Effect of different plant substrates and growth hormones on the development characteristics and nutrient content of *Pleurotus ostreatus* in semi-arid conditions

SM Sihlangu
0000-0003-1155-6971

National Diploma in Agriculture in Plant Production (Lowveld College of Agriculture, 2008)

Bachelor of Science in Agriculture in Crop Science (North-West University, Mafikeng Campus, 2014)

Dissertation submitted in fulfilment of the requirements for the degree Master of Science in Agriculture in Crop Science at the Mafikeng Campus of the North-West University

Supervisor: Dr K Ramachela

Graduation: October 2017

Student no: 23200359

Declaration

I, Sydwell Mcebo Sihlangu, duly declare that this dissertation for the Master of Science in Agriculture - Crop Science at the North-West University (Mafikeng Campus) hereby submitted, has not been previously tendered by me for a degree at this institution or any other University. I further declare that this dissertation is my own work in design, structure and that all material and sources contained herein have been acknowledged.

Candidate: Sydwell Mcebo Sihlangu	Signature	Date
Supervisor: Dr Khosi Ramachela	Signature	Date

Dedication

This thesis is dedicated to my exquisite and noteworthy mother, Ms Sitani Magareth Nkosi who never got the opportunity to go to school; I will forever be gratified for being the greater source of support to pursue my dream of studying further despite the poverty. To my brother Ishmael, sister Clodia, daughter Khensani and father Irvin Sihlangu thank you for understanding my goal of studying, believing in my vision and your support during my studying. Lastly to all the young people who come from disadvantage background “with education you are advantaged and diversity can only make you stronger, it cannot rob you the power to dream and to reach your dreams”.

“Education is the most powerful weapon which you can use to change the world”

– Nelson Mandela

Acknowledgements

My first acknowledgment goes to my creator the almighty God for being the endless source of my strength, wisdom and guidance throughout my education till to this very day. It is through you that the word impossible is alien in my life, words can never be enough for the appreciation and yes we did it again.

I would like to express my heartfelt gratitude to my preeminent supervisor Dr Khosi Ramachela, for his supervision, guidance and support in facilitating the completion of this research. He stood by me throughout this research despite the uncertainty of the implementation of the first trial; he was patient and optimistic to the very end. It is through your supervision that I can safely say “in you I have found a father”.

I would also like to thank North-West University in particular Mafikeng campus for admitting, allowing and supported me to further my studies in such a prestigious educational institution such as this one.

There are not enough words in my heart to express my profound and genuine gratitude to Department of Agriculture, Forestry and Fisheries (DAFF) [Food Security and Safety Niche Area]; it was your two year financial support that enabled the completion of this phenomenal work.

To the two hard working individuals whom I worked with since day one till the last day of this research, Ms Neo Makhobela and Ms Elander Nkosi you stunning ladies are the champions in this work. You have made possible for this research to be solid and recognized by sixteen different media across the globe, this was through your outstanding assistance on the field and in the laboratory. I am humbled before you; it has been a privilege to work with you and thank you.

To New Start Eco-Gardens, Dolph and Joe Swanepoel your indispensable contribution and assistance during my research has made it all possible. I will forever be grateful for such a remarkable relationship you have given me since day one till now and a family was established.

When I was seeking for assistance the following individuals in these departments welcomed me with warm hands when I knocked on their laboratory doors. Crop Science in particular Prof Veronica Ngole, Animal Health: Ms Mpho Tseole, Biochemistry: Dr Ademola Ayeleso and Animal Science: Mr Sibusiso Hadebe. You all made an enormous contribution to this research work therefore, with no measure to my appreciation I thank you all.

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P. ostreatus	*Pleurotus ostreatus*
P. sajor-caru	*Pleurotus sajor-caru*
G. frondasa	*Grifola frondosa*
U. panicoides	*Urochloa panicoides*
Z. mays	*Zea mays*
D. stramonium	*Datura stramonium*
S1	Substrate – Liverseed grass
S2	Substrate – Maize grass
S3	Substrate – Thorn apple
H0	No hormones – Control
H1	Hormone – Cytokinins
H2	Hormone – Auxins
H3	Hormone – Gibberellins
IAA	Indole-3-acetic acid
NAA	Naphthaleneacetic acid
GA_3	Gibberellic acid
AgriLASA	Agri-Laboratory Association of Southern Africa
PPM	Parts per million
SDM	Solar-dried mushrooms
SLS	Sea Land Services
MCD	Mycelial colony diameter
HSD	Honest significant difference
NWU	North-West University
UNSSCN	United Nations Systems Standing Committee on Nutrition

Abstract

With the global and local need to identify low-cost sustainable sources of protein and essential minerals for human nutrition the study was undertaken to investigate effect of different plant growth substrates and hormones on the yield and nutrient content of *Pleurotus ostreatus*. The other objectives were to analyse the transfer efficiency of macro- and micro-nutrients by the respective substrates and plant growth hormones and also investigate possible post-harvest mushroom processing technologies. The experiment was laid out in a 3 X 4 factorial treatment combinations. The experimental factors were as follows: three (3) substrates - *Urochloa panicoides*, *Zea mays* and *Datura stramonium* and four (4) hormones - cytokinins; auxins; gibberellins and control (no hormone). The treatment combinations were replicated three times making a total of thirty six (36) experimental units. Treatments were arranged in a Randomized Complete Block Design (RCBD). The amount of substrate used on each mushroom dome was determined by the volume of the mushroom dome structure and their texture i.e. *Urochloa panicoides* (1kg), *Zea mays* (1.23kg) and *Datura stramonium* (1.1kg). Dosage of hormonal treatment used to treat the respective substrates were as follows: auxins (PoMaxa): 31.6%, cytokinins (MaxCel): 31.6% and gibberellins: 11.1% and for the control - no hormones. The pH levels on respective substrates were analysed using 1:25 probe and meter procedure with water. Macro- and micro-nutrient content on various substrates were analyzed using the dry-ashing macro- and micro-nutrient procedure provided by AgriLASA. *Pleurotus ostreatus* production process on various substrates with different hormonal treatment was evaluated by assessing mycelial development, fruiting body initiation and total biomass. Fruit cap diameter and style length of mushrooms were measured using a tape measure at each harvest and conversion efficiency ratio from different treatment combinations into total biomass was determined by using the following formula: Mushroom Biomass / Substrate biomass X 100 = Convention Ratio (%). Analysis of variance indicated that there was a significant difference on effect of substrates and hormones on total biomass however, there was no significant difference from the ‘substrates X hormones’ interaction ($P \leq 0.05$). Hormones also had significant effect on fruit cap size and style length ($P \leq 0.05$). Transfer efficiency of macro- and micro-nutrient, protein content and heavy metals by the respective substrates and plant growth hormones were

analyzed using the dry-ashing macro- and micro-nutrient procedure provided by AgriLASA. Polyphenols content of mushrooms fruiting bodies were analyzed using the Folin-Ciocalteu colorimetric method and flavonoidsand determined by a colorimetric method. The results on transfer efficiency of macro- and micro-nutrient content of *Pleurotus ostreatus* fruiting bodies indicated that pH, substrate and phytochemicals have a significant effect on nutrients transfer efficiency of *Pleurotus ostreatus*. Mushrooms harvested from *Urochloa panicoides* had high levels of micro-nutrient composition on Fe and Zn compared to *Zea mays* and *Datura stramonium.* All the hormones had no significant influence on transfer efficiency ratio of *Pleurotus ostreatus*. Protein content percentage (per 100g) was relatively high on treatment combination S1H3 (*Urochloa panicoides* + Gibberellins) and S2H2 (*Zea mays* + Auxins). The levels of total polyphenols and flavonoids on mushrooms harvested from treatment combination S3H2 (*Datura stramonium* + Gibberellins) were found to be significantly higher than treatment combination S2H1 (*Zea mays* + Auxins) and all other treatment combinations. Accumulation coefficient of macro- and micro-nutrient content and heavy metals in *Pleurotus ostreatus* fruiting bodies had high levels of Fe, Zn and Mn on mushrooms harvested from substrate *Urochloa panicoides*. The nutrient content of the processed solar-dried *Pleurotus ostreatus* was analyzed using the dry-ashing macro- and micro-nutrient procedure provided by AgriLASA. A participatory assessment technique was used to determine the palatability and acceptability of the three different mushroom biscuits. The biscuits were made from the following ingredients: one egg, 250g of wheat-flour, 2.5g salt, and 200ml of water with three different ratios of solar-dried oyster mushrooms i.e. 50g, 100g and 150g. A random selection of 33% of students was carried out in a residence of a total one hundred and fifty. The selected students were used for the palatability and acceptability survey. The results on the macro- and micro-nutrient content of the different biscuits grades, i.e., ‘Mushroom-Wheat Flour’ mixing ratios, indicated an increase in levels of micro-nutrient Mn in all biscuits compared to the solar-dried mushrooms. The palatability survey on taste and acceptability of the biscuits indicated respondents preferred biscuits A which had the lowest mushroom nutrient content compared to biscuit B and C. In conclusion, the general finding of this study was that there is potential to improve oyster mushroom yield by identifying and use of appropriate substrates. It also showed that there is potential to improve nutritive value of mushrooms by amending the respective substrates by use of plant growth

hormones. Furthermore, the study showed that oyster mushrooms had relatively low levels of heavy metals, i.e., Cr, Cd and Pd. These heavy metal levels were noted to be of acceptable safe standard for human consumption. This was noted for both the solar-dried mushrooms and the mushroom biscuits.

Keywords**:** Mycelial, pinning, biomass, substrates, hormones, minerals, biochemical, transfer efficiency.

Chapter One
General introduction

1.1 Introduction

Oyster mushroom (*Pleurotus ostreatus*) is a widely grown edible mushroom. It was first cultivated in Germany as a subsistence measure during the Second World War and has since been grown commercially around the world as a source of food (Eger *et al.,* 1976). These species belongs to the kingdom: Fungi; phylum: Basidiomycetes; class: Agaricaceaea; order: Agaricales; family: *Pleurotaceae* (Eger *et al.,* 1976). *Pleurotus ostreatus* is reported as a commonly sought wild mushrooms, though it can also be cultivated on straw and other growth media (Chitamba *et al.,* 2012). A typical *Pleurotus ostreatus* can grow in several places, but some other related species, such as the branched oyster mushroom, grow only on trees (Shah *et al.,* 2004; Fanadzo *et al.*, 2010). Whereas an atypical mushroom is the lobster mushroom, which is a deformed with cooked-lobster-colored parasitized fruit body of a *Lactarius*, colored and deformed by the mycoparisitic (Volk, 2001). *Pleurotus ostreatus* is a saprophyte, feeding on dead and decaying matter. Fruit cap size usually ranges between 5 to 25cm in diameter and are shaped like an oyster (Maniruzzaman, 2004). Fruit caps are rolled into a convex shape when young and flatten out and turn up as the mushroom ages (Eger *et al.,* 1976). This mushroom specie varies in color from white to yellow, brown, tan, and even pink (Gupta, 1986). *Pleurotus ostreatus* is one of the few mushrooms species that are also known to be carnivorous. Its mycelium has been reported to kill and digest nematodes; this is believed to be the means by which the mushroom obtains nitrogen (Banik and Nandi, 2004).

Pleurotus ostreatus is reported as a delicacy in several countries, such as Bangladesh, Japan, Korea and China (Nasim *et al.,* 2001; Dey *et al.,* 2007; Suman and Sharma, 2007). It is commonly served on its own but also in soups, stuffed, or in stir-fry recipes with soy sauce (Davis and Aegerter, 2000). Occasionally these mushrooms are made into a sauce, used in Asian cooking, which is similar to oyster sauce. It has a mild taste with a slight smell similar to anise (Quimio *et al.,* 1990). It is always advisable to pick the oyster mushroom when young, the flesh becomes tough and the flavour becomes acrid and unpleasant as the mushroom ages (Suman and

Sharma, 2007). *Pleurotus ostreatus* has been reported to be of importance not only for consumption but also the usage of spent mushroom substrate in the production of vegetables and other crops as an organic matter to facilitate metabolic process in the production of organic material (Islam *et al.,* 2007). They secrete enzymes that break down the organic bonds in wood or organic material into smaller molecules. The carbon-hydrogen bonds in wood are similar to those found in oil and pesticides (Royse, 2002). Thus, oyster mushrooms are also efficient in breaking down the organic bonds in toxic chemicals (Chang and Miles, 2004). Oyster mushrooms mycelium is vigorous, i.e., it can eat through wood, paper, coffee grounds, and even petroleum products (Baysal *et al.,* 2003). A company in the USA has proposed using the mycelium together with the growing substrate as a substitute for petroleum derived expanded polystyrene packing material (Fanadzo *et al.*, 2010). Furthermore, it has been reported that mycelia have the potential to be used to absorb and digest oil spills and other petroleum products (Patil *et al.,* 2010). These mushrooms are also grown to play a role in mycorestoration which is the process of using mushrooms to remediate pollution levels in a given area. It was also reported in studies in Mexico that oyster mushrooms can break down disposable diapers (Baysal *et al.,* 2003; Kayole *et al.,* 2015).

Pleurotus ostreatus can be produced in large quantities over a short period of time, and they provide more protein per unit area than many other crop (Gupta, 1986). Production of mushrooms is generally known to be influenced by various factors such as growth media and other biochemical factors such as plant growth hormones (Khandakar, 2004). There are numerous plant growth hormones that are used to enhance several stages of plant growth, development and yield. Among these hormones are auxins, cytokinins and gibberellins which are known mitotic division in plant cells (Cheng *et al.,* 2007). Mushrooms are plants with no chlorophyll and they do not require photosynthesis to produce their own food (Sarker and Chowdhury, 2013). It is therefore, logical to presume their effect on mushroom development as several researchers have used them to enhance the production of mushrooms. Plant growth hormones are involved in several stages of plant growth and development. Auxins, gibberellins and cytokinins are plant growth hormones known to produce a variety of responses in plants (Dey *et al.,* 2007). These responses include fruit setting, changes in vegetative growth, regulated cell division, stimulated auxiliary and

adventitious shoot proliferation, regulated differentiation, inhibition of root formation, activated RNA synthesis, and stimulation of protein and enzyme activity (Maniruzzaman, 2004). It is therefore deduced that hormones would have similar effects on mushrooms.

Other than supplementing with hormonal, the type of substrate used is also one of the major factors affecting the yield and quality of oyster mushrooms (Chitamba *et al.,* 2012). This is because different substrates have different structure and nutrient composition thus influencing nutrient availability and release from the different respective substrates (Buah *et al.,* 2010). It has further been reported that higher spawn concentrations generally provide more energy for mycelial growth and development (Islam *et al.,* 2007). As much as seed quality is important to crop production, so is spawn quality to mushroom production (Khandakar, 2004; Siddant *et al.,* 2013). Spawn is pure culture of mycelium growing on a solid substrate such as cereal grain. A more rapid spawn run would reduce the time non-colonized substrate is exposed to competitors such as weed molds and bacteria (Royse, 2002). More inoculum points, available from increased spawn levels, would provide faster substrate colonization and therefore, more rapid completion of the production cycle. In addition, the cultivation of oyster mushrooms needs preparation of substrate and composite; preparation of spawn and seeding of the spawn on suitable substrate for mycelial growth and production of fruiting bodies (Meera, 2004). Ayodele and Okhuoya (2007) reported that an increase in spawn rates from 1.25% substrate wet mass to 5% may result in yield increases by nearly 50%.

Oyster mushrooms are also reported to be good sources of macro- and micro-nutrients such as P, K, Zn and Fe which are essential for humans (Chang and Miles, 2004). Oyster mushrooms are also renowned for their healthy attributes such as low fat content and high levels of proteins, macro- and micro-nutrients and dietary fiber (Manzi *et al.,* 1999; Manzi and Pizzoferrato, 2000). Dietary fiber includes components of fungal cell walls such as chitin, other hemi-celluloses and beta glucans, which play vital roles in several human health requirements. These consist of enhancement of macrophage functions and human resistance to many bacterial, viral, fungal and parasitic infections, activation of a non-specific immune stimulation, reduction of blood cholesterol and a reduction of blood glucose levels (Cheung,

2009). These mushrooms are also reported to help improve human health as they have been reported to reduce cholesterol levels and mitigate against cancer development (Chang, 1999). Oyster mushrooms naturally produce compounds called statins. Statin is a drug which reduces cholesterol by stimulating receptors in the liver to clear the blood cholesterol from the blood-stream (Chang and Miles, 2004). Studies have shown a link between consuming *Pleurotus ostreatus* and a lowering of cholesterol levels, probably because of the statins they produce; however, at this point no research has revealed exactly how much mushroom to eat to get these effects. Other medicinal benefits that have been reported, include its anti-cancerous effects. Research has shown a possible anti-tumor effect from polysaccharides in oyster mushrooms which lead to the lowering of cancer (Khan and Tamia, 2012). Specific polysaccharides, known as beta-D-glucans, are suspected to stimulate the immune system to fight cancer (Chang and Miles, 2004). The β-D-glucan isolated from oyster mushrooms is called pleuran. Studies are ongoing into the effects of pleuran for cancer treatment (Khan and Tamia, 2012). However, so far such research has not been experimented on human subjects but laboratory animal trials have been carried out.

Production of oyster mushrooms is undertaken throughout the year under controlled environment conditions (Pirc, 1999). Mushrooms like fruits and vegetables respire, mature and senesce at post-harvest which affects produce quality and shelf-life significantly (Rai, 2009). The knowledge and understanding of the post-harvest methods and physiological processes which have a negative impact on the quality and shelf-life of oyster mushrooms is essential (Arumuganathan *et al.,* 2004). The harvesting of these mushrooms is continuous and growers usually harvest in a three-day interval depending on the maturity and size of the fruit caps (Rai and Arumuganathan, 2008). Mushrooms which are packed loose in conventional cardboard boxes are often maintained/stored in cool conditions in order to reduce loss of quality. Loss of quality include: mass loss, shrinkage, browning (Chi *et al.,* 1998). The quality and shelf-life of mushrooms after harvest is critical and it is influenced by various factors such as packaging, storage and preservation techniques (Roy *et al.,* 2000). When storing mushrooms they should be stored and preserved without damage and change in their appearance, taste and nutrient content (Wang *et al.,* 2004). Oyster mushrooms have a shelf-life of one to three days

at suitable controlled temperature (Xiao and Zhang, 2003). Oyster mushrooms are generally delicate in texture and have proven to be a challenge when stored for more than twenty four hours without controlled conditions prevailing in the tropics where temperatures ranges from 20 to 35°C (Chi *et al.,* 1998). The lower consumption of oyster mushrooms by the poor rural communities has been caused by the high-cost of mushrooms because of its traditional expensive production technology. However, there is a low-cost mushroom production technology that has been developed to enable the poor rural population to produce these mushrooms. This is an important possible intervention for addressing the silent hunger as oyster mushrooms contain relatively high levels of most macro- and micro-nutrients (Cheung, 2009). Silent hunger is a term that is used by the World Health Organization to refer to macro- and micro-nutrient deficiencies in poor rural communities which often go un-noticed.

1.2 Problem statement

Mafikeng is a dry region with high temperatures which can reach up to 39°C, this generally results in a low relative humid of 15-40%. Such climatic conditions are typical of semi-arid regions and leaves farmers with few options to cultivate crops. Crop production under rain fed conditions has been noted to be risky in localities where smallholders live causing food and nutritional insecurity. Usually, this leads to farmers resorting to livestock farming and little crop production. Those who cultivate crops often grow crops such maize, sunflower and soybeans under irrigation which is generally expensive to establish and therefore not accessible to the majority of smallholder farmers. These are all seasonal crops and therefore are grown during the period of October to March leaving the months of April to September a non-productive period, where the land remains fallow. In between cropping seasons, there are gaps in production and thereby resulting in rural communities with no source of food or income. There have been initiatives of growing vegetables during this period but this is mostly under tunnels because of the harsh climatic conditions. It is therefore necessary to identify an alternative appropriate crop which may bridge this hunger gap by providing the nutritional requirements of the local communities and also offer opportunities of generating income for these households. Such a crop should at least have four months of production duration and be able to withstand the harsh climatic conditions of this region. It is therefore imperative that alternative crop production systems that would bridge this food production gap be developed.

1.3 Study justification

The output of this study will be an alternative technology that enables smallholder to produce oyster mushrooms at a lower cost and to economically able to use their small piece of land efficiently. It will provide knowledge and awareness that agro-waste found on the farm can be converted into mushrooms with high protein content. Producers will be able to identify other potential substrates that can be used to obtain high mushroom yield. The ability to produce oyster mushrooms in these harsh climatic regions will enable rural communities to address the problems of malnutrition, food security and macro- and micro-nutrients deficiencies. This study will also generate post-harvest technology that would improve mushroom keeping qualities and shelf-life.

1.4 Study objectives and research hypotheses

1.4.1 Main objective

To investigate effects of different plant substrates and growth hormones on the development, characteristics and nutrient content of *Pleurotus ostreatus* in semi-arid conditions.

1.4.2 Specific objectives

(i) To investigate the effects of various hormonal treated plant substrates on the development and yield of *Pleurotus ostreatus*

(ii) To analyze the transfer efficiency of macro- and micro-nutrient and biochemical content in *Pleurotus ostreatus* fruiting bodies from different substrates

(iii) To carry out comparative analyses of nutrient content and acceptability of processed *Pleurotus ostreatus* from two different processing technologies

1.4.3 Research hypotheses

(i) Hormonal treated plant substrates will enhance the development and total biomass of *Pleurotus ostreatus*.

