COMPARATIVE STUDY ON THE QUALITY OF NAMIBIAN COMMERCIAL OYSTER MUSHROOMS CULTIVATED ON ENCROACHER BUSHES AS SUBSTRATES

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ABSTRACT

Humans need a wide range of nutrients to have a well-balanced diet, nutritious edible mushrooms can be part of a healthy lifestyle. The objectives of the study were to evaluate the yield and yield components, to determine antioxidant activity, total phenolic content (TPC), total flavonoid content (TFC) and nutrient composition of different mushroom species cultivated on four different bush encroacher substrates. To provide mushroom cultivation training to farmers in Okondjatu district. Mushrooms were cultivated in 2kg bags of dry substrate, harvested, dried and powdered. Mushrooms powder was extracted in 99.9% methanol. Antioxidant activity of the mushroom extracts were analysed by reducing power and 2, 2 Diphenylpicrylhydrazyl (DPPH) assay. The TPC and TFC were quantified. Crude protein was calculated (N x 6.25) from determining the amount of nitrogen (N) in mushroom powder using the Dumas combustion method. Potassium and Phosphorus were determined by using the flame emission spectroscopy, while Phosphorus was determined by colorimetry. Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) were analyzed using the Ankom 220 fibre analyzer unit. Mushroom cultivation training was conducted at three randomly selected villages. There was a significance difference in weight of fresh mushrooms, days to first harvest and number of fruiting bodies which was due to mushroom species used at $p=0.002$, $p<0.001$ and $p=0.019$ respectively. *Pleurotus florida* (PF) yielded more mushrooms with 350 g, followed by PS with 322 g, followed by *Pleurotus ostreatus* (PO) with 314 g, and the least was on HK 35 with 243 g. The inhibitory concentration at 50% (IC50) values ranged from 0.380±0.098 mg/ml to 0.780±0.007 mg/ml on different mushroom species grown. The inhibition of the DPPH radical by PO extracts cultivated on *Terminelia sericea*

(0.380±0.007 mg/ml) showed the highest activity. All Mushroom species grown on different bushes showed an appreciable ability to reduce ferricyanide complex to ferrous form at different concentrations (0.063–1.00 mg/mL). The TPC varied from 3.93 mg GAE/g to 8.016 mg GAE/g. TFC ranged from 0.515 mg QE/g to 12.1 mg QE/g, which showed a significant difference on the bush species (p-value < 0.01). Crude protein content ranged from 28.0% to 39.0%. A significant difference in % NDF on mushroom species was found, where a variation was detected in HK35 while PF, PO, and PS showed a similar performance in % NDF. The study showed that ash content ranged from 5.77% to 17.9%. Percentage moisture ranged from 86.0% to 87.5%. A significant difference in mineral composition, with PO showing a high content of potassium (2.292%), PF showing a high content of sodium (0.059%), and PS showing a high content of phosphorus (0.746%) as compared to other mushroom species. A total of 45 community members participated in the training. In conclusion, all four encroaching bushes can be used as the substrate for mushroom cultivation. All mushroom species showed antioxidant activity and appreciable nutrient and mineral composition, thus can be used as a remedy to illnesses and a supplement to human diets. Therefore, it is essential to promote awareness and train farmers on mushroom cultivation.

Keywords: Bush encroachment, Minerals, Mushrooms, Nutrients, Training

LIST OF PUBLICATION(S)/CONFERENCE(S) PROCEEDINGS

1. Haukongo K.N, Horn L.N, Tjiurutue M.C Effects of different substrates as medium for mushroom cultivation. Acad. J. Food. (2021) 9(2): 032-037.
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LIST OF ABBREVIATIONS AND ACRONYMS

°C:	Degree Celsius
µg:	Microgram
µL:	Microliter
ADF:	Acid Detergent Fibre
AIDS:	Acquired Immune Deficiency Syndrome
ANOVA:	Analysis of Variance
BHA:	butylated hydroxyanisole
BHT:	Butylated Hydroxytoluene
CaCO3:	Calcium Carbonate
COVID 19:	Coronavirus disease 2019
CUPRAC:	Cupric reducing antioxidant capacity
DDT:	Dichlorodiphenyltrichloroethane
DMSO:	Dimethyl sulfoxide
DNA:	Deoxyribonucleic acid
DPPH:	1,1-diphenyl-2-picrylhydrazyl
FAO:	Food and Agriculture
FC:	Folin-Ciocaliteu
FMB:	Forest Management Body
FRAP:	Ferric ion reducing antioxidant power
G:	Gram
GIZ:	Deutsche Gesellschaft für Internationale Zusammenarbeit
H_2O_2:	Hydrogen peroxide

HCl:	Hydrochloric acid
HIV:	Human Immunodeficiency Virus
HORAC:	Hydroxyl Radical Antioxidant Capacity
HPLC:	High Pressure Liquid Chromatography
IC50:	50 % Inhibitory concentration
LDL:	Low-density lipoprotein
MCI:	Mild Cognitive Impairment
Mg:	Milligram
Ml:	Millilitre
NDF:	Neutral Detergent Fibre
Nm:	Nanometre
ORAC:	Oxygen Radical Absorbance Capacity
PC:	Phenolic compounds
PH:	Potential of hydrogen
ROS:	Reactive Oxygen Species
RUFORUM:	Regional University Forum
SCFA:	Short Chain Fatty acids
SE:	Standard Error
SMS:	Spent Mushroom Substrates
SPSS:	Statistical Package of Social Sciences
TRAP:	Telomeric repeat amplification protocol
UK:	United Kingdom
UNAM:	University of Namibia
USA:	United States of America

UV:	Ultraviolet
ZERI:	Zero Emission Research Initiative

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DEDICATION

Firstly, I would like to dedicate this study to the Almighty God for his divine wisdom, a healthy life and strength in order to carry out this study. He has been my great lifter and pillar during the times I felt like giving up.

I would like to wholeheartedly dedicate this work to my friends and family, who have been a great inspiration who continually provide their spiritual, emotional, and financial support to finish this study. My parents Wilbard Haukongo and Susan Paulus, thank you for motivating me to work hard and for always advising me not to give up even at times where it seems impossible. May God bless you!

DECLARATION

I, Kristine Ndeuyamunye Haukongo, hereby declare that this research work is a true reflection of my own work. Information derived from other sources have been duly referenced and acknowledged explicitly in the text. No part of this work has been written for me by another person nor copied from any student or other sources.

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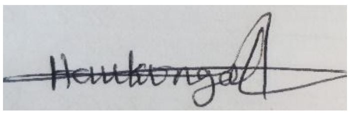

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Kristine Ndeuyamunye Haukongo

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The swift increase in bush encroachment and unabated drought has raised concerns on how the government, scientists and business leaders are going to overcome these crises in Namibia [1]. In addition, malnutrition is among the problems Namibia is currently facing and it is of great significance that every human being has access to a balanced diet, food security and healthy living conditions. In the past, many people depended on traditional farming for animal feed, human food, and income. However, due to natural and artificial disasters there is a gradual decrease in traditional farming that can no longer sustain sufficient fodder for livestock and produce enough food for the growing population [2, 3]**.** In addition, the rise in unemployment and abnormal increase of food prices is alarming. Therefore, it is critical to develop and innovate alternative methods to overcome these challenges [4]. For instance, finding alternative methods to cultivate mushrooms while increasing yield, nutrition and simultaneously reducing the bush encroachment problem. This ensures food security, reduce competition for livestock and substrate for mushrooms can be transformative.

Bush encroachment is of huge concern to Namibian farmers, as it reduces the availability of fodder/grass for livestock consumption [1]. Generally, Namibian savannas are very dry with poor soil fertility, resulting in limited grass for animal grazing [5]. Moreover, different substrates such as grass and manure used for mushroom cultivation creates competition between livestock, plants and mushroom cultivation. However, an alternative management method would involve using encroacher bushes as substrate for growing mushrooms with more economical incentives [1, 5]. Currently, encroacher bushes are locally used for firewood, make droppers for construction, power generations, bio-oil or bio-gases at the industrial level, animal feed, compost for agricultural application, but not for mushroom cultivation [6, 7]. Various organizations such as Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), Regional Universities Forum (RUFORUM) in collaboration with the University of Namibia, and the Namibian Government have initiated projects to combat bush encroachment problems in one of the most economically viable processes for the bioconversion of bush encroacher wastes is the cultivation of edible mushrooms [5, 7]. One such project to combat bush encroacher is based in the Okondjatu constituency of the Otjozondjupa Region in Namibia (UNAM-RUFORUM PROJECT).

Mushrooms, which can improve food security, are highly nutritious, can have great therapeutic effects and can contribute to reduction of bush encroachment in Namibia. Among some of the importance of mushrooms are their nutritional and medicinal properties as well as a wide variety of antioxidant activities that comprises phenolic compounds [8]. Oxidative stress is a result of highly reactive oxygen species (ROS) that contain an unpaired electron [9]. An accumulation of ROS in the human body causes ageing, cancer, cellular injury, hepatic, neurodegenerative, renal disorders and cardiovascular disease [9]. There are numerous factors that contribute to the production of ROS, this includes environmental pollutants such as heavy metals, chemicals and toxins, deep fried foods, radiation, and stress [10]. The human body produces antioxidant substances such as vitamin C and E that counteract potential harmful oxidizing agents [11]. However, natural antioxidants cannot sustain excessive generation of oxidizing agents which lends the body into oxidative stress. In addition, ROS leads to the formation of abnormal proteins which results in the depletion of antioxidants in the immune system [9, 11].

Phenolic compounds have the ability to neutralize free radicals which cause oxidative damage in the human body [12]. Phenolics includes stilbenes, coumarins, hydrolysable, phenolic acids. These secondary metabolites act as protective agents against UV light, contributing to plant pigmentation and antioxidants [13]. Phenolics and flavonoids constitute an aromatic ring having at least one hydroxyl group responsible for facilitating free radical scavenging. Furthermore, phenolic compounds have the ability to inhibit peroxide decomposition, inactivate metals and scavenge oxygen in biological systems [12, 14].

Mushroom's nutrient and phenolic content can be different depending on species and also depending on the substrate on which they are grown [15]. Table 1 illustrates results obtained by Gowda et al. [21], on the biochemical composition of mushroom species grown on different substrates. In addition, Flieger et al. [16], emphasised that the antioxidant activity varies depending on morphological parts and difference in the natural resources used during growth. Mushrooms can grow on various substrates including straw, and, importantly bush encroacher species and are increasingly being used as healthy food sources containing proteins, carbohydrates as well as vitamins [17]. Furthermore, mushrooms have been reported as having a wide range of medical benefits such as the ability to halt the spread of breast cancer, colon cancer , immune booster, as well as minimizing constipation [18]. For example, *Ganoderma lucidum* is a large woody and medicinal mushroom commonly known as the Reishi, known to have anti-oxidative properties, antiallergenic, antiviral, antidiabetic and anticancer effects [19, 20].

Table 1: Effect of different substrates on the biochemical constituents of mushrooms. Source: Gowda et al. [21]

Biochemical Parameter	*Pleurotus florida*		*Hypsozygous ulmarius*	
	Paddy Straw	Redgram Husk	Paddy Straw	Redgram Husk
Protein (%)	58.71	28.65	64.10	24.14
Carbohydrates (%)	36.83	29.31	51.76	38.00
Amino acids (%)	18.83	12.76	19.08	08.53

Hence, the present study was undertaken with the overall aim to demonstrate the importance of mushroom cultivation to the farmers in Otjozondjupa region with the objective of analysing the effects of bush encroachment species on mushroom biochemical and yield component aspects to combat the threat of food insecurity and malnutrition diseases. Specifically, the study focuses mainly on nutritional content, total phenolic content, and antioxidant activity of different oyster mushrooms (*Pleurotus florida, Pleurotus sojar caju, Pleurotus ostreatus* and HK35) grown on different bush encroacher substrates from four common encroacher bushes that occur in Namibia. These bushes include *Senegalia mellifera, Terminalia sericea. Grewia flava,* and *Combretum collinum.*

1.2 STATEMENT OF THE PROBLEM

Bush encroachment is widespread in Namibia as stated by de Klerk, [22]. Due to drought, farmers are losing hope in producing food as the majority of farmers depend on rainfall to produce food for both consumption and income. Wild mushroom consumption is limited in Namibia as they are only harvested once during the rainy season. Utilizing grass, maize or millet straw as substrate for mushroom cultivation increases competition between livestock and mushroom producers. Additionally, no thorough study has been thus far conducted on antioxidant, total phenolic content, total flavonoid content, mineral and nutritional composition of mushrooms grown on the four selected bush encroacher species.

1.3 OBJECTIVES OF THE STUDY

a) To evaluate the yield and yield components of different mushrooms grown on four different encroacher bush substrates

b) To determine antioxidant activities of different strains of mushrooms grown on four different encroacher bush substrates.

c) To determine mycochemicals of different mushroom species grown on four different encroacher bush substrates.

d) To determine nutritional composition and mineral content of different mushroom species grown on four different encroacher bush substrates.

e) To train local farmers, especially women and youth on how to produce and manage mushroom production using encroacher bush substrates.

1.4 SIGNIFICANCE OF THE STUDY

The study will provide important insight on how the four different bush encroacher substrates may influence nutritional properties of the mushrooms grown on them. The use of encroacher bushes as mushroom growth substrate will reduce competition between mushroom cultivation and grazing animals. Once people are educated on alternative ways to grow food at their homestead it will make a positive impact to the economy and development of the country, especially given the alarming rise in food prices. In addition, knowledge on the nutritional value and antioxidant potential of different mushroom species will be beneficial in promoting their consumption by local people [23]. This is especially important for the marginalised people in the villages who have lost hope of cultivating foods for themselves due to dry seasons, poverty and lack of know-how of new methods. Communities can be educated on the results of this study through training, awareness raising via YouTube videos and scientific and popular science publications including in open-access online papers. The identification of mushrooms grown on different bush encroachers that are enriched with phenolic compounds, nutrients and minerals may lead to the development of new value-added food products for the Namibian and international food markets.

1.5 LIMITATIONS OF STUDY

Budgetary constraints negatively affected the transportation of adequate encroacher bushes materials required for mushroom substrates. Furthermore, transportation of substrate from Okondjatu to University of Namibia (UNAM) main campus costs are high, due to the quantity required. Lacking efficient analysis equipment, some samples were analysed at the Ministry of Agriculture, Water, and Land Reform (MAWLR). To confirm antioxidant activity, various *in vitro* tests were not conducted such as Oxygen Radical Absorbance Capacity (ORAC), Hydroxyl Oxygen Radical Absorbance Capacity (HORAC), Telomeric Repeat Amplification Protocol (TRAP), Reducing Antioxidant Capacity (CUPRAC) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), assays, due to lack of reagents and equipment necessary to conduct multiple assays which would enable an even more meaningful comparison of the antioxidant activity of different mushroom extracts on various assays. However, in the current study, analysis on antioxidants of mushrooms used DPPH and reducing power assay.

1.6 DELIMITATIONS OF STUDY

Even though there are more bush encroaching species found in the study area, this study only considered four common species of bushes including *S. mellifera, T. sericea, G. flava* and *C. collinum*. The study focused on conducting mushroom cultivation training to one region out of the 14 regions in Namibia. There are many nutritional aspects of mushrooms, however the study focused on five nutritional aspects (crude protein, neutral detergent fibre, acid detergent fibre, ash and moisture) and three major mushroom mineral aspects (phosphorus, potassium and sodium). These delimitations were allowed for a more comprehensive analysis of the different mushrooms within the boundaries of the duration of the study and budget or funds allocated to this specific research.

CHAPTER 2: LITERATURE REVIEW

2.1 MORPHOLOGICAL, TAXONOMICAL AND ECOLOGICAL DISTRIBUTION OF MUSHROOMS

Mushrooms belong to the kingdom of Fungi, a group entirely distinct from that of plants, animals, insects and bacteria [24]. Most fungi do not appear to have the ability to use energy from the sun since they lack chlorophyll, however there is ample evidence that some fungi can indeed thrive using "hard light" in the X- and gamma-ray wavelengths to drive their metabolic processes thus making them in a way "photosynthetic" [25]. Most common fungi including those used for this study depend on other organisms for food, absorbing nutrients from the organic material in which they live [21, 26]. The living body of the fungus is called the mycelium and it is made out of a web of micron-sized threads (or filaments) called hyphae. Under specific conditions, sexually compatible hyphae will fuse and start to form spores which later develop into a mushroom [23]. Fungi multiply by producing millions of spores; the haploid spore is produced by mitosis from the haploid parent cell [24]. When a spore undergoes an ideal suitable environment (moisture, temperature, and nutrients), it can germinate and branch to form mycelium. When two sexually compatible mycelia meet, they may fuse to form a so-called secondary mycelium, which is capable of forming fruiting bodies [23, 26]. Cultivated mushrooms are the fruiting bodies of basidiomycetes which are saprobic, meaning they feed on dead, decaying organic matter [27].

Mushroom cultivation, production, and import is encountering great development in various world regions. According to Sharma *et al.* [28], scientific cultivation of mushrooms began in the early 20th century when pure cultures of mushrooms were prepared from spores and mushroom tissue. In 2016, the global production of mushrooms was over 10 million metric tons, including almost 8 million tons in China. In 2020, China was the highest mushroom producer with 9 million, followed by Japan, USA, Poland and Netherland as illustrated in Figure 1 below [29]. Africa has the least production of mushrooms of about 0.4% to the global market [30]. Moreover, mushroom cultivation is now an ongoing practice worldwide due to an increase in food demand and due to its enriched medicinal features to alleviate emerging and persistence of diseases globally [29]. Celik *et al*. [31], stated that crop and animal farming is a major practice in African countries which is mostly done on a small scale in rural areas and with the continually growth in the population, rural farming can no longer satisfy the population. Thus, the diversification of farming to these areas is crucial in order to reduce unemployment and secure food security and improve the economy in developing countries [32]. Every year, South African farmers make about 21 000 tonnes of mushrooms. This accounts for roughly 11% of all global production and is the second-highest quantity in Africa after Nigeria. Although South Africa provides the majority of its own needs and is a net exporter, it also exports to nations like Namibia and Mauritius [33]. It has a well-established mushroom industry, with a large number of commercial mushroom farms producing a variety of mushroom species, including button mushrooms, shiitake mushrooms, oyster mushrooms, and portobello mushrooms. Other African countries where mushrooms are grown include Kenya, Uganda, Tanzania, Rwanda, Nigeria, and Ghana [29].

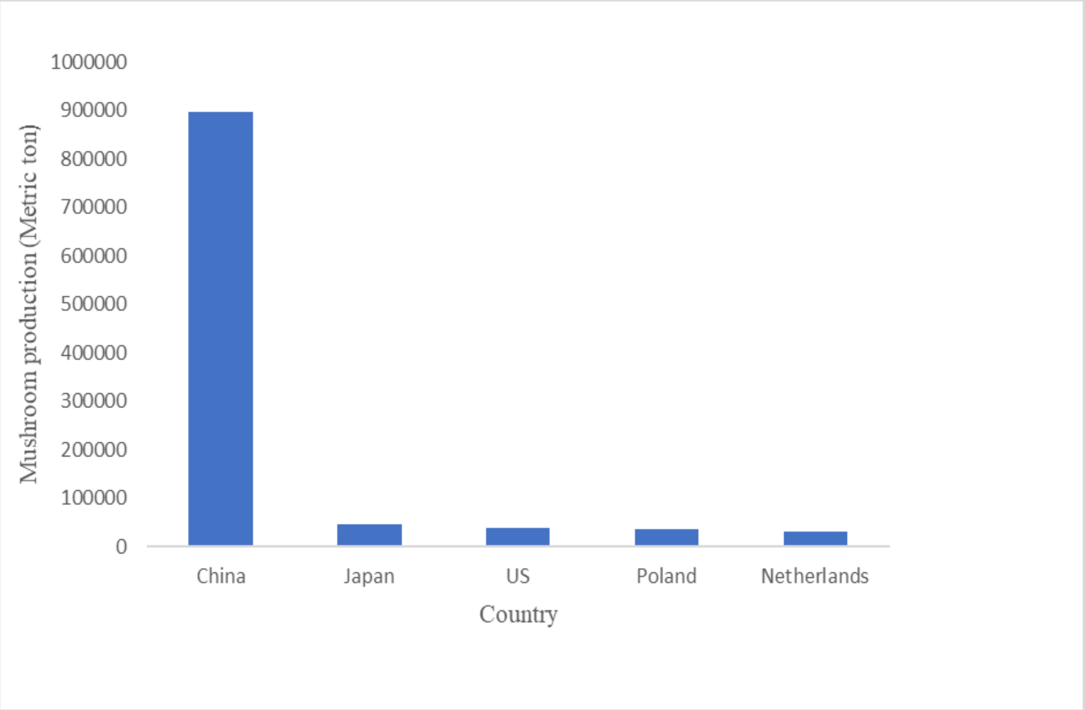

Figure 1: Mushroom and truffles production volume in 2019 (in MT). Source: 2020 industry report: Mushroom [28]

2.2 MUSHROOM PRODUCTION IN NAMIBIA

Mushroom cultivation in Namibia is not a common practice, however the Zero Emissions Research Initiative (ZERI) at the University of Namibia promotes research on edible and medicinal mushrooms, cultivation, and value addition of indigenous mushrooms [35]. Mushroom projects such as Tulonga community that was based in Henties Bay in the Erongo region stopped operating due lack of adequate sustainability, electricity, funds and water. Many farmers fail in the production of mushrooms because they do not have enough funds to keep the farm operating, lack of good spawn, poor sustainability or negligence which leads to high contamination rate [32, 33]. Furthermore, many people who are interested in mushrooms have little or no information on how to grow and manage mushrooms. Some farmers encounter termites, mites, unsuitable temperatures or weather leading to fluctuations in relative humidity and some travel long distances for spawn suppliers and a lot are missing out on the preservation methods of the fruiting bodies [2]. This gives a clear indication as to why mushrooms have not been commonly adopted as a cash crop among Namibian farmers. Mushroom farming could alleviate poverty among Namibian youth and women due to small space requirements, fast growth and no weeding requirement [32]. Mushrooms can be harvested several times per year which increases the rate of investment returns, but this is hardly ever attained due to the standards of living and household conditions exacerbated by Namibia being such an arid country [2, 31]. The annual income for most individuals is very low, which stands in the way to afford the upfront investment in equipment and education necessary to start growing lucrative mushrooms [36]. Additionally, people lack proper education on the benefits of mushroom cultivation and consumption which may result in stigma to those people who have interest in mushroom cultivation by receiving negativity from other community members might minimizes their interest in producing mushrooms [37].

2.3 BUSH ENCROACHMENT AND MYCO-REMEDIATION

Bush encroachment is one of the major ecological problems that is so far reported to have affected over 26 million hectares of grazing land which resulted in economical loss of more than N$ 700 million in Namibia [38]. The objective of this research was to use local encroacher bushes as substrates to grow mushroom varieties, while at the same time creating value for the locals by reducing encroacher bushes' impact on grazing land. The invasion of bushes reduces the availability of grass needed by animals to feed [39]. The environment is congested with dead bushes which are of less importance to our local people and in most cases the dead bushes are either burnt, let to decay or cut down to make fire wood [38]. The main species causing the encroachment problem are *S. mellifera* commonly known as black thorn (Figure 2), *Dichrostachys cinerea* (Sickle bush), *G. flava* (Figure 2), *T. sericea* with a common name Silver terminalia (Figure 3) [40, 41, 42], *Terminalia prunioides* (Purple-pod terminalia), *Acacia erubescens* (Blue thorn), *Acacia reficiens* (False umbrella thorn1), *C. collinum* (Figure 3) and *Colophospermum mopane* (Mopane) [43, 44]. These bushes are native to parts of Africa, including Angola, Botswana, Kenya, Malawi, Mozambique, Namibia, South Africa, Tanzania, Zambia, and Zimbabwe. Within these regions, they are typically found in savannas, woodlands, and grasslands, growing on a range of soils, including sandy, rocky, and clay soils. It is often found growing in association with other woody species, particularly in the understory of larger trees. The distribution of *S. mellifera* is relatively widespread in its native range, although it may be more common in certain areas than others. It is also sometimes cultivated outside of its native range as an ornamental tree, particularly in areas with a similar climate [45].

Nevertheless, value can be added to these encroaching bushes when they are used to produce something useful to the community such as mushrooms. The unwanted bushes can be chopped or milled into sawdust, on which mushroom mycelium can feed to obtain energy and nutrition [44]. Educating communities on alternative bush handling methods and creating awareness campaigns to gain interest for the cultivation of mushrooms among community members can contribute to the economic development of Namibia and reduce bush encroachment [38]. A Namibian project called Mycohab is transforming *S. mellifera* into biomaterials (making bricks made from mushroom waste with the aim of building low-cost houses), producing mushrooms which is contributing to food security in Namibia and storing carbon in the bricks which reduces carbon footprint [46]. Carbon footprint refers to the total amount of greenhouse gases, mainly carbon dioxide, that are released into the atmosphere as a result of human activities [47]. These activities can include burning fossil fuels such as coal, oil, and gas, as well as deforestation, agriculture, and transportation. Reducing carbon footprint is important to mitigate the impacts of climate change, and can be achieved through a range of strategies, such as increasing energy efficiency, using renewable energy sources, reducing waste, and changing transportation habits [48]. The cultivation of mushrooms can help to reduce the demand for meat, which is a significant source of greenhouse gas emissions due to the energy required for feed production, transportation, and processing [48]. Many governments, organizations, and individuals are taking steps to reduce their carbon footprint, in order to promote sustainability and protect the environment. In addition, mushroom cultivation has a potential to reduce the carbon footprint of agriculture by providing a sustainable source of protein [45, 47].

