oyster mushroom spawn

The ranking of substrates in terms of suitability for oyster mushroom production from a study conducted at the ARC-TSC Nelspruit is as follows (Table 1):

Table 1: Suitability ranking of different substrates following drying of substrates for a period of one month after being collected and pasteurised for planting and harvesting of oyster mushrooms as determined by a study at the ARC-TSC, Nelspruit

Substrate	Drying period	Ranking
Wheat straw	One month	Very high rate of colonisation, free from contamination after pasteurisation and very high final yield (completely filling the bag)
Thatch grass	One month	High rate of colonisation, free from contamination after pasteurisation and high final yield
Wood chips	One month	Medium rate of colonisation, free from contamination after pasteurisation and medium final yield

Figure 19: White wash (Gluex) lime for pasteurising substrates and for the control of pH level of water and of substrate. Pasteurisation is using heat to eliminate contamination

Figure 20: Filling of 10 kg of substrate into a drum of 225 L (1:22.5, substrate: volume of water) for pasteurisation and wetting of growing media

Figure 21: Adding of white wash (gluex) lime for pasteurising wheat straw in a drum of 225 L filled with water

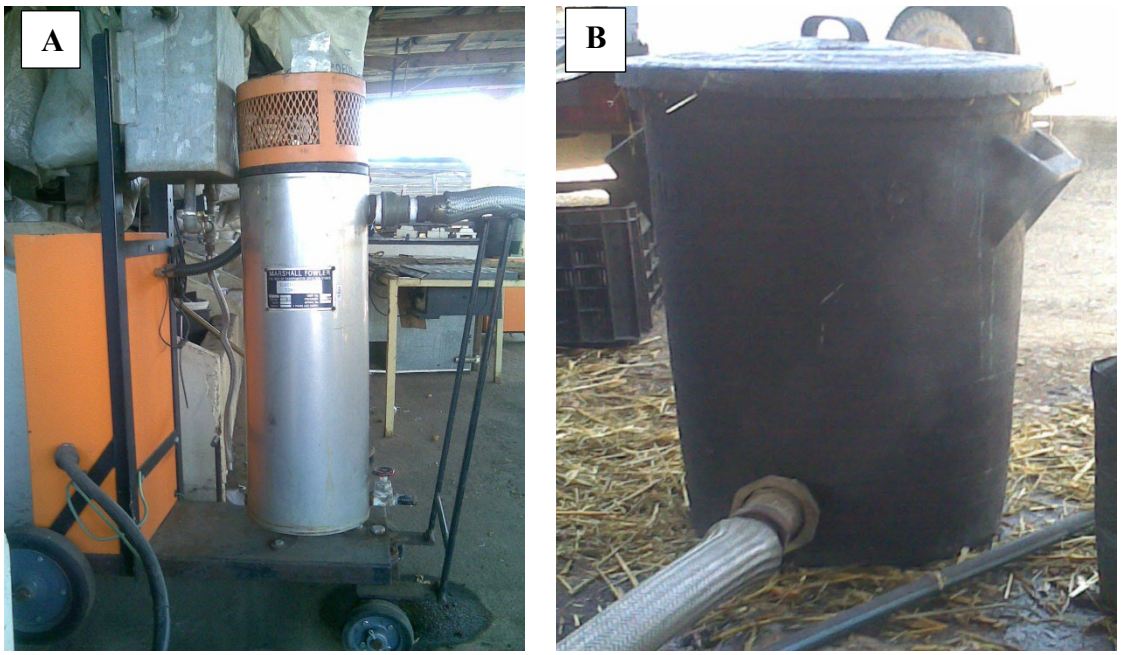


Figure 22A: Electrode steam conditioner machine and **22B:** a 50 L of rubber plastic dust bin used for pasteurising growing media

There are various methods to kill undesirable micro-organisms present in the straw and favour the growth of *Pleurotus* mycelium as shown in Figures 19; 21; 22A & 22B. If the substrate is not sterilised or pasteurised properly, the invader parasitic fungi can colonise the growing medium before the oyster mushroom spawn does. In addition, if invader parasitic fungi can colonise the planter bags first, all contaminated bags should be discarded. Proper sterilisation or pasteurisation ensures good conditions for oyster mushroom spawn to colonise the substrates with ease, as the competitors are killed during the pasteurisation process. The substrate can be pasteurised using various methods such as boiling, steam (Figure 22A & 22B) or chemical sterilisation (Figure 19 & 21) as shown in Table 2. When boiling the substrate for pasteurisation, the substrate should be drained for a period of 1 hour to make sure that the excess extracts have leached. The oyster mushroom spawn is sensitive to excess water, although the substrates need to be wet so that it can be able to colonise or grow on the growing media with ease. Draining of substrates for 1 hour makes sure excess leachate is gotten rid of while keeping the growing media with suitable moisture for optimal colonisation of oyster mushroom spawn.