(ii) The transfer efficiency of macro- and micro-nutrient, heavy metals and biochemical content of *Pleurotus ostreatus* fruiting bodies from different hormonal treated plant substrates will vary.

1.5 Description of study area

1.5.1 Chapter Three: Effects of various hormonally treated plant substrates on development and yield of *Pleurotus ostreatus*

The study was carried out during the 2015 winter cropping season at Molelwane farm, North-West University, North-West, South Africa, located 6.44km from the North-West University- Mafikeng Campus. The geographical location is 25’ 47’ 54’’ South, 25’ 32’ 52’’ North. In this study, African hut mushroom dome structures covered with a fabric cloth was used to create a growth environment/chamber for the oyster mushrooms. Analysis of macro- and micro-nutrient content and pH on respective substrates were carried out and determined at NWU Animal Health and Crop Science laboratory.

1.5.2 Chapter Four: Transfer efficiency of macro- and micro-nutrient and selected biochemical content of *Pleurotus ostreatus* fruiting bodies grown on different substrates treated with plant growth hormones

In this study, three different analyses were carried out in the three NWU laboratories namely: Animal Health, Crop Science and Biochemistry laboratory. An in-vitro analysis of macro- and micro-nutrients of harvested dried oyster mushrooms was carried out at the NWU Animal Health laboratory and the protein content analysis of dried oyster mushrooms was carried out at the NWU at Crop Science Molelwane laboratory. Polyphenols and flavonoids were analyzed at NWU Biochemistry laboratory.

1.5.3 Chapter Five: Comparative analyses of solar-dried and biscuits nutrient content and consumer acceptability of processed *Pleurotus ostreatus*

In this study, three different analyses were carried out at three NWU facilities: Animal Health, Crop Science and student residence.

Chapter Two

Literature review

2.1 Climatic conditions of oyster mushrooms

Pleurotus ostreatus requires a temperature that ranges between 24-30°C with 26°C being the optimum temperature for optimal growth and yield (Quimio, 1990). This type of mushroom performs well under relative humidity between 80-95% and this range of relative humidity enhances optimal growth, good quality and high yield of oyster mushroom (Zervakis *et al.,* 2001). The temperatures of Mafikeng ranges between 20-39°C and this generally results in low relative humid of 15-40% thus, results creating unsuitable conditions for the growth and development of oyster mushrooms using conventional methods.

2.2 Plant growth substrates

Pleurotus ostreatus production systems that use crop stover or any plant material as a substrate are an appropriate food security enterprise for smallholder farmers in arid and semi-arid regions (Buah, 2010). The type of substrates used for inoculation of spawn is critical on the mycelial development, pinning and total production biomass of mushrooms (Shah *et al.,* 2004; Das and Mukherjee, 2006). Various studies have been carried out on the use of different types of crop stover as substrate for oyster mushroom production (Baysal *et al.,* 2003; Hasan *et al.*, 2010; Fanadzo *et al.*, 2010). The results of such studies have however been variable. Baysal *et al.* (2003) reported that oyster mushroom can convert 100g of dry waste into 50-70g of fresh mushrooms, while other studies gave different dry waste conversion rates (Nasim *et al.,* 2001). Usage of maize straw (*Zea mays*) as a substrate has been investigated by several researchers and has widely been used because of its high rate of fruiting. This has been attributed to its high percentage of carbohydrate and protein content (Chitamba *et al.,* 2012).

There are other potential plant materials which can be utilized as a substrates for the production of oyster mushrooms and these include: Liverseed grass (*Urochloa panicoides*), Thorn apple (*Datura stramonium*) and various other plant species. In the North-West province in particular Mafikeng region liverseed grass and thorn apple are locally available in large quantities and therefore have potential to be used

as substrates for the production of oyster mushrooms. However, there are no previous studies reported to have used liverseed grass (*Urochloa panicoides*) and thorn apple (*Datura stramonium*) as substrates for the production of oyster mushrooms which lead to difficulties comparing it with the current existing substrates. Liverseed grass is often used as fodder for cattle and horses (MacLachlan *et al.,* 2013). It is generally considered to be a weed and a potential seed contaminant. It has however been known to contain high levels of crude protein and therefore has been considered to have potential to be used as source of livestock feed (MacLachlan *et al.,* 2013). Thorn apple on the other hand has been known for being the source of blue-green dye from the leaves which is traditionally used by local communities to decorate interiors of huts (Le Bourgeois *et al.,* 2008). It is also used traditionally to treat asthma, headaches and wounds. In some cases, it is used as an ornamental plant in the gardens. Sreenivasa *et al.* (2012) have also reported that it contains high levels of fatty acids and vitamin E and therefore, has a potential to be used for various other purposes such as in the cosmetic industry.

2.3 Spawn

Spawn is defined as “any substance that can be inoculated with mycelium for the vegetative growth of fungus” (Royse, 1995). As mushrooms are fungus therefore, spawn is required for their production. The vegetative growth of mushroom spawn is called mycelium, usually carried out on grain material (Mbogoh *et al.*, 2011). The most frequently used grains for spawn production of oyster mushrooms are maize, sorghum and wheat grain (Mbogoh *et al.*, 2011). Mbogoh *et al.* (2011) used maize, wheat and millet grains and linear mycelium extension was measured and recorded after every three days. From this study, Mbogoh *et al.* (2011) revealed that linear mycelial growth was significantly affected by the type of grain used for spawn production ($P>0.05$). There was a significant difference in mycelial growth with maize grain being the best substrate, followed by wheat, and millet grains were the least.

As the spawn rate increases, the number of days to production decreases. By using a spawn rate of 5% of the wet substrate weight, it is possible to reduce the time to production by more than seven days compared with a spawn rate of 1.25% (Royse, 1995). Generally the optimum inoculation ratio of spawn with substrate-spawn ratio of 1:3 (Siddant *et al.,* 2013). With higher spawn rates 10 to 15%, growers can

therefore complete the crop cycle faster minimizing the exposure of the production substrate to pest infestations (Khandakar, 2004). One of the ways to increase the production of mushrooms in a unit area is to optimally use the potential power of the growth medium to provide mushroom nutrient requirements (Islam *et al.,* 2007). This can include use of the following: carbon substances, growth controlling hormones and microorganisms existing in composites. Carbon substances, i.e., cellulose, glucose and mannose have been noted to be important in enhancing the growth of mycelial which results in better absorption of substances required for mushroom growth and therefore improve mushroom productivity (Fanadzo *et al.*, 2010).

2.4 Plant growth hormones

In an effort to improve the productivity of growth media, addition of plant growth hormones was investigated by Sarker and Chowdhury (2013). Auxin is a plant growth hormone that has the ability to stimulate differential growth in response to gravity or light stimuli (Expósito-Rodríguez *et al.,* 2007). Khandakar (2004) reported that auxins are involved in cell growth and cell expansion. It is primarily produced in parts of the plant that are actively growing such as the stem, specifically at the very top of the stem. The concentration of auxin is highest at the top of the plant and decreases as it get closer to the roots, this controls the overall shape of the plant and helps keep the primary stem of a plant in position (Grieneisen *et al.,* 2007). Auxins maintain apical dominance preventing lots of lateral buds and branches from growing on the sides of the stem (Barlier *et al.,* 2002). The application of indole-3-acetic acid (IAA) or synthetic auxins to plants causes profound changes in plant growth and development (Barlier *et al.,* 2002). Maniruzzaman (2004) found the best mycelial colony diameter (MCD) on different application rates of IAAi.e. 5 ppm IAA + 0 ppm NAA. Much of the knowledge of the physiological roles of auxins in plants is derived from studies on how plants respond to excess exogenous auxin (Barlier *et al.*, 2002).

Oyster mushrooms as a macro-fungi are in fact plants without chlorophyll it is therefore logical to assume that plant growth hormones would also have an effect on their growth (Cheng *et al.,* 2007). Mukhopadhyay *et al.* (2004) studied the enhancement of biomass production of the edible mushroom *Pleurotus sajor-caju* grown in substrate that had been treated with plant growth hormones and it was noted that indole-3-acetic acid enhanced biomass yield and mycelia protein. The

findings by Khandakar (2004) indicated that the mean values for mycelial colony diameter of the species were found to be statistically significant for different hormonal treatments. These findings further indicated that the colony diameter increased gradually during the growth of all mushroom species. *P. ostreatus* exhibited clear better growth rate over the other two species which were *P. sajor-caru* and *A. bisporus* throughout the entire cultivation period.

The results were similar to the results of Islam *et al.,* (2007) who observed the best colony production in *P. ostreatus* compared *G. frondosa* and the *A. bisporus* exhibited inferior performance with regard to colony diameter. Furthermore, Carabelli *et al.* (2007) highlighted that cytokinins affect many aspects of plant growth and development, including cell division, shoot initiation and growth, leaf senescence, apical dominance, sink/source relationships, nutrient uptake, phyllotaxis, and vascular, gametophyte, and embryonic development, as well as the response to biotic and abiotic factors. Cytokinins are produced in the root apical meristems and taken up the stem through the xylem to the apical structure (Dey *et al.,* 2007). In plants cytokinins help delay senescence in leaf tissues or the natural aging process and it is also known that cytokinins promote mitosis and stimulate the differentiation of the meristem into shoots and roots. Cytokinins are also reported to assist in repairing the plant when wounded and the plants can fix themselves with the aid of cytokinins and auxins (Srivastava, 2002). Cytokinins have also been reported to have a significant role for various growth processes in the growth and development of mushrooms (Mukhopadhyay *et al.,* 2004).

The other plant growth hormone that is important is the gibberellic acid (also called gibberellin A_3, GA and GA_3) which plays a significant role in several developmental stages in plants. It is known to be responsible for making stems longer (Barlier *et al.,* 2002). Gibberellins enhance stem elongation between nodes on the stem. Grieneises *et al.* (2007) investigated the effect of gibberellic acid (GA_3) in oyster mushroom production. The study established that at an application rate of 10mg/L of gibberellic acids on a 500g spawn packet the fresh economic yield was increased to about 34% during the primordial initiation stage. At the second and third harvest the increase was 80% and 115% dry weights respectively. GA_3 showed a positive effect on the number of effective fruiting body, stalk length, pileus, diameter, biological yield

and economic yield. Sarker and Chowdhury (2013) also assessed the effect GA_3 primordial at initiation stage on the growth and yield of oyster mushrooms. The results suggested that GA_3 at 10ppm/packet would be the best possible concentration for production of oyster mushrooms. Some growth controlling hormones in different densities affect the development and umbrella/cap size of the mushroom. All these hormones have been reported to play a significant role in the laboratory growth of mushroom mycelial colony (Maniruzzaman, 2004). Siddiqui (2002) also reported that the diameter of culture mycelium colony was influenced by different growth media and hormones. Therefore the study sought to investigate effect of various hormonally treated substrates on the mycelial development, pinning and production fruiting bodies i.e., testing effect of different hormones on various substrates.

2.5 Macro- and micro-nutrients of oyster mushrooms

Mineral content is an important component of nutritional value of mushroom species including *Pleurotus ostreatus* (Kalogeropoulos *et al.,* 2013). Their fruiting bodies are characterized by high level of mineral constituents (Dundar *et al.,* 2008). They are reported to contain macro- and micro-nutrients such as K, P, Na, Ca, Mg, Cu, Zn, Fe, Mo (Bano *et al.,* 1981). However, the macro- and micro-nutrient content of *Pleurotus ostreatus* is reported to vary according to substrate composition (Parashare *et al.,* 2013). *Pleurotus* spp. provide a reasonable amount of micro-nutrients in comparison with vegetables (Sun and Liu, 2004; Ahmed *et al.,* 2013). Furthermore, Guillamón *et al.* (2010), reported variation of mineral content between various *Pleurotus* strains. Macro- and micro-nutrient proportions have been reported to vary depending on the species, age and also shape of the fruiting body (Kayode *et al.,* 2015). Among the micro-nutrients measured, Fe and Zn are the most abundant nutrients found in *Pleurotus* spp. (Shah *et al.,* 1997). These micro-nutrients play several significant roles in a human body. It has been reported that micro-nutrients control important biological processes, facilitating the binding of molecules to receptor sites on cell membranes (Chang and Miles, 2004). Some of these donate or accept electrons in the reactions of reduction and oxidation which results in the generation and utilization of metabolic energy (Bano *et al.,* 1981).

2.6 Protein content of oyster mushrooms

Pleurotus spp. are known to be a good source of protein, especially for vegetarians providing essential amino acids for human dietary needs (Shah *et al.,* 1997; Wani *et al.,* 2010). *Pleurotus* spp. have 90% of absorbable protein by the human body which is similar to that of plants - 90% and comparable with meat - 99% (Wani *et al.,* 2010). On a dry mass basis, oyster mushrooms have a substantial protein content ranging from 15-35% and in addition contain free amino acids, folic acid, vitamins such as A & B while are rich in fiber (Banik and Nandi, 2004). The protein content ranges between 10 and 40%, and has been reported to vary widely among and within the species (Patil *et al.,* 2010). The average protein content of *Pleurotus* spp. however, ranges between 20 and 25%. Colak *et al.* (2009) highlighted that the protein content of mushrooms is dependent on several factors, such as the type of mushroom, the stage of maturation, mushroom part and availability of nitrogen in the medium, as well as the time of harvest. It is also reported that the mushroom protein content is significantly affected by the availability of nitrogen from the substrate (Colak *et al.,* 2009). Substrates produced under high and low fertility conditions with regards to nitrogen levels resulting to variation of mushroom protein content. Colak *et al.* (2009) showed that the protein content of different *Pleurotus ostreatus* growth in different substrates ranged between 17-42%.

2.7 Post-harvest of oyster mushrooms

Oyster mushrooms are extremely delicate and therefore deterioration of fruit caps and browning begins soon after harvest (Lescane, 1984). In order to address this loss of quality, oyster mushrooms are often cooled as quickly as possible after picking and kept cool 6 to 10°C in cold-rooms (Rai and Arumuganathan, 2004). Using low temperatures to store oyster mushrooms has been found to be an excellent method to minimize deterioration of harvested mushrooms (Minamide *et al.,* 1980). Murr and Morris (1975a) reported that the maturation and textural changes in mushrooms were controlled when refrigerated at 0°C, ensuring the maintenance of excellent quality. Minamide *et al.* (1980) observed that the shelf-life of mushrooms was about 14-20 days when stored at 1°C, about 10 days at 6°C and 2 to 3 days at 20°C. It was noted that polyphenol oxidase activity and respiration rate were enhanced at 20°C storage. Baker *et al.* (1981) observed that forced-air-cooling of mushrooms resulted in a 2.5% mass loss within 15-30 minutes.

It is not a general practice to store fresh mushrooms at 0°C, perishable products generally stored at temperature between 6°C to10°C (Torringa *et al.,* 2001). Storage of fresh mushrooms in plastic bags or in airtight containers is not advisable as this results in fast spoilage. It is recommended that a paper bag or open container is used for storage (Gonzalez *et al.,* 2000). The shelf-life of fresh mushrooms under refrigerated conditions is about 4 days in commercial super markets. The reduction in temperature to 0°C is known to prevent increased protease activity, but often causes greater accumulation of free amino acids (Murr and Morris, 1975a), however, temperatures from 18 to 20°C are known to result in the reduction of protein content with accumulation of free amino acids due to activated protease enzyme activity (Murr and Morris, 1975b).

Fresh oyster mushrooms are generally packed in perforated polythene bags, some are stored and some are immediately sent to the nearest market (Wang *et al.,* 2004). It is reported that harvested mushrooms can be stored at low temperature (0-5°C) for 1-2 weeks without loss in quality, appearance and taste (Minamide *et al.,* 1980). It was also noted that the size and shape of the packaging materials play significant role in the selection of the cooling room and the cooling system used (Wang *et al.,* 2004). It has been observed that there are various challenges when oyster mushrooms are packaged in packs of more than 10kg or with 15cm thick layers (Anantheswaran *et al.,* 1994). Varszegi (2003) conducted an experiment to determine the relationship between the bacterial growth on mushroom cap and the pre-cooling methods, i.e., forced wet cooling and vacuum cooling. It was found that vacuum cooling provided the longest period of time needed to reach the highest threshold of microbial population while this method was found beneficial for quality maintenance.

Oyster mushrooms contain approximately 90% moisture at the time of harvesting and are often dried to a moisture levels below 10-12% (Torringa *et al.,* 2001). They are commonly dried using an oven dryer at a temperature of 55-60°C. At this temperature any insects and microbes found in or on the mushrooms are killed in a few hours and the remnant is the dehydrated final product of lower moisture content with longer shelf-life (Gonzalez *et al.,* 2000). Ambient temperature, moisture content of the mushroom and relative humidity of the air affect the color of the dried product

(Yapar *et al.,* 1990). Dehydrated mushrooms are used as a key ingredient in several food formulations including instant soups, pasta, snacks seasonings, casseroles, as well as meat and rice dishes (Arumuganathan *et al.,* 2004).

Most mushrooms, except for button mushrooms, are traditionally dried for long-term storage, e.g. oyster, shiitake, paddy straw, *Auricularia* etc. (Tuley, 1996; Gothandapani *et al.,* 1997). In case of button mushrooms, it is the blackening and irreversible change of texture, which often discourages the use of this simple technique of preservation (Ahlawat *et al.,* 2000b). The shelf-life of dried mushrooms can be 3-4 months and this can only be possible with a moisture content of 2-4% retained in the mushrooms (Arumuganathan *et al.,* 2004). For adequate preservation mushrooms should be in sealed plastics without any change in taste (Rai and Arumuganathan, 2008). The dried produce can be rehydrated in warm water (40-50°C) within 20-30 minutes to give 80-90% of the original mass (Torringa *et al.,* 2001).

2.8 Gaps with respect to the research problem

From the literature review it is noted that climatic conditions of Mafikeng region are not ideal for the production of oyster mushrooms and other mushroom species using a conventional technology unless high technology is put place. These communities do not have access to current technology which enables them to produce under such harsh climatic conditions and this is due to its high-cost hence the need to develop a suitable low-cost technology affordable for farmers. It is also noted that the type of substrates mostly readily available to these farmers has not been used previously to produce mushrooms and their performance compared to the currently mostly used is not known. Furthermore, plant growth hormones are reported to play a significant role in the growth and development stages of plants and fungi therefore, it is of important to determine their significant role on transfer efficiency of the macro- and micro-nutrients, protein content and growth stages of the mushrooms. Mushrooms are very delicate and deteriorate easily when not stored properly henceforth it is critical to investigate different techniques of post-harvest which would increase the shelf-life.

Chapter Three

Effects of various hormonally treated plant substrates on development and yield of *Pleurotus ostreatus*

3.1 Introduction

For more than a century, mushrooms have been harvested from the wild and some have been cultivated outdoors, i.e., without controlled environmental conditions (Gupta, 1986). Over the past five decades growers who sought to increase production, initiated growing mushrooms indoors. They designed and built structures that enabled them to control growing conditions. Building such structures provides a suitable micro-climate throughout the year and therefore, enables continuous production of mushrooms (Sarker and Chowdhury, 2013). There are numerous species of mushrooms that produce well when grown indoors, among these species is the *Pleurotus ostreatus,* commonly known as oyster mushroom. Oyster mushroom is one of the most important macro-fungi that produces high levels of protein content when grown on various agro-wastes or forest wastes (Banik and Nandi, 2004).

Oyster mushrooms are mainly cultivated in the temperate and subtropical regions of the world (Quimio *et al.,* 1990). The initiative of using crop residues to grow oyster mushrooms stems from the realization that the incorporation of non-conventional crop production systems into the existing agricultural systems can help to improve the socio-economic well-being of small scale farmers (Mukhopadhyay *et al.,* 2004). This mushroom is highly tolerant to variations in temperature, humidity, light levels and carbon dioxide levels, and therefore, an ideal crop for poor-resourced smallholder farmers who often reside in unstable environmental conditions (Atikpo *et al.,* 2008). Furthermore, oyster mushrooms can be produced on a small piece of land and have shorter production cycles compared to other mushrooms species and also require low production inputs (Gupta, 1986; Maniruzzaman, 2004).

In addition, these mushrooms can be produced after the main field crop has been harvested and therefore are an ideal hunger gap-bridging food enterprise. It is also a potentially good income generating enterprise for the landless poor. Diversification of agriculture to high value crops and transformation of smallholder agriculture from subsistence to commercial business enterprises offers promising options for

revitalization of agriculture and wealth creation among the rural poor. The success of commercialization of oyster mushroom lies in the development of technology that is easily adoptable by smallholder farmers, particularly using of locally available resources such as plant crop stover and other farm biomass. The objective of this study was therefore to investigate the effects of various plant substrates such as *Urochloa panicoides, Zea mays* and *Datura stramonium* inoculated with plant growth hormones such as cytokinins, auxins and gibberellins on the development and yield of oyster mushrooms in semi-arid conditions.

Communal and smallholder farmers in semi-arid regions of South Africa generally experience low crop yields due to various biotic and abiotic factors. The biotic factors include the use of low-yielding crop varieties some of which may not be climatically suited to the area while abiotic factors include, among others, low rainfall, averaging 450 to 550mm per annum, and the relatively nutrient deficient soils. These soils are not able to sustain crop growth for many seasons without the application of commercial fertilizers (Shah *et al.,* 2004). With this low land productivity, it is advisable that alternative non-land-based food production technologies be developed and introduced for adoption by the communal farmers in these dry regions. The aim of the study was to contribute towards the development of a low-cost technology that is readily and easily adoptable by smallholder farmers in order to address food insecurity at the household level.