Figure 2: Common encroaching bushes S.mellifera (left) and G.flava (right)

Figure 3: Common encroaching bushes T. sericea (left) and C. collinum (right)

Among ways to combat bush encroachment is the application of myco-remediation by Harnessing fungi's natural ability to break down materials for a beneficial effect of mushroom production and cleaning the environment [24]. This involves the usage of mycelium to break down, remove toxins and get rid of heavy metals from the environment by producing lignocellulosic enzymes and use the degraded products to produce fruiting bodies as stated by Stamets [26]. As a result, mushrooms are part of the fungal substances that can be regarded as a significant biotechnological process for reducing agro-industrial waste [49]. Lignocellulosic waste is a main constituent of renewable biomass, containing three major components (Figure 4): cellulose, hemicelluloses and lignin [50]. Cellulose is a polysaccharide that contains lignocellulosic materials that consist of β-1, 4 linked D-glucose units in unbranched linear chains. Hemicellulose is arranged in forms of strips tightly linked with cellulose and lignin in plant cell walls; it contains branched polysaccharides, mainly xylans and mannans. It contains branched heterogeneous polysaccharides of pentose (xylose and arabinose), hexoses (glucose, mannose, and galactose) and sugar acids. Lignin has long chained carbon-hydrogen which enables the wood to be more resistant to decomposition. The chemical structure of lignin comprises three precursors namely, p-coumaryl, coniferyl and sinapyl alcohols [50, 51]. Lignin illustrated in Figure 5 helps in the protection of woody tissues from microbial and fungal attack covering the carbohydrate structure, causing reduced availability of enzymes for hydrolysis [46]. Partial solubility and complexity of the lignin makes it tough for degradation by microorganisms. However, mushrooms secrete enzymes such as quinone reductases strong enough to break down the lignin with carbon dioxide and water being the by-products [52]. Fungal metabolism reduces complex structures like lignin and cellulose into simple forms like chitin, proteins, enzymes, polysaccharides and alcohols [53]. A mushroom web of mycelium attached to the wood shown in Figure 6 feeds on the lignocellulosic wood until all the nutrients are depleted from the substrate [49]. In addition, the mushroom hyphae form aggregations which enables it to perform a variety of ecological functions, including acting as a nutrient and water transport network. Strands of parallel hyphae form the mycelium, a root-like structure that seeks out the fungus's resources until it becomes a fully developed fruiting body [24].

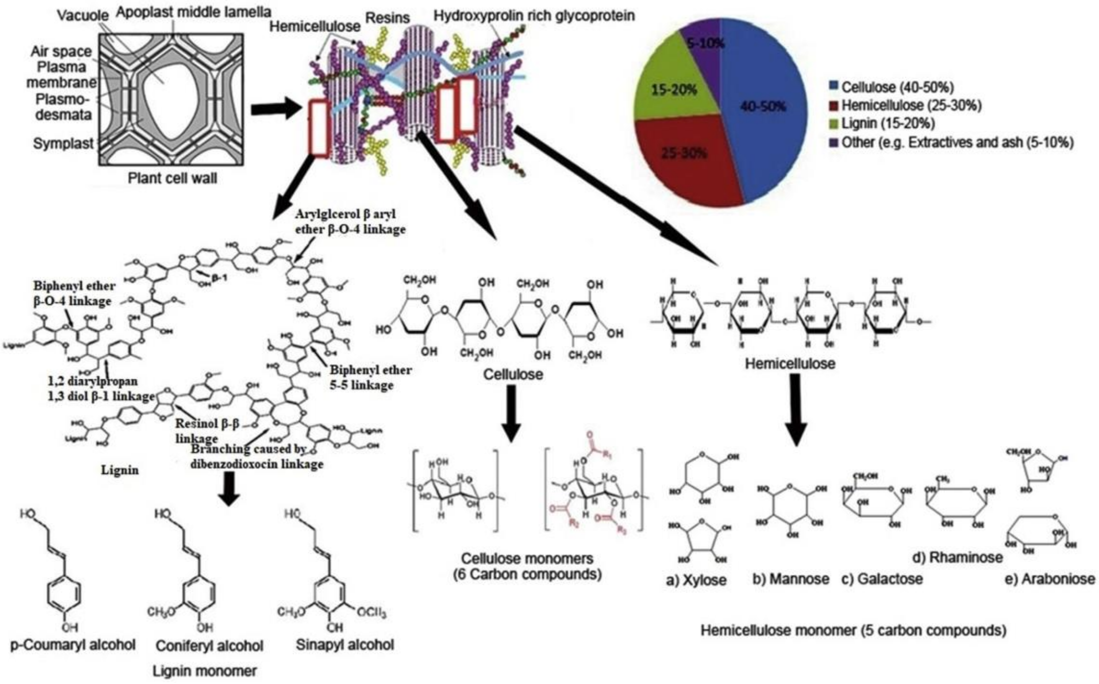

Figure 4: Composition of lignocellulosic waste and structure of primary monomer, cellulose, hemicelluloses and lignin structure and primary monomers: the most frequent bonds are indicated. Sources: Kumar et al. [49]

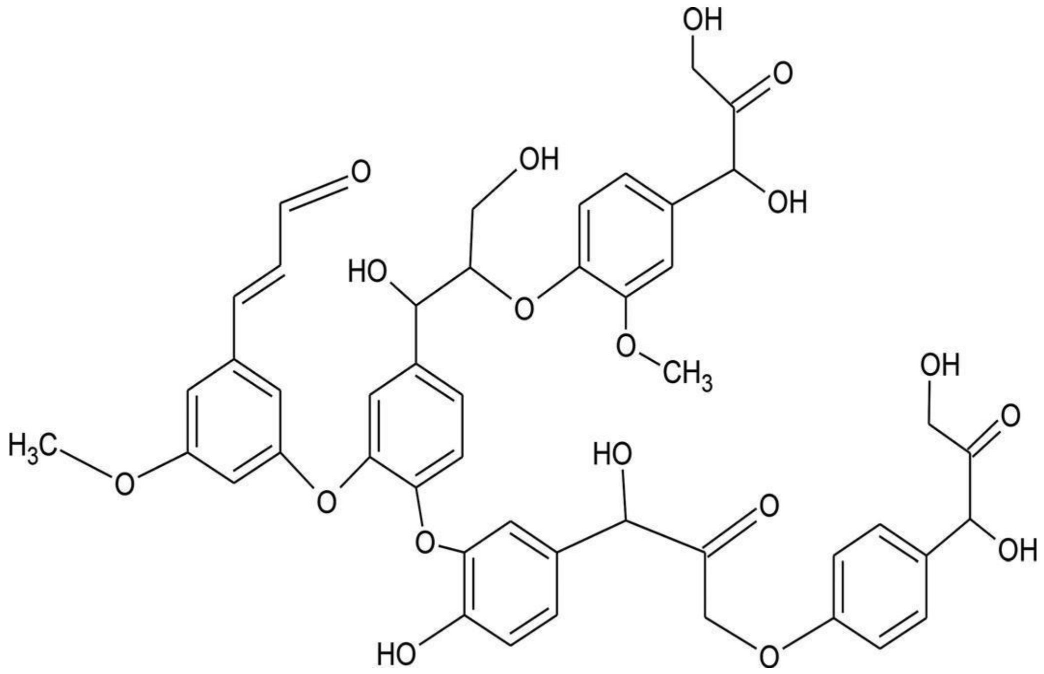

Figure 5: Chemical structure of lignin. Source: Mahmood et al. [50]

Figure 6: Microscopic picture of Mycelium feeding on Senegalia mellifera x10 magnification (left) and x30 magnification (right

2.4 STAGES IN MUSHROOM PRODUCTION

2.4.1 SPAWN PREPARATION

Sharma and Kumar [37] stated that spawn was first introduced at the Pennsylvania State University further explaining that grain spawn is better than manure spawn as it could easily be mixed and in 1962 Stoller improved the process of making spawn which remained unaltered up to date [37]. Mushroom spawn is commonly referred to as mycelium growing on a given substrate, it serves as "mushroom seeds" for planting in mushroom cultivation [53]. To produce spawn, cultivators obtain grains such as wheat grains, rye or sorghum grains and mix with a piece from mycelium culture of a particular mushroom species, the process is called inoculation [54, 55]. According to Stanely et al. [55], after the culture has colonised throughout the grains and becomes fully white, it is called spawn. The process includes the soaking of grains in water overnight, then the grain will be drained of excess water. The grains are then supplemented with wheat bran that is added to facilitate attachment of the mycelium to the grains [56, 57]. The pH can also be controlled by adding chalk ($CaCO_3$) to achieve the preferred value of 6.5 according to Khan et al. [57]. The grain is sterilized or pasteurized prior to the inoculation with mycelial cultures. Once grains are inoculated, incubation can take between 10 and 15 days but this may depend on the weight of grains and mycelia culture inoculated. Spawning can be carried out in transparent bags or containers or bottles for easy inspection [57, 58].

2.4.2 SUBSTRATE PREPARATION AND SPAWNING

A substrate is a bulk material that the mushroom mycelium gets its energy and nutrition from. A good substrate is needed for the mycelium to grow and produce fruit. Common substrates used for growing mushrooms usually involve straw or sawdust, although there are many other good options to be considered [58]. According to Owaid et al. [53], oyster mushrooms can be cultivated using substrate such as rice straws, paddy straw, wheat straw, paper, coffee pulp, cotton waste, cotton seed hulls, corn cobs waste, bean straw, crushed bagasse, and molasses wastes, most of these represent different types of a generalized notion of “agri-waste” (sometimes referred to as “agro wastes”) [54]. Substrate preparation includes the soaking of substrate in water to improve the moisture content and making it easier for the mycelium to break down lignins and cellulose as well as nutrient absorption [55]. Furthermore, soaking helps to destroy all aerobic organisms that might interfere in the colonisation of the mycelium on the substrate [56]. The substrate can be soaked in water for as long as 10 to 12 hours after which it is necessary to drain the excess water. When the substrate is ready, prepared spawning can be carried with caution to prevent contamination by other microorganisms, thus it is performed under sterile conditions [56, 58]. The inoculated substrate is then incubated in a dark room because mycelium colonises best under dark conditions and it inhibits early pinning of the mushrooms (mimics nature) [54, 59]. Fully colonised bags are then transferred to the fruiting room for mushrooms' fruiting bodies to start growing (Light is kept in the fruiting room to promote primordial initiation). The different mushroom cultivation stages are illustrated in Figure 7 below. [59].

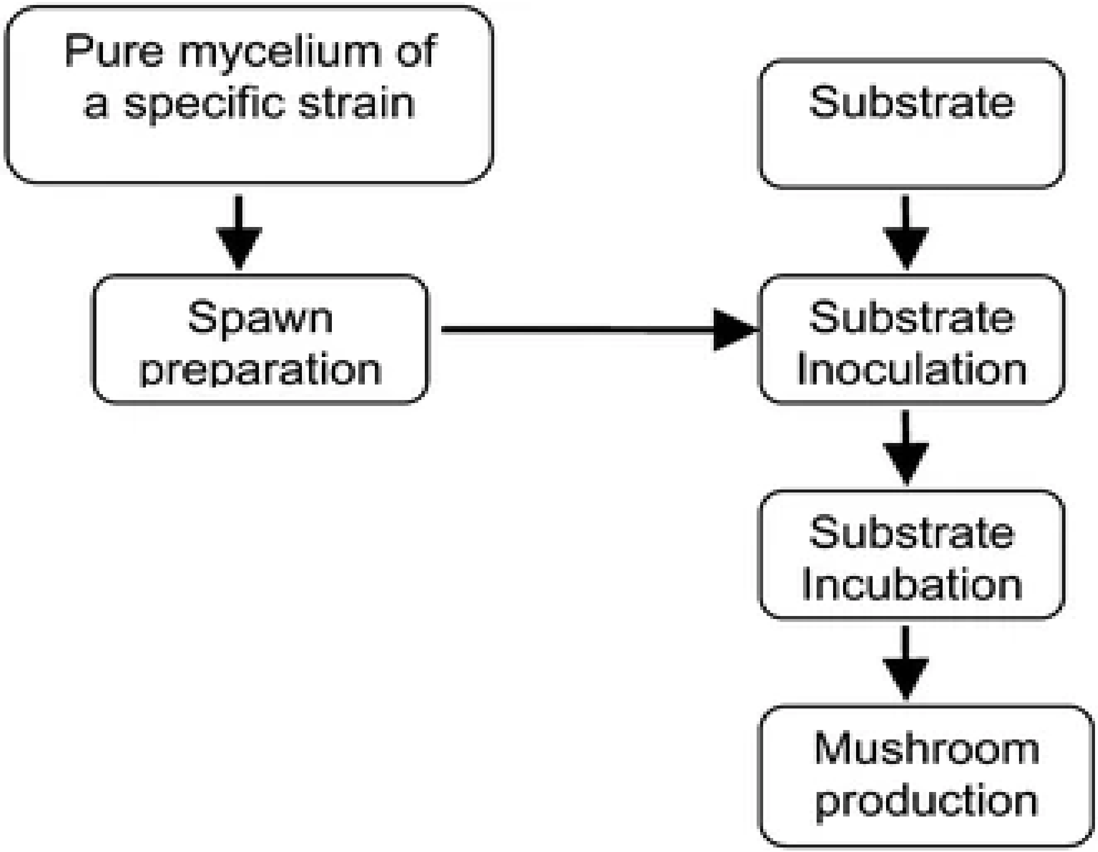

Figure 7: Mushroom cultivation process. Source: Sánchez et al.,[59]

2.5 NUTRITIONAL AND HEALTH BENEFITS OF MUSHROOMS

Edible mushrooms are biological and genetic resources with nutritional value and biotechnological potential [60]. This is applicable for both wild and cultivated mushrooms, nutritionally, mushrooms are low in total calories (energy) and fat but high in protein, carbohydrate and dietary fibre [61]. Furthermore, mushrooms contain a variety of trace elements and minerals; they are exceptionally rich sources of potassium and selenium amongst others. Studies have proven that potassium can help lower high blood pressure, while selenium is an antioxidant that protects cells from free radicals [61, 62]. According to Cheung [62], mushrooms are an important source of biologically active compounds with potential additional medicinal benefits. There are a large number of publications consistently demonstrating the variety of ways in which mushrooms are useful in preventing and treating serious health conditions [63]. The fruiting bodies of edible mushrooms (e.g., *Lentinus edodes*) are mainly consumed by cooking them in their fresh or dried and rehydrated form [64]. While medicinal mushrooms (e.g., *Ganoderma lucidum*) are considered "non-edible" fungi that have biopharmaceutical applications due to the bioactive components such as polysaccharides and triterpenoids [65, 66] and can be prepared as ethanol suspensions, tinctures or teas and brews from whole mushroom powders or spores, alone or in mixtures of several species. In the United States there is a resurgence of interest in powdered mushroom mixes as coffee replacement e.g., Mud Watr company [67].

Various researches have demonstrated that, sufficient dietary fibre intake has benefits for health maintenance and disease prevention including cardiovascular disease, diabetes, cancer and weight regulation [68, 61]. Furthermore, mushrooms are an excellent source of naturally occurring antibiotics, glucans on their cell walls contain immunomodulatory effects. Many secondary metabolites produced by the mycelia inhibit bacterial and viral pathogenic species [69]. According to Rathee et al [67], mushrooms are anti-viral, anti-allergic, anti-inflammatory, hepatoprotective and hypoglycemic agents. Additionally, the mycelial exudates commonly known as "mushroom pee" from the mushrooms show activity against protozoa, for example, *Plasmodium falciparum* which causes malaria [70,71]. The absence of starch in mushrooms makes them ideal for persons with diabetic conditions and it can be recommended to people who wish to lose weight [72]. In addition, mushrooms act as prebiotics to stimulate the growth of gut microbiota [73].

Several studies and clinical trials have provided evidence that the preventive effects on cancer could be mediated by the immunomodulatory capability of mushrooms [70].

Several nutrients and bioactive health ingredients, such as amino acids, proteins, dietary fibres, and active polysaccharides, are present in mushroom mycelia and fruiting bodies [71]. Mushrooms are considered anticancer agents, traditionally, they have been used in treating cancers, of which most trials were concerned with the treatment of prostate cancer as outlined by Zhu et al. [69]. Mushroom b-glucan is an effective and ideal component for infusing immune health benefits into any kind of foods, baked goods, beverage and dietary supplements [69]. Zhu et al. [69] emphasised that higher basidiomycete's mushrooms contain various types of biologically active, high-molecular complexes such as beta glucan and low-molecular compounds (e.g., triterpenes, lectins, steroids) in fruiting bodies, spores, culture mycelia and culture broth, with suggested anticarcinogenic effects [69]. In addition, mushrooms contain various compounds that intervene in signalling pathways of tumour-specific proliferation, regulation of apoptosis, cancer-specific metabolism, angiogenesis, metastasis and key functions of the immune system [73, 74]. According to Divyia [71], mushrooms can be blended with or processed into other food products to transform into other new products, numerous value-added mushroom products are therefore commercially available in the market, such as sauces, seasonings, extracts, and mushroom supplements.

Gupta et al. [72] outlined that edible mushrooms have been used as human food and appreciated for texture and flavour since 600-800 AD in China and other Asian countries. Polysaccharopeptides found in mushrooms can benefit general health by inducing enzymes that remove free radicals and reduce the oxidative damage [74, 75]. *Ganoderma lucidum* triterpenes may act as potential prebiotics to modulate gut microbiota composition and promote the relative abundance of short chain fatty acids

(SCFA)-producing bacteria [76]. Mushrooms are rich in polysaccharide probiotics, which assist in the prevention of constipation and maintenance of a healthy gut [77]. Furthermore, epidemiological studies found that mushrooms assist in reducing mild cognitive impairment (MCI) [75]. Several mushrooms with antiplasmodial properties have been proved as sources for novel antiplasmodial compounds [78]. Beta glucan from *Lentinus edodes* is an effective immunostimulatory drug which is reported to induce protective immune responses to control the proliferation of malaria parasites during the blood-stage [74]. Additionally, in a recent study by Sevindik et al. [76], it was reported that mushrooms can help in the treatment of the ongoing COVID-19 pandemic by taking them in as supplements to support the immune system.

Mushrooms contributes to the treatments of skin diseases, heart ailments, rheumatoid arthritis, cholera besides intermittent fevers, diaphoretic, diarrhoea, dysentery, cold, anaesthesia, liver disease, gallbladder diseases and are used as vermicides [72, 75]. The Food and Agricultural Organization (FAO) has recommended mushroom foods to assist in solving the problem of malnutrition [77]. Furthermore, mushrooms have been proved to; boost health; inhibit tumour growth; avert viruses; bacteria, and fungi, and support the body's detoxification mechanisms. They have also been shown to be good for the heart as they carry minimal amounts of sodium and are rich in potassium thus regulating salt balance and maintaining blood circulation within humans [78]. Mushrooms are rich in vitamins, they contain a high amount of ergosterol, provitamin D_2 which can be converted to vitamin D_2 by ultraviolet radiation [79]. The enrichment of vitamin D provides the advantage that calcium in foods can be more available to children, elderly people and menopausal mothers [75].

2.6 ADDITIONAL USES OF MUSHROOMS AND SPENT MUSHROOM SUBSTRATE

Inclusion of mushrooms as functional food can help in the early intervention of sub-healthy states in humans and it might prevent the consequences of life-threatening disease [80]. Several value-added mushroom products are commercially available in the market, such as sauces, seasonings, extracts, and mushroom supplements [81]. Additionally, the use of mushroom products has the capability to enhance nutrition and has the possibility to boost mushroom's industrial value without compromising product acceptability [82]. Mushrooms are majorly used in flour-based goods, and their powder is being used in several food products, such as muffins, bread, pasta, and snacks to improve the nutritional quality. They improve the antioxidant and nutrient value of pasta, by reducing the starch's digestibility [83]. Mushroom extracts, including shiitake mushroom extract, have not only antioxidant properties but also anti-irritant properties, thus the right combination of mushroom extracts could be ideal constituents in anti-aging formulations [84]. During the past decade, many researchers sought out environment-friendly approaches to maintaining aquatic animal well-being, focusing on feed additives, and among those feed additives, mushrooms and their derivatives are considered the most promising source of functioning feed additives [85].

Spent mushroom substrate (SMS) can be employed in a number of green technology endeavours, and the enzymes recovered are potentially useful for the bioremediation of pollutants and other industrial biotechnology purposes [86]. Due to the problem of economic waste and environmental pollution, the mushroom industry has paid much attention to the handling and reprocessing of the spent substrate [87]. SMS, as a lignocellulosic substance, could be a potential source of reducing sugars to produce biofuels and cultivate microorganisms, [88]. To date, SMSs have been applied widely in environmental remediation such as soil remediation, bioremediation of an insecticide Dichlorodiphenyltrichloroethane (DDT), acid mine drainage treatment and spilled petroleum removal. Correspondingly, Spent Mushroom Substrates (SMSs) could be used to produce animal feedstock fertilizes, feed additives, enzymes and antioxidants [89]. Mushrooms can be used in broiler chicken production as they were found to have a positive impact by increasing growth performance, improve microbiota status in the gut, modulate immune response, exert tissue antioxidant activity, influence intestinal morphology and improve lipid profile in the chicken [89].

2.7 ANTIOXIDANTS, TOTAL PHENOLICS AND TOTAL FLAVONOIDS

Antioxidants are compounds that can help prevent or slow down damage to cells caused by free radicals, which are unstable molecules produced as a natural by-product of metabolic processes in the body or as a result of exposure to environmental toxins like cigarette smoke and air pollution [11]. Antioxidants can help neutralize free radicals by donating an electron, thereby preventing the chain reaction and reducing the potential damage [11, 90]. The measurement of the amount of total phenolic compounds as well as the identification of the main phenolics in mushrooms, have both great importance in their nutritional and functional characterization [90]. Mushrooms are a source of phenolic compounds (antioxidants) such as variegatic acid, nicotic acid and diboviquinone and they are an easily accessible source of natural antioxidants and as a possible food supplement [91]. Phenolic compounds include different subclasses as illustrated in Figure 8 demonstrating a large variety of structures, some of which may escape the usual methodologies of analysis, commonly carried out by High Performance Liquid Chromatography (HPLC) coupled to detection devices [92]. Phenols are important plant constituents because of their scavenging ability, and can act as free radical scavengers and oxygen quenchers [92]. They were found to have excellent antioxidant activity in the inhibition of low-density lipoprotein (LDL) oxidation. Investigation of mushrooms and their extracts antioxidant activity and content of antioxidant compounds is of scientific importance [93, 94]. Antioxidant capacities can be related to the total phenolic content of the mushroom extracts due to the combinational effect of different phenolic compounds [96]. A moderate correlation between phenolic content and antioxidant activities is an indication that phenolics contribute to the antioxidant activities of the mushroom extracts [97, 96]. It has been found that polyphenols are one of the major contributors to the antioxidant activity of fruits, vegetables and mushrooms [98, 99].

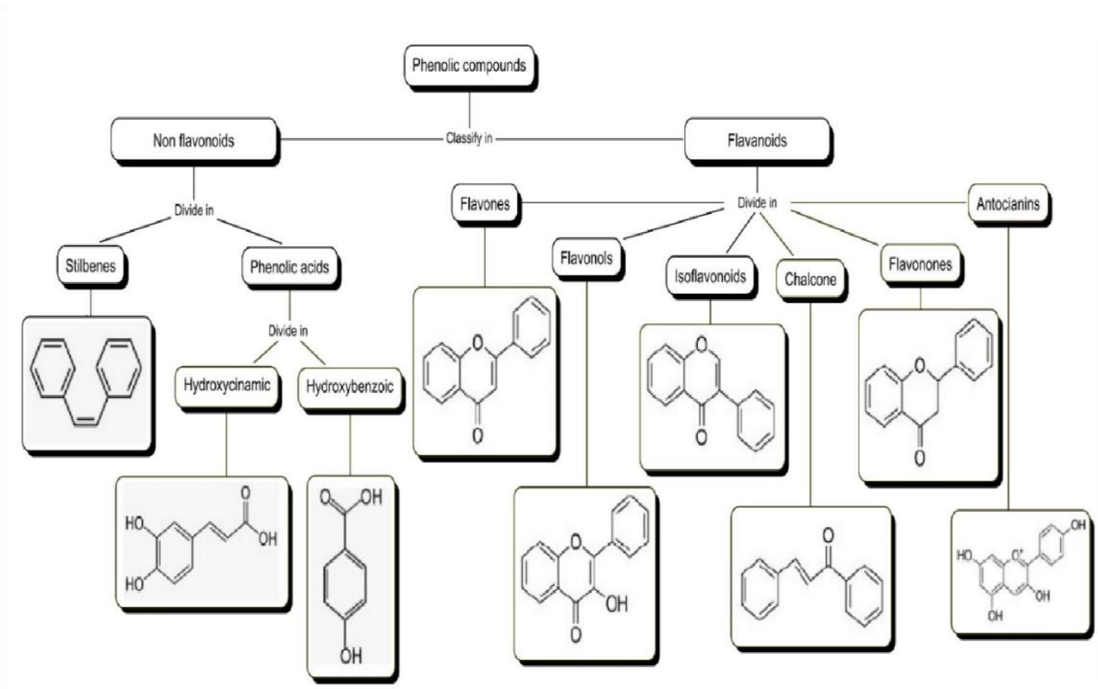

Figure 8 Phenolic compounds (PC) classification. Source: Halake et al. [92]:

Among the biologically active substances present in mushrooms, phenolics have attracted much attention due to their superb properties as antioxidant, anti-inflammatory, anti-tumour agents, amongst others [100]. In addition, they can be classified as free radical inhibitors, peroxide decomposers, metal inactivators and oxygen scavengers due to their antioxidant activity [101]. Various studies have ventured into quantifying phenolic compounds from different mushroom species for cosmetic reasons such as producing nutritional supplements and anti-aging creams or lotions [8]. Phenolic compounds and their derivatives (Figure 9) with antioxidant potential for example, caffeic, ferulic, gallic, *p*-hydroxynzoic, homogentisic, protocatechuic acid, and myricetin, were identified in different species of edible mushrooms such as *A. bisporus*, *Boletus edulis*, *Calocybe gambosa*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Hygrophorus marzuolus*, *Lactarius deliciosus, Lentinus edodes, Pleurotus eryngii, Pleurotus ostreatus* [8]. Phenolics are involved in growth and reproduction and provide plants with resistance against pathogens and predators, protect the body from excessive free radicals which helps slow down the formation of many chronic diseases, and they can also be added to food products containing lipids and their associated foods which hinders lipid peroxidation and lengthens the shelf life of food products. [102, 103]. Antioxidant activity depends on the concentration of the extracts, with stronger inhibition of lipid oxidation occurring at higher concentrations of the extracts [100]. The food industry has long used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as preservatives in the food industry in order to prevent lipid oxidation which leads to malodours in lipid-rich foods [104]. However, synthetic antioxidants in food are now restricted due to their carcinogenic effects and have also been suspected to cause liver damage, and this has led to increased interest in antioxidant substances from natural resources [100, 105]. Natural antioxidant substances are generally considered safe because they are found abundantly in vegetables, fruits and many foods of plant origin. Numerous naturally occurring substances have been studied and found to possess antioxidant activity [106, 107].

