



Effects of substrate biofortification with broiler litter and phyto-mediated-nano-ZnO on *Pleurotus ostreatus* production and degradation of substrate contaminants

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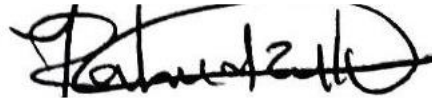
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Graduation: July 2024

1.DECLARATION

I, Imani Ravhudzulo, duly declare that this dissertation titled **Effects of substrate biofortification with broiler litter and phyto-mediated-nano-ZnO on Pleurotus ostreatus production and degradation of substrate contaminants** submitted for the Master of Science in Crop Science at the North-West University (Mafikeng Campus) has not previously been submitted for a degree at this school or any other University. I further certify that this dissertation is my work in terms of design and organization and that all material and sources included within have been properly acknowledged.



2023/12/04

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Supervisor: Prof Khosi Ramachela

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Date

2. DEDICATION

This dissertation is dedicated to myself for not giving up and for embracing opportunities to learn more.

3. ACKNOWLEDGEMENT

My initial thanks go to my creator, the almighty God, for being an infinite source of strength, knowledge, and direction throughout my schooling and up to this day. It is because of you that the word "impossible" has become foreign to me; words can never express how grateful I am, and yes, we did it again.

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8. LIST OF ACRONYMS AND ABBREVIATIONS	

<i>P. ostreatus</i>	<i>Pleurotus ostreatus</i>
AgriLASA:	Agri-Laboratory Association of Southern Africa
ANOVA:	Analysis of variance
BL:	Broiler litter
CRD:	Completely Randomized Design

FAO:	Food and Agriculture Organization
FTIR:	Fourier Transform Infrared
GLM:	General linear model
HIV/AIDS:	Human Deficiency Virus/ Acquired Immunodeficiency Syndrome
LSM:	Least square Means
NanoC:	Nanoparticle concentration
NPs:	Nanoparticles
SEM:	Standard error of the mean
SMS:	Spent mushroom substrate
WHO:	World Health Organization
XRD:	X-Ray diffraction analysis
ZnO:	Zinc Oxide

9.ABSTRACT

The increase in human population and the effects of climate change have resulted in food insecurity, and this makes communities living in poor environments have no access to nutritious food. These communities suffer from protein and mineral deficiencies. The study was therefore undertaken to investigate the effect of biofortification of substrate with broiler litter and phyto-mediated-nano-ZnO on the yield and growth of *Pleurotus ostreatus* as a means of addressing these challenges. The other objective was to investigate the safety of *Pleurotus ostreatus* fruiting bodies for human consumption, and the safety of spent mushroom substrate for livestock feed by analysing for presence of heavy metals. The experiment was laid out in 5 X 6 factorial treatment combinations. The experimental factors were as follows: *Urochloa panicoides* (1 kg) was used as substrate with five (5) broiler litter levels- 0%, 1.25%, 2.5%, 5% and 10% BL and six (6) nanoparticle concentration- 0 mg/kg, bulk-ZnO 2 mg/kg, conventional ZnO 8 mg/kg, green-nano-ZnO 2

mg/kg, green-nano-ZnO 10 mg/kg and green-nano-ZnO 50 mg/kg. The treatments were replicated four times making a total of 30 treatment combinations and 120 experimental units. The treatments were arranged in a completely randomized design (CRD). The mycelial growth, fruiting body initiation, and total biomass of *Pleurotus ostreatus* on *Urochloa panicoides* substrate with varied broiler litter and nanoparticle concentrations were assessed. At each harvest, the fruit cap diameter and style length of mushrooms were measured using a tape measure, and the conversion efficiency ratio of substrate to fruiting bodies/biomass was calculated. Macro- and micro-nutrient content on various substrates were analyzed using the dry-ashing macro- and micro-nutrient procedure provided by AgriLASA. The analysis of variance indicated a significant difference in the impact of nanoparticle concentration and broiler litter on total biomass ($P < 0.05$), but there was no significant difference in the interaction. Broiler litter had a statistically significant effect on fruit cap size and style length ($P < 0.05$), but there was no significant effect of the different nanoparticle concentrations. AgriLASA's dry-ashing macro- and micronutrient approach was utilised to evaluate the transfer efficiency of macro- and micronutrients, protein and heavy metals. Mushrooms and wasted mushroom substrate recovered from 5 BL green-nano-ZnO 50 mg/kg, 10 BL control, and 10 BL green-nano-ZnO 50 mg/kg had increased levels of Fe and Zn micronutrients compared to other treatments. The transfer macro- nutrient and heavy metal efficiency ratio of *Pleurotus ostreatus* was unaffected by any nanoparticle concentration. The accumulation coefficient of macronutrient (N, P, Ca, Mg) and micronutrient content (Fe, Zn, Mn) were significantly different in *Pleurotus ostreatus* fruiting bodies harvested from different broiler litter treatments. Finally, the study established that treatment; '5 BL green-nano-ZnO 2 mg/kg' had the highest conversion ratio of 51.61%, followed by '5 BL green-nano-ZnO 50 mg/kg' with 33.59% and 0 BL control at 25.35%. The lowest biological efficiency was obtained from 2.5 BL green-nano-ZnO 10 mg/kg at 0.58 %. The results therefore indicate that it is possible to boost oyster mushroom yield by substrate biofortification with appropriate broiler litter and nanoparticle concentrations. The study also established that the heavy metals Cr, Cd, and Se that were found in oyster mushrooms were within the acceptable human food safety level. Biofortifying liverseed grass with broiler litter and nanoparticle concentrations in oyster mushroom cultivation can contribute to the boosting of fruiting body crude protein content.

Keywords: Mycelial, Pinning, biomass, substrates, broiler litter, nanoparticle concentration and heavy metals.

1. General Introduction

1.1 Background

Despite there being enough food to feed the world's population, 815 million people still suffer from hunger, the majority of whom live in developing countries, particularly in sub-Saharan Africa (SSA) (FAO *et al.*, 2017). There is therefore an urgent need to improve the production of food particularly of high nutritional value to feed the rapidly growing human population and protect the masses from malnutrition. One of the best food resources of high nutritional value is the mushrooms. Mushrooms contain 19-35% protein on a dry weight basis as compared to 7.3% in rice, 13.2% in wheat and 25.2% in milk (Chang & Miles, 1989) and are rich in essential minerals and trace elements (Chandha & Sharma, 1995). The mushroom is the most well-known and documented edible forest product (Chamberlain *et al.*, 1998) highly valuable as a source of multiple nutrients, fibre, and phytochemicals that provide both nutritional and health benefits (Cheung, 2010) and as a source of medicine (Lakhanpal & Rana, 2005; Mshandete, 2011). Their medicinal values include wound-healing, immunity-enhancement, anticancer, antibiotic, anti-inflammatory and antiviral activities, and blood lipid-lowering effects (Chang, 1999; Dai *et al.*, 2009; Lavi *et al.*, 2010). They have also shown extraordinary positive results in trials for HIV/AIDS patients in Africa (Chang, 2006).

Oyster mushrooms (*Pleurotus ostreatus*) which were originally harvested from the wild are now widely cultivated in large quantities. These large quantities are grown in a short period and provide more protein per unit area than any other crop (Gupta, 1986). They are organoleptic fast-growing macro fungi with distinctive fruiting bodies. They are a unique biota which assembles its food by secreting degradative mycelial enzymes that decompose the complex organic materials including fibre and toxins to generate simpler compounds in the substrate that serve as a source of nutrition to the mushroom (Chang & Miles, 1991). Their attractive feature is that they can utilize a wide variety of agricultural waste products and transform the lignocellulosic biomass into high-quality food (Bano & Rajarathanam, 1982). One of the most important aspects of a good mushroom mycelial growth is the substrate source, which directly affects the bio-conversion ability of raw material and consequently the productivity. For good growth of *P. ostreatus*, it is necessary to find sources of carbon and nitrogen as well as macro-minerals (S, Ca, Mg, P and K) and

to some extent lower concentrations of micro-minerals; Fe, Zn, Mn, Cu and Mo [Chang & Miles, 2009; Romero *et al.*, 2010], with an ash content of between 2.5 to 15.7% (Sánchez & Royse, 2002). Mushroom mycelia produce a series of enzymes (Saavedra, 2007; Gutiérrez-Rojas *et al.*, 2014) which drive the biochemical processes that convert the organic matter into edible fruiting bodies. *Pleurotus* spp. degrade complex lignin-cellulose-hemicellulose structures from which they absorb nutrients for their development (Guzmán *et al.*, 2008; Grilli *et al.*, 2015; Saucedo Castillo *et al.*, 2015). They also require nitrogen, and many studies have shown the need to supplement nitrogen-poor substrates with richer sources of this element (e.g. soya beans, lucerne, sunflower, etc.) (Chegwin *et al.*, 2013). Whilst enrichment of substrates with too much nitrogen can lead to negative effects, in addition to increasing production costs (Silva *et al.*, 2007; Zied *et al.*, 2011; Siqueira *et al.*, 2012), biofortification of substrates with nitrogen is, however also important to increase the protein content in the mushrooms (Curvetto *et al.*, 2002).

Biofortification of mushroom substrates with animal protein-rich biowastes such as chicken manure, and dung manure has the potential to increase mushroom yield and productivity (Morimoto *et al.*, 1981; Royse *et al.*, 2004). Mwita *et al.* (2011) showed that chicken manure enhanced cap lengths and diameters, stipe lengths and diameters and dry weights of *Coprinus cinereus* mushrooms grown on substrates of sisal dust, sisal fibres and sisal decortication leaf wastes (Mwita *et al.*, 2011). Also, the biofortification of straw substrates with poultry manure increased the yields of *Agaricus bisporus* mushrooms (Noble *et al.*, 2002). Further, Hasan *et al.* (2010) obtained the highest *P. ostreatus* mushroom yield and economic return from rice straw treated with 10% poultry litter with 1% lime. Furthermore, it was found by Carter (2017) that the addition of 25% broiler litter into wheat straw substrate increased *P. ostreatus* mushroom yields compared to 100% wheat straw. This is not unexpected as broiler litter, a mixture of bird excrement, spilled feed, and feathers (Nunes *et al.*, 2016), is a rich source of nitrogen (26% crude protein) as well as macro (Ca, P, K, Mg and S) and micro (Cu, Mn and Zn) minerals (Bolan *et al.*, 2010).

Notwithstanding, the utilization of broiler litter as mushroom substrate biofortificant can be a potential threat to the health of people who consume mushrooms that sequester antibiotic residues and toxic substances from substrates that are biofortified with chicken manure containing antibiotic residues. In this situation, most antimicrobials orally administered to chickens are not fully absorbed in the chicken gut and up to 90% of them can be excreted in the faeces (Kumar *et al.*, 2005b) and ultimately be found in the litter (Boxall *et al.*, 2003; Rooklidge, 2004). Since plants bioaccumulate some antimicrobial

agents and can be sources of exposure to such compounds (Kumar *et al.*, 2005a), it is also very likely that mushrooms can pose a potential risk to human health through similar mechanisms of antibiotic bioaccumulation. Indeed, feed additives such as bacitracin, chlortetracycline, monensin, narasin, nicarbazine, penicillin, salinomycin, and virginiamycin that are often added to poultry feed in commercial farms, and controlled trials have been found in poultry litter which can pose harmful effects (Furtula *et al.*, 2010). Kyakuwaire *et al.* (2019) have further reported the presence of not only antibiotics such as fluoroquinolones, sulfonamides and tetracyclines and pathogenic microbes with antibiotic-resistant genes but also pathogenic microorganisms, bacteria, fungi, viruses, parasitic protozoa and helminths, heavy metals, growth and sex hormones, estrogens: 17 β -estradiol and testosterone, and pesticides dioxins, furans, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons. Only 5-15% of heavy metals ingested with chicken feeds are absorbed by the birds and the bulk is excreted through faeces and urine into chicken litter (Bolan *et al.*, 2010). High concentrations of Cd, Pb, As, and Hg would pose serious health risks to human beings if poultry litter is used as a mushroom substrate. In addition, mycotoxins, especially aflatoxin B₁, have been detected in excess levels in poultry litter (Westlake, 1985; Manafi *et al.*, 2011; Viegas *et al.*, 2012). Also, traces of aflatoxins and their metabolites (AFM₁, AFM₂, AFP₁, and aflatoxicol) have been found in the litter from layer hens fed diets contaminated with 30 and 500 μ g per kg of AFB₁ (Cortés *et al.*, 2010).

Against this background, there is a need for technology to enhance the bioremediation capacity of mushrooms cultivated on poultry litter-biofortified substrates in order to ensure mushroom safety for human consumers. In this regard, mushroom-forming fungi can secrete biodegradative enzymes such as laccase, lignin peroxidase, cellulase and xylanase (Phan & Sabaratnam, 2012; Wan & Li, 2012) that can biodegrade not only lignocellulosic materials but environmental toxins including mycotoxins as well. White rot fungi including *P. ostreatus* exhibit high activity of laccase and manganese-peroxidase enzymes that have the potential to biodegrade AFB₁ and other combined mycotoxins (Alberts *et al.*, 2009; Yehia, 2014; Loi *et al.*, 2018). It is therefore hypothesized herein that enhancement of the mushroom mycelial capacity to secrete more of these bioremediation enzymes will further improve the capacity of mushrooms to remediate a combination of antibiotic residues, mycotoxins, and other toxic substances in poultry litter-biofortified mushroom substrates.

Recently, the application of nanotechnology in mushroom production has demonstrated phenomenal improvements in mushroom yields (Guo *et al.*, 2015; Prasad *et al.*, 2017; Owaid, 2019). In this regard, the application of small doses of nano-urea (3 and 5 mg/kg substrate) induced the highest number of *P. ostreatus* mushroom flushes at a faster rate (15 days) as well as the highest biological and economic yields (Naim *et al.*, 2020a). This is, however, contrary to early findings where high doses of nitrogen caused inhibition of mushroom growth and production. Nanoparticles have also been reported to improve mushroom protein, fibre, total carbohydrates, fructose, essential and non-essential amino acids, and nickel and lead whilst decreasing the sucrose, glucose, Ca, K, Na, Cu, Fe and Zn contents (Sassine *et al.*, 2021). Furthermore, Naim *et al.* (2020b) found a nano-fertilizer (Lithovit®-Amino25) with a mixture of 25% L-amino acids when applied at 3 or 5 g/kg to improve the biological and economic yield, proteins, and essential amino acids (threonine, valine, isoleucine, leucine, and histidine) of *P. ostreatus* mushrooms. In this study nano-fertilizer application also enhanced substrate lignin degradation, which suggested the capacity of nanotechnology to improve the biodegradative capacity of oyster mushrooms. It was therefore hypothesized that substrate biofortification with broiler litter and phyto-mediated zinc oxide nanoparticles would improve the growth, productivity, nutritional composition, and heavy metal degradation capacity of oyster mushrooms and the chemical composition of the spent mushroom substrate.

1.2 Problem statement

Sub-Saharan Africa is generally known to have a large population that experiences hunger, poverty, and malnutrition (Clover, 2003; Bain *et al.*, 2013; Adeyeye *et al.*, 2017; Giller *et al.*, 2020). A similar situation exists in South Africa (Nwani *et al.*, 2020). This is projected to worsen due to population increase and a decline in rainfall as a result of climate change, worsening food insecurity.

Oyster mushroom has been identified as a malnutrition alleviator of deficiency of protein, and trace elements such as Zn, K, and Mg which the World Health Organization has referred to as silent hunger (Chang & Miles, 2004). This is because these trace elements are given in modest quantities by regularly cultivated crops, and these crops take longer to mature. The conventional cropping season where there is a food gap in between the food growing season leaves most of the rural communities experiencing food deficiencies.

However, mushrooms cannot grow well on substrates that are only nutritionally carbon-based, they also require a source of nitrogen. Broiler litter is one of the commonly used

cheap and abundantly available sources of nitrogen that can be used to biofortify mushroom substrates. Unfortunately, very often this broiler litter contain antibiotic residues and heavy metals. Broiler litter poses a risk to both human and animal health if it gets into the food chain [Boxall *et al.*, 2003; Rooklidge, 2004; Kumar *et al.*, 2005b; Bolan *et al.*, 2010; Furtula *et al.*, 2010; Kyakuwairu *et al.*, 2019]. Similarly, this risk also includes the production of mycotoxins (Westlake, 1985; Cortés *et al.*, 2010; Manafi *et al.*, 2011; Viegas *et al.*, 2012).

1.3 Justification

Oyster mushrooms have been recognized to be rich in protein and trace elements such as Zn, Fe, K and Mg which are important for the human diet (Atikpo *et al.*, 2008; Ashraf *et al.*, 2013). Furthermore, oyster mushroom has also been noted to play an important role in climate change agriculture adaptation initiatives in that it does not utilize large volumes of water, and it does not require large land (Gupta, 1986; Maniruzzaman, 2004). They are produced in between the traditional cropping seasons. The yield of oysters can be improved by the biofortification of substrates with several organic and inorganic materials (Zięba *et al.*, 2020; Leong *et al.*, 2022; Souza *et al.*, 2023). Organic material would include chicken manure and inorganic material will use different trace elements including nanotechnology.

Recently, the use of nanotechnology has been reported to enhance the growth and yield of crops. Furthermore, food treated with nanotechnology improves the biochemical processes that aid in scavenging free radicals from the human body and inhibiting the growth of cancer cells. It is on this basis that it is hypothesised that nanoparticle-treated oyster mushrooms would contain similar properties (Chellaram *et al.*, 2014; Hafeez *et al.*, 2015; Mali *et al.*, 2020; Zahedi *et al.*, 2020; Salama *et al.*, 2021). This study was undertaken to investigate the effects of biofortification with broiler litter and nano-ZnO NPs on mushroom establishment, growth and bioremediation. Broiler litter management and disposal is a major problem for farmers, businesses, and the public, due to rising environmental pollution concerns (Garces, 2013). Hence, its use as a mushroom biofortificant should contribute to addressing this problem.

The capacity of oyster mushrooms to secrete biodegradative enzymes (Gaitán-Hernández *et al.*, 2005) is a characteristic that can be exploited to curtail the transmission of antibiotics and mycotoxins to humans who consume mushrooms produced from the broiler litter biofortified substrate. The use of oyster mushrooms is intended to diminish antibiotic

resistance among microorganisms that infect humans, as well as diseases linked with mycotoxicosis.

1.4 Aim and objectives.

1.4.1 Main objective

To investigate biofortification effects of broiler litter and phyto-mediated-nano-ZnO on *Pleurotus ostreatus* production and spent substrate remediation.

1.4.2 Specific objectives

- a) To synthesize and characterize *Vachellia erioloba* seed-based ZnO nanoparticles
- b) To determine the effects of substrate biofortification with broiler litter and phyto-mediated-Nano-ZnO on oyster mushroom establishment, growth and yield.
- c) To examine the effects of substrate biofortification with broiler litter and phyto-mediated-nano-ZnO on mushroom and spent substrate heavy metal composition.
- d) To analyze the effects of substrate biofortification with broiler litter and phyto-mediated-Nano-ZnO on the chemical composition of spent mushroom substrate.

1.5 Hypotheses

For specific objective 1

Null Hypothesis: *Vachellia erioloba* seeds will not improve the physico-chemical properties of ZnO nanoparticles.

Alternative Hypothesis: *Vachellia erioloba* seeds will improve the physico-chemical properties of ZnO nanoparticles.

For specific objective 2

Null hypothesis: application of phyto-mediated-nano-ZnO and broiler litter will not increase the establishment and yield of oyster mushrooms.

Alternative hypothesis: The application of phyto-mediated-nano-ZnO and broiler litter will increase the establishment and yield of oyster mushrooms.

For specific objective 3

Null hypothesis: application of phyto-mediate-nano-ZnO will not assist in degrading heavy metals.

Alternative hypothesis: The application of phyto-mediate-nano-ZnO will assist in degrading heavy metals.

For specific objective 4

Null hypothesis: application of phyto-mediated-nano-ZnO and broiler litter will not improve spent mushroom substrates.

Alternative hypothesis: application of phyto-mediated-nano-ZnO and broiler litter will improve spent mushroom substrate chemical composition.

11. CHAPTER TWO: LITERATURE REVIEW

2.1 The Value of Oyster Mushrooms as a Source of Nutritious Food in South Africa's Rapidly Growing Human Population Era.

South Africa is diverse in population, and it is a relatively industrialized country in Africa. With an ever-growing population, it is estimated that the food demand might outpace the domestic food supply in developing countries (Cook, 2020). Furthermore, low-income members of the community are living below the poverty level. Moreover, these community members come across obstacles such as diet-related health concerns such as the increased prevalence of non-communicable diseases (NCDs) such as obesity, nutrient deficiencies, stunting and the persistence of hunger. Mushrooms are one of the foods that can be used for the provision of protein and essential micronutrients such as Zn, P and K. Mushrooms are fruiting bodies of fungi mainly of the Class Ascomycetes. Furthermore, it is also recognized that mushrooms are a household necessity in the combat against poverty and malnutrition (Chang, 2007). In various communities, oyster mushrooms are not only of popular culinary value but also have the potential to enrich human diets, particularly in underdeveloped nations where meat is rare and costly (Dube, 2019).

A report by FAO (1995), recommended the use of edible mushrooms as a possible cost-effective source of protein for developing countries which are very often heavily cereal-diet dependent (Boa, 2010). The nutritional composition of edible mushrooms demonstrates that they are abundant in essential oils, protein, vitamins, minerals, lectins, fibre and bioactive substances (including all essential amino acids) (Rezaeian *et al.*, 2016). In addition, edible mushrooms provide varying quantities of a strong antioxidant selenium that helps to maintain the immune system and prevent cell and tissue damage (Khatua *et al.*, 2013). There is also folic acid in the oyster mushroom which can be used to treat anaemia. People with hypertension, obesity and diabetes can safely eat oyster mushroom because it is low in sodium: potassium ratio, carbohydrates, fat, and calorific value, and alkaline ash and high fibre content make them good for consumption with hyperacidity and cholesterol-inhibiting constipation mushrooms (Randive, 2012). Furthermore, the delicious taste of *Pleurotus* mushrooms may improve meal quality (Lavelli *et al.*, 2018). *Pleurotus osreatus* includes all of the necessary amino acids, except for sulfur-containing amino acids, cysteine and methionine (Dabbour & Takruri, 2002).

Dried oyster mushrooms and goods containing them are an excellent source of iron, zinc, and copper for those who avoid animal products (Dwyer 1999; Kala & Svoboda 2000;

Isiloglu *et al.*, 2001). The problem of a perpetually rising population can be solved by providing food for the world's constantly rising population, as well as waste management, which are two of the world's key problematic issues. The growing of oyster mushrooms on agro-industrial waste is a key aspect in converting low-value non-edible wastes into higher-value products that can be used as food. Mushroom growing is a suitable bio-conversion strategy for the management of agricultural and agro-industrial waste. Because of its simplicity and low-cost growing methods, oyster mushrooms have grown in popularity.

2.2 Nutritional and Substrate Requirements for Efficient Oyster Mushroom and Mycelial Growth.

As mushrooms do not photosynthesize, they acquire their nutritional requirement through the saprophytic process where organic material supplies their needs such as carbon and various macro and micronutrients. Oyster mushrooms can be grown on a wide range of substrates (Ngezimana *et al.*, 2007). The substrate quality is the major determining factor for producing mushrooms as it supplies all the energy and nutrients that mushrooms demand during their development process (Tavarwisa *et al.*, 2021). Agro-industrial waste materials with low nitrogen content have generally been viewed as a limiting factor in oyster mushroom cultivation. Different substrates can be selected for use for mushroom production in whichever region depending on whatever is available in the respective areas (Cohen *et al.*, 2002). Oyster mushrooms have the potential to produce high yields with optimum conversion efficiency substrates under variable environmental conditions. Oyster mushrooms also have a potential bioremediation effect on agricultural wastes and crop residues.

The survival and growth of mushrooms are linked to a variety of specific elements that can interact with each other. The chemical, physical and biological factors that are important for oyster mushroom production include processes that involve water activity, carbon and nitrogen cycles, mineralization, pH, moisture, antimicrobial agents and the interactions between microorganisms (Eira, 2003). Appropriate substrates' optimum pH for oyster mushroom production is in the range of 6 to 8, depending on the species of oyster mushroom (James, 2009).

2.3 Broiler litter as biofortificant in Oyster mushroom production: Nutritional composition and effects on mushroom production and nutritional composition.

Broiler litter has for decades been used as a source of nutrients for plant growth. Satisfactory crop yields are produced by applying poultry waste at an acceptable agricultural rate (Wood *et al.*, 1993). Bedding material, feathers, manure, and spilt feed are the big components of broiler litter (Tasistro *et al.*, 2004). This litter contains plant nutrients such as N, P and K, and trace elements such as Cu, and Zn. The nutrients available in broiler litter are required for oyster mushroom nutrient growth. Adding broiler litter to soils also enhances their physical, chemical and organic fertility (McGrath *et al.*, 2009). In addition to helping solve disposal challenges, adding boiler litter to soils can also increase the physical, chemical, and biological fertility of soils (Friend *et al.*, 2006), urthermore, it can be used as a substrate for growing mushrooms in order to reduce pollution. Alternative to the use of these inorganic fertilizers, various animal manures have also been used. More specifically, broiler litter has been noted to contain the correct nutrients for the cultivation of various crops. It could also be considered a valuable source of carbon and nitrogen in oyster mushroom production.

2.4 Heavy Metals in Broiler Litter: Health Implications of heavy metals and their toxicity

Broiler litter, which is an important source of nutrients for crop production, may also be contaminated with a variety of pathogenic bacteria such as Salmonella and Campylobacter, posing a risk to humans who may consume contaminated mushrooms produced from broiler litter-fortified substrates (Nunes *et al.*, 2016). Antibiotics are generally utilized as preventative or curative treatments in biosecurity treatments in poultry feed, aquaculture, animal feed, and human or veterinary medicinal products (Ramirez, 2018). Extensive use of antibiotics in chicken production systems has been reported to cause microbial selection pressure which results in the development of antibiotic-resistant microorganisms. This gets scientists concerned about the harm to the health of both human beings and animals.

Furthermore, the widespread distribution of antibiotic-resistant foodborne bacteria in chicken litter or chicken litter-based organic fertilizers poses a severe environmental risk (Ghorbani *et al.*, 2016). Under some circumstances, horizontal transfer of mobile antibiotic resistance genes from one bacterium to another may occur (Rensing *et al.*, 2002).

Antibiotic-resistant bacteria can be traced in high concentrations in chicken litter or chicken litter-based organic fertilizers, with some species, such as *E. coli*, *Enterococcus* and *Providencia*, being multi-resistant to multiple antibiotics (Kyakuwairwa *et al.*, 2019).

Other than microbial contamination, chicken manure has been reported to contain heavy metals (Verma *et al.*, 2019). Heavy metals are non-biodegradable and in the food chain are usually biomagnified (Singh *et al.*, 2008). When translocated through the food chain, they pose a risk to human health.

2.5 Mushroom Mycelial Enzyme Biodegradation of Heavy Metals

Some of the possibilities for employing fungi, namely white-rot fungi, to clean up polluted soil were explored by several workers (Jebapriya *et al.*, 2013; Varjani *et al.*, 2017; Tirsit, 2020). The ability of white-rot fungi to be effective in degrading a wide variety of organic compounds is due to the inherent characteristics that release extracellular lignin-modifying enzymes. They generally can biodegrade a wide range of molecules that are found in plant materials. The enzymes used in the lignin degradation system include lignin-peroxidase (LiP), manganese peroxidase (MnP), different H₂O₂-generating enzymes, and laccase.

Mushrooms and other fungi have enzymatic machinery for waste/pollutant biodegradation because they can be used to treat a wide range of contaminants (Purnomo *et al.*, 2013; Kulshreshtha *et al.*, 2013). What separates mushroom species from vegetable crops is their ability to synthesize dietary protein in the form of fruiting bodies formed from diverse wastes through a variety of hydrolyzing and oxidizing enzymes (Kuforiji & Fasidi, 2008; Zhu *et al.*, 2013). With their large biomass and strong texture, these mycelia act as biological filters and effective sorbents (Volesky & Holan 1995). The extracellular enzymes and acids released by fungal biomass aid in the degradation of lignin and cellulose while also aiding in solubilization and metal complexing (Sesli *et al.*, 2008; Singh & Sharma 2013; Damodaran *et al.* 2014; Mani & Kumar 2014). They are great candidates for pollution remediation because their cell wall is composed of polysaccharides and proteins with important functional groups that aid in the binding of organic and inorganic contaminants (Igiri *et al.*, 2018). They are also recognised as good degraders because of characteristics like aggressive growth, a large hyphal network, resistance to changing environmental circumstances, the presence of metal-binding proteins, and extracellular enzyme systems (Khan *et al.*, 2019).

The action of enzymes capable of breaking down the refractory heavy metals may result in heavy metal degradation. White-rot fungi are known to have highly effective enzyme

systems for the breakdown of polycyclic aromatic hydrocarbons (Pickard *et al.*, 1999). Specific enzymes isolated from *Pleurotus spp.*, a genus that contains numerous edible and cultivable mushroom species, produced encouraging results in heavy metals breakdown (Dhanker *et al.*, 2021; Gupta *et al.*, 2021; Deshpande *et al.*, 2022).

2.6 The role of Zinc in enzymatic activities in the bioremediation process.

This micronutrient is required by most organisms and is involved in many bio-metabolic activities. For the action of more than 300 enzymes, Zinc has been reported to be necessary for their various biochemical processes (McCall *et al.*, 2000; Frassinetti *et al.*, 2006). In addition to its catalytic, structural, and regulatory functions, (Stefanidou *et al.*, 2006), Zinc was shown to be an essential cofactor in many enzymes (Maret, 2013). Zinc is known to enhance the structure and function of biological membranes and antioxidant enzymes (Ambali *et al.*, 2010). According to Cordova and Alvarez-mona (1995), the role of Zinc as a component can be divided into three categories: catalytic – Zinc is required to stimulate the enzymatic activity of various enzymes such as carbonate anhydrase, carboxypeptidase, thermolysin, and aldolase; structural – Zinc stabilizes protein structure; and regulatory – Zinc can be an activator or inhibitor of enzymatic activity.

The most important enzymatic activity in the soil environment is played by oxidoreductases: dehydrogenases, nitrate reductase, polyphenol oxidases, catalase, peroxidases, hydrolases: esterases, alkaline phosphatase and acid phosphatase, phosphodiesterase, aryl phosphatase, cellulase, -glucosidase, invertase and protease [Kucharski, 1997; Moreno *et al.*, 2001]. Their activity as evaluated using 2,3,5 triphenyl tetrazolium chloride (TTC) as an oxidant reflects that of the total microbial community (Kucharski, 1997), Zinc, as a protein component, serves as a functional, structural, or regulatory cofactor for a wide range of enzymes. Many of the physiological changes caused by Zn deficit are connected with disruptions in normal enzyme activity; consequently, Zn deficiency-induced photosynthesis suppression coincides with a drop-in activity of important photosynthetic enzymes. It should be emphasized, however, that Zn, as a component of metalloenzymes, may both hinder and promote enzymatic activity. This element is required for the normal operation of enzymes such as carbonate anhydrase, carboxypeptidase, thermolysine and aldolase. Zinc metalloenzymes have a role in plant growth regulation by acting indirectly on auxin metabolism, membrane integrity, protein synthesis, glucose metabolism, and photosynthesis regulation (Gondal *et al.*, 2021 & Solanki, 2021). On the other hand, it has indirect effects on stomatal opening and shutting

as well as reduction of oxidative species detoxification. Zinc deficiency may influence the catalytic activity of a variety of enzymes and hence the metabolic pathways in which they are engaged as a result of these.