3.2 Study objectives

3.2.1 Main objective

To investigate the effects of various hormonally treated plant substrates on the development and yield of *Pleurotus ostreatus*

3.2.2 Specific objectives

(i) To determine the effects of different plant species on the growth characteristics of *Pleurotus ostreatus*

(ii) To determine the influence of different plant growth hormones on the mycelial development, pinning, total biomass and fruiting bodies of *Pleurotus ostreatus* production on different substrates

(iii) To analyse the conversion efficiency ratio of different plant substrates to *Pleurotus ostreatus*

3.3 Materials and methods

3.3.1 Experimental layout

The experiment was laid out in a 3 X 4 factorial treatment combination, i.e., three substrates by four hormonal treatments. The treatment combinations were replicated three times making a total of thirty six experimental units. The three growth substrates: *Urochloa panicoides* – liverseed grass (S1), *Zea mays* – maize straw (S2) and *Datura stramonium* – thorn apple (S3) and the hormone treatments were: 6-Benzyladenine –a cytokinin (H1); 2-4D- an auxin (H2); Gibberellic acid -a gibberellin (H3) and control– no hormone (H0). The experimental units were arranged in a Randomized Complete Block Design (RCBD). The twelve treatment combinations were: S1H0, S1H1, S1H2, S1H3, S2H0, S2H1, S2H2, S2H3, S3H0, S3H1, S3H2 and S3H3. The substrates from different treatment combinations were placed in each of the 70cm diameter mushroom domes and each was inoculated with 0.327kg of spawn. The inoculated growth media were kept humid by using the scorpion irrigation system (Fig. 3.1). A water delivery of 1.5 litre per hour was supplied in the form of a drip by the water outlet of the scorpion irrigation system. The irrigation/watering schedule was not fixed due to the dependence on temperature variations. For temperatures less than 30°C, irrigation took place in two day interval for a period of two hours and for temperatures above 30°C the irrigation intervals and scheduling were subjected to change to a one day interval.

Figure 3.1 Scorpion water outlet

3.3.2 Description of the experiment

3.3.2.1 Preparation of growth media

The individual substrates, that is, *U. panicoides*, *Z. mays* and *D. stramonium* were milled, and solar-dried. The dry substrates were weighed before wetting and the amount of substrate used was determined by the volume of the African hut mushroom dome structure and their texture, i.e., *U. panicoides* (1kg), *Z. mays* (1.23kg) and *D. stramonium* (1.1kg) (Siddant *et al.,* 2013). All substrates were sterilized using an autoclave at a temperature of 121°C for 30 minutes at 100kpa. After sterilization of the respective substrates, they were allowed to cool in sterilized containers for sixteen (16) hours. The following dosage of hormonal treatment were used to treat the respective substrates: auxins (PoMaxa): 31.6%, cytokinins (MaxCel): 31.6% and gibberellins: 11.1% and for the control- no hormones (Khandakar, 2004; Sarker and Chowdhury, 2013). Distilled water was used to wet the hormonal treated substrates, before inoculation with *Pleurotus ostreatus* spawn.

3.3.2.2 Spawn preparation

The preparation of the spawn was carried out in the laminar flow. The ratio of spawn inoculation with substrates was 1:3 and a total spawn of 11.77kg was used in all the thirty six treatment combinations (Siddant *et al.,* 2013). For the thirty six treatment combinations, a quantity of 0.327kg was weighed, packed and stored for use for inoculating the respective substrates.

3.3.2.3 pH analysis of various substrates

pH analysis of all three individual substrates was carried out using the 1:25 probe and meter procedure with water (Soil Analysis Handbook of Reference Method, 1999). The pH was tested before the inoculation with oyster mushrooms and after harvest. The following materials were used bench pH-Meter, reference, buffer solution pH 7.0, buffer solution pH 4.00, refillable electrode, distilled water, 150ml glass beaker, magnetic stirrer, stir bar (20mm) and a 100ml graduated cylinder.

3.3.2.4 Macro- and micro-nutrient analysis of various substrates

Macro- and micro-nutrient content of respective substrates were analyzed using the dry-ashing macro- and micro-nutrient procedure provided by the Agri-Laboratory Association of Southern Africa guidelines (AgriLASA, 1998). The substrates were dried at room temperature (26°C) for 72 hours and milled thereafter; one gram (1g) of substrate was placed into crucibles and further dried for 24 hours, by crucibles with dry samples into an ashing oven at 600°C for a period of 8 hours. The 4:1 ICP plant extract procedure was used, 8ml Nitric acid (HNO_3) and 2ml hydrochloric acid (HCl) were used as reagents and the samples were incubated in a Microwave Reaction System Model 3000 for 45 minutes. Placed digested samples were cooled and transferred into respective filling the volumetric flasks (100ml) which were topped-up to 100ml with distilled water and left standing for 24 hours. Samples were slowly transferred to McCartney bottles without disturbing the sediment. An ICP Mass Spectrometer (Perkin-Elmer, 1982, NexION 300Q) was used to analyze the macro- and micro-nutrients of the different substrates. Macro- and micro-nutrients analyzed were Fe, Zn, Mg, Mn, K, Cl, Se, Cd, Pb, Cu, and Cr and these were calculated in mg/100g.

3.3.2.5 Assessment of mycelial development, pinning and biomass accumulation in the different treatment combinations

Mushroom growth on different substrates and hormones was evaluated by assessing mycelial development, fruiting body initiation (pinning) and total biomass production. The data on mycelial development was collected at two-day intervals. The surface cover of mycelial development was measured inside each of the 70cm diameter African hut mushroom dome structures and measured as percentages dome cover. Data on pinning was also recorded at an interval of two days. The pinning heads inside the African hut mushroom dome were measured for surface cover in percentage. The fruit bodies (fresh mass) were weighed immediately after harvest using an electronic balance at three decimal places and recording the total biomass in grams (g) for each harvest.

3.3.2.6 Fruit cap and styles length

At each harvest, the fruit cap diameter and style length of all mushrooms were measured using a tape measure.

3.3.2.7 Conversion efficiency ratio

Conversion Efficiency (CE) is defined as the percentage ratio of fresh mass of harvested mushroom over dry mass of substrate (Pokhrel and Ohga 2007). The means of spawn, substrates and total biomass of mushroom for the three replicates for each treatment was calculated in order to determine the conversion efficiency ratio. The following equation was used:

$$\text{Conversion efficiency ratio} = \frac{\text{Mushrooms harvested (g)}}{\text{Substrate (g)}} \text{ X } 100$$

3.3.2.8 Temperature and relative humidity

The climatic conditions of temperature (°C) and relative humidity (percentage) in the domes were also recorded at an interval of two days from the first day of inoculation until harvesting day using a multi-meter.

3.3.2.9 Statistical analysis

All the collected and recorded data on mycelial development, pinning and total biomass production of the different treatment combinations were subjected to Analysis of Variance (ANOVA). All interpretations were based on mean comparisons and standard deviations for the different treatment combinations. A Tukey's HSD (honest significant difference) test was used to determine the significances of inter- and intra-treatments variations on growth characteristics and macro- and micro-nutrient composition of substrates for the mean separation and standard deviation. The SAS 9.4 software package Version 2.0 (SAS Institute, Cary, NC, USA, 2002-2012) was used to process and analyze all data.

3.4 Results

3.4.1 pH analysis of various substrates

The pH determination showed that there was an increase in pH levels assessed initially at pre-inoculation and later assessed at post-harvest for all the respective substrates. *U. panicoides* had a pH level of 6.66 before inoculation and 7.20 after harvest followed by *Z. mays* which had a pH level of 6.83 before inoculation and 7.93 after harvest. *D. stramonium* had the highest pH level of 8.23 before inoculation and 8.69 after harvest (Table 3.1).

Table 3.1 pH analysis of various substrates

Substrates	pH levels before inoculation with spawn	pH levels after harvest/ (spent substrates)
Urochloa panicoides	6.66	7.20
Zea mays	6.83	7.93
Datura stramonium	8.23	8.69

3.4.2 Macro-and micro-nutrient analysis of various substrates (mg/100g)

Analysis of variance of macro- and micro-nutrients indicated that there was a statistically significant differences in the macro- and micro-nutrients levels ($P \leq 0.05$). The following micro-nutrients; Fe, Zn, Mg, Mn were statistically significantly higher in *D. stramonium* than in *U. panicoides* and *Z. mays.* The two nutrients N and K were however significantly higher in *U. panicoides* and *Z. mays* than in *D. stramonium*. All substrates had relatively low levels of the following heavy metals; Pb, Cd, Se and Cr (Table 3.2).

Table 3.2 Analysis of macro- and micro-nutrient composition on different substrates (mg/100g)

Component (per 100g portion)	Fe mg/100g Mean ± SD	Zn mg/100g Mean ± SD	Mg mg/100g Mean ± SD	Mn mg/100g Mean ± SD	K mg/100g Mean ± SD	Cl mg/100g Mean ± SD	Ca mg/100g Mean ± SD	N mg/100g Mean ± SD	Se mg/100g Mean ± SD	Cd mg/100g Mean ± SD	Pb mg/100g Mean ± SD	Cu mg/100g Mean ± SD	Cr mg/100g Mean ± SD
Urochloa panicoides	3.69 ± 0.25b	0.11 ± 0.028b	49.05 ± 3.20ab	0.53 ± 0.049b	41.95 ± 2.74a	45.68 ± 7.34a	0.31 ± 0.34c	78.78 ± 2.35a	0.0008 ± 0.0002c	0.00003 ± 0.00001a	0.0087 ± 0.0001a	0.06 ± 0.014a	0.007 ± 0.00014b
Zea mays	4.62 ± 0.42ab	0.17 ± 0.028b	39.55 ± 4.85b	0.41 ± 0.064b	40.08 ± 1.36a	49.21 ± 4.47a	0.46 ± 0.56b	81.55 ± 1.43a	0.0051 ± 0.0005b	0.00002 ± 0.00001a	0.0017 ± 0.0009b	0.05 ± 0.007b	0.015 ± 0.00282a
Datura stramonium	5.03 ± 0.01a	0.30 ± 0.021a	63.37 ±1.98a	0.73 ± 0.014a	29.72 ± 1.80b	59.75 ± 2.24a	0.73 ± 1.25a	55.58 ± 1.66b	0.0078 ± 0.0003a	0.00006 ± 0.00002a	0.0021 ± 0.0002b	0.20 ± 0.014a	0.014 ± 0.00014a

The results were expressed as mean ± SD and as mg/100g.

3.4.3 Effect of different plant growth substrates and hormones on mycelial development of *Pleurotus ostreatus* (dome surface cover %)

3.4.3.1 Analysis of variance on mycelial development percentage

Data analysis on the effect of different treatment combinations indicated that there was a statistically significant differences on the effects of substrates on the mycelial development (P≤0.0001). Plant growth hormones and 'substrates X hormones' interaction were also noted to have significant effect on the mycelial development (P≤0.05).

Table 3.3 Analysis of variance on mycelial development over a period of 28 days after inoculation (dome surface cover)

Source	DF	ANOVA SS	Mean Square	F Value	Pr>F
Substrates	2	62824.96296	31412.48148	163.73	<.0001****
Hormones	3	1924.54630	641.51543	3.34	0.0226*
Replicates	2	631.35185	315.67593	1.65	0.1986 ns
Assessments	13	14455.40741	7227.70370	37.67	<.0001****
Substrates X Hormones	11	4272.14815	712.02469	3.71	0.0024*

*** - Significant at (P≤0.05)**

****** - Significant at (P≤0.0001)**

ns- Significant at (P≤0.05)

3.4.3.2 Mycelial development rate assessed over a period of 28 days after inoculation

Mycelial development in substrate *U. panicoides* had the highest percentage surface cover of 100% followed by *Z. mays* with 84.78% and *D. stramonium* with 36.31% [Fig. 3.2(a)].

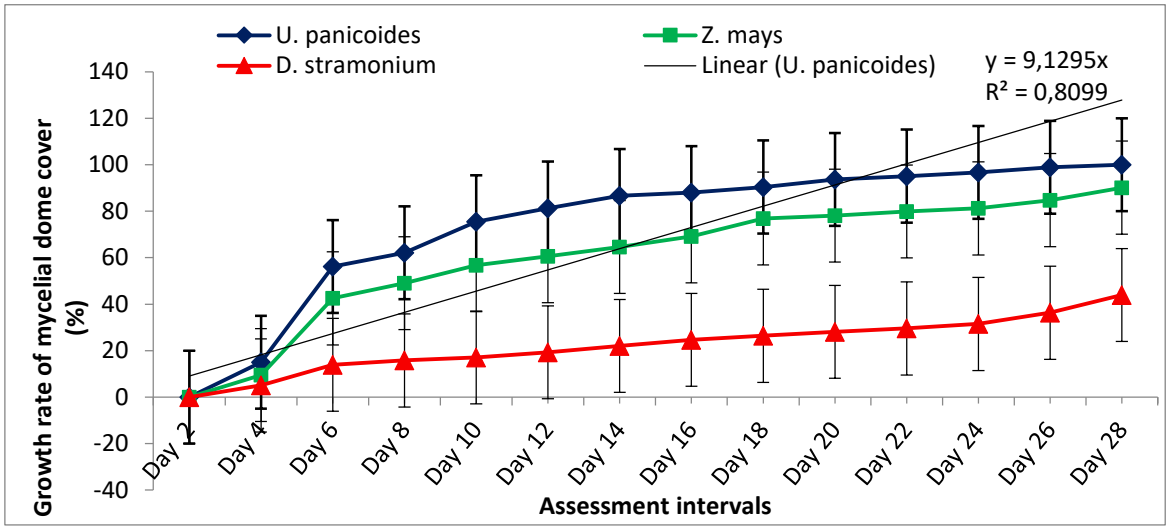

Figure 3.2(a) Mycelial growth rate of *Pleurotus ostreatus* on different substrates assessed in 2-day interval over a period of 28 days after inoculation (%)

3.4.3.3 Effect of different substrates on mycelial development assessed over a period of 28 days after inoculation

Figure 3.2(b) shows that there are statistically significant differences in the mycelial development between the different substrates (P≤0.0001). *U. panicoides* had statistically significantly higher mycelial development (74.08%) than in *Z. mays* (60.03%) and *D. stramonium* (17.36%).

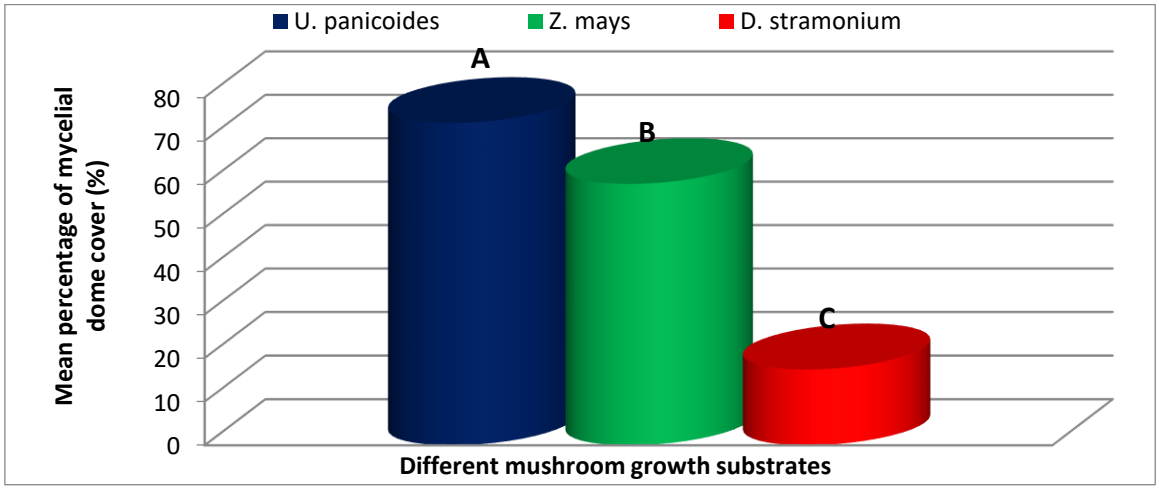

Figure 3.2(b) Effect of different substrates on mycelial development over a period of 28 days after inoculation (%)

3.4.3.4 Effect of different plant growth hormones on mycelial development assessed over a period of 28 days after inoculation

Figure 3.2(c) shows statistically significant difference between the effects of auxins and cytokinins on the mycelial development (P≤0.05). There was however, no significant differences between auxins, gibberellins and control (no hormonal treatment).

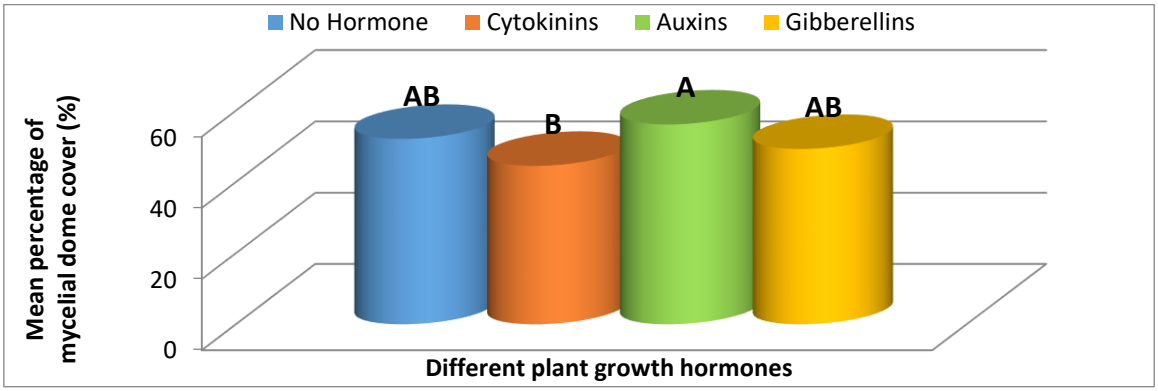

Figure 3.2(c) Effect of different plant growth hormones on mycelial development assessed over a period of 28 days after inoculation (%)

3.4.3.5 Effect of 'substrates X hormones' interaction on mycelial development assessed over a period of 28 days after inoculation

Analysis of variance (Table 3.3) showed statistically significant effect of 'substrates X hormones' interaction on the mycelial development. Mycelial development in the following treatment; S1H0, S1H1, S1H2, S1H3, and S2H0 were statistically significantly higher than the following treatments; S2H1, S2H2, S3H0, S3H1, S3H2 and S3H3 [Fig. 3.2(d)].

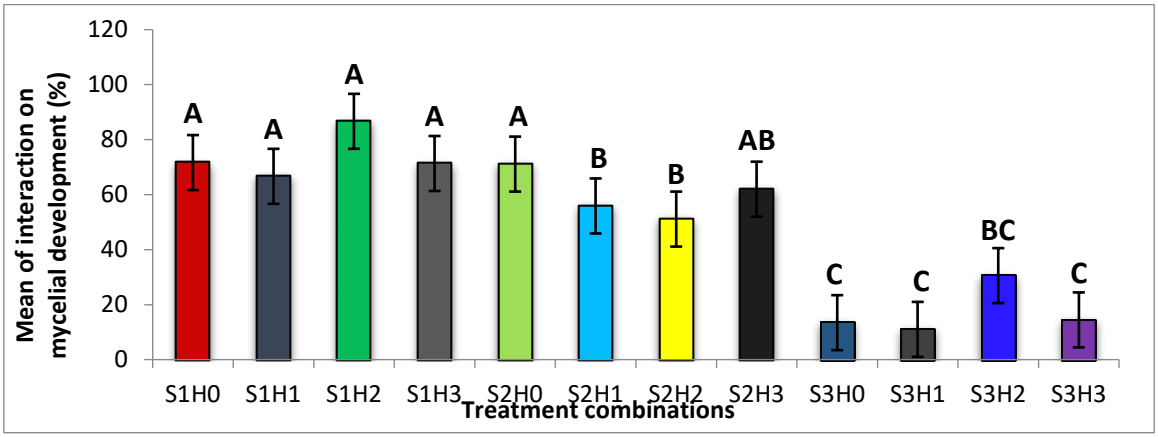

Figure 3.2(d) Effect of different treatment combinations 'substrates X hormones' interaction on mycelial development over a period of 28 days after inoculation (%)

Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*, H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

3.4.4 Effect of different plant growth substrates and hormones on pinning percentage of *Pleurotus ostreatus* (dome surface cover %)

3.4.4.1 Analysis of variance on pinning percentage

Data analysis showed that there were statistically significant differences on the effects of substrates on the pinning rate (P≤0.0001). Plant growth hormones and 'substrates X hormones' interaction were also noted to have significant effect on the pinning rate (P≤0.05).

Table 3.4 Analysis of variance on pinning percentage over a period of 64 days

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Substrates	2	21006.72222	10503.36111	24.44	<.0001****
Hormones	3	5956.51852	1985.50617	4.62	0.0047*
Replicates	2	10840.16667	5420.08333	12.61	<.0001*
Assessments	13	26918.00000	13459.00000	31.31	<.0001****
Substrates X Hormones	11	6169.64815	1028.27469	2.39	0.0341*

*** - Significant at (P≤0.05)**

****** - Significant at (P≤0.0001)**

3.4.4.2 Pinning rate assessed over a period of 64 days after inoculation

There was a variation on mycelial pinning rate in all the respective substrates. At 64 days after inoculation, *U. panicoides* had 100% pinning/fruiting followed by *Z. mays* with 86.87% and lastly *D. stramonium* with 58.89% [Fig. 3.3(a)].