Table 2: Suitability ranking of different pasteurisation methods for different substrates as determined by a study at the ARC-TSC, Nelspruit

Pasteurisation method	Period	Ranking
Boiling – 10 kg of bale in 225 L of water [Wheat straw:Water (1:2.25 L)]	Maintain boiling point for 1 hour	Very high
Steaming – 10 kg of bale exposed to steam for 5 minutes	5 minutes (100°C)	High
Liming (1.339% substrate mass/v) – 3.01 kg in 225 L of water	Soaked overnight	Medium to high
Sodium hypochlorite (3.5% v/v) – 7.875 L made up to 225 L with water	Soaked overnight	Medium

5. Inoculation of planter bags

Industrial polyethylene bags (410 mm x 750 mm x 45 µm, width, length and thickness) from retailers such as Plastilon (Gezina, Pretoria) should be used with the pasteurised substrates (Figures 24; 25 & 26), e.g. (5.2 kg) (wet) wheat straw rye, brown rice, white rice, wild bird seed and even popcorn together with spawn (1 kg, must be split into three equal portions, i.e. ≈0.33 kg to ensure adequate distribution of spawn throughout the bag, one portion at the bottom, middle and top parts of the polyethylene bag) to make up a total weight of 6.20 kg as shown in Figure 23 and production cycle per year thoroughly explained in Table 3. Alternatively, normal white refuse bags (750 mm x 950 mm x 20 or 28 micron) from any wholesalers or retailers in South Africa can be used. White or clear colour plastic bags allow the farmer to see the rate of mycelia colonisation inside the bags. Cuts [making small crosses (X) from top to bottom after fully colonisation] can then be made in time for oyster mushroom

clusters to sprout. All substrates must be drained for an appropriate time as checked by complete drainage, because the substrate cannot be too wet or too dry for the spawn to colonise the substrate properly. A wet substrate can influence the rate of colonisation by parasitic fungi which can invade the plastic bag. The moisture content is adjusted between 65% to 75% using moisture analyser balance and scale after pasteurisation or disinfection, although this is an expensive apparatus for small-scale farmers. For small-scale farmers, they can try to squeeze the substrates by hand after an hour to check whether the medium is still releasing water or not. Before inoculating the bags, all proper hygiene processes should be strictly adhered to, for example, protective clothing and hairnets should be worn and hands should be washed regularly with detergent. All planted bags should have their edges (at the bottom corners of planter bags) cut using a scissor to make sure that excess water can drain from the bag (Figure 14).

Figure 23: Oyster mushroom spawn measured using a weighing scale before spawning

Figure 24: Planting of oyster mushroom spawn with pasteurised wheat straw into an Industrial polyethylene bag (410 mm x 750 mm x 45 µm, width, length and thickness) on a planting table

Figure 25: Weighing of planted plastic bag using a measuring scale (1 kg of oyster mushroom spawn and 5.2 kg of pasteurised wheat straw) in order to know the amount of substrate used and spawn for uniformity. It can assist the farmer to determine the input – output and the income from the production

Figure 26: Planting bags filled with wood chips and tied with rubber bands to seal the tops of the bags in order to protect them from contamination

6. Crop management (incubation) of oyster mushroom

Before bags are placed in the growing chamber, the room should be completely sterilised using sodium hypochlorite solution (3.5% bleach spray). All the walls, floors and benches should be sprayed. There must be more than sufficient aeration, which could be enhanced by the use of window frame without windowpanes but shielded by plastic or a fan. Direct sunlight should be avoided at all cost, but there must be light for sufficient yield. Once again, this can be achieved by the window frame without windowpanes. The relative humidity within the chamber should