In another study that dealt with the mushroom extract from *Scleroderma verrucosum* demonstrated a wide range of special metabolites of medical, biological and agricultural importance, as well as expressive contents of total flavonoids and phenolics that influence the ability to reduce the free radical DPPH, making this mushroom species attractive for future tests in biological production of medicines, lotions, topical emulsions, as well as in biotechnological use due to the presence of reducing sugars [107]. Oxidative stress imposed by reactive oxygen species (ROS) can cause damage to biomolecules such as DNA, proteins, lipids and carbohydrates, which may be the contributing factors for many diseases such as cardiovascular diseases, cancer, arthritis and inflammation, and due to adverse effects caused by the use of synthetic antioxidants chemicals on human health, there has been an increase in the discovery and application of natural antioxidants [108]. Mushrooms are becoming ever more widely recognized as functional foods and as a source of physiologically beneficial components, they have been shown to boost heart health; lower the risk of cancer; promote immune function; ward off viruses, bacteria, and fungal infections, combat allergies; support the body’s detoxification mechanism and balance blood sugar levels [109].

The antioxidant investigations on different mushroom species discovered that most mushrooms possess high reductive potential, metal chelation, and hydroxyl radical scavenging activities, with high concentration of total phenol and total flavonoids. These bioactive compounds together with the high antioxidant activities obtained in those species of mushrooms may be responsible for their nutritional and therapeutic uses [105]. Mushrooms usually contain a wide variety of free radical scavenging molecules, such as polysaccharides and polyphenols, dietary antioxidants such as vitamin C, E and carotenoids that have generated particular interest as defences against degenerative diseases [106]. Although living organisms can combat free radical–induced damage by the action of enzymes, those systems become insufficient when the balance between oxidative stress and antioxidant activity is disturbed by different oxidative agents such as radiation or poisoning. Therefore, antioxidant supplements in the diet acquire significant importance in preventing or reducing oxidative damage [107].

The fruiting bodies of Honey mushrooms (*Armillaria mellea*) in terms of the content of total phenols, total flavonoids, β-carotene, lycopene and β-glucans, (all have antioxidant properties), the results obtained confirmed the presence of those substances in the fruiting body of Honey mushrooms and fruiting bodies of Honey mushrooms (*Armillaria mellea*). This knowledge makes these mushrooms a functional food with the possibility of using their antioxidant potential in the pharmaceutical industry [108]. In most studies, determination of total phenolic in mushrooms was determined by using the Folin-Ciocalteu colorimetric assay, as stated by Gan et al. [109]. Although mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids, among the antioxidant compounds, polyphenols have gained importance due to their large array of biological actions [110].

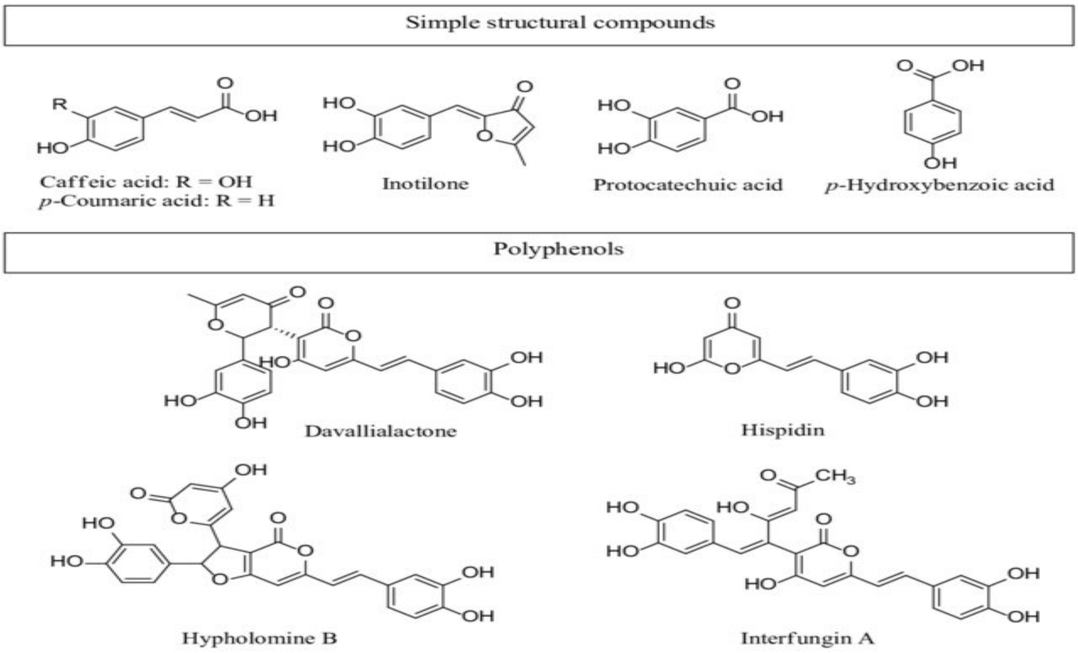

Figure 9: *Chemical structures of some beneficial cosmetic compounds isolated from mushrooms. Source: Sujarit [8]*

2.8 ANTIOXIDANT ASSAYS

Number of procedures are applied to perform in vitro determination of antioxidant potential, total phenolic content and flavonoid content. These tests are different from one another in terms of the reagents, substrates, conditions of the experiment, the medium for the reaction, and the accepted analytical assessment techniques. DPPH is a dark-coloured crystalline powder containing stable free radical molecules [12, 15]. The DPPH assay is based on the reduction of the purple DPPH (2,2-diphenyl-1-picryl hydrazyl) ˙ to 2, 2-diphenyl-1-picryl hydrazine which is illustrated in Figure 10 [14, 104]. It is a simple and inexpensive assay that can be used to test a wide range of compounds. However, it does not differentiate between different types of antioxidants, and the results can be affected by pH and solvent [111].

The in vitro determination of polyphenols includes Folin-Ciocalteu (FC) assay (Figure 11), the extract reacts with the FC reagent forming a blue complex that is quantified by the visible -light spectrophotometry. The Folin-Ciocalteu (FC) assay is a low-cost and highly sensitive method. It can be used to measure the total phenolic content in a wide range of samples, including food, plants, and biological fluids [12, 15]. However, the FC assay has some limitations. It is not specific to phenolic compounds and can also detect other reducing agents, leading to overestimation of the phenolic content [111]. The assay can also be affected by interference from other substances present in the sample, such as lipids, pigments, and vitamins [15]. Additionally, the lack of a standardized protocol for the FC assay can make it difficult to compare results between different studies. To minimize these limitations, it is important to carefully optimize the assay conditions and use appropriate controls to account for any interfering substances in the sample [111].

The ABTS assay is based on the generation of a blue/green $ABTS^{\cdot+}$ that can be reduced by antioxidants as shown in Figure 12. It is more sensitive than the DPPH assay and can be used to measure the antioxidant activity of both hydrophilic and lipophilic compounds [16, 111]. However, unlike the DPPH assay, it is more expensive and it can be affected by pH and solvent. [112]. This reduction results in a change in color that can be measured spectrophotometrically. The extent of the color change is proportional to the reducing power of the sample, which is an indication of its antioxidant activity [113]. The RP test is a quick and affordable way to measure a sample's antioxidant activity. The non-specificity, interference, and lack of standardization are some of its shortcomings and potential sources of error that researchers should put into consideration. It is crucial to properly optimise the assay conditions and employ suitable controls to account for any interfering chemicals in the sample in order to reduce these constraints [111, 114].

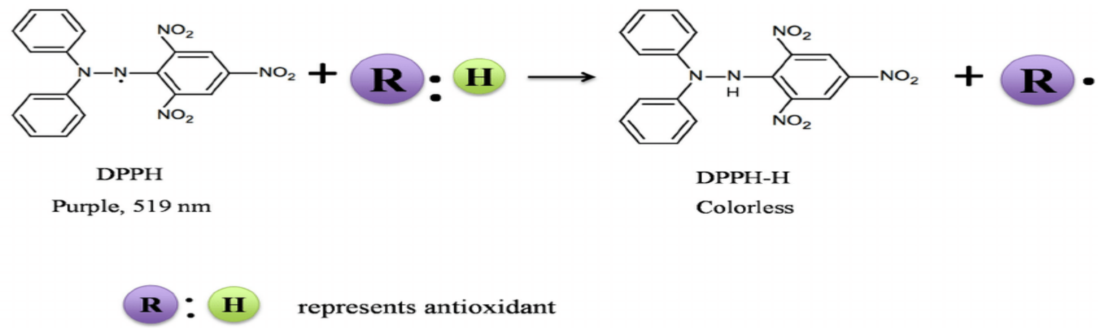

Figure 10: Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with antioxidants. Source: Liang et al. [104] R: H = antioxidant radical scavenger; R = antioxidant radical.

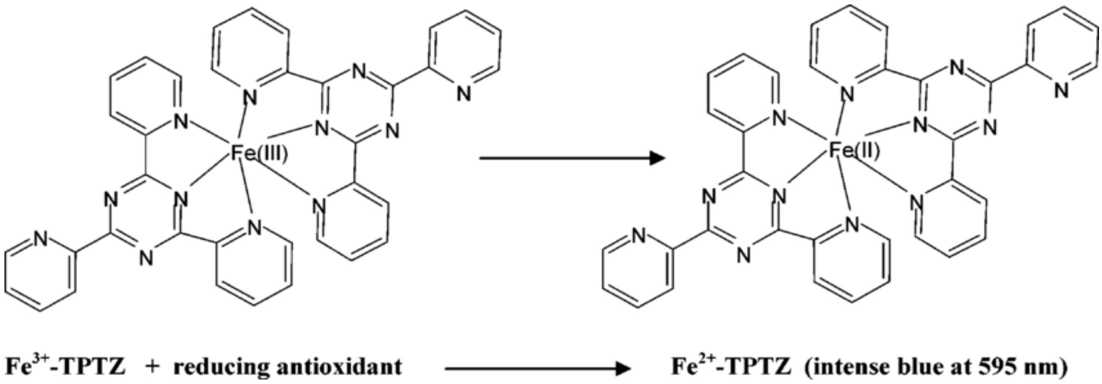

Figure 11: Reaction for FRAP assay. Source: Proestos et al. [105]

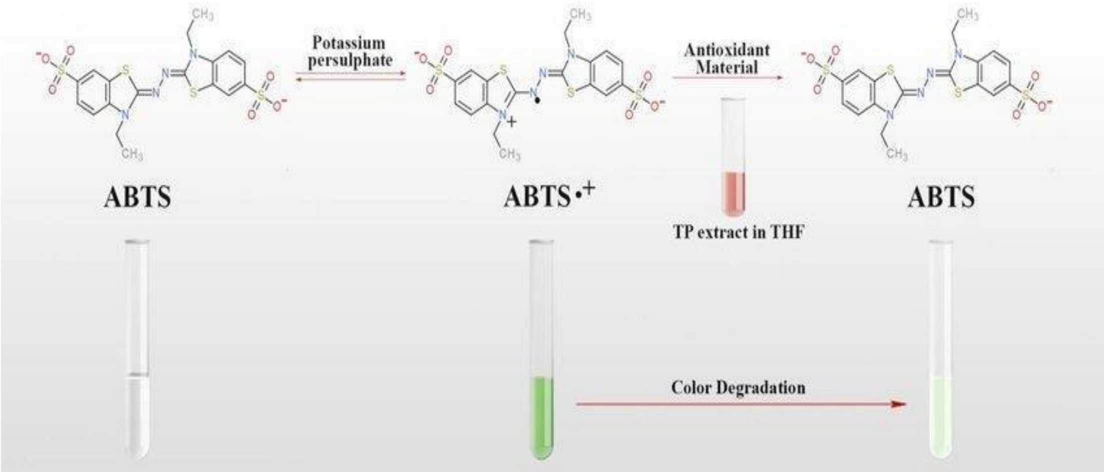

Figure 12: ABTS radical scavenging reaction. Source: Üstündaş et al. [106]

2.9 DIFFERENT CULTIVATED PLEUROTUS MUSHROOMS

Pleurotus species are from the family *Tricholomataceae* that belongs to a group called white rot fungi, they are one of the most widely cultivated oyster mushrooms in the world, with a growing demand due to their versatility in cooking, high nutritional value, and potential health benefits [115]. According to Raman et al., [116], the production of *Pleurotus* mushrooms has increased significantly in recent years, reaching approximately 27% of global production. Some of the most widely cultivated *Pleurotus* species include *Pleurotus ostreatus*, *Pleurotus eryngii*, *Pleurotus florida*, *Pleurotus citrinopileatus*, *Pleurotus soja-caju* and *Pleurotus pulmonarius*. These species are commonly grown in Asia, Europe, and North America, with China, India, and Italy being some of the leading producers and they grow widely in tropical and subtropical areas [117, 118]. In addition to their widespread cultivation, *Pleurotus* mushrooms are also a popular choice for small-scale and home-based cultivation, due to their relatively low cost, low maintenance requirements, and fast growth rate [117]. With the increasing popularity of plant-based diets and the growing demand for functional foods, the production of *Pleurotu*s mushrooms is likely to continue to increase in the coming years [118, 119]. Compared to other mushrooms, *Pleurotus* mushrooms are relatively low in contamination due to their ability to grow fast and high resistance to disease which makes it difficult for harmful pathogens to grow and thrive [120]. Each *Pleurotus* species has its unique morphology, nutritional value, and potential health benefits as discussed below.

2.8.1 Pleurotus ostreatus: This is one of the most commonly cultivated and consumed species. It has a soft, violet black to brownish grey in colour, fan-shaped cap and a tender, flavourful flesh. *Pleurotus ostreatus* is a rich source of protein, fibre, vitamins, and minerals, and is also believed to have anti-tumour properties and to enhance the immune system [118]. According to Haseeb et al., [121], *Pleurotus ostreatus* are good sources of vitamin B12, which may potentially reduce brain Shrinkage. Furthermore, this mushroom contains long-chain omega-3 fatty acids which contributes to healing of depression [121].

2.8.2 Pleurotus eryngii: This species is commonly known as the king oyster mushroom and is widely cultivated in Asia [121]. It has a large, meaty stem which is relatively bigger than its tender flavourful cap [122]. *Pleurotus eryngii* is a rich source of protein, fiber, vitamins, and minerals, and is also believed to regulate blood sugar levels and reduce cholesterol levels [123].

***2.8.3 Pleurotus citrinopileatus*:** This species is known for its lemon-yellow cap, commonly referred to as a golden oyster mushroom and is widely cultivated in Asia. *Pleurotus citrinopileatus* mushroom is a significant source of micronutrients and antioxidants components. Like other oyster mushrooms, it contains a reasonable amount of protein, fiber, vitamins, and minerals, and is also believed to have anti-tumour properties [124].

***2.8.4 Pleurotus pulmonarius*:** This species is commonly known as the phoenix oyster mushroom and is widely cultivated in Europe and North America. It is a mushroom that resembles *Pleurotus ostreatus,* the pearl oyster, but with a few key variations [125]. Pulmonarius has a grayish coloured sporophore with a fleshy texture and it develops a stem. It has a soft, tender lung shaped cap and a flavourful flesh [126]. *Pleurotus pulmonarius* is a rich source of protein, fiber, vitamins, and minerals, and is also believed to have anti-inflammatory properties [125].

***2.8.5 Pleurotus florida*:** This species is commonly known as the elm oyster mushroom and is widely cultivated in tropical West Africa and Southern part of Asia. It has a tender, flavourful cap ranging from 1.5 to 7.5cm diameter and a stipe ranging from 0.5cm to 2.5cm long, in addition, the annulus is absent, and the spore print is cream – white in colour and a soft, delicate flesh. It is a good source of protein, fiber, vitamins B and C, and minerals like potassium and iron [127].

***2.8.6 Pleurotus soja caju*:** This species is also known as the cashew oyster mushroom and is known for its nutty flavour and meaty texture. It is having a light brown colour and grows on a variety of substrates, including cashew shells, sawdust, and straw. It is identified to have a white spore print, gills attachment and usually observed with an eccentric stipe. In terms of nutrition, *Pleurotus soja caju* is a good source of protein, fiber, vitamins B and C, and minerals like potassium and iron [128].

2.8.7 Hk35: This species is a hybrid of *Pleurotus* species and is known for its meaty texture, mild flavour, and fast growth. It has a creamy white to light brown colour and is commonly grown on straw and sawdust. In terms of nutrition, hk35 is a good source of protein, fibre, vitamins B and C, and minerals like potassium and iron [129].

2.10 FACTORS AFFECTING THE YIELD AND NUTRITIONAL COMPOSITION OF MUSHROOMS

Mushroom survival and multiplication are dependent on a variety of factors, which may act independently or in concert [118]. Constraints commonly encountered in a mushroom production include low mushroom shelf life, a lack of marketing facilities, a lack of technical knowledge, mushroom house not bearable or conducive for mushroom production, poor technical guidance, a lack of information flow, a lack of availability of quality straw, and a lack of commitment of farmers. Apart from the above-mentioned factors, environmental conditions contribute greatly to yield in a mushroom farm [118, 130]. Thus, increasing bio-efficiency, or getting a high mushroom yield from fewer raw materials, is necessary to reduce production costs [130, 131]. In order to get a good harvest, mushroom cultivators ensure an optimal combination of the major factors listed in Figure 13, which are best air temperature, moisture, nutrient conditions, and other variables. Many nutrients are essential for fungi, at least in trace amounts. Here we will focus on a few particularly significant ones.

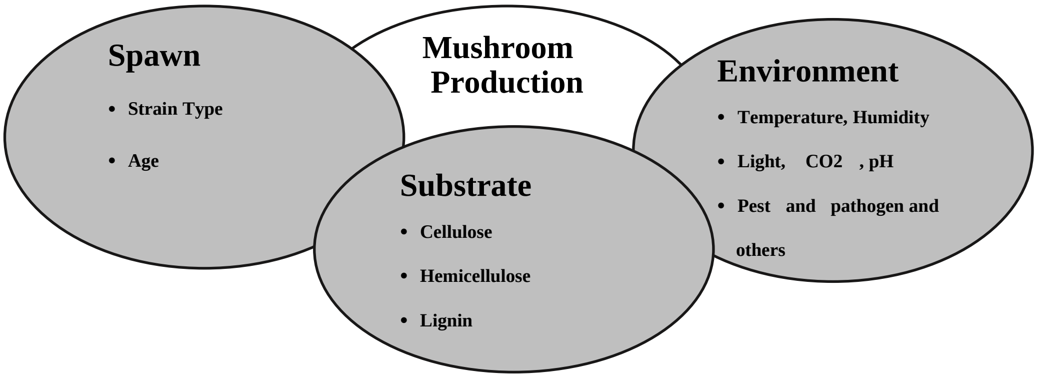

Figure 13: An overview of the major factors involved in mushroom production. Sources: Rai et al. [130]

2.9.1 COMPOSITION OF SUBSTRATES

Substrates used in mushroom cultivation contribute to the chemical, functional, and sensory properties of mushrooms. According to Stamets [24], mushrooms draw nutrients from the substrate via their mycelium, acquiring substances required for growth such as carbon, nitrogen, vitamins, and minerals. The ability of fungus to secrete enzymes involved in utilizing lignocellulosic substrates will determine its ability to use different types of substrates [26]. Mushrooms degrade the substrate by producing enzymes, and the first signs of mushroom growth appear within the first three days of inoculation [129]. Particle size of the substrate plays a role in substrate degradation by the enzymes, very small particles result in a compressed substrate, interfering with the aeration system and in oxygen used by microorganisms as stated by Bellettini et al. [118]. On the other hand, particles with large size cause an increase in space between particles, thus improving the movement of oxygen which allows a smooth metabolic process in mushrooms. Rouven [131], found that particle sizes between < 4.0 mm and > 0.85 mm produced a high biological efficiency and everything below 0.85 mm reduced the biological efficiency as illustrated in the Figure 14 below.

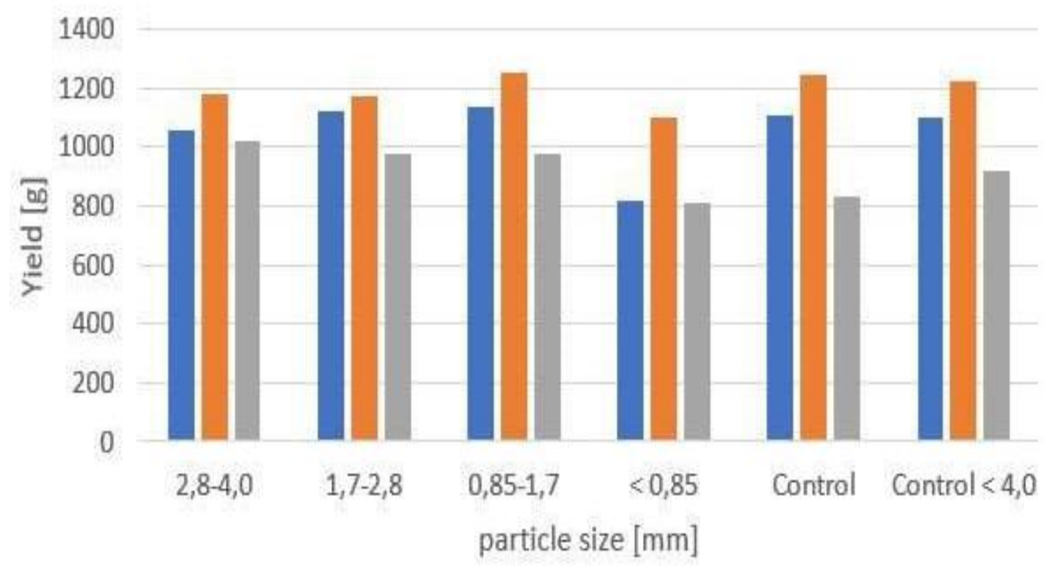

Figure 14: Influence of wood-chip particle size on mushroom yield. Source: Rouven [131]

2.9.2 NITROGEN SOURCES AND RATIO OF CARBON TO NITROGEN (C/N)

Nitrogen sources are referred to as any material that supplies nitrogen elements in mushrooms [24]. Farmers are strongly encouraged to supplement nitrogen to their substrates because this improves the nutritional value of the mushrooms. Purine synthesis, nucleic acid synthesis, and polysaccharide synthesis all depend on nitrogen [26]. Previous studies have indicated that Amino acids, urea, ammonia, along with nitrate on occasion, are the main sources of nitrogen [26]. Furthermore, Nune at al. [132] discovered that *P. ostreatus* in nitrogen-enriched substrates showed increased mushroom productivity, glucan content as well as good quality mushrooms [132]. Farmers typically add wheat bran, oat bran, and other bran to substrates to increase the nitrogen content. The mole number ratio between carbon atoms in culture medium and nitrogen atoms in nitrogen source is known as the carbon nitrogen ratio [116,132]. According to Carrasco et al. [133], carbon and nitrogen are the two primary macronutrients needed by fungi for structural and energy requirements. The ratio of carbon to nitrogen in the substrate must be balanced for mushrooms to grow. Additionally, adding cereal bran to the substrate or combining different ingredients may encourage the fungus to produce more and be more effective biologically. Rhaman et al. [116], found different ratio of C/N in different agricultural biomass (Table 2), He stated that low C/N ratio can lead to better production performance of fruiting bodies and the yield of the mushroom.