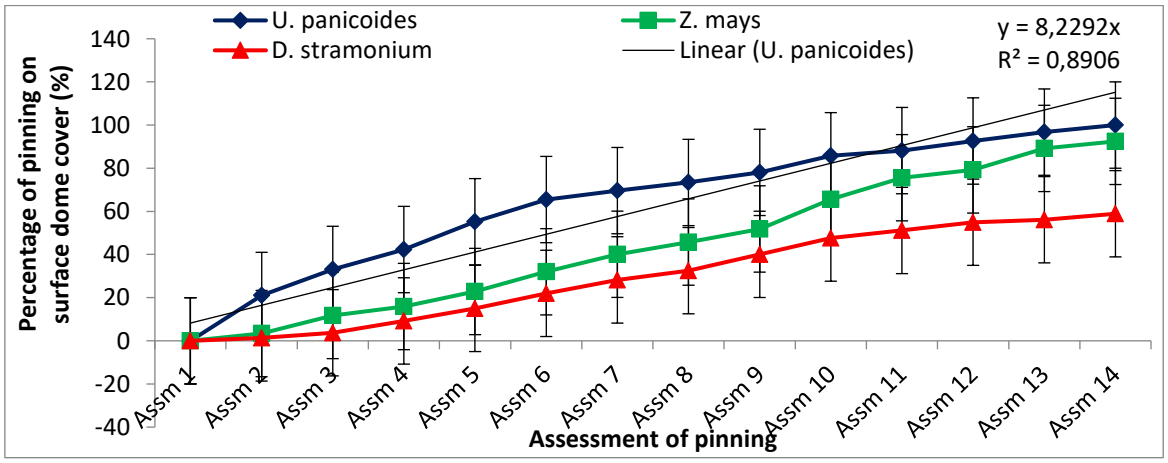

Figure 3.3(a) Pinning rate of *Pleurotus ostreatus* on various substrates assessed over a period of 64 days after inoculation (%)

3.4.4.3 Effect of different substrates on pinning percentage assessed over a period of 64 days after inoculation (%)

Analysis of variance (Table 3.4) showed that there were statistically significant differences in the effect of different substrates on mycelial pinning (P≤0.0001). *U. panicoides* had the highest pinning percentage with 48.31% followed by *Z. mays* with 28.02% and *D. stramonium* with 14.37% [Fig. 3.3(b)].

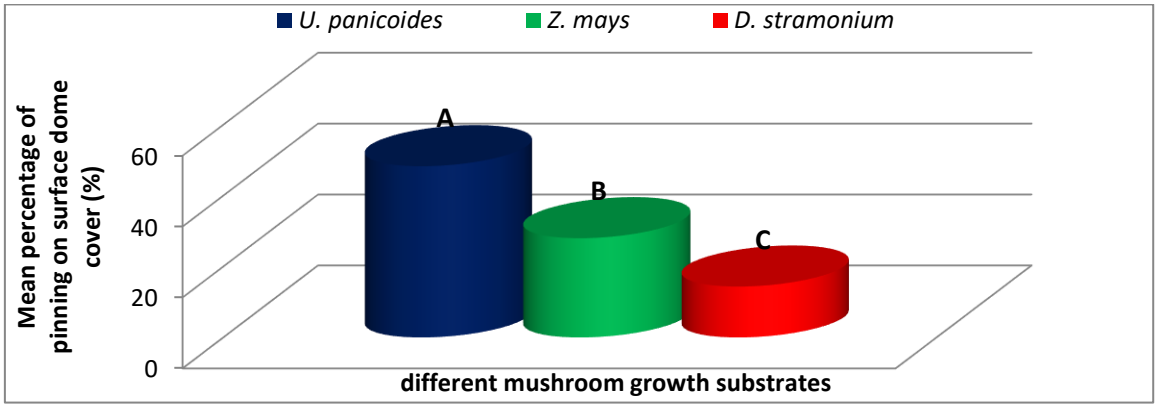

Figure 3.3(b). Effect of different substrates on pinning percentage assessed over a period of 64 days after inoculation (%)

3.4.4.4 Effect of various plant growth hormones on pinning percentage assessed over a period of 64 days after inoculation.

Results of data analysis showed that there were statistically significance differences on the effect of different hormones on the pinning percentage (P≤0.05). Auxins had statistically significant higher pinning percentage than cytokinnis and gibberellins. There was however, no significant difference between auxins and the control - no hormone treatment [Fig. 3.3(c)].

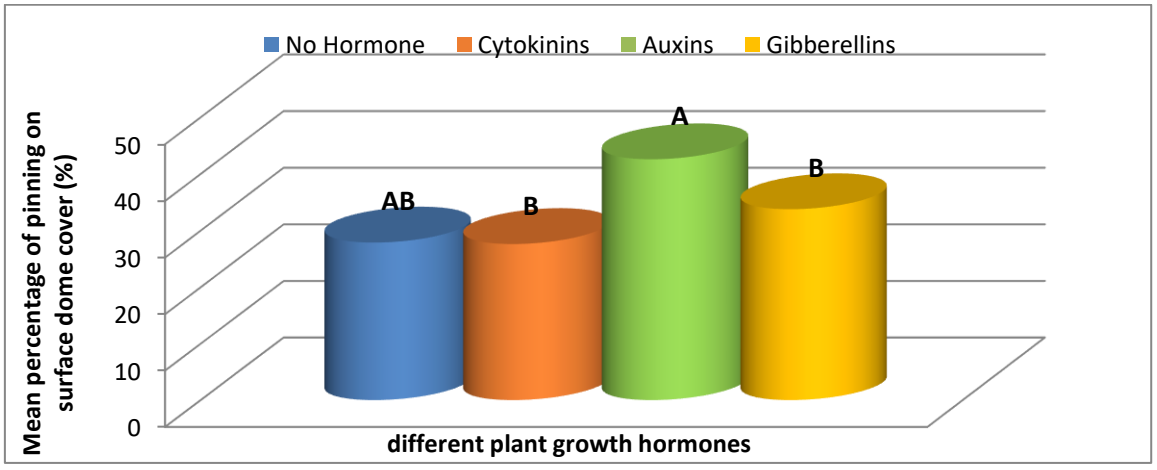

Figure 3.3(c) Effect of different plant growth hormones on pinning percentage assessed over a period of 64 days after inoculation (%)

3.4.4.5 Effect of 'substrates X hormones' interaction on pinning percentage assessed over a period of 64 days after inoculation

Data analysis results showed that there were statistically significant differences on the effects of 'substrates X hormones' interaction on the pinning percentage. Treatment combination S1H2 had statistically significantly higher pinning percentage than all other treatment combinations with the exception of treatment combination S1H0 (P≤0.05). The lowest pinning rate was noted in the following treatment combinations S3H0, S3H1 and S3H3. Pinning percentage of treatment combination of S3H2 was comparable to the following treatment combinations: S1H0, S1H1, S1H3, S2H0, S2H1, S2H2, S2H3 and S3H2 [Fig. 3.3(d)].

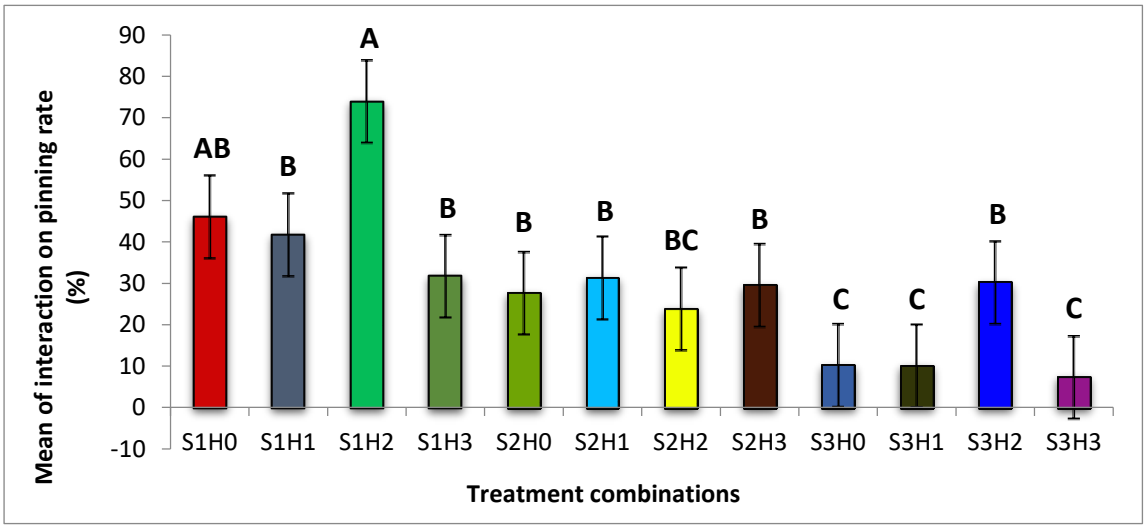

Figure 3.3(d) Effect of different treatment combinations 'substrates X hormones' interactions on pinning percentage assessed over a period of 64 days after inoculation

Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*, H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

3.4.5 Effect of different plant growth substrates and hormones on total biomass of *Pleurotus ostreatus*

3.4.5.1 Analysis of variance on total biomass of *Pleurotus ostreatus*

Results of analysis of variance (Table 3.5) showed that there was statistically significant difference on the effect of different substrates on the total biomass (P≤0.0001). Plant growth hormones also had significant effect on the mushroom total biomass (P≤0.05). However, there was no significant difference on the effect of 'substrates X hormones' interaction.

Table 3.5 Analysis of variance on total biomass of *Pleurotus ostreatus* over a period of 16 weeks after inoculation

Source	DF	ANOVA SS	Mean Square	F Value	Pr> F
Substrates	2	761122.3423	380561.1712	14.42	<.0001****
Hormones	3	213331.7802	71110.5934	2.69	0.0504*
Replicates	2	50276.8837	25138.4419	0.95	0.3899 ns
Assessments	7	64621.6403	32310.8202	1.22	0.2986 ns
Substrates X Hormones	11	284722.1808	47453.6968	1.80	0.1084 ns

*** - Significant at (P≤0.05)**

****** - Significant at (P≤0.0001)**

ns- Significant at (P≤0.05)

3.4.5.2 Effect of different substrates on the total biomass of *Pleurotus ostreatus* fruiting bodies assessed over a period of 16 weeks after inoculation

Total biomass production of *P. ostreatus* was noted to be highest on *U. panicoides* (7609.56g) followed by *Z. mays* (7296.42g) and *D. stramonium* (4368.15).

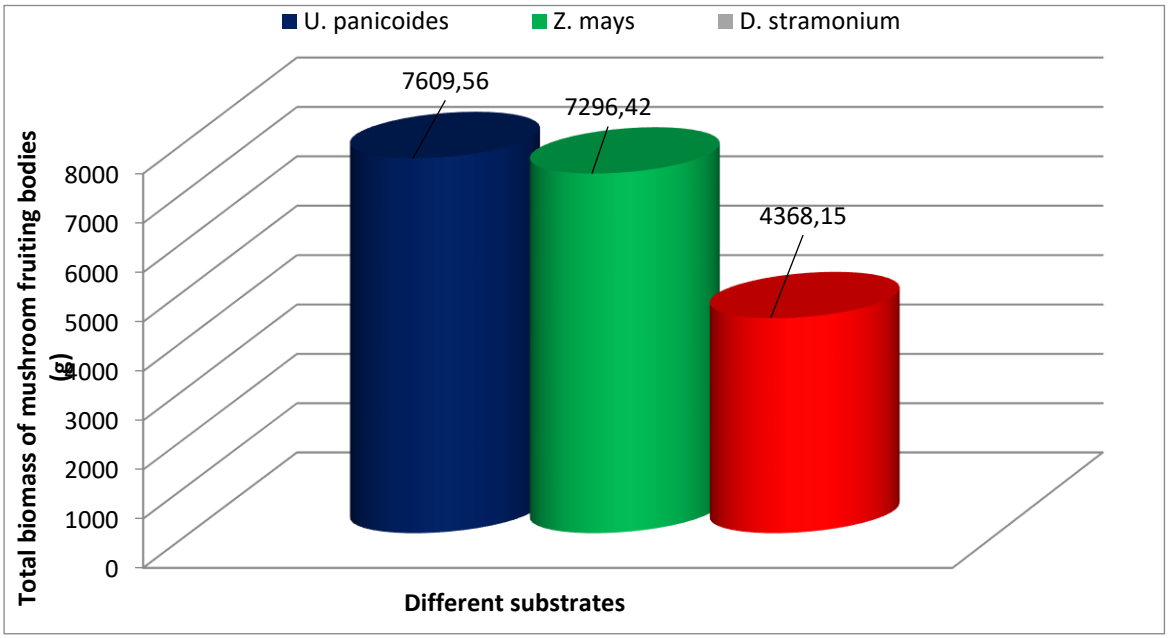

Figure 3.4(a) Effect of different substrates on the total biomass of *Pleurotus ostreatus* fruiting bodies assessed over a period 16 weeks after inoculation (g)

3.4.5.3 Effect of different substrates on the total biomass of *Pleurotus ostreatus* fruiting bodies over a period of 16 weeks after inoculation

Total biomass of fruiting bodies harvested from substrate *U. panicoides* and *Z. mays* were statistically significantly higher than the harvest from *D. stramonium* substrate [Fig. 3.4(b)].

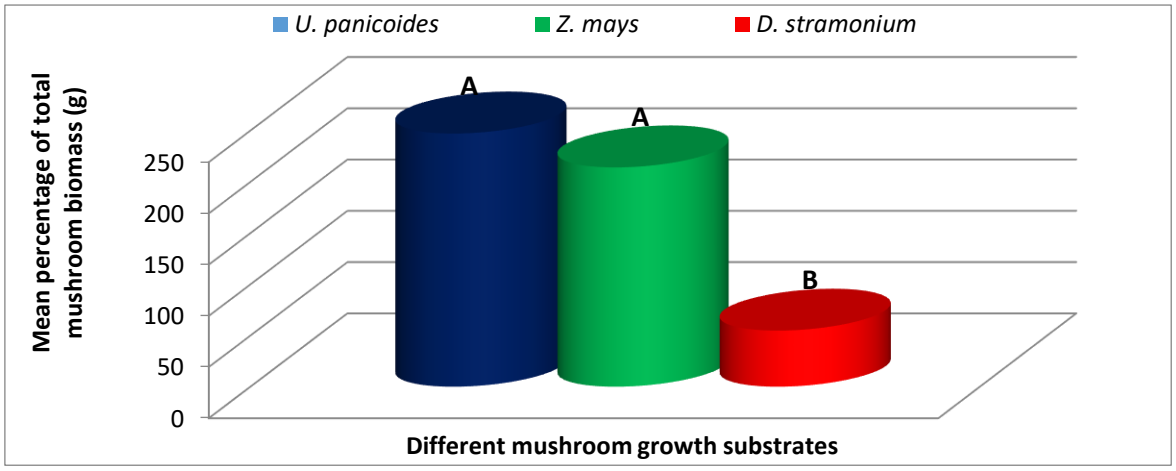

Figure 3.4(b) Effect of different substrates on total biomass of *Pleurotus ostreatus* fruiting bodies assessed over a period of 16 weeks after inoculation (g)

3.4.5.4 Effect of different plant growth hormones on the total biomass of *Pleurotus ostreatus* fruiting bodies over a period of 16 weeks after inoculation

The effect of the control (no hormonal treatment) on the total mushroom biomass was statistically significant different from that of gibberellins treatment. However, the effect of the control-no hormonal, cytokinins and auxins were not statistically significant different from each other [Fig. 3.4(c)].

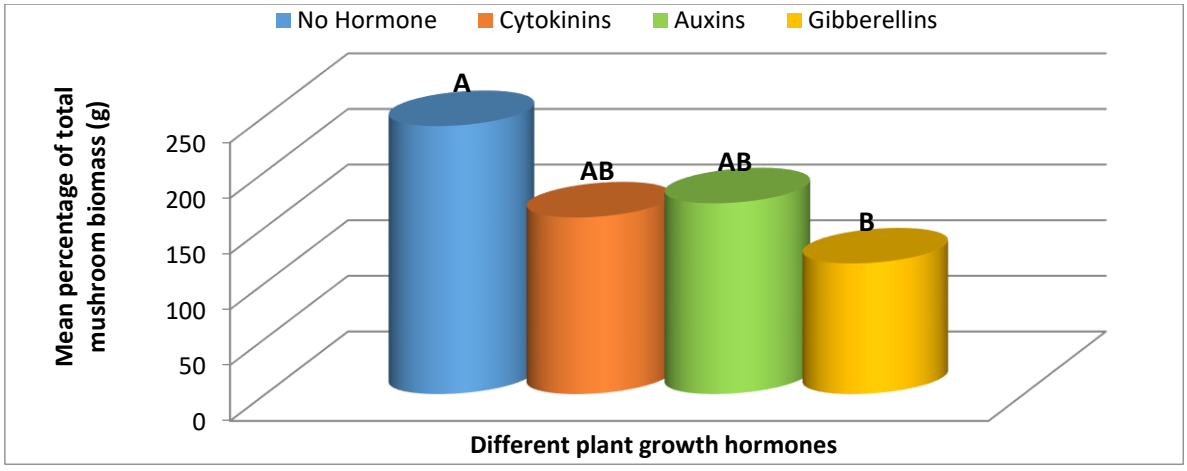

Figure 3.4(c) Effect of different plant growth hormones on total biomass of *Pleurotus ostreatus* fruiting bodies over a period of 16 weeks after inoculation (g)

3.4.6 Effect of hormonally treated substrates on the growth characteristics of *Pleurotus ostreatus*

3.4.6.1 Fruit cap and style length

There were statistically significant differences in fruit cap diameter and style length on mushrooms which were grown in different substrates treated with different hormones (P≤0.05). In general, substrates treated with auxins produced mushrooms with large fruit caps with a total average mean of 13.42cm followed by those treated with cytokinins with 9.9cm and gibberellins with 7.13cm (Table 3.6).

Table 3.6 Fruit cap (diameter cm) and style length (cm) over a period of 16 weeks.

Substrates	No Hormone		Cytokinins		Auxins		Gibberellins	
	Fruit caps (Diameter cm)	Style lengths (cm)	Fruit caps (Diameter cm)	Style lengths (cm)	Fruit caps (Diameter cm)	Style lengths (cm)	Fruit caps (Diameter cm)	Style lengths (cm)
Urochloa panicoides	8.3^{a}	9.4^{a}	12.2^{a}	10.3^{a}	16.9^{a}	8.9^{a}	8.9^{a}	13^{a}
Zea mays	5.7^{b}	6.9^{ab}	10.3^{ab}	8.9^{ab}	13.25^{b}	6.7^{ab}	7.4^{b}	10.6^{ab}
Datura stramonium	7.1^{a}	5.8^{b}	7.2^{b}	7.3^{b}	10.12^{c}	5.2^{b}	5.1^{c}	9.5^{b}
Total Average (cm)	**7.03**	**7.3**	**9.9**	**8.83**	**13.42**	**6.93**	**7.13**	**11.03**

The results are presented as means of fruit cap diameters and style lengths from week seven to sixteen of harvests (cm). The values in the same column not sharing common superscript letter(s) are significantly different (P≤0.05).

3.4.7 Conversion efficiency ratio

Treatment combination S1H0 had the highest conversion ratio of 96.3%, followed by S2H0 with 74.1% and S1H2 with 71.1% (Table 3.7). The lowest convention ratio was recorded with treatment combination S3H3 (12.3%)

Table 3.7 Conversion efficiency ratio of various treatment combinations

Treatment combinations	Spawn* (g)	Substrate* (g)	Harvested Mushrooms* (g)	Conversion Efficiency* %
S1H0	981	3000	2888.14	96.3
S1H1	981	3000	1955.48	65.2
S1H2	981	3000	2130.53	71.1
S1H3	981	3000	759.41	25.3
S2H0	981	3690	2735.19	74.1
S2H1	981	3690	1368.25	37.1
S2H2	981	3690	1632.06	44.2
S2H3	981	3690	1532.51	41.5
S3H0	981	3300	-	-
S3H1	981	3300	-	-
S3H2	981	3300	505.92	15.3
S3H3	981	3300	406.36	12.3

Key: *- Mean averages of treatments combination: S1 – *U. panicoides*: S2 – *Z. mays*: S3 – *D. stramonium*: H0 – No hormone: H1 – Cytokinins: H2 – Auxins: H3 – Gibberellins

3.5 Discussion

The differences in pH levels of the different substrates (Table 3.1) could have contributed to different mycelial development rate that is exhibited in Fig. 3.2(a). The 100% mycelial dome cover that was recorded with *U. panicoides* is indicative that this substrates had characteristics that better influenced mycelial development than the other two substrates. The poor mycelial development on the substrate *D. stramonium* was probably due to the high pH levels and high levels of micro-nutrients Mg, Ca and Cl (Table 3.1 and 3.2). As indicated in Table 3.1, the pH of *U. panicoides* was 6.66 and according to Chitamba *et al.* (2012) this fungus grows best in a pH ranging from 4.5 to 7 but the optimum pH is 6.5. The high pH level of 8.23 of the *D. Stramonium* substrates could have had a suppressive effect on mycelial growth. This could have subsequently caused delay on pinning and fruiting resulting in poor biomass production (4368.15g). Fanadzo *et al.* (2010) indicated that fungal growth is less prolific under high alkaline conditions. Furthermore, Buah *et al.* (2010) and Jonathan *et al.* (2013) both reported that pH of the substrate is one of the key factors that affect mycelial growth and subsequently the sporophore yield.