2.7 Application of Green (phyto-mediated) Nanotechnology in mushroom production: Nano-ZnO

Algae, microorganisms, plants, heterotrophic human cell lines and numerous biocompatible agents are portrayed as efficient and ecologically benign green nanofactories, which have been specifically created for the generation of various inorganic Nanoparticles (NPs) NPs because they are easily accessible (Sharama *et al.*, 2015; Heuer-Jungemann *et al.*, 2019; Huang *et al.*, 2020). Highly stable organisms such as phototrophic eukaryotes may convert metal ions to metal NPs. So, by using these phototrophic eukaryotes, bacteria, plants, and biomolecules because they are harmless and ecologically friendly, green-produced metal NPs have been employed in a variety of applications such as medicine, agriculture, and allied industrial sectors. Rahaiee *et al.* (2020) and Satpathy *et al.* (2018) investigated the use of green-generated NPs in applications such as anticancer activity, antioxidant activity, and agriculture. The huge financial expenditures associated with manufacturing and processing nanoproducts are a limitation of the widespread use of modern nanotechnology. The majority of the described nanoparticle synthesis processes are not cost-effective. An innovative way to improve agriculture is to employ nanotechnology to increase productivity (Fraceto *et al.*, 2016; Kim *et al.*, 2018). They have been found to improve crop growth, yield, and quality indices in some studies (Duhan *et al.*, 2017; Sassine, 2019). The use of chemically synthesized nanomaterials is increasingly regarded to be harmful to the environment. However, nanomaterials may be synthesized from plant systems, which is referred to as green nanotechnology (Prasad, 2014). Green nanotechnology, in addition to being ecologically friendly, reduces greenhouse gas emissions and is a safe method since it employs less material and renewable inputs whenever feasible (Aithal & Aithal, 2021; Fulekar *et al.*, 2014; Nasrollahzed *et al.*, 2019). Because these items are made from renewable resources, they have little negative impact on the environment (Prasad *et al.*, 2014, 2016).

Securing a high-tech agricultural system with nanotools might be a great way to revolutionize agricultural methods, decrease or even eliminate the environmental impacts of modern agriculture as well and improve yields (Sekhon, 2014; Liu & Lal, 2015). While nanomaterials catalyse waste and hazardous material degradation, they also help

microorganisms degrade waste and harmful materials more effectively. The use of nano-ZnO improves mushroom protein, fibre, total carbohydrates, fructose, essential and non-essential amino acids whilst it decreases the sucrose, glucose, Ca, K, Na, Cu, Fe and Zn contents (Sassine et al., 2021).

References

- Adeyeye, S.A.O., Adebayo-Oyetoro, A.O. and Tiamiyu, H.K., 2017. Poverty and malnutrition in Africa: a conceptual analysis. *Nutrition & Food Science*, 47(6), pp.754-764.
- Alberts JF, Gelderblom WCA, Botha A, van Zyl WH. 2009. Degradation of aflatoxin B1 by fungal laccase enzymes. *International Journal Food Microbiol*, 135(1):47e52.
- Aithal, S. and Aithal, P.S., 2021. Green and eco-friendly Nanotechnology–concepts and industrial prospects. *International Journal of Management, Technology, and Social Sciences* , 6(1), pp.1-31.
- Ambali SF, Abubakar AT, Shittu M, Yaqub LS, Anafi SB, Abdullahi A. 2010. Chlorpyrifos-induced alteration of hematological parameters in wistar rats: ameliorative effect of zinc. *Resource Journal for Environment Toxicology*, 4, pp.55–66
- Ashraf, J., Ali, M. A., Ahmad, W., Ayyub, C. M., and Shafi, J. 2013. Effect of Different Substrate Supplements on Oyster Mushroom (*Pleurotus spp.*) Production, *Food Science and Technology*, 1(3), pp.44-51. doi: 10.13189/fst.2013.010302.
- Atikpo, M. et al. 2008. ‘Sustainable mushroom production in Africa: A case study in Ghana’, *African Journal of Biotechnology*, 7(3), pp. 249-253. doi: 10.5897/AJB07.640.
- Bain, L.E., Awah, P.K., Geraldine, N., Kindong, N.P., Siga, Y., Bernard, N. and Tanjeko, A.T., 2013. Malnutrition in Sub–Saharan Africa: burden, causes and prospects. *Pan African Medical Journal*, 15(1).
- Bano, Z., Rajarathanam, S., 1982. *Pleurotus* mushrooms as a nutritious food. In: *Tropical mushrooms –Biological Nature and cultivation methods*, (
- Boa, E. 2008. *Wild edible fungi a global overview of their use and importance to people*, Biology. Zomba, Malawi, Forest Research Institute of Malawi and CABI Bioscience. P. 61
- Bolan, N. S. et al. 2010. Uses and management of poultry litter, *World’s Poultry Science Journal*, 66(4), pp. 673-698. doi: 10.1017/S0043933910000656.
- Boxall, A. B. A., D. W., Kolpin, B. Halling-Súrensen, and J. Tolls. 2003. Are veterinary medicines causing environmental risks? *Environment Science and Technology journal*, 37, pp.265A-294A.

- Branà, M.T, Cimmarusti, M.T, Haidukowski M, Logrieco, A.F, Altomare, C. 2017. Bioremediation of aflatoxin B1-contaminated maize by king oyster mushroom (*Pleurotus eryngii*). *PLoS ONE* 12(8), doi.org/10.1371/journal.pone.0182574
- Carter, J.B. III. 2017. A laboratory-scale study on the production of high-value products from broiler litter involving solid-state anaerobic digestion and mushroom cultivation. MSC Thesis, University of Arkansas. Theses and Dissertations. 2377. <http://scholarworks.uark.edu/etd/2377>.
- Chamberlain, J.; Bush, R. and Hammett, A. 1998. Non-Timber Forest Products: The Other Forest Products. *Forest Product Journal.*, 48, pp.10-19.
- Chandha, K.L and Sharma, S.R. 1995. *Advances in Horticulture Mushroom*, Malhotra Publication house, New Delhi. 13, pp.649.
- Chang, S.T. and P.G. Miles. 1989. *Edible Mushrooms and their Cultivation*. CRC Press. Florida.
- Chang, S.T. 1999. World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* in China. *International Journal Medicine. Mushrooms*, 1, pp.291-300.
- Chang, S.T. 2006. The world mushroom industry: Trends and technological development. *International Journal of Medecinal Mushrooms*, 8, pp.297-314.
- Chang. S. T. 2007. Mushroom cultivation using the “ZERI” principles: potential for application in Brazil’. *Micolology Application International*. 19, pp. 33-34.
- Chang, S.; Miles, P. 2009. 2nd ed. *Mushrooms Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact*. Washington. pp. 477.
- Chegwin, C.; Ivonne, J.; Nieto, R. 2013. Influencia del medio de cultivo en la producción de metabolitos secundarios del hongo comestible *Pleurotus ostreatus* cultivado por fermentación en estado líquido empleando harinas de cereales como fuente de carbono. *Rev. Mexicana de Micología*. 37, pp. 1-9.
- Chellaram, C. Murugaboopathi, G. John, A. Sivakumar, R. Ganesan, S. Krithika, S. Priya, G. 2014 Significance of Nanotechnology in Food Industry, *APCBEE Procedia*, 8, pp. 109-113. doi.org/10.1016/j.apcbee.2014.03.010.
- Cheung, P.C.K. 2010. The nutritional and health benefits of mushrooms. *Nutrition Bulletin.*, 35, pp. 292-299.

- Claiborne, A., and Fridovich, I. 1979. Purification of the o-dianisidine peroxidase from *Escherichia coli*. Physicochemical characterization and analysis of its dual catalatic and peroxidatic activities. *Journal of Biological Chemistry*. 254, pp. 4245-4252.
- Clover, J., 2003. Food security in sub-Saharan Africa. *African security review*, 12(1), pp.5-15.
- Cohen R, Persy L, Hadar Y. 2002. Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. *Applied Microbiology and Biotechnology*, 58, pp. 582-594.
- Cook, N. 2020. South Africa: Current issues, economy, and U.S. relations', Congressional Research Services, pp. 1-33. Available at: <https://fas.org/sgp/crs/row/R45687>.
- Cordova A., Alvarez-mon M. 1995. Behavior of zinc in physical exercise: a special reference to immunity and fatigue. *Neuroscience and Biobehavioral Revised.*, 19(3), pp. 439-445.
- Cortés, G., Carvajal, M., Méndez-Ramírez, I., Ávila-González, E., Chilpa-Galván, N., Castillo-Urueta, P. & Flores, C.M. 2010. Identification and quantification of aflatoxins and aflatoxicol from poultry feed and their recovery in poultry litter. *Poultry Science*, 89, pp. 993-1001. DOI: 10.3382/ps.2009-00019.
- Curvetto, N.; Figlas, D.; Devalis, R.; Delmastro, S. 2002. Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls supplemented with N-NH₄⁺ and/ or Mn (II). *Revised Bioresource Technology*. 84, pp. 171-176
- D'Mello, J. P. F. 2003. Mycotoxins in cereal grains, nuts and other plant products', In D'Mello, J. P. F. (ed), *Food Safety Contaminants and Toxins*, Cromwell Press, Trowbridge, pp. 65-90.
- Dabbour, I.R and Takruri, H.R. 2002. Protein digestibility using corrected amino acid score method (PDCAAS) of four types of mushrooms grown in Jordan. *Plant Foods for Human Nutrition*. 2002;57(1), pp.13–24.
- Dada TA, Ekwomadu TI and Mwanza M. 2020. Multi Mycotoxin Determination in Dried Beef Using Liquid Chromatography Coupled with Triple Quadrupole Mass Spectrometry (LC-MS/MS). *Toxins* 12: 357. DOI:10.3390/toxins12060357
- Dai, Y.C.; Yang, Z.L.; Cui, B.K.; Yu, C.J. and Zhou, L.W. 2009. Species diversity and utilization of medicinal mushrooms and fungi in China (Review). *International Journal of Medicinal Mushrooms*, 11, pp.287-302

- Deshpande, A.G. and Arya, A., 2022. Mushroom Biotechnology: Developing Cultivation Protocol for Four Different Mushrooms and Accessing Their Potential in Pollution Management. *In Biology, Cultivation and Applications of Mushrooms*, pp. 457-485. Singapore: Springer Singapore.
- de Souza, D.F., da Silva, M.D.C.S., de Souza, T.C., Rocha, G.C., Kasuya, M.C.M. and Eller, M.R., 2023. Effect of selenium-enriched substrate on the chemical composition, mineral bioavailability, and yield of edible mushrooms. *Biological Trace Element Research*, 201 (6), pp. 3077- 3087.
- Trace Element Research*, 201 (6), pp. 3077- 3087.
- Dhanker, R., Tyagi, P., Kamble, S.S., Gupta, D. and Hussain, T., 2021. Advances in fungi: Rejuvenation of polluted sites. *In Fungi bio-prospects in sustainable agriculture, environment and nano-technology*, pp. 251-275. Academic Press.
- Dube, E. 2019. Rarity or Reality of Some Theories of Poverty in Explaining the Poverty in Africa', *Journal of Rural and Community Development*, 14(3), pp. 48-60.
- Duhan, J. Kumar, R. Kumar, N. Kaur, P. Nehra, K and & Duhan, S. 2017. Nanotechnology: The new perspective in precision agriculture. *Biotechnology Reports*. 15, pp.11-23.
- Dwyer J., 1999. Convergence of plant-rich and plant-only diets. *American Journal of Clinical Nutrition*. 70, pp. 620- 622.
- Eira, A F. 2003. Cultivo do cogumelo medicinal (first ed.), Editora Aprenda Fácil, Viçosa (in Portuguese)
- FAO .1995. Trade restrictions affecting international trade in non-wood forest products, by M. Iqbal. Non-wood Forest Products, No. 8. Rome. FAO (Food and Agriculture Organization), IFAD (International Fund for Agricultural Development), UNICEF (United Nations Children's Fund), WFP (World Food Programme) and WHO (World Health Organization). 2017. The state of food security and nutrition in the world 2017: Building resilience for peace and food security. Rome: FAO. Accessed February 15, 2019. <http://www.fao.org/3/a-l7695e.pdf>.
- [org/3/a-l7695e.pdf](http://www.fao.org/3/a-l7695e.pdf).
- FAO (Food and Agriculture Organization), IFAD (International Fund for Agricultural Development), UNICEF (United Nations Children's Fund), WFP (World Food Programme) and WHO (World Health Organization). 2017. The state of food security

and nutrition in the world 2017: Building resilience for peace and food security. Rome: FAO. Accessed February 15, 2019. <http://www.fao.org/3/a-l7695e.pdf>.

Fraceto, L.F., Grillo, R., de Medeiros, G.A., Scognamiglio, V., Rea, G. and Bartolucci, C., 2016. Nanotechnology in agriculture: which innovation potential does it have? *Frontiers in Environmental Science*, 4, p.20.

ulture: which innovation potential does it have? *Frontiers in Environmental Science*, 4, p.20.

Frassinetti, S., Bronzetti, G.L., Caltavuturo, L., Cini, M. and Della Croce, C., 2006. The role of zinc in life: a review. *Journal of environmental pathology, toxicology and oncology*, 25(3).

Friend, A.L., Roberts, S.D., Schoenholtz, S.H., Mobley, J.A. & Gerard, P.D. 2006. Poultry litter application to loblolly pine forests: growth and nutrient containment. *Journal of Environmental Quality*, 35 (3), pp. 837-848.

Fulekar, M.H., Singh, A., Dutta, D.P., Roy, M., Ballal, A. and Tyagi, A.K., 2014. Ag incorporated nano BiPO 4: sonochemical synthesis, characterization and improved visible light photocatalytic properties. *Rsc Advances*, 4(20), pp.10097-10107.

Furtula, V., Farrell, E.G., Diarrassouba, F., Rempel, H., Pritchard, J. & Diarra, MS. 2010. Veterinary pharmaceuticals and antibiotic resistance of Escherichia coli isolates in poultry litter from commercial farms and controlled feeding trials. *Poultry Science*, 89, 180-188. doi: 10.3382/ps.2009-00198.

009-00198.

Gaitán-Hernández, R., Esqueda, M., Gutiérrez, A., Sánchez, A., . Beltrán-García, M and Mata, G. 2005. Bioconversion of agrowastes by *Lentinula edodes*: the high potential of Viticulture residues, *Applied microbiology biotechnology journal*, 71, pp. 432-439. doi: 10.1007/s00253-005-0241-1.

Garcês, A ., Afonso, S. M. S., Chilundo, A., and Jairoce, C. T. S. 2013. Evaluation of different litter materials for broiler production in a hot and humid environment: 1. Litter characteristics and quality', *Journal of Applied Poultry Research*, 22(2), pp. 168-176. doi.org/ 10.3382/japr.2012-00547.

Poultry Research, 22(2), pp. 168-176. doi.org/ 10.3382/japr.2012-00547.

Gondal, A.H., Zafar, A., Zainab, D., Toor, M.D., Sohail, S., Ameen, S., Ijaz, A.B., Ch, B.I., Hussain, I., Haider, S. and Ahmad, I.A., 2021. A detailed review study of zinc

- involvement in animal, plant and human nutrition. *Indian Journal of Pure & Applied Biosciences*, 9(2), pp.262-271.
- Ghorbani, B. et al. 2016. Effect of Antibiotics Overuse in Animal Food and its Link with Public Health Risk', *International Journal of Scientific Research in Science and Technology*, 1(2), pp. 46-50.
- Giller, K.E., 2020. The food security conundrum of sub-Saharan Africa. *Global Food Security*, 26, p.100431.
- Grilli, D.; Egea, V.; Paez Lama, S.; Carcaño, D.; Allegretti, L; Sosa Escudero, M.; Arenas, G. N. 2015. Degradación y utilización de la hemicelulosa contenida en especies forrajeras por *Pseudobutyrvibrio ruminis* y *Pseudobutyrvibrio xylanivorans*. *Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina*. 47(2), pp. 231-243.
- Gupta, R S. 1986. Mushroom cultivation, *Indian Horticulture*. 31(1), p.1.
- Gupta, S., Summuna, B., Gupta, M. and Annepu, S.K., 2018. Edible mushrooms: cultivation, bioactive molecules, and health benefits. *Bioactive molecules in food*, 1, pp.1-33.
- Guo, C., Liao, W., Li, Z., Sun, L. and Chen, C., 2015. Easy conversion of protein-rich enoki mushroom biomass to a nitrogen-doped carbon nanomaterial as a promising metal-free catalyst for oxygen reduction reaction. *Nanoscale*, 7(38), pp.15990-15998.
- Gutiérrez-Rojas, I.; Moreno-Sarmiento, N.; Montoya, D. 2014. Mecanismos y regulación de la hidrólisis enzimática de celulosa en hongos filamentosos: casos clásicos y nuevos modelos. *Rev Iberoamericana de Micología*. 32(1), pp. 1-12.
- Guzmán, G.; Mata, G.; Salmenes, D. 2008. 2ed. El cultivo de los hongos comestibles. Xalapa. México. 56 p.
- Hafeez, A., Razzaq, A., Mahmood, T. and Jhanzab, H.M., 2015. Potential of copper nanoparticles to increase growth and yield of wheat. *Journal of Nanoscience with Advanced Technology*, 1(1), pp.6-11.
- Hall, C. et al. 2017. The impact of population growth and climate change on food security in Africa: looking ahead to 2050', *International Journal of Agricultural Sustainability*. Taylor and Francis Ltd., 15(2), pp. 124-135. doi: 10.1080/14735903.2017.1293929.

- Hasan, M.N., Rahman, M.S., Nigar, S., Bhuiyan, M.Z.A. & Ara, N. 2010. Performance of oyster mushroom (*Pleurotus ostreatus*) on different pretreated substrates. *International Journal of Sustainable Crop Production*, 5 (4), pp.16 - 24.
- Heuer-Jungemann, A., Feliu, N., Bakaimi, I., Hamaly, M., Alkilany, A., Chakraborty, I., Masood, A., Casula, M.F., Kostopoulou, A., Oh, E. and Susumu, K., 2019. The role of ligands in the chemical synthesis and applications of inorganic nanoparticles. *Chemical reviews*, 119(8), pp.4819-4880.
- 4880.
- Huang, H., Feng, W., Chen, Y. and Shi, J., 2020. Inorganic nanoparticles in clinical trials and translations. *Nano Today*, 35, p.100972.
- Igir, F. G., & Tampi, J. R. E. (2018). Pengaruh Kualitas Produk Dan Harga Terhadap Keputusan Pembelian Mobil Daihatsu Grand Max Pick Up. 6(2), pp. 86–96.
- Imtiaj, A. and Rahman, S. A. 2014. Economic viability of mushrooms cultivation to poverty reduction in Bangladesh Universidad Autónoma de Yucatán', (June).
- Isiloglu M., Yilmaz F., Merdivan M., 2001. Concentrations of trace elements in wild edible mushrooms. *Food Chemistry*. 73, pp. 169-175.
- Jebapriya, G.R. and Gnanadoss, J.J., 2013. Bioremediation of textile dye using white rot fungi: a review. *International Journal of Current Research and Review*, 5(3), p.1.
- Kabak B, Dobson AD, Var I. 2006. Strategies to prevent mycotoxin contamination of food and animal feed': a review. *Critical Reviews in Food Science and Nutrition*, 46, pp. 593-619.
- Kabata-Pendias, A., & Mukherjee, A. B. 2007. Trace elements from soil to human'. Springer, Verlag, Berlin. <https://doi.org/10.1007/978-3-540-32714-1>.
- Kalač P., Svoboda L., 2000. A review of trace element concentrations in edible mushrooms. *Food Chemistry*, 69, pp. 273-281.
- Kellner, H., Luis, P., and Francois, B. 2007. Diversity of laccase-like multicopper oxidase genes in Morchellaceae: identification of genes potentially involved in extracellular activities related to plant litter decay. *Microbiol and Ecology*, 61, pp. 153-163. doi: 10.1111/j.1574-6941.2007.00322.x.

- Khatua, S, Paul, S and Acharya, A. 2013. Mushroom as the potential source of new generation of antioxidant: a review," *Research Journal of Pharmacy and Technology*, 6, pp. 496–505.
- Kim, D.Y., Kadam, A., Shinde, S., Saratale, R.G., Patra, J. and Ghodake, G., 2018. Recent developments in nanotechnology transforming the agricultural sector: a transition replete with opportunities. *Journal of the Science of Food and Agriculture*, 98(3), pp.849-864.
- Kuforiji OO, Fasidi IO. 2008. Enzyme activities of *Pleurotus tuber-regium* (Fries) Singer, cultivated on selected agricultural wastes. *Bioresource Technology*, 99, pp. 4275-4278. doi:10.1016/j.biortech.2007.08.053
- Kulshreshtha S, Mathur N, Bhatnagar P .2013a. Mycoremediation of paper, pulp and cardboard industrial wastes and pollutants. In: Goltapeh EM, Danesh YR, Varma A (eds) *Fungi as Bioremediators: Soil Biology*. Springer Berlin, Heidelberg, pp. 77-116
- Kumar, K., S. C. Gupta, S. K. Baidoo, Y. Chander, and C. J. Rosen. 2005a. Antibiotic uptake by plants from soil fertilized with animal manure. *Journal of Environmental Quality*. 34 pp. 2082-2085.
- Kumar, K., S. C. Gupta, Y. Chander, and A. K. Singh. 2005b. Antibiotic use in agriculture and their impact on the terrestrial environment. *Advances in Agronomy*. 87, pp.1-54.
- Kumar, S. et al. (2019) 'Crop biofortification for iron (Fe), zinc (Zn) and vitamin A with transgenic approaches', *Heliyon*. Elsevier Ltd, 5(6), p. e01914. doi: 10.1016/j.heliyon.2019.e01914.
- Kucharski J. 1997. Relationships between soil enzymatic activity and fertility. In: *Microorganisms in the natural environment: occurrence, activity and significance*. Ed. W. Barabasz. AR Kraków, pp. 327-342.
- Kyakuwaire, M. et al. 2019. How safe is chicken litter for land application as an organic fertilizer? A review, *International Journal of Environmental Research and Public Health*, 16(19). doi: 10.3390/ijerph16193521.
- Lakhanpal, T.N. and Rana, M. 2005. Medicinal and nutraceutical genetic resources of mushrooms. *Plant Genetic Resources*, 3, pp. 288-303.
- Latif R, Malek M, Mirmonsef H. 2013. Cadmium and lead accumulation in three endogeic earthworm species, *Bulletin of Environmental Contamination and Toxicology journal*, 90(4), pp. 456-459. <https://doi.org/10.1007/s00128-012-0941-z>.

- Lavelli V, Proserpio C, Gallotti F, et al. 2018 Circularreuse of bio-resources: the role of *Pleurotusspp.* in the development of functional foods. *Food Function*, 9(3), pp. :1353–1372
- Leong, Y.K., Ma, T.W., Chang, J.S. and Yang, F.C., 2022. Recent advances and future directions on the valorization of spent mushroom substrate (SMS): A review. *Bioresource Technology*, 344, p.126157.
- Liu, R., and Lal, R. 2015. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. *Science of the Total Environment*, 514, pp. 131-139. doi: 10.1016/j.scitotenv.2015.01.104
- , pp. 131-139. doi: 10.1016/j.scitotenv.2015.01.104
- Loi, M.; Fanelli, F.; Cimmarusti, M.T.; Mirabelli, V.; Haidukowski, M.; Logrieco, A.; Caliandro, R.; Mule, G. 2018. In vitro single and combined mycotoxins degradation by Ery4 laccase from *Pleurotus eryngii* and redox mediators. *Food Control*, 8, p. 245.
- Loi M, Fanelli F, Zucca P, Liuzzi VC, Quintieri L, Cimmarusti MT, et al. 2016. Aflatoxin B1 and M1 Degradation by Lac2 from *Pleurotus pulmonarius* and Redox Mediators. *Toxins*.; <https://doi.org/10.3390/toxins8090245> PMID: 27563923
- Mali, S.C., Raj, S. and Trivedi, R., 2020. Nanotechnology a novel approach to enhance crop productivity. *Biochemistry and Biophysics Reports*, 24, p.100821.
- Manafi, M., Mohan, K. and Ali, M.N., 2011. Effect of ochratoxin A on coccidiosis-challenged broiler chicks. *World Mycotoxin Journal*, 4(2), pp.177-181.
- Maniruzzaman, M. (2004). Influence of media composition and growth regulators on mycelial growth and spawn production of three mushroom species. MS Thesis, Department of Biotechnology, BAU, Mymensingh.
- Manzano, A. M., Torres, G., González, A., Banguela, A., Ramos-González, P. L., Valiente, P. A., et al. 2013. Role of laccase isozymes in textile dye decolorization and diversity of laccase genes from *Ganoderma weberianum* B-18. *Journal of Applied Sciences & Environmental Management*.. 8, pp. 237-242.
- Maret, W. 2013. Zinc Biochemistry: From a Single Zinc Enzyme to a Key Element of Life, *Advances in Nutrition*, 4, pp. 82-91
- McCall, K.A., Huang, C.C. and Fierke, C.A., 2000. Function and mechanism of zinc metalloenzymes. *The Journal of nutrition*, 130(5), pp.1437S-1446S.

- Mcgrath, S., Maguire, R.O., Tacy, B.F. and Kike, J.H. 2009. Improving soil nutrition with poultry litter application in low-input forage systems. *Agronomy Journal*, 102, pp. 48-54.
- Mohammed, A.S., Kapri, A., Goel, R. 2011. Heavy metal pollution: source, impact, and remedies', *Biomanagement of metal-contaminated soils*, pp.1-28.
https://doi.org/10.1007/978-94-007-1914-9_1.
- Moreno J.L., Garcia C., Landi L., Falchini L., Pietramellara G., Nannipieri P. 2001. The ecological dose value (ED50) for assessing Cd toxicity on ATP content and dehydrogenase and urease activities of soil. *Soil Biology and Biochemistry*, 33(4-5), pp.483-489.
- Morimoto, N., Suda, S. and Sagara, N. 1981. Effect of ammonia on fruiting body induction of *Coprinus cinerius* in darkness. *Plant Cell Physiology* 22, pp. 247-254.
- Murugesan, K., In-Hyun, N., Kim, Y., and Chang, Y. 2007. Decolorization of reactive dyes by a thermostable laccase produced by *Ganoderma lucidum* in solid state culture. *Enzyme Microbiology*, 4, pp. 1662-1672. doi: 10.1016/j.enzmictec.2006.08.028
- Murugesan GR, Ledoux DR, Naehrer K, Berthiller F, Applegate TJ, Grenier B, Phillips TD, Schatzmayr G. 2015. Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. *Poultry Science*, 94, pp.1298-1315, doi:10.3382/ps/pev075.
- Mwita, L.N., Lyantagaye, S.L. & Mshandete, A.M. 2011. The effect of the interaction of varying chicken manure supplement levels with three different solid sisal wastes substrates on sporocarp cap lengths and diameters, stipe lengths and diameters and dry weights of *Coprinus cinereus* (Schaeff) S. Gray s.lat. *African Journal of Biotechnology*, 10 (7), pp.1172 -1180.
- Naim, L., Alsanad, M.A., El Sebaaly, Z., Shaban, N., Fayssal, S.A. & Sassine, Y.N. 2020a. Variation of *Pleurotus ostreatus* (Jacq. Ex Fr.) P. Kumm. (1871) performance subjected to different doses and timings of nano-urea. *Saudi Journal of Biological Sciences*, 27, pp.1573-1579. doi.org/10.1016/j.sjbs.2020.03.019.
- Naim, L., Alsanad, M.A., Shaban, N., El Sebaaly, Z., Fayssal, S.A. & Sassine, Y.N. 2020b. Production and composition of *Pleurotus ostreatus* cultivated on Lithovit®-Amino25 supplemented spent substrate. *AMB Expr* 10: 188 <https://doi.org/10.1186/s13568-020-01124-1>.

- Nasrollahzadeh, M., Sajadi, S.M., Sajjadi, M. and Issaabadi, Z., 2019. An introduction to nanotechnology. *In Interface science and technology*, 28, pp. 1-27.
- Ngezimana, W. Mtaita, T. A and Mtukwa, I. 2007. Potential of organic residues in producing oyster mushroom," *Pleurotus Ostreatus* Fr. (Polyporaceae) *International Journal of Biological and Chememical Sciences*, 2, pp. 108-120.
- Noble, R., Hobbs, P.J., Mead, A. & Dobrovin-Pennington, A. 2002. Influence of straw types and nitrogen sources on mushroom composting emissions and compost productivity. *Journal of Industrial Microbiology & Biotechnology*, 29, pp. 99-110. doi:10.1038/sj.jim.7000292.
- Nunes, J. C. et al. 2016. Effect of litter substrates on the performance, carcass traits, and environmental comfort of red-winged tinamou (*Rhynchotus rufescens*)', *Revista Brasileira de Ciencia Avicola*, 18, pp. 41-49. doi: 10.1590/1806-9061-2015-0142.
- Nwani, Stanley Emife; Osuji, Emeka. 2020. Poverty in Sub-Saharan Africa: The dynamics of population, energy consumption and misery index, *International Journal of Management, Economics and Social Sciences*, 9(4), pp. 247-270. doi: org/10.32327/IJMESS/9.4.2020.13
- Owaid, M.N., 2019. Green synthesis of silver nanoparticles by *Pleurotus* (oyster mushroom) and their bioactivity. *Environmental Nanotechnology, Monitoring & Management*, 12, p.100256.
- Phan CW, Sabaratnam, V. 2012. Potential uses of spent mushroom substrate and its associated lignocellulosic enzymes. *Applied Microbiol Biotechnology*, 96, pp. 863-873.
- Pickard MA, Roman R, Tinoco R Vazquez-Duhalt R. 1999. Polycyclic aromatic hydrocarbon metabolism by white rot fungi and oxidation by *Corioloropsis gallica* UAMH 8260 Laccase. *Applied Environment and Microbiology*, 65, pp. 3805-3809.
- Prasad AS. 2008. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. *Exp Gerontol*. 43(5), pp. 370-377. doi:10.1016/j.exger.2007.10.013.
- Prasad, R. 2014. Synthesis of silver nanoparticles in photosynthetic plants. *Journal of Nanoparticles*. 2014:963961. doi: 10.1155/2014/963961
- Prasad, R., Kumar, V., and Prasad, K. S. 2014. Nanotechnology in sustainable agriculture: present concerns and future aspects. *African Journal of Biotechnology*. 13, pp. 705-713. doi: 10.5897/AJBX2013.13554

- Prasad, R., Pandey, R., and Barman, I. 2016. Engineering tailored nanoparticles with microbes: quo vadis. WIREs Nanomed. *Nanobiotechnology*, 8, pp. 316-330. doi: 10.1002/wnan.1363
- Prasad R, Bhattacharyya, A and Nguyen, Q. D. 2017. Nanotechnology in Sustainable Agriculture: Recent Developments, Challenges, and Perspectives. *Frontier Microbiology*, 8, p.1014. doi: 10.3389/fmicb.2017.01014
- Purnomo AS, Mori T, Putra SR, Kondo R. 2013. Biotransformation of heptachlor and heptachlor epoxide by white-rot fungus *Pleurotus ostreatus*. *International Biodeterioration and Biodegradation*, 82, pp. 40–44, doi:10.1016/j.ibiod.2013.02.013
- Rahaiee, S., Ranjbar, M., Azizi, H., Govahi, M. and Zare, M., 2020. Green synthesis, characterization, and biological activities of saffron leaf extract-mediated zinc oxide nanoparticles: a sustainable approach to reuse an agricultural waste. *Applied Organometallic Chemistry*, 34(8), p.e5705.
- Ramirez, A. A. 2018. Use of antibiotics in broiler production : Global impacts and alternatives', 4, pp. 170-178. doi: 10.1016/j.aninu.2018.03.002.
- Randive, S. D. 2012. Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis', 3(4), pp. 1938-1949.
- Reddy, K. R.N, Salleh, B., Saad, B., Abbas, H. K., Abel, C. A and Shier, W. T. 2010. An overview of mycotoxin contamination in foods and its implications for human health, *Toxin Reviews*, 29(1), 3-26. Doi: 10.3109/15569541003598553.
- Rensing, C.; Newby, D.T.; Pepper, I.L. 2002. The role of selective pressure and selfish DNA in horizontal gene transfers and soil microbial community adaptation', *Soil Biology Biochemistry*, 34, pp. 285-296.
- Rezaeian S, Saadatmand S, Sattari TN, Mirshamsi A. 2016. Antioxidant potential and other medicinal properties of edible mushrooms naturally grown in Iran', *Biomedical Research International*, 27, pp. 240-247.
- Romero, O.; Huerta, M.; Damián, M.; Macías, A.; Tapia, A.; Parraguirre, J.; Juárez, J. 2010. Evaluación de la capacidad productiva de *Pleurotus ostreatus* con el uso de hoja de plátano (*Musa paradisiaca*) L.; CV. Roatan) deshidratada, en relación con otros sustratos agrícolas. *Rev Agronomía Costarricense, Colegio de Postgraduados, Puebla, México*, 34(1), pp. 53-63.