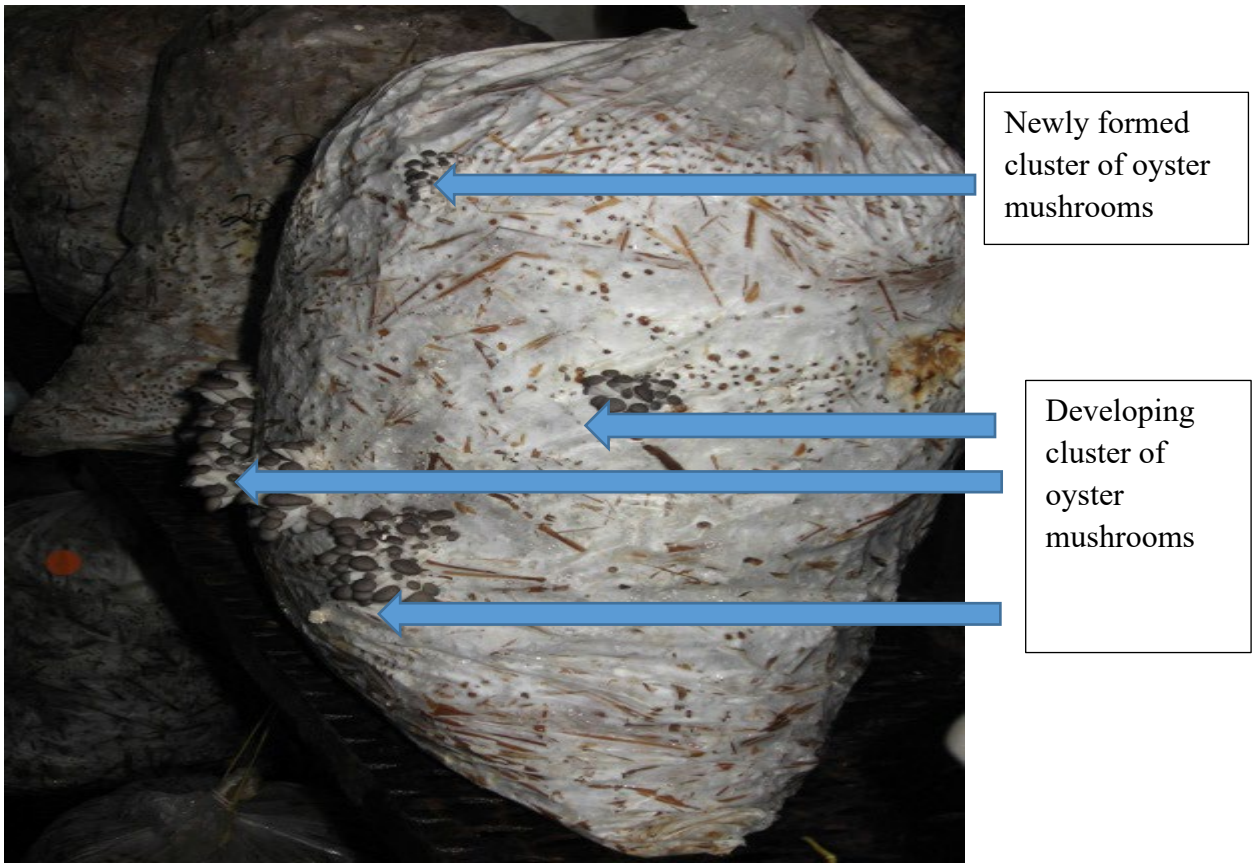
be quite high and this can be achieved by watering the planter bags and growth chamber floor using over-head micro-jet sprays (if there is irrigation infrastructure) on a daily basis in the morning and late afternoon. For a farmer without irrigation infrastructure in place, the sand floor must be watered to field capacity with clean water using buckets in the morning and late afternoon on a daily basis.

After approximately 20 days, full colonisation on the substrate will have occurred (Figure 27A & 27B). Once this has been achieved, a sterilised blade should be used to cut the industrial bag [making small crosses (X) from top to bottom after fully colonisation], so that the clusters can sprout out of the bag without any physical restriction as shown in Figure 28A & 28B. If using sterile scalpel blades or razor blades, they should be discarded after each bag to avoid cross-contamination between bags. Micro-jet sprayers are used to adjust daily average temperature (20 - 30°C) and average relative humidity (70 – 80%) in a hut house. A HOBO data logger or thermometer is used to measure temperature and relative humidity on a daily basis and proper adjustment effected when required as shown in Figure 29A & 29B and Figure 30.