The increase in pH levels of substrates from pre-inoculation level to the post-harvest level for all the respective substrates could have occurred as result of uptake and utilization of CO_2 by fungal mycelium. Mycelial development of oyster mushroom is known to utilize CO_2 during its early stages of establishment (Urben, 2004; Meera, 2004). Since CO_2 reacts with water to form carbonic acid, an increase in CO_2 results in acid condition, conversely, a decrease CO_2 by it being used by the mycelium resulted in an increase in pH. Variation in post-harvest pH increase levels between the different Spent Mushroom Substrates (SMS) could have been influenced by the inherent different biological characteristics of the respective substrates. These characteristics could have influenced their different rates of carbon dioxide extraction and resultantly influencing the pH levels of the SMS.

Other than pH, the high percentage of mycelial development (Fig. 3.2a) and pinning rate on substrate *U. panicoides* and *Z. mays* could have been caused by low levels of anti-fungal micro-nutrients, such as Zn, Mn and Cu (Table 3.2). Micro-nutrients such as Zn, Mn, and Cu are known to have anti-fungal properties and, therefore, are suppressive to fungal growth (Royse, 1995; Shah *et al.,* 2004; Parashare *et al.,*

2013). The high levels of Zn, Fe, Ca, and Mn on the *D. stramonium* substrate could have caused poor mycelial development. Several workers have reported that these micro-nutrients had anti-fungal effect (Shah *et al.,* 2004; Parashare *et al.,* 2013). The poor mycelial development noted in *D. stramonium* substrate could probably be because of low levels of N and K (Table 3.2). N and K are known to be important nutrient for mycelial development (Meera, 2004). It can therefore be deduced that deficiency of these macro-nutrients in a substrate, mycelial development would have contributed to the compromised mycelial development and mushroom yield.

Furthermore, the high levels of mycelial development, pinning and total biomass on both substrates of *U. panicoides* straw and *Z. mays* could have been caused by the low levels of phytochemicals such as phenols, flavoids and alkanoids that are generally found in the various substrates in different quantities (Dewanto *et al.,* 2002; MacLachlan *et al.,* 2013). It is also possible that the poor mycelial development which led to poor pinning on *D. stramonium,* might have been caused by the high content of these phytochemicals that are found in *D. stramonium* (Soni *et al.,* 2012). There is a negative correlation between flavonoid and phenolic content and the mushroom yield (Sreenivasa *et al.,* 2012). The higher the flavonoid, phenolic and alkaloid content of the substrate, the lower the mushrooms yield (Chang, 1999).

Although *U. panicoides* was observed to have better supported mycelial development and mushroom yield better than the other substrates, there was however, general observation of the delay on mycelial colonization for all the substrates. The delay on mycelial colonization in all three substrates could have been due to the high temperatures which resulted in low humidity (Fig. 3.5). There is a relationship between temperature and relative humidity which greatly influences the mycelial development in oyster mushrooms. During the mycelial development phase of this study, temperatures were ranging between 20-30°C and relative humidity of 45-60% (Fig. 3.5). The mycelial development of oyster mushroom is reported to be optimal at temperatures ranging from 18-28°C and relative humidity of 70-90% (Zervakis *et al.,* 2001; Islam *et al.,* 2007).

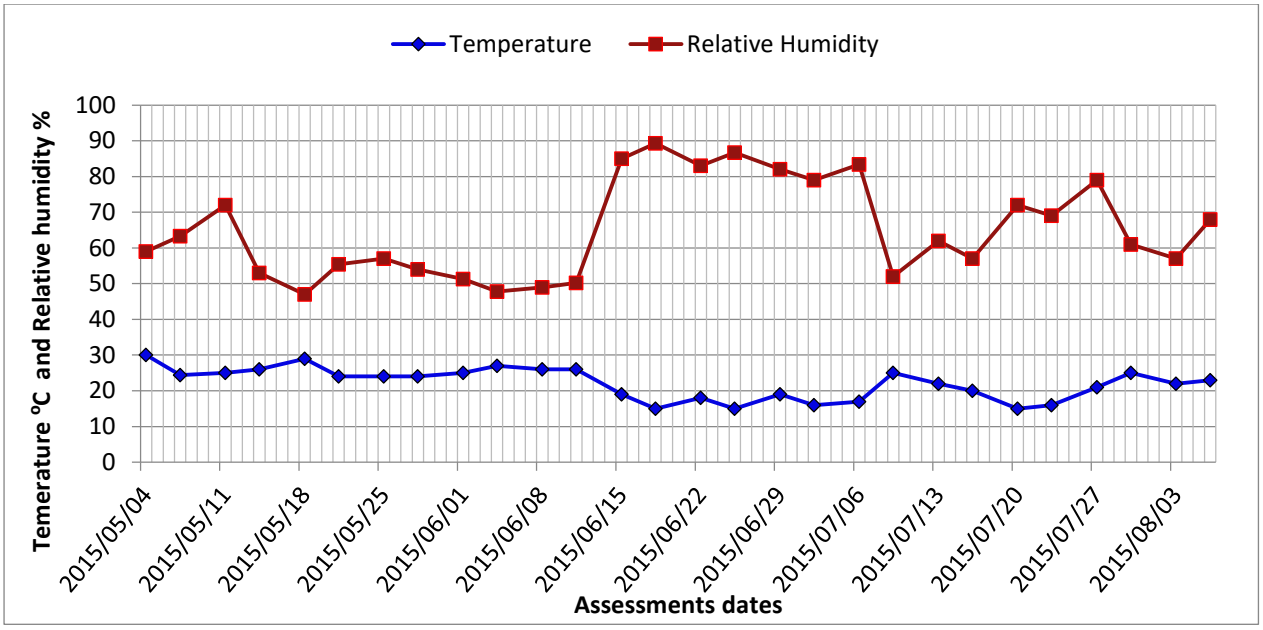

Figure 3.5 Mean temperature (°C) and Relative humidity (%) recorded from 04 May 2015 to 03 August 2015 inside the African hut mushroom domes during the experiment.

In addition to the good mycelial establishment, *U. panicoides* was also noted to produce better mushroom fruiting bodies. The big size of fruiting caps on substrate *U. panicoides* could have been caused by high levels of macro-nutrient composition such as N, and K. High levels of macro-nutrients such as N and K on substrates are known to enhance fruiting cap diameter, style thickness and length which contribute to increases in mass, yield, protein, fiber and ash of the mushroom (Hoa *et al.,* 2015). High levels of N also have a close correlation with the total mycelial colonization period, mushroom mass together with mushroom protein content (Hoa *et al.,* 2015).

Other than influence of macro- and micro-nutrients on the fruit cap diameter and style thickness, the study also investigated influence of plant growth hormones. The results indicated that mushrooms from substrates that were not hormonally treated had smaller fruit caps and shorter styles when compared to the hormonally treated substrates. However, non-hormone treatment had the highest mushroom total dry biomass compared to all mushrooms harvested from the hormonally treated substrates (Fig. 3.4c). The large diameter of fruit caps (Table 3.6) on mushrooms grown in auxin treated substrates was probably caused by the enhanced cell elongation which is known to occur in plants treated with auxins (Grieneisen *et al.,* 2007). Mushrooms harvested from *U. panicoides* exhibited positive interaction with gibberellins in the influence of style length recording the longest styles (Table 3.6).

Gibberellins in plants are known to be responsible for making stems longer and enhance stem elongation between nodes (Barlier *et al.,* 2002).

The treatment combination S1H2 (*U. panicoides* + Auxins) was observed to be the best treatment combination for mycelial development and pinning rate (Fig. 3.2d and 3.3d). These results are similar to those reported by Islam *et al.* (2007) who observed the best oyster mushroom mycelial development. This was probably due to the both the low content of anti-fungal micro-nutrients found in the substrate *U. panicoides* (Table 3.2) and auxin which is known to enhance mitotic cell division (Khandakar, 2004). As oyster mushrooms are biologically classified as plants without chlorophyll it is logical to assume that plant growth hormones have similar an effect on their growth (Cheng *et al.,* 2007). Auxin hormones in plants are known to be responsible for cell elongation and control the differentiation of meristem into vascular tissue and promote leaf development and arrangement (Khandakar, 2004). In plants, auxins are known to stimulate shoot elongation, seed germination, and fruit and flower maturation (Sarker and Chowdhury, 2013).

Generally, it was observed that mushroom produced in substrates that were not treated with hormones had the highest total biomass compared to the hormonal treated substrates (Fig. 3.4c). The low total biomass of mushroom from hormonally treated substrates could possibly be explained by that the increased mitotic division occurring as a result of auxins stimulation resulted in higher pinning rate i.e. many smaller fruiting caps. More pinning could therefore be deduced to have resulted in many smaller fruiting bodies as opposed to few fully developed fruiting bodies which was noted with substrates that were not treated with hormones.

The finding of this study is that highest conversion efficiency ratio of treatment combination S1H0 – *U. panicoides* + no hormone (96.3%) could have been caused by the fewer pinnings which could have resulted in less competition between pinnings and therefore, more biomass accumulation for the few fruiting bodies. Increased mitotic cell division in the hormonally treated substrates could have created nutrient competition in-between the many individual pinnings and fruiting bodies including mycelial result in reduction of harvestable yield. Although the results in Table 3.7 highlight that treatment combination S1H0 (*U. panicoides* + No hormone) had the highest conversion ratio it is essential that mushrooms harvested

from the various treatments be analysed for their nutrient content. The determination of their nutritional value i.e. macro- and micro-nutrients, phytochemicals and protein content would contribute to the knowledge base for addressing nutritional deficiencies and food insecurities.

Chapter Four

Transfer efficiency of macro- and micro-nutrient and selected biochemical content of *Pleurotus ostreatus* fruiting bodies grown on different hormonally treated substrates

4.1 Introduction

With the high nutritional value and high levels of macro- and micro-nutrients oyster mushrooms contained, they also have the potential to be incorporated into the global efforts to address the extensive nutrients deficiencies, particularly in developing countries (Kalogeropoulos *et al.,* 2013). The World Health Organization (WHO) reported that malnutrition is one of the nutritional challenges facing many developing countries around the world (Penelope *et al.,* 2006; Unknown, 2006). One of the main causes of malnutrition is a deficiency of macro- and micro-nutrients such as Fe, Zn, Ca and or P and this affects billions of people (Islam, 2007). According to the WHO millions of people mostly children, all over the world are affected by poverty and malnutrition. The poor, particularly the rural poor, tend to subsist on a diet of staple crops, such as maize, rice and wheat which are low in micro-nutrients (Penelope *et al.,* 2006). A large number of these people cannot afford to efficiently cultivate nor buy sufficient fruit, vegetables or meat products that are sources of adequate levels of these nutrients (McClafferty and Islam, 2007).

Many people, particularly children, consume non-nutritious food, such as fast food, sugared sodas, and foods rich in fat (Johns and Pablo, 2007). Young (2002) revealed that the consumption of non-nutritious food is mostly common in developing countries and is often associated with high food prices. Furthermore, the bulk of the poor population in these regions, i.e., rural communities are not able to access nutritiously balanced foods. According to Pray *et al.* (2007), increasing the macro- and micro-nutrients levels in staple crops can aid in reducing deficiencies of micro-nutrients. There are several efforts that have been undertaken by the WHO to improve nutrition of people in these vulnerable communities. These initiatives include fortification, enrichment and bio-fortification (De Benoist *et al.,* 2008). Food fortification is defined as a practice to intentionally increase the content of macro- and micro-nutrients and vitamins in food (Bailey, 2015). Fortification is the addition of macro- and micro-nutrients to the food as a form of improving the food quality. Food

fortification is commonly practiced in cereal-based products, milk products, fats, oils, tea and other beverages (Penelope *et al.,* 2006). On the other hand, enrichment is defined as addition of macro- and micro-nutrients to food which are lost during processing and synthetic biology is the addition of probiotic bacteria to foods. Bio-fortification differs from the other technologies, it focusses on manipulating a growing plant to produce nutritious food products, while growing rather than adding nutrients to the foods when they are being processed and these technologies are practiced for the purpose of macro- and micro-nutrients supplementation (Bailey, 2007). Of all the four initiatives that have been employed by the WHO and FAO to address macro- and micro-nutrients deficiencies at the global level, food fortification has been found to be the second best strategy, after bio-fortification (Unknown, 2015).

The goal of bio-fortification is to produce crop cultivars with increased nutritional value (Young, 2002). This process can be achieved either through conventional selective breeding, or through genetic engineering (Pray *et al.,* 2007). This approach is viewed as the most feasible strategy that can provide these critical nutrients to the rural poor, who rarely have access to commercially fortified foods (Islam, 2007). Bio-fortification of crops with micro-nutrients can be achieved without changing their taste or appearance (Penelope *et al.,* 2006). In the case of Fe, the WHO estimated that bio-fortification could assist curing about two billion people suffering from Fe deficiency-induced anemia (De Benoist *et al.,* 2008; Unknown, 2010). Crop scientists, nutritionists, economists and behavioral-change experts all work jointly to ensure that nutrient-rich crops are effective in meeting the nutritional needs of consumers. The United Nations System Standing Committee on Nutrition (UNSSCN) identified the following crops to have been successfully bio-fortified with various micro-nutrients: (i) Zn bio-fortification of wheat, rice, beans, sweet potato and maize; (ii) Febio-fortification of rice, beans, sweet potato, cassava and legumes; (iii) Provitamin A carotenoid-bio-fortification of sweet potato, maize and cassava; and (iv) Amino acid and protein-bio-fortification of sorghum and cassava (Beyer *et al.,* 2002; Unknown, 2004; Pray *et al.,* 2007).

Although these strategies have been effective in producing crops that are rich in these macro- and micro-nutrients and other factors contributing to a healthy living, their lack of availability to the rural poor communities has been a limiting factor. The

high cost of the technology and patenting processes; that is employed to produce these crop cultivars, make them too expensive for the rural poor (Sun and Lui, 2004; Bailey, 2007; Katsule, 1999). Poor rural populations have limited access and resources to purchase bio-fortified cultivars (Young, 2002). Furthermore, the techniques used for genetic engineering on crops may compromise consumer's immune systems, i.e., the risk of allergic reactions (Pray *et al.,* 2007). Lastly, these micro-nutrients are delivered individually not in a wholesome or bulk, which will lead to the consumer being compelled to purchase different crops in order to meet a balanced nutritional requirements of different micro-nutrients, such as Fe and Zn (Johns and Pablo, 2007). Such approaches often mean that one macro- and micro-nutrient problem is addressed and leaves the consumers deficient in one or more of other macro- and micro-nutrients (Unknown, 2012). There are a handful of other strategies that may be employed to overcome these challenges; these include education to increase consumption of higher nutritional value foods/crops (Chang and Miles, 2004). Among these crops is the oyster mushroom which is known for its high levels of micro-nutrients essential for human nutritional requirements (Chang and Miles, 2004). In some countries such as Uganda and China, the oyster mushroom is a poor man's type mushroom because of its ability to survive harsh conditions (Oei, 1996). These mushroom species are highly productive and have high nutritional values (Shah *et al.,* 2004). The primary socio-economic importance of oyster mushroom cultivation is its use as food and also as a form of income generation (Banik and Nandi, 2004).

In South Africa, at least 3 million people under the age of 15 are suffering from malnutrition (Ayodele *et al.,* 2011). The 2010 national food consumption survey in South Africa showed that 15% of children in rural areas are consuming wild leaves (Ayodele *et al.,* 2011). Proteins are one of the main macromolecules contained in oyster mushroom (Bano *et al.,* 1981; Cheung 2009). Proteins are known to help in growth and repair of cells, the construction of strong structures in the body (bones, muscles) and are part of a balanced diet (Haytowitz, 2004; Kayode *et al.,* 2015). Cultivation of oyster mushrooms has therefore an important role in the national food security program. Inclusion of *Pleurotus* spp. in the diet could be one of the strategies for combating Fe, Zn and other micro-nutrients deficiencies (Mattila *et al*., 2002; Haytowitz, 2004). It has been reported that the use of different substrates to

produce mushrooms has some influence on the macro-and micro-nutrient content of mushrooms (Mbogoh *et al.*, 2011). Stihi *et al.* (2011) reported that growing mushrooms on substrates containing high concentrations of various heavy metals might result in mushroom fruiting bodies sequestering these heavy metals. The high concentration of these heavy metals found in mushrooms can be toxic to humans hence the need to assess the levels of macro- and micro-nutrients and heavy metals, for human consumption. Although oyster mushrooms have been reported to contain these macro- and micro-nutrients, their levels have been noted to be highly variable, possibly influenced by the type of substrates used and climatic conditions grown under. This study was therefore undertaken to analyze the macro- and micro-nutrient content, biochemical compounds and heavy metals of mushrooms raised indifferent hormonally treated substrates.

4.2 Study objectives

4.2.1 Main objective

To determine the transfer efficiency of macro- and micro-nutrients and selected biochemical content in *Pleurotus ostreatus* fruiting bodies grown on substrates treated with different plant growth hormones.

4.2.2 Specific objectives

(i) To analyze macro- and micro-nutrient and protein content of *Pleurotus ostreatus* grown on substrates treated with different plant growth hormones

(ii) To assess the selected pharmacological and biochemical status of *Pleurotus ostreatus* grown on different substrates

(iii) To determine the accumulation coefficient of macro- and micro-nutrients and heavy metals in *Pleurotus ostreatus* fruiting bodies

4.3 Materials and methods

4.3.1 Description of the experiment

4.3.1.1 Macro- and micro-nutrient analysis of mushrooms harvested from different treatment combinations

Macro- and micro-nutrient content from the respective treatment combinations were analyzed using the dry-ashing macro- and micro-nutrient procedure provided by the Agri-Laboratory Association of Southern Africa guidelines

(AgriLASA, 1998). The mushrooms harvested from different treatments were dried at room temperature (26°C) for 72 hours and then milled. One gram (1g) of dried mushroom was placed into a crucibles and further dried for 24 hours, by placing the crucibles with dry mushrooms into an ashing oven at 600°C for a period of 8 hours. The ICP 4:1 plant extract procedure was used, i.e., 8ml Nitric acid (HNO_3) and 2ml hydrochloric acid (HCI) were used as reagents and incubated in a Microwave Reaction System Model 3000 for 45 minutes. Placed digested samples were cooled and transferred into respective filling the volumetric flasks (100ml) which were topped-up to 100ml with distilled water and left standing for 24 hours. Samples were slowly transferred to McCartney bottles without disturbing the sediment. Thereafter, an ICP Mass Spectrometer (Perkin-Elmer, 1982, NexION 300Q) was used to analyze for macro- and micro-nutrients of mushrooms harvested from the different treatment combinations. The macro- and micro-nutrients analyzed were: Fe, Zn, Mn, Mg, K, Ca, Na, P, Cu, Cr, Cd, Co, and Pb and these were expressed as mg/100g.

4.3.1.2 Protein analysis

Samples were analyzed for N content using the same procedure for macro- and micro-nutrient analysis of mushrooms described in paragraph 4.3.1.1. The total N derived from the macro- and micro-nutrient analysis was converted into protein by multiplying by a factor 6.25 that takes into account the N content of a known amino acids composition. The protein content of foodstuffs is commonly estimated by analysis of total N according to the method of Kjeldahl (Ezeibekwe *et al.,* 2009). The conversion factor from total N to protein is Nitrogen x 6.25 (Ezeibekwe *et al.,* 2009).

4.3.1.3 Polyphenol analysis

Total polyphenolic content in the aqueous mushrooms extracts was determined using the Folin-Ciocalteu's phenol reagent according to the method described by (Singleton *et al.,* 1998; Dewanto *et al.,* 2002). A gallic acid of (0.040g) was added to 50ml of 10% ethanol and the reagent that was used was Folin ($NaCO_3$). A 100ml of standard/sample, 625ml folin reagent was added and incubated for 5 minutes at 25°C temperature. Thereafter, 500ml $NaCO_3$ was added and incubated for two hours at room temperature. The absorbance was

measured at 760nm using a thermo atomic absorption spectrometer. All extracts were analyzed for two replicates and expressed as mgGAE/g.

4.3.1.4 Flavonoid analysis

Total flavonoids were extracted from the mushrooms and were determined by a colorimetric method described by (Marinova *et al.,* 2005; Marnewick *et al.,* 2011). 600µl was used of sample/standard thereafter, a 2% of $AlCl_3$ in ethanol was added and the mixture was incubated for 1 hourat ambient temperature (25°C). Subsequently, the solution was thoroughly mixed and change in color was measured at 420nm using a thermo atomic absorption spectrometer. Two replicates were used for all extracts, there were analyzed and expressed as mg catechin standard equivalents (CE) per gram sample (mgQE/g).