- Rooklidge, S. J. 2004. Environmental antimicrobial contamination from terraccumulation and diffuse pollution pathways. *Science of the Total Environment*, 325, pp.1-13.
- Royse D J, Rhodes T W, Ohga S, Sanchez, J E. 2004. Yield, mushroom size and time to production of *Pleurotus cornucopiae* (oyster mushroom) grown on switch grass substrate spawned and supplemented at various rates. *Bioresource Technology*, 91, pp.85-91.
- Rubert, J.; Soler, C.; Mañes, J. Application of an HPLC–MS/MS method for mycotoxin analysis in commercial baby foods. *Food Chemistry*, 2012, 133, pp. 176-183.
- Saavedra, M. 2007. Biodegradación de alperujo utilizando hongos del género *Pleurotus* y anélidos de la especie *Eisenia foetina*. Tesis doctoral. Universidad de Granada. Instituto de Biotecnología. Granada. México.
- Sadh, P.K.; Duhan, S.; Duhan, J.S. 2018. Agro-industrial wastes and their utilization using solid state fermentation', A review. *Bioresource and Bioprocess*, 5, p1.
- Salama, D.M., Abd El-Aziz, M.E., Rizk, F.A. and Abd Elwahed, M.S.A., 2021. Applications of nanotechnology on vegetable crops. *Chemosphere*, 266, pp.129026.
- Sánchez, A.; Royse, B. 2002. La biología y el cultivo de *Pleurotus* spp. Editorial Limusa S. A. México. P.288 .
- SAS. 2012. SAS User's Guide: 10th edition. SAS Institute Inc. Cary, North Caroline 27513-2414, USA.
- Sassine YN, Al Turki SM, Sebaaly E, Bachour Z, Masri L. 2019. Finding alternatives for Dormex (Hydrogen Cyanamid) as dormancy breaking agent. *Fresen Environment Bulletin*, 28,pp.10214-10224.
- Sassine, Y.N., Naim, L., El Sebaaly, Z., Fayssal, SA, Alsanad, M.A. & Yordanova, M.H. 2021. Nano urea effects on *Pleurotus ostreatus* nutritional value depending on the dose and timing of application. *Scientific Reports*, 11, p.5588. <https://doi.org/10.1038/s41598-021-85191-9>.
- Satpathy, S., Patra, A., Ahirwar, B. and Delwar Hussain, M., 2018. Antioxidant and anticancer activities of green synthesized silver nanoparticles using aqueous extract of tubers of *Pueraria tuberosa*. *Artificial cells, nanomedicine, and biotechnology*, 46(3), pp.71-85.
- Saucedo Castillo, O.; de Mello Prado, R.; Castellanos González, L.; Ely, N.; Silva Campos, C. N.; Pereira Da Silva, G.; Assis, L. C. 2015. Efecto de la fertilización fosfatada con

cachaza sobre la actividad microbiana del suelo y la absorción de fósforo en caña de azúcar (*Saccharum* spp.). *Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina.* 47(1), pp. 33-42

Sekhon, B. S. 2014. Nanotechnology in agri-food production: an overview.

Nanotechnology, Science and Applications, 7, 31-53. doi: 10.2147/NSA.S39406

Sharma, V.K., Filip, J., Zboril, R. and Varma, R.S., 2015. Natural inorganic nanoparticles—formation, fate, and toxicity in the environment. *Chemical Society Reviews*, 44(23), pp.8410-8423.

Shen X, Chi Y, Xiong K. 2019. The effect of heavy metal contamination on humans and animals in the vicinity of a zinc smelting facility. *PLoS One*, 14(10):e0207423.

Silva, E. and Stefanou, S.E., 2007. Dynamic efficiency measurement: theory and application. *American Journal of Agricultural Economics*, 89(2), pp.398-419.

Singh, D K. 2008. Biodegradation and bioremediation of pesticide in soil: Concept, method and recent developments. *Indian Journal of Microbiology*, 48(1), pp. 35-40.

Siqueira, F.; Maciel, W.; Martos, E.; Duarte, G.; Miller, R.; DA Silva, R.; Dias, E. 2012. Cultivation of *Pleurotus* mushrooms in substrates obtained by short composting and steam pasteurization. *Revised Journal of Biotechnology*, 11(53), pp.11630-11635.

Solanki, M., 2021. The Zn as a vital micronutrient in plants. *Journal of microbiology, biotechnology and food sciences*, 11(3), pp.e4026-e4026.

Stefanidou, M., Maravelias, C., Dona, A. and Spiliopoulou, C. (2006) Zinc: a multipurpose trace element. *Archives of Toxicology*, 80, pp. 1-9.

Steindor, K. A, Franiel, I. J, Bierza, W. M, Pawlak, B, Palowski, B. F. 2016. Assessment of heavy metal pollution in surface soils and plant material in the post-industrial city of Katowice', *Journal of environmental science and health*, 51(5), p. 371. doi: 10.1080/10934529.2015.1120509.

Srivastava, A. K., Prabhat, F. and Soreng, K. 2012. An effort to domesticate wild edible mushrooms growing in the forest of Jharkhand', *International Journal of Recent Trends in Science And Technology*, 3(3), pp. 88-92. Available at: https://www.statperson.com/Journal/ScienceAndTechnology/Article/Volume3Issue3/IJRTSAT_3_3_7

:

https://www.statperson.com/Journal/ScienceAndTechnology/Article/Volume3Issue3/IJRTSAT_3_3_7

- Tavarwisa, D. M., Govera, C., Mutetwa, M and Ngezimana, W. 2021. 'Evaluating the Suitability of Baobab Fruit Shells as Substrate for Growing Oyster Mushroom (*Pleurotus ostreatus*)', *International Journal of Agronomy*, 10, pp. 1-7. doi: 10.1155/2021/6620686.
- Teerapatsakul, C., Abe, N., Bucke, C., and Chitradon, L. 2007. Novel laccases of *Ganoderma* sp. KU-Alk4, regulated by different glucose concentration in alkaline media. *World Journal of Microbiology and Biotechnology*, 23, pp. 1559-1567. doi: 10.1007/s11274-007-9401-z.
- Tirsit, T.B., 2020. Biotechnological applications of white rot fungi: a review. *GSC Advanced Research and Reviews*, 5(2), pp.097-103.
- Torres Farradá,,G. Manzano, A. Rineau, F. Ramos, L. M. Thijs, S. Jambon, I. Put, J. Czech, J. Rivera, G. Carleer, R and Vangronsveld, J. 2019. Biodegradation of polycyclic Aromatic hydrocarbons by native *Ganoderma* sp. strains: identification of metabolites and proposed degradation pathways. *Applied Microbiology and Biotechnology*. P. 103. 10.1007/s00253-019-09968-9.
- Varjani, S.J. and Patel, R.K., 2017. Fungi: a remedy to eliminate environmental pollutants. *Mycoremediation and Environmental Sustainability*, 1, pp.53-67.
- Venter, F. and Venter, J. 1996. Making the most of indigenous trees. Priza Publications. Pretoria, South Africa.
- Verma, R., Vijayalakshmy, K and Chaudhry, V. 2018. 'Detrimental impacts of heavy metals on animal reproduction: A review', *Journal of Entomology and Zoology Studies*, 6(6), pp. 27-30.
- Viegas, C., Carolino, E., Malta-Vacas, J., Sabino, R., Viegas, S. and Verissimo, C., 2012. Fungal contamination of poultry litter: a public health problem. *Journal of Toxicology and Environmental Health*, 75 (22-23), pp. 1341-1350. DOI: 10.1080/15287394.2012.721165.
- Wan C, Li Y .2012. Fungal pretreatment of lignocellulosic biomass. *Biotechnol Adv* 30, pp.1447-1457.

- Westlake, K., 1985. The incidence of mycotoxins in litter, feed and livers of chickens in Natal. *South African Journal of Animal Science*, 15 (4), pp. 175-177.
- Wood CW, Torbert HA, Delaney DP. 1993. Poultry litter as a fertilizer for bermudagrass: effects on yield and quality', *Journal of Sustainable Agriculture*, 3, pp. 21-36.
- Wood DA, Hartley RD. 1988. Conclusions and recommendations. In: Zadrazil F, Reiniger P (eds), *Treatment of lignocellulosics with white-rot fungi*. London: Elsevier Applied Science, pp.115-117.
- Wright D, G. 2010. 'Antibiotic resistance in the environment: a link to the clinic', *Current opinion in microbiology*, 13(5), 589-594. doi: 10.1016/j.mib.2010.08.005.
- Yehia, R.S. Aflatoxin detoxification by manganese peroxidase purified from *Pleurotus ostreatus*. *Brazilian Journal of Microbiology*, 2014, 45, pp. 127-134.
- Zahedi, S.M., Karimi, M. and Teixeira da Silva, J.A., 2020. The use of nanotechnology to increase quality and yield of fruit crops. *Journal of the Science of Food and Agriculture*, 100(1), pp.25-31.
- Zięba, P., Kała, K., Włodarczyk, A., Szewczyk, A., Kunicki, E., Sękara, A. and Muszyńska, B., 2020. Selenium and zinc biofortification of *Pleurotus eryngii* mycelium and fruiting bodies as a tool for controlling their biological activity. *Molecules*, 25(4), p.889.
- Zied, D.; Savoie, A. ; Pardo-Giménez. 2011. Soybean the main nitrogen source in cultivation substrates of edible and medicinal mushrooms. Soybean and nutrition. *In Technology Europe*, pp.307-317
- Zhang, Y., Ge, F., Hou, F., Sun, W., Zheng, Q., Zhang, X., et al. 2017. Transcription factors responding to Pb stress in maize. *Genes* 8, p.231. doi: 10.3390/genes8090231
- Zhu MJ, Du F, Zhang GQ, Wang HX, Ng TB (2013) Purification a laccase exhibiting dye decolorizing ability from an edible mushroom *Russula virescens*. *International Biodeterioration and Biodegradation*, 82, pp. 33-39, doi:10.1016/j.ibiod.2013.02.010

3.1 Introduction

The study of materials whose sizes are in the nanometric dimension has gained increased attention in different fields (Mbenga *et al.*, 2022). These materials have the potential to bridge the gap between bulk materials and atomic structures. They are referred to as nanoparticles when the size of their dimensions is between 1 – 100 nm. Nanoparticles could be applied in different areas such as electronics, cosmetics, coatings, packaging, and biotechnology (Anand *et al.*, 2019). Therefore, the synthesis of NPs is an important and active area of study. Different methods have been used for their synthesis and are broadly divided into bottom-up and top-down approaches. In the bottom-up approach, structures are constructed atom by atom, molecule by molecule, and cluster by cluster. Biological and chemical procedures could be used to synthesize nanoparticles in this approach. Its advantage is that it can obtain NPs with comparatively fewer defects and homogenous chemical composition. The top-down approach uses physical and chemical means. Due to its high ratio, it impacts the chemistry and physical properties of the NPs (Kulkarni & Feng, 2013). Physical and chemical approaches are expensive and consume a lot of energy (Parveen *et al.*, 2016), while the green approach to NPs is often low in cost and does not require lots of energy. In addition, the chemical approach has the disadvantage of contamination from precursors and possible hazardous by-products (Sagadevan *et al.*, 2022).

The highlighted shortcomings of the physical and chemical methods encouraged the need to introduce a low-cost, non-toxic and environmentally friendly method for NP synthesis (Shaik *et al.*, 2018). The biological method for synthesizing NPs has been applauded due to the availability of natural resources, which could be plants, algae, fungi, yeast, bacteria, and or viruses that are used as reducing and/or stabilizing agents. Metal oxide NPs have been widely explored in materials chemistry (Roopan *et al.*, 2014), and ZnO-NP is one of the semiconducting metal oxides that have a wide area of applications and antimicrobial activities (Maryanti *et al.*, 2014; Thema *et al.*, 2015). It is non-toxic, naturally abundant and has good photocatalytic properties (Mallika *et al.*, 2014). Zinc oxide nanoparticles are commonly employed in agriculture, gas sensors, catalysts, batteries, high-temperature superconductors, solar energy conversion, surfactants, antimicrobials, antioxidants, antibacterial, and anticancer agents (Rajeshkumar *et al.*, 2018; Gao *et al.*, 2019; Shobha

et al., 2019; Abdelhakim *et al.*, 2020; Abdelghany *et al.*, 2022; Al-Ghamdi *et al.*, 2022;). A variety of ways are available for the synthesis of ZnO nanoparticles, but the use of plant materials as a mediating agent is a very good alternative and environmentally beneficial method.

Different studies have reported the use of a variety of plants in the synthesis of ZnO nanoparticles (Mutukwa *et al.*, 2022; Sahin *et al.*, 2022; Zeghoud *et al.*, 2022). Suresh *et al.* 2018 described a green solution combustion route to ZnO nanoparticles using aqueous *Cassia fistula* plant extract as fuel. The photodegradative, antibacterial, and antioxidant properties of the NPs were investigated and they were found to contain reducing components such as polyphenols (11%), flavonoids, and phenolic acids. A ZnO NP of hexagonal wurtzite structure has been produced by using extracts of *Euphorbia hirta* leaves (Ahmad & Kalra, 2020). Vanathi *et al.* 2014 adopted a green chemistry technique using *Eichhornia crassipes* leaf extract to synthesize ZnO NPs, thereby reporting a low-cost approach to ZnO NPs rather than a more expensive and environmentally unfriendly method. During the synthesis of nanoparticles, the aqueous leaf extract acts as either a reducing or/and capping agent to afford an eco-friendly alternative to the chemical synthesis of ZnO NPs.

As yet, only a few initiatives have exploited *Vachellia erioloba* seed extracts in the green production of nanoparticles. *Vachellia erioloba* is a native plant found throughout Southern Africa. *Vachellia erioloba* is a legume that belongs to the Fabaceae family of plants. It thrives in tough, arid environments and it is frost and heat-resilient in the summer (Barnes *et al.*, 1997). It has a deep root system that extends about 6 meters down, allowing it to seek underground water for survival (Schulze *et al.*, 1998). It is very beneficial for different purposes. Their pods may be used as fodder for cattle and consumed by wild animals, and their timber is sturdy enough to be used as firewood (Coe, 1998; Materechera & Materechera, 2001; Timberlak *et al.*, 1999). The powder from their pods can be used to treat ear infections, and the roots can be used to treat toothaches (Coe, 1998; Palgrave, 2015). In addition, this plant contains phytochemicals that have biological importance such as antioxidants (Semenya & Maroyi, 2018) and have antibacterial potency (Du Preez & Mumbengegwi, 2014). For example, the stem bark extract of *Vachellia erioloba* contains tannins, saponins and sterols. The ethanol and methanol extracts of the stem bark have antibacterial efficacy against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* strains (Okoro *et al.*, 2012).

Apart from the biological relevance, the phytochemicals of plants could be utilized in the synthesis of nanoparticles, since they contain some functional groups that are capable of acting as mediators or capping agents in the synthesis of nanoparticles (Dauthal & Mukhopadhyay, 2016; Regulacio *et al.*, 2020; Vaid *et al.*, 2020). In this study, the aqueous extracts of *Vachellia erioloba* have been used for the synthesis of ZnO nanoparticles.

3.2 Objectives

3.2.1 Main objective

To synthesize ZnO nanoparticles using *Vachellia erioloba* seed extract..

3.2.2 Specific objective

- i. To characterize ZnO NP formation using XRD, TEM SEM and UV-vis.
- ii. To investigate anticancer and antioxidant medical properties of ZnO-Nps.

3.3 Materials and Methods

3.3.1 Plant collection and chemicals

Vachellia erioloba pods were collected from the North-West University's Molelwane farm and shredded (scatter) to obtain the seeds. Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) and sodium hydroxide (NaOH) were purchased from Merck, Germany.

3.3.2 Preparation of seed extract

In order to obtain the extracts, *Vachellia erioloba* pods were pounded inside a bag and the seeds were removed from the pods. The seeds were placed in a milling machine to grind them into a moderately fine powder. Thereafter, 800 g of the powder was placed in a beaker and 1000 mL of de-ionized water was mixed with it in a blender. The mixture of seeds and de-ionized water was blended to a fine texture. Finally, the mixtures were filtered, and the resulting extracts were stored in a refrigerator for later use. Figure 3.1 presents the process of seed extraction.

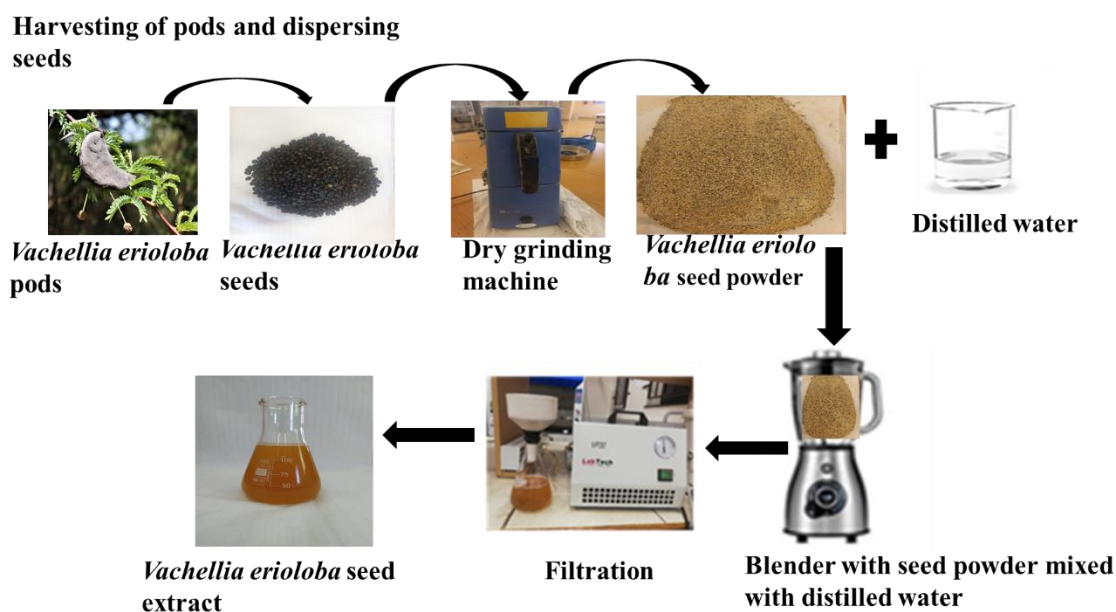


Figure 3.1: Preparation of aqueous extract of *Vachellia erioloba* seeds

3.3.3 Synthesis of ZnO nanoparticles

The ZnO NPs were synthesized by mixing 4.4 g of zinc acetate with 30 mL distilled water which acted as a precursor for Zn. This was carried out with different volumes of the *Vachellia erioloba* aqueous extracts, 20 and 30 mL. The NaOH solution was used to adjust the pH to 7. These suspensions were then stirred using a magnetic stir for 2 hours at 85 °C. After the reaction, the product obtained was centrifuged at 5000 rpm for 15 min and washed with distilled water and ethanol. Following that, the product was dried overnight before being calcined at 400 °C for 2 hours (Figure 3.2) to obtain zinc oxide nanoparticles designated as ZnO (20) and ZnO (30) for nanoparticles using 20 and 30 mL extracts, respectively.

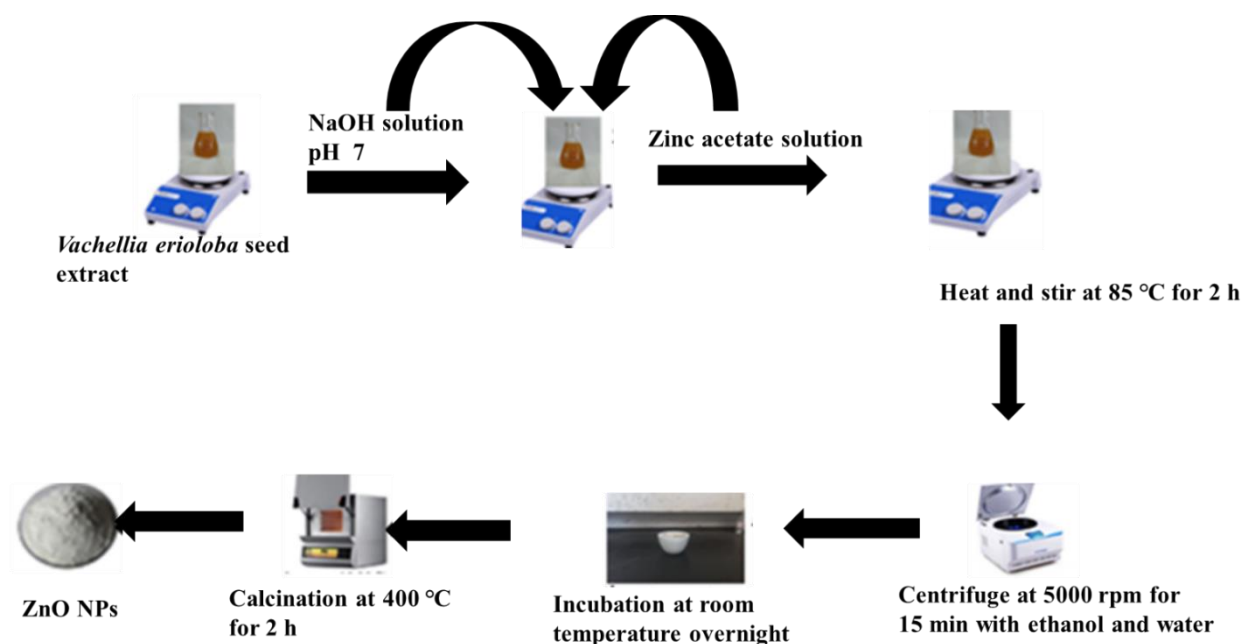


Figure 3.2: Green synthesis of Nano-ZnO using extracts of *Vachellia erioloba* seeds

3.3.4 Characterization of nanoparticles

A Bruker D8 advanced X-ray diffraction (XRD) with Cu K α radiation ($k = 1.5405$; nickel filter), fitted with a proportional counter and recorded in the range $2\theta = 20$ to 80° was utilized to confirm the crystalline phase of the nanoparticles. A FTIR measurement of *Vachellia erioloba* seed powder was obtained using a Cary 670 FTIR spectrometer in the wavelength range of 400 - 4000 cm^{-1} (Agilent Technologies) (Ravichandrika *et al.*, 2012). A Quanta FEG 250 scanning electron microscope (SEM) with a 30 kV acceleration voltage was used to analyze the external morphology of the ZnO nanoparticles. A TECNAI G2 (ACI) transmission electron microscope (TEM) with a 200 kV accelerating voltage was used to examine the nanoparticles' internal shape and sizes. Absorbance properties of the synthesized ZnO nanoparticles were measured from 200 to 800 nm (Agilent Technologies) using a Cary 30 UV-vis spectrophotometer.

3.3.5 Anticancer assay

The ability of *V. erioloba* mediated ZnO nanoparticles to suppress cancer cells was performed on two cell lines: immortal human embryonic kidney (HEK 293) and carcinoma cervical cancer (HeLa) cell. The cells were sourced from ATCC, Manassas, VA, USA. They were cultured in a 25 cm^2 tissue flask using Dulbecco's Modified Eagle's Medium (DMEM) (Lonza BioWhittaker, Walkersville, MD, USA) with 10% fetal bovine serum, $100\text{ }\mu\text{g/mL}$ streptomycin, and 100 U/mL penicillin. The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-

diphenyltetrazolium bromide (MTT) assay was used to analyze the cells in a 96 well plate that contained 2.5×10^2 cells/well in 100 μL DMEM and the cells were cultured for 24 hours as described by Adeyemi *et al.* (2020). Prior to incubation, the cells were incubated for 4 hours at 37 °C. After incubation, the cells were treated with different concentrations of ZnO NPs (10, 25, 50, and 100 $\mu\text{g mL}^{-1}$). The treated cells were cultured at 37 °C for 48 hours before the MTT assay. Fluorouracil (5-FU) was used as the standard. The assay medium was replaced with a fresh solution containing 10% MTT reagent and incubated for another 4 hours at 37 °C. The insoluble formazan crystals were dissolved in 100 μL of DMSO, and the absorbance was measured at 570 nm using DMSO as a blank. These tests were performed three times to obtain the average value of absorbance. These tests were done because NPs have numerous advantageous properties, such as simultaneous delivery of antigens and adjuvants to the same antigen-presenting cells (APCs); protection of bioactive cargo molecules from enzymes-induced ZnO which can be transferred to oyster mushrooms that will enhance anticancer properties, NPs have immunotherapy (Gomes, 2021; Thakur *et al.*, 2020).

3.3.6 Antioxidant assay

The radical scavenging activity of the ZnO-NPs was carried out using DPPH assay following methods adopted from Dousti *et al.* (2021), however, with minor modification. DPPH solution was prepared by dissolving 6.4 mg of DPPH in 25 mL of ethanol to obtain a 0.016 mM concentration of DPPH. The solution was placed in the dark for 30 min. After incubation, 1 mL of DPPH solution was added to the ZnO solutions, which were prepared by dissolving different concentrations of ZnO-NPs (3.13, 6.25, 12.5, 25, and 50 $\mu\text{g/mL}$) in ethanol. The blank solution contained only ethanol and DPPH. The study was carried out three times and the absorbance was recorded at 540 nm. Thereafter, the % inhibition of ZnO-NPs against free radicals was estimated using the following equation:

$$\% \text{ Inhibition} = [(A_c - A_s)/A_c] \times 100\% \dots\dots\dots (3.1)$$

(A_c) represents the absorbance of the control, while (A_s) is the absorbance of the test sample.

The antioxidant assay was done because NPs reduce the risk of heart disease, fight degenerative illnesses, and extend the life of the product by obstructing or delaying oxidation processes, which oyster mushrooms also have.

3.4 Results

3.4.1 FTIR of *Vachellia erioloba* seed powder

The FTIR spectrum showed (Figure 3.3) transmittance peaks at 3277, 2921, 2093, 1739, 1632, 1533, 1338, 1243, 1035, and 522 cm^{-1} .

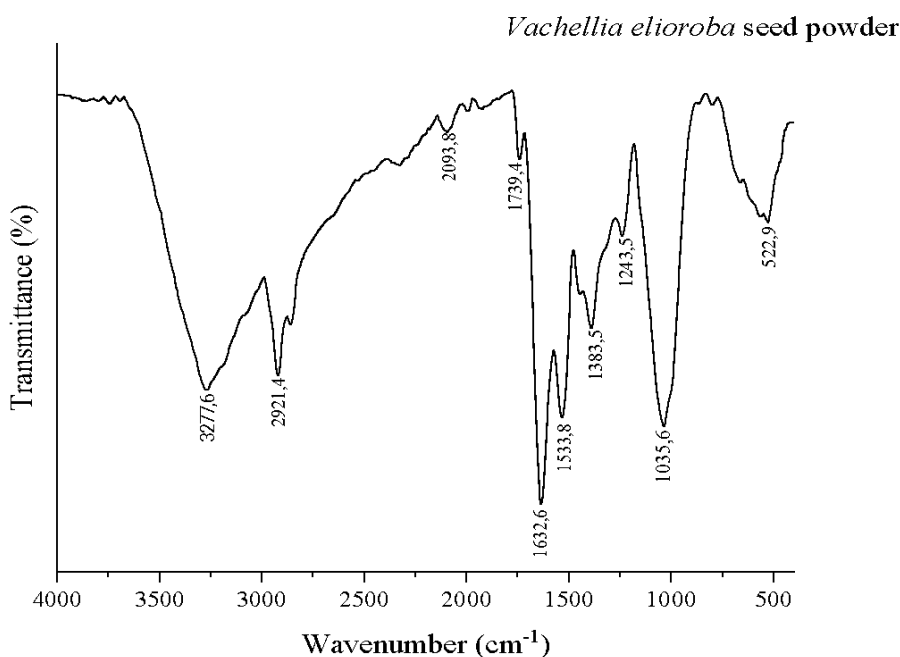


Figure 3.3: The FTIR analysis of the *Vachellia erioloba* seed powder showing transmittance peaks

3.4.2 X-ray diffraction studies of *Vachellia erioloba* ZnO nanoparticles

Figure 3.4a and 3.4b shows the diffraction pattern of the ZnO nanoparticles. The diffracted beam's intensity was plotted as a function of diffraction angles in the diffractogram.