Figure 27A: Full colonisation planter bag, **27B:** Planter bag being cut with blade making small crosses (X) from top to bottom after fully colonisation

Table 3: Production cycle per year for oyster mushrooms. This table shows the ideal season for optimal oyster mushroom production

Number of planted bags	Productions per year					
	April – June months			September – November months		
	Number of clusters	Number of caps	Fresh mass (g)	Number of clusters	Number of caps	Fresh mass (g)
6.20 kg x 4 bags	13	115	1432.15	32	308	2783.43



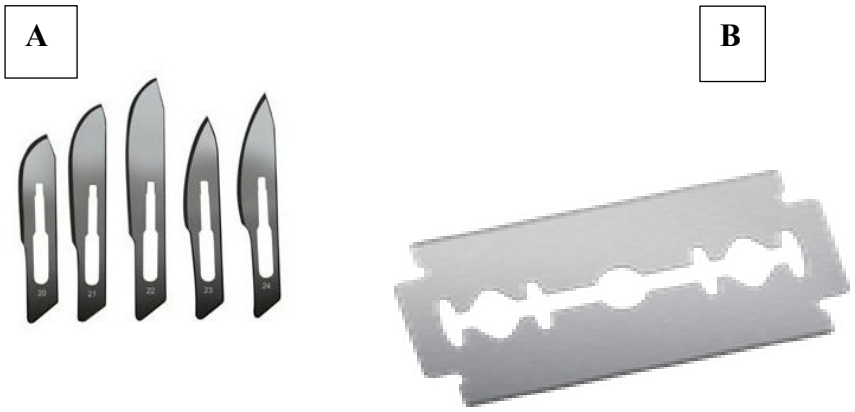


Figure 28A: Fully colonised plastic bag (showing dense white threads) and **28B:** Scalpel Blades (Carbon Steel) 100/Packet or ThumbsUp - Bluebeards Revenge Safety Razor Blades (10 Pack) used for cutting small crosses on the plastic bags from top to bottom to facilitate the growth of mushroom clusters

Figure 29A: An Onset HOBO U14-001 LCD Temperature Humidity Data Logger Kit at a cost of R3 997.00 can be used by small-scale farmers who can afford it. This device can be set to record both temperature and relative humidity data on daily basis, hourly or in seconds and the data can be stored for longer period and downloaded to a computer afterwards. Both the temperature and relative humidity can be monitored with ease as it is shown on the digital display screen for adjustment as required; **29B**: Maximum and minimum thermometer

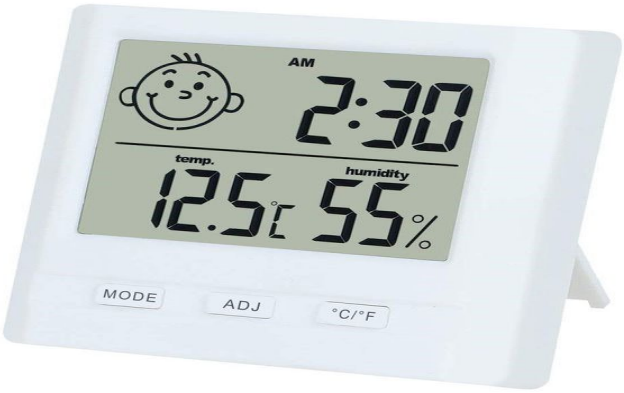


Figure 30: Digital Indoor Thermometer Temperature Humidity Clock at a cost of R150.00 can be used by small-scale farmers. The device makes it easy to monitor both the temperature and relative humidity. The gadget, however, cannot store data collected.

7. Contamination in planted bags

If the bags containing the substrate and spawn are not colonised quickly enough, then invader species, such as *Trichoderma* species (green mould), can colonise the planted bags resulting in a loss of production, as these bags need to be immediately discarded as green mould cannot be controlled from this point onwards. The issue of hygiene and proper pasteurisation plays a very big role in avoiding the contamination from early stages of oyster mushroom colonisation. The personnel who are planting must wash their hands regularly after visiting the bathroom and after food breaks to avoid this problem. The level of contamination is shown in Figures 31A & B.