4.3.1.5 Heavy metals analysis

The samples were analyzed for heavy metals using the same procedure for macro- and micro-nutrient analysis of the mushrooms described in paragraph 4.3.1.1. The method of Stihi *et al.* (2011) was used to estimate the heavy metal content using the following equation: $K_a = \frac{C_m}{C_s}$, were C_m is concentration of heavy metal in the mushroom and C_s is the concentration of heavy metal in the mushroom substrate. The coefficient of accumulation of Fe, Cu, Mn, Ni, Zn, Se, Cd, Pb and Cr in mushroom fruiting bodies were analyzed and presented as mg/100g.

4.3.1.6 Statistical analysis

The mean values for the various macro- and micro-nutrients and heavy metals were analyzed from the harvested mushrooms and their respective standard deviations were determined. A Tukey's HSD (honest significant difference) test was used to determine the significances of inter- and intra-treatments variations of the samples for the mean separation and standard deviations. Statistical analyses were accomplished by using SAS 9.4 software package Version 2.0 (SAS Institute, Cary, NC, USA, 2002-2012).

4.4 Results

4.4.1 Macro- and micro-nutrient analysis of *Pleurotus ostreatus* fruiting bodies

4.4.1.1 Effect of different substrates on macro- and micro-nutrient composition of *Pleurotus ostreatus* (mg/100g)

Data analysis results indicated that there was a statistically significant effect of different substrates on the *P. ostreatus* nutrient composition for both macro- and micro-nutrients (P<0.05). The following micro-nutrient levels; Zn, Mn, Mg, and Ca. were significantly higher in *Z. mays* than in the other two substrates, *U. panicoides* and *D. stramonium*. There was however, no significant differences on Fe, Na and Cu levels. Macro-nutrient K level was significantly higher in *U. panacoides* than in *Z. mays* and *D. stramonium,* whereas P was significantly higher in *Z. mays* (Table 4.1).

Table 4.1 Macro- and micro-nutrient composition of *Pleurotus ostreatus* harvested from different substrates (mg/100g)

Substrates	Fe mg/100g Mean ± SD	Zn mg/100g Mean ± SD	Mn mg/100g Mean ± SD	Mg mg/100g Mean ± SD	K mg/100g Mean ± SD	Ca mg/100g Mean ± SD	Na mg/100g Mean ± SD	P mg/100g Mean ± SD	Cu mg/100g Mean ± SD	Cr mg/100g Mean ± SD	Cd mg/100g Mean ± SD	Co mg/100g Mean ± SD	Pb mg/100g Mean ± SD
S1	2.52 ± 0.529a	0.35 ± 0.052a	0.16 ± 0.049a	27.37 ± 5.48a	41.07 ± 1.82a	0.39 ± 0.18b	5.73 ± 1.87a	44.69 ± 18.62b	0.13 ± 0.037a	0.064 ± 0.017b	0.00007 ± 0.00003a	0.0026 ± 0.0013b	0.0014 ± 0.0007b
S2	2.29 ± 0.751a	0.39 ± 0.111a	0.19 ± 0.129a	27.89 ± 8.81a	36.93 ± 0.83b	0.51 ± 0.19ab	5.29 ± 1.67a	67.05 ± 17.31a	0.17 ± 0.070a	0.067 ± 0.016b	0.00002 ± 0.00001b	0.0023 ± 0.0020b	0.0015 ± 0.0002b
S3	2.06 ± 0.547a	0.25 ± 0.061b	0.13 ± 0.035b	18.69 ± 13.78b	31.29 ± 0.72b	0.69 ± 0.14a	5.64 ± 1.59a	63.31 ± 81.85a	0.14 ± 0.039a	0.086 ± 0.018a	0.00007 ± 0.00009a	0.0046 ± 0.0039a	0.0025 ± 0.0006a

The results are expressed as mean ± SD and as mg/100g.
Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*,

4.4.1.2 Effect of different plant growth hormones on macro- and micro-nutrient composition of *Pleurotus ostreatus* (mg/100g)

Table 4.2 shows various nutrient content of mushrooms harvested from substrates treated with different plant growth hormones. Analysis of variance of the data indicated that there was a statistically significant effect of different plant growth hormones on the fruiting bodies nutrient composition for both macro- and micro-nutrients levels (P≤0.05). Micro-nutrient Zn level was significantly higher in mushrooms harvested from the following treatments: control (no hormone),

cytokinins, and gibberellins treated substrates than those harvested from auxin treated substrates. Mg was significantly higher in the mushroom harvested from the following treatments; control (no hormone), cytokinins, auxin than those harvested from the gibberellin treated substrates. Heavy metals were generally low in mushroom fruiting bodies harvested from all the experimental units.

Table 4.2 Macro- and micro-nutrient composition of *Pleurotus ostreatus* harvested from substrates treated with different plant growth hormones (mg/100g)

Hormones	Fe mg/100g Mean ± SD	Zn mg/100g Mean ± SD	Mn mg/100g Mean ± SD	Mg mg/100g Mean ± SD	K mg/100g Mean ± SD	Ca mg/100g Mean ± SD	Na mg/100g Mean ± SD	P mg/100g Mean ± SD	Cu mg/100g Mean ± SD	Cr mg/100g Mean ± SD	Cd mg/100g Mean ± SD	Co mg/100g Mean ± SD	Pb mg/100g Mean ± SD
H0	2.32 ± 0.612a	0.37 ± 0.085a	0.12 ± 0.045b	29.85 ± 8.09a	39.82 ± 3.94a	0.40 ± 0.21b	4.60 ± 1.85a	61.94 ± 24.05a	0.17 ± 0.068a	0.056 ± 0.012b	0.00007 ± 0.00004a	0.0011 ± 0.0006c	0.0012 ± 0.0004a
H1	2.65 ± 0.487a	0.39 ± 0.062a	0.15 ± 0.047b	28.10 ± 4.89a	39.21 ± 2.18a	0.39 ± 0.06b	6.29 ± 1.23a	62.51 ± 18.01a	0.15 ± 0.052a	0.074 ± 0.009a	0.00009 ± 0.00008a	0.0026 ± 0.0013b	0.0019 ± 0.0009a
H2	2.18 ± 0.782a	0.29 ± 0.097b	0.14 ± 0.051b	24.80 ± 7.44a	36.76 ± 4.59a	0.41 ± 0.19b	4.83 ± 1.93a	43.98 ± 15.53b	0.12 ± 0.033a	0.067 ± 0.016ab	0.00002 ± 0.00001b	0.0023 ± 0.0020b	0.0019 ± 0.0008a
H3	2.30 ± 0.626a	0.35 ± 0.109a	0.20 ± 0.145a	22.69 ± 13.31a	35.41 ± 3.44a	0.71 ± 0.13a	6.36 ± 1.09a	64.24 ± 21.47a	0.16 ± 0.069a	0.078 ± 0.025a	0.00005 ± 0.00003a	0.0046 ± 0.0039a	0.0016 ± 0.0004a

The results are expressed as mean ± SD and as mg/100g.
Keys: H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

4.4.1.3 Effect of 'substrates X hormones' interaction on macro- and micro-nutrient composition of *Pleurotus ostreatus* (mg/100g)

Data analysis results showed that there was a statistically significant effect of 'substrates X hormones' interaction on the macro- and micro-nutrient oyster mushroom composition ($P<0.05$). Micro-nutrient Fe was significantly higher in the mushrooms harvested from the following treatment combinations: S1H1, S1H2, S1H3, S2H0, S2H1, S2H3 and S3H2 than the other two treatment combinations, i.e. S2H2 and S3H3. Zn levels were only significantly higher in the mushroom harvested from the two treatment combinations S1H2 and S2H1 than the rest of the other treatment combinations. The other nutrient that is nutritionally important is the K and it was noted to be significantly higher in the mushroom harvested from the following treatment combinations: S1H0, SH1, S1H2, S1H3 and S2H2 than the rest of other treatment combinations. Table 4.3 shows comparison of the significant differences of various nutrients.

Table 4.3 Macro- and micro-nutrient composition of *Pleurotus ostreatus* (mg/100g)

Component (per 100g portion)	Fe mg/100g Mean ± SD	Zn mg/100g Mean ± SD	Mn mg/100g Mean ± SD	Mg mg/100g Mean ± SD	K mg/100g Mean ± SD	Ca mg/100g Mean ± SD	Na mg/100g Mean ± SD	P mg/100g Mean ± SD	Cu mg/100g Mean ± SD	Cr mg/100g Mean ± SD	Cd mg/100g Mean ± SD	Co mg/100g Mean ± SD	Pb mg/100g Mean ± SD
S1H0	1.91 ± 0.34b	0.32 ± 0.035b	0.09 ± 0.028c	23.94 ± 4.09b	43.61 ± 2.16a	0.22 ± 0.042d	3.41 ± 1.68c	42.94 ± 18.26c	0.14 ± 0.049b	0.047 ± 0.007c	0.00004 ± 0.00001a	0.0001 ± 0.0003e	0.0009 ± 0.0002c
SIH1	2.96 ± 0.09a	0.36 ± 0.064b	0.15 ± 0.049b	29.46 ± 4.17a	40.46 ± 0.81a	0.40 ± 0.085c	7.06 ± 0.57a	60.72 ± 27.01b	0.13 ± 0.049c	0.079 ± 0.007b	0.00003 ± 0.00001a	0.0037 ± 0.0007c	0.0011 ± 0.0002c
S1H2	2.85 ± 0.37a	0.40 ± 0.049a	0.19 ± 0.035b	27.01 ± 8.34b	41.80 ± 2.32a	0.26 ± 0.064d	5.56 ± 1.73b	30.52 ± 13.39d	0.14 ± 0.042b	0.080 ± 0.008b	0.00002 ± 0.00001a	0.0053 ± 0.0014a	0.0012 ± 0.0002c
S1H3	2.38 ± 0.57a	0.32 ± 0.057b	0.11 ± 0.035c	29.06 ± 8.29a	39.38 ± 0.57a	0.62 ± 0.13b	6.92 ± 1.09a	44.34 ± 16.67c	0.11 ± 0.042c	0.050 ± 0.011c	0.00002 ± 0.00001a	0.0012 ± 0.0005d	0.0014 ± 0.0003c
S2H0	2.74 ± 0.57a	0.32 ± 0.099b	0.15 ± 0.049b	35.77 ± 6.28a	37.27 ± 0.41b	0.57 ± 0.11b	5.79 ± 1.34b	80.69 ± 3.34a	0.20 ± 0.085a	0.065 ± 0.007b	0.00001 ± 0.00001a	0.0012 ± 0.0009d	0.0015 ± 0.0003b
S2H1	2.35 ± 0.57a	0.42 ± 0.063a	0.15 ± 0.064b	26.74 ± 6.86b	37.71 ± 1.51b	0.36 ± 0.062c	5.53 ± 1.39b	64.31 ± 15.19b	0.17 ± 0.064b	0.069 ± 0.011b	0.00003 ± 0.00001a	0.0028 ± 0.0009	0.0016 ± 0.0004b
S2H2	1.29 ± 0.22b	0.24 ± 0.071c	0.09 ± 0.028c	17.79 ± 3.87c	38.13 ± 1.24a	0.35 ± 0.78c	3.24 ± 1.69c	43.47 ± 5.83c	0.11 ± 0.035c	0.051 ± 0.012c	0.00003 ± 0.00002a	0.0014 ± 0.0006d	0.0016 ± 0.0001b
S2H3	2.81 ± 0.54a	0.34 ± 0.085b	0.13 ± 0.099b	31.24 ± 9.46a	36.75 ± 0.82b	0.72 ± 0.099a	6.62 ± 0.56a	79.75 ± 0.21a	0.13 ± 0.078c	0.085 ± 0.015b	0.00003 ± 0.00002a	0.0095 ± 0.0007a	0.0016 ± 0.0002b
S3H0	-	-	-	-	-	-	-	-	-	-	-	-	-
S3H1	-	-	-	-	-	-	-	-	-	-	-	-	-
S3H2	2.41 ± 0.54a	0.24 ± 0.078c	0.19 ± 0.042b	29.61 ± 6.21a	30.89 ± 1.41c	0.65 ± 0.65a	5.72 ± 2.27b	57.97 ± 15.44c	0.16 ± 0.035b	0.071 ± 0.008b	0.00004 ± 0.00001a	0.0020 ± 0.0007d	0.0029 ± 0.0003a
S3H3	1.72 ± 0.38b	0.27 ± 0.064c	0.38 ± 0.035a	16.78 ± 5.31c	31.61 ± 1.21c	0.75 ± 0.75a	5.57 ± 1.55b	68.65 ± 26.73b	0.23 ± 0.049a	0.101 ± 0.002a	0.00002 ± 0.00001a	0.0032 ± 0.0009c	0.0020 ± 0.0002b

The results are expressed as mean ± SD and as mg/100g.

Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*, H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

4.4.2 Effect of different plant growth substrates and hormones on the protein content of *Pleurotus ostreatus* per 100g (%)

4.4.2.1 Effect of various substrates on protein content percentage of *Pleurotus ostreatus* per 100g (%)

Analysis of variance results showed that there was a statistically significant effect of different substrates on the protein content of the harvested mushrooms ($P<0.05$). Substrate *Z. mays* (S2) was noted to have the highest protein content

with 50.97% followed by *D. stramonium* (S3) with 49.23% and the lowest was *U. panicoides* (S1) with 47.55% (Table 4.4).

Table 4.4 Protein content percentage of *Pleurotus ostreatus* harvested from different substrates per 100g (%)

Substrates	Protein content percentage (%) Mean ± SD
S1	47.55 ± 4.24c
S2	50.97 ± 1.81a
S3	49.23 ± 1.61b

Protein content is expressed as (%/100g) ± SD
Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*,

4.4.2.2 Effect of different plant growth hormones on protein content percentage of *Pleurotus ostreatus* per 100g (%)

Data analysis results showed that there was a statistically significant effect of different plant growth hormones on the protein of the harvested mushrooms ($P<0.05$). Mushroom harvested from the gibberellin treated substrates and the control had significantly higher protein than those harvested from the cytokinins and auxin treated substrates. Table 4.5 shows protein content levels of mushroom harvested from the various treatments.

Table 4.5 Protein content percentage of *Pleurotus ostreatus* harvested from substrates treated with different plant growth hormones per 100g (%)

Hormones	Protein content percentage (%) Mean ± SD
H0	50.58 ± 1.28a
H1	46.59 ± 2.17b
H2	48.73 ± 4.69b
H3	50.66 ± 2.26a

Protein content is expressed as percentages (%/100g) ± SD
Keys: H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

4.4.2.3 Effect of 'substrates X hormones' interaction on protein content percentage of *Pleurotus ostreatus* per 100g (%)

Data analysis results showed that there was statistical significant effect of 'substrates X hormones' interaction on the protein content of the mushrooms harvested from the various treatment combinations (P<0.05). Mushroom harvested from the following treatment combinations: S1H3, S2H0, S2H2, S2H3 and S3H2 and had significantly higher protein content than mushroom harvested from various other treatment combinations (Table 6).

Table 4.6 Protein content percentage of *Pleurotus ostreatus* per 100g (%)

Component (per 100g portion)	**S1H0 Mean ± SD**	**S1H1 Mean ± SD**	**S1H2 Mean ± SD**	**S1H3 Mean ± SD**	**S2H0 Mean ± SD**	**S2H1 Mean ± SD**	**S2H2 Mean ± SD**	**S2H3 Mean ± SD**	**S3H0 Mean ± SD**	**S3H1 Mean ± SD**	**S3H2 Mean ± SD**	**S3H3** Mean ± SD
Protein content %	49.6 ± 1.35b	44.6 ± 1.23c	42.7 ± 1.86c	52.9 ± 0.94a	51.2 ± 1.18a	48.9 ± 1.46b	52.5 ± 0.89a	51.1 ± 1.05a	-	-	50.5 ± 1.14ab	48.1 ± 1.78b

Protein content is expressed as percentages (%/100g) ± SD
Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*, H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

4.4.3 Effect of various plant growth substrates and hormones on total polyphenols of *Pleurotus ostreatus*

4.4.3.1 Effect of different substrates on total polyphenols content of *Pleurotus ostreatus* (mgGAE/g)

Data analysis results showed that there was statistically significant effect of different substrates on total polyphenols in the harvested mushrooms (P<0.05). Table 4.7 indicates that mushroom harvested from *D. stramonium* (S3) had was significantly higher total polyphenol content than those harvested from *U. panicoides.*

Table 4.7 Total polyphenols of *Pleurotus ostreatus* harvested from different substrates (mgGAE/g)

Substrates	**Total polyphenols cont. Mean ± SD**
S1	189.313 ± 21.07b
S2	186.043 ± 45.16ab
S3	194.973 ± 62.21a

Total polyphenols are expressed as Mean ± SD as mgGAE/g
Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*

4.4.3.2 Effect of different plant growth hormones on total polyphenols content of *Pleurotus ostreatus* (mgGAE/g)

Analysis of data showed that there was statistically significant effect of different plant growth hormone on the total polyphenols in the harvested mushrooms ($P<0.05$). Table 4.8 shows that mushrooms harvested from the cytokinins treated substrate (H1) had significantly higher total polyphenol content than those harvested from *D. stramonium* (H3) and control (no hormone – H0).

Table 4.8 Total polyphenols of *Pleurotus ostreatus* harvested from substrates treated with different plant growth hormones (mgGAE/g)

Hormones	**Total polyphenols cont. Mean ± SD**
H0	193.901 ± 22.23b
H1	219.043 ± 29.23a
H2	178.201 ± 56.52ab
H3	176.961 ± 27.61b

Total polyphenols are expressed as Mean ± SD as mgGAE/g
Keys: H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

4.4.3.3 Effect of 'substrates X hormones' interaction on total polyphenols content of *Pleurotus ostreatus* (mgGAE/g)

Figure 4.1(a) shows that mushroom harvested from the treatment combinations S2H1 and S3H2 had significantly higher total polyphenol content than those harvested from all the other treatment combinations. Polyphenol content levels of mushrooms harvested from the rest of the other treatment combinations were not statistically significantly different from each other.

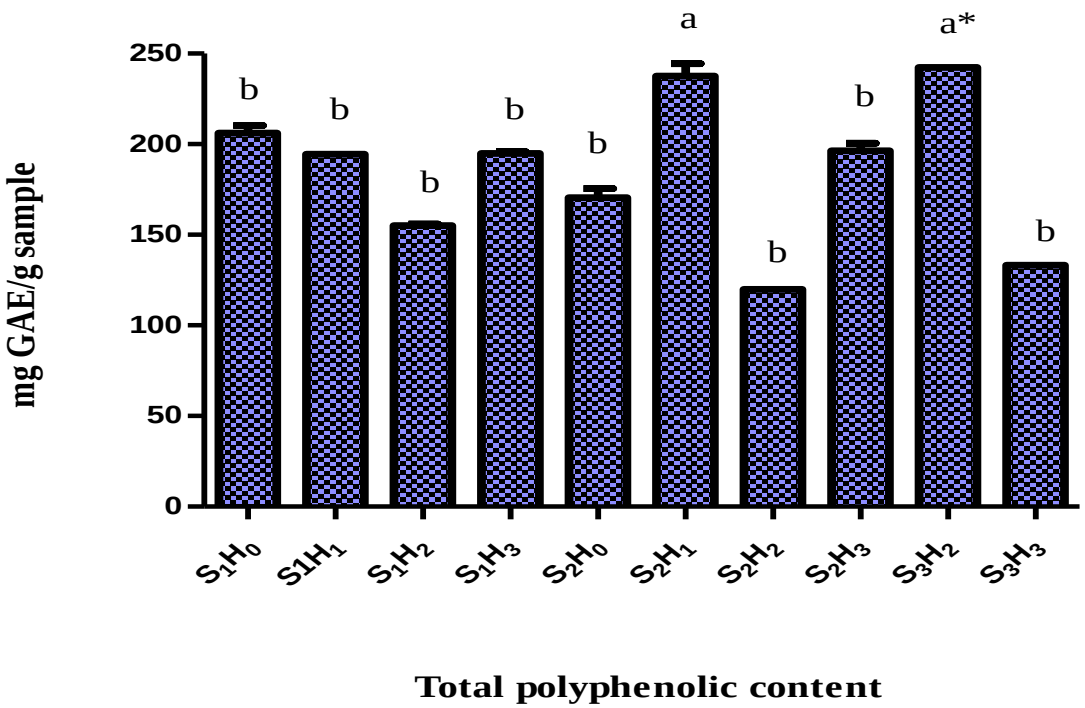

Figure 4.1(a) Total polyphenols of various treatment combinations (mgGAE/g)

The results are expressed as a mean in milligrams per gram (mg/g). The treatment combinations which has (a*) had the highest polyphenols and those which are not sharing a common superscript letter(s) are significantly different from one another (P≤0.05).

Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*, H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

4.4.4 Effect of various plant growth substrates and hormones on total flavonoids of *Pleurotus ostreatus*

4.4.4.1 Effect of different substrates on total flavonoids content of *Pleurotus ostreatus* (mgQE/g)

Table 4.9 shows total flavonoids of mushrooms harvested from all the three substrates i.e. *U. panicoides* (S1), *Z. mays* (S2) and *D. stramonium* (S3) were statistically significantly different. The mushrooms harvested from *D. stramonium* had the highest flavoids level.