The Debye-Scherer equation (Equation 3.2) was used to estimate the average crystallite size of both ZnO(20)-NPs and ZnO(30)-NPs seed extract, and the nanoparticle sizes were found to be 9.45 and 12.00 nm, respectively.

$$D = \frac{K\lambda}{\beta \cos \theta} \dots \dots \dots (2)$$

Where, D= crystalline size (nm)

K= 0.9 (Scherrer constant)

λ = 0,15406 (wavelength of the X-ray)

β = FWHM (radians)

θ = peak position (radians)

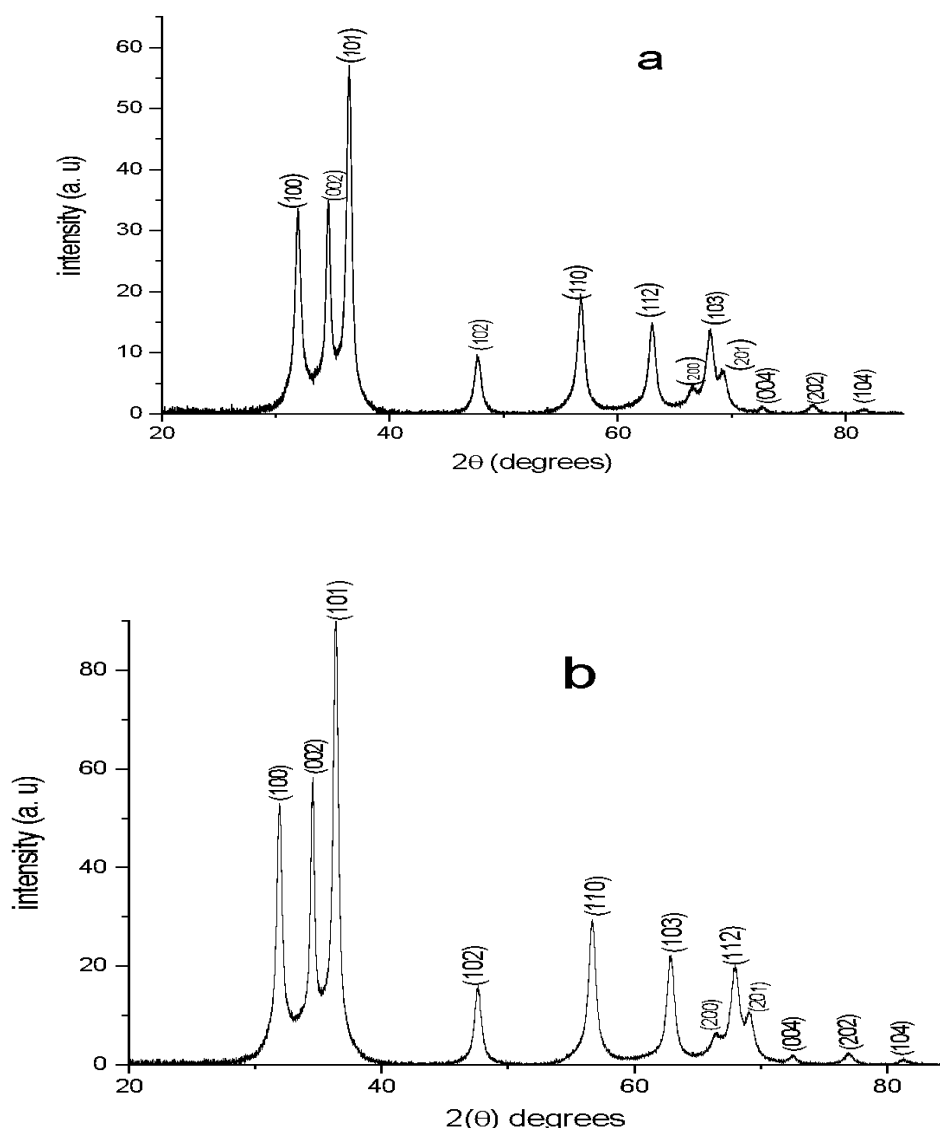


Figure 3.4: ZnO NPs synthesized using (a) 20 and b (30) mL extracts of *Vachellia erioloba* seed

3.4.3 SEM, TEM and particle size distribution histogram of *Vachellia erioloba* mediated ZnO nanoparticles

The size of nanoparticles has a significant impact on the overall characteristics of the material. As a result, studying the size of semiconducting nanoparticles is critical for understanding the material's characteristics (Talam *et al.*, 2012). The SEM micrographs show the external morphology of ZnO(20) and ZnO(30)-NPs in Figure 3.3a and Figure 3.3b, respectively. The particles showed a spherical form and were closely packed. The

TEM analysis was conducted in order to better understand the nanoparticles' shape and size (Arvizo *et al.*, 2011; Suthar *et al.*, 2023).

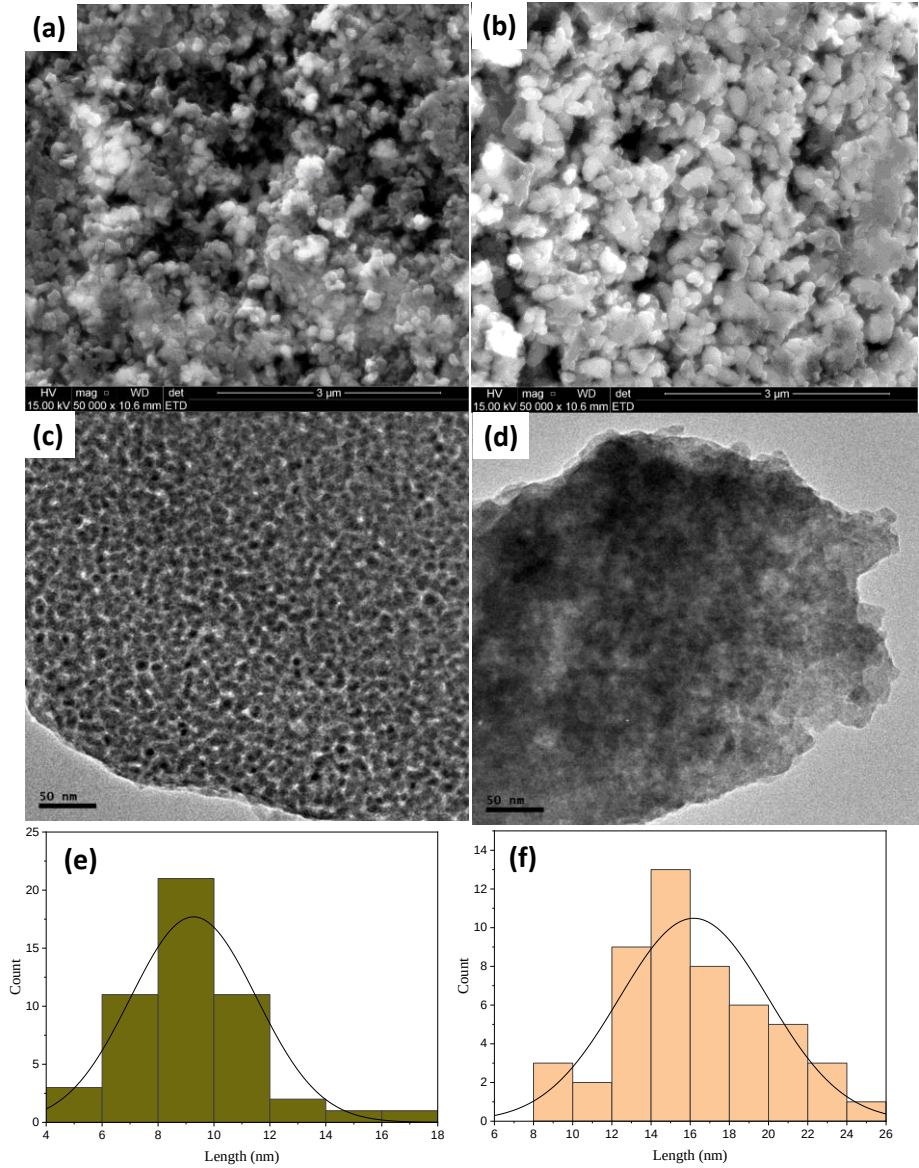


Figure 3.5:(a, (b) SEM, (c), (d) TEM, and (e), (f) particle size distribution histogram for ZnO nanoparticles prepared using 20 and 30 mL seed extract of *Vachellia erioloba*, respectively.

3.4.4 UV-visible spectroscopic studies of ZnO nanoparticles prepared using *Vachellia erioloba* seed extract

UV-visible spectroscopy is commonly utilized to investigate the optical characteristics of nanoscale particles. The (Figure 3.6a) and (Figure 3.6b) spectra of the nanoparticles showed the absorption peaks at 207 and 220 nm for the ZnO (20) and ZnO (30)-NPs, respectively.

The band gap energies of the ZnO from both routes were determined from the Tauc plot using equation 3.

$$\alpha h\nu = A(h\nu - E_g)^n \dots\dots\dots (3.3)$$

where α is the absorption coefficient, A is a constant, h is Plank's constant, E_g is the optical band gap energy and n is $\frac{1}{2}$ for direct band gap and 2 for indirect band gap semiconductor.

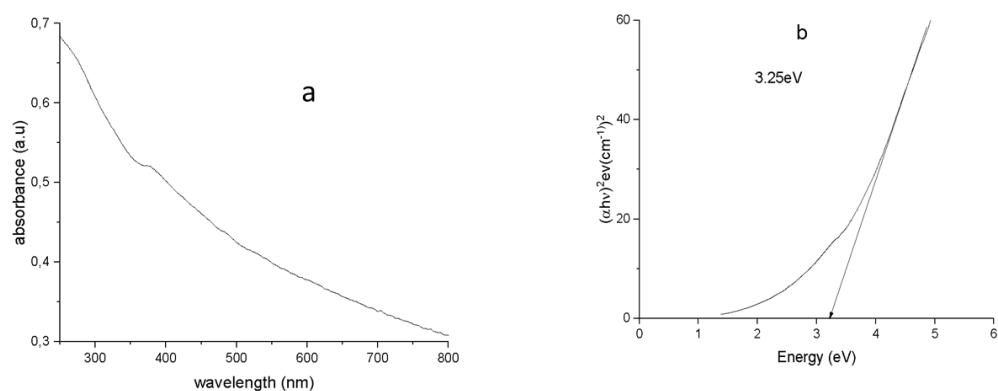


Figure 3.6: (a) UV-vis absorption peak of ZnO (20), and (b) its corresponding Tauc plot for the band gap estimation.



Figure 3.6: (a) UV-vis absorption peak and (b) its corresponding Tauc plot for the band gap estimation

3.4.5 Anticancer activity

The HEK 293 and HeLa cell lines were used to test the anticancer efficacy of *Vachellia erioloba*-mediated ZnO NPs as well as the seed extract. Table 1 illustrates the activity of the ZnO NPs obtained using various concentrations of the plant extract as well as the calculation of minimum inhibitory concentration (IC₅₀) values for the cell lines which can be used to enhance the anticancer activities on oyster mushrooms.

Both the HEK 293 and HeLa have IC₅₀ values of 5.27 µg/mL for ZnO (20) and 11.4 µg/mL for ZnO (30), respectively, which are higher than most values reported in the literature (Ray, 2021). Apart from the established potency of ZnO, the particles exist in nano form which enhances the activity of the material.

Table 1: Cytotoxic activity if the green ZnO NPs and *Vachellia erioloba* seed powder

Cell lines	Test samples	ZnO Concentration (µg/mL)				IC ₅₀ (µg/mL)
		10	25	50	100	

HEK 293	5-FU	77.36	±	51.92	±	35.34	±	11.33 ± 0.016	6.05
		0.047		0.003		0.010			
	ZnO (20)	45.15	±	30.91	±	23.99	±	20.73 ± 0.004	5.27
		0.055		0.041		0.023			
	ZnO (30)	64.74	±	27.12	±	18.68	±	11.15 ± 0.011	14.19
		0.031		0.036		0.038			
	Seed powder	69.35	±	49.78	±	25.65	±	12.40 ± 0.016	22
		0.033		0.028		0.061			
	5-FU	78.40	±	58.89	±	50.47	±	37.99 ± 0.02	17.48
		0.03		0.04		0.02			
HeLa	ZnO (20)	58.61	±	38.28	±	24.06	±	17.41 ± 0.043	14.4
		0.045		0.039		0.011			
	ZnO (30)	54.76	±	31.84	±	15.84	±	10.51 ± 0.027	11.14
		0.041		0.032		0.047			
	Seed powder	73.56	±	59.27	±	42.28	±	12.69 ± 0.023	29.72
		0.032		0.072		0.093			

Values are expressed as mean cell viability (%) ± standard deviation (n=3). Fluorouracil is a standard.

3.4.6 Antioxidants activities

The radical scavenging ability of ZnO-NPs was determined using a DPPH assay. It was observed that the free radical scavenging ability of ZnO-NPs and ascorbic acid increased in a concentration-dependent manner. The percentage of DPPH activity increased with increasing concentration of the samples (Table 2). A similar observation was reported by Mthana *et al.* (2022), whereby increasing ZnO-NPs, which were synthesized using plant extract of *Capsicum chinense*, increased the radical scavenging activity. The deep violet colour of the DPPH solution gradually changed to pale yellow in the presence of ZnO-NPs and ascorbic acid used as reference/standard, which indicates the antioxidant capacity of ZnO-NPs.

The obtained IC₅₀ values of 64.57 and 22.91 µg/mL for green ZnO(30) and Zn(20) –NPs, respectively, were higher than that of ascorbic acid (20.89 µg/mL). These results are in line with previous studies, which reported lower IC₅₀ values of ascorbic acid than green-synthesized ZnO-NPs (Arumugam *et al.*, 2021).

Table 2: Antioxidant activity (%) of green ZnO NPs

Test samples	Sample concentration (µg/mL)					IC ₅₀ (µg/mL)
	3.13	6.25	12.5	25	50	
Ascorbic acid	12.26 ± 0.040	35.95 ± 0.011	40.04 ± 0.080	52.21 ± 0.082	65.20 ± 0.064	20.89
ZnO(20) NPs	9.99 ± 0.067	26.91 ± 0.072	29.40 ± 0.028	38.300 ± 0.037	46.16 ± 0.014	64.57
ZnO(30) NPs	17.44 ± 0.031	32.27 ± 0.052	41.85 ± 0.014	54.03 ± 0.078	58.35 ± 0.052	22.91

Values are expressed as mean inhibition (%) ± standard deviation (n=3). Ascorbic acid is a standard.

3.5 Discussion

The FTIR showed the transmittance peak at 3277 cm^{-1} , corresponding to the stretching vibration of the O–H group from either phenols, polysaccharides, or proteins. The stretching vibrations of the (C–H) group of a saturated carbon are responsible for the peaks at 2921.4 cm^{-1} . The peaks at 1632 and 1533 cm^{-1} were associated with the hydroxyl (OH) and amine (N–H) groups respectively of proteins or enzymes found in *Vachellia erioloba* seed powder. The stretching vibrations of the C–O bond in the seed powder were responsible for the bands at 1035 cm^{-1} . Furthermore, peaks at 532 may correspond to the C–N stretch (Sagar Raut & Thorat, 2015) in Figure 3.3. The X-ray diffraction peaks for ZnO nanoparticles synthesized using 20 ml seed extract Figure 4a appeared at 2θ values of 32.01°, 34.67°, 36.46°, 47.75°, 56.79°, 63.04°, 67.52°, 68.14°, 69.25°, 72.73°, 77.14° and 81.57°, which corresponded to the lattice planes of (100), (002), (101), (102), (110), (103), (112), (200), (201), (004), (202) and (104) respectively. Similarly, the crystalline structure of ZnO nanoparticles synthesized using 30 ml seed extract also showed peaks at 31.93°, 34.57°, 36.38°, 47.63°, 56.67°, 62.89°, 67.51°, 68.0°, 69.12°, 72.56°, 76.98° and 82.12° (figure 4b), that are indexed as (100), (002), (101), (102), (110), (103), (112), (200), (201), (202), (004) and (104) planes, respectively. These peaks correspond to that of a hexagonal phase ZnO crystal structure with a space group of P6₃mc and a reference code of (00-036-1451) (McMurdie *et al.*, 1986).

The TEM images of ZnO confirmed that the particles are spherical with a small thickness variation, which is consistent with the SEM result. The average particle size was found to be 9.2 (ZnO(20)) and 16.3 (ZnO(30)) -NPs nm from the particle size distribution histogram presented in Figures 3.5e and 3.5f, respectively. Generally, depending on the changes in nanoparticle size, the absorption peak could shift to a higher or lower wavelength with respect to the peak of absorption of the bulk (Dobrucka *et al.*, 2019; Khatak *et al.*, 2021). The calculated band gap energy values of the ZnO (20) and ZnO (30) NPs were 3.25 and 3.31 eV respectively shown in Figure 3.5b and Figure 3.6b. These values are lower than the band gap of bulk ZnO (3.38 eV), which is the consequence of the size effect similar to earlier reports (Stan *et al.*, 2015; Luque *et al.*, 2018; Khan *et al.*, 2019; Kumar *et al.*, 2019; Ramesh *et al.*, 2021). A reduction in the band gap value with respect to bulk has been reported for green synthesized ZnO NPs using leaves of *Phoenix roebelenii* palm and this observation was attributed to the effect of native defects (zinc interstitials and oxygen

vacancies). These defects could produce localized electronic states within the energy gap, which could result in the shortening of the energy band gap (Aldeen et al., 2022).

The results reveal a concentration-dependent profile with some promising actions against the cell lines tested. Furthermore, because of their profound reactive oxygen species - generating potential during dark and light exposure, ZnO NPs would boost the exceptional anticancer activity in oyster mushrooms (Bhardway et al., 2020). The ZnO samples displayed very good toxicity (*in-vitro*) on both cancer cell lines (HEK 293 and HeLa). The ZnO NPs (20) extract was able to suppress half of the HEK 293 cell lines more than the standard, whereas samples from the ZnO-NPs (30) extract were able to suppress half of the HeLa cell line. It could be inferred that green synthesized ZnO-NPs are more effective in the treatment of cancer cells than the plant extract which can help in increasing the anticancer properties in plants. The performance of ZnO NPs at high concentrations could be due to the presence of biologically active compounds found in the *Vachellia erioloba* seed extract that was used to synthesize the ZnO. The scavenging activity of ascorbic acid (standard) was higher than that of both samples of ZnO-NPs (Table 3.2); this, indicates that the standard was more effective in capturing the DPPH's free radicals compared to both ZnO-NPs. However, the ZnO(30) -NPs showed higher inhibition potency compared to the ZnO(20)-NPs. Since ZnO(30) -NPs were shown to have a smaller particle size, they have larger surface area to volume ratio than the ZnO(20) -NPs (Jacob, 2019). Generally, a large surface area to volume ratio of NPs results in high material performance due to the domination of materials' surface by atoms.

3.6 Conclusion

ZnO-NPs were successfully produced from *Vachellia erioloba* pods. XRD, SEM, TEM and UV-vis were used to characterize the nanoparticle formation. The FTIR study on *Vachellia erioloba* seed powder indicated the presence of biomolecules, which may have played an important role in the creation of ZnO NPs. TEM pictures clearly demonstrated that *Vachellia erioloba* produced ZnO-NPs. It formed a spherical shape with an average particle size of 9.2 nm for ZnO (20) NPs and 16.3 nm for ZnO (30) NPs. They have shown band gap energy of 3.25 and 3.31 eV for ZnO (20) NPs and ZnO (30) NPs respectively. The anticancer test was carried out to assess the biological activity of the produced ZnO NPs, they were active against HEK 293 and HeLa cell line and the DPPH assay revealed that ZnO (20) NPs and ZnO (30) NPs possess antioxidant activity with an IC₅₀ value of

64.57 and 22.91 $\mu\text{g/mL}$. Our findings show that the aqueous seed extract of *V. erioloba* might be used to synthesize stable ZnO-NPs and because ZnO-NPs were shown to be more effective on plant development, they will be used to boost the growth and production of oyster mushrooms and enhance the anticancer and antioxidants properties on oyster mushroom (Šebesta *et al.*, 2021). Nanoparticles have the potential to alter the agriculture and food industries by providing different revolutionary tools for disease control, illness detection, and increasing plant nutrient absorption capacity (Goswami & Mathur, 2019).

Nanotechnology offers enormous promise and benefits in agronomy and biotechnology and such understanding is required before employing nanoparticles in future plant science research (Avellan *et al.*, 2019). The size of NPs influences their interaction and accumulation within plants and ZnO NPs which were formed were within safe measures for application in oyster mushroom production. Even though there are new techniques for plant development, employing NPs has proven to be effective, although NPs have various fatal consequences for plant growth and survival rate. (Mishra *et al.*, 2014). As a result, research on NPs is needed, specifically on their interactions with plants and their physiological, biochemical, and molecular impacts. Hence the ZnO NPs synthesized will be used for *Pleurotus ostreatus* growth and development.

References

- Abdelghany, T.M., Al-Rajhi, A.M., Yahya, R., Bakri, M.M., Al Abboud, M.A., Yahya, R., ... Salem, S.S. 2022. Phytofabrication of zinc oxide nanoparticles with advanced characterization and its antioxidant, anticancer, and antimicrobial activity against pathogenic microorganisms. *Biomass Conversion and Biorefinery*, pp.1-14.
- Abdelhakim, H., El-Sayed, E. & Rashidi, F. 2020. Biosynthesis of zinc oxide nanoparticles with antimicrobial, anticancer, antioxidant and photocatalytic activities by the endophytic *Alternaria tenuissima*. *Journal of Applied Microbiology*, 128(6)1, pp.634-1646.
- Adeyemi, J.O., Onwudiwe, D.C., Nundkumar, N. & Singh, M. 2020. Diorganotin (iv) benzyldithiocarbamate complexes: Synthesis, characterization, and thermal and cytotoxicity study. *Open Chemistry*, 18(1), pp.453-462.
- Ahmad, W. & Kalra, D. 2020. Green synthesis, characterization and anti microbial activities of ZnO nanoparticles using *Euphorbia hirta* leaf extract. *Journal of King Saud University-Science*, 32(4), pp.2358-2364.
- Al-Ghamdi, S., Alkathiri, T., Alfarraj, A., Alatawi, O.M., Alkathiri, A., Panneerselvam, C., ... Pasha, A. 2022. Green synthesis and characterization of zinc oxide nanoparticles using *Camellia sinensis* tea leaf extract and their antioxidant, anti-bactericidal and anticancer efficacy. *Research on Chemical Intermediates*, 48(11), pp.4769-4783.
- Aldeen, T.S., Mohamed, H.E.A. & Maaza, M. 2022. ZnO nanoparticles prepared via a green synthesis approach: Physical properties, photocatalytic and antibacterial activity. *Journal of Physics and Chemistry of Solids*, 160:110313.
- Anand, G.T., Renuka, D., Ramesh, R., Anandaraj, L., Sundaram, S.J., Ramalingam, G., ... Kaviyarasu, K. 2019. Green synthesis of ZnO nanoparticle using *Prunus dulcis* (Almond Gum) for antimicrobial and supercapacitor applications. *Surfaces and Interfaces*, 17:100376.
- Arumugam, V., Subramaniam, S. & Krishnan, V. 2021. Green synthesis and characterization of zinc oxide nanoparticles using *Berberis tinctoria* Lesch. leaves and fruits extract of multi-biological applications. *Nanomedicine Research Journal*, 6(2), pp.128-147.
- Arvizo, R.R., Rana, S., Miranda, O.R., Bhattacharya, R., Rotello, V.M. and Mukherjee, P., 2011. Mechanism of anti-angiogenic property of gold nanoparticles: role of nanoparticle size and surface charge. *Nanomedicine: Nanotechnology, Biology and Medicine*, 7(5), pp.580-587.

- Avellan, A., Yun, J., Zhang, Y., Spielman-Sun, E., Unrine, J.M., Thieme, J., Li, J., Lombi, E., Bland, G. and Lowry, G.V., 2019. Nanoparticle size and coating chemistry control foliar uptake pathways, translocation, and leaf-to-rhizosphere transport in wheat. *ACS nano*, 13(5), pp.5291-5305.
- Bangroo, A., Malhotra, A., Sharma, U., Jain, A. & Kaur, A. 2022. Biosynthesis of Zinc Oxide Nanoparticles Using *Catharanthus roseus* Leaves and Their Therapeutic Response in Breast Cancer (MDA-MB-231) Cells. *Nutrition and Cancer*, 74(4), pp.1489-1496.
- Barnes, R., Fagg, C. & Milton, S. 1997. *Acacia erioloba: monograph and annotated bibliography*. Oxford Forestry Institute, University of Oxford.
- Bhardwaj, K., Sharma, A., Tejwan, N., Bhardwaj, S., Bhardwaj, P., Nepovimova, E., Shami, A., Kalia, A., Kumar, A., Abd-Elsalam, K.A. and Kuča, K., 2020. Pleurotus macrofungi-assisted nanoparticle synthesis and its potential applications: a review. *Journal of Fungi*, 6(4), p.351.
- Coe, M. 1998. Some aspects of the interaction between mammalian herbivores and *Acacia erioloba* E. Mey. *Transactions of the Royal Society of South Africa*, 53(2), pp.141-147.
- Dauthal, P. and Mukhopadhyay, M., 2016. Noble metal nanoparticles: plant-mediated synthesis, mechanistic aspects of synthesis, and applications. *Industrial & Engineering Chemistry Research*, 55(36), pp.9557-9577.
- Dobrucka, R., Kaczmarek, M., Łagiedo, M., Kielan, A. & Długaszewska, J. 2019. Evaluation of biologically synthesized Au-CuO and CuO-ZnO nanoparticles against glioma cells and microorganisms. *Saudi Pharmaceutical Journal*, 27(3), pp.373-383.
- Dousti, B., Habibi, A. & Nabipour, F. 2021. Biosynthesis of zinc oxide nanoparticles using *Fumaria parviflora* extract and evaluation of their antibacterial and antioxidant activities. *BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology*, 102(1),
- Du Preez, I.C. & Mumbengegwi, D.R. 2014. Phytochemical investigation on Namibian plants for anti-malaria compounds. *Journal for studies in humanities and social sciences*, pp.147-158.
- Gao, Y., Arokia Vijaya Anand, M., Ramachandran, V., Karthikkumar, V., Shalini, V., Vijayalakshmi, S. & Ernest, D. 2019. Biofabrication of zinc oxide nanoparticles from *Aspergillus niger*, their antioxidant, antimicrobial and anticancer activity. *Journal of Cluster Science*, 30(4), pp.937-946.

- Gomes, H.I., Martins, C.S. and Prior, J.A., 2021. Silver nanoparticles as carriers of anticancer drugs for efficient target treatment of cancer cells. *Nanomaterials*, 11(4), p.964.
- Jaswal, V.S., Chaudhary, A., Thakur, P., Sharma, D., Arora, A.K., Khanna, R. & Tuli, H.S. 2019. ZnO nanoparticle with promising antimicrobial and antiproliferation synergistic properties. In. *Comprehensive Analytical Chemistry*. 87: Elsevier. pp. 251-262.
- Khan, M.M., Saadah, N.H., Khan, M.E., Harunsani, M.H., Tan, A.L. & Cho, M.H. 2019. Potentials of *Costus woodsonii* leaf extract in producing narrow band gap ZnO nanoparticles. *Materials Science in Semiconductor Processing*, 91, pp. 194-200.
- Khatak, S., Wadhwa, N. & Jain, P. 2021. Monometallic Zinc and Bimetallic Cu-Zn Nanoparticles Synthesis Using Stem Extracts of *Cissusquadrangularis* (Haddjod) and Proneness as Alternative Antimicrobial Agents. *Biosciences Biotechnology Research Asia*, 17(4), pp.763-774.
- Kumar, M.A., Ravikumar, C., Nagaswarupa, H., Purshotam, B., Gonfa, B., Murthy, H.A., ... Tadesse, S. 2019. Evaluation of bi-functional applications of ZnO nanoparticles prepared by green and chemical methods. *Journal of Environmental Chemical Engineering*, 7(6), p.103468.
- Kulkarni, S.A. and Feng, S.S., 2013. Effects of particle size and surface modification on cellular uptake and biodistribution of polymeric nanoparticles for drug delivery. *Pharmaceutical research*, 30, pp.2512-2522.
- Luque, P., Soto-Robles, C., Nava, O., Gomez-Gutierrez, C., Castro-Beltran, A., Garrafa-Galvez, H., ... Olivas, A. 2018. Green synthesis of zinc oxide nanoparticles using *Citrus sinensis* extract. *Journal of Materials Science: Materials in Electronics*, 29(12), pp. 9764-9770.
- Mallika, A., RamachandraReddy, A., SowriBabu, K. & Reddy, K.V. 2014. Synthesis and optical characterization of aluminum doped ZnO nanoparticles. *Ceramics International*, 40(8), pp.12171-12177.
- Maryanti, E., Damayanti, D. & Gustian, I. 2014. Synthesis of ZnO nanoparticles by hydrothermal method in aqueous rinds extracts of *Sapindus rarak* DC. *Materials Letters*, 118, pp.96-98.
- Materechera, E. & Materechera, S. 2001. Breaking dormancy to improve germination in seeds of *Acacia erioloba*. *South African Journal of Plant and Soil*, 18(4), pp.142-146.
- Mbenga, Y., Mthiyane, M., Botha, T.L., Horn, S., Pieters, R., Wepener, V. & Onwudiwe, D.C. 2022. Nanoarchitectonics of ZnO Nanoparticles Mediated by Extract of

- Tulbaghia violacea and Their Cytotoxicity Evaluation. *Journal of Inorganic and Organometallic Polymers and Materials*, pp.1-11.
- McMurdie, H.F., Morris, M.C., Evans, E.H., Paretzkin, B., Wong-Ng, W., Ettlinger, L. & Hubbard, C.R. 1986. Standard X-ray diffraction powder patterns from the JCPDS research associateship. *Powder Diffraction*, 1(2), pp. 64-77.
- Mishra, V., Mishra, R.K., Dikshit, A. and Pandey, A.C., 2014. Interactions of nanoparticles with plants: an emerging prospective in the agriculture industry. In *Emerging technologies and management of crop stress tolerance*, pp. 159-180. Academic press.
- Mthana, M.S., Mthiyane, D.M., Onwudiwe, D.C. & Singh, M. 2022. Biosynthesis of ZnO Nanoparticles Using Capsicum chinense Fruit Extract and Their In Vitro Cytotoxicity and Antioxidant Assay. *Applied Sciences*, 12(9), pp. 4451.
- Mutukwa, D., Taziwa, R. and Khotseng, L.E., 2022. A review of the green synthesis of ZnO nanoparticles utilising Southern African indigenous medicinal plants. *Nanomaterials*, 12(19), p.3456.
- Okoro, S., Kawo, A. & Arzai, A. 2012. Phytochemical screening, antibacterial and toxicological activities of Acacia senegal extracts. *Bayero Journal of Pure and Applied Sciences*, 5(1), pp.163-170.
- Palgrave, K.C. 2015. *Palgrave's trees of southern Africa*. Penguin Random House South Africa.
- Rajeshkumar, S., Kumar, S.V., Ramaiah, A., Agarwal, H., Lakshmi, T. & Roopan, S.M. 2018. Biosynthesis of zinc oxide nanoparticles using Mangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. *Enzyme and microbial technology*, 117, pp.91-95.
- Parveen, K., Banse, V. and Ledwani, L., 2016, April. Green synthesis of nanoparticles: Their advantages and disadvantages. In *AIP conference proceedings*. 1724, (1). AIP Publishing.
- Phukan, M.M., Sangma, S.R., Kalita, D., Pankaj, P.P., Das, P.P., Bora, P., Saha, J., Manoj, K., Hazarika, N. and Kataki, R., 2023. Next-generational biosurfactant and their practical application in the food industry. In *Applications of Next Generation Biosurfactants in the Food Sector*, pp. 361-389. Academic Press.
- Ramesh, P., Saravanan, K., Manogar, P., Johnson, J., Vinoth, E. & Mayakannan, M. 2021. Green synthesis and characterization of biocompatible zinc oxide nanoparticles and evaluation of its antibacterial potential. *Sensing and Bio-Sensing Research*, 31:100399.