Table 2: C/N ratio in each of the agriculture biomass for mushroom substrates. Source: Rhaman et al. [116]

Agriculture Biomass	C/N Ratio
Sawdust	0.9721
Paddy straw	0.5907
Oil palm frond	1.6074

2.9.3 pH OF THE SUBSTRATE

In mushroom cultivation, controlling the pH level of the substrate is crucial for several reasons. Mushroom species have a specific pH range in which they grow best, thus controlling the pH of the substrate, the farmer ensures that the mushroom mycelium is growing in an environment that is optimal for its growth and development [134]. In addition, controlling pH can reduce the risk of contamination and increase nutrient availability. If the pH is too low or too high, certain essential nutrients may not be available to the mushroom mycelium, leading to slow growth or even death [130, 134]. Studies have indicated that the substrate needs to be slightly acidic, with a pH level of about 5 – 6.5, Some mushrooms, like oyster mushrooms, can tolerate a pH up to about 8 [130]. According to Rai et al. [130], various mushroom species can grow in a wide range of pH conditions (Table 3). Furthermore, adding lime (calcium hydroxide) to the substrate can raise the pH, while adding sulfur can lower the pH. Calcium carbonate is a common supplement used to adjust the pH control in the substrate as stated by Kalmis et al [134]. buffers such as calcium carbonate (chalk), magnesium carbonate, and potassium carbonate can maintain a stable pH [132, 134].

Table 3: Important edible mushrooms and optimum pH range for their mycelial growth. Source: Rai et al. [130]

Species	pH range
Agaricus bisporus	6.8 - 7.0
Auricularia polytricha	5.0 - 5.4
Agaricus bitorquis	6.0 - 6.4
Hericium erinaceus	4.0
Auricularia auricula	4.5 - 7.5
Pholiota nameko	5.0 - 7.0
Flammulina velutipes	4.0 - 8.0
Lentinula edodes	4.7 - 4.8
Pleurotus ostreatus	5.4 - 6.0
Pleurotus sajor-caju	6.0 - 6.5
Volvariella volvacea	7.5
Tremella fuciformis	5.0 - 6.0

2.9.4 MOISTURE AND HUMIDITY

Water is essential to the life cycle and nutritive process of oyster mushrooms. It does not only maintain cell rigidity, but it is also the best solvent and medium for biochemical reactions to occur in cells [26]. Furthermore, water is required for vegetative growth as well as the development phase of the mushroom fruiting body [130]. High moisture content in the substrate makes gaseous exchange difficult for the mycelium, inhibiting perspiration and making fruiting body development impossible, even with increased inoculum amounts or the number of holes in mushroom cultivation packages, resulting in the development of undesirable organisms such as bacteria and other fungal contaminants [118]. Mushrooms have a short shelf life due to its moisture content of 85%-95% [136]. The optimal moisture content for growth and substrate utilization is determined by the organism and the cultivation substrate [133]. It is believed that increasing the moisture level reduces the porosity of the substrate, limiting oxygen transfer. As a result, the use of high moisture content limited growth throughout the entire substrate, resulting in surface growth [122].

2.9.5 PEST AND DISEASES

Mushrooms are not immune to pest or disease infestations. Mushrooms, like any other cultivated crop, are vulnerable to pathogens, viruses, mites and pests [26]. These biotic agents feed on the mycelium which reduces output and yield in a mushroom farm. In addition, Pathogens for example green mould compete for space and nutrients with cultivated mushrooms [24]. Depending on the stage of infection, compost quality, and environmental conditions, crop failure can occur. Lack of proper pasteurization of the substrate and unhygienic conditions becomes a major source of infection of fungi and bacteria in mushroom cultivation [118]. Once the disease has been introduced, it attracts pests on the smell of decaying mushrooms. Hygiene and proper pasteurization not only prevent pests and pathogens, but also reduces the use of chemicals as a way to control pests and diseases which is highly discouraged in food farming [26, 118]. To minimize the risk of contamination, it is important to follow good agricultural practices and strict hygiene measures, including proper sterilization of the growing medium, proper storage of the mushrooms, and proper handling and packaging of the final product. Additionally, it is important to ensure that the growing environment is well-ventilated and free from contaminants, such as chemicals and other pollutants [137]

CHAPTER 3: RESEARCH METHODOLOGY

3.1 STUDY AREAS

The study was conducted in two phases. The first phase was done at the University of Namibia (UNAM), where experimental analysis on antioxidant activity, yield and yield components was performed at the UNAM main campus. Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) analysis was performed at the UNAM Neudamm campus, while mineral and nutritional composition were performed at the Ministry of Agriculture, Water and Land Reform (MAWLR) in Windhoek. The second phase of the study was conducted at Okondjatu district in Okakarara constituency, Otjozondjupa region, where harvesting of bush encroachers and mushroom cultivation training was conducted.

3.2 RESEARCH DESIGN

A 4x4 factorial arrangement in a completely randomized design (CRD) setting was done to determine the yield and yield components of different mushroom species cultivated on different bush encroachers at the ZERI mushroom house. Furthermore, each factor-level combination was replicated three times, as illustrated in Table 4.

Table 4: Experimental design for mushroom cultivation at UNAM ZERI mushroom house

Bush encroacher	Mushroom species			
Combretum collinum	HK35 x3	*Pleurotus florida x3*	*Pleurotus Ostreatus x3*	*Pleurotus soja-caju x3*
Grewia flava	HK35x3	*Pleurotus florida x3*	*Pleurotus Ostreatus x3*	*Pleurotus soja-caju x3*
Terminalia Sericea	HK35x3	*Pleurotus florida x3*	*Pleurotus Ostreatus x3*	*Pleurotus soja-caju x3*
Senegalia mellifera	HK35x3	*Pleurotus florida x3*	*Pleurotus Ostreatus x3*	*Pleurotus soja-caju x3*

3.3 MUSHROOM CULTIVATION

3.3.1 SPAWN PREPARATION

Wheat grains were soaked in water overnight; excess water was then drained, and the grains were packed in bottles. Bottles containing the grains were autoclaved for 2 hours and allowed to cool. The bottles were inoculated with different species of mushroom mycelium cultures under sterile conditions and incubated until fully colonized [138].

Figure 15: Wheat grains before inoculation

Figure 15 above shows bottles kept under the laminar flow after sterilization in the autoclave for two hours. This was done to allow the grains to cool to room temperature before inoculation with mushroom mycelium. The burning lamp was used in order to keep the surrounding air minimal to microorganisms that might cause contaminations.

Figure 16: Wheat grain inoculation

Figure 16 illustrates a student inoculating mycelium culture into the wheat grains under the laminar flow by adding 8 spoons of fully colonised grain spawn to the newly autoclaved grains, the bottles were labelled with the specific mushroom species used. Inoculated bottles were then incubated in cupboards until fully colonized. In addition, this step was done with precaution, the student made sure the working station is clean or sterile to prevent contaminations and producing poor quality spawn.

Figure 17: Wheat grains after inoculation

Fully colonized bottles shown in Figure 17 were kept in the refrigerator when not in use for substrate inoculation. This was done to prevent mycelium overgrowth at the same time retaining the vigour and strength of the mycelium.

3.3.2 HARVESTING OF ENCROACHING BUSHES FOR MUSHROOM CULTIVATION

Harvesting of substrate was done in the adversely bush-encroached Okondjatu district due to easy access to machinery for cutting and milling the bush encroacher at the African Wild Dog Conservancy hub (Figure 18). Different trees that form part of bush encroachment were collected or harvested (*S. mellifera*, *G. flava*, *C. collinum*, and *T. serecea*) from the community and milled. The leaf part of the bushes was used to make animal feed and the hard or stem parts of the bush was used for mushroom cultivation.

The milled bushes were air dried as illustrated in Figure 19, packed in bags, and transported to the University of Namibia**.**

Figure 18: Harvesting of bush encroachers in Okondjatu African wild dog conservancy camp

Figure 19: Community members and students air drying milled bushes

3.3.3 SUBSTRATE PREPARATION

The milled bush (substrate) was soaked overnight in drums (Figure 20) and drained excess water, 3kg of wheat bran was then added to 24 kg of each bush encroacher (substrate). The substrate mixed with wheat bran was then packed in plastic bags and pasteurized for 3 hours (all the bags weighed 2 kg) as illustrated Table 5. The bags were cooled to room temperature for about 4 hours before inoculation with different species of mushroom spawn. The inoculated bags were then incubated in a dark room at room temperature until they were fully colonized, at which point they were transferred to the harvesting room. Small holes were slitted around the bag to allow primordial initiation. The slitted bags were then watered three times a day to maintain a humidity of 70%-80%, and a temperature of 17°C -25 °C for the mushrooms to start fruiting. The number of days to fully colonize bags, mushroom cap diameter, stipe size, and weight of fresh and dried mushrooms were recorded [139].

Table 5: Substrate preparation and inoculation

Substrate treatment	Pasteurisation and curing time	Inoculation
24kg *C. collinum* + 3kg wheat bran	3 hours + 4 Hours	200g spawn/2kg substrate
24 kg *G. flava* + 3kg wheat bran	3 hours+ 4 hours	200g spawn/2kg substrate
24kg *T. sericea* + 3kg wheat bran	3 hours+ 4 hours	200g spawn/2kg substrate
24 kg *S. mellifera* + 3kg wheat bran	3 hours+ 4 hours	200g spawn/2kg substrate

Figure 20: Students soaking the substrate (top left) and on the right is mushroom varieties growing on milled bush substrate at ZERI mushroom house

3.4 COMMUNITY MUSHROOM CULTIVATION TRAINING

The second phase of the study relating to training in mushroom cultivation was carried out in the Okondjatu district. Three potential growing houses at three different villages were randomly selected, one built with bricks and cement in Okatupapa village, and another one was built with corrugated iron sheets and insulated inside with cow dung mud in Orukune village. The main mushroom house was built with rocks and cement at a local training centre that was strategically established at the Okondjatu African Wild Dog Conservancy. The house was built with the help of skilled community members recruited to do the construction of the project. A questionnaire was issued to the trainees to examine their purpose of interest in mushroom cultivation, their income, and any challenges they might encounter in starting up a mushroom cultivation farm. 45 participants were interviewed and data collected was analysed using excel, results obtained were presented in the form of bar charts, tables and pie charts.

3.5 LABORATORY ANALYSIS

3.5.1 EXTRACTION OF MUSHROOM POWDER

Dried mushrooms were ground into a fine powder. The weighed powder was then mixed with 99.9% of methanol in a flask and left in a shaker for 24 hours. After this, the sample was filtered with Whatman Grade 1 paper. Methanol was evaporated under laminar flow. The dried extracts were stored at 4 °C in bottles until analysis [140].

3.5.2 DPPH RADICAL SCAVENGING ACTIVITY

About 100µl of distilled water (autoclaved) was added to all the wells in the plate. 100 µl of the 1 mg/ml extract was then added from the stock solution and diluted from the first row downwards (the plate contained different concentrations in each well, from

1 mg/ml to 0.5 mg/ml to 0.25 mg/ml to 0.125 mg/ml). A total of 100 µl of vitamin C was added and serially diluted downward in the control wells. 100 µl of ethanol was added to the wells of the extract blank, and then 100 µl of DPPH from the stock solution was added to all the wells except the extract blank wells and plate blank wells. The plate was then covered with foil and incubated for 30 min, and plate absorbance was read at 520 nm [141]. IC50 was calculated using graphpad prism (Graphpad software Inc. California, USA). % inhibition was calculated using the following formulae:

$$\textbf{(\%) Inhibition} = (A_0 - A_1) / A_0 \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

3.5.3 REDUCING POWER OF MUSHROOM EXTRACTS

The reducing power of the extracts were analysed according to Archaria et al. [142], with few modifications. Different reagents were prepared as follows:

3.5.3.1 Preparation of potassium ferricyanide solution (1%)

About 1mg of potassium ferricyanide was added in a 50ml volumetric flask, which was dissolved in a small amount of autoclaved distilled water. The volumetric flask was then filled to mark with the autoclaved distilled water.

3.5.3.2 Preparation of trichloroacetic acid solution (10%)

Trichloroacetic acid (10mg) was added in a 50ml volumetric flask and dissolved in small amount of autoclaved distilled water. The volumetric flask was further filled to mark with autoclaved distilled water.

3.5.3.3 Preparation of ferric chloride solution (0,1%)

About 0,1mg of ferric chloride was added in 50ml volumetric flask, dissolved in small amount of autoclaved distilled water. Then filled the volumetric flask to mark with autoclaved distilled water.

3.5.3.4 Preparation of phosphate buffer (0,2M)

An amount of 3.48g potassium hydrogen phosphate and 2.72g potassium dihydrogen phosphate was added in 100ml flask, which was then dissolved in small amount of autoclaved distilled water. The flask was later filled with autoclaved distilled water to the mark.

3.5.3.5 Preparation of ascorbic acid solution

About 1mg of ascorbic acid was dissolved into 10ml autoclaved distilled water to make concentration of the solution of 0.1mg/ml = 100ug/ml (stock solution).

3.5.3.6 Preparation of mushroom extract solution

Different mushroom extracts (1mg) were dissolved into 10ml of autoclaved distilled water to make concentration of 0.1mg/ml=100ug/ml.

3.5.3.7 Procedure

In 96-well plates, 10 µl of mushroom extract of different concentrations (1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.063 mg/ml) was added to the wells for extracts. 25 µl of buffer and 25 µl of 1% potassium ferricyanide was further added to wells of extract. The plate was then incubated for 20 min at room temperature.

Furthermore, 25 µl of 10% trichloroacetic acid (TCA) solution was added to stop the reaction n. 85 µl of distilled water and 8.5 µl of 0.1% ferric chloride were added to each well of the extracts. The solution was thoroughly mixed, then incubated for another 15 minutes at room temperature. Absorbance of the solutions was measured at 700 nm. Ascorbic acid was used as the positive control at concentrations 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.063 mg/ml [142].

3.5.4 DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

The TPC of the extracts were analysed according to Saeed et al. [140] with slight modifications. About 0.5 ml of the sample (1 mg/ml) was mixed with 0.5 ml of Folin-Ciocalteu's phenol reagent and incubated for 5 minutes at room temperature. 5 mL of a 7% Na2CO3 solution and 6.5 mL of deionized distilled water were added, respectively. The mixture was left in the dark for 60 minutes at room temperature. Gallic acid was prepared as a positive control at different concentrations (0.063 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml, and 1 mg/mL). The absorbance was measured at 750 nm, and a standard curve from different concentrations of gallic acid was then calibrated to determine the total phenolic content. The total phenolic content was expressed in milligrams of gallic acid equivalents (GAE) per gram of dr= y matter [140], which was calculated by using the following equation: $C = \frac{cV}{m}$

Where, C = Total phenolic content in mg/g in gallic acid equivalent (GAE), c = concentration of gallic acid obtained from the calibration curve in mg/ml, V = volume of extract in mL, m = weight of extract in mg

3.5.5 DETERMINATION OF TOTAL FLAVONOID CONTENT (TFC)

This procedure was carried out as stipulated by Josipovic et al. [143]. Firstly, 0.4 ml of distilled water and 0.03 ml of a 5% sodium nitrite solution were added to 0.1 ml of an extract solution (1 mg/ml) and incubated for 5 minutes. Afterwards, 0.03 ml of 10% aluminium chloride was added, and the mixture was further incubated for 6 minutes. 0.2 ml of 1 M sodium hydroxide was added, and the volume was adjusted to 1 ml by adding 0.24 ml of distilled water. The absorbance was measured immediately against the reagent blank at 510 nm. Quercetin was prepared as a positive control at different concentrations (0.063, 0.125, 0.25, 0.5, and 1 mg/ml), a standard curve was prepared from different concentrations of quercetin, and the total flavonoid content was expressed in milligrams of quercetin equivalents (QE) per gram of dry matter, which was calculated by using the following equation: $\mathbf{C = \frac{cV}{m}}$

Where, C = Total flavonoid content in mg/g in Quercetin equivalent (QE), c = concentration of quercetin obtained from the calibration curve in mg/ml, V = volume of extract in mL, m = weight of extract in mg

3.5.6 MOISTURE CONTENT OF FRESH MUSHROOMS

Fresh mushroom samples were collected, weighed, and documented. The mushrooms were then air dried, and the dry mass was recorded. The loss in weight after drying is known as the moisture content, which was calculated by using the following equation [144].

Moisture% = (Initial Weight-Final Weight) *100/Sample's Initial Weight

3.5.7 ASH OF CONTENT MUSHROOMS

The dry crucible and lid were placed in a hot air oven for 20 minutes at 105 degrees Celsius. The crucible and lid were cooled in the desiccator for 20 minutes. The masses of the crucible and lid were taken and recorded. Three (3g) of dried mushroom samples were placed in the crucible and placed in the furnace at 700 °C for 2 hours. The crucibles were then removed and cooled, and the weight of the crucible with ash was recorded [145]. The total ash was calculated as follows:

Ash % = (weight of crucible with ash (g) – weight of crucible (g)) ×100/ Weight of sample (g)

3.5.8 NEUTRAL DETERGENT FIBER (NDF) AND ACID DETERGENT FIBER (ADF)

NDF and ADF were performed at the UNAM Neudamm campus. The ANKOM 220 Fiber Analyzer unit (ANKOM Technology Corporation, Macedon, NY, USA) was used to analyse neutral detergent fiber (NDF) and acid detergent fiber (ADF) [147]. The Ankom filter bags were labelled and weighed. The bags were then filled with 0.50 g of mushroom powder, and the bags were heat sealed and weighed. Furthermore, the bags were placed in a bag suspender and transferred to an Ankom machine. The machine was then filled with approximately 2 L of NDF/ADF solution, which covered the top level of the bag suspender. The lid was tightly closed and agitated for 75 minutes for NDF and ADF. The solution was removed from the machine, and boiling distilled water was added to the reservoir to rinse the bags by agitating for 5 minutes. The bags were then removed from the suspender, and excess water was manually removed. The bags were placed in a 100-degree Celsius oven for 6 hours, and the dry bags were weighed using a desiccator. % NDF or ADF was calculated as following:

% NDF/ADF = 100 x (W3- (W1 X C1))/W2

Where: W1 = Bag tare weight, W2 = Sample weight W3 = Dried weight of bag with fiber after extraction process C1 = Blank bag correction (running average of final oven-dried weight divided by original blank bag weight)

3.5.9 CRUDE PROTEIN OF MUSHROOMS

Mushroom powder samples were taken and analysed at the Ministry of Agriculture, Water, and Land Reform (MAWLR). The determination of total nitrogen by the Dumas combustion method and crude protein was obtained by calculating N x factor 6.25. The Dumas combustion method for nitrogen determination replaces the previous laborious and hazardous wet-chemistry acid digestion method of Kjeldahl. The dry combustion method is based on the transformation of the sample to a gas phase by extremely rapid and complete flash combustion of the sample material [148]. Figure 21 illustrates the analyser for total nitrogen determination using Dumas. 0.20 g of the mushroom powder was weighed into a tin foil cup, placed into an automated sample loader. The sample was burned in a high-temperature furnace at roughly 1000 °C with the addition of pure oxygen (O_2).The resulting gas combination was then transmitted via a carrier gas, where nitrogen oxide was reduced to elemental nitrogen on a copper surface in step 2, followed by the separation of water and carbon dioxide in steps 3 and 4. Additionally, the carrier gas circulates throughout the entire system while being analyzed and quantified in step 5 using a thermal conductivity detector (TCD). The combination of carrier gas and N2 was then measured by the TCD. The TCD can measure the voltage difference caused by the variation in carrier gas composition, which is then used to determine the amount of nitrogen content of the sample [149].

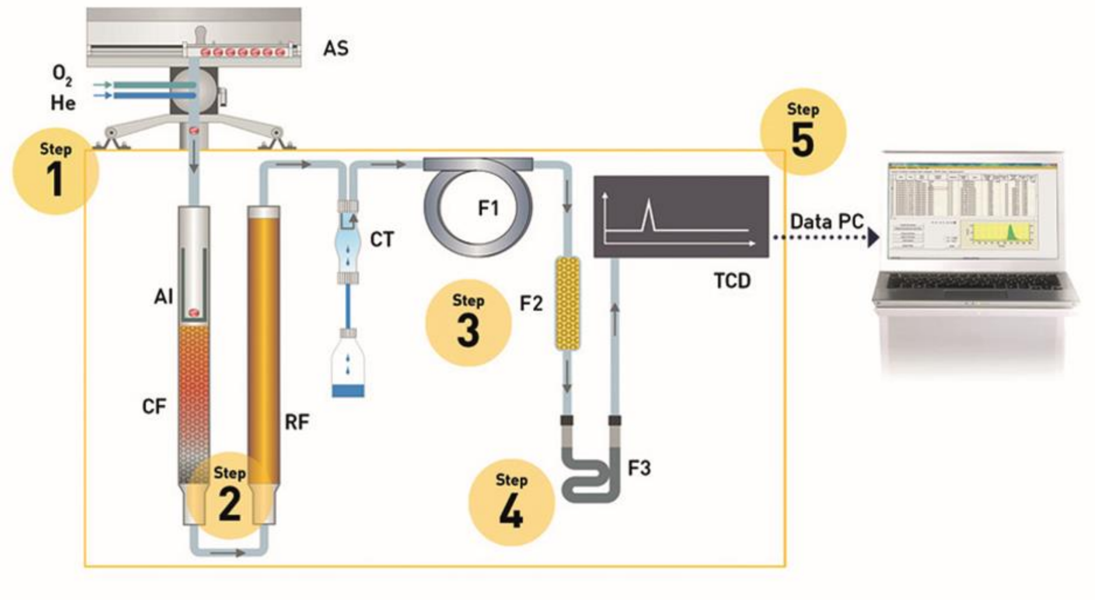

Figure 21: Analyzer for determination of total nitrogen using Dumas Source: Oeno [144]

3.5.10 MINERAL COMPOSITION OF MUSHROOMS

Mushroom powder samples were taken and analysed at the Ministry of Agriculture, Water, and Land Reform (MAWLR). Flame emission spectroscopy was used for the determination of potassium and sodium contents. The sample solution is nebulized into an air-propane flame, where it is vaporized. Potassium and sodium are atomized and excited, which then emit radiation, whose intensity is measured at a wavelength of 766.5 nm for potassium and 589.0 nm for sodium. The intensity of emission is proportional to the concentrations of potassium and sodium in the sample. Phosphorus was determined by colorimetry, phosphorus concentration in the solution of the digested sample is determined spectrophotometrically as the yellow phospho-vanado-molybdate complex [150].

a) Potassium

To prepare the potassium standard solutions, 0, 2.00, 4.00, 6.00, 8.00, 10.00, and 12.00 cm3 of the potassium stock standard solution were individually pipetted into 100 cm3 volumetric flasks. Next, an equal volume of reagents used for the digestion of the samples were added, followed by the addition of deionised, distilled water to make up the volume. To obtain the diluted standards, 1.0 cm3 of each standard solution was diluted with 9.0 cm3 of cesium solution. The resulting diluted standards had potassium concentrations of 0, 8, 16, 24, 32, 40, and 48 mg dm-3. The emission intensity of the diluted standard series was subsequently measured at a wavelength of 766.5 nm utilizing an air-propane flame, and a calibration graph was then constructed [150].

b) Sodium

Using a pipette, the volumes of 0, 2.00, 4.00, 6.00, 8.00, 10.00, and 12.00 cm3 of the sodium stock standard solution were measured and transferred into 100 cm3 volumetric flasks. An equivalent volume of reagents utilized for the digestion of the samples was added, and the volume was made up with deionised, distilled water. In parallel with the dilution of the sample digests, 1.0 cm3 of each standard was diluted with 9.0 cm3 of cesium solution, yielding diluted standards with sodium concentrations of 0, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 mg dm-3, respectively. The emission intensity of the diluted standards was subsequently measured at a wavelength of 589.0 nm, employing an air-propane flame. Subsequently, a calibration graph was drawn based on the measured emission intensities [150].

i) Mushroom powder (Sample)

To begin the analysis, 1.0 cm3 of the mushroom powder digest was pipetted into 100 cm3 volumetric flasks, followed by the addition of 9.0 cm3 of cesium solution. The diluted blank and samples were then subjected to emission intensity measurements at a wavelength of 766.5 nm for potassium and 589.0 nm for sodium, utilizing an air-propane flame. The corresponding potassium and sodium concentrations were subsequently determined by reference to the corresponding standard [150].