Table 4.9 Total flavonoids of *Pleurotus ostreatus* harvested from different substrates (mgQE/g)

Substrates	Total flavonoids cont. Mean ± SD
S1	3.493 ± 0.95c
S2	4.243 ± 2.55b
S3	5.495 ± 4.03a

Total flavonoids are expressed as Mean ± SD as mgGAE/g
Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*

4.4.4.2 Effect of different plant growth hormones on total flavonoids content of *Pleurotus ostreatus* (mgQE/g)

Table 4.10 shows that there is statistically significant difference in the effect of different plant growth hormones on total flavonoids ($P<0.05$). Mushroom harvested from cytokinins treated substrates had the highest flavonoids content and the gibberellin treated substrates had the lowest content.

Table 4.10 Total flavonoids of *Pleurotus ostreatus* harvested from substrates treated with different plant growth hormones (mgQE/g)

Hormones	Total flavonoids cont. Mean ± SD
H0	3.295 ± 1.40c
H1	5.928 ± 2.34a
H2	4.675 ± 3.45b
H3	3.153 ± 1.13d

Total flavonoids are expressed as Mean ± SD as mgGAE/g
Keys: H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

4.4.4.3 Effect of 'substrates X hormones' interaction on total flavonoids content of *Pleurotus ostreatus* (mgQE/g)

Figure 4.1(b) shows that there was a statistically significant effect of 'substrates X hormones' interaction on the mushroom total flavonoids content (P<0.05). Mushrooms harvested from the two treatment combinations; S2H1 and S3H2 had significantly higher flavonoids content than the mushrooms harvested form the rest of the other treatment combinations. It was also observed that treatment combinations S1H0, S1H1, S1H2, S1H3, S2H0, S2H2, S2H3 and S3H3 were not statistically significant different from each other.

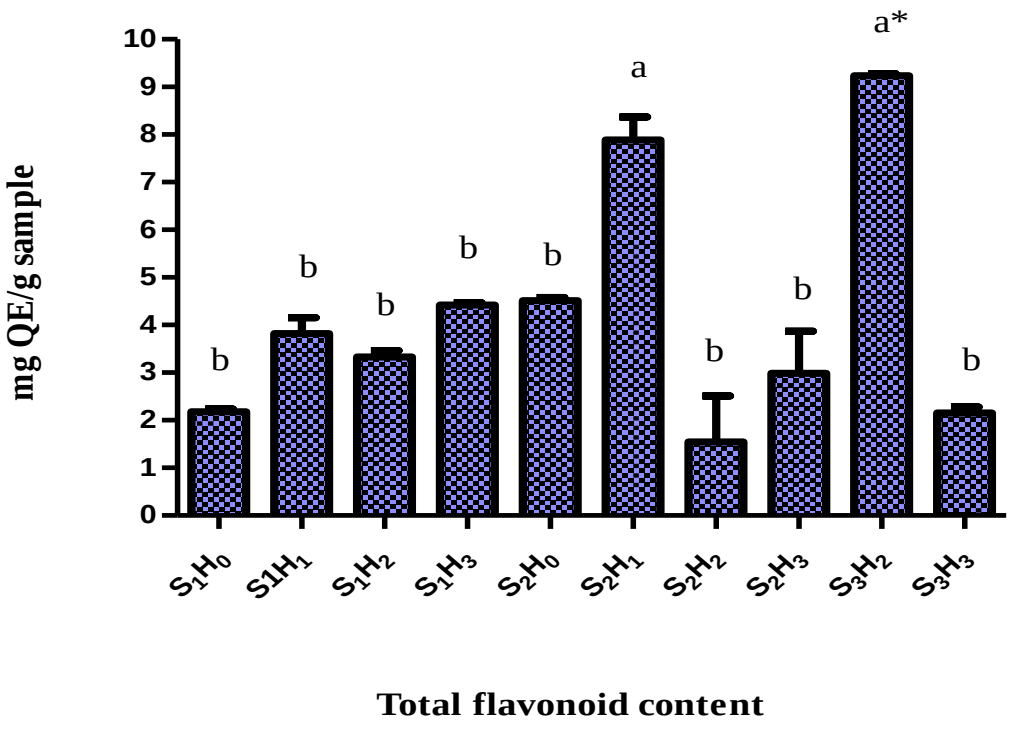

Figure 4.1(b) Total flavonoids of various treatment combinations (mgQE/g)

The results are expressed as mean in milligrams per grams (mg/g). The treatment combinations which has (a*), had the highest flavonoids and those which are not sharing a common superscript letter(s) are significantly different from one another (P≤0.05).

Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*, H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

4.4.5 Effects of various plant growth substrates and hormones on accumulation coefficient of macro- and micro-nutrient and heavy metals in *Pleurotus ostreatus* fruiting bodies (mg/100g)

4.4.5.1 Effect of different substrates on accumulation coefficient of macro- and micro-nutrient and heavy metals in *Pleurotus ostreatus* fruiting bodies (mg/100g)

Table 4.11 shows that there was a statistically significant effect of different substrates on the accumulation coefficient of heavy metals, macro- and micro-nutrients of *P. ostreatus* fruiting bodies. On analysis of important micro-nutrients, it is noted that *U. panicoides* (S1) had significantly higher accumulation coefficient of Fe and Zn than other substrates. However, *Z. mays* (S2) and *D. stramonium* (S3) had significantly higher accumulation coefficient of heavy metal Pb.

Table 4.11 Accumulation coefficient of macro- and micro-nutrient and heavy metals in *Pleurotus ostreatus* fruiting bodies from different substrates (mg/100g)

Substrates	Fe mg/ml Mean ± SD	Mg mg/ml Mean ± SD	Cu g/ml Mean ± SD	Mn mg/ml Mean ± SD	Ni mg/ml Mean ± SD	Zn mg/ml Mean ± SD	Se mg/ml Mean ± SD	Cd mg/ml Mean ± SD	Pb mg/ml Mean ± SD	Cr mg/ml Mean ± SD
S1	0.66 ± 0.12a	0.52 ± 0.06a	2.02 ± 0.31b	0.23 ± 0.09b	1.02 ± 0.72b	3.05 ± 0.28a	0.0001 ± 0.00008a	1.19 ± 1.36a	0.11 ± 0.05 b	0.78 ± 0.41a
S2	0.45 ± 0.15b	0.61 ± 0.18a	3.21 ± 0.89a	0.40 ± 0.23a	0.36 ± 0.09c	1.80 ± 0.58b	0.0001 ± 0.00009a	0.33 ± 0.21b	1.15a ± 0.28	0.52 ± 0.27b
S3	0.40 ± 0.10b	0.46 ± 0.27a	0.51 ± 0.32c	0.17 ± 0.04b	2.23 ± 0.87a	0.69 ± 0.22c	0.00001 ± 0.00001a	0.00 ± 0.00c	1.10 ± 0.25a	0.59 ± 0.05b

The results are expressed as mean ± SD and as mg/100g.

Keys: S1 – *U. panicoides*, S2 – *Z. mays*, S3 – *D. stramonium*,

4.4.5.2 Effect of different plant growth hormones on accumulation coefficient of macro- and micro-nutrient and heavy metals in *Pleurotus ostreatus* fruiting bodies (mg/100g)

Table 4.12 shows that there was a statistically significant effect of various plant growth hormones on the accumulation coefficient of various macro- and micro-nutrients in mushroom harvested from various substrates treated with different plant growth hormones ($P<0.05$). Accumulation coefficient of Fe was significantly higher in mushroom harvested from the following treatments: control – no hormonal treatment (H0), cytokinins (H1), and gibberellins (H3) than those harvested from auxins treated substrates (H2). Accumulation coefficients of Zn were also higher in mushroom harvested from the control – no hormone treatment and cytokinins treated substrates than mushroom harvested from the gibberellin treated substrates. There was however no significant differences in the accumulation coefficient of Pb which is an important heavy metals. Accumulation coefficient of all heavy metals of mushrooms harvested from all experimental units was generally low.

Table 4.12 Accumulation coefficient of macro- and micro-nutrient and heavy metals in *Pleurotus ostreatus* fruiting bodies from different plant growth hormones (mg/100g)

Hormones	Fe mg/ml Mean ± SD	Mg mg/ml Mean ± SD	Cu g/ml Mean ± SD	Mn mg/ml Mean ± SD	Ni mg/ml Mean ± SD	Zn mg/ml Mean ± SD	Se mg/ml Mean ± SD	Cd mg/ml Mean ± SD	Pb mg/ml Mean ± SD	Cr mg/ml Mean ± SD
H0	0.52 ± 0.49ab	0.59 ± 0.21a	2.81 ± 0.73a	0.24 ± 0.12b	0.16 ± 0.18d	2.30 ± 0.69a	0.00015 ± 0.00009a	1.31 ± 0.36a	0.71 ± 0.69 a	0.51 ± 0.43a
H1	0.63 ± 0.18a	0.59 ± 0.06a	2.57 ± 0.69a	0.29 ± 0.06ab	0.62 ± 0.33c	2.85 ± 0.32a	0.00012 ± 0.00007a	1.09 ± 0.41b	0.53 ± 0.49a	0.77 ± 0.41b
H2	0.47 ± 0.23b	0.53 ± 0.14a	1.62 ± 0.81b	0.24 ± 0.09b	1.21 ± 0.73a	1.77 ± 1.25ab	0.00004 ± 0.00005ab	0.00 ± 0.00c	0.79 ± 0.58a	0.61 ± 0.35ab
H3	0.51 ± 0.15ab	0.59 ± 0.23a	2.11 ± 1.79ab	0.37 ± 0.31a	1.59 ± 1.14b	1.72 ± 1.03b	0.00008 ± 0.00005b	0.00 ± 0.00c	0.80 ± 0.59a	0.68 ± 0.19ab

The results are expressed as mean ± SD and as mg/100g.

Keys: H0 – No hormone, H1 – Cytokinins, H2 – Auxins, H3 – Gibberellins

4.4.5.3 Effect of 'substrates X hormones' interaction on accumulation coefficient of macro- and micro-nutrient and heavy metals in *Pleurotus ostreatus* fruiting bodies (mg/100g)

Table 4.13 shows that there was a statistically significant difference on the effect of different treatment combinations on the accumulation coefficient of macro- and micro-nutrients of mushrooms harvested from different combinations. Treatment combinations S1H1, S1H2 and S2H1 had significantly higher accumulation coefficient of Fe than the rest of treatment combinations. Mushrooms with significant higher accumulation coefficient of heavy metal Pb was with those harvested from the following treatments; S2H0 and S2H2 when compared to mushrooms harvested from the rest of the other treatment combinations.

Table 4.13 Accumulation coefficient of macro- and micro-nutrient and heavy metals in *Pleurotus ostreatus* fruiting bodies (mg/100g)

Component (per 100g portion)	Fe mg/ml Mean ± SD	Mg mg/ml Mean ± SD	Cu mg/ml Mean ± SD	Mn mg/ml Mean ± SD	Ni mg/ml Mean ± SD	Zn mg/ml Mean ± SD	Se mg/ml Mean ± SD	Cd mg/ml Mean ± SD	Pb mg/ml Mean ± SD	Cr mg/ml Mean ± SD
S1H0	0.51 ± 0.02b	0.47 ± 0..03b	2.27 ± 0.08c	0.14 ± 0.05c	0.44 ± 0.08e	2.89 ± 0.04ab	0.00002 ± 0.00001d	2.61 ± 0.08a	0.09 ± 0.02c	0.14 ± 0.02c
SIH1	0.77 ± 0.05a	0.57 ± 0.56b	2.02 ± 0.21c	0.25 ± 0.04b	0.90 ± 0.08bc	3.08 ± 0.26a	0.00015 ± 0.00007b	2.18 ± 0.21a	0.11 ± 0.02 c	1.11 ± 0.11a
S1H2	0.73 ± 0.07a	0.55 ± 0.51b	2.17 ± 0.23c	0.35 ± 0.02b	1.85 ± 0.07b	3.34 ± 0.43a	0.00010 ± 0.00008b	0.00 ± 0.00b	0.08 ± 0.07c	0.98 ± 0.12a
SIH3	0.63 ± 0.07ab	0.53 ± 0.09b	1.63 ± 0.28c	0.19 ± 0.02c	1.31 ± 0.08b	2.88 ± 0.06ab	0.00015 ± 0.00007b	0.00 ± 0.00b	0.14 ± 0.03c	0.91 ± 0.03a
S2H0	0.54 ± 0.07b	0.70 ± 0.28a	3.36 ± 0.63b	0.33 ± 0.06b	0.32 ± 0.6d	1.72 ± 0.23b	0.00030 ± 0.00014a	0.00 ±	1.31 ± 0.04a	0.88 ± 0.06a
S2H1	0.78 ± 0.04a	0.68 ± 0.28a	3.11 ± 0.41b	0.34 ± 0.06b	0.35 ± 0.06d	2.63 ± 0.16b	0.00007 ± 0.00002b	0.00 ± 0.00b	0.95 ± 0.16b	0.42 ± 0.05b
S2H2	0.24 ± 0.06d	0.45 ± 0.64c	2.11 ± 0.16c	0.19 ± 0.04c	0.30 ± 0.06d	1.24 ± 0.24b	0.00001 ± 0.00001d	0.00 ± 0.00b	1.00 ± 0.08a	0.25 ± 0.21c
S2H3	0.57 ± 0.57b	0.74 ± 0.08a	4.26 ± 0.48a	0.76 ± 0.13a	0.49 ± 0.05c	1.63 ± 0.24b	0.00002 ± 0.00001d	0.00 ± 0.00b	1.36 ± 0.49 a	0.53 ± 0.08b
S3H0	-	-	-	-	-	-	-	-	-	-
S3H1	-	-	-	-	-	-	-	-	-	-
S3H2	0.48 ± 0.06b	0.69 ± 0.07a	0.59 ± 0.09d	0.18 ± 0.04c	1.49 ± 0.22b	0.74 ± 0.08c	0.00002 ± 0.00001c	0.00 ± 0.00b	1.31 ± 0.011a	0.58 ± 0.06b
S3H3	0.33 ± 0.06c	0.26 ± 0.04d	0.42 ± 0.53d	0.16 ± 0.04c	2.99 ± 0.16a	0.65 ± 0.35c	0.00001 ± 0.00001c	0.00 ± 0.00b	0.89 ± 0.077b	0.59 ± 0.05b

4.5 Discussion

Pleurotus spp. as a saprophytic macro-fungi it extracts its nutrients from the substrate, i.e., grasses, wood and agricultural residues through its mycelium, obtaining substances necessary for its development, such as carbon, nitrogen, minerals and vitamins (Urben, 2004). The significantly higher levels of Zn, Mn, and Mg (Table 4.1) micro-nutrients levels recorded in *Z. mays* than in the other two substrates, *U. panicoides* and *D. stramonium* is indicative that substrate *Z. mays* had better transfer efficiency of these nutrients than the other growth media. Transfer efficiency could have been influenced by factors such as substrate, pH and various biochemical processes that influence uptake and transfer of nutrients in fungal mycelia (Gençcelep *et al.,* 2009; Owaid *et al.,* 2015). The high levels of micro-nutrient composition of Mn, Ca and Cu on substrate S3H3 (*D. stramonium* + Gibberellins) were noted not to have a corresponding response in the levels of these micro-nutrients in the oyster mushroom fruiting bodies. This was probably because the micro-nutrient composition of the substrate *D. stramonium* was not efficiently transferred to the mushroom fruit caps during the development phase. Although it has been established in this study that biotic and abiotic factors do influence distribution and deposition of minerals in oyster mushrooms, it has been reported that deposition of these minerals to the various parts of the mushroom parts including fruit bodies is also considerably influenced by the age of mycelium and by the interval between the formation of different fruiting bodies. Minerals are known to be distributed unevenly within fruiting bodies which grow at different times (Stihi *et al.,* 2011; Skiba *et al.,* 2016).

In plants uptake of Zn has been reported to be influenced by soil pH and it is also reported that it influences increase or decrease in concentration of other micro-nutrients. Zn is often referred to as having antagonistic effect to Fe or Cu (Chilian *et al.,* 2015). This is evident in the mushroom nutrient (Table 4.3) where Zn level is high and Fe and Cu levels are evidently low. Furthermore, it is possible that nutrient transfer efficiency could have been influenced by other factors such as parent material. Plant material would determine the total quantity of the nutrients in the soil but their availability to plants is known to be influenced by several biotic and abiotic factors. The abiotic factors would include; solubility, mobility, and bioaccumulation

properties of a particular nutrient (Robinson *et al.,* 2005). It is therefore possible that higher nutrient transfer efficiency recorded with the mushroom grown in *Z. mays* could be indicative that this substrate enhanced solubility, mobility and bioaccumulation of these particular elements. Weng *et al.* (2002) also reported that dissolving of organic matter promoted nutrients solubility and mobility in plants. This however, needs to be investigated in oyster mushrooms. Generally the mushrooms harvested from substrate *Z. mays* had high levels of P and this could probably be the results of phosphate fertilizer applications during the growth of *Z. mays* (Table 4.3). The less percentage of P in the mushroom harvested from *U. panicoides* was most likely because this grass is often grown as a fallow crop after maize and therefore would be growing in P depleted soils. *D. stramonium* as a weed would also have limited access to P as it is often weeded out from the fields.

Influence of the abiotic factors on the nutrient transfer efficiency as noted in Tables 4.12 and 4.13, could have resulted from the hormonal treatment of substrates. This could possibly highlight that plant growth hormones can also have influence on the nutrient transfer efficiency in oyster mushroom. The low levels of Zn in mushroom harvested from the hormone auxin treated substrate could have been because of the cellular oxidation relationship between Zn and auxins. This process could have reduced levels of Zn in the fruiting caps in favour of its accumulation in the mitotic active mycelium. (Table 4.2). It has been reported that auxins promote cell elongation and Zn play an important role in cell oxidation. The need for Zn in these actively growing mycelium could have generated physiological attraction of Zn to the actively growing sites (Khandakar, 2004; Dey *et al.,* 2007). Kobyłecka and Skiba (2008) working on plants established that treating plants with auxins may affect either mineral uptake from the soil environment or their further migration within the plant body. However, the high levels of macro-nutrient K on mushrooms harvested from treatment combination (*U. panicoides* + no hormone) was probably indicative that transfer efficiency of different nutrients are not all influenced by hormones.

Pleurotus spp. other than being important sources of minerals, they are also known to be good sources of protein. The high percentage of protein content on mushrooms harvested from substrate S1H3 (*U. panicoides* + Gibberellins) and S2H2 (*Z. mays* + Auxins) was probably because of the high N content in *Z. mays* and *U. panicoides*

substrates (Table 3.2 in chapter 3). Nitrogen is an important component of amino acids which are the main building block of proteins (Shah *et al.,* 1997; Sun and Liu, 2004; Mukhopadhyay and Guha, 2015). However, further research work needs to be undertaken to determine appropriate N forms and their optimum levels that can be used to bio-fortify various substrates that are commonly found in the semi-arid regions.

In addition to the nutritional importance of oyster mushrooms, they mushrooms are also reported to have medicinal properties (Shang *et al.,* 2015). Total polyphenols and flavonoids were high in mushroom harvested from treatment combination S3H2 and S2H1 (Fig. 4.1a and Fig. 4.1b). This could probably be as a result of the higher transfer efficiency of these phytochemicals in the two substrates *D. stramonium* and *Z. mays*than with in *U. panicoides*. It has been reported that *D. stramonium* and *Z. mays* contain high quantities of these phytochemicals (Dewanto *et al.,* 2002; Son *et al.,* 2012). Finding of this study indicated that pH, substrate and phytochemicals have a significant role on transfer efficiency of nutrients in *P. ostreatus* fruiting bodies.

Despite the nutritional benefit of oyster mushrooms, its continuous availability to both producers and consumers is affected by its high perishability. Therefore, there is a need to develop and identify post-harvest processing technologies that will help improve shelf-life and keeping qualities of these mushroom species.

Chapter Five

Comparative analyses of solar-dried and biscuits nutrient content and consumer acceptability of processed *Pleurotus ostreatus*

5.1 Introduction

With all challenges surrounding the after harvest, storage and keeping quality of *Pleurotus ostreatus*, post-harvest practices have been developed to extend their shelf-life (SLS, 1999). There are several practices which have been explored to achieve advancement of their storage and improvement in their shelf-life. These practices include cooling and refrigeration, drying, irradiation and vacuum-cooling (Rai, 2009). The most commonly used method is the cooling and refrigeration (Ahlawat *et al.,* 2000). Cooling and refrigeration to preserve mushrooms can be classified into two categories: refrigeration and freezing. Generally, storage at 0°C and 95% RH has been reported to be the optimum condition to extend mushrooms shelf-life (SLS, 1999). Household and commercial refrigerators usually run at 4 to 7°C. Freezing of mushrooms take place at temperature of below -18°C (Minamide *et al.,* 1980). Cooling storage generally preserves perishables for days or weeks and frozen storage, i.e., deep-freezing, preserves mushrooms for several months or even years. Low temperature retards the growth of micro-organisms, reduces the rate of post-harvest metabolic activities of the mushroom tissues and minimizes moisture loss (Rai and Arumuganathan, 2008).

Other methods that are used for the preservation of oyster mushrooms include irradiation technique. Irradiation is a process whereby sterilization is used to preserve the mushroom without a noticeable change in their physical natural characteristics. This method not only extends the shelf-life of mushrooms, but also lowers the microbial contamination (Lescane, 1984; Benoit *et al.,* 2000). It is a process which should be applied immediately after harvest for optimum benefits (Roy and Bahl, 1984). Various types of beneficial effects of radiation have been observed in preserving button mushrooms (Roy *et al.,* 2000). The use of irradiation has been found to reduce the loss of water, change in color, flavor, texture and finally the quality (Benoit *et al.,* 2000). It has, however, been noted that irradiation damages the mushrooms by breaking up molecules and also creates free radicals which kill some bacteria and can destroy vitamins and enzymes (Benoit *et al.,* 2000).