- Ravichandrika, K., Kiranmayi, P. & Ravikumar, R. 2012. Synthesis, characterization and antibacterial activity of ZnO nanoparticles. *International Journal of Pharmacy Sciences*, 4,
- Ray, P. 2021. Curing Cancer with Nanotherapy Continues to be an Elusive Goal. *UMBC Faculty Collection*,
- Regulacio, M.D., Yang, D.P. and Ye, E., 2020. Toward greener methods of producing branched metal nanostructures. *CrystEngComm*, 22(3), pp.399-411.
- Roopan, S.M., Surendra, T.V., Elango, G. & Kumar, S.H.S. 2014. Biosynthetic trends and future aspects of bimetallic nanoparticles and its medicinal applications. *Applied microbiology and biotechnology*, 98(12), pp. 5289-5300.
- Sagadevan, S., Imteyaz, S., Murugan, B., Anita Lett, J., Sridewi, N., Weldegebrieal, G.K., Fatimah, I. and Oh, W.C., 2022. A comprehensive review on green synthesis of titanium dioxide nanoparticles and their diverse biomedical applications. *Green Processing and Synthesis*, 11(1), pp.44-63.
- Sagar Raut, D. & Thorat, R. 2015. Green synthesis of zinc oxide (ZnO) nanoparticles using *OcimumTenuiflorum* leaves. *International journal of science and research*, 4(5), pp.1225-1228.
- Şahin, B., Aydin, R., Soylu, S., Türkmen, M., Kara, M., Akkaya, A., Çetin, H. and Ayyıldız, E., 2022. The effect of thymus syriacus plant extract on the main physical and antibacterial activities of ZnO nanoparticles synthesized by SILAR method. *Inorganic Chemistry Communications*, 135, p.109088.
- Schulze, E.-D., Caldwell, M.M., Canadell, J., Mooney, H.A., Jackson, R.B., Parson, D., ... Trimborn, P. 1998. Downward flux of water through roots (ie inverse hydraulic lift) in dry Kalahari sands. *Oecologia*, 115(4), pp.460-462.
- Šebesta, M., Kolenčík, M., Sunil, B.R., Illa, R., Mosnáček, J., Ingle, A.P. and Urík, M., 2021. Field application of ZnO and TiO₂ nanoparticles on agricultural plants. *Agronomy*, 11(11), p.2281.
- Semenya, S.S. & Maroyi, A. 2018. Ethnomedicinal Uses of Fabaceae Species for Respiratory Infections and Related Symptoms in the Limpopo Province, South Africa. *Journal of Pharmacy and Nutrition Sciences*, 8(4), pp.219-229.
- Shaik, M.R., Khan, M., Kuniyil, M., Al-Warthan, A., Alkhathlan, H.Z., Siddiqui, M.R.H., ... Khan, M. 2018. Plant-extract-assisted green synthesis of silver nanoparticles using *Origanum vulgare* L. extract and their microbicidal activities. *Sustainability*, 10(4), p.913.

- Shobha, N., Nanda, N., Giresha, A.S., Manjappa, P., Sophiya, P., Dharmappa, K. & Nagabhushana, B. 2019. Synthesis and characterization of Zinc oxide nanoparticles utilizing seed source of *Ricinus communis* and study of its antioxidant, antifungal and anticancer activity. *Materials Science and Engineering: C*, 97, pp.842-850.
- Stan, M., Popa, A., Toloman, D., Dehelean, A., Lung, I. & Katona, G. 2015. Enhanced photocatalytic degradation properties of zinc oxide nanoparticles synthesized by using plant extracts. *Materials Science in Semiconductor Processing*, 39, pp.23-29.
- Suthar, J.K., Vaidya, A. and Ravindran, S., 2023. Toxic implications of silver nanoparticles on the central nervous system: A systematic literature review. *Journal of Applied Toxicology*, 43(1), pp.4-21.
- Suresh, J., Pradheesh, G., Alexramani, V., Sundrarajan, M. & Hong, S.I. 2018. Green synthesis and characterization of zinc oxide nanoparticle using insulin plant (*Costus pictus* D. Don) and investigation of its antimicrobial as well as anticancer activities. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 9(1):015008.
- Talam, S., Karumuri, S.R. & Gunnam, N. 2012. Synthesis, characterization, and spectroscopic properties of ZnO nanoparticles. *International Scholarly Research Notices*, 2012,
- Tetty, C.O. and Shin, H.M., 2019. Evaluation of the antioxidant and cytotoxic activities of zinc oxide nanoparticles synthesized using *Scutellaria baicalensis* root. *Scientific African*, 6, p.e00157.
- Thakur, N., Thakur, S., Chatterjee, S., Das, J. and Sil, P.C., 2020. Nanoparticles as smart carriers for enhanced cancer immunotherapy. *Frontiers in Chemistry*, 8, p.597806.
- Thema, F., Manikandan, E., Dhlamini, M. & Maaza, M. 2015. Green synthesis of ZnO nanoparticles via *Agathosma betulina* natural extract. *Materials Letters*, 161, pp.124-127.
- Timberlak, J., Fagg, C. & Barnes, R. 1999. *Field guide to the Acacias of Zimbabwe*. Department for International Development (DFID).
- Vaid, P., Raizada, P., Saini, A.K. and Saini, R.V., 2020. Biogenic silver, gold and copper nanoparticles-A sustainable green chemistry approach for cancer therapy. *Sustainable Chemistry and Pharmacy*, 16, p.100247.
- Vanathi, P., Rajiv, P., Narendhran, S., Rajeshwari, S., Rahman, P.K. & Venckatesh, R. 2014. Biosynthesis and characterization of phyto mediated zinc oxide nanoparticles: a green chemistry approach. *Materials Letters*, 134, pp.13-15.

VINOY JACOB, R.P. 2019. IN VITRO ANALYSIS: THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ZINC OXIDE NANOPARTICLES FROM CURCUMA LONGA. *IN VITRO*, 12(1),

Zeghoud, S., Hemmami, H., Seghir, B.B., Amor, I.B., Kouadri, I., Rebiai, A., Messaoudi, M., Ahmed, S., Pohl, P. and Simal-Gandara, J., 2022. A review on biogenic green synthesis of ZnO nanoparticles by plant biomass and their applications. *Materials Today Communications*, p.104747.

13. CHAPTER FOUR: BIOFORTIFICATION OF SUBSTRATES WITH BROILER LITTER

Determination of effects of biofortification of substrates with broiler litter and phyto-mediated-Nano-ZnO on oyster mushroom establishment growth and yield

4.1 Introduction

Mushroom is reported to have been cultivated dating back to the 1800s (Dinssa *et al.*, 2020). The need to cultivate mushrooms has been increasing as a result of population growth, market expansion, changes in consumer behaviour, and developments in the manufacturing industries, transportation, and retailing (Dinssa *et al.*, 2020). Oyster mushroom cultivation can help in managing organic waste whose disposal has been reported to have become a problem (Das & Mukherjee, 2007). These mushroom species are a rich source of protein (Sher *et al.*, 2010). *Pleurotus ostreatus* contain about 25-40% protein, high levels of vitamin B1, B2, B6, C, and D, and are rich in minerals that are essential for human health (Atikpo *et al.*, 2008). Oyster mushrooms can be cultivated using different plant substrates (Tesfay *et al.*, 2020). Deficiency of micronutrients such as Zn and Fe particularly in developing countries is reported to be widespread and affecting almost three billion people (Kumar *et al.*, 2019). Micronutrient deficiency which is also called 'hidden hunger' increases the general risk of infectious illnesses in people living in low-income countries (Kumar *et al.*, 2019). These have a negative impact on health performance and productivity and are a substantial impediment to economic progress (Manwaring *et al.*, 2016).

The nutritional content of oyster mushrooms is known to be influenced by the nutrient composition of the substrates they are grown in. It is therefore projected that the biofortification of substrates with micronutrients has the potential to improve the mineral nutritional content of oyster mushrooms. Biofortification is a technique that aims at increasing the nutrient content of edible parts, and endosperm while increasing their bioavailability (Shahzad *et al.*, 2014). Zn and Fe are important nutrients in the human diet and are known to boost the immune system and have anti-fungal properties (Maqbool & Beshir, 2019). Iron is particularly required in large amounts in the human body (Connorton *et al.*, 2019). The objective of this study was therefore to investigate the effect of bio-fortification of substrates with broiler litter and phyto-mediated-nano-ZnO on the growth and yield of oyster mushrooms.

4.2 Study objectives

4.2.1 Major objective

To investigate the effects of different broiler litter levels and nanoparticles on the establishment, growth, and yield of *Pleurotus ostreatus*.

4.2.2 Specific objectives

- i. To determine the effects of broiler litter and nanoparticles on the growth characteristics of *Pleurotus ostreatus*.
- ii. To determine the influence of broiler and phyto-mediated-nano-ZnO 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 broiler litter and phyto-mediated-nano-ZnO biofortified substrate to fruiting bodies.

4.3 Materials and methods

4.3.1 Description of the study area

The study was carried out at the North-West University, Molelwane farm, North West, South Africa with the geographical location is 25° 47' 54" South, 25° 32' 52" East. The African hut dome structure for mushrooms covered with a fabric cloth was used to create a growth environment for the oyster mushrooms. The study was conducted inside an enclosed environment where temperature and relative humidity were controlled.

4.3.2 Experimental layout

Broiler litter was mixed with 1 kg liver seed grass (*Urochloa panicoides*) at various ratios: 0% (no broiler litter), 1.25%, 2.5%, 5%, and 10%. These substrates were treated with the following concentrations of nanoparticle solutions: Control (liver seed grass), bulk ZnO (8 mg/kg), conventional or chemically synthesized Nano-ZnO (2 mg/kg), and green Nano-ZnO (2, 10, and 50 mg/kg). The experiment was laid into a 5 x 6 factorial experiment in a completely randomized design with 36 treatment combinations of 4 replicates each. Each replicate was 1 kg. Distilled water was used to wet the nano-treated substrates before inoculation with *P. ostreatus* spawn. Each experimental unit was filled into 10 × 23 cm autoclave

plastic bags. After cooling the substrates to room temperature, it was inoculated with oyster mushroom spawn. The experimental units were watered with 2 litres per hour which was supplied in the form of a drip by the water outlet of the scorpion irrigation system (Figure 4.1). Depending on the temperature variations irrigation schedule was not fixed. For temperatures less than 30 °C, irrigation took place in the two-day interval for two hours, and for temperatures above 30 °C the irrigation intervals and scheduling were subjected to change to a one-day interval.



Figure 4.1: Irrigation water outlet used to maintain relative humidity in the treatments.

4.3.1 Description of the experiment

4.3.1.1 Preparation of growth media

The test substrate, i.e., *U. panicoides* was dried at room temperature and milled. The dry substrates were weighed before wetting and the amount of substrate used was determined by the volume of the autoclave bags and their texture, i.e., *U. panicoides* (1 kg) and broiler litter (Siddhant *et al.*, 2013). Liver seed grass was sterilized using hot water in a drum at a temperature of 121 °C for 2 hours at 100kpa. After sterilization of the respective substrates, they were allowed to cool in sterilized containers for Four (4) hours. The following dosage of ZnO NPs treatment was used to treat the respective substrates: Control (un-treated), bulk ZnO (8 mg/kg), conventional or chemically synthesized Nano-ZnO (2 mg/kg), and green-nano-ZnO (2, 10, and 50 mg/kg) (Khandakar, 2004; Sarker & Chowdhury, 2013). Distilled water was used to wet the treated substrates before inoculation with *Pleurotus ostreatus* spawn.

4.3.1.2 Spawn preparation

Forty kilograms of spawn was purchased from Eco-Agro Enterprise (Pty) Ltd in Nelspruit, Mpumalanga. The ratio of spawn inoculation with substrates was 1:3 and a total spawn of 40 kg was used in all 120 experimental units (Siddhant *et al.*, 2013). For the 120 treatment combinations, a quantity of 250 grams was weighed and inoculated to each experimental unit.

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

Mushroom growth from different treatment combinations was evaluated by assessing mycelial development, fruiting body initiation (pinning) and total biomass production. The data on mycelial development was collected at seven-day intervals. The plastic cover of mycelial growth were measured as a centimetre cover within each of the autoclave bag designs. Data on pinning were also recorded at an interval of seven days (1 week). The pinning was measured by calculating the number of pinheads outside the plastic. The fruit bodies (fresh mass) were weighed immediately after each harvest using an electronic balance at two decimal places and recording the total biomass in grams (g) for each harvest.

4.3.1.4 Fruit cap diameter and style length

The fruit cap diameters and style lengths of all mushrooms were measured using a tape measure at each harvest on a 7-day interval.

4.3.1.5 Conversion efficiency ratio

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

$$\text{Conversion efficiency ratio} = \frac{\text{mushroom harvested (g)}}{\text{substrate (g)}} \times 100 \dots\dots\dots (4.1)$$

4.3.1.6 Temperature and relative humidity

Temperature (°C) and relative humidity (%) in the domes were recorded at an interval of two days from the first day of inoculation until harvesting day using a multi metre. This was done to determine the amount of water needed to maintain the recommended temperature and relative humidity.

4.3.1.7 Statistical analysis

The general linear model (GLM) procedure of Minitab (2000) was used to determine the effect of the inclusion level of broiler litter and the form of ZnO on the growth and yield of oyster mushrooms. The following GLM model was used:

$$Y_{ij} = \mu + B_i + Z_j + (B \times Z)_{ij} + E_{ij}$$

Where Y_{ij} = The response variable

μ = population mean

B_i = Effect of broiler litter level

Z_j = Effect of form of ZnO

$(B \times Z)_{ij}$ = Effect of interaction between broiler litter level and form of ZnO

E_{ij} - Random error associated with observation ij .

For all statistical tests, the significance will be accepted at $P \leq 0.05$.

4.4 Results

4.4.1 Effects of Broiler Litter X Nanoparticle Concentration on *Pleurotus ostreatus* mycelial development (autoclave bag cover) assessed over 5 weeks after inoculation.

4.4.1.1 Analysis of Variance on Mycelial Development

The data analysis on the effect of different treatment combinations on mycelial growth indicated that there was a statistically significant difference in different broiler litter levels ($P \leq 0.001$) but there was however non-significance in mycelium grown in NanoC treated substrates ($P \geq 0.05$) (Table 3).

Table 3 : Analysis of Variance of effects of broiler litter and nanoparticle concentration on mycelial development assessed over 5 weeks after inoculation

Source	DF	Mean SS	Adj MS	F	P- value
BL	4	177.25	44.31	4.08	0.004**
NanoC	5	62.97	12.59	1.16	0.336 ^{ns}
BL X NanoC	20	618.95	30.95	2.85	0.000***

P value indicates significance. ***-Significant at ($P \leq 0.001$), **-Significant at ($P \leq 0.01$), *-Significant at ($P \leq 0.05$) and ns-significant at ($P > 0.05$). BL- Broiler litter. NanoC- Nanoparticles concentration.

4.4.1.2 Effects of broiler litter X Nanoparticle concentration interaction on mycelial development assessed over 5 weeks after inoculation.

The Analysis of variance (Table 4) showed that there was a statistically significant effect of broiler litter X nano-type interaction on mycelial development. Mycelial development in the following treatment; 0 BL Bulk-ZnO, 0 BL Green-Nano-ZnO 2mg/kg, 0 BL Green-Nano-ZnO 50 mg/kg, and 1.25 BL Conv-Nano-ZnO 2 mg/kg exhibited statistically significant differences ($P \leq 0.05$).

Table 4.: Effects of different levels of broiler litter and nanoparticles on oyster mushroom mycelial development (cm)

Treatments		Mycelial development recorded in cm over time (weeks)				
BL	NanoC	week 1	week 2	week 3	week 4	week 5
0	Control	17.75 ^a	22 ^{ab}	24.5 ^a	26.2 ^a	26.75 ^a
0	Bulk-ZnO 8 mg/kg	16 ^{ab}	22.3 ^{ab}	24.5 ^a	26.00 ^a	26.25 ^{ab}
0	Conv-Nano-ZnO 2 mg/kg	8 ^{abc}	10 ^{c-g}	16 ^{a-e}	16.00 ^{a-d}	22.00 ^{abc}
0	Green-Nano-ZnO 2mg/kg	3.8 ^c	4 ^g	8 ^{de}	9.00 ^d	16.75 ^c
0	Green-Nano-ZnO 10mg/kg	8.5 ^a	14.5 ^{a-f}	18.8 ^{abc}	20.50 ^{ab}	23.25 ^{abc}
0	Green-Nano-ZnO 50mg/kg	15 ^{ab}	21.5 ^{ab}	23.5 ^{ab}	24.75 ^{ab}	26.50 ^a
1.25	Control	8.8 ^{abc}	11.8 ^{b-g}	15.3 ^{a-e}	18.50 ^{a-d}	19.75 ^{abc}
1.25	Bulk-ZnO 8 mg/kg	11 ^{abc}	13 ^{a-g}	19.3 ^{abc}	21.25 ^{ab}	22.25 ^{abc}
1.25	Conv-Nano-ZnO 2mg/kg	5 ^{bc}	9.8 ^{c-g}	13.3 ^{b-e}	17.00 ^{a-d}	17.25 ^{bc}
1.25	Green-Nano-ZnO 2mg/kg	7.3 ^{abc}	14 ^{a-g}	17.3 ^{a-e}	19.00 ^{a-d}	19.50 ^{abc}
1.25	Green-Nano-ZnO 10mg/kg	6.8 ^{abc}	8.3 ^{d-g}	13.8 ^{b-e}	14.75 ^{bcd}	25.00 ^{abc}

1.25	Green-Nano-ZnO 50mg/kg	10.5 ^{abc}	14.5 ^{a-f}	21.8 ^{abc}	22.00 ^{ab}	23.50 ^{abc}
2.5	Control	10 ^{abc}	12.5 ^{a-g}	17.3 ^{a-e}	18.75 ^{a-d}	21.00 ^{abc}
2.5	Bulk-ZnO 8 mg/kg	7.8 ^{abc}	10 ^{c-g}	15 ^{a-e}	16.75 ^{a-d}	17.75 ^{abc}
2.5	<i>Conv-Nano-ZnO</i> 2mg/kg	5.3 ^{bc}	6.8 ^{fg}	7.8 ^e	9.75 ^{cd}	21.50 ^{abc}
2.5	Green-Nano-ZnO 2mg/kg	9.8 ^{abc}	14.5 ^{a-f}	18.5 ^{a-d}	20.00 ^{abc}	24.00 ^{abc}
2.5	Green-Nano-ZnO 10mg/kg	5 ^{bc}	10 ^{c-g}	17.5 ^{a-e}	18.50 ^{a-d}	19.50 ^{abc}
2.5	Green-Nano-ZnO 50mg/kg	9 ^{abc}	16.3 ^{a-f}	17.5 ^{a-e}	19.75 ^{abc}	22.25 ^{abc}
5	Control	5.8 ^{bc}	7.8 ^{efg}	11.3 ^{cde}	15.00 ^{bcd}	21.25 ^{abc}
5	Bulk-ZnO 8 mg/kg	9.5 ^{abc}	14 ^{a-g}	15.5 ^{a-e}	20.00 ^{abc}	23.00 ^{abc}
5	<i>Conv-Nano-ZnO</i> 2mg/kg	10 ^{abc}	17.3 ^{a-e}	19.3 ^{abc}	20.75 ^{ab}	23.50 ^{abc}
5	Green-Nano-ZnO 2mg/kg	13.8 ^{abc}	19 ^{abc}	22 ^{ab}	24.25 ^{ab}	24.75 ^{abc}
5	Green-Nano-ZnO 10mg/kg	10.5 ^{abc}	13.5 ^{a-g}	19.5 ^{abc}	21.75 ^{ab}	23.50 ^{abc}
5	Green-Nano-ZnO 50mg/kg	5.3 ^{bc}	18.5 ^{a-d}	21.3 ^{abc}	21.50 ^{ab}	24.25 ^{abc}
10	Control	13 ^{abc}	18.5 ^{a-d}	21.3 ^{abc}	22.50 ^{ab}	23.75 ^{abc}

10	Bulk-ZnO 8 mg/kg	8.5 ^{abc}	9 ^{c-g}	13.5 ^{b-e}	15.75 ^{a-d}	20.25 ^{abc}
10	<i>Conv-Nano-ZnO</i> 2mg/kg	6.3 ^{bc}	14 ^{a-g}	21.8 ^{abc}	22.75 ^{ab}	25.00 ^{abc}
10	Green-Nano-ZnO 2mg/kg	8.3 ^{abc}	12.8 ^{a-g}	19 ^{abc}	20.50 ^{ab}	25.75 ^{abc}
10	Green-Nano-ZnO 10mg/kg	12.8 ^{abc}	16.8 ^{a-f}	20.3 ^{abc}	23.50 ^{ab}	25.50 ^{abc}
10	Green-Nano-ZnO 50mg/kg	9.3 ^{abc}	12 ^{a-g}	14.5 ^{a-e}	16.25 ^{a-d}	22.25 ^{abc}
Pooled SEM		1.04	0.95	0.96	0.98	0.82
P-values	Broiler litter	P≤0.05	P=0.001	P<0.05	P<0.05	P< 0.01
	NanoC	P≤0.05	P<0.01	P<0.05	P>0.05	P>0.05
	Broiler litter X NanoC	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Mean with different letters in a row are significantly different at $P \leq 0.05$ via Tukey's means separation. NanoC- Nanoparticle concentrations. Reported values are averages of three replications. Significance: ns-not significant at $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, or *** $P \leq 0.001$, respectively.

4.4.2 Effect of broiler litter levels and nanoparticle concentration on pinning rate of *Pleurotus ostreatus* (autoclave bag cover)

4.4.2.1 Analysis of variance on pinning rate

Nanoparticle concentration and nanoparticle and broiler litter showed a statistical difference in pinning rate at ($P=0.001$) in Table 5

Table 5: Analysis of Variance of effects of broiler litter and nanoparticle concentration on pinning rate over 5 weeks after inoculation

Source	DF	Seq SS	Adj MS	F	P-value
BL	4	1.6667	0.4167	2.21	0.075 ^{ns}
NanoC	5	4.0667	0.8133	4.31	0.001 ^{***}
BL X NanoC	20	9.9333	0.4967	2.63	0.001 ^{***}

P value indicates significance. ***-Significant at ($P\leq 0.001$), **-Significant at ($P\leq 0.01$), *-Significant at ($P\leq 0.05$) and ns-significant at ($P>0.05$). BL- Broiler litter. NanoC- Nanoparticles concentration.

4.4.2 Effect of 'broiler litter X Nano type' interaction on pinning rate assessed over 6 weeks after inoculation.

There was a variation in the pinning rate in some of the respective treatments at 5 weeks after inoculation, 0 BL control had the highest pinning from week 1 to week 2 and week 4 to week 5, and 10 BL had the highest pinning on week 3, and week 4. Overall, 0 BL control had the highest pinning for 5 weeks. Treatment combination 0 BL control had a statistically significantly higher pinning rate than all other treatment combinations ($P\leq 0.05$) (Table 6)

Table 6: Effects of different levels of broiler litter and nanoparticle concentration on oyster mushroom pinning rate.

Treatments		Mean of pinheads recorded over time (weeks)					
BL	NanoC	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
0	Control	2.50 ^a	2.50 ^a	0.00	1.75 ^a	1.75 ^a	0.25 ^a
0	Bulk-ZnO 8 mg/kg	0	0.00	2.7 ^a	0.00	0.25 ^b	0.00
0	Conv-Nano-ZnO 2mg/kg	0.00	0.00	0.00	0.00	0.00	0.00
0	Green-Nano-ZnO 2mg/kg	0.00	0.00	0.00	0.00	0.00	0.00
0	Green-Nano-ZnO	0.00	0.00	0.50 ^a	0.00	0.00	0.00

0	10mg/kg Green-Nano-ZnO	0.00	0.00	0.00	0.00	0.25 ^b	0.00
1.25	50mg/kg Control	0.00	0.00	0.00	0.00	0.25 ^b	0.00
1.25	Bulk-ZnO 8 mg/kg	0.00	0.00	0.00	0.00	0.00	0.00
1.25	Conv-Nano-ZnO	0.00	0.00	0.00	0.00	0.00	0.00
1.25	2mg/kg Green-Nano-ZnO	0.00	0.00	0.00	0.00	0.00	0.00
1.25	2mg/kg Green-Nano-ZnO	0.00	0.00	0.00	0.00	0.00	0.00
1.25	10mg/kg Green-Nano-ZnO	0.00	0.00	0.75 ^a	0.00	0.00	0.00
2.5	50mg/kg Control	0.00	0.00	0.00	0.00	0.00	0.00
2.5	Bulk-ZnO 8 mg/kg	0.00	0.00	0.00	0.50 ^{ab}	0.00	0.00
2.5	Conv-Nano-ZnO	0.00	0.00	0.00	0.00	0.00	0.00
2.5	2mg/kg Green-Nano-ZnO	0.00	0.00	0.00	0.75 ^{ab}	0.00	0.00
2.5	2mg/kg Green-Nano-ZnO	0.0	0.00	0.00	0.00	0.00	0.00
2.5	10mg/kg Green-Nano-ZnO	0.00	0.00	0.00	0.50 ^{ab}	0.50 ^b	0.50 ^a
5	50mg/kg Control	0.00	0.00	0.00	0.50 ^{ab}	0.00	0.00
5	Bulk-ZnO 8 mg/kg	0.00	0.00	0.00	0.00	0.00	0.00
5	Conv-Nano-ZnO	0.00	0.00	0.25 ^a	0.00	0.00	0.00
5	2mg/kg Green-Nano-ZnO	1.25 ^b	1.25 ^b	0.75 ^a	0.00	0.75 ^{ab}	0.25 ^a
5	2mg/kg Green-Nano-ZnO	1.00 ^b	0.50 ^b	2.50 ^a	0.25 ^b	0.00	0.25 ^a
5	10mg/kg Green-Nano-ZnO	0.00	0.00	0.50 ^a	0.50 ^{ab}	0.00	0.25 ^a
10	50mg/kg Control	0.00	0.00	0.75 ^a	0.25 ^b	0.75 ^{ab}	1.00 ^a
10	Bulk-ZnO 8 mg/kg	0.00	0.00	0.00	0.00	0.00	0.00
10	Conv-Nano-ZnO	0.00	0.00	0.00	0.00	0.00	0.00
10	2mg/kg Green-Nano-ZnO	0.00	0.00	0.00	0.00	0.00	0.00
10	2mg/kg Green-Nano-ZnO	0.00	0.00	0.50 ^a	0.00	0.25 ^b	0.00
10	10mg/kg Green-Nano-ZnO	0.00	0.00	0.00 ^a	0.50 ^{ab}	0.00	0.00
10	50mg/kg Green-Nano-ZnO	0.00	0.00	0.00 ^a	0.50 ^{ab}	0.00	0.00
Pooled SEM		0.09	0.09	0.28	0.13	0.11	0.09
P-values	Broiler litter	P<0.001	P<0.001	P>0.05	P>0.05	P>0.05	P>0.05
	NanoC	P<0.001	P<0.001	P>0.05	P>0.05	P=0.001	P>0.05
	Broiler litter X NanoC	P<0.001	P<0.001	P>0.05	P<0.01	P=0.001	P>0.05

Mean with different letters in a row are significantly different at $P \leq 0.05$. Significance: $P > 0.05$ = not significant, * $P \leq 0.05$, ** $P \leq 0.01$ or *** $P \leq 0.001$, respectively using Tukey's means separation procedure. NanoC- Nanoparticle concentrations. Reported values are averages of three replications.

4.4.3 Effect of different broiler litter levels and nanoparticles on total biomass of *Pleurotus ostreatus*

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

The Results of the analysis of variance (Table 7) showed that there was a statistically significant difference in the effect of different substrates on the fresh weight ($P \leq 0.05$). However, there was no significant difference in the effect of the broiler litter X Nano-type interaction.

Table 7: Analysis of Variance on Total Biomass on *Pleurotus ostreatus* for over 5 weeks after inoculation

Source	DF	Seq SS	Adj SS	F	P
BL	4	119.17	29.79	0.88	0.482
NanoC	5	294.19	58.84	1.73	0.136
BL X NanoC	20	691.11	34.56	1.02	0.452

P value indicates significance. ***-Significant at ($P \leq 0.001$), **-Significant at ($P \leq 0.01$), *-Significant at ($P \leq 0.05$) and ns-gnificant at ($P > 0.05$). BL- Broiler litter. NanoC- Nanoparticles concentration.

4.4.3.2 Effect of different Broiler litter X NanoC on the total biomass of *Pleurotus ostreatus* fruiting bodies over a period of 5 weeks after inoculation

The fresh weight and dry weight of fruiting bodies harvested was not statistically significantly.

Table 8 : Effect of different levels of broiler litter and nanoparticles on oyster mushroom fruiting bodies fresh weight (g)

Treatments		Time				
BL	NanoC	FW 1	FW 2	FW3	FW4	FW5
0	Control	28.8 ^a	0	7.1 ^a	7.1 ^a	2.1 ^a
0	Bulk-ZnO 8 mg/kg	0	7.7 ^a	0	0	0

0	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
0	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
0	Green-Nano-ZnO 10mg/kg	0	3.3 ^a	0	0	0
0	Green-Nano-ZnO 50mg/kg	0	0	7 ^a	7 ^a	0
1.25	Control	0	0	0	0	0
1.25	Bulk-ZnO 8 mg/kg	0	0	0	0	0
1.25	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
1.25	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
1.25	Green-Nano-ZnO 10mg/kg	0	0	0	0	0
1.25	Green-Nano-ZnO 50mg/kg	0	0	0	0	0
2.5	Control	0	0	0	0	0
2.5	Bulk-ZnO 8 mg/kg	0	0	0	0	0
2.5	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
2.5	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
2.5	Green-Nano-ZnO 10mg/kg	0	0	0.9 ^a	0.9 ^a	0
2.5	Green-Nano-ZnO 50mg/kg	0	15.8 ^a	0	0	14.2 ^a
5	Control	0	17.5 ^a	4.4 ^a	4.4 ^a	0
5	Bulk-ZnO 8 mg/kg	0	0	0	0	0
5	Conv-Nano-ZnO 2mg/kg	0	4.5a	0	0	0
5	Green-Nano-ZnO 2mg/kg	35.2 ^a	24.7 ^a	11.9a	0	5.6a
5	Green-Nano-ZnO 10mg/kg	3.7 ^b	14.5 ^a	8.5 ^a	0	0

5	Green-Nano-ZnO 50mg/kg	0	16 ^a	26.5 ^a	8.5 ^a	8 ^a
10	Control	0	16.1 ^a	0	26.5 ^a	3.6 ^a
10	Bulk-ZnO 8 mg/kg	0	0	0	0	0
10	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
10	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
10	Green-Nano-ZnO 10mg/kg	0	0	0	0	0
10	Green-Nano-ZnO 50mg/kg	0	0	8.2 ^a	14.4 ^a	0
Pooled		1.9	3.7	2.9	3	1.5
SEM						
P-values	Broiler litter	P<0.01	P<0.05	P<0.05	P>0.05	P>0.05
	NanoC	P<0.01	P>0.05	P>0.05	P>0.05	P>0.05
	Broiler litter X NanoC	P<0.001	P>0.05	P>0.05	P>0.05	P>0.05

Mean with different letters in a row are significantly different at $P \leq 0.05$. NanoC- Nanoparticle concentrations. Reported values are averages of Four replications. Significance: $P > 0.05$ is not significant, * $P \leq 0.05$, ** $P \leq 0.01$ or *** $P \leq 0.001$, respectively. Means followed by different letters within each column are significantly different at $P \leq 0.05$ using Tukey's means separation procedure. FW- fresh weight. FW1-FW5 (fresh weight from first flush to last flush).