The potassium and sodium content of the sample, in percentage is calculated by using the following equation:

% (K or Na) = (((a - b) × D)/m) *100

Where:

a = Concentration of element in the diluted sample, in mg dm^{-3}

b = Concentration of element in the diluted blank, in mg dm^{-3}

V = Total volume of the sample solution at the end of the digestion

Procedure, i.e., 100 cm^3

m = Mass of the sample, in g

D = Dilutions, if any

C) Phosphorus

The mushroom sample solution was prepared by wet digestion. The digest was transferred to 100 cm^3 volumetric flask and make up with water. 10 cm3 of each phosphorus working standard solution was then pipetted into a 50 cm^3 Volumetric flask. To each flask 5 cm^3 of 5 M hydrochloric acid and 5 cm^3 of ammonium (molybdate-ammonium metavanadate reagent) was added and diluted to 50 cm^3. The mixture was allowed to stand for 30 min. The absorbance in a 10 mm optical cell at 400 nm was read and a graph was calibrated, relating absorbance to phosphorus concentration present, i.e., 2, 4, 6, 8 and 10 μg cm^{-3} respectively. About 10 cm^3 of mushroom solution was pipetted into a 50 cm^3 volumetric flask. 5 cm^3 of 5M hydrochloric acid and 5 cm^3 of ammonium molybdate-ammonium metavanadate reagent was added. The mixture was Diluted to 50 cm^3 and allowed to stand for 30 min. The absorbance was measured in a 10 mm optical cell at 400 nm. A blank was prepared and absorbance was read [150]. The Phosphorus content of the sample, in percentage is calculated by using the following equation:

% P = ((C x V x 50)/ 10 x m) *100

C = Difference between the sample and blank concentrations as read from the graph, in μg cm^3

V = Total volume of the sample digest solution, (here 100 cm^3)

m = Mass of the sample, in g

10 = Sample aliquot (here 10 cm^3)

50 = Dilution of sample aliquot (10 cm^3) to 50 cm^3

3.5.11 STATISTICAL ANALYSIS

A general linear model analysis of variance was used for the analysis, using the Statistical Package for Social Sciences version 25 (SPSS). The means (yield components, nutritional compositions and phytochemicals) that differ significantly were detected using the Duncan multiple range comparison, and the equation for the general linear model used was y_{ijk}=u + M_i + MB_{ij} + E_{ijk}. Where, y_{ijk}= the K^{th} replicate of i^{th} mushroom species in the j^{th} bush substrates

u = Overall mean

Mi = Effect of the i^{th} mushroom species

Bj = Effect of the j^{th} encroaching bush

MiBj = Effect of the interaction between i^{th} mushrooms and j^{th} bush substrate.

The significant difference was tested at p-value $\leq$ 0.05. Normality assumption was checked using the Shapiro-Wilk's test and the histogram residuals, Homogeneous variance was tested using Levine's test and the plot of residuals versus fitted values. Independent assumption was tested using a plot of residuals versus observation order. The minimum inhibitory concentration at 50% (IC50) was calculated using a graph pad prism (Graph pad software Inc. California, USA).and data collected from the survey was analysed using Microsoft excel. A pilot survey was contacted to pre-test the questionnaire for validity and reliability.

3.6 RESEARCH ETHICS

Research permission was requested from the Centre for Postgraduate Studies (CPGS) and an ethical Clearance Certificate was issued from the University of Namibia in order to carry out the research. A harvesting permit was requested and granted by the Forest Management Body (FMB) of the African wild dog community forest in

Okondjatu community. COVID-19 safety protocols and regulations were adhered to during mushroom cultivation training in the community. Information provided in the survey by community members was treated as private and confidential. Full personal protective equipment (PPE) was worn at all times during laboratory work, in addition all laboratory rules and regulations were strictly followed to prevent accidents and ensure correct disposal of chemicals to prevent either water or land pollution.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 YIELD AND YIELD COMPONENTS

The yield performance of different mushroom varieties grown on different encroaching bush substrates was evaluated by measuring the performance of each mushroom species and encroaching bush substrate. The results obtained and presented in Table 6 show that there was no significant difference in yield performance when bush substrates and mushroom species interacted. The parameters that showed high significance indicate that the source of variation for mushroom cultivation varied among the different mushroom species used in the experiment, as shown in Table 7.

Table 6: The effects of Interaction of bush substrates and mushroom species on yield components

Bush Species Substrate	Mushroom Species	Weight (g)	Stipe (cm)	Diameter (cm)	Days To First Harvest	Number Of Fruiting Bodies
	HK 35	203	2.97	5.97	39.0	2.67
	PF	319	3.00	5.95	59.0	7.33
Combretum collinum	PO	353	3.40	7.40	48.0	8.00
	PS	340	2.90	6.63	60.7	6.33
	HK 35	263	2.73	7.27	40.0	3.00
	PF	408	3.30	9.17	39.0	3.67
Grewia flava	PO	328	2.77	6.43	38.0	2.67
	PS	313	2.90	7.73	38.3	6.67
	HK 35	250	2.47	7.73	39.3	2.33
	PF	288	2.73	7.63	33.3	5.33
Terminelia sericea	PO	294	2.33	7.53	34.0	3.33
	PS	287	2.73	6.80	29.7	6.67

	HK 35	257	3.17	6.87	36.3	3.00
	PF	384	4.40	6.60	41.3	6.00
Senegalia	PO	282	3.27	8.03	39.0	2.33
mellifera	PS	347	4.03	4.63	38.3	5.67
SEM		37.0	0.674	0.824	5.16	1.63
P-VALUE		0.496	0.989	0.161	0.254	0.655

NB: PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Table 7: The main effects of bush substrates and mushroom species on yield components

Bush Species Substrate	Weight (g)	Stipe (cm)	Diameter (cm)	Days To First Harvest	Number Of Fruiting Bodies
Combretum collinum	304	2.83	6.96	38.67	6.08
Grewia flava	328	3.36	7.34	43.17	4.00
Terminalia sericea	280	2.94	7.35	39.75	4.41
Senegalia mellifera	318	3.14	6.45	41.75	4.25
SEM	18.5	0.34	0.41	2.58	0.82
P-Value	0.300	0.70	0.38	0.62	0.28
Mushroom Species					
Hk 35	243^{b}	3.07	6.49	51.7^{a}	2.75^{b}
Pf	350^{a}	2.93	7.65	38.8^{b}	5.58^{a}
PO	314^{a}	2.57	7.43	34.1^{b}	4.08^{ab}
PS	322^{a}	3.72	6.53	38.8^{b}	6.33^{a}
SEM	18.5	0.337	0.412	2.58	0.816
P-Value	0.002	0.129	0.117	<0.001	0.019

NB: Mean values that bear different superscripts letters are significantly different ($\alpha \leq 0.05$). PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

According to the results, *G. flava* provided a conducive environment for the growth of PF mushrooms, with a yield of 408g of fruiting bodies harvested per 2kg of dry substrate, which was higher compared to other encroaching bushes. Conversely, the lowest yield of mushrooms was harvested from HK 35 cultivated on *C. collinum* with a yield of 205g as illustrated in table 6. The main effects shown in table 7 demonstrated that mushrooms could grow on *C. collinum* in a short period of time, with only 39 days required for fruiting. Additionally, *C. collinum* produced more fruiting bodies (6.08) compared to other bush substrates, whereas *G. flava* produced a lower number of fruiting bodies (4). However, the significant difference in yield performances was not attributed to variations in the substrates used in the experiment. No significant difference was recorded for the size of the mushroom stipe and diameter among the four different bush species, which ranged from 2.83cm to 3.36cm and 6.45cm to 7.35cm, respectively. The largest stipe and diameter were observed in mushrooms grown on *S. mellifera*, with PF exhibiting a stipe size of 4.4cm and PO exhibiting a diameter of 8.03cm. However, these differences were not statistically significant. These yields were similar to those observed in Dlamini et al [151], where the diameter size ranged from 13.3 mm in banana leaves to 51.0 mm in maize stover and cobs. Additionally, the pileus diameter for sugarcane tops was 35.3 mm, 13.8 mm in maize stover, 51.0 mm in maize stover and cobs, and 113.3 mm in banana leaves. Table 7 illustrates the significant differences detected among the mushroom species used in the experiment for weight, days to first harvest, and number of fruiting bodies, with p-values of 0.002, <0.001, and 0.019, respectively. PF, PO, and PS exhibited similar performance in terms of weight and number of days to first harvest. PF yielded the highest weight of mushrooms with 350g, followed by PS with 322g, PO with 314g, and the lowest was observed in HK 35 with 243g. Horn [152] demonstrated different results compared to the current study. In the study, using 2kg of composted substrate resulted in a yield of 2kg of mushrooms during the first flush. Additionally, a harvest of 1.8kg was obtained from straws, cobs, and haulm. Among the mushroom species, PS yielded the highest number of fruiting bodies (6.33) and took the least number of days to fruit compared to other species as illustrated in table 7. This could be attributed to the quality of the spawn used, which resulted in the mycelium taking a shorter time to mature or fully colonize the substrate [153]. Previous studies have also reported variations in the number of days to harvest, which could be influenced by differences in the chemical composition and carbon-to-nitrogen ratio (C: N) of the substrates used [154]. Environmental conditions such as temperature and humidity may also contribute to variations in the number of days to harvest [155]. In summary, the results indicate that the choice of mushroom species and encroaching bush substrate significantly influenced the yield and fruiting characteristics of the mushrooms. *G. flava* provided a favourable environment for PF mushroom growth, while *C. collinum* supported faster fruiting and higher fruiting body production. The results suggest the importance of selecting appropriate mushroom species and substrates to optimize yield in mushroom cultivation. However, further research is needed to explore the underlying factors contributing to these differences and to optimize the cultivation conditions for each mushroom species.

4.2 DPPH FREE RADICAL SCAVENGING ASSAY

The inhibitory concentration at 50% of the methanolic extracts of mushrooms cultivated on different encroaching bushes was summarized in Table 8. As the concentration of the extract increased, the scavenging activity toward DPPH radicals increased. The results were expressed as extract concentrations capable of inhibiting 50% of the radical solution (IC_{50} value), whereby the extract with the lowest IC_{50} value contains the most antioxidant-holding extracts. As illustrated in figure 22, the % inhibition at 0.5mg/ml and 1 mg/ml for different mushroom extracts cultivated on different bushes ranged from 51.9 % to 61.2% and 66.8% to 79.1% respectively.

Table 8: Inhibitory concentration at 50% (IC50) of different mushroom species cultivated on different encroaching bushes

Bush species substrate	Mushroom Species	IC50 (mg/ml)
Combretum collinum	HK 35	0.530±0.900
	PF	0.723±0.017
	PO	0.770±0.029
	PS	0.703±0.001
Grewia flava	HK 35	0.575±0.103
	PF	0.476±0.098
	PO	0.727±0.028
	PS	0.676±0.090
Terminelia sericea	HK 35	0.607±0.014
	PF	0.520±0.017
	PO	0.780±0.007
	PS	0.380±0.098
Senegalia mellifera	HK 35	0.607±0.014
	PF	0.562±0.014
	PO	0.478±0.025
	PS	0.703±0.104
Vitamin C		0.0235±0.053

PS = Pleurotus sajor-caju, PO = Pleurotus ostreatus, PF = Pleurotus florida

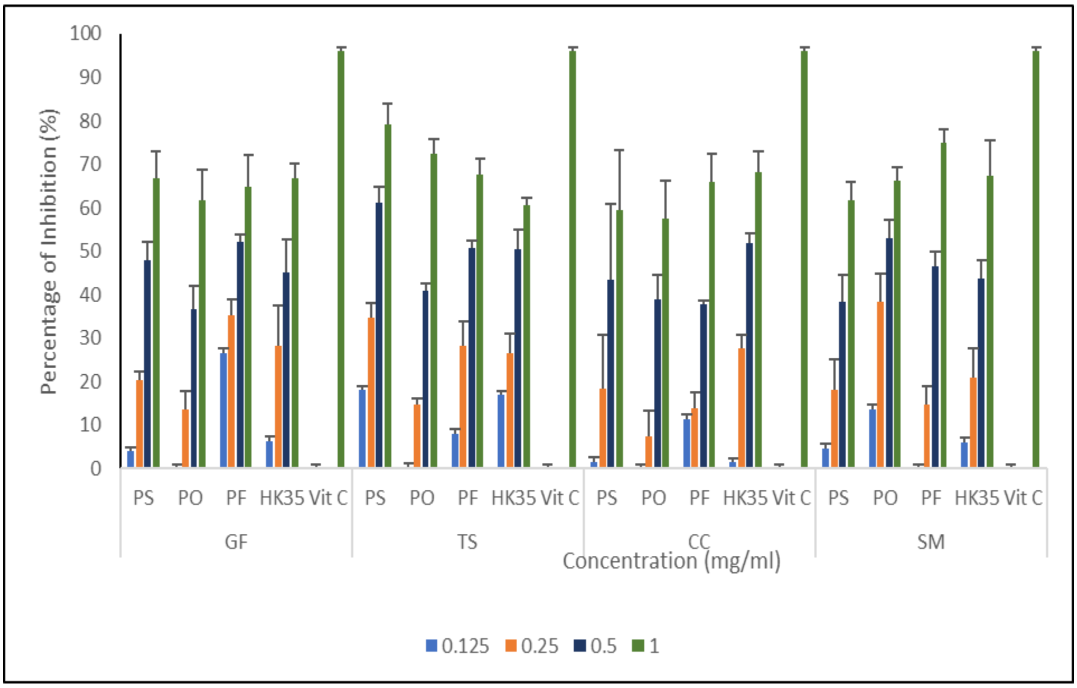

Figure 22: *DPPH radical scavenging activity of different mushroom species cultivated on encroaching bushes*

PS= *Pleurotus sajor-caju*, PO= *Pleurotus ostreatus*, PF= *Pleurotus florida, GF= Grewia flava, TS= Terminalia sericea, CC= Combretum collinum, SM= Senegalia mellifera*

The inhibitory concentration at 50% (IC_{50}) values ranged from 0.380±0.098 mg/ml to 0.780±0.007 mg/ml on different mushroom species grown (Table 8). These results were similar to those reported by Panthong et al. [156], with an IC_{50} ranging from 0.28 mg/ml to 11.45 mg/ml. However, the IC_{50} values reported were different from those reported by Naglot et al. [157] ranging from (1053.07±30.49 µg /ml)- 1531.59±50.47 µg /ml) cultivated on locally available substrate *Eleusine coracana* (finger millet) straw for the cultivation of different *Pleurotus* species. The inhibition of the DPPH radical by PS extracts cultivated on *T. sericea* (0.380±0.098 mg/ml) showed the highest activity compared to other mushroom extracts cultivated on the four bush encroachers. PO grown *T. sericea* showed the least activity (0.780±0.007 mg/ml). However, the IC_{50} of standard ascorbic acid (0.023±0.026mg/ml) was higher than that of all extracts tested. DPPH scavenging activity is one of the most frequently used in vitro tests to investigate the antioxidant activity of mushroom species [158].

Figure 22 shows that the mushroom extracts cultivated on different encroaching bushes scavenged free radicals in a concentration-dependent manner. In addition, lower concentrations of the extracts showed less or no colour change, while high concentrations showed strong colour changes. The results revealed that all the concentrations of the mushroom extracts in each encroaching bush substrate and the control (Vitamin C) showed antioxidant activity. However, the maximum antioxidant activity was observed at the highest concentration (1.00 mg/ml), followed by lower concentrations such as 0.500 mg/ml, 0.250 mg/ml, and 0.125 mg/ml, respectively, for all the mushroom species. Among the extracts of mushroom tested, PS cultivated of *T. sericea* (79.1%) showed high percentage of radical scavenging activity at 1mg/ml. The least % inhibition at 1 mg/ml was detected in HK35 (66.8%) cultivated on *G. flava*. These results were higher than those of Prabu [157], who demonstrated a % inhibition of 21.90±0.88 % and 23.13±1 at the concentration of 1000 µg/ml of *Pleurotus florida* and *Calocybe indica* respectively.

There is a wide variation in the antioxidant activity of mushroom species reported by different researchers. This could be due to genetic variations, different substrates, different cultivation methods, and different environmental conditions, particularly humidity and temperature [154, 157]. The dry mushroom extracts showed positive antioxidant activity by changing the dark blue colour of the DPPH solution to pale yellow [159]. The scavenging activities of radicals were in direct proportion to the concentrations of the extracts. Furthermore, results were expressed in different ways depending on what worked for the author. Thus, it is a challenge to compare and classify the results of different species with other studies. However, these results demonstrated that the mushroom species used are potential good antioxidants.

4.3 REDUCING POWER ASSAY

The antioxidant activity of mushroom extracts can be determined by assessing their reducing power. This is measured using a Fe^{3+} to Fe^{2+} assay, where the color of the test solution changes from yellow to various shades of green and blue based on the reducing power of each sample. Therefore, higher absorbance indicates a higher reductive potential of the mushroom extracts. All the mushroom species showed an appreciable ability to reduce ferricyanide complex to ferrous form at different levels of concentration (0.063–1.00 mg/mL). Moreover, reducing power ability increases with concentration, as illustrated in Figure 23.

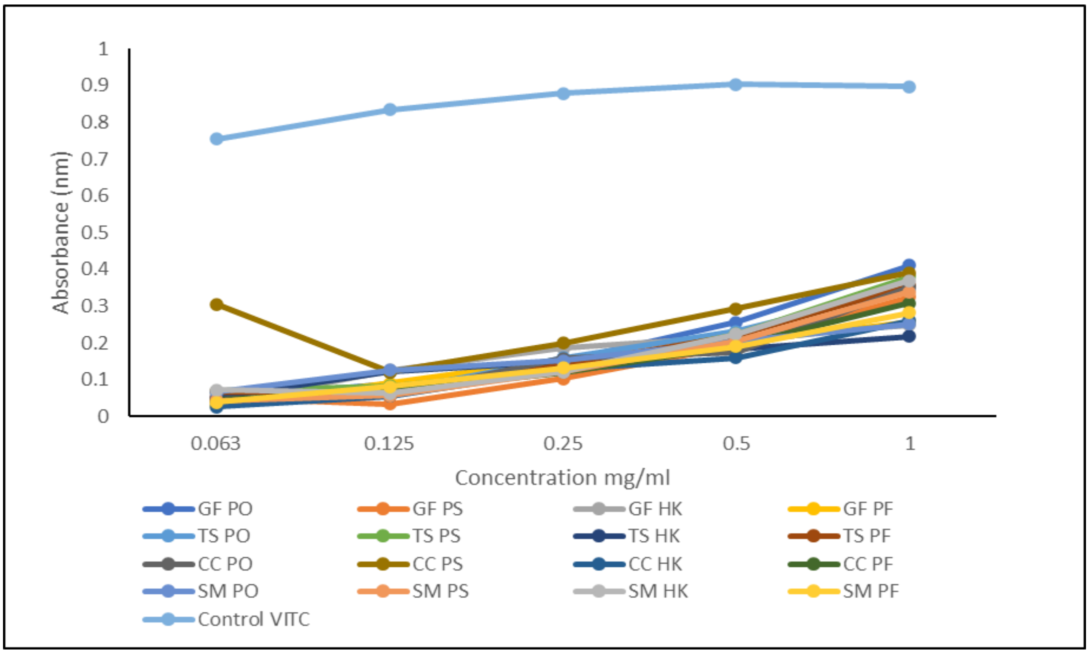

Figure 23: Reducing power of different mushroom extracts cultivated on different encroaching bush substrates

Note: PS= Pleurotus sajor-caju, PO= Pleurotus ostreatus, PF= Pleurotus florida, GF= Grewia flava, TS= Terminalia sericea, CC= Combretum collinum, SM= Senegalia mellifera

According to figure 23, the greatest ability for reducing the ferricyanide complex to ferrous form was observed in PO cultivated on *G. flava* extracts at a concentration of 0.411nm at 1.00 mg/ml. These results were similar to that of Kim et al. [161], with a reducing power of 0.41nm at 1.0 mg/mL of yellow strains of oyster mushrooms. HK35 on *T. sericea* showed the least ability (0.21 nm at 1.00 mg/ml) to reduce the ferricyanide complex. However, the results of reducing ability by Vitamin C (0.90nm at 1 mg/ml) that served as a positive control were significantly higher than the activity of mushroom extracts. Furthermore, the results showed no significant difference in reducing power among various mushroom species cultivated in different encroaching bushes. This is a significant finding indicating that the reducing power is not significantly affected by the combination of mushroom and substrate species in this setting, therefore it is advisable to choose the combination based on other practical parameters including availability, speed of growth, and hardiness of species. According to Arbaayah et al. [158], in order to reduce ferricyanide (Fe3+) to ferrocyanide (Fe2+) the mushroom extracts donate an electron which results in a change of colour from a yellow solution of test compound containing ferric ion to Pearl's Prussian blue or green to intense blue colour solution [160]. As illustrated in Figure 24, an intense green colour is observed in the vitamin C wells (control), whereas the wells of PO *G. flava* (extract) showed a light green colour at higher concentrations and remained yellow as you decreased the concentration.

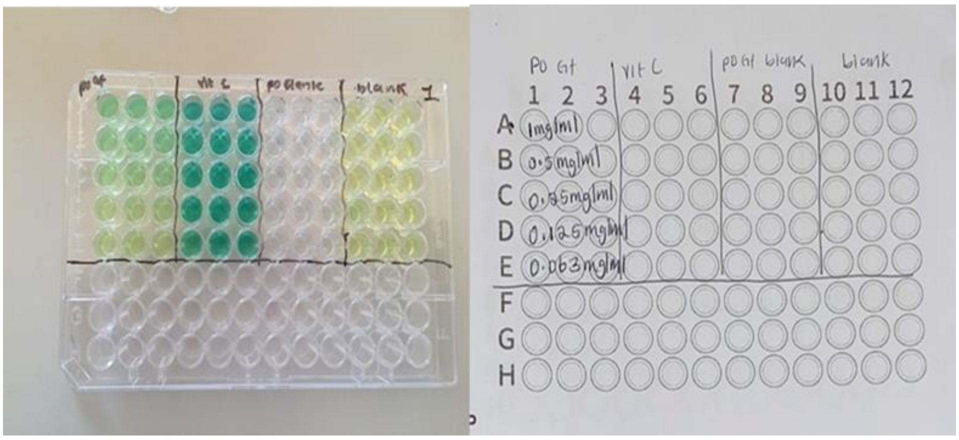

Figure 24: Reduction of ferricyanide (Fe3+) to ferrocyanide (Fe2+) (left) and right is the plate labelling of how the concentrations were added in the well

4.4 MYCOCHEMICALS ASSAYS

Flavonoids and phenolics are natural antioxidants that are commonly found in mushrooms and have redox properties that act as hydrogen donors, reducing agents, and free radical scavengers [160]. TPC and TFC values were obtained from the calibration curve y = mx + c, where x is the absorbance and y is the concentration of gallic acid solution (mg/mL) expressed as mg GAE/g. According to the results presented in Table 9, there was no significant difference in the interaction of bush species and mushroom species on total phenolic content (TPC) and flavonoid content (TFC). The capacity of flavonoids to act as antioxidants highly depends on their chemical structure [160]. The main effects of bush substrates and mushroom species on phytochemicals shown in Table 10 showed that the significance difference in TPC was due to both bush substrates and mushrooms species used at p-values <0.01 and 0.18 respectively. While significance difference for TFC was only detected in bush species substrate used at a p-value <0.01.

Table 9: The effects of Interaction of bush substrates and mushroom species on mycochemicals

Bush species Substrate	Mushroom Species	TPC (mg GAE/g)	TFC (mg QE/g)
Combretum collinum	HK 35	5.21	10.16
	PF	3.93	7.40
	PO	4.49	9.07
	PS	4.37	12.1
Grewia flava	HK 35	5.34	3.64
	PF	4.42	6.01
	PO	4.93	2.72
	PS	4.87	2.78
Terminelia sericea	HK 35	7.37	1.46
	PF	6.11	.970
	PO	6.22	.760
	PS	5.84	.510
Senegalia mellifera	HK 35	7.18	1.19
	PF	5.88	1.02
	PO	6.91	.980
	PS	8.02	.890
SEM		0.741	1.44
P-value		0.912	0.48

NB: PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Table 10: The main effects of bush substrates and mushroom species on Mycochemicals

Bush Species substrate	TPC (mg GAE/g)	TFC (mg QE/g)
Combretum collinum	4.50^b	9.68^a
Grewia flava	4.89^b	3.79^c
Terminelia Sericea	6.38^a	$.922^b$
Senegalia mellifera	7.00^a	1.017^c

SEM	0.371	0.718
P-value	<0.01	<0.01
Mushroom Species		
HK 35	6.28[a]	4.11
PF	5.09[c]	3.85
PO	5.64[ab]	3.38
PS	5.77[ab]	4.07
SEM	0.37	0.72
P-value	0.18	0.88

NB: Mean values that bear different superscripts letters are significantly different (α ≤ 0.05). PS = Pleurotus sajor-caju, PO = Pleurotus ostreatus, PF = Pleurotus florida

In the present study, the total phenolic of different oyster mushroom species varied from 3.93 mg GAE/g to 8.016 mg GAE/g. The TPC found in this study were comparable to those reported by Rajoriya et al. [163], for medicinal wild *Ganoderma lucidum* extracts ranging from 8.44–11.60 mg GAE/g [164]. The total phenolic contents of *Pleurotus ostreatus* grown on farm waste, sawdust, and peanut waste ranged from 2.672 ± 0.003 to 1.073 ± 0.028 as reported by Yilmaz et al. [164], which were relatively lower compared to the current study [162]. However, the current results were contrary to the results of Sifat et al. [165], whose study showed that the total phenolic content and total flavonoid content of different mushroom species ranged from 10.5-83.5 mg GAE/g and 51.9-184.8 mg QE/g respectively [165]. The arrangement of hydroxyl groups and the other properties in the chemical configuration of flavonoids are contributing factors to their free radical scavenging activities and antioxidant properties [163]. TFC ranged from 0.515 mg QE/g to 12.1 mg QE/g, which showed a significant difference on the main effects of the bush species at a p-value of less than 0.01 (Table 10). There was a similar performance in TFC between *G. flava* and *S. mellifera*, while *C. collinum* and *T. serecea* performed differently. *T. serecea* contributed less TFC (0.922 mg QE/g) compared to other bush species, while *C. collinum* showed the highest TFC (9.680 mg QE/g) as illustrated in Table 10. All mushroom extracts showed antioxidant activity and thus could serve as easily accessible natural foods rich in antioxidants, which may protect the immune system against oxidative damage and be used as potential sources of therapeutic agents [164].