Another commonly used method for mushrooms preservation is drying. This technique is popular with both commercial and small-scale producers. Drying is possibly the oldest food processing technique known to mankind for preservation of agricultural produce (Torringa *et al.,* 2001). It is the process whereby the removal of moisture from the produce is made to such a low level that microbial and biochemical activities are suppressed by the reduction of water activity. This produces products that are suitable for safe storage and protection against attack by microorganisms (Torringa *et al.,* 2001). It is reported that when stored properly, dried mushrooms can enhance stronger flavor than fresh ones. The drying of mushroom is conducted in various ways such as using solar dryers, drying at room temperature at 26°C for at least 74 hours and pre-heated (cooked) at 100°C for 20 minutes (Arumuganathan *et al.,* 2004).

As oyster mushroom production is highly dependent on fruit cap formation, harvesting is often carried out in two-day intervals. Therefore, growers should have sufficient storage and proper preservation methods for their produce. Due to the high perishability of mushrooms, smallholder producers face challenges of producing mushrooms which will be at the risk of deterioration and loss of quality; hence there is a need to explore and develop other appropriate preservation alternatives that are cost-effective and easy to handle for poor smallholder producers. Most rural communities do not have access to high technology for the purpose of storing and preserving the harvested produce, this is due to the high cost of advanced technology (Meera, 2004). Like any other highly perishable horticultural produce, profitability of oyster mushrooms is highly dependent on the control of the deterioration process. Loss of quality owing to poor storage conditions very often correspondingly results in a drop in price or rejection by the market (Baker *et al.,* 1981; Ahlawat *et al.,* 2000). Oyster mushrooms get spoiled due to browning, wilting, liquefaction, loss of texture, aroma, flavor, etc, making it not suitable for the market and consumers (Anantheswaran *et al.,* 1994). The primary cause of spoilage on fresh mushroom are enzymatic reactions and the action of bacteria in the living tissue. The browning of mushrooms is caused by a combination of auto enzymatic and microbial action (Gonzalez *et al.,* 2000). This study was, therefore, carried out to investigate the effect of different low cost post-harvest processing technologies on the preservation and retention of valuable nutrients.

5.2 Study objectives

5.2.1 Main objective

To analyze nutrient content of the processed *Pleurotus ostreatus* from two different processing techniques and consumer acceptability

5.2.2 Specific objectives

(i) To analyse the nutrient content of solar-dried mushrooms and *Pleurotus ostreatus* biscuits

(ii) To assess the palatability of *Pleurotus ostreatus* biscuits made from different 'Mushroom-Wheat Flour' mixing ratios

5.3 Materials and methods

5.3.1 Description of the experiment

5.3.1.1 Preparation of processed oyster mushrooms

5.3.1.1.1 Preparation of solar-dried mushrooms (SDM)

The fresh mushrooms were solar-dried for 120 hours at a temperatures ranging between 26 to 34°C using a solar-drier, thereafter, they were milled using a blender. The mushrooms were dried for a period of five (5) days those specific temperatures was to properly dry and allowing the mushrooms to only retain less than 10% of moisture content.

5.3.1.1.2 Preparation of *Pleurotus ostreatus* biscuits

The following biscuit mixing ratios were prepared:

i. Biscuit A: 1 egg: 50g of SDM: 250g of wheat-flour: 2.5g salt: 200ml of water
ii. Biscuit B: 1 egg: 100g of SDM: 250g of wheat-flour: 2.5g salt: 200ml of water
iii. Biscuit C: 1 egg: 150g of SDM: 250g of wheat-flour: 2.5g salt: 200ml of water

The biscuits were baked using an ordinary domestic oven:

Keys: SDM* - Solar-dried mushrooms

Figure 5.1 Biscuits of three different mushroom-wheat flour mixing ratios.

5.3.1.1.3 Description analysis of solar-dried mushrooms and *Pleurotus ostreatus* biscuits

5.3.1.1.3.1 Analysis of macro- and micro-nutrient composition in solar-dried mushrooms

Macro-and micro-nutrient composition was analysed in solar-dried mushrooms using the dry-ashing macro- and micro-nutrient procedure provided by the Agri-Laboratory Association of Southern Africa guidelines (AgriLASA, 1998). The mushrooms were dried using a solar dryer for 120 hours at a temperatures ranging between 26 to 34°C and then milled. One gram (1g) solar-dried mushroom was placed into a crucible. Crucibles with the dry sample were placed in an ashing oven at 600°C for a period of 8 hours. The ICP 4:1 plant extract procedure was used, i.e., 8ml nitric acid (HNO_3) and 2ml hydrochloric acid (HCl) were used as reagents and the samples incubated in a Microwave Reaction System Model 3000 for 45 minutes. Samples were digested for 45 minutes, cooled and transferred into respective volumetric flasks (100 ml) which were eventually topped-up to volume with distilled water and left standing for 24 hours. The samples were slowly transferred to McCartney bottles without disturbing the sediment. Thereafter, an ICP Mass Spectrometer (Perkin-Elmer, 1982, NexION 300Q) was used to analyze for trace elements in mushroom harvested from the different treatment combinations. The analyzed trace elements were Fe, Zn, Mn, Mg, K, Ca, Na, P, Cu, Cr, Cd, Co, and Pb and these were measured in mg/100g.

5.3.1.1.3.2 Analysis of macro- and micro-nutrient composition in *Pleurotus ostreatus* biscuits

The biscuits were milled and crucibles were used as containers for ashing. The samples were placed into an MRC laboratory ash equipment ELF 11/23 Model at 600°C for a period of 4 hours. One gram (1g) of each sample was weighed out. A hot plate was used to perform a wet digestion of the biscuits using a mixture $HN0_3$:HCl (8:4) (12ml for 1.0g sample) (Narin *et al.,* 2005; Gopalani *et al.,* 2007). The mixture was heated to 130°C for 3 hours. After cooling, 5ml distilled water was added and was mixed. The residue was filtered through Whatmann filter paper No. 42 and the sample was then diluted with distilled water to 50ml. Blank digestions were also performed. Samples were analyzed using an ICP Mass Spectrometer (Perkin-Elmer, 1982, NexION 300Q) and macro- and micro-nutrient from all sample solutions were run in triplicate until they became clear. The analysed macro- and micro-nutrient were Fe, Zn, Mn, Mg, K, Ca, Na, P, Cu, Cr, Cd, Co, and Pb and were expressed as mg/100g.

5.3.1.1.3.3 Assessment on palatability of *Pleurotus ostreatus* biscuits: 'Mushroom-Wheat Flour' mixing ratios

A participatory assessment technique was used to determine the palatability and acceptability of the three mushroom biscuits type and from one egg, 250g of flour, 2.5g salt, and 200ml of water with three different ratios of solar dried oyster mushrooms i.e. 50g, 100g and 150g. A random selection of 33% of students was done in a resident of a total one hundred and fifty. Thereafter a total of fifty students were given three biscuits each and a questionnaire (Appendix B). Each student was requested to taste the three mushroom biscuits and to use the questionnaire to rank the processed products on taste perception and acceptability using the following scale:

Taste perception assessment: **1 to 5** (1-unappealing and 5-excellent)
Acceptability comment: **1 to 5** (1-very poor and 5-very good)

5.3.1.1.4 Statistical analysis

The mean values for the various macro- and micro-nutrients and heavy metals were analyzed from the solar dried mushrooms and different *Pleurotus ostreatus* biscuits and their respective standard deviations were determined. A Tukey's HSD (honest significant difference) test was used to determine the significances of inter- and intra-treatments variations of the samples for the mean separation and standard deviations. Statistical analyses were accomplished by using SAS 9.4 software package Version 2.0 (SAS Institute, Cary, NC, USA, 2002-2012).

All statistical data analyses on palatability survey of *Pleurotus ostreatus* biscuits were performed using SPSS version 24.0 (IBM Corp, 2016) and frequency of the given samples and their percentages were determined.

5.4 Results

5.4.1 Analysis of macro- and micro-nutrient of solar-dried mushrooms (mg/100g)

Table 5.1 shows macro- and micro-nutrient composition of solar-dried mushrooms (SDM).

Table 5.1 Macro- and micro-nutrient composition of solar-dried mushrooms (mg/100g)

Component (per 100g portion)	Fe mg/100g Mean ± SD	Zn mg/100g Mean ± SD	Mn mg/100g Mean ± SD	Mg mg/100g Mean ± SD	K mg/100g Mean ± SD	Ca mg/100g Mean ± SD	Na mg/100g Mean ± SD	P mg/100g Mean ± SD	Cu mg/100g Mean ± SD	Cr mg/100g Mean ± SD	Cd mg/100g Mean ± SD	Co mg/100g Mean ± SD	Pb mg/100g Mean ± SD
Solar Dried Mushrooms	2.96 ± 0.012	0.37 ± 0.029	0.14 ± 0.022	29.17 ± 0.33	44.89 ± 2.12	0.52 ± 0.009	5.63 ± 1.15	69.52 ± 1.87	0.27 ± 0.018	0.022 ± 0.0039	0.0005 ± 0.00011	0.0026 ± 0.00041	0.0012 ± 0.0001

5.4.2 Analysis of macro- and micro-nutrient of *Pleurotus ostreatus* biscuits (mg/100g)

Table 5.2 shows that there is statistically significant difference in the nutrient levels of the three Biscuit types. Biscuit A had significant higher Fe level than Biscuit B and C. However, Biscuit B had higher significant higher levels of Na than the other two types of biscuits i.e. A and C. It is evident that there is generally low levels of heavy metals in all respective biscuits.

Table 5.2 Macro- and micro-nutrient composition for three types of *Pleurotus ostreatus* biscuits (mg/100g)

Component (per 100g portion)	Fe mg/100g Mean ± SD	Zn mg/100g Mean ± SD	Mn mg/100g Mean ± SD	Mg mg/100g Mean ± SD	K mg/100g Mean ± SD	Ca mg/100g Mean ± SD	Na mg/100g Mean ± SD	P mg/100g Mean ± SD	Cu mg/100g Mean ± SD	Cr mg/100g Mean ± SD	Cd mg/100g Mean ± SD	Co mg/100g Mean ± SD	Pb mg/100g Mean ± SD
Biscuit A	2.96 ± 0.035a	0.38 ± 0.014a	0.62 ± 0.049a	15.38 ± 0.27a	46.54 ± 1.52a	0.16 ± 0.007a	5.68 ± 0.08b	32.43 ± 1.28a	0.15 ± 0.014a	0.018 ± 0.0042b	0.0004 ± 0.00014a	0.0025 ± 0.00056a	0.0002 ± 0.00007b
Biscuit B	2.47 ± 0.057b	0.41 ± 0.070a	0.63 ± 0.091a	20.13 ± 0.69a	43.33 ± 4.26a	0.22 ± 0.014a	3.89 ± 0.64a	38.83 ± 0.049a	0.15 ± 0.007a	0.027 ± 0.0035a	0.0005 ± 0.00007a	0.0038 ± 0.00049a	0.0002 ± 0.00007b
Biscuit C	2.31 ± 0.049b	0.39 ± 0.049a	0.60 ± 0.064a	18.26 ± 2.09a	40.95 ± 2.39a	0.16 ± 0.014a	2.71 ± 0.035c	37.53 ± 2.86a	0.15 ± 0.007a	0.019 ± 0.0021b	0.0007 ± 0.00014a	0.0033 ± 0.00014a	0.0006 ± 0.00007a

5.4.3 Assessment on palatability of *Pleurotus ostreatus* biscuits: 'Mushroom-Wheat Flour' mixing ratios.

Data analysis results from the fifty questionnaires indicated that there was statistically significant difference in the three biscuit types as scored for their tastes and acceptability. Best overall biscuit percentage were as follows: sixty percent (60%) respondents preferred Biscuit A, twenty eight percent (28%) preferred Biscuit B and only twelve percent (12%) preferred Biscuit C.

Table 5.3 Palatability assessment of three biscuits types.

Taste Score ⬇	**Biscuit A F (%)**		**Biscuit B F (%)**		**Biscuit C F (%)**	
Unappealing	0		0		8 (16)	
Poor	0		2 (4)		31 (62)	
Fair	12 (24)		23 (46)		5 (10)	
Good	19 (38)		13 (26)		2 (4)	
Excellent	19 (38)		12 (24)		4 (8)	
Acceptability Score ⬇	**F (%)**		**F (%)**		**F (%)**	
Very poor	0		0		0	
Poor	4 (8)		12 (24)		13 (26)	
Acceptable	11 (22)		11 (22)		22 (44)	
Good	16 (32)		17 (24)		11 (22)	
Very good	19 (38)		10 (20)		0	
Detection of difference among samples ➡	**Yes F (%)** 46 (92)	**No F (%)** 4 (8)	**Yes F (%)** 47 (94)	**No F (%)** 3 (6)	**Yes F (%)** 48 (96)	**No F (%)** 2 (4)
Best overall biscuit on palatability ➡	**F (%)** 30 (60)		**F (%)** 14 (28)		**F (%)** 6 (12)	

* F – Frequency in a given sample
* % – Percentages

5.5 Discussion

The increase in levels of micro-nutrient Mn in all different oyster mushroom ratios biscuits highlighted in Table 5.2 could have been because of the supply of Mn from the egg that was used in the backing of all the three biscuits. A chicken egg is reported to contain high levels of various micro-nutrients including Mn (Abduljaleel and Shuhaimi-Othman, 2011). Variation of levels in micro-nutrients Fe, Mg, Ca and Cu might have been as a result of the different eggs used which were probably collected from different chickens feeding on different feeds. The levels of macro- and micro-nutrient content on commercial eggs is determined with the type feeds consumed by chicken during the egg production (Abduljaleel *et al.,* 2011). The high levels of macro-nutrient P in biscuit B could have been because of the higher proportion of dried mushroom added to the recipe (100g mushroom 250g flour) that lead to the probability that biscuit A with lower mushroom proportion of “50g mushroom: 250g flour” was insufficient to increase P content. On the other hand the high quantity “150g mushrooms: 250g flour” in biscuit C was noted not to have a corresponding response on the content of macro-nutrient P. The low levels of micro nutrient Zn and Mg in biscuit A could be probably due the less quantity of mushroom that was added. It was also noted that the levels of following macro- and micro-nutrients: Mg, Ca, P and Cu in the biscuits (Table 5.2) were general lower compared to the macro- and micro-nutrients in the solar dried mushrooms (Table 5.1).

It is particularly important to note that heavy metals Cr, Cd and Pd from both biscuits and solar dried mushrooms were of acceptable safe levels for human consumption (Skiba *et al.,* 2016). High levels of heavy metals are known to be toxic for human consumption (Stihi *et al.,* 2011).

The high percentage on taste score (38%), acceptability score (38%) and best overall biscuits on palatability (60%) for the preference for Biscuit A by respondents could have been influenced by the taste score (38%) and detection of difference among samples (92%). Respondents pointed out that taste and detection of difference among samples as the factors that influenced the decisions of preference. It can be deduced that the less the dried mushroom added to a 250g flour, the more presentable and appetizing the biscuit was to the respondents. The poor ratings on biscuits B (24%) and C (8%) was probably due to the dark color and the strong

undesirable taste which was caused by the addition of solar dried mushroom high quantity levels of 100g and 150g respectively. There is therefore a need for further research in the determination of the optimum mixing ratios that would improve attractiveness, palatability and nutritional value of the oyster mushroom biscuits. There is also need to investigate other processing technologies that will contribute to the improvement of keeping quality of the processed mushrooms. Various other technologies can be investigated and these include the following: making mushroom noodles, sausages etc. Dry noodles can be kept for 1-2 years when properly stored under suitable conditions.

Chapter Six

General discussion

The study on the effect of various hormonally treated plant substrates on the development and yield of *Pleurotus ostreatus* revealed that substrates that were not treated with plant growth hormones generally had higher mushroom yield than the treated substrates with *Urochloa panicoides* recording the highest total yield after 90 days producing mean yield of 247.14g (Fig. 3.4b). It is evident that treating the respective substrates with plant growth hormones had suppressive effect on the mushroom biomass accumulation. The highest conversion efficiency ratio was noted with untreated substrate *Urochloa panicoides* with 96.3% conversion ratio. The reduction in the biomass accumulation in the hormone treated substrates could possibly be explained by the fact that all the three hormones cytokinins, auxins, and gibberellins which are known to enhance mitotic cell division in plants could have exerted similar effect on the oyster mushroom. The enhanced mitotic cell division could have resulted in more mycelial growth and greater number of pinnings which resulted in the creation of intra-competition between the different mushroom growth components, i.e., pinnings and aggressively growing mycelia. Biomass produced from these respective hormonally treated substrates was most probably partitioned and sequestered in many small mushroom fruit caps resulting in the low overall yield.

Although treatments of all the substrates with hormones resulted in the reduction of mushroom yield, it is however noted that the respective hormones had positive effect on transfer efficiency of macro- and micro-nutrients from substrates to mushroom fruiting bodies. Table 4.3 shows the accumulation coefficient values of macro- and micro-nutrients and heavy metals from the different treatment combinations. The accumulation coefficient values for micro-nutrients such as Fe, Zn and Mg which are important for human nutrition were noted to be significantly high in the following hormonally treated substrates i.e. S1H1 (*Urochloa panicoides* + cytokinins) and S1H2 (*Urochloa panicoides* + auxin). It is of importance to note that treatment combinations S1H3, S3H2 and S3H2 had the low accumulation coefficient values for heavy metals. These levels are of acceptable food safety standard (Stihi *et al.,* 2011). Treatments of substrates with hormones was also noted to have had increased transfer efficiency of phytochemicals such as polyphenols on S2H1

(240mgGAE/g) and S3H2 (245mgGAE/g). Flavoids levels in mushrooms harvested from various treatment combinations were as follows: S2H1 (8mgQE/g) S3H2 (9mgQE/g) [Fig. 4.1(a) and Fig. 4.1(b)]. Several workers have reported oyster mushrooms to be of medicinal importance and it is possible that the reported medicinal properties of oyster mushrooms could be attributed to these phytochemicals.

The general finding of this study is that type of substrate that are used to cultivate oyster mushroom has great influence on the yield. In this study *Urochloa panicoides* which is abundantly available in the study area had 98.8% conversion ratio. It is important that various other extensively locally available organic materials should investigated for their potential use as oyster mushroom substrate. Although use of plant growth hormones did not show positive effect on the mushroom yield, further work needs to be undertaken to establish the optimum concentration rates of the respective hormones in the various many other plant materials. In addition to investigating the effects of plant growth hormones, bio-fortification of substrates with various macro- and micro- nutrients can be explored in view of improving nutritional values of the mushroom. Bio-fortification studies could also involve use of organic manures such chicken, pig, cow and goat manure. Furthermore, there is also a need to carry out research on the development of low cost processing technologies that will improve the shelf-life and keeping qualities of mushroom produce. This could include working on the improvement of palatability, taste and keeping qualities of oyster mushroom biscuits that were produced in this study. Among many other potential research areas, another interesting area that can be explored is the production of dried oyster mushroom noodles.

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List of appendices

Appendix A

Figure 6.1(a) *Urochloa panicoides*

Figure 6.1(b) *Zea mays*

Figure 6.1(c) *Datura stramonium*

Figure 6.2(a) Mushroom domes

Figure 6.2(b) pH analysis

Figure 6.2(c) Spawn preparation

Figure 6.2(d) *Pleurotus ostreatus*

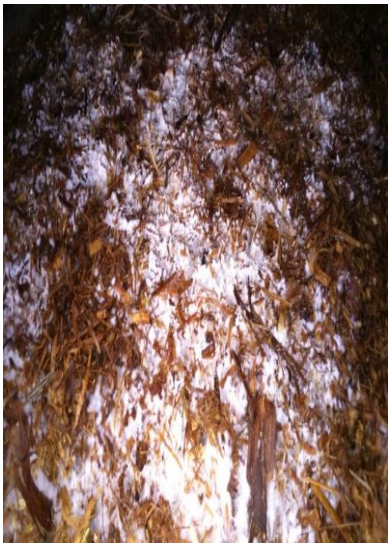

Figure 6.3(a) Mycelial dev.

Figure 6.3(b) Pinning

Figure6.3(c) Fruit cap dev.

Figure 6.3(d) Fruit cap maturity

Figure 6.4(a) Measuring fruit cap

Figure 6.4b: Measuring style length

Figure 6.4c. Packaging

Figure 6.4d. Mushroom dome

Appendix B

Palatability test survey questionnaire

Please **Taste** following oyster mushroom biscuits samples and rate them for **Taste perception** and **Acceptability** on a scale of 1 – 5. Kindly circle your choice.

	Taste score					**Acceptability score**					
	(Unappealing)	(Poor)	(Fair)	(Good)	(Excellent)		(Very poor)	(Poor)	(Acceptable)	(Good)	(Very good)
Sample A	1	2	3	4	5	Comment:	1	2	3	4	5
Sample B	1	2	3	4	5	Comment:	1	2	3	4	5
Sample C	1	2	3	4	5	Comment:	1	2	3	4	5

1. Could you detect a difference among the samples' **palatability**? (please circle) **Yes** **No**

2. Over Best Sample for **palatability:** (please circle) **A** **B** **C**