4.4.4 Effect of nanoparticles and broiler litter on the growth characteristics of *Pleurotus ostreatus*

4.4.4.1 Analysis of variance on fruit style length of *Pleurotus ostreatus* fruiting bodies

There was statistical significance difference between BL and NanoC on fruit cap diameter and style length ($P > 0.05$) (Tables 9 and 10)

Table 9: Analysis of Variance in Fruit Style Length on *Pleurotus ostreatus* for over 5 weeks after inoculation

Source	DF	Seq SS	Adj MS	F	P
BL	4	2.367	0.592	1.45	0.224 ^{ns}
NanoC	5	3.975	0.795	1.95	0.094 ^{ns}
BL X NanoC	20	14.233	0.712	1.74	0.040*

P value indicates significance. ***-Significant at ($P \leq 0.001$), **-Significant at ($P \leq 0.01$), *-Significant at ($P \leq 0.05$) and ns-significant at ($P > 0.05$). BL- Broiler litter. NanoC- Nanoparticles concentration.

Table 10 : Analysis of Variance of the effect of broiler litter and nanoparticle concentration on cap diameter over a period of 5 weeks

Source	DF	Seq SS	Adj MS	F	P
BL	4	7.717	1.929	1.26	0.291
NanoC	5	15.842	3.168	2.07	0.076
BL X NanoC	20	48.283	2.414	1.58	0.076

P value indicates significance. ***-Significant at ($P \leq 0.001$), **-Significant at ($P \leq 0.01$), *-Significant at ($P \leq 0.05$) and ns-significant at ($P > 0.05$). BL- Broiler litter. NanoC- Nanoparticles concentration.

4.4.4.2 Fruit cap and style length

There was no statistically significant difference in fruit cap diameter for mushrooms grown in different substrates. There was, however, a significant effect of interaction between “broiler litter levels and nanoparticle” concentration ($P \leq 0.05$) on week 1. In general, liver seed grass biofortified with broiler litter produced larger fruit caps than those produced by nanoparticle-treated substrates.

Table 11: Effect of different levels of broiler litter and nanoparticle on cap diameter of oyster mushroom at each harvest (cm)

Treatments	Cap diameter (cm)

BL	NanoC	Cap1	Cap2	Cap3	Cap4	Cap5
0	Control	8 ^a	0	4.9 ^a	4.9 ^a	1.3 ^a
0	Bulk-ZnO 8 mg/kg	0	4.5 ^a	0	0	0
0	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
0	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
0	Green-Nano-ZnO 10mg/kg	0	1.3 ^a	0	0	0
0	Green-Nano-ZnO 50mg/kg	0	0	1.3 ^{ab}	1.3 ^{ab}	0
1.25	Control	0	0	0	0	0
1.25	Bulk-ZnO 8 mg/kg	0	0	0	0	0
1.25	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
1.25	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
1.25	Green-Nano-ZnO 10mg/kg	0	0	0	0	0
1.25	Green-Nano-ZnO 50mg/kg	0	0	0	0	0
2.5	Control	0	0	0	0	0
2.5	Bulk-ZnO 8 mg/kg	0	0	0	0	0
2.5	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
2.5	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
2.5	Green-Nano-ZnO 10mg/kg	0	0	0	0	0
2.5	Green-Nano-ZnO 50mg/kg	0	2.8 ^a	0	0	0
5	Control	0	2.5 ^a	2.8 ^{ab}	2.8 ^{ab}	0
5	Bulk-ZnO 8 mg/kg	0	0	0	0	0

5	Conv-Nano-ZnO 2mg/kg	0	1.5 ^a	0	0	0
5	Green-Nano-ZnO 2mg/kg	1.8 ^b	2 ^a	2 ^{ab}	2 ^{ab}	2.3 ^a
5	Green-Nano-ZnO 10mg/kg	0	3.8 ^a	2 ^{ab}	2 ^{ab}	0
5	Green-Nano-ZnO 50mg/kg	1.5 ^b	2 ^a	1.8 ^{ab}	1.8 ^{ab}	1.8 ^a
10	Control	0	1.3 ^a	0	1.3 ^{ab}	0.8 ^a
10	Bulk-ZnO 8 mg/kg	0	0	0	0	0
10	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
10	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
10	Green-Nano-ZnO 10mg/kg	0	0	0	0	0
10	Green-Nano-ZnO 50mg/kg	0	2 ^a	1.5 ^{ab}	1.5 ^{ab}	0
Pooled SEM	SEM	0.26	0.55	0.42	0.43	0.31
P-values	Broiler litter	P<0.001	P<0.05	P<0.01	P<0.05	P>0.05
	NanoC	P<0.001	P>0.05	P<0.05	P<0.01	P>0.05
	Broiler litter X NanoC	P<0.001	P>0.05	P>0.05	P>0.05	P>0.05

Cap- Cap diameter. NanoC- Nanoparticle concentrations. Reported values are averages of four replications. Significance: P>0.05 is non-significant, * P ≤0.05, ** P ≤ 0.01 or *** P ≤0.001, respectively. Means followed by different letters within each column are significantly different at P ≤0.05 via Tukey's means separation procedure. Cap1 to Cap5 is the cap diameter from the first flush to the last flush.

Table 12 : Effects of different levels of broiler litter and nanoparticles on pleurotus ostreatus style length at each harvest (cm)

Treatments		Style length (cm)				
BL	NanoC	STYL1	STYL2	STYL3	STYL4	STYL5
0	Control	4.3 ^a	0	2.5 ^a	2.5 ^a	1 ^a
0	Bulk-ZnO 8 mg/kg	0	2.3 ^a	0	0	0
0	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
0	Green-Nano-ZnO 2mg/kg	0	0	0	0	0

0	Green-Nano-ZnO 10mg/kg	0	1.8 ^a	0	0	0
0	Green-Nano-ZnO 50mg/kg	0	0	1.3 ^a	1.3 ^a	0
1.25	Control	0	0	0	0	0
1.25	Bulk-ZnO 8 mg/kg	0	0	0	0	0
1.25	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
1.25	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
1.25	Green-Nano-ZnO 10mg/kg	0	0	0	0	0
1.25	Green-Nano-ZnO 50mg/kg	0	0	0	0	0
2.5	Control	0	0	0	0	0
2.5	Bulk-ZnO 8 mg/kg	0	0	0	0	0
2.5	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
2.5	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
2.5	Green-Nano-ZnO 10mg/kg	0	0	0	0	0
2.5	Green-Nano-ZnO 50mg/kg	0	2.3 ^a	0	0	0
5	Control	0	2.5 ^a	1.3 ^a	1.3 ^a	0
5	Bulk-ZnO mg/kg	0	0	0	0	0
5	Conv-Nano-ZnO 2mg/kg	0	1 ^a	0	0	0
5	Green-Nano-ZnO 2mg/kg	1.5 ^b	2.3 ^a	1 ^a	1 ^a	1.5 ^a
5	Green-Nano-ZnO 10mg/kg	0	3 ^a	2 ^a	2 ^a	0
5	Green-Nano-ZnO 50mg/kg	1 ^b	1.3 ^a	2.5 ^a	2.5 ^a	1 ^a
10	Control	0	1.3 ^a	0.8 ^a	0.8 ^a	0.5 ^a
10	Bulk-ZnO mg/kg	0	0	0	0	0
10	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0
10	Green-Nano-ZnO 2mg/kg	0	0	0	0	0
10	Green-Nano-ZnO 10mg/kg	0	0	0	0	0
10	Green-Nano-ZnO 50mg/kg	0	1.3 ^a	1 ^a	1 ^a	0
Pooled SEM	SEM	0.17	0.43	0.36	0.36	0.16
P-values	Broiler litter	P<0.001	P<0.01	P<0.05	P<0.05	P>0.05
	NanoC	P<0.001	P>0.05	P>0.05	P>0.05	P>0.05
	Broiler litter X NanoC	P<0.001	P>0.05	P>0.05	P>0.05	P<0.05

STYL- Style length. The STYL1 to STYL5 is the style length from the first flush to the last flush. Reported values are averages of four replications. Significance: P>0.05=not

significant, * $P \leq 0.05$, ** $P \leq 0.01$ or *** $P \leq 0.001$, respectively. Means followed by different letters within each column are significantly different at $P \leq 0.05$ via Tukey's means separation procedure.

4.4.5 Conversion efficiency ratio

The treatment combination of '5 BL Green-Nano-ZnO 2 mg/kg' had the highest conversion ratio of 51.61%, followed by '5 BL Green-Nano-ZnO 50 mg/kg' with 33.59% and 0 BL control at 25.35%. The lowest biological efficiency was obtained from 2.5 BL Green-Nano-ZnO 10 mg/kg at 0.58 %.

Table 13: Effect of different levels of broiler litter and nanoparticle concentration treatment combinations on oyster mushroom conversion efficiency

Treatment combinations		Spawn (g)	Substrate (g)	Harvested mushroom (g)	Conversion efficiency (%)
BL	Nanoparticle concentration				
0	Control	200	600	152.08	25.35
0	Bulk-ZnO 8mg/kg	200	600	30.95	5.16
0	Conv-Nano-ZnO 2mg/kg	200	600		0
0	Green-Nano-ZnO 2mg/kg	200	600		0
0	Green-Nano-ZnO 10mg/kg	200	600	13.21	2.20
0	Green-Nano-ZnO 50mg/kg	200	600	27.68	4.61
1.25	Control	200	600		0
1.25	Bulk-ZnO 8 mg/kg	200	600		0
1.25	Conv-Nano-ZnO 2mg/kg	200	600		0
1.25	Green-Nano-ZnO 2mg/kg	200	600		0
1.25	Green-Nano-ZnO 10mg/kg	200	600		0
1.25	Green-Nano-ZnO 50mg/kg	200	600		0
2.5	Control	200	600		0
2.5	Bulk-ZnO 8 mg/kg	200	600		0
2.5	Conv-Nano-ZnO 2mg/kg	200	600		0
2.5	Green-Nano-ZnO 2mg/kg	200	600		0
2.5	Green-Nano-ZnO 10mg/kg	200	600	3.45	0.58
2.5	Green-Nano-ZnO 50mg/kg	200	600	63.11	10.52
5	Control	200	600	87.86	14.64
5	Bulk-ZnO 8 mg/kg	200	600		0
5	Conv-Nano-ZnO 2mg/kg	200	600	17.99	3.0
5	Green-Nano-ZnO 2mg/kg	200	600	309.63	51.61
5	Green-Nano-ZnO 10mg/kg	200	600	107.1	17.85
5	Green-Nano-ZnO 50mg/kg	200	600	201.55	33.59
10	Control	200	600	78.83	13.14

10	Bulk-ZnO 8 mg/kg	200	600		0
10	Conv-Nano-ZnO 2mg/kg	200	600		0
10	Green-Nano-ZnO 2mg/kg	200	600		0
10	Green-Nano-ZnO 10mg/kg	200	600		0
10	Green-Nano-ZnO 50mg/kg	200	600	32.6	5.43

BL- broiler litter treatments. NanoC- nanoparticle concentrations.

4.5 Discussion

The time it took for the liver seed grass biofortified with both broiler litter and nanoparticle concentration to fully colonize ranged from 30 to 90 days. On the 0% BL control, the shortest intervals (30 days) were recorded (Table 4). The longest duration to complete colonization was found to be 80 days. However, the following treatments '0% BL Green-Nano-ZnO 2 mg/kg', 'conv-Nano-ZnO' and '2.5 BL ZnO 8 mg/kg' failed to fully colonize. Looking at the growth patterns in Table 4, there was a progressive drop in mycelial development when the broiler litter level was increased from 0% to 5, and then 10%, since the lowest mycelial development occurred at 10% BL and 5% BL. The proliferation of mycelia in the substrate is an important aspect of mushroom production. In this study, 0 BL (control) had the highest mycelial growth rate since it required the least amount of time to complete a spawning run compared to the other substrate treatments studied. This fast mycelial running rate might be due to liver seed grass having a greater C: N ratio, which favoured rapid mycelial development (Gregori *et al.*, 2007;Tisdale, 2004). .

The difference in pinning rate was statistically significant at $P \leq 0.001$ for nanoparticle concentration (Table 5). The lack of a significant difference in BL might be attributed to the presence of bacteria in broiler litter and ZnO NPs as they also pose antifungal effects. Almost 80% of the treatments did not result in pinning formation. The 0 BL control had the quickest pinning rate for flush 1. All flush 1 pinning times were much longer than flushes 2, 3, 4, and 5 pinning times (Table 6). Considering the production of primordia or pinheads is closely tied to the availability of carbon and nitrogen (C: N) from the lignocellulose substrate (Narain *et al.*, 2008), the decrease in pinning rate might be ascribed to a reduction in carbon and nitrogen. For flush 2, the lowest pinning was on 5 BL Conv-Nano-ZnO 2 mg/kg and the highest was 5 Green-Nano-ZnO 2 mg/kg. In flush 3, the lowest pinning was on 2.5 BL Green-Nano-ZnO 10 mg/kg and the highest pinning was on 5 BL Green-Nano-ZnO 50 mg/kg. For the lowest pinning formation, humidity and CO₂ levels might have contributed to poor pinhead development. Temperature is a key factor in

pinhead development. Pinhead evolution failed in these treatments because the temperatures were not optimal, and the relative humidity was extremely low during primordia development. During primordia production, relative humidity must be more than 90% (Tesfaw *et al.*, 2015).

The '0 BL control' had the largest cap diameter and style length, while the 10 BL control had the lowest (Tables 11 and 12), the presence of nitrogen in broiler litter may have resulted in a short cap diameter and style length. The size of cap diameter and style length decreased as broiler litter increased respectively among other substrates, in a comparable habitat and cultural behaviours. There was no statistical difference in cap diameter as well as style length between BL and NanoC. The cap diameter and style length are the growth characteristics of oyster mushrooms. The 5 Green-Nano-ZnO 50 mg/kg acquired cap diameter and style length would not match market criteria because of their modest size.

The impact of varied broiler litter levels and nanoparticle concentrations on overall *Pleurotus ostreatus* yield was low. Treatment '5 BL Green-Nano-ZnO 2 mg/kg' resulted in the lowest yield. This means that mushrooms grown on substrates containing 5 BL Green-Nano-ZnO 2 mg/kg agricultural waste absorbed available nutrients more efficiently than mushrooms cultivated in 2.5 BL Green-Nano-ZnO 10 mg/kg and 0 BL Green-Nano-ZnO 10 mg/kg. As a result, the substrate containing 5 BL Green-Nano-ZnO 2 mg/kg had a higher percentage of biological efficiency, which may have resulted in nanoparticles and greater litter levels. At a certain level, nanoparticles are antifungal, and broiler litter has a high nitrogen concentration, which can reduce oyster mushroom output. The statistical analysis reveals that broiler litter and nanoparticle concentration (Table 8) did not affect the overall yield of mushrooms produced from the various treatments. There is a positive association between BE and cellulose and hemicellulose degradation, whereas a negative relationship between BE and lignin degradation was discovered. In terms of yield and BE, the substrate treatments 0 BL control and 5 BL Green-Nano-ZnO 2 mg/kg appear to be more favourable for mushroom production than other substrates. The term biological efficiency refers to the efficiency with which substrates are converted into fruiting bodies. BE value should be greater than 50% from an economic standpoint (Sardar *et al.*, 2016; Wiafe-Kwagyan *et al.*, 2023). Only 5 BL Green-Nano-ZnO 2mg/kg achieved a 50% BE level in this investigation since this substrate produced the most fruiting bodies in each packet (Table 13). Oyster mushrooms grown on a 2.5 BL Green-Nano-ZnO 10 mg/kg substrate have a substantially lower BE.

The size of the mushroom is determined by substrates low in cellulose, hemicelluloses, and lignin, which act as physical barriers and are difficult to break down in the absence of lignin-degrading enzymes. The changes in quality traits seen may be related to variances in the pace of growth and development of the pins, where rapid growth may have limited the mushroom caps' exposure to insects and other diseases.

4.6 Conclusion

In terms of yield, 5 BL Green-Nano-ZnO 2 mg/kg, 5 BL Green-Nano-ZnO 50 mg/kg, and 0 BL control (liver seed grass) substrates were established to be appropriate for producing the mushroom *P. ostreatus*. However, it was found that cultivating *Pleurotus ostreatus* using broiler litter and nanoparticle concentrations did not provide an effective technique to boost the potential yield and growth of *Pleurotus ostreatus* as predicted. Further study should be carried out to examine the effects of nanoparticle concentrations and broiler litter substrates on the growth and development of *Pleurotus ostreatus* enzyme activities present in broiler litter. Further research should be carried out to identify the appropriate amount of ZnO nanoparticles and broiler litter for use in *Pleurotus ostreatus* production. Mushroom growing is one of the most efficient methods of recycling waste. This is important for farmers who may not have readily available mushroom substrates. Therefore blending substrates that are in short supply might help farmers achieve their desired yields. Substrate mixing may aid in the optimisation of substrate properties such as water holding capacity, enhanced texture, porosity that increases water penetration and gaseous exchange, and an optimal C: N ratio that enhances substrate efficiency. As a result, it is critical to identify the N content of each raw agro-industrial by-product employed in the substrate mix for mushroom cultivation and then settle on appropriate combination proportions. These findings may aid in the long-term management of liver seed grass waste.

14. REFERENCES

- Adedokun, O.M.; Akuma, A.H. 2013. Maximizing agricultural residues: Nutritional properties of straw mushroom on maize husk, waste cotton, and plantain leaves. *Natural Resources*, 4, pp. 534–537
- Akter, M., Halawani, R.F., Aloufi, F.A., Taleb, M.A., Akter, S. and Mahmood, S., 2022. Utilization of agro-industrial wastes for the production of quality oyster mushrooms. *Sustainability*, 14(2), p.994.
- Ashraf, R., F. Shahid and T. A. Ali 2007 Association of fungi, bacteria, and actinomycetes with different composts. *Pakistan Journal of Botany*, 39(6), pp. 2141–2151.
- Atikpo, M., Onokpise, O., Abazinge, M., Louime, C., Dzomeku, M., Boateng, L., & Awumbilla, B. 2008. Sustainable mushroom production in Africa: A Case study in Ghana. *African Journal Of Biotechnology*, 7, pp. 249–253
- Bhattacharjya, D. K., Paul, R. K., Miah, M. N., & Ahmed, K. U. 2014. Effect of different saw dust substrates on the growth and yield of oyster mushroom (*Pleurotus ostreatus*), *Journal of Agriculture and Veterinary Science*, 7, pp. 38-46.
- Carvalho, C. S. M., Sales-Campos, C., & De Andrade, M. C. N. 2010. Mushrooms of the *Pleurotus* genus: A review of cultivation techniques. *Interciencia*, 35(3), pp. 177-182.
- Connorton, J.M. and Balk, J., 2019. Iron biofortification of staple crops: lessons and challenges in plant genetics. *Plant and Cell Physiology*, 60(7), pp.1447-1456.
- Chukwurah, N.F., Eze, S.C., Chiejina, N.V., Onyeonagu, C.C., Okezie, C.E.A., Ugwuoke, K.I., Ugwu, F.S.O., Aruah, C.B., Akobueze, E.U. and Nkwonta, C.G., 2013. Correlation of stipe length, pileus width, and stipe girth of oyster mushroom (*Pleurotus ostreatus*) grown in different farm substrates. *Journal of Agricultural Biotechnology and Sustainable Development*, 5(3), pp.54-60.
- Das N., Mukherjee M. 2007. Indoor Cultivation of *P. ostreatus*. *Philosophy Agriculture*, 61, pp.253-262.
- Dinssa, B., Temesgen, S. and Mosisa, W., 2020. Effect of Substrate (Waste Paper, Leaves of *P. Juliflora* and Sugar Cane Bagasse) on Nutritional Composition of *P. ostreatus* at Oda Bultum University, Chiro, Ethiopia. DAS

- Dlamini, B. E., Earnshaw, D. M., & Masarirambi, M. T. 2012. Growth and yield response of Oyster Mushroom (*Pleurotus ostreatus*) grown on different locally available substrates, *Current research journal of biological sciences*, 4(5), pp. 623-629.
- Estrada, A. E. R., del Mar Jimenez-Gasco, M., & Royse, D. J. 2009. Improvement of yield of *Pleurotus eryngii* var. *eryngii* by substrate supplementation and use of a casing overlay. *Bioresources and Technology*, 100(21), pp. 5270-5276.
- Fanadzo, M., Zireva, D. T., Dube, E., & Mashingaidze, A. B. 2010. Evaluation of various substrates and supplements for biological efficiency of *Pleurotus ostreatus sajour-caju* and *Pleurotus ostreatus*. *African Journal of Biotechnology*, 9, pp. 2756-2761.
- Gregori, A., Švagelj, M. and Pohleven, J., 2007. Cultivation techniques and medicinal properties of *Pleurotus* spp. *Food Technology and Biotechnology*, 45(3), pp.238-249.
- He, L., Liu, Y., Mustapha ,A., Lin, M. 2011. Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. *Microbiology Research*, 166, pp. 207–215
- Jafarpour, M., Jalalizand, A., & Eghbalsaied, S. 2011. High fiber media as the most efficient substrates for *Pleurotus florida* culture. *Archives of biological sciences.*, 63(3), pp. 889-895.
- Kadiri, M., & Fasidi, O. 1993 . Use of agricultural wastes for the cultivation of *Lentinus submidus* in Nigeria. *Revista de Biología Tropical.*, 41, pp. 41-415.
- Khandakar, J. 2004. Effect of media composition and growth regulators on mycelial growth and spawn production of three mushroom species (MS Thesis). Department of Biotechnology, BAU, Mymensingh.
- Kortei, J. N. K. 2015. Comparative Effect of Steam and Gamma Irradiation Sterilization of Sawdust Compost on the Yield, Nutrient and Shelf-Life of *Pleurotus Ostreatus* (Jacq.Ex. Fr) Kummer Stored in Two Different Packaging Materials. Ph. D Thesis. Department of Nuclear Agriculture and Radiation Processing University of Ghana. pp. 7-17.
- Kumar, S., Palve, A., Joshi, C. and Srivastava, R.K., 2019. Crop biofortification for iron (Fe), zinc (Zn) and vitamin A with transgenic approaches. *Heliyon*, 5(6).
- Limayema, A.; Ricke, S.C. Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects. *Progress in Energy and Combustion Science*. 2012, 38, pp. 449–46

- López-Arevalo, A., Huerta-Palacios, G., & Sánchez-Vázquez, J. E. 1996. Contamination encountered during various phases of cultivation of *Pleurotus ostreatus* in Tropical Mexico. Paper presented at the Proceed. II *International Conference on Mushroom Biology and Mushroom Products*. The Pennsylvania State University, USA.
- Mangat, M., Khanna, P. K., Kapoor, S., & B.S, S. 2008. Biomass and extracellular lignocellulolytic enzyme production by *Calocybe indica* strains. *Global Journal of Biotechnology and Biochemistry*, 3, pp. 98–104.
- Miller, M. W., & Jong, S. C. 1987. Commercial cultivation of shiitake in sawdust-filled plastic bags. *African Crop Science Journal*., 10, pp. 421-426.
- Manwaring, H.R., Bligh, H.F.J. and Yadav, R., 2016. The challenges and opportunities associated with biofortification of pearl millet (*Pennisetum glaucum*) with elevated levels of grain iron and zinc. *Frontiers in Plant Science*, 7, p.1944.
- Maqbool, M.A. and Beshir, A., 2019. Zinc biofortification of maize (*Zea mays L.*): Status and challenges. *Plant breeding*, 138(1), pp.1-28.
- Moore-Landecker, E. 1982. Fundamentals of the fungi (2nd Ed.). Prentice-Hall Inc.,-USA.
- Naraian, R., Sahu, R., Kumar, S., Garg, S., Singh, C., & Kanaujia, R. 2009. Influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on corn cob substrate. *Environmentalist*, 29(1), pp. 1-7.
- Narain, R., Sahu, R. K., Kumar, S., Garg, S. K., Singh, C. S., & Kanaujia, R. S. 2008. Influence of different nitrogen rich supplements during cultivation of *Pleurotus florida* on maize cobs substrate. *Environmentalist*, 29, pp. 1-7.
- Obodai, M., & Vowotor, K. (2002). Performance of different strains of *Pleurotus* species under Ghanaian conditions. *J. Food Tech. in Africa*, 7(3), pp. 98-100.
- Oseni, T. O., Dube, S. S., Wahome, P. K., Masarirambi, M. T., & Earnshaw, D. 2012a. Effect of wheat bran supplement on growth and yield of oyster mushroom (*Pleurotus ostreatus*) on fermented pine sawdust substrate. *Experimental Agriculture and Horticulture*., 30, p. 40.
- Oseni, T.O., Dlamini, S.O., Earnshaw, D.M. and T MASARIRAMBI, M.I.C.H.A.E.L., 2012(b). Effect of substrate pre-treatment methods on oyster mushroom (*Pleurotus ostreatus*) production. *International journal of agriculture and biology*, 14(2).

- Pardo-Giménez, A., Pardo, J.E., Dias, E.S., Rinker, D.L., Caitano, C.E.C., Zied, D.C. 2020. Optimization of cultivation techniques improves the agronomic behavior of *Agaricus subrufescens*. *Scientific Reports*, 10, p.8154
- Patrick, F., Mtui, G. Y. S., Mshandete, A. M., & Kivaisi, A. K. 2014. Ligninolytic Enzymes Activities of *Pleurotus sapidus* P969 during Vegetative Growth and Fruit Development on Sugarcane Residues-Based Substrates. *International Journal of Biotechnology*, 4(3), pp. 58-71.
- Pokhrel, C.P., Ohga, S. 2007. Cattle bedding waste used as substrate in the cultivation of *Agaricus blazei* Murill. *Journal Faculty Agriculture Kyushu University*, 52: 295298.
- Pokhrel, Kalyan, N., Budathoki, U., & Yadav, R. K. P. 2013. Cultivation of *Pleurotus sajor-cajun* using different agricultural residues. *International Journal of Agricultural Policy and Research*, 1(2), pp. 19-23.
- Pokhrel, Yadav, R., & Ohga, S. 2009. Effects of physical factors and synthetic media on mycelial growth of *Lyophyllum decastes*. *Journal of Ecobiotechnology*, 1, pp. 46-50.
- Ponmurugan, P., Nataraja Sekhar, Y., & Sreesakthi, T. R. 2007. Effect of various substrates on the growth and quality of mushrooms. *Pakistan Journal of Biological Science*, 10, pp. 171-173.
- Przybylowicz, P., & Donoghue, J. 1990. *Shiitake Growers Handbook*. Kendall/Hunt Publishing company. USA.
- Rühl, M., Fischer, C., & Kües, U. 2008. Ligninolytic enzyme activities alternate with mushroom production during industrial cultivation of *Pleurotus ostreatus* on wheat straw-based substrate. *Current Trends in Biotechnology and Pharmacy*, 2(4), pp.478 - 492.
- Savi, G. D., Bortoluzzi, A. J., Scussel, V. M. 2013. Antifungal properties of zinc-compounds against toxigenic fungi and mycotoxin. *International Journal of Food Science & Technology*, 48, pp.1834–1840.
- Sawai J. 2003. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO, and CaO) by conductimetric assay. *Journal of Microbiology Methods*, 54, pp.177–182.
- Shah, Z. A., Ashraf, M., & Ishtiaq, C. H. 2004. Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates wheat straw, leaves, sawdust. *Pakistan Journal of Nutrition*, 3, pp. 158-160.

- Shahzad, Z., Rouached, H., & Rakha, A. 2014. Combating mineral malnutrition through iron and zinc biofortification of cereals. *Comprehensive Reviews in Food Science and Food Safety*, 13, pp. 329–346. <https://doi.org/10.1111/1541-4337.12063>
- Shankar, A. H., & Prasad, A. S. 1998. Zinc and immune function: The biological basis of altered resistance to infection. *American Journal of Clinical Nutrition*, 68, 447S–63. <https://doi.org/10.1093/ajcn/68.2.447S>
- Sharma, V., & Kumar, S. 2011. Spawn production technology, Mushrooms Cultivation. Marketing and Consumption, Directorate of Mushroom Research (ICAR), Chambaghat, Solan. <http://www.dhruvagro.com/spawn.pdf>.
- Sardar, H.; Anjum, M.A.; Nawaz, A.; Ejaz, S.; Ali, M.A.; Khan, N.A.; Nawaz, F.; Raheel, M. 2016. Impact of various agro-industrial wastes on yield and quality of *Pleurotus sajor-caju*. *Pakistan Journal of Phytopathology*, 28, pp. 87–92.
- Sarker, N. C. 2004. Oyster mushroom (*Pleurotus ostreatus*) Production technology suitable for Bangladesh and its nutritional and postharvest behavior, Ph.D. Thesis, Bangabandhu Sheikh MujiburRahman Agricultural University, Gazipur, Bangladesh
- Sarker R. R. and. Chowdhury, A. K. M. S. H. 2013. Effect of different doses of GA3 application at primordia initiation stage on the growth and yield of Oyster mushroom. *Journal of the Bangladesh Agricultural University*. 11(1), pp. 5–10.
- Siddhant Yadav S, Singh CS. Spawn and spawning strategies for the cultivation of *Pleurotus ostreatus*. *International Journal of Pharmacy and Chemical Sciences*. 2013; 2(3), pp.1494-1500.
- Sher, H., Al-Yemeni, M., Bahkali A.H.A., Sher H., 2010. Effect of Environmental Factors on the Yield of Selected Mushroom Species Growing in Two Different Agro-Ecological Zones of Pakistan. *Saudi Journal of Biological Sciences*. 4, pp. 321-326.
- Tesfaw, A., Tadesse, A. and Kiros, G., 2015. Optimization of oyster (*Pleurotus ostreatus*) mushroom cultivation using locally available substrates and materials in Debre Berhan, Ethiopia. *Journal of Applied Biology and Biotechnology*, 3(1), pp.015-020.
- Tesfay, T., Godifey, T., Mesfin, R. and Kalayu, G., 2020. Evaluation of waste paper for cultivation of oyster mushroom (*Pleurotus ostreatus*) with some added supplementary materials. *AMB Express*, 10(1), pp.1-8.
- Tisdale, T.E., 2004. Cultivation of the oyster mushroom (*Pleurotus sp.*) on wood substrates in Hawaii (Doctoral dissertation).