4.5 NUTRIENT COMPOSITION OF MUSHROOMS

Edible mushrooms are known to be a good source of protein, usually ranging from 28.93% to 39.1% of their dry weight, as stated by Ragunathan et al. [166]. According to the results illustrated in Table 11, there was a significant difference in the interaction of bush substrates and mushroom species on crude protein and ash content at p-values of 0.01 and 0.015, respectively. Table 11 shows the main effects of bush substrates and mushroom species on nutrient composition, which illustrated a significant difference in % NDF on mushroom species at p-value of 0.008. These results did not indicate any significance difference for % moisture of the bush substrates or mushroom species as shown in Table 11 and 12.

Table 12: The effects of Interaction of bush substrates and mushroom species on nutrient composition

Bush species substrate	Mushroom Species	% Crude Protein	% NDF	% ADF	% Ash	% Moisture
Combretum collinum	HK 35	37.4^{ab}	33.5	32.0	6.30^{d}	86.4
	PF	30.1^{c}	30.5	30.9	16.2^{ab}	87.1
	PO	29.7^{cd}	30.1	30.8	5.77^{d}	87.2
	PS	38.0^{ab}	28.8	34.3	8.53^{bcd}	86.3
Grewia flava	HK 35	31.1^{c}	34.8	31.6	7.20^{d}	85.9

	PF	36.6^{b}	28.1	29.4	6.30^{d}	87.4
	PO	36.1^{b}	30.8	29.9	8.37^{bcd}	86.7
	PS	37.3^{ab}	30.7	32.0	8.10^{bcd}	87.4
Terminalia sericea	HK 35	38.3^{ab}	33.7	31.4	17.20^{a}	86.5
	PF	39.0^{a}	28.9	30.0	6.43^{d}	87.4
	PO	36.3^{b}	31.7	34.2	10.4^{abcd}	87.0
	PS	36.2^{b}	29.5	30.0	7.87^{cd}	86.7
Senegalia mellifera	HK 35	28.0^{d}	33.5	31.4	17.9^{a}	86.8
	PF	31.2^{c}	27.7	29.4	15.7^{abc}	87.1
	PO	31.2^{c}	30.9	32.0	12.6^{abcd}	87.5
	PS	36.4^{b}	30.6	30.3	8.20^{bcd}	87.0
SEM		0.690	2.02	2.64	2.47	0.755
P-Value		<0.01	0.990	0.966	0.015	0.986

NB: Mean values that bear different superscripts letters are significantly different (α ≤ 0.05). PS = Pleurotus sajor-caju, PO = Pleurotus ostreatus, PF = Pleurotus florida

Table 13: The main effects of bush substrates and mushroom species on nutrient composition

Bush species substrate	% Crude Protein	% NDF	% ADF	% Ash	% Moisture
Combretum collinum	33.8^{c}	30.7	32.0	9.2^{b}	86.7
Grewia flava	35.3^{b}	31.1	30.7	7.5^{b}	86.9
Terminalia Sericea	37.5^{a}	30.9	31.4	10.5^{ab}	86.9
Senegalia mellifera	31.7^{d}	30.7	30.8	13.6^{a}	87.1

SEM	0.346	1.009	1.32	1.24	0.377
P-value	<0.01	0.988	0.890	0.011	0.906
Mushroom Species					
HK 35	33.7^{b}	33.9^{a}	31.6	12.1^{a}	86.4
PF	34.2^{b}	28.8^{b}	29.9	11.2^{ab}	87.5
PO	33.3^{b}	30.9^{b}	31.7	9.30^{ab}	87.1
PS	37.0^{a}	29.9^{b}	31.6	8.18^{b}	86.9
SEM	0.346	1.009	1.32	1.236	0.377
P-value	<0.01	0.008	0.726	0.121	0.425

NB: Mean values that bear different superscripts letters are significantly different ($\alpha \leq 0.05$). PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

In the current study, crude protein content ranged from 28.0% to 39.0%, with PF grown on *T. sericea* having a high protein content. These findings are consistent with those reported by Johnsy et al. [167], who found that protein content ranged from 28.9±0.60% to 39.1±0.46%. The interaction of *C. collinum* and mushroom species revealed (Table 11) that there was no significant difference in the percentage of crude protein of HK35 and PS, whereas PF and PO showed a variation in percentage of crude protein. Mushrooms cultivated on *G. flava* showed similar performance on PF, PO, and PS, whereas HK35 showed a significant difference with the least crude protein (31.1%). PO and PF grown on *S. mellifera* showed no significant difference in crude protein content, whereas HK35 and PS did. Fibre contributes to the cleansing of the digestive tract, prevents constipation, maintains consistency in faeces and helps in reducing blood sugar [166]. Elattar et al. [168], reported ADF and NDF content of *Pleurotus ostreatus* within a range of 22.8%–28.5% and 48.7%–59.4%, respectively. Comparing these results to the current study, ADF content was in proximity, ranging from 29.4% to 34.2%, while NDF content was relatively higher than that of the current study (27.7% to 34.8%). Table 11 revealed that there was no statistically significant difference in %NDF and % ADF on the effects of bush and mushroom species interaction. However, the main effect is shown in Table 12, which illustrated a significant difference in % NDF on mushroom species, where a variation was detected in HK35 while PF, PO, and PS showed a similar performance in % NDF.

The study showed that ash content ranged from 5.77% to 17.9% (Table 11). These results are like those of Naem et al. [169], who reported % ash of edible mushrooms in the range 6.2%–8.26%, and Bhattacharjya et al. [171], whose study showed % ash of PO grown on sawdust to be in a range 9.0%–13.0%. The current results showed that different bush species performed differently on the ash content of different mushroom species. *S. mellifera* showed the highest ash content in HK35 (17.9%), while the least ash content was detected in PO (5.77%), cultivated on *C. collinum*. According to Alam et al. [171], % moisture ranged from 86.0% to 87.5% which were like those of the current study. According to the results, there was no significant difference in the moisture content of the mushroom species cultivated on different bush species. PO grown on *S. mellifera* showed the highest percentage of moisture content (87.5%), whereas PS grown on *C. collinum* had the least percentage of moisture (86.4%). Moisture of the mushrooms is greatly affected by relative humidity and temperature. The mushroom house was not temperature-controlled, and the humidity distribution was uneven [170, 171]. Grains are coated with $CaCO_3$ to raise the pH above 7 accelerating the growth of the spawn. Slow growth of spawn could be attributed to the use of cheaper grade of $CaCO_3$ or French chalk [172].

4.6 MINERAL COMPOSITION OF MUSHROOMS

Mineral content is one of the most important factors in human nutrition. Minerals are essential for the formation of healthy bones, metabolic reactions, water regulation, and nerve impulse transmission [173]. It is very important to determine the chemical and nutrient composition of the substrates, especially those used for commercial purposes [174]. Table 13 shows that the interaction between different bush substrates cultivated on different mushroom species and mineral composition showed no significant difference. However, the main effects of bush substrates and mushroom species on mineral composition illustrated in table 14 showed a significance difference at a p-value of <0.001.

Table 14: The effects of Interaction of bush substrates and mushroom species on mineral composition

Bush species substrate	Mushroom Species	%Potassium	% Sodium	%Phosphorus
Combretum collinum	HK	2.41	.033	.760
	PF	2.11	.077	.723
	PO	2.28	.050	.727
	PS	2.23	.043	.753
Grewia flava	HK	1.89	.060	.710
	PF	2.04	.053	.713
	PO	2.19	.040	.743

	PS	2.02	.050	.733
Terminelia sericea	HK	2.21	.040	.730
	PF	1.91	.050	.740
	PO	2.29	.040	.720
	PS	1.98	.040	.757
Senegalia mellifera	HK	2.02	.040	.530
	PF	1.86	.057	.433
	PO	2.40	.040	.737
	PS	1.90	.043	.740
SEM		0.019	0.002	0.014
P-value		<0.001	< 0.001	<0.001

NB: PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Table 15: The main effects of bush substrates and mushroom species on mineral composition

Bush Species substrate	% Potassium	% Sodium	% Phosphorus
Combretum collinum	2.26[a]	.051[a]	.741
Grewia flava	2.04[c]	.051[a]	.725
Terminelia Sericea	2.10[b]	.042[b]	.737
Senegalia mellifera	2.05[c]	.045[b]	.610
SEM	0.009	0.001	0.007
P-value	<0.001	<0.001	<0.001
Mushroom Species			
HK 35	2.13[b]	.043[b]	.683[b]
PF	1.98[d]	.059[b]	.653[a]
PO	2.29[a]	.042[b]	.732[a]
PS	2.03[c]	.044[a]	.746[a]
SEM	0.009	0.001	0.007

P-value	<0.001	<0.001	<0.001

NB: Mean values that bear different superscripts letters are significantly different ($\alpha \leq 0.05$). PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

The mineral composition of mushrooms grown on *C. collinum* ranged from 2.107% to 2.407% potassium, 0.033% to 0.077% sodium, and 0.760-0.723% phosphorus. The interaction of the mineral composition of different encroaching bush substrates and mushrooms on *G. flava* ranged from 1.890 to 2.193% potassium, 0.060-0.040% sodium, and 0.743-0.710% phosphorus. *T. Sericea* ranged from 2.287-1.913% of potassium and 0.040-0.050% of sodium and 0.740-0.710% of phosphorus. According to Mallikarjuna et al. [173], mushroom species, morphology of mushroom species, quality of mycelium used, and biochemical composition of substrate affect mineral accumulation in mushrooms [173]. The main effects detected in bush substrates and mushroom species as presented in Table 14 showed a significant difference at a p-value <0.001. The potassium mean content of encroaching bushes as substrates was *C. collinum* > *T. sericea* > *S. mellifera* > *G. flava*, while sodium was *C. collinum* > *G. flava* > *S. mellifera* > *T. sericea*, and phosphorus was *C. collinum* > *T. sericea* > *G. flava* > *S. mellifera*. Moreover, the main effects detected in different mushroom species (Table 14) showed a significant difference in mean mineral composition, with PO showing a high content of potassium (2.292%), PF showing a high content of sodium (0.059%), and PS showing a high content of phosphorus (0.746%) as compared to other mushroom species. Overall, among the four different bushes, *C. collinum* (potassium 2.256%, sodium 0.05%, and phosphorus 0.74%) had the greatest effect on the mineral composition of the different mushrooms cultivated. The minimal content of sodium and high potassium in mushrooms promotes salt balance and blood circulation in the human body [176].

4.7 MUSHROOM CULTIVATION TRAINING

A mushroom cultivation training was conducted at Okondjatu dristrict of Otjozondjupa region, Namibia. Due to a high rate of contamination in mushroom cultivation, a limited number of 15 people participated in the training at three different villages. A pilot survey was conducted prior to the training to test for validity and reliability of a survey which was conducted after the training. Table 15 shows the age and gender of the respondents. The survey assesed wether the respondents would like to grow mushrooms for income or consumption (figure 25), Challenges that might prevent respondents s from starting a mushroom cultivation (figure 26) and the monthly income of the trainees as shown in figure 27.

Table 16: Age of trainees in mushroom cultivation

Age range	No. of Males	No. of Females	Total No. of Trainees
15-25	3	1	4
26-45	14	7	21
46-70	9	4	13
Unknown	3	4	7
Total	29	16	45

Mushroom cultivation can be done at any age, as long as the person is physically able to perform the necessary tasks involved in mushroom cultivation, such as lifting bags or containers of substrate, maintaining the appropriate temperature and humidity levels, and harvesting the mushrooms. However, some types of mushroom cultivation may require more specialized knowledge or equipment, which could limit the ability of older individuals to participate. Table 15 indicates the number and age of trainees in the Okondjatu mushroom cultivation training. A total number of 45 people participated in the training, of which 29 were males. Among the male participants, 14 were in the age range of 26–45 years, 9 were in the age range of 46–70, and 3 were in the range of 15–25. Three male participants did not provide their ages. 37.8% (15-45 years) of male participants are youth or young adults, which brings a high potential for more farmers to participate in mushroom farming. A total of 16 females attended the training, with 7 being between the ages of 26 and 45, 4 being between the ages of 46 and 70, and 1 being between the ages of 14 and 25. The main objective of the study, to train youth and women, was achieved, as 55.6% of trainees were 15–45 years old, while 28.9% were older than 45 years.

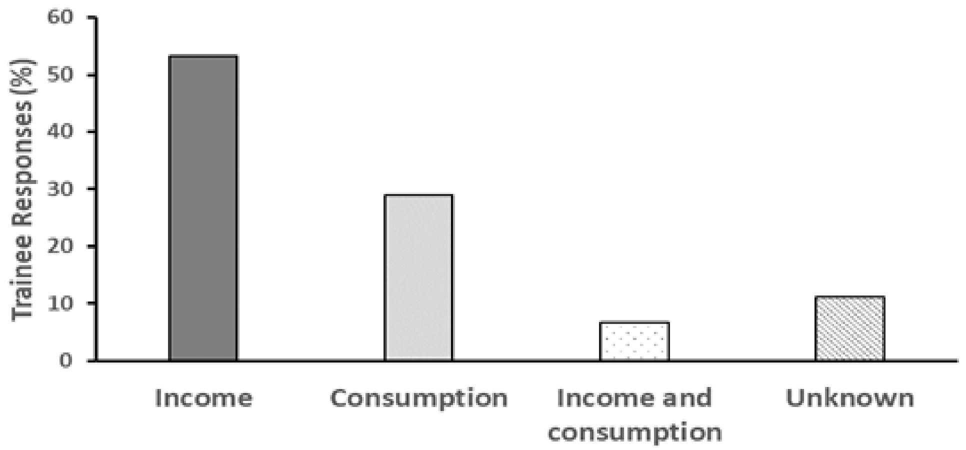

Figure 25: Response of trainees on whether they would like to grow mushrooms for income or consumption

The above results (Figure 25) represent the percentage of respondents who wanted to cultivate mushrooms for either income or consumption. Most trainees preferred to grow mushrooms for income generation (53.3%), followed by 28.9% of trainees who preferred mushroom cultivation for consumption, while 6.7% of trainees preferred it for both consumption and income. About 11.1% of the trainees did not indicate their motive for wanting to do mushroom cultivation.

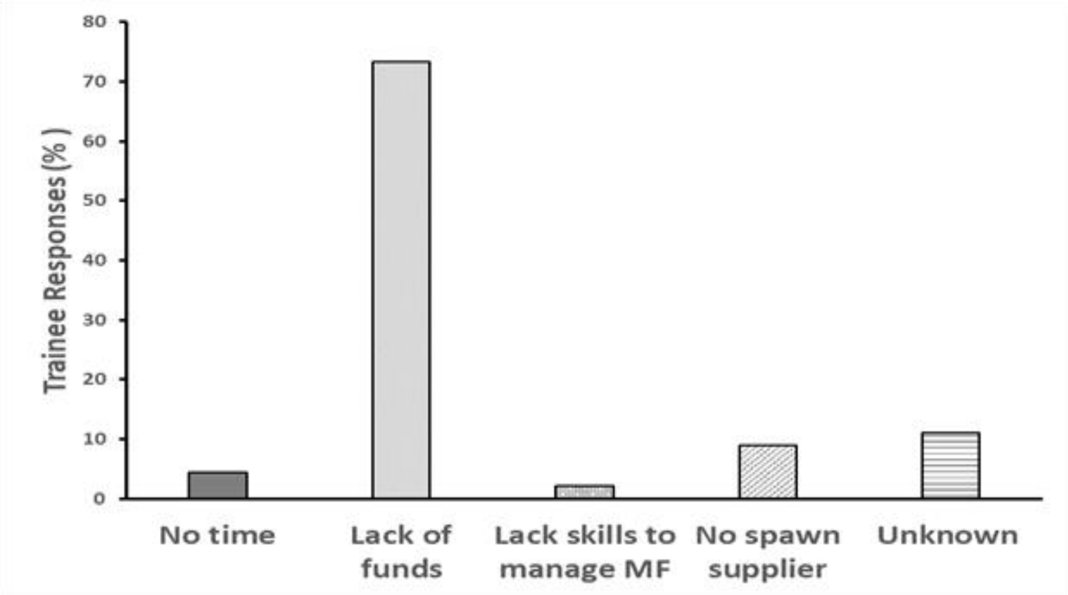

Figure 26: Challenges preventing farmers from starting a mushroom cultivation

MF= Mushroom farm

Mushroom cultivation requires for one to have knowledge, start-up capital and a consistent supply of quality spawn [2]. Figure 26 shows Challenges preventing farmers from starting a mushroom cultivation. According to the results obtained from the survey conducted, about 73.3% of the trainees indicated that they were unable to start mushroom cultivation due to a lack of funds, with 2.2% indicating that they lacked knowledge on how to manage a mushroom farm. 4.4% was due to a lack of time, while 8.9% was due to a lack of spawn suppliers in their area, and 11.1% did not indicate the challenges they might encounter starting mushroom cultivation.

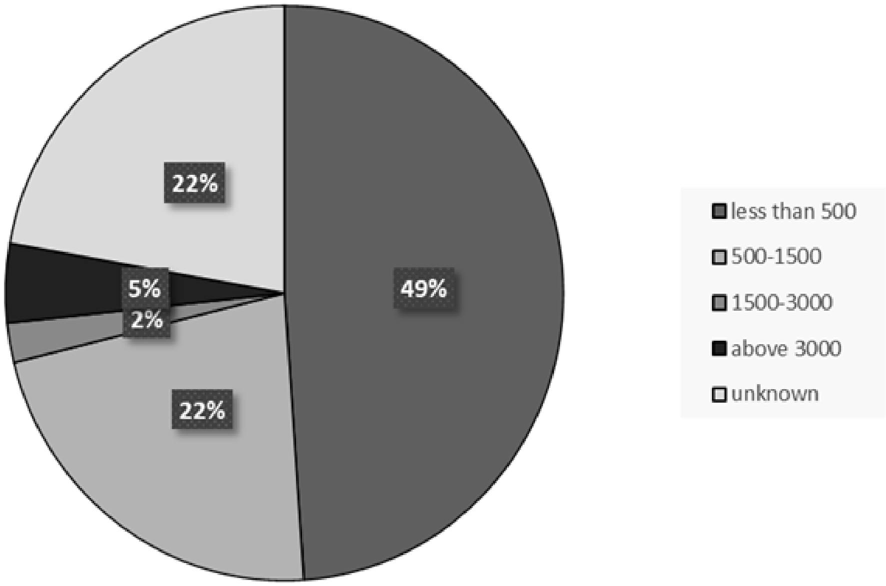

Figure 27: Monthly income of trainees

The amount of start-up capital required for mushroom cultivation at home can vary depending on the type of mushrooms farm one plans to grow and the scale of your operation. Figure 27 illustrates the monthly income of trainees. The study found that 49% of the trainees have a monthly income of less than N$500, 22% have a monthly income of N$500-N$1500, 2% have a monthly income of 1500-3000, and 4.4% have a monthly income above N$3000, while the monthly income of 22% of the trainees is unknown.

CHAPTER 5: GENERAL CONCLUSIONS AND RECOMMENDATIONS

Numerous studies have shown that mushrooms contain beneficial bioactive substances like dietary fibres, polysaccharides, fatty acids, oligosaccharides, peptides, amino acids, and phenolic compounds. The presence of these substances in mushroom extracts is influenced by several variables, including the type of substrate used, the species of mushroom used, and the environmental conditions [175]. The current experiment showed that the interaction between encroaching bush species and different mushroom species did not have a significant difference on the yield and yield components studied. The study showed a high yield of mushrooms in PF (408 g) on *G. flava* substrate with no significant difference in comparison to other encroaching bush substrates and mushroom species. On the other hand, the main effects on weight were further observed among mushroom species with PF, PS and PO (350 g, 322 g and 314 g respectively) not significantly different, while HK35 (243 g) was significantly lower by average weight harvested as compared to other mushroom species. The main effects among mushroom species in the number of days recorded to first harvest showed that HK35 took significantly longer to the first harvest (51.8days). However, the main effects on encroaching bush substrates did not indicate a significant difference on the yield and yield components. All four encroaching bushes used as substrate to cultivate various mushroom species in the current study have proven to be feasible to grow mushrooms at both a small-scale and commercial level in Namibia. On the other hand, mushroom species PF, PO, PS are concluded to be better performing mushroom species that can potentially produce a good harvest in a mushroom cultivation in Namibia. The strains of HK35 used yielded a reasonable number of mushrooms, it is least recommended not only because of the low weight of mushrooms that was harvested compared to other mushroom species, but also because it can easily get contaminated and takes longer to harvest. However, supplementation with $CaCO_3$ adjusts the pH to 7; accelerates the growth and yield of mushrooms [172]. Therefore, the study concluded that yield and yield components that showed statistically significant differences indicate that variation for the cultivation of mushrooms was due to different types of mushroom species used in the experiment.

The antioxidant assay performed in the study showed antioxidant activity among mushroom species cultivated on different substrates, PO proved to exhibit good antioxidant activities with high percentage inhibition (79.1%) and the lowest IC50 value (0.380 mg/ml), greatest reducing power was observed in PS. The study proved that mushrooms are crucial in the bioconversion of encroaching bushes that are reducing the amount of grazing pasture for livestock, in addition to their antioxidant and nutritional benefits. Demonstrating the antioxidant activity in these mushroom extracts, the chemical properties of the antioxidative components in these extracts will benefit from further investigation. While these studies provide valuable insights into the antioxidant potential of mushrooms, there is a need for more in vivo studies to determine the bioavailability, metabolism, and overall effects of mushroom antioxidants in living organisms. Additional research is necessary to fully understand the mechanism and metabolic pathways of mushroom bioactive compounds that are responsible for their bio-activity. It is also suggested that other potential antioxidant mechanisms and extraction methods be used to investigate the antioxidant activity of mushrooms grown on encroaching bushes. In complimentary to the current study, it is recommended to use multiple antioxidant assays to gain a comprehensive understanding of the antioxidant activity of mushrooms. Furthermore, studying the impact of factors like cultivation conditions, harvesting methods, and post-harvest processing on the antioxidant activity can help optimize the production and preservation of mushroom antioxidants as well as improved yields. As a result, it can be said that the study objectives were accomplished because mushrooms that were grown on various encroaching bushes as substrates showed substantial yields which can contribute to food security and income generation. More remote studies on other nutritional components of mushrooms need to be conducted with equipment capable of addressing additional aspects in a practical way.

Among the objectives of the study was to train farmers in Okondjatu district on mushroom cultivation. A total of 45 trainees, of which 29 were male and 16 were female, participated in the mushroom cultivation training in Okondjatu district, Otjozondjupa region. The Okondjatu community is greatly affected by bush encroachment. Therefore, it is recommended to participants or farmers who want to practice mushroom cultivation to use the common encroaching bushes in their area. To build a mushroom house, it is recommended that they use locally available materials such as rocks, cow dung, or thatch. The mushroom house should have three sections to allow for the rotation of the mushroom bags. Mushroom houses must be ventilated and have humidity and light in the harvesting room. It is recommended to use oyster mushroom species like PF. However, farmers who want to grow mushrooms for medicinal purposes are recommended to grow PO, as it showed high antioxidant potential compared to other species cultivated in this study. Farmers who participated in the training are recommended to become self-reliant and educate the next generation on mushroom cultivation in their community.

Mushroom cultivation is slowly gaining popularity in Namibia due to a slight shift in mushroom cultivation training using low-cost farmer-friendly methods [177]. However, additional research on other encroaching bushes as substrate for mushroom cultivation, marketing strategies for produced mushrooms, and other challenges such as pest and disease outbreaks in mushroom farms are required. The government together with Namibia Standard Institutes (NSI) and commercial mushroom stakeholders such as Mycohab and Agricon should implement spawn quality control labs and create mushroom standards. This will promote high quality spawn production in Namibia. Strengthening technical support to the farmers, such as educating them on time management during training, in order for them to accommodate mushroom cultivation as a new farming activity. In addition, the government should implement strategies with minimal restrictions to provide loans as start-up capital for citizens earning a low income. In conclusion, this study has proven the presence of antioxidant activity and various nutritional and mineral compositions in mushroom species cultivated on different encroaching bushes. Therefore, it is concluded that mushrooms are potential therapeutic agents, and mushroom cultivation could be among various ways to combat bush encroachment and improve livelihood by reducing unemployment in the Okondjatu community and Namibia at large.

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APPENDICES

APPENDIX 1: ETHICAL CLEARANCE CERTIFICATE

UNAM

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SOS-0008 Date: 25 October 2021

This Ethical Clearance Certificate is issued by the University of Namibia Ethics Committee (REC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the ethics committee.

Title of Project: COMPARATIVE STUDY ON VALUE CHAIN, YIELD, AND NUTRITIONAL ASPECT OF NAMIBIAN COMMERCIAL MUSHROOMS CULTIVATED ON POST-HARVEST ENCROACHER BUSHES AS SUBSTRATES IN OTJOZONDJUPA REGION

Student: KRISTINE NDEUYAMUNYE HAUKONGO

Student Number: 201511909

Supervisor(s): DR LYDIA HORN (UNIVERSITY OF NAMIBIA)

Centre for Research Services

Take note of the following:

1. Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the ethics committee. An application to make amendments may be necessary.
2. Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the ethics committee
3. The Principal Researcher must report issues of ethical compliance to the ethics committee (through the Chairperson) at the end of the Project or as may be requested by the ethics committee
4. The ethics committee retains the right to:

i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,

ii) Request for an ethical compliance report at any point during the course of the research.