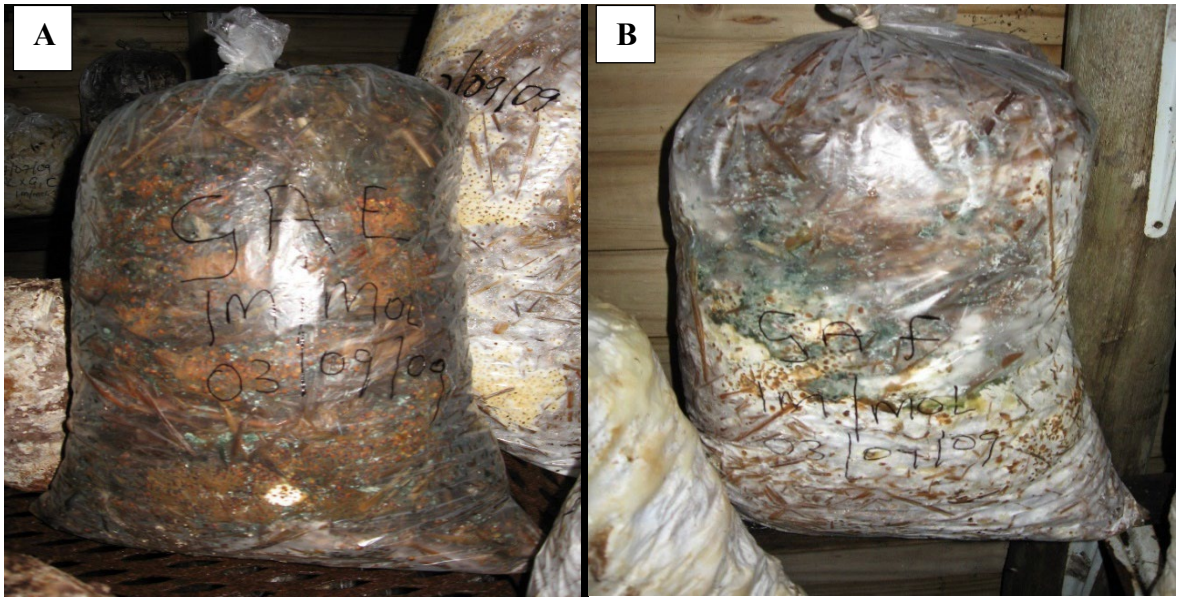


Figure 31: Two planted bags invaded by the parasitic fungi *Trichoderma* spp., causing green mould

8. Harvesting of oyster mushrooms

Harvesting should be done when the clusters are mature. This is approximately 3 to 4 days after flushing as denoted by regular sizes of the caps, with no splitting at the edges of the cap. When harvesting the mushroom, remove the oyster mushroom completely by twisting firmly at the base. The oyster mushroom should be very carefully harvested after reaching an appropriate degree of development in accordance with criteria relevant to the variety and to the area in which they are grown as shown in Figure 32.

Figure 32: Mature oyster mushrooms that have reached an appropriate degree of development in accordance with criteria relevant to the variety in a hut-house growth chamber

The development and condition of the oyster mushroom must be such as to enable them:

- to withstand transport and handling; and

- to arrive in a satisfactory condition at the final destination.

Harvested oyster mushroom should be stored in brown bags to prevent moisture loss and damage. After the straw ceases to produce mushrooms, it can be fed to livestock or composted.

9. Pests of oyster mushroom

There are a few pests that attack the oyster mushrooms, and include snails and rats as shown in Figure 33A & B. Oyster mushrooms attacked by snails are eaten at the edges, whereas rats are able to destroy the whole oyster mushroom crop. There are currently no control measures to prevent snail attacks, so constant observations are important, followed by the removal of any

snails by hand. The ease with which the pests can reach the crop can also be reduced by hanging planted bags or putting planted bags on shelves.