- Sopit, V. 2006. Oyster mushroom cultivation on different cellulosic substrates. *Research journal of agriculture and biological sciences*, 2(6), pp. 548-551.
- kumarUkoima, H. N., Ogbonnaya, L., Anikpo, G. E., & Ikpe, F. 2009. Cultivation of mushrooms on various farm wastes in Obubra local. *Pakistan Journal of Nutrition*, 8(7), pp. 1059-1061.
- Vetayasuporn, S., Chutichudet, P., & Cho-Ruk, K. 2006. Bagasse as a possible substrate for *Pleurotus oesterotus* (Fr.) Kummer cultivation for the local mushroom farms in the Northeast of Thailand. *Pakistan Journal of Biological Sciences.*, 9, pp. 2512-2515.
- Wiafe-Kwagyan, M., Odamtten, G. T., & Kortei, N. K. 2022. Influence of substrate formulation on some morphometric characters and biological efficiency of *Pleurotus ostreatus* EM-1 (Ex. Fr) Kummer grown on rice wastes and “wawa” (*Triplochiton scleroxylon*) sawdust in Ghana. *Food Science & Nutrition*, 10(6), pp.1854-1863. <https://doi.org/10.1002%2Ffsn3.2802>.
- Wiafe-Kwagyan, M., 2023. Role of Mushroom Pathogen *Trichoderma harzianum* Rafai in the Reduction of Growth Yield, Nutrient Quality, and Morphometric Measurements of the Fruiting Body of *Pleurotus ostreatus* EM-1 From Seven Oyster Mushroom Cultivation Farms in Ghana. *International Journal of Food Science & Technology*, 4(1), pp.1-12.
- Yang. 2000. Cultivation of Edible Mushroom.China Agriculture Press; Beijing: p. 36.
- Yang, Guo, F. L., & Wan, Z. J. 2013. Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. *Saudi Journal of Biological Sciences*, 20(4), pp.333-338.
- Yildiz, S., Yildiz, U. C., Gezer, E. D., & Temiz, A. 2002. Some lignocellulosic wastes used as raw material in cultivation of the *Pleurotus ostreatus* culture mushroom. *Process Biochemistry*, 38, pp. 301-106.
- Zadrazil, F. 1980. Influence of Ammonium Nitrate and Organic Supplements on The Yield of *Pleurotus Sajor-Caju* (Fries), Singer. *Eur. Journal of Applied Microbiology and Biotechnology*, 9, pp. 31-34.

Effects of biofortification with broiler litter and phyto-mediated-Nano-ZnO on bioremediation of heavy metal in *Pleurotus ostreatus* fruiting bodies and SMS

5.1 Introduction

Due to its bulky size, broiler litter is not economically feasible to transport to places where crop nutrients are needed (Waldrip *et al.*, 2015). Poultry litter can be used as a partial replacement for mineral fertilizers to supplement the nutrients available in neighbouring croplands (Lourenço *et al.*, 2013; Bolan *et al.*, 2010). Repeated applications of poultry litter can lead to nutrient buildup in the soil, which can increase the risk of leaching and runoff which can lead to pollution of groundwater (Sharpley *et al.*, 2003; Gascho & Hubbard, 2006; He *et al.*, 2009a; Waldrip *et al.*, 2015). It is difficult to determine the appropriate rate of application to the soil due to the various factors that affect its chemical composition. Some of these include the type and quantity of material used for bedding and the number of spills. Besides these, other factors such as the length of time that the animals have been housed and the season of the year also affect its composition (Tasistro *et al.*, 2004; Dao & Zhang, 2007; Ávila *et al.*, 2008).

Previously, broiler litter was used to feed cattle (Ngodigha & Owen, 2009). Farmers were asked to stop feeding litter to livestock because it contains harmful heavy metals that can endanger livestock (Mangalgiri *et al.*, 2015; Pender, 2016; Hubbard *et al.*, 2020). Heavy metals, compared to many organic wastes, cannot be biodegraded to innocuous compounds. Mushrooms absorb heavy metals from their substrate through their extensive mycelium. Mushrooms, as opposed to green plants, may bio-accumulate more heavy metals in their fruit bodies since some of these heavy metals are naturally occurring components of the earth's crust. Because of their mycelial structure, mushrooms have been proven to be capable of bio-accumulating a wide range of molecules (Dermibas, 2000). Heavy metal concentrations have been found in the fruiting bodies of mushrooms collected near heavy metal smelters (Kalac *et al.*, 1996) and landfills in sewage sludge emission zones (Cibulka *et al.*, 1996). South Africa has had a viable mushroom business since the 1990s (Khan *et al.*, 2013). Rice straw, wheat straw, cotton seed waste, aquatic weeds, and sawdust from trees such as *Triplochiton scleroxylon* from mixed redwood of *Terminalia ivorensis* and *Chlorophora excelsa* are the principal substrates utilized in commercial mushroom cultivation in South Africa (Obodai *et al.* 2000; Sithole *et al.*, 2017; Sithole *et al.*, 2022). However, certain mushroom species grow only on termite and ant mounds.

There is extensive study on mushrooms' nutritional and medicinal properties, but there is not much or no information on their heavy metals bio-accumulation in South Africa. The goal of this study was to determine the heavy metal content of fruiting bodies of *Pleurotus ostreatus* when grown in boiler litter and nanoparticle biofortified substrates, as well as their safety for human consumption, and their use as fertilizer and animal feed.

5.2 Study objectives

5.2.1 Main objectives

To analyse the biochemical physiological processes of *Pleurotus ostreatus* on transfer efficiency of micro-nutrients and bio-remediation heavy metals on broiler litter fortified substrates treated with different nanoparticles.

5.2.2 Specific objectives

- i. To investigate the accumulation coefficient of macro- and micro-nutrients and bio-remediation of heavy metals in *Pleurotus ostreatus* fruiting bodies and SMS.
- ii. To determine the chemical composition of *Pleurotus ostreatus* SMS

5.3 Materials and Methods

5.3.1 Study area and resources

The research was carried out at the North-West University's animal health laboratory in Northwest Province, South Africa. The mushroom fruiting bodies and spent mushroom substrate were analyzed at North-West University's Centre for Applied Radiation Science and Technology and chemical analysis was carried out at Molelwane farm at the Animal Science Laboratory.

5.3.2 Description of the experiment

5.3.1.1 Macro- and micro-nutrient analysis of mushrooms harvested from different treatments.

Macro- and micro-nutrient content of fruiting bodies from the respective treatment combinations in chapter three were analysed by use of 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 crucible and further dried for 24 hours, by placing the crucibles with dry mushrooms into an ashing oven at 600 °C for 8 hours. The ICP 4:1 plant extract procedure was used, i.e., eight (8) millilitres of Nitric acid (HNO₃) and two (2) millilitres of hydrochloric acid (HCl) 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 volumetric flasks (100ml) which were topped up to 100 ml 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, and heavy metals that were analyzed were as follows: Fe, Zn, Mn, N, Mg, Ca, P, Cu, Cr, Cd, Se, and Pb, and these were expressed as mg/100g.

5.3.1.2 Heavy Metals Analysis

The samples were analyzed for heavy metals using the same procedure for macro- and micro-nutrient analysis of the mushrooms (AGRILASA). The method of Stihl *et al.* (2011) was used to estimate the heavy metal content using the following equation:

$$K_a = C_m / C_s,$$

where C_m = Concentration of heavy metal in the mushroom and C_s = concentration of heavy metal in the mushroom substrate.

The coefficient of accumulation of Fe, Cu, Mn, Zn, Se, Cd, Pb and Cr in mushroom fruiting bodies was analysed and presented as mg/100g.

5.3.1.3 Chemical analysis

The SMS samples were dried in an oven at 60 °C for 48 hours before being pulverized with a Wiley mill to pass a 1.0 mm screen. AOAC (1990) techniques 934.01, 976.05, 920.39, and 942.05, respectively, were used to analyze dry matter, crude protein, ether extract and crude ash. The weight loss during ashing was used to determine organic matter (OM). The acid detergent fibre (ADF) (Robertson & VanSoest 1981) and neutral detergent fibre (NDF) with a heat-stable amylase expressed exclusive of residual ash (Van Soest *et al.*, 1991) were determined. Lignin (sa) was measured by dissolving cellulose in sulphuric acid (Robertson & Van Soest 1981). Milled SMS samples (0.45-0.5

g) were refluxed with neutral detergent and acid detergent solutions for 60 and 75 minutes, respectively, using the ANKOM 2000 fibre analyzer (ANKOM Technology, New York), according to the method described by Van Soest *et al.* (1991) The NDF was calculated using a heat-stable amylase that did not include sodium sulphite. The acid detergent lignin (ADL) concentration was evaluated by comparing ADF residues treated in ANKOM F57 bags with 72% sulfuric acid to break down cellulose.

5.3.1.4 Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA) using the Minitab (2000) software package and was presented as Least Square Means (LSM) with respective pooled SEMs where significant differences ($P \leq 0.05$) between treatments were observed, LSM was compared using Tukey's test. The general linear model (GLM) procedure of Minitab (2000) was used to determine the effect of the inclusion level of broiler litter and the form of ZnO on the chemical composition and minerals of oyster mushroom fruiting bodies and spent mushroom substrate. The following GLM model was used:

$$Y_{ij} = \mu + B_i + Z_j + (B \times Z)_{ij} + E_{ij}$$

Where Y_{ij} = The response variable

μ = population mean

B_i = Effect of broiler litter level

Z_j = Effect of form of ZnO

$(B \times Z)_{ij}$ = Effect of interaction between broiler litter level and form of ZnO

E_{ij} - Random error associated with observation ij .

For all statistical tests, the significance was accepted at $P \leq 0.05$.

5.4 Results

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

5.4.1.1 Effect of different broiler litter levels and nanoparticle concentration on macro- and micro-nutrient composition of *Pleurotus ostreatus* (mg/kg)

Data analysis results indicated that there was a statistically significant effect of different broiler litter levels, nanoparticle concentration and interaction between broiler litter and nanoparticle concentration on the *P. ostreatus* nutrient composition for both macro- and

micro-nutrients ($P < 0.05$). The following macro-nutrient (N, P, Ca, Mg) and micro-nutrient (Fe, and Zn) levels were statistically significantly higher in oyster mushrooms produced on 5 BL (Green-Nano-ZnO 2 mg/kg, Green-Nano-ZnO 10 mg/kg and Green-Nano-ZnO 50 mg/kg) There was, however, no significant differences in N level in interaction between broiler litter and nanoparticle concentrations (Table 14). Heavy metals were within the acceptable level (0.5 mg/kg)for human food safety in fruiting bodies from all the experimental units.

Table 14: Effect of different levels of broiler litter and nanoparticle concentration on oyster mushroom macro- and micro-nutrient (mg/kg)

Treatments		Minerals					
BL	NanoC	N	P	Ca	Mg	Fe	Zn
0	Control	1559.5 ^a	23.2 ^{ab}	37.8 ^{ab}	6.7 ^{ab}	0.4 ^a	0.1 ^a
0	Bulk-ZnO	963.9 ^a	11.9 ^{ab}	15.9 ^{ab}	3.2 ^{ab}	0.2 ^a	0.03 ^a
0	Conv-Nano-ZnO	0	0	0	0	0	0
	2mg/kg						
0	Green-Nano-ZnO	0	0	0	0	0	0
	2mg/kg						
0	Green-Nano-ZnO	395.6 ^a	4.9 ^{ab}	9.6 ^{ab}	1.2 ^b	0.1 ^a	0.02 ^a
	10mg/kg						
0	Green-Nano-ZnO	483 ^a	6.3 ^{ab}	9.8 ^{ab}	1.6 ^{ab}	0.1 ^a	0.02 ^a
	50mg/kg						
1.25	Control	0	0	0	0	0	0
1.25	Bulk-ZnO	0	0	0	0	0	0
1.25	Conv-Nano-ZnO	0	0	0	0	0	0
	2mg/kg						
1.25	Green-Nano-ZnO	0	0	0	0	0	0
	2mg/kg						
1.25	Green-Nano-ZnO	0	0	0	0	0	0
	10mg/kg						
1.25	Green-Nano-ZnO	0	0	0	0	0	0
	50mg/kg						
2.5	Control	0	0	0	0	0	0
2.5	Bulk-ZnO	0	0	0	0	0	0
2.5	Conv-Nano-ZnO	0	0	0	0	0	0
	2mg/kg						
2.5	Green-Nano-ZnO	0	0	0	0	0	0
	2mg/kg						
2.5	Green-Nano-ZnO	0	0	0	0	0	0
	10mg/kg						
2.5	Green-Nano-ZnO	875.1 ^a	12.1 ^{ab}	22.5 ^{ab}	3.2 ^{ab}	0.4 ^a	0.04 ^a
	50mg/kg						
5	Control	945.5 ^a	12 ^{ab}	18.2 ^{ab}	3.4 ^{ab}	0.2 ^a	0.04 ^a
5	Bulk-ZnO	0	0	0	0	0	0
5	Conv-Nano-ZnO	467.4 ^a	5.5 ^{ab}	12.8 ^{ab}	1.6 ^{ab}	0.1 ^a	0
	2mg/kg						

5	Green-Nano-ZnO 2mg/kg	1873.7 ^a	21.3 ^{ab}	38.4 ^{ab}	5.9 ^{ab}	0.4 ^a	0.1 ^a
5	Green-Nano-ZnO 10mg/kg	1214.4 ^a	17 ^{ab}	36.6 ^{ab}	4.7 ^{ab}	0.4 ^a	0.1 ^a
5	Green-Nano-ZnO 50mg/kg	3362.3 ^a	5.3 ^{ab}	8.4 ^{ab}	1.6 ^{ab}	0.1 ^a	0.02 ^a
10	Control	3557.8 ^a	32.6 ^{ab}	58.9 ^{ab}	10 ^a	0.5 ^a	0.1 ^a
10	Bulk-ZnO	0	0	0	0	0	0
10	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0	0
10	Green-Nano-ZnO 2mg/kg	0	0	0	0	0	0
10	Green-Nano-ZnO 10mg/kg	0	0	0	0	0	0
10	Green-Nano-ZnO 50mg/kg	2596.7 ^a	16.9 ^{ab}	19.6 ^{ab}	4.2 ^{ab}	0.2 ^a	0.04 ^a
Pooled SEM		411.53	2.81	4.69	0.79	0.05	0.01
P-values	Broiler litter	P<0.05	P<0.01	P=0.05	P<0.01	P<0.01	P=0.001
	NanoC	P<0.05	P<0.01	P<0.01	P<0.01	P<0.05	P<0.05
	Broiler litter X NanoC	P>0.05	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01

NanoC-nanoparticles concentration. Reported values are averages of four replications. Significance: P>0.05-not significant, * P ≤0.05, ** P ≤ 0.01 or *** P ≤0.001, respectively. Means followed by different letters within each column are significantly different at P ≤0.05 via Tukey's means separation procedure. N- Nitrogen, P- Phosphorus, Ca- Calcium, Mg- Magnesium, Fe- Iron, and Zn- Zinc.

Table 15: Effect of different levels of broiler litter and nanoparticle concentration on *Pleurotus ostreatus* heavy metals (mg/kg)

Treatments		Minerals						
BL	NanoC	Mn	Cl	Se	Cd	Pb	Cu	Cr
0	Control	0.03 ^{ab}	3505.3 ^a	0.38 ^a	0.19 ^a	2.67 ^{ab}	0.03 ^a	0.04 ^a
0	Bulk-ZnO	0.01 ^{ab}	1835.2 ^a	0.15 ^a	0.07 ^a	1.92 ^{ab}	0.01 ^a	0.01 ^a
0	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0	0	0
0	Green-Nano-ZnO 2mg/kg	0	0	0	0	0	0	0
0	Green-Nano-ZnO 10mg/kg	0.004 ^b	867.1 ^a	0.08 ^a	0.03 ^a	0.62 ^{ab}	0.01 ^a	0.003 ^a
0	Green-Nano-ZnO 50mg/kg	0.004 ^b	824.2 ^a	0.09 ^a	0.03 ^a	0.79 ^{ab}	0.01 ^a	0.01 ^a
1.25	Control	0	0	0	0	0	0	0
1.25	Bulk-ZnO	0	0	0	0	0	0	0
1.25	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0	0	0
1.25	Green-Nano-ZnO 2mg/kg	0	0	0	0	0	0	0

1.25	Green-Nano-ZnO 10mg/kg	0	0	0	0	0	0	0
1.25	Green-Nano-ZnO 50mg/kg	0	0	0	0	0	0	0
2.5	Control	0	0	0	0	0	0	0
2.5	Bulk-ZnO	0	0	0	0	0	0	0
2.5	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0	0	0
2.5	Green-Nano-ZnO 2mg/kg	0	0	0	0	0	0	0
2.5	Green-Nano-ZnO 10mg/kg	0	0	0	0	0	0	0
2.5	Green-Nano-ZnO 50mg/kg	0.03 ^{ab}	1666.8 ^a	0.17 ^a	0.08 ^a	1.7 ^{ab}	0.02 ^a	0.05 ^a
5	Control	0.01 ^{ab}	1846.7 ^a	0.2 ^a	0.17 ^a	0.65 ^{ab}	0.02 ^a	0.01 ^a
5	Bulk-ZnO	0	0	0	0	0	0	0
5	Conv-Nano-ZnO 2mg/kg	0.005 ^b	756.8 ^a	0.09 ^a	0.07 ^a	0.7 ^{ab}	0.01 ^a	0.01 ^a
5	Green-Nano-ZnO 2mg/kg	0.02 ^{ab}	3024.8 ^a	0.35 ^a	0.18 ^a	4.7 ^a	0.03 ^a	0.01 ^a
5	Green-Nano-ZnO 10mg/kg	0.02 ^{ab}	2480.8 ^a	0.3 ^a	0.21 ^a	3.95 ^{ab}	0.03 ^a	0.03 ^a
5	Green-Nano-ZnO 50mg/kg	0	746.6 ^a	0.21 ^a	0.03 ^a	0.56 ^{ab}	0.01 ^a	0.004 ^a
10	Control	0.05 ^a	5336.9 ^a	0.45 ^a	0.16 ^a	1.71 ^{ab}	0.04 ^a	0.01 ^a
10	Bulk-ZnO	0	0	0	0	0	0	0
10	Conv-Nano-ZnO 2mg/kg	0	0	0	0	0	0	0
10	Green-Nano-ZnO 2mg/kg	0	0	0	0	0	0	0
10	Green-Nano-ZnO 10mg/kg	0	0	0	0	0	0	0
10	Green-Nano-ZnO 50mg/kg	0.02 ^{ab}	3528.9 ^a	0.29 ^a	0.06 ^a	1.25 ^{ab}	0.02 ^a	0.01 ^a
Pooled SEM		0.04	488.15	0.04	0.022	0.4	0.004	0.005
P-values	Broiler litter	P>0.05	P<0.05	P<0.01	P<0.001	P<0.01	P<0.01	P>0.05
	NanoC	P<0.05	P<0.05	P<0.01	P>0.05	P>0.05	P<0.01	P>0.05
	Broiler litter X NanoC	P<0.05	P=0.05	P=0.05	P<0.05	P<0.01	P<0.01	P<0.05

NanoC-nanoparticles concentration. The mean with different letters is significantly different at $P \leq 0.05$. Mean with different letters in a row are significantly different at $P \leq 0.05$. Reported values are averages of four replications. Significance: $P > 0.05$ -not significant, * $P \leq 0.05$, ** $P \leq 0.01$ or *** $P \leq 0.001$, respectively. Means followed by different letters within each column are significantly different at $P \leq 0.05$ via Tukey's means separation procedure. Mn- Manganese, Cl- Chlorine, Se- Selenium, Cd- Cadmium, Pb- Lead, Cu- Copper and Cr- Chromium.

5.4.1.2 Effect of broiler litter X nanoparticle concentration interaction on accumulation coefficient of macro- and micro-nutrient and heavy metals in *Pleurotus ostreatus* spent substrates (mg/kg)

Table 16 shows data analysis results that indicate that there was a statistically significant effect of different broiler litter levels, nanoparticle concentration, and interaction between broiler litter and nanoparticle concentration on the *P. ostreatus* nutrient composition for both macro- and micro-nutrients ($P < 0.05$). The following macro-nutrient (N, P, Ca, Mg)and micro-nutrient (Fe, and Zn) levels were statistically significantly different in spent mushroom on broiler litter levels and N, Zn, Pb, and Cr showed statistical differences in nanoparticle concentration.

Table 16: Effects of different broiler litter levels and nanoparticle concentration on SMS macro- and micro-nutrient (mg/kg)

Treatments		Minerals				
BL	NanoC	N	Ca	Mg	Fe	Zn
0	Control	7283.2 ^a	655 ^{bc}	25.4 ^b	20.7 ^{ab}	0.2 ^{ab}
0	Bulk-ZnO	6342.2 ^{abc}	607.8 ^{bc}	22 ^b	15.8 ^{ab}	0.2 ^b
0	Conv-Nano-ZnO	6202.3 ^{abc}	514.5 ^{bc}	19.8 ^b	25.9 ^{ab}	0.2 ^b
0	2mg/kg					
0	Green-Nano-ZnO	7321.3 ^a	688.8 ^{bc}	21.7 ^b	20.4 ^{ab}	0.2 ^b
0	2mg/kg					
0	Green-Nano-ZnO	6526.7 ^{ab}	768.7 ^{abc}	19.1 ^b	19.2 ^{ab}	0.2 ^b
0	10mg/kg					
0	Green-Nano-ZnO	6970.7 ^{ab}	617.1 ^{bc}	24 ^b	17.3 ^{ab}	0.4 ^{ab}
0	50mg/kg					
1.25	Control	5837.7 ^{abc}	843.8 ^{abc}	25.3 ^b	17.7 ^{ab}	0.2 ^b
1.25	Bulk-ZnO	6146.3 ^{abc}	971.9 ^{abc}	23.1 ^b	14.5 ^{ab}	0.3 ^{ab}
1.25	Conv-Nano-ZnO	5220.0 ^{bcd}	794.6 ^{abc}	17.4 ^a	27.8 ^a	0.4 ^{ab}
1.25	2mg/kg					
1.25	Green-Nano-ZnO	3766.2 ^{def}	1515 ^a	25.8 ^b	21.7 ^{ab}	0.3 ^{ab}
1.25	2mg/kg					
1.25	Green-Nano-ZnO	3319.3 ^{d-h}	984 ^{abc}	22 ^b	23.3 ^{ab}	0.3 ^{ab}
1.25	10mg/kg					
1.25	Green-Nano-ZnO	3069.9 ^{e-h}	1212.7 ^{ab}	22.3 ^b	19.4 ^{ab}	0.6 ^a
1.25	50mg/kg					
2.5	Control	3429.3 ^{d-h}	917 ^{abc}	21.5 ^b	14.1 ^{ab}	0.2 ^{ab}
2.5	Bulk-ZnO	3637.2 ^{d-g}	782 ^{abc}	21.4 ^b	14.3 ^{ab}	0.3 ^{ab}
2.5	Conv-Nano-ZnO	2644.0 ^{e-h}	1062.3 ^{abc}	21.7 ^b	19 ^{ab}	0.3 ^{ab}
2.5	2mg/kg					
2.5	Green-Nano-ZnO	3426.8 ^{d-h}	855.2 ^{abc}	22.9 ^b	15.3 ^{ab}	0.2 ^{ab}
2.5	2mg/kg					
2.5	Green-Nano-ZnO	3307.6 ^{d-h}	773.1 ^{abc}	25 ^b	16.8 ^{ab}	0.3 ^{ab}
2.5	10mg/kg					
2.5	Green-Nano-ZnO	3228.9 ^{e-h}	811 ^{abc}	23.2 ^b	17.1 ^{ab}	0.6 ^a

	50mg/kg					
5	Control	2465.1 ^{e-h}	840.4 ^{abc}	19.5 ^b	10.3 ^b	0.2 ^b
5	Bulk-ZnO	2825.5 ^{e-h}	678.2 ^{bc}	17.9 ^b	20.9 ^{ab}	0.2 ^b
5	Conv-Nano-ZnO	2871.7 ^{e-h}	826.5 ^{abc}	20.5 ^b	13.7 ^{ab}	0.2 ^b
	2mg/kg					
5	Green-Nano-ZnO	4391.6 ^{cde}	741.3 ^{bc}	22.4 ^b	18 ^{ab}	0.2 ^b
	2mg/kg					
5	Green-Nano-ZnO	3398.6 ^{d-h}	773.8 ^{abc}	17.3 ^b	17.7 ^{ab}	0.1 ^b
	10mg/kg					
5	Green-Nano-ZnO	3306.9 ^{d-h}	674.9 ^{bc}	22.7 ^b	15 ^{ab}	0.4 ^{ab}
	50mg/kg					
10	Control	2633.4 ^{e-h}	740.2 ^{bc}	20 ^b	14.1 ^{ab}	0.2 ^b
10	Bulk-ZnO	2419.8 ^{fgh}	896.4 ^{abc}	18.9 ^b	13.9 ^{ab}	0.2 ^b
10	Conv-Nano-ZnO	2024.2 ^{fgh}	608.2 ^{bc}	15.8 ^b	12 ^{ab}	0.2 ^b
	2mg/kg					
10	Green-Nano-ZnO	1924.7 ^{fgh}	365.3 ^c	13.4 ^b	12.7 ^{ab}	0.1 ^b
	2mg/kg					
10	Green-Nano-ZnO	1716.7 ^{gh}	520.1 ^{bc}	15.6 ^b	11.5 ^b	0.1 ^b
	10mg/kg					
10	Green-Nano-ZnO	1483.7 ^h	508.4 ^{bc}	22.8 ^b	12 ^{ab}	0.4 ^{ab}
	50mg/kg					
Pooled SEM		180.13	69.47	13.38	1.47	0.03
P-values	Broiler litter	P< 0.001	P<0.001	P>0.05	P<0.001	P<0.001
	NanoC	P< 0.01	P>0.05	P>0.05	P>0.05	P<0.001
	Broiler litter X NanoC	P= 0.001	P<0.05	P>0.05	P>0.05	P>0.05

NanoC- nanoparticles concentration. Mean with different letters in a row are significantly different at $P \leq 0.05$. Reported values are averages of four replications. Significance: $P > 0.05$ -not significant, * $P \leq 0.05$, ** $P \leq 0.01$ or *** $P \leq 0.001$, respectively. Means followed by different letters within each column are significantly different at $P \leq 0.05$ via Tukey's means separation procedure. N- Nitrogen, Ca- Calcium, Mg- Magnesium, Fe- Iron, and Zn- Zinc.

Table 17: Effects of different broiler litter levels and nanoparticles on SMS on micro-nutrient and heavy metals (mg/kg)

Treatments		Minerals						
BL	NanoC	Mn	Cl	Se	Cd	Pb	Cu	Cr
0	Control	1 ^{a-d}	7591.7 ^{a-f}	1.3 ^{a-d}	0.1 ^a	0.003 ^{abc}	0.02 ^{bc}	0.11 ^a
0	Bulk-ZnO	0.8 ^{bcd}	6799.6 ^{a-g}	1.3 ^{bcd}	0.2 ^a	0.003 ^{abc}	0.01 ^c	0.13 ^a
0	Conv-Nano-ZnO	1 ^{a-d}	7031.3 ^{a-g}	1.5 ^{a-d}	0.1 ^a	0.005 ^{abc}	0.03 ^{abc}	0.24 ^a
	2mg/kg							
0	Green-Nano-ZnO	1 ^{a-d}	7682.9 ^{a-f}	1.3 ^{a-d}	0.1 ^a	0.004 ^{abc}	0.02 ^c	0.18 ^a
	2mg/kg							
0	Green-Nano-ZnO	0.8 ^{bcd}	5885.5 ^{b-g}	1.3 ^{abcd}	0.1 ^a	0.004 ^{abc}	0.02 ^{bc}	0.16 ^a
	2mg/kg							

0	10mg/kg Green-Nano-ZnO	1 ^{a-d}	8133.7 ^{a-e}	1.3 ^{bcd}	0.2 ^a	0.005 ^{abc}	0.02 ^c	0.10 ^a
1.25	50mg/kg Control	1.2 ^{a-d}	7368.6 ^{a-g}	1.5 ^{a-d}	0.1 ^a	0.005 ^{abc}	0.03 ^{abc}	0.10 ^a
1.25	Bulk-ZnO	1 ^{a-d}	8057.9 ^{a-e}	1.5 ^{a-d}	0.2 ^a	0.006 ^{abc}	0.04 ^{abc}	0.11 ^a
1.25	Conv-Nano-ZnO	1.5 ^a	10358.7 ^a	2.1 ^{ab}	0.2 ^a	0.004 ^{abc}	0.05 ^{a^bc}	0.22 ^a
1.25	2mg/kg Green-Nano-ZnO	1.4 ^{ab}	7021.9 ^{a-g}	2 ^{abc}	0.2 ^a	0.004 ^{abc}	0.06 ^a	0.19 ^a
1.25	2mg/kg Green-Nano-ZnO	1.3 ^{abc}	7672.3 ^{a-f}	1.8 ^{a-d}	0.1 ^a	0.003 ^{abc}	0.05 ^{abc}	0.21 ^a
1.25	10mg/kg Green-Nano-ZnO	1.2 ^{a-d}	6347.7 ^{b-g}	1.8 ^{a-d}	0.2 ^a	0.003 ^{bc}	0.04 ^{abc}	0.19 ^a
2.5	50mg/kg Control	1 ^{a-d}	8756.1 ^{a-d}	1.5 ^{a-d}	0.3 ^a	0.007 ^{abc}	0.03 ^{abc}	0.12 ^a
2.5	Bulk-ZnO	1 ^{a-d}	6877.4 ^{a-g}	1.7 ^{a-d}	0.3 ^a	0.011 ^a	0.04 ^{abc}	0.14 ^a
2.5	Conv-Nano-ZnO	1.2 ^{a-d}	7727.9 ^{a-f}	1.9 ^{a-d}	0.2 ^a	0.003 ^{abc}	0.05 ^{ab}	0.15 ^a
2.5	2mg/kg Green-Nano-ZnO	0.9 ^{a-d}	9696.7 ^{ab}	2.2 ^a	0.2 ^a	0.003 ^{abc}	0.04 ^{abc}	0.15 ^a
2.5	2mg/kg Green-Nano-ZnO	0.9 ^{a-d}	9596.1 ^{abc}	1.8 ^{a-d}	0.1 ^a	0.004 ^{abc}	0.03 ^{abc}	0.22 ^a
2.5	10mg/kg Green-Nano-ZnO	0.9 ^{bcd}	7108.1 ^{a-g}	2 ^{abc}	0.2 ^a	0.009 ^{ab}	0.04 ^{abc}	0.17 ^a
5	50mg/kg Control	0.8 ^{cd}	6639.5 ^{a-g}	1.4 ^{a-d}	0.2 ^a	0.004 ^{abc}	0.03 ^{abc}	0.08 ^a
5	Bulk-ZnO	0.9 ^{bcd}	7700.4 ^{a-f}	1.6 ^{a-d}	0.2 ^a	0.005 ^{abc}	0.03 ^{abc}	0.17 ^a
5	Conv-Nano-ZnO	0.9 ^{bcd}	6600.3 ^{a-g}	1.6 ^{a-d}	0.2 ^a	0.004 ^{abc}	0.03 ^{abc}	0.13 ^a
5	2mg/kg Green-Nano-ZnO	1 ^{a-d}	8115.3 ^{a-e}	1.8 ^{a-d}	0.2 ^a	0.004 ^{abc}	0.02 ^{abc}	0.17 ^a
5	2mg/kg Green-Nano-ZnO	0.8 ^{bcd}	6606.2 ^{a-g}	1.6 ^{a-d}	0.1 ^a	0.0003 ^{bc}	0.03 ^{abc}	0.22 ^a

5	Nano-ZnO 10mg/kg Green-Nano-ZnO	0.8 ^{bcd}	6439.7 ^{b-g}	1.7 ^{a-d}	0.1 ^a	0.005 ^{abc}	0.02 ^{bc}	0.20 ^a
g10	50mg/kg Control	0.9 ^{a-d}	5445.7 ^{d-g}	1.4 ^{a-d}	0.1 ^a	0.002 ^{bc}	0.03 ^{abc}	0.09 ^a
10	Bulk-ZnO	1 ^{a-d}	5808.8 ^{c-g}	1.6 ^{a-d}	0.1 ^a	0.003 ^{abc}	0.03 ^{abc}	0.11 ^a
10	Conv-Nano-ZnO	0.8 ^{bcd}	4608.2 ^{efg}	1.2 ^{cd}	0.2 ^a	0.003 ^{abc}	0.02 ^{bc}	0.08 ^a
10	2mg/kg Green-Nano-ZnO	0.7 ^d	4391.5 ^{efg}	1.3 ^{bcd}	0.1 ^a	0.003 ^{bc}	0.02 ^{bc}	0.14 ^a
10	2mg/kg Green-Nano-ZnO	0.6 ^d	3881.6 ^{fg}	1 ^d	0.1 ^a	0.001 ^c	0.02 ^c	0.04 ^a
10	10mg/kg Green-Nano-ZnO	0.7 ^d	3559 ^g	1.1 ^{cd}	0.1 ^a	0.003 ^{bc}	0.03 ^{abc}	0.08 ^a
	50mg/kg							
Pooled SEM		0.055	352.28	0.08	0.0385	0.0005	0.003	0.019
P-values	Broiler litter	P<0.001	P<0.001	P<0.001	P<0.05	P<0.01	P<0.001	P<0.01
	NanoC	P>0.05	P>0.05	P>0.05	P>0.05	P<0.05	P>0.05	P<0.05
	Broiler litter X NanoC	P>0.05	P<0.01	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

NanoC-nanoparticles concentration. The mean with different letters is significantly different at $P \leq 0.05$. Mean with different letters in a row are significantly different at $P \leq 0.05$. Reported values are averages of four replications. Significance: $P > 0.05$ -not significant, * $P \leq 0.05$, ** $P \leq 0.01$ or *** $P \leq 0.001$, respectively. Means followed by different letters within each column are significantly different at $P \leq 0.05$ via Tukey's means separation procedure. Mn- Manganese, Cl- Chlorine, Se- Selenium, Cd- Cadmium, Pb- Lead, Cu- Copper and Cr- Chromium.