The ethics committee wishes you the best in your research.

Dr. Zivayi Chiguvare (Chairperson Ethics Committee)

Prof. Davis Mumbengegwi (Head, Multidisciplinary Research)

APPENDIX 2: HARVESTING PERMIT

COMMUNITY FORESTRY IN NAMIBIA

0051

FOREST MANAGEMENT BODY (FMB)
African Wild Dog Community Forest
P.O. Box: 302, Okakarara

TYPE OF PERMIT:	**Harvesting**		**Transport** *(For Regional use only)*		**Marketing**
	Commercial	~~Own use~~	Commercial	~~Own use~~	Valid within the CF area

Permission is hereby granted in terms of the Forest Act, 2001 (Act No. 12 of 2001) as amended Act No. 13 of 2005, in connection with the approved Forest Management Plan and the Block Permit to:

Permit Holder Name	University of Namibia (Ruform Project)
Namibia ID/Passport	
Address	P Bag 13188
Name of ~~Village~~ or Town	Windhoek
Region	Khomas
Contact Telephone No.	

To Harvest / Transport / Market ***(encircle applicable)*** Forest Produce specific below, subject to the conditions on the reverse side

Species	**Product**	**Quantity** (to be written in Figures and Words) (number, length, diameter, kg, ton)
Senegalia Mellifera	Animal feed	3000 Kg
Combretum Collinum		
Terminalia Sericea		
Grewia flava		

Harvesting Point or Harvesting Location	Okondjatu
Loading Point Address	/
Destination Address	/
Transport mode/Vehicle Registration No.	

THIS PERMISSION IS VALID FROM 18/05/2022 **UNTIL** 18/08/2022

African Wild Dog Community Forest
04 MAY 2022
P.O. Box 302 Okakarara

Amount Paid: N$ 100 Receipt Number:

Name of licensing FMB Member: G. Tjiparanga

Signature of licensing FMB member: [signature]

Place: Okondjatu

Date: 04/05/2022

I declare that the information provided is true and correct, that I have read and understand the conditions stated overleaf, that I am able to identify the forest produce which I have a permission for, that I clearly know and respect the boundaries of **African Wild Dog Community Forest,** and I acknowledge that a failure to observe these conditions shall constitute an offence.

[signature] 04/05/2022

Signature of Permit Holder Place and Date

APPENDIX 3: SURVEY QUESTIONNAIRE

A. General information

Date_____________

Region___________________________

Constituency_________________________

Village/sublocation_____________________

Name of respondent_____________________ ☐ M ☐ F

Age (range) ___________________________

1. Level of Education:

☐ Illiterate

☐ grade 1-7

☐ grade 7-10

☐ Grade 10-12

☐ Graduate

☐ Post graduate

2. Monthly income N$:

☐ less than 500

☐ 500-1500

☐ 1500-3000

☐ Above 3000

3. Are you currently employed?

☐ YES

☐ NO

Section B: Mushroom cultivation

4. Did you enjoy the mushroom cultivation training?

□ YES

□ NO

5. Would you like to grow mushrooms for your own consumption or income?

□ Consumption

□ Income

6. Do you think mushroom cultivation is rewarding?

□ Yes

□ NO

7. After participating in the training are you interested in starting a mushroom Cultivation?

□ YES

□ NO

8. What challenges do you think can prevent you from starting a mushroom cultivation?

□ No time

□ Lack of funds

□ Lost interest

 No idea how to manage a mushroom farm

 No spawn supplier

APPENDIX 4: MUSHROOM CULTIVATION TRAINING NOTES

Okondjatu Training Notes

Compiled By: Kristine Haukongo

1. **Spawn**

Figure 1: Fully colonised/white Spawn

Spawn in simple words is known as mushroom seeds.

Materials needed

- Mother spawn/ culture
- Wheat grains or sorghum
- Savanna bottles/ mayonnaise or plastic bags
- Sanitiser/methylated spirits
- Dishwasher/hand soap
- Gloves (preferably)
- Face musk

Basic step for making spawn

1. Soak your wheat grains/sorghum in water overnight
2. Drain excess water from the grains to the right moisture

3. Pack the grains in plastics or bottles
4. pasteurise for 2 hours in a pressure cook or pot.

NOTE:

- Wear clean clothes
    - No talking or unnecessarily moving in the inoculation room
    - Keep the doors closed to avoid dust and dirt in the room

Pasteurisation is the process of reducing possible contaminations in the spawn.

5. Inoculate pasteurised spawn with mycelium cultures or from grain spawn in a clean/sterile environment.

Inoculation means the introduction or planting of spawn to substrate in order to initiate its growth and development of the mycelium.

6. Incubate in a sterile dark place for 7-8 days until fully white
7. Spawn is then stored in the fridge or under cool environment

Figure 2: Inoculation of spawn

Substrate

Mushroom substrate is a material were mushroom mycelium feed on on to grow into a mushroom

Materials needed

- Substrate (sawdust/straw)
- Transparent heat resistant plastic bag
- Tying ropes
- Spade
- Garden folk
- 200l drum
- Firewood
- Stone for making fire

Basic steps of substrate preparation

A) Substrate of choice (Wood chips, straws or leaves) is soaked in water over night, excess water is drained the next day

B) The soaked substrate is mixed with wheat bran and then packed in plastic bags.

C) The packed bags are then placed in pasteurisation drums, where they are steamed at 100 degrees Celsius for 4 hours.

NB! Keep fire burning to make sure the steam there is steam throughout pasteurization.

Figure 3: Preparation and Pasteurisation of substrate

2. **Inoculation, Incubation and Harvesting**

This process involves moving plastic bags from pasteurisation drums into a clean room and allowed to cool to room temperature.

- The bags are then inoculated with spawn and sealed or tied with strings.
- All the inoculated bags are marked (name and date) and transported to incubation room (dark room).
- They stay incubated for three weeks maximum until fully white.
- Fully colonised bags are moved to fruiting rooms, and make small openings/slits to allow mushroom pinning.
- Once mushrooms are matured enough, they are Harvested and taken to the market or dried.

Figure 4: Mushrooms ready for Harvest

3. **Factors affecting the production of Mushrooms**

   - Unhygienic conditions
   - Contamination (Green/black mould)
   - Insect pest infestation
   - Poor aeration
   - Lack of sufficient nutrients
   - Insufficient or excess water supply
   - Poor mushroom house management

APPENDIX 5: RAW DATA FOR YIELD AND YIELD COMPONENTS

Mushroom Spp.	Substrate/Treatment	weight of fresh mushrooms (g)	size of stipe (cm)	Size of diameter (cm)	days to first harvest	Number of fruiting bodies
PF	Sub1	307	2.8	7.4	39	7
PF	Sub1	351.1	3.5	7.8	41	11
PF	Sub1	299.78	1.9	6.6	40	4
PF	Sub2	464.4	4.7	8.5	43	8
PF	Sub2	268.25	2.7	6.5	39	2
PF	Sub2	490	2.5	12.5	35	1
PF	Sub3	265.9	3.8	7.3	39	5
PF	Sub3	302.2	2.6	6.7	36	5
PF	Sub3	295	1.9	5.3	39	6
PF	Sub4	420	3.4	7.2	44	4
PF	Sub4	478.4	3.7	7.7	35	12
PF	Sub4	254.6	1.6	8.3	36	2
PO	Sub1	351.8	4	7.8	36	12
PO	Sub1	360	1.7	7.7	41	6
PO	Sub1	348.15	1.7	7.7	41	6
PO	Sub2	278.8	4.2	8.5	36	1
PO	Sub2	402.8	2.7	7.2	34	4
PO	Sub2	302.83	1.3	7.2	30	3
PO	Sub3	302	2.6	5.9	33	4
PO	Sub3	281.4	2.4	9.3	41	4
PO	Sub3	298.2	2	7.4	28	2
PO	Sub4	284.8	4	6.6	30	2
PO	Sub4	292.8	2.5	7	29	2
PO	Sub4	268.4	1.7	6.8	30	3
PS	Sub1	402.67	2.7	5.3	32	6
PS	Sub1	315	3.9	6.7	36	11
PS	Sub1	302.7	2.9	8.6	41	2
PS	Sub2	278.3	4.3	6.8	38	3
PS	Sub2	275.5	4.6	6.3	39	7
PS	Sub2	386.15	4.3	6.7	47	10
PS	Sub3	212.6	3.8	8.7	33	6
PS	Sub3	395.5	4.1	7.6	40	10
PS	Sub3	253.97	1.9	7.8	44	4
PS	Sub4	453.4	3.1	5.7	42	7
PS	Sub4	278.3	7.2	1.8	38	2
PS	Sub4	310.2	1.8	6.4	35	8

HK35	Sub1	229.2	3.4	7	42	4
HK35	Sub1	261.6	3.5	8	38	3
HK35	Sub1	120	2	2.9	37	1
HK35	Sub2	251.6	3.5	6.1	39	2
HK35	Sub2	274.3	2.5	5.8	79	4
HK35	Sub2	262.95	3	5.95	59	3
HK35	Sub3	278.2	3	7.4	37	3
HK35	Sub3	248	3	7.4	66	2
HK35	Sub3	224.6	4.2	7.4	41	2
HK35	Sub4	231.7	3.3	6	44	2
HK35	Sub4	265	3.6	8	84	3
HK35	Sub4	273.2	1.8	5.9	54	4

PS = Pleurotus sajor-caju, PO = Pleurotus ostreatus, PF = Pleurotus florida , sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

APPENDIX 6: RAW DATA FOR NUTRIENT COMPOSITION

Mushroom Spp.	Substrate	%CRUDE PROTEIN	%NDF	%ADF	%ASH	%MOISTURE
PF	Sub1	30.9	32.44	33.2	20.3	85.04886
PF	Sub1	29.1	32.92	25.87	17.3	88.32242
PF	Sub1	30.4	26.04	33.52	11	87.92448
PF	Sub2	37.1	31.38	32.7	7.3	87.1447
PF	Sub2	36.4	24.81	27.12	7.3	87.13886
PF	Sub2	36.2	28.2	28.5	4.3	87.87755
PF	Sub3	39	30.9	30.33	3.7	86.64912
PF	Sub3	38.7	28.92	36.38	8.3	86.20119
PF	Sub3	39.2	26.79	23.13	7.3	89.32203
PF	Sub4	31.6	29.66	31.84	16.7	86.61905
PF	Sub4	30.8	29.35	30.57	14	87.83445
PF	Sub4	31.2	24	25.72	16.3	86.95994
PO	Sub1	30.6	30.77	32.09	7.3	86.46958
PO	Sub1	28.7	33.48	32.32	4.3	87.13889
PO	Sub1	29.8	26.05	28.11	5.7	87.96496
PO	Sub2	37.8	32.19	29.69	8.7	87.58967
PO	Sub2	38.1	31.33	34.56	8.7	85.77458
PO	Sub2	32.3	28.87	25.4	7.7	86.72523
PO	Sub3	36.8	30.98	35.46	7.3	86.19205
PO	Sub3	36	37.81	35.97	20	87.13575
PO	Sub3	36.2	26.25	31.28	4	87.59222
PO	Sub4	32.8	32.89	32.53	9.3	88.48315
PO	Sub4	28.9	32.87	35.5	18.3	87.36339

PO	Sub4	31.8	26.79	28	10.3	86.62444
PS	Sub1	38.2	30.28	35.52	9.3	87.28487
PS	Sub1	38.2	30.7	42.15	12.3	84.88889
PS	Sub1	37.5	25.46	25.08	4	86.68649
PS	Sub2	37.8	32.72	32.76	6.3	87.7111
PS	Sub2	37	32.74	36.57	7.3	87.18693
PS	Sub2	37.1	26.62	26.54	10.7	87.31063
PS	Sub3	37.5	31.13	35.24	9	83.96049
PS	Sub3	36.9	31.38	32.53	4.3	88.82427
PS	Sub3	34.2	26.03	22.15	10.3	87.20321
PS	Sub4	36.2	32.01	28.07	8.3	87.5386
PS	Sub4	36.8	33.86	32.82	8	87.63924
PS	Sub4	36.3	25.8	29.97	8.3	85.91231
HK35	Sub1	37.1	34.23	31.76	3.3	84.1623
HK35	Sub1	37.8	35.83	34.79	7.3	85.66514
HK35	Sub1	37.3	30.29	29.53	8.3	89.33333
HK35	Sub2	31.2	36.02	37.29	6.3	84.61844
HK35	Sub2	29.8	37.93	30.54	10.6	86.76631
HK35	Sub2	32.3	30.42	26.91	4.7	86.33787
HK35	Sub3	38.6	35.3	31.57	24.3	85.90942
HK35	Sub3	37.9	34.83	34.15	8.3	87.05645
HK35	Sub3	38.4	30.82	28.4	19	86.48293
HK35	Sub4	27.3	34.02	32.26	23.3	85.97324
HK35	Sub4	28.7	37.12	33.82	20	87.28302
HK35	Sub4	27.9	29.45	28.15	10.3	87.26208

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida , sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera*

APPENDIX 7: RAW DATA FOR MINERAL COMPOSITION

Mushroom Spp.	Substrate/Treatment	%PHOSPHORUS	POTASSIUM	%SODIUM
PF	Sub1	0.73	2.1	0.08
PF	Sub1	0.69	2.13	0.07
PF	Sub1	0.75	2.09	0.08
PF	Sub2	0.71	2.05	0.05
PF	Sub2	0.7	2.04	0.06
PF	Sub2	0.73	2.04	0.05
PF	Sub3	0.75	1.91	0.05
PF	Sub3	0.74	1.89	0.05
PF	Sub3	0.73	1.94	0.05
PF	Sub4	0.36	1.89	0.06
PF	Sub4	0.53	1.82	0.05
PF	Sub4	0.41	1.88	0.06
PO	Sub1	0.72	2.27	0.05

PO	Sub1	0.73	2.29	0.05
PO	Sub1	0.73	2.29	0.05
PO	Sub2	0.74	2.2	0.04
PO	Sub2	0.74	2.18	0.04
PO	Sub2	0.75	2.2	0.04
PO	Sub3	0.72	2.3	0.04
PO	Sub3	0.72	2.26	0.04
PO	Sub3	0.72	2.3	0.04
PO	Sub4	0.74	2.42	0.04
PO	Sub4	0.73	2.39	0.04
PO	Sub4	0.74	2.4	0.04
PS	Sub1	0.76	2.21	0.04
PS	Sub1	0.74	2.25	0.05
PS	Sub1	0.76	2.22	0.04
PS	Sub2	0.74	2.02	0.05
PS	Sub2	0.73	2.01	0.05
PS	Sub2	0.73	2.04	0.05
PS	Sub3	0.75	1.93	0.04
PS	Sub3	0.76	2.03	0.04
PS	Sub3	0.76	1.99	0.04
PS	Sub4	0.74	1.91	0.05
PS	Sub4	0.74	1.87	0.04
PS	Sub4	0.74	1.91	0.04
HK35	Sub1	0.76	2.31	0.03
HK35	Sub1	0.76	2.5	0.04
HK35	Sub1	0.76	2.41	0.03
HK35	Sub2	0.71	1.89	0.06
HK35	Sub2	0.72	1.88	0.06
HK35	Sub2	0.7	1.9	0.06
HK35	Sub3	0.74	2.22	0.04
HK35	Sub3	0.74	2.2	0.04
HK35	Sub3	0.71	2.2	0.04
HK35	Sub4	0.54	2.04	0.04
HK35	Sub4	0.53	2.01	0.04
HK35	Sub4	0.52	2.00	0.04

PS = Pleurotus sajor-caju, PO = Pleurotus ostreatus, PF = Pleurotus florida , sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

APPENDIX 8: RAW DATA FOR % INHIBITION

Concentration	Psts	psts 2	psts 3	PS AVERAGE	TS	SE (±)
0.125 mg/ml	14.75318	14.22164	24.82295	17.93259		3.448595

0.25 mg/ml	32.00373	30.93547	41.40573	34.78164	3.326369
0.5 mg/ml	54.21603	62.81032	66.55383	61.19339	3.652223
1 mg/ml	71.46658	77.73136	88.16088	79.11961	4.868962
conc.	ps cc 1	pscc 2	ps cc 3	AVERAGE	SE (±)
0.125 mg/ml	-17.2748	16.83318	4.966371	1.508238	9.996801
0.25 mg/ml	-5.82271	35.19862	25.34408	18.24	12.3631
0.5 mg/ml	10.64427	69.72736	50.03786	43.46983	17.3691
1 mg/ml	32.6732	78.63481	66.72754	59.34518	13.77186
conc.	Pots	po ts	po ts3	AVERAGE	SE (±)
0.125 mg/ml	-4.22098	1.777211	2.529326	0.284954	2.135812
0.25 mg/ml	12.35738	9.243197	14.12512	14.60776	1.427045
0.5 mg/ml	32.19751	27.72959	33.73656	40.74345	1.80146
1 mg/ml	73.50867	62.61054	72.47067	72.29066	3.472661
conc.	pssm 1	pssm 2	ps sm 3	AVERAGE	SE (±)
0.125 mg/ml	-8.10219	13.46432	8.626588	4.662905	6.533547
0.25 mg/ml	4.644674	28.07689	21.55425	18.09194	6.982309
0.5 mg/ml	26.60065	40.63111	47.91056	38.38077	6.253693
1 mg/ml	60.20935	54.66531	69.90469	61.59312	4.453307
conc.	hk sm 1	hk35 2	hk35 3	AVERAGE	SE (±)
0.125 mg/ml	-2.03372	13.33231	6.984099	6.094227	4.45805
0.25 mg/ml	8.989647	32.30476	21.34871	20.88104	6.734554
0.5 mg/ml	34.96752	48.41158	47.61926	43.66612	4.355312
1 mg/ml	50.9487	76.41854	74.35749	67.24158	8.168135
conc.	pogf 1	po gf 2	po gf 3	AVERAGE	SE (±)
0.125 mg/ml	-5.53094	-4.7534	6.097263	-1.39569	3.753195
0.25 mg/ml	5.902949	14.43027	20.61339	13.64887	4.264474
0.5 mg/ml	27.65833	45.84184	36.5958	36.69866	5.249377
1 mg/ml	64.38536	72.30442	48.09384	61.59454	7.126934
conc.	hk gf 1	hk gf 2	hk gf3	AVERAGE	SE (±)
0.125 mg/ml	-3.88605	11.7602	10.96088	6.278345	5.087435
0.25 mg/ml	13.22279	25.7398	45.31463	28.0924	9.338497
0.5 mg/ml	31.60714	47.27041	56.79422	45.22392	7.34253
1 mg/ml	65.7398	61.62415	73.05272	66.80556	3.341903
conc.	pocc 1	po cc 2	po cc 3	AVERAGE	SE (±)
0.125 mg/ml	-14.999	-3.2568	-1.51515	-6.59033	4.234307
0.25 mg/ml	-3.37008	16.86224	8.883187	7.458452	5.883851
0.5 mg/ml	40.99014	28.06973	47.44624	38.83537	5.696331
1 mg/ml	40.67814	60.46769	70.89443	57.34675	8.861173
conc.	hk cc1	hk cc2	hk cc3	AVERAGE	SE (±)
0.125 mg/ml	2.568027	-8.35884	10.0085	1.405896	5.333941
0.25 mg/ml	27.80612	22.2534	32.78061	27.61338	3.040472
0.5 mg/ml	49.79592	49.46429	56.38605	51.88209	2.254018
1 mg/ml	59.5068	68.17177	76.62415	68.10091	4.941479
conc.	pf cc 2	pfcc 2	pf cc 3	AVERAGE	SE (±)

0.125 mg/ml	31.13095	4.94716	-1.79502	11.4277	10.04204
0.25 mg/ml	18.57993	16.38145	6.823749	13.92838	3.608561
0.5 mg/ml	36.31803	39.23592	37.42372	37.65922	0.850513
1 mg/ml	70.82483	73.75054	52.92415	65.83317	6.509535
conc.	ps gf 1	psgf 2	ps gf 3	AVERAGE	SE (±)
0.125 mg/ml	1.053448	6.881108	3.897377	3.943978	1.682462
0.25 mg/ml	20.88078	23.62317	16.17745	20.22714	2.174099
0.5 mg/ml	43.09826	56.40147	44.15839	47.88604	4.268699
1 mg/ml	55.02183	76.44677	68.51811	66.66223	6.254073
conc.	hk ts 1	hk ts 2	hk ts 3	AVERAGE	SE (±)
0.125 mg/ml	28.80102	10.09354	11.7602	16.88492	5.977444
0.25 mg/ml	33.57993	18.27381	27.44048	26.43141	4.447209
0.5 mg/ml	55.2466	41.64116	54.82143	50.56973	4.465973
1 mg/ml	62.61054	57.5085	61.7602	60.62642	1.578165
conc.	pf gf1	pf gf2	pf gf3	AVERAGE	SE (±)
0.125 mg/ml	26.04592	38.54592	15.02551	26.53912	6.794234
0.25 mg/ml	33.63095	42.28741	29.56633	35.16156	3.75116
0.5 mg/ml	54.80442	48.92007	52.28741	52.00397	1.704568
1 mg/ml	67.93367	51.07993	75.48469	64.83277	7.21364
conc.	pf ts 1	pfts 2	pf ts 3	AVERAGE	SE (±)
0.125 mg/ml	5.089831	9.210315	9.616498	7.972215	1.445954
0.25 mg/ml	21.37183	39.16534	23.86085	28.13267	5.562928
0.5 mg/ml	48.65851	49.01859	54.31384	50.66365	1.828055
1 mg/ml	70.49057	60.31172	71.91216	67.57148	3.653008
conc.	pf sm 1	pf sm 2	pf sm 3	AVERAGE	SE (±)
0.125 mg/ml	-7.02894	3.803731	0.944279	-0.76031	3.241189
0.25 mg/ml	6.443628	21.1528	16.51151	14.70265	4.341425
0.5 mg/ml	50.16567	49.75264	39.54835	46.48889	3.472316
1 mg/ml	76.86972	79.19947	68.9457	75.00497	3.103381
conc.	po sm 1	po sm 2	po sm 3	AVERAGE	SE (±)
0.125 mg/ml	28.54592	0.348639	12.08333	13.6593	8.177905
0.25 mg/ml	47.71259	41.13095	26.26701	38.37018	6.342833
0.5 mg/ml	60.87585	51.0119	47.32143	53.06973	4.045844
1 mg/ml	72.20238	64.73639	61.30102	66.07993	3.217853
conc.	Vitc 1	Vitc 2	Vitc 3	AVERAGE	SE (±)
0.125 mg/ml	96.91815	95.46769	79.19469	90.52684	5.681525
0.25 mg/ml	95.5954	95.2466	95.75075	95.47016	0.149064
0.5 mg/ml	96.84996	95.70578	95.88437	95.53092	0.355388
1 mg/ml	96.31814	96.0119	94.08044	96.14671	0.700461

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*, *sm = Senegalia mellifera*, *cc = Combretum collinum*, *ts = Terminelia sericea*, *gf = Grewia flava*

APPENDIX 9: RAW DATA FOR REDUCING POWER

	1 mg/ml	0.5 mg/ml	0.25 mg/ml	0.125 mg/ml	0.063 mg/ml
PO GF	0.3183	0.2194	0.1029	0.0866	0.0493
PO GF	0.427333	0.2446	0.135833	0.073233	0.0344
PO GF	0.4875	0.305833	0.207767	0.104667	0.07
average	0.411044	0.256611	0.148833	0.088167	0.051233
Standard error (±)	0.049518	0.025664	0.030962	0.009108	0.010322
PS GF	0.203533	0.178633	0.1083	0.0171	0.001733
PS GF	0.3347	0.209633	0.1384	0.081833	0.044333
PS GF	0.4368	0.185	0.0598	0.003367	0.108933
average	0.325011	0.191089	0.102167	0.0341	0.051667
Standard error	0.067512	0.009453	0.022896	0.024194	0.031162
HK GF	0.244667	0.2141	0.186867	0.108533	0.0352
HK GF	0.260667	0.216367	0.197433	0.138267	0.043267
HK GF	0.240867	0.2192	0.171267	0.132333	0.024
average	0.248733	0.216556	0.185189	0.126378	0.034156
Standard error	0.006067	0.001475	0.0076	0.009085	0.005586
PF GF	0.3004	0.1838	0.109567	0.0465	0.021933
PF GF	0.233933	0.2118	0.1744	0.0815	0.025067
PF GF	0.238867	0.212367	0.182433	0.1441	0.037267
average	0.257733	0.202656	0.155467	0.0907	0.028089
Standard error	0.021381	0.009429	0.023067	0.028548	0.004677
PS SM	0.311233	0.167	0.0792	0.0247	0.024033
PS SM	0.349567	0.2208	0.156367	0.0818	0.0437
PS SM	0.3552	0.225867	0.1302	0.0653	0.061167
average	0.338667	0.204556	0.121922	0.057267	0.042967
Standard error	0.013813	0.018835	0.022657	0.016966	0.010726
HK35 SM	0.3317	0.2275	0.131433	0.0625	0.0551
HK35 SM	0.405267	0.2505	0.172833	0.094367	0.065133
HK35 SM	0.371333	0.194267	0.0597	0.034133	0.095733
average	0.369433	0.224089	0.121322	0.063667	0.071989
Standard error	0.021258	0.016323	0.033048	0.017398	0.01222
PF SM	0.283933	0.172633	0.110567	0.044467	0.022633
PF SM	0.2413	0.171333	0.128033	0.1045	0.018
PF SM	0.323033	0.224433	0.1566	0.095033	0.0757
average	0.282756	0.189467	0.131733	0.081333	0.038778
Standard error	0.023602	0.017487	0.013417	0.018635	0.01851
PO SM	0.2413	0.171333	0.128033	0.1045	0.018
PO SM	0.265633	0.222067	0.198433	0.165767	0.126733