Figure 33: Fresh oyster mushrooms eaten by snail pest (**A**) and (**B**) snail feeding on mushroom

10. Storage of harvested oyster mushrooms

The final harvested product (oyster mushrooms) should be packaged in FT11 containers and covered with a plastic wrapper (Econo) from Lowveld Packaging, Mbombela or from any other supplier as shown in Figure 34A & B and Figure 35A & B. The mushrooms can be stored at 2 - 5°C in a refrigerator for approximately 2 weeks, after which point they start to show signs of deterioration, as indicated by a colour change. Darker patches appear on the mushrooms and other fungi start to grow on the mushrooms. They also lose their rigidity and become watery and slimy with a pungent smell.

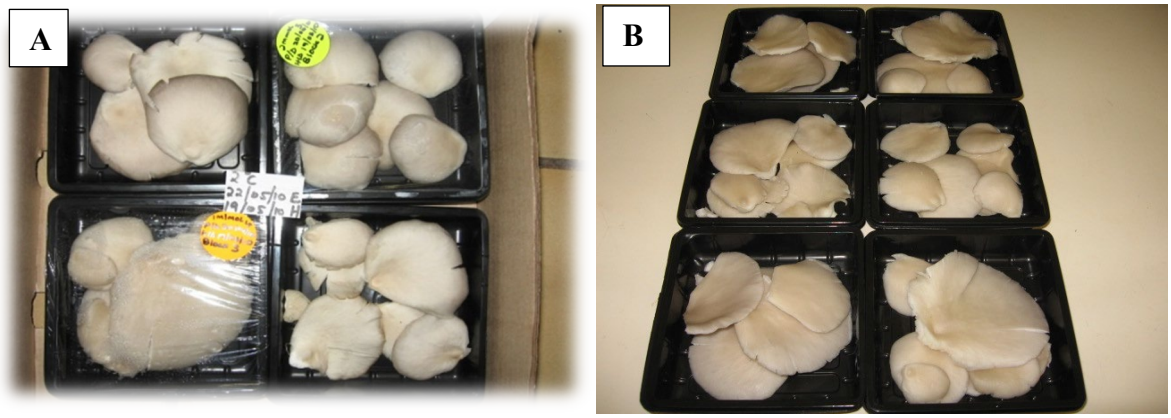


Figure 34: Harvested fresh oyster mushroom packaged in black (white could also be used) FT11 containers covered with a plastic Econo wrapper (**A**) and (**B**) FT11 containers uncovered as determined by a study at the ARC-TSC, Nelspruit

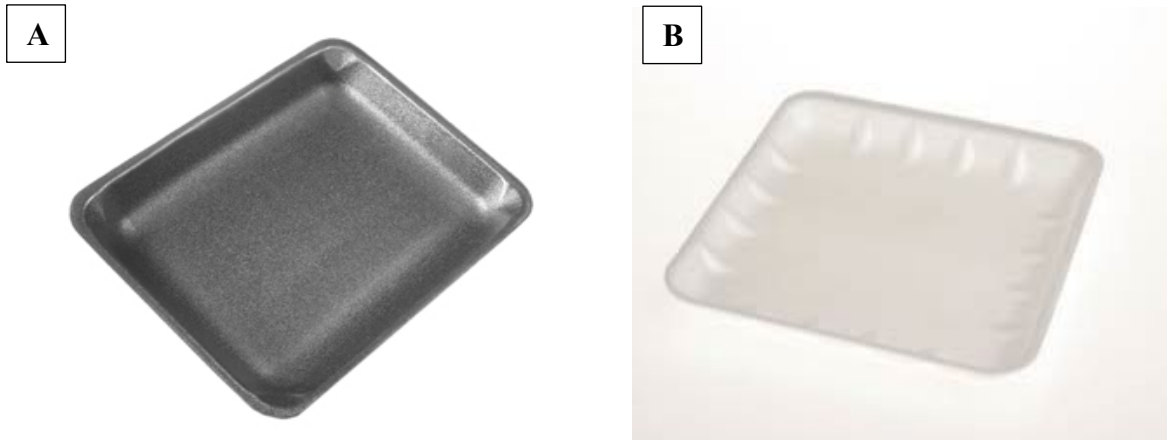


Figure 35: Foam trays (e.g. Rectangular Black or White No.73) can be used for fresh harvested oyster mushroom with a plastic Econo wrapper. These are easily available, economical and widely used so much that customers are familiar with them