5.4.2 Chemical analysis

Table 18 shows the chemical composition of the effects of broiler litter and phyto-mediated-nano-ZnO on spent oyster mushroom substrate where broiler litter did not

showed statistical differences in DM, CP, EE, ASH, NDF, ADF, and ADL. Nanoparticle concentration did not show any statistical difference and interaction between Broiler litter and Nanoparticle concentration showed a statistical difference in EE and CP.

Table 18: Effects of different broiler litter levels and nanoparticles on SMS chemical composition (%)

Treatments		Chemical composition (%)						
BL	NanoC	DM	CP	EE	Ash	NDF	ADF	ADL
0	Control	98.07 ^a	45.5 ^a	1.19 ^{ab}	1.93 ^a	88.83 ^a	81.22 ^a	67.03 ^a
0	Bulk-ZnO	97.97 ^a	39.6 ^{abc}	1.04 ^{ab}	2 ^a	91.98 ^a	83.28 ^a	63.1 ^a
0	Conv-Nano-ZnO 2mg/kg	98 ^a	38.8 ^{abc}	0.6 ^b	2.03 ^a	87.94 ^a	70.59 ^a	68.59 ^a
0	Green-Nano-ZnO 2mg/kg	97.96 ^a	45.8 ^a	0.32 ^b	2.04 ^a	93.06 ^a	83.69 ^a	73.97 ^a
0	Green-Nano-ZnO 10mg/kg	97.9 ^a	40.8 ^{ab}	0.32 ^b	2.1 ^a	91.73 ^a	86.47 ^a	74.32 ^a
0	Green-Nano-ZnO 50mg/kg	97.91 ^a	43.6 ^{ab}	0.87 ^{ab}	2.09 ^a	90.95 ^a	81.8 ^a	71.7 ^a
1.25	Control	97.7 ^a	36.5 ^{abc}	1.1 ^{ab}	2.3 ^a	88.12 ^a	80.84 ^a	67.69 ^a
1.25	Bulk-ZnO	97.61 ^a	38.5 ^{abc}	1.52 ^{ab}	2.13 ^a	92.86 ^a	83.57 ^a	71.71 ^a
1.25	Conv-Nano-ZnO 2mg/kg	97.87 ^a	32.7 ^{bcd}	1.29 ^{ab}	2.4 ^a	93.13 ^a	78.75 ^a	67.3 ^a
1.25	Green-Nano-ZnO 2mg/kg	98.4 ^a	23.6 ^{def}	1.27 ^{ab}	1.6 ^a	93.08 ^a	79.64 ^a	69.72 ^a
1.25	Green-Nano-ZnO 10mg/kg	98.11 ^a	20.8 ^{d-h}	1.95 ^{ab}	1.89 ^a	90.05 ^a	80.28 ^a	68.95 ^a
1.25	Green-Nano-ZnO 50mg/kg	97.92 ^a	19.2 ^{e-h}	1.54 ^{ab}	2.08 ^a	94.14 ^a	79 ^a	66.54 ^a
2.5	Control	96.4 ^a	21.5 ^{d-h}	1.07 ^{ab}	3.6 ^a	95.64 ^a	85.03 ^a	72.58 ^a
2.5	Bulk-ZnO	96.9 ^a	22.8 ^{d-g}	1.29 ^{ab}	2.27 ^a	88.26 ^a	78.56 ^a	67.92 ^a
2.5	Conv-Nano-ZnO 2mg/kg	97.73 ^a	16.6 ^{e-h}	0.62 ^b	3.1 ^a	90.05 ^a	82.03 ^a	71.94 ^a
2.5	Green-Nano-ZnO 2mg/kg	97.61 ^a	21.4 ^{d-h}	1.84 ^{ab}	2.39 ^a	89.43 ^a	81.82 ^a	67.28 ^a
2.5	Green-Nano-ZnO 10mg/kg	96.22 ^a	20.7 ^{d-h}	0.89 ^{ab}	3.78 ^a	93.54 ^a	82.56 ^a	67.21 ^a
2.5	Green-Nano-ZnO 50mg/kg	96.74 ^a	20.2 ^{e-h}	1.84 ^{ab}	3.26 ^a	94.04 ^a	85.64 ^a	69.54 ^a
5	Control	96.36 ^a	15.4 ^{e-h}	0.49 ^b	3.64 ^a	88.76 ^a	82.67 ^a	71.58 ^a
5	Bulk-ZnO	96.48 ^a	17.7 ^{e-h}	0.52 ^b	3.66 ^a	88.32 ^a	84.5 ^a	69.35 ^a

5	Conv-Nano-ZnO 2mg/kg	96.71 ^a	17.9 ^{e-h}	0.62 ^b	3.52 ^a	94.23 ^a	84.56 ^a	69.81 ^a
5	Green-Nano- ZnO 2mg/kg	96.59 ^a	27.4 ^{cde}	0.72 ^b	3.29 ^a	92.05 ^a	81.31 ^a	68.47 ^a
5	Green-Nano- ZnO 10mg/kg	96.89 ^a	21.2 ^{d-h}	0.77 ^b	3.11 ^a	90.01 ^a	81.61 ^a	70.11 ^a
5	Green-Nano- ZnO 50mg/kg	96.25 ^a	20.7 ^{d-h}	1.07 ^{ab}	3.75 ^a	90.68 ^a	82.92 ^a	76.44 ^a
10	Control	96.19 ^a	16.5 ^{e-h}	0.8 ^{ab}	3.81 ^a	88.14 ^a	80.99 ^a	74.69 ^a
10	Bulk-ZnO	98.75 ^a	15.2 ^{fgh}	1.19 ^{ab}	3.53 ^a	90.79 ^a	83.37 ^a	74.84 ^a
10	Conv-Nano-ZnO 2mg/kg	96.47 ^a	12.7 ^{fgh}	0.7 ^b	1.25 ^a	88.52 ^a	83.7 ^a	70.09 ^a
10	Green-Nano- ZnO 2mg/kg	96.6 ^a	12.0 ^{fgh}	0.5 ^b	3.4 ^a	87.23 ^a	85.23 ^a	75.48 ^a
10	Green-Nano- ZnO 10mg/kg	96.42 ^a	10.7 ^{gh}	0.62 ^b	3.57 ^a	87.54 ^a	85.87 ^a	75.51 ^a
10	Green-Nano- ZnO 50mg/kg	96.36 ^a	9.3 ^h	0.75 ^b	3.64 ^a	85.73 ^a	84.6 ^a	73.33 ^a
Pooled SEM		0.26	1.13	0.16	0.26	0.97	1.07	1.32
P- values	Broiler litter	P<0.001	P<0.0001	P<0.001	P<0.001	P=0.05	P=0.05	P<0.01
	NanoC	P>0.05	P<0.01	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
	Broiler litter X NanoC	P>0.05	P<0.0001	P<0.05	P>0.05	P>0.05	P>0.05	P>0.05

Mean with different letters in a row are significantly different at $P \leq 0.05$ via Tukey's test. DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin. NanoC- nanoparticles concentration. Reported values are averages of four replications. Significance: $P > 0.05$ =not significant, * $P \leq 0.05$, ** $P \leq 0.01$ or *** $P \leq 0.001$, respectively.

5.5 Discussion

In general, the mineral components required for mushroom fruiting are the same as those required by any other cultivated plant, including macro and micronutrients (Molena, 1986). N, P, K and Mg are macronutrients required for the development of certain fungi (Miles & Chang, 1997). Ca was named as one of these elements by Molena (1986). Minerals such as Mg, Cl, and Ca, according to Kurtzman and Zadrazil (1984), encourage mycelium development and the creation of fruiting bodies. Macro

minerals found in fruiting bodies on various treatments were as follows: N was the highest ranging from 397.6- 3557.8 mg/kg, followed by Ca (8.4- 37.8 mg/kg), P (5.3- 23.2) and Mg (1.6- 6.7 mg/kg). Among the micro minerals found in the mushrooms growing on the various treatments studied, Cl had the greatest amount, ranging from 746.6 to 3505 mg/kg, followed by Fe (0.1- 0.5 mg/kg), Zn (0.02-0.1) and Mn (16.00- 23.00). According to Sturion and Ranzani (2000), although having the highest value among macro minerals found in mushrooms, P has been reported to be low in most other human diets. This characteristic, along with the fact that it is also high in Ca, makes this mushroom of interest in the treatment of hypertension sufferers. The heavy metals found ranged from Pb (0.56- 2.67 mg/kg), Se (0.08- 0.38 mg/kg), Cr (0.003- 0.04) and Cd (0.03- 0.19) which are considered to fall within the acceptable human food safety level.

The mean zinc concentration in the analysed mushroom sample is less than the recommended limit of 0.3-1.0 mg/kg in diet (FAO/WHO,2004). The mean content of 0.03 mg/kg is less than the FAO/WHO (2004) permitted threshold of 0.05-0.5 mg/kg in meals. The average zinc content of 0.03 mg/kg is recommended since zinc is one of the most critical elements required by our body systems owing to its association with protein and carbohydrate-rich diets.

Among the heavy metals that are of concern in food safety, Pb has been reported to cause health problems such as insomnia, fatigue, hearing loss, and weight loss. Unfortunately, the Pb contents in all of the treatments were significantly greater than the allowable limits in food. The FAO/WHO (2004) acceptable weekly lead consumption is 0.025 mg/kg body weight. This result might be due to Pb in the air from automotive and industrial pollutants. Pb from the air is frequently deposited on natural vegetation.

However, the findings for several metals from the oyster mushroom species studied in this study varied substantially. This variation in metal content could be attributed to differences in the composition of the substrate from which the mushrooms derived their nutrients, the location where the broiler litter was collected, the age of the fruiting bodies and mycelium, and the distance from the source of pollution. Furthermore, metal concentrations in mushroom samples may have been influenced primarily by the pH or organic matter content of the substrates. Although minerals were not analysed in the different development phases of the fungus in the current study, a significant increase in their concentration was noted in the substrate degraded by the fungus.

Mushroom SMS has the potential to be beneficial to the animal feed and fertilizer industries. There is a widespread interest in the use of mushroom SMS as fertiliser for vegetable cultivation (Maher, 1991) and as a component in animal feeding (Alborés *et al.*, 2006).

The lowest N concentration was detected at 0 BL Green-Nano-ZnO 10 mg/kg, whereas the maximum was reported at '10 BL Control' on fruiting bodies. This might have come from the fruiting body bioaccumulating the minerals. The 10 BL have a high nitrogen concentration because as ammonia is produced in broiler litter, it returns to its native chemical form of nitrogen and hydrogen. On fruiting bodies, 0 BL Green-Nano-ZnO 10 mg/kg had the lowest phosphorus concentration, whereas 10 BL Control had the greatest. The high value may have contributed to the high concentration reported in broiler litter. Fruiting bodies from 5 BL Green-Nano-ZnO 10 mg/kg had the lowest calcium concentration, whereas those from 5 BL Green-Nano-ZnO 2 mg/kg had the greatest. Fruiting bodies harvested from Both 10 BL control and 0 Green-Nano-ZnO had statistically significantly higher magnesium content than other treatments.

The dry matter composition of SMS differed significantly due to the use of various broiler litter levels and nanoparticle concentrations. The 10 BL Bulk-ZnO had the greatest dry matter content (98.75%), whereas the 10 BL Control had the lowest (96.19%). The addition of broiler litter as an organic material source might have had a statistically significant impact on the dry matter composition of the spent mushroom substrate. *Pleurotus* spp. is regarded as a high-quality protein source with evenly distributed essential amino acids (Patil *et al.*, 2010). Protein levels were strongly impacted by broiler litter and nanoparticle concentrations of 1.25 BL Conv-Nano-ZnO 2 mg/kg and 5 BL Green-Nano-ZnO 2 mg/kg. There was a significant variation between 10 BL Control, 10 BL Green-Nano-ZnO 10 mg/kg, and 10 BL Green-Nano-ZnO 50 mg/kg. The largest quantity of protein (45.8%) was discovered in 0 Green-Nano-ZnO 2 mg/kg, while the lowest amount of protein (9.3%) was found in 10 BL Green-Nano-ZnO 50 mg/kg. The high values of protein might have been caused by the high levels of protein that *Pleurotus ostreatus* contains. These increases in the nutritional composition of the substrates point to an alternative biotransformation of agro-waste for the generation of aggregate products as a source of animal protein, particularly since the use of agro-waste for animal feed is limited by low digestibility and nutrient availability (Sanchez & Royse, 2001).

Different broiler litter and nanoparticle concentrations substantially altered the quantity of ether extract, although all treatments were inconsequential. The maximum quantity of ether extract (3.81%) and the lowest amount of ether extract (0.32%) were detected in the 10 BL control. The percentage fat content of spent mushrooms generated by broiler litter levels and nanoparticle concentrations demonstrates that mushrooms are low in fat and cholesterol. The ash content varied significantly due to the use of different broiler litter levels, however, there was no significant change in nanoparticle concentrations. The highest ash level was obtained at 10 BL control (3.81%), while the lowest ash content was reported at 10 BL Conv-Nano-ZnO 2 mg/kg (1.25%). Because broiler litter is organic, it affects the ash concentration. The ash level of this spent mushroom substrate was quite low which may be utilised as a substitute for animal manure and feedstuff.

The use of various broiler litter resulted in a highly significant change in neutral detergent fibre and acid detergent fibre concentration. The greatest neutral detergent fibre content was established to be from the diet of 5 BL Conv-Nano-ZnO (94.23%), while the lowest was found to be 10 BL Green-Nano-ZnO (85.73%). The highest acidic detergent fibre concentration was obtained in 10 BL Green-Nano-ZnO 10 mg/kg (85.87%) and the lowest in 0 BL Conv-Nano-ZnO 2 mg/kg (70.59%). The findings in their fibre content are equally noteworthy and exciting, as fibre content aids digestion in animals.

5.6 Conclusion

The findings of this study revealed that samples of *Pleurotus ostreatus* fruiting bodies and SMS produced using substrate inorganic and organic culture techniques such as broiler litter and nanoparticle concentration methods were high in macro- and micronutrients such as N, P, Mg, and Ca. They were also rich in trace minerals including Fe, Zn, Cu, and Mn. The heavy metals that were found were in levels falling within the acceptable human food safety levels. From the results, the various substrates utilised in this study generated mushrooms high in K, P, Mg, and Fe, all of which are vital for human nutrition and health.

There are various advantages to using agricultural waste as a feed element. It is a cost-effective utilization of local resources. This application will aid in the resolution of environmental issues that may arise as a consequence of the accumulation of agricultural waste. It may provide economic incentives for agribusiness to investigate these leftovers

as important resources and build new businesses to exploit them to make nutritional mushroom products. As a result, mushroom growing might become one of the most successful agribusinesses, producing food items from various substrates and assisting in their disposal in an environmentally beneficial manner.

16. REFERENCES

- AOAC (Association of Official Analytical Chemists), 1990. Official methods of analysis
- Ávila VS, Oliveira U, Figueiredo EAP, Costa CAF, Abreu VMN, Rosa PS. 2008. Avaliação de materiais alternativos em substituição à maravalha como cama de aviário. *Revista Brasileira de Zootecnia*, 37, pp.273-7.
- Alborés, S., Pianzolla, M., Soubes, M., Cerdeiras, M.P. 2006. Biodeterioration of agroindustrial wastes by *Pleurotus* spp. for its use as ruminant feed. *Electronic Journal of Biotechnology*. 9, pp.215-220.
- Bhuvnesh, S, Shilpi, T., Khasa, Y.P., Akshaya, G., Puniya, A.K., Kuhad, R.C. 2011. White-rot fungal conversion of wheat straw to energy-rich cattle feed. *Biodegradation*, 22(4), pp.823–831
- Bolan, N.S., Szogi, A.A., Chudasavathi, T., Seshadri, B., Rothrock, M.J., Panneerselvam, P. 2010. Uses and management of poultry litter. *World's Poultry Science Journal*, 66, pp.673-98. doi:10.1017/S0043933910000656
- Chang, S. T. and Miles, P.G. 1992. Mushroom biology—a new discipline. *The Mycologist* 6, pp.64-65
- Cibulka, J., Sisak, L., Pulkrab, K., Miholova, D., Szakova, J., Fucikova, A et. al. 1996. Cadmium, lead, mercury and caesium levels in wild mushrooms and forest berries from different localities of the Czech Republic. *Scientia Agriculturae Bohemica*, 27, pp. 113– 129.
- Dao TH, Zhang H. Rapid composition and source screening of heterogeneous poultry litter by x-ray fluorescence spectrometry. 2007. *Annals of Environmental Science and Toxicology*. ,1, pp.69-79.
- Demirbas, A. 2000. Accumulation of heavy metals in some edible mushrooms from Turkey. *Food Chemistry*, 68, pp. 415–419
- Falandysz, J., Szymczyk, K., Ichihashi, H., Bielawski, L., Gucia, M., Frankowska, A., Yamasaki, S. 2001. ICP/MS and ICP/AES elemental analysis (38 elements) of edible wild mushrooms growing in Poland. *Food Additives & Contaminants*. 18(6), pp. 503–513.
- FAO/WHO, Joint Expert Committee on Food Additives, Fiftyninth meeting. 2004. P (www.fao.org/es/ESN/Jecfa/59corr.pdf) accessed 20th June, 2017

- Food and Agricultural Organization (FAO). 2012. The State of Food Insecurity in the World. (F.-c. d. repository, ed.). Rome, Italy: Food and Agriculture Organization, Communication Division.
- Gascho, G.J., and R.K. Hubbard. 2006. Long-term impact of broiler litter on chemical properties of a Coastal Plain soil. *Journal Of Soil Water Conservation*, 61, pp.65–74.
- Gebrelibanos, M., Megersa, N., Taddesse, A.M. 2016. Levels of Essential and Non-Essential Metals in Edible Mushrooms Cultivated in Haramaya, Ethiopia. *International Journal Of Food Contaminants*, 3, p. 2.
- He, Z., C.W., Honeycutt, I.A., Tazisong, Z.N., Senwo, and D. Zhang. 2009a. Nitrogen and phosphorus accumulation in pasture soil from repeated poultry litter application. *Communications in Soil Science and Plant Analysis*. 40, pp. 587–598.
- Hubbard, L.E., Givens, C.E., Griffin, D.W., Iwanowicz, L.R., Meyer, M.T. and Kolpin, D.W., 2020. Poultry litter as potential source of pathogens and other contaminants in groundwater and surface water proximal to large-scale confined poultry feeding operations. *Science of The Total Environment*, 735, p.139459.
- Jin, Z., Hou, Q. and Niu, T., 2020. Effect of cultivating *Pleurotus ostreatus* on substrates supplemented with herb residues on yield characteristics, substrates degradation, and fruiting bodies' properties. *Journal of the Science of Food and Agriculture*, 100(13), pp.4901-4910.
- Kalac, P., Niznanska, M., Berilaqua, D., and Staskova, I., 1996. Concentrations of mercury, copper, cadmium and lead in the fruiting bodies of edible mushrooms in the vicinity of a mercury smelter and copper smelter. *Science of the Total Environment*. 177, 251,p. 8.
- Khan, M.W., Ali, M.A., Khan, N.A., Khan, M.A., Rehman, A. and Javed, N.J.P.J.B., 2013. Effect of different levels of lime and pH on mycelial growth and production efficiency of oyster mushroom (*Pleurotus spp.*). *Pakistan Journal of Botany*, 45(1), pp.297-302.
- Kurtzman RH, Zadrzil F (1982) Physiological and taxonomic considerations for cultivation of *Pleurotus* mushrooms. In Chang ST, Quimio TH (Eds.) *Tropical Mushrooms: Biological Nature and Cultivation Methods*. Chinese University Press. Hong Kong, China. pp.299-348.
- Lourenço, K.S., Corrêa, J.C, Ernani, P.R., Lopes, L.D.S., Nicoloso, R.D.S. 2013. Nutrient uptake and yield of common bean fertilized with poultry litters and mineral nutrients.

Revista Brasileira de Ciência do Solo, 37, pp. 462-71. doi:10.1590/S0100-06832013000200017

- Maher, M.J. 1991. Spent mushroom compost (SMC) as a nutrient in peat based potting substrates. In Maher MJ (Ed.) *Science and Cultivation of Edible Fungi*. Balkema. Rotterdam, Holland. pp. 645-650.
- Mangalgiri, K.P., Adak, A. and Blaney, L., 2015. Organoarsenicals in poultry litter: detection, fate, and toxicity. *Environment International*, 75, pp.68-80.
- Micha, R., Wallace, S.K. & Mozaffarian, D. 2010. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus a systematic review and meta-analysis. *Circulation*, 121, pp. 2271-2283.
- Miles, P.G. and Chang, S.T. 1997. *Mushrooms Biology: Concise Basics and Current Developments*. Word Scientific. Singapore. 194.
- Mohamed, M.F., Refaei, E.F., Abdalla, M.M. and Abdelgalil, S.H., 2016. Fruiting bodies yield of oyster mushroom (*Pleurotus columbinus*) as affected by different portions of compost in the substrate. *International Journal of Recycling of Organic Waste in Agriculture*, 5, pp.281-288.
- Molena, O. 1986. *O Moderno Cultivo de Cogumelos*. Nobel. São Paulo, Brazil. P.170
- Nas, F.S. and Ali, M. 2018. The effect of lead on plants in terms of growing and biochemical parameters: a review. *Ecology & Environmental Sciences*, 3(4), pp.265-268.
- Nasehi, M., Torbatinejada, N.M., Zerehdaranb S., Safaiee, A.S. 2017. Effect of solid-state fermentation by oyster mushroom (*Pleurotus florida*) on nutritive value of some agro by-products. *Journal of Applied Animal Research*, 45(1), pp.221–226
- Ngodigha, E.M. and Owen, O.J. 2009. Evaluation of the bacteriological characteristics of poultry litter as feedstuff for cattle. *Scientific Research Essays*, 4(3), pp.188-190.
- Nuorteva P, Autio S, Lehtonen J, Lepistö A, Ojala S, Seppänen A, Tulisalo E, Veide P, Viipuri J, & Willamo R. 1986 – Levels of iron, aluminium, zinc, cadmium and mercury in plants growing in the surroundings of an acidified and a non– acidified lake in Espoo, Finland. *Annales Botanici Fennici*, 23, pp.333–340.
- Obodai, M., Sawyerr LCB, and Johnson, P.N.T. 2000. Yield of seven strains of oyster mushrooms (*Pleurotus* spp.) grown on composted sawdust of *Triplochiton scleroxylon*. *Tropical Science*, 40 (2), pp.95– 99.

- Pavel K, Lubomír S, Božena H. 2004. Contents of detrimental metals mercury, cadmium, and lead in wild growing edible mushrooms, A review. *Energy Education Science and Technology*, 13(1), pp.31– 38
- Pender, C., 2016. The importance of gut health in antibiotic-free production. *Science and Solutions*, 27, pp.2-5.
- Phillips R. 2006 – Mushrooms. Pub. McMillan, ISBN 0– 330– 44237– 6. P. 266.
- Pollak, M and Favoino E. 2004. Heavy metals and organic compounds from wastes used as organic fertilisers. Final Report. Env. A.2./ETU/2001/0024
- Robertson, J. B and P. J. VanSoest.1981.The detergent analysis system and its application to human food. *The Analysis of Dietary Fiber*, pp. 123-129.W.P.T.James and O.The ander, edited by Marcel Dekker, New York, NY
- Rogeri, D.A., Ernani, P.R., Mantovani, A., Lourenço, K.S. 2016. Composition of Poultry Litter in Southern Brazil. *Rev Bras Cienc Solo*. 40:e0140697
- Sharpley, A.N., T. Daniel, T. Sims, L. Lemunyon, R. Stevens, and R. Parry. 2003. Agricultural phosphorus and eutrophication. 2nd ed. ARS-149. USDA– ARS, Pasture Systems & Watershed Management Research Unit, University Park, PA.
- Shepherd, M.W.; Liang, P.; Jiang, X.; Aoyla, M.P.; Erickson, M.C. 2007. Fate of *Escherichia coli* 0157:H7 during on-farm dairy manure-based composting. *Journal of Food Protection*, 70, pp.2708–2716.
- Sithole, S.C., Mugivhisa, L.L., Amoo, S.O., Olowoyo, J.O.,2017. Pattern and concentrations of heavy metal sin mushrooms harvested from heavy metal polluted soils in Pretoria, South Africa. *South African Journal of Botany*,108, pp.315–320.
- Sithole, S.C., Agboola, O.O., Mugivhisa, L.L., Amoo, S.O. and Olowoyo, J.O., 2022. Elemental concentration of heavy metals in oyster mushrooms grown on mine polluted soils in Pretoria, South Africa. *Journal of King Saud University-Science*, 34(2), p.101763.
- Tasistro, A.S., Kissel, D.E., Bush, P.B. 2004. Spatial variability of broiler litter composition in a chicken house. *Journal of Applied Poultry Research*,13, pp.29-43.
- Tyler, G. 1980. Metals in sporophores of basidiomycetes. *Transactions of the British Mycological Society*.74(1), pp.41–49.

- Van Soest, P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*. 1991; 74, pp.3583–3597. PMID:1660498
- Waldrip, H.M., Pagliari, P.H., He, Z., Harmel, R.D., Cole, N.A. and Zhang, M., 2015. Legacy phosphorus in calcareous soils: Effects of long-term poultry litter application. *Soil Science Society of America Journal*, 79(6), pp.1601-1614.
- WHO. Exposure to Cadmium: A Major Public Health Concern; WHO: Geneva, Switzerland, 2019
- Wilkinson, K.G.; Tee, I.E.; Tomkins, R.B.; Hepworth, G.; Premier, R. Effect of heating and aging of poultry litter on the persistence of enteric bacteria. *Poultry Science*, 2011, 90, pp.10–18.
- Yilka, T. 2015. Role of white rot fungi as a biological treatment of low quality animal feeds: Review. *Scholarly Journal of Agricultural Science*, , 5(7), pp.247–255.
- Zhang, T., Y. Yang, L. Liu, Y. Han, G. Ren, G. Yang. 2014. Improved Biogas Production from Chicken Manure Anaerobic Digestion Using Cereal Residues as Co-substrates. *American Chemical Society. Energy Fuels*, 28, pp. 2490-2495.

17. CHAPTER SIX

6.1 General Discussion

The study on the effect of biofortification of liver seed grass with different broiler litter levels and nanoparticle concentration on the development and yield of *Pleurotus ostreatus* revealed that liver seed grass biofortified by broiler litter and nanoparticle concentration (5 Green-Nano-ZnO 2mg/kg) generally had higher mushroom yield than other biofortified substrates with broiler litter and nanoparticle concentration recording the highest total yield after 90 days producing a mean yield of 309.63 (51.61%). Biofortifying liver seed grass with sufficient broiler litter and nanoparticle concentration has a significant impact on

mushroom biomass accumulation. The non-biofortified treatments had an overall conversion efficiency ratio of 25.35% at liver seed grass. The decrease in biomass accumulation in other biofortified treatments could be explained by the fact that most of the broiler litter and nanoparticle concentrations, which are known to have high nitrogen content, and nanoparticles have an antifungal effect, could have had a similar effect on the oyster mushroom. Oyster mushroom production is hindered by excessive nitrogen levels in the substrate. The high nitrogen levels may have resulted in higher mycelial growth and a greater number of pinnings, resulting in intra-competition between the various mushroom growth components, namely pinnings and aggressively developing mycelia. The biomass produced by these nanoparticle concentrations and broiler litter treatments was most likely partitioned and sequestered in a large number of tiny mushroom fruit caps, resulting in a poor overall yield.

This low yield was also quite evident in that 80% of the experimental units did not produce fruiting bodies. Furthermore, SMS from these treatments were found to have accumulated high levels of both macro and micro-nutrients. The accumulation coefficient values of macro- and micronutrients, as well as heavy metals, are shown in Table 4.8 for the various treatment combinations. The accumulation coefficient values for micronutrients such as Fe, Zn, and Mg, which are crucial for human nutrition, were considerably higher in the biofortified treatments 0 BL Control and 5 Green-Nano-ZnO 2mg/kg. It is worth noting that the treatment combinations 0 Green-Nano-ZnO 10mg/kg, 0 Green-Nano-ZnO 50mg/kg, 5 Green-Nano-ZnO 50mg/kg, and 5 Conv-Nano-ZnO 2mg/kg had the lowest heavy metal accumulation coefficient values. These values are within the acceptable food safety standards (Stihi *et al.*, 2011). Biofortified treatments with broiler litter and nanoparticle concentration were also found to have increased transfer efficiency of chemical composition such as neutral detergent fibre (5 Conv-Nano-ZnO 2 mg/kg), acid detergent fibre (10 Green-Nano-ZnO 10 mg/kg), acid detergent lignin (5 Green-Nano-ZnO 50 mg/kg), ash (10 BL Control), ether (10 BL Control), dry matter (10 BL Bulk-ZnO). The study established that oyster SMS has the potential for use as livestock feed for both poultry and other farm animals.

The overall conclusion of this study is that the type of substrate utilised to produce oyster mushrooms has a significant impact on production. In this study, the conversion ratio of liver seed grass biofortified with 5% broiler litter and conventional-Nano-ZnO in the study region was 51.61%. It is critical to study several alternative widely accessible organic materials for their possible usage as oyster mushroom substrates. Although the use of

broiler litter and nanoparticles did not influence mushroom yield, more research is needed to determine the appropriate combination rates of the relevant broiler litter and nanoparticles produced from various plant parts.

References

- Stihi, C., Radulescu, C., Buscuiou, G., Popescu, I.V., Gheboianu, A., and Ene, A. (2011). Studies on accumulation of heavy metals from substrate to edible wild mushrooms. Bucharest. Romanian Journal of Physics, 56 (1-2), pp. 257-264.