PO SM	0.248	0.218033	0.127933	0.105333	0.055633
Average	0.251644	0.203811	0.151467	0.1252	0.066789
Standard error	0.007257	0.016281	0.023483	0.020285	0.03188
PO TS	0.399233	0.2623	0.1568	0.045567	0.0209
PO TS	0.194733	0.171067	0.1413	0.073667	0.0465
PO TS	0.451833	0.261967	0.182267	0.0844	0.0595
average	0.3486	0.231778	0.160122	0.067878	0.0423
Standard error	0.078417	0.030356	0.011942	0.011578	0.011339
PS TS	0.347033	0.219867	0.1382	0.079733	0.048133
PS TS	0.392833	0.255967	0.1725	0.0967	0.083
PS TS	0.399133	0.1962	0.073867	0.070533	0.074767
average	0.379667	0.224011	0.128189	0.082322	0.068633
Standard error	0.016418	0.017377	0.02891	0.007664	0.010522
PF TS	0.207	0.178733	0.1189	0.051033	0.008467
PF TS	0.392833	0.255967	0.1725	0.0967	0.083
PF TS	0.466333	0.2245	0.126833	0.044333	0.112167
average	0.355389	0.219733	0.139411	0.064022	0.067878
Standard error	0.077169	0.022422	0.016702	0.016453	0.030876
HK TS	0.2259	0.194833	0.150167	0.102833	0.022933
HK TS	0.180567	0.150033	0.1239	0.115233	0.103567
HK TS	0.24741	0.2094	0.1743	0.146367	0.024267
average	0.217959	0.184756	0.149456	0.121478	0.050256
Standard error	0.0197	0.017863	0.014554	0.012949	0.026658
PF CC	0.286967	0.172	0.103467	0.045	0.020567
PF CC	0.329267	0.2003	0.1219	0.069033	0.0357
PF CC	0.306967	0.204967	0.1326	0.0923	0.077233
average	0.307733	0.192422	0.119322	0.068778	0.0445
Standard error	0.012217	0.0103	0.008508	0.013655	0.01694
PS CC	0.371667	0.3415	0.176233	0.077233	0.0427
PS CC	0.412333	0.253267	0.248167	0.171533	0.7729
PS CC	0.3912	0.283133	0.174633	0.1156	0.100733
average	0.391733	0.292633	0.199678	0.121456	0.305444
Standard error	0.011742	0.02591	0.024249	0.027379	0.234327
PO CC	0.399	0.196	0.2548	0.046433	0.040867
PO CC	0.311467	0.138267	0.085467	0.0389	0.024867
PO CC	0.346833	0.192133	0.135767	0.086933	0.066233
average	0.352433	0.175467	0.158678	0.057422	0.043989
Standard error	0.025423	0.018633	0.050207	0.014915	0.012043
HK CC	0.263	0.1624	0.0824	0.0477	0.0274
HK CC	0.2788	0.165	0.1877	0.0422	0.0137
HK CC	0.2386	0.153333	0.1149	0.077633	0.0353
average	0.260133	0.160244	0.128333	0.055844	0.025467
Standard error	0.011693	0.003536	0.031131	0.01101	0.00631
VIT C	0.787733	0.832167	0.7949	0.729233	0.568967

VITC	1.029489	1.025156	1.024189	0.988922	0.966656
VIT C	0.871483	0.852717	0.81655	0.78455	0.72815
average	0.896235	0.903346	0.878546	0.834235	0.754591
Standard error	0.070878	0.061193	0.073089	0.078975	0.115562

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida, sm = Senegalia mellifera, cc = Combretum collinum, ts = Terminelia sericea, gf = Grewia flava*

APPENDIX 10: RAW DATA FOR TPC

Mushroom Spp.	Substrate/Treatment	TPC (mg GAE/g)	TFC (mg QE/ g)
PF	Sub1	2.979127	6.00557
PF	Sub1	5.755078	3.180874
PF	Sub1	7.813761	0.356177
PF	Sub2	4.40009	0.340545
PF	Sub2	3.957185	0.77016
PF	Sub2	3.765251	5.440848
PF	Sub3	5.035053	10.74386
PF	Sub3	5.943606	0.390631
PF	Sub3	8.987339	1.091158
PF	Sub4	3.112415	11.68699
PF	Sub4	4.581455	2.159683
PF	Sub4	4.695467	1.192583
PO	Sub1	5.111628	12.38691
PO	Sub1	4.526646	1.209924
PO	Sub1	6.116131	0.968673
PO	Sub2	4.484817	6.650806
PO	Sub2	5.213256	3.801813
PO	Sub2	6.637061	0.95282
PO	Sub3	3.871675	8.174811
PO	Sub3	6.130085	0.926558
PO	Sub3	6.271907	0.884022
PO	Sub4	5.059782	4.223544
PO	Sub4	6.398438	0.37843
PO	Sub4	7.818088	1.092312
PS	Sub1	3.42307	11.88765
PS	Sub1	4.353629	1.588232
PS	Sub1	6.129403	0.993317
PS	Sub2	4.941131	11.9349
PS	Sub2	5.66061	0.051931
PS	Sub2	6.436605	1.210718
PS	Sub3	4.730254	12.44198
PS	Sub3	6.106006	0.016662
PS	Sub3	8.114732	0.81142

PS	Sub4	4.608142	6.713012
PS	Sub4	5.26954	3.675256
PS	Sub4	9.496218	0.637501
HK35	Sub1	6.175236	9.691521
HK35	Sub1	4.244752	0.561379
HK35	Sub1	9.927119	1.39151
HK35	Sub2	3.979031	8.793651
HK35	Sub2	6.014967	0.443077
HK35	Sub2	7.831676	1.113214
HK35	Sub3	5.482552	11.98536
HK35	Sub3	5.550887	0.446362
HK35	Sub3	5.914759	1.092203
HK35	Sub4	5.771843	9.90099
HK35	Sub4	6.623606	2.534653
HK35	Sub4	7.802904	1.361745

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida, sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera*

APPENDIX 11: MUSHROOM CULTIVATION TRAINING

Figure 1: Community members collecting stones to build a mushroom house

Figure 2: Okatupapa village Participants standing in front of the mushroom house

Figure 3: Orukune village and participants standing in front of the mushroom house

Figure 4: Okondjatu conservancy center and participant undergoing theoretical training

Appendix 12: POST HOC TESTS

Substrate: Weight of fresh mushrooms

Duncan[a,b]

		Subset
Substrate/Treatment	N	1
Sub3	12	279.7975
Sub1	12	304.0833
Sub4	12	317.5667
Sub2	12	327.9900
Sig.		.101

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Mushroom species:Weight of fresh mushrooms

Duncan[a,b]

		Subset	
Mushroom Spp.	N	1	2
HK	12	243.3625	
PO	12		314.3317
PS	12		322.0242
PF	12		349.7192
Sig.		1.000	.211

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate: Size of stipe

Duncan[a,b]

Substrate/Treatment	N	Subset 1
Sub1	12	2.8333
Sub3	12	2.9417
Sub4	12	3.1417
Sub2	12	3.3583
Sig.		.324

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Mushroom species: Size of stipe

Duncan[a,b]

		Subset	
Mushroom Spp.	N	1	2
PO	12	2.5667	
PF	12	2.9250	2.9250
HK	12	3.0667	3.0667
PS	12		3.7167
Sig.		.331	.126

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate/Treatment	N	Subset 1
Sub4	12	6.4500
Sub1	12	6.9583
Sub2	12	7.3375
Sub3	12	7.3500
Sig.		.168

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Mushroom species: Size of diameter

Duncan[a,b]

Mushroom Spp.	N	Subset 1
HK	12	6.4875
PS	12	6.5333
PO	12	7.4250
PF	12	7.6500
Sig.		.076

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate: Days to first harvest

Substrate/Treatment	N	Subset 1
Sub1	12	38.6667
Sub3	12	39.7500
Sub4	12	41.7500
Sub2	12	43.1667
Sig.		.270

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Duncan[a,b]

Mushroom Spp.	N	Subset	
		1	2
PO	12	34.0833	
PS	12	38.7500	
PF	12	38.8333	
HK	12		51.6667
Sig.		.229	1.000

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Mushroom species: Number of fruiting bodies

Duncan[a,b]

Mushroom Spp.	N	Subset 1	2
HK	12	2.7500	
PO	12	4.0833	4.0833
PF	12		5.5833
PS	12		6.3333
Sig.		.257	.074

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate: Number of fruiting bodies

Duncan[a,b]

Substrate/Treatment	N	Subset 1
Sub2	12	4.0000
Sub4	12	4.2500
Sub3	12	4.4167
Sub1	12	6.0833
Sig.		.108

Duncan[a,b]

Substrate/Treatment	N	Subset 1	2	3	4
Sub4	12	31.6917			
Sub1	12		33.8000		
Sub2	12			35.2583	
Sub3	12				37.4500
Sig.		1.000	1.000	1.000	1.000

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Mushroom species: % Crude proten

Duncan[a,b]

		Subset	
Mushroom Spp.	N	1	2
PO	12	33.3167	
HK	12	33.6917	
PF	12	34.2167	
PS	12		36.9750
Sig.		.091	1.000

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate: % NDF

Duncan[a,b]

		Subset
Substrate/Treatment	N	1
Sub4	12	30.6517
Sub1	12	30.7075
Sub3	12	30.9283
Sub2	12	31.1025
Sig.		.777

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Duncan[a,b]

Mushroom Spp.	N	Subset 1	2
PF	12	28.7842	
PS	12	29.8942	
PO	12	30.8567	
HK	12		33.8550
Sig.		.179	1.000

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Sustrate: Days to first harvest

Substrate/Treatment	N	Subset 1
Sub2	12	30.7150
Sub4	12	30.7708
Sub3	12	31.3825
Sub1	12	31.9950
Sig.		.538

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Mushroom species: % ADF

Duncan[a,b]

Mushroom Spp.	N	Subset 1
PF	12	29.9067
HK	12	31.5975
PS	12	31.6167
PO	12	31.7425
Sig.		.378

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Duncan[a,b]

Substrate/Treatment	N	Subset 1	Subset 2
Sub2	12	7.4917	
Sub1	12	9.2000	
Sub3	12	10.4833	10.4833
Sub4	12		13.5917
Sig.		.115	.085

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Mushroom species: % Ash

Duncan[a,b]

Mushroom Spp.	N	Subset 1	Subset 2
PS	12	8.1750	
PO	12	9.3000	9.3000
PF	12	11.1500	11.1500
HK	12		12.1417
Sig.		.117	.134

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate: % Moisture

Duncan[a,b]

Substrate/Treatment	N	Subset 1
Sub1	12	86.7409
Sub2	12	86.8485
Sub3	12	86.8774
Sub4	12	87.1244
Sig.		.519

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Mushroom species: % Moisture

Duncan[a,b]

Mushroom Spp.	N	Subset 1
HK	12	86.4042
PS	12	86.8456
PO	12	87.0878
PF	12	87.2536
Sig.		.155

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate Treatment	N	Subset 1	2
Sub1	12	4.4982	
Sub2	12	4.8934	
Sub3	12		6.3825
Sub4	12		6.9970
Sig.		.456	.250

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mel

Duncan[a,b]

Mushroom Spp.	N	Subset 1	Subset 2
PF	12	5.0855	
PO	12	5.6366	5.6366
PS	12	5.7724	5.7724
HK	12		6.2766
Sig.		.225	.258

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate: % TFC

Duncan[a,b]

Substrate Treatment	N	Subset 1	Subset 2	Subset 3
Sub3	12	.9223		
Sub4	12	1.0175		
Sub2	12		3.7875	
Sub1	12			9.6782
Sig.		.926	1.000	1.000

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Mushroom species: % TFC

Duncan[a,b]

Mushroom Spp.	N	Subset 1
PO	12	3.3805
PF	12	3.8493
PS	12	4.0660
HK	12	4.1096
Sig.		.519

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate: % Potasium

Duncan[a,b]

Substrate/Treatment	N	Subset 1	2	3
Sub2	12	2.0375		
Sub4	12	2.0450		
Sub3	12		2.0975	
Sub1	12			2.2558
Sig.		.577	1.000	1.000

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Mushroom species: % Potasium

Duncan[a,b]

Mushroom Spp.	N	Subset 1	2	3	4
PF	12	1.9817			
PS	12		2.0325		
HK	12			2.1300	
PO	12				2.2917
Sig.		1.000	1.000	1.000	1.000

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate: % Phosphorus

Duncan[a,b]

		Subset	
Substrate/Treatment	N	1	2
Sub4	12	.6100	
Sub2	12		.7250
Sub3	12		.7367
Sub1	12		.7408
Sig.		1.000	.146

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

Mushroom species: % Phosphorus

Duncan[a,b]

		Subset		
Mushroom Spp.	N	1	2	3
PF	12	.6525		
HK	12		.6825	
PO	12			.7317
PS	12			.7458
Sig.		1.000	1.000	.168

PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

Substrate: % Sodium

Duncan[a,b]

Substrate/Treatment	N	Subset	
		1	2
Sub3	12	.0425	
Sub4	12	.0450	
Sub2	12		.0508
Sub1	12		.0508
Sig.		.093	1.000

sub1 = Combretum collinum, sub2 = Grewia flava, sub3 = Terminalia sericea, sub4 = Senegalia mellifera

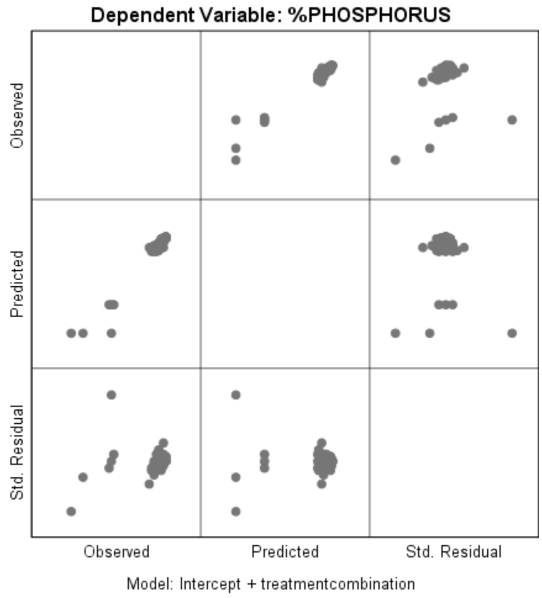
Mushroom species: % Sodium

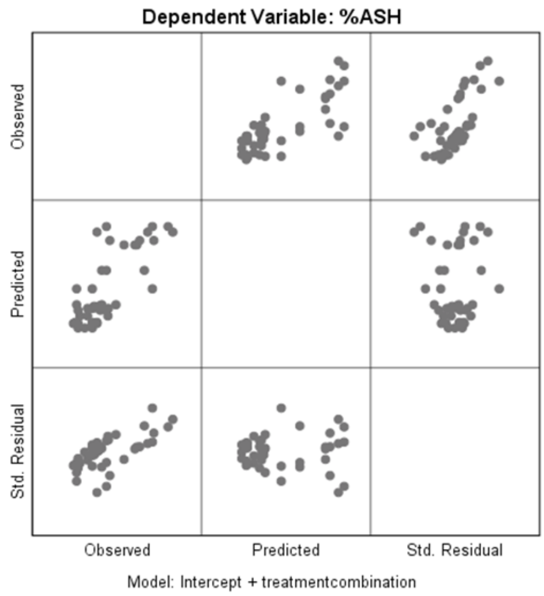
Duncan[a,b]

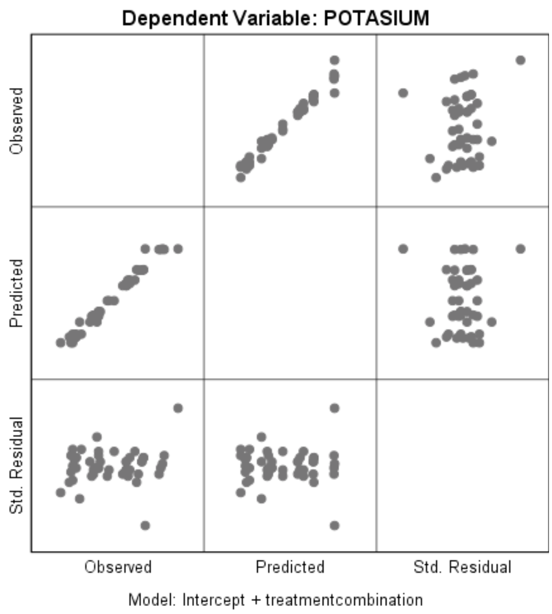
Mushroom Spp.	N	Subset	
		1	2
PO	12	.0425	
HK	12	.0433	
PS	12	.0442	
PF	12		.0592
Sig.		.285	1.000

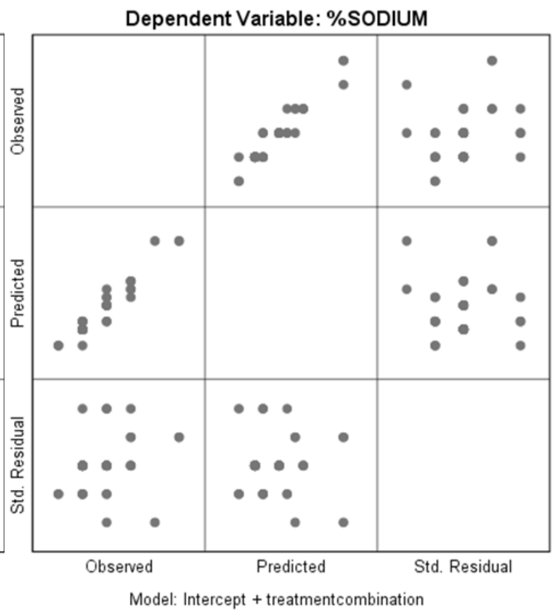
PS = *Pleurotus sajor-caju*, PO = *Pleurotus ostreatus*, PF = *Pleurotus flo*

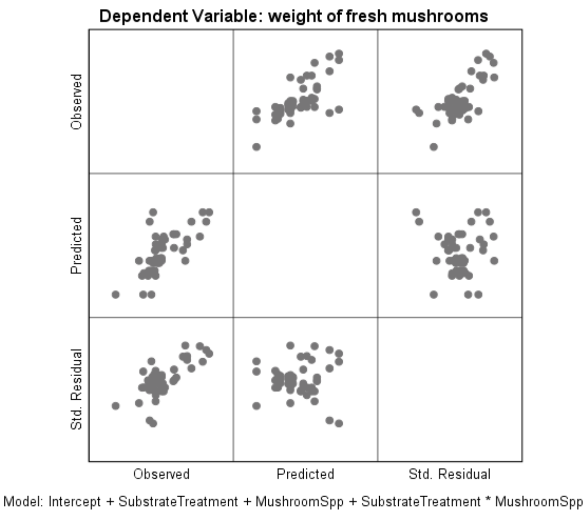
APPENDIX 13: PLOT OF RESIDUALS

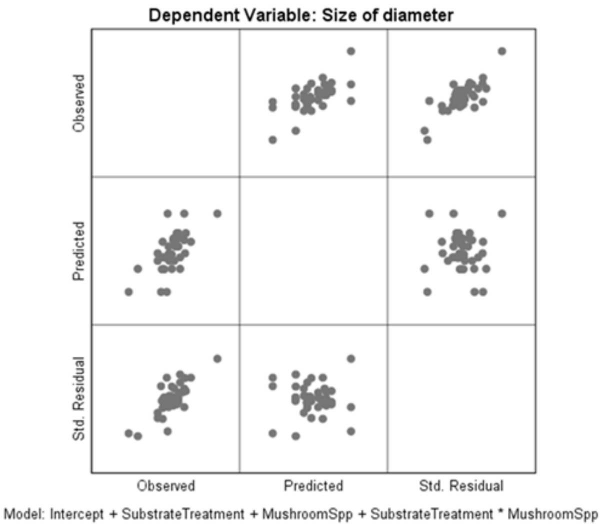




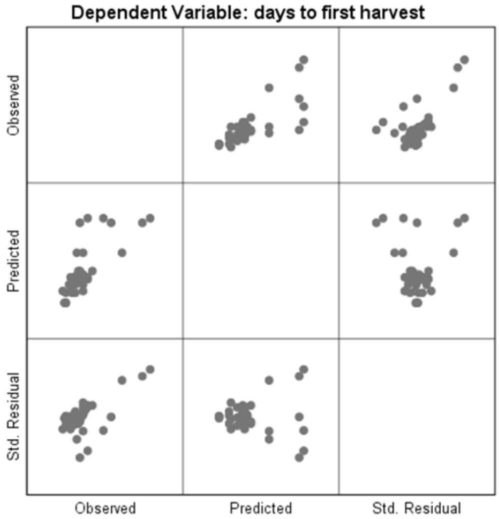





Dependent Variable: weight of fresh mushrooms
Observed
Predicted
Std. Residual
Observed
Predicted
Std. Residual
Model: Intercept + SubstrateTreatment + MushroomSpp + SubstrateTreatment * MushroomSpp

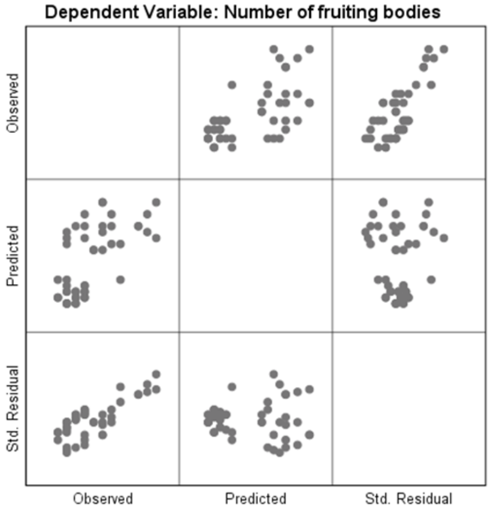

Dependent Variable: Size of diameter
Observed
Predicted
Std. Residual
Observed
Predicted
Std. Residual
Model: Intercept + SubstrateTreatment + MushroomSpp + SubstrateTreatment * MushroomSpp

Dependent Variable: days to first harvest

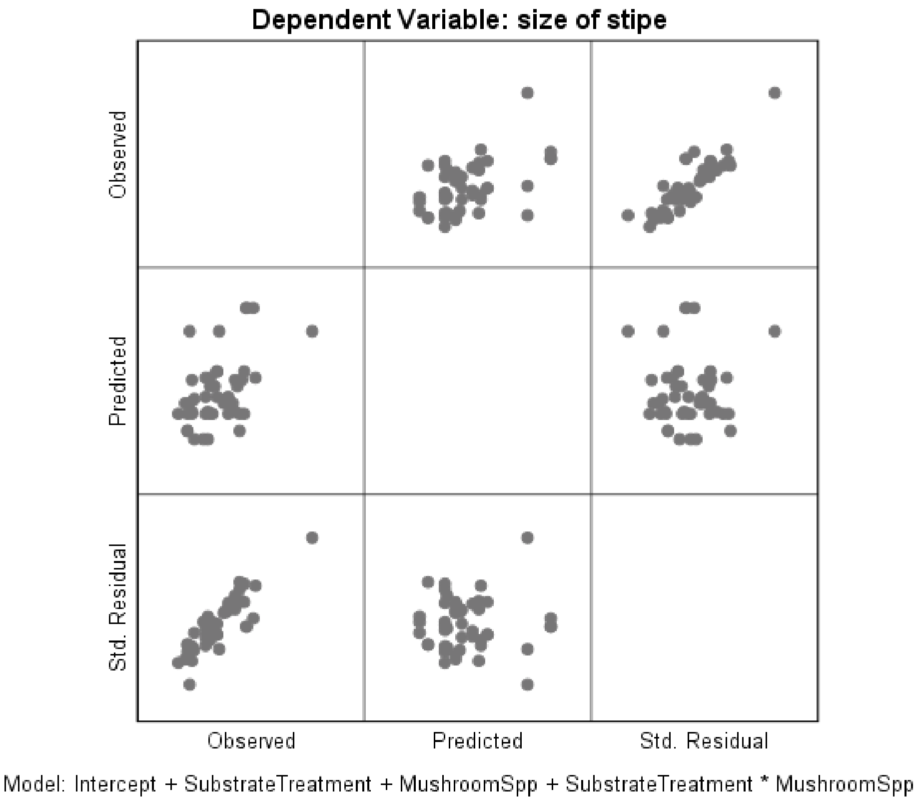


Model: Intercept + SubstrateTreatment + MushroomSpp + SubstrateTreatment * MushroomSpp

Dependent Variable: Number of fruiting bodies



Model: Intercept + treatmentcombination


Dependent Variable: size of stipe
Observed
Predicted
Std. Residual
Observed
Predicted
Std. Residual
Model: Intercept + SubstrateTreatment + MushroomSpp + SubstrateTreatment * MushroomSpp

APPENDIX 14: PHYTOCHEMICAL PROFILE PLOTS