11. Curing/drying of oyster mushrooms

Once harvested, oyster mushrooms may be used fresh or dried by either placing them in the sun or by using an electric dehydrator. Both methods do not require the use of preservatives. For sun drying, 48 hours is required for proper drying as shown in Figure 37A & B. Fresh produce is popular among fruit and vegetable retailers, restaurants and home based food preparation. Dried oyster mushrooms are sold in retailers, fast food outlets for soup making and on the go food packages for the Army. Oyster mushrooms should be placed on trays with gills facing upwards for proper exposure to sunlight. They should be covered with veils (such

as fine netting) to prevent flies and dust from landing on the mushrooms. The electric dehydrator (Commercial scale hot-air forced convection dryer – AD 3000 Agri-Dryer from Dryer for Africa, White River, South Africa) as shown in Figure 36 can be set for 9 h with three steps in between that lasts for 3 h each. Settings are as follows: first three hours (60°C, 40% RH), second three hours (60°C, 27% RH) and last three hours (55°C, 18% RH). Dried oyster mushrooms are left on trays for approximately 1 day to cool down and unsticking to the trays, to avoid breaking of mushrooms before being packaged and sealed in plastic bags.

Figure 36: Electrical dehydrator (Commercial scale hot-air forced convection dryer – AD 3000 Agri-Dryer) with fresh oyster mushrooms ready for drying

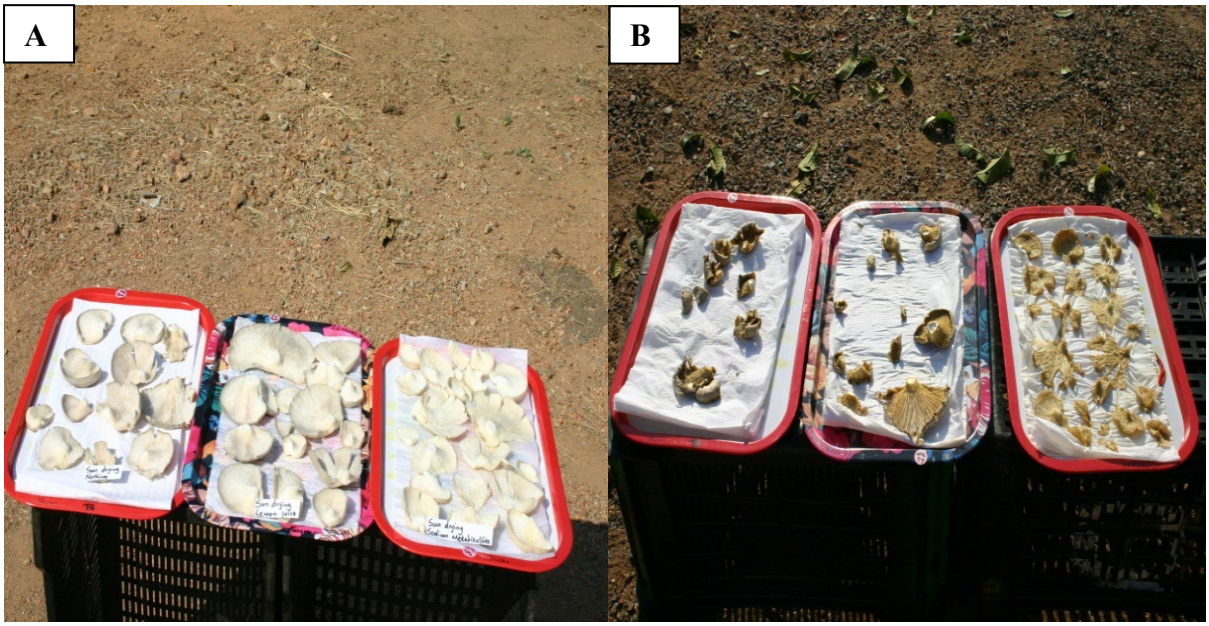


Figure 37: Trays containing fresh (**A**) and sun-dried (**B**) oyster mushrooms on paper towels

12. Marketing of the crop

Before a small-scale farmer can start cultivating oyster mushrooms, the intended market must be determined. The small-scale farmer can sign an off-take agreement with hotels, restaurants and wholesalers in the surrounding areas. The oyster mushrooms are scarce in every province of South Africa, which makes it easy to market because there are very few competitors with similar types of mushrooms. The current well established mushroom market in South Africa is for button mushrooms and brown mushrooms.

13. References

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