

UNIVERSITY OF BOTSWANA
BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES



**VALUE-ADDITION OF CEREAL CROP RESIDUES USING LOW TECHNOLOGY
OYSTER MUSHROOM (*PLEUROTUS* SPP.) PRODUCTION TO IMPROVE
SMALL-SCALE FARMERS' INCOME AND NUTRITION IN BOTSWANA**

**A dissertation submitted in partial fulfilment of the requirements for the award of MSc
in Crop Science (Crop Protection Stream)**

By

Thobo Motlhalamme

ID NO: 201100119

August 2019

Main Supervisor: Prof E.B. Khonga

Co-Supervisor: Dr T.V. Balole

Co-Supervisor: Dr D.S. Marumo

**Department of Crop and Soil Sciences
Botswana University of Agriculture and Natural Resources**

CERTIFICATION

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Supervisor's name and signature

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Date

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Co- supervisor's name and signature

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Date

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Co- supervisor's name and signature

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Date

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Head of Department's name and signature

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Date

APPROVAL

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Supervisor's name and signature

Date

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Co- supervisor's name and signature

Date

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Co- supervisor's name and signature

Date

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Head of department's name and signature

Date

.....

Dean of Faculty's name and signature

Date

STATEMENT OF ORIGINALITY

The work contained in this dissertation was compiled by the author at the University of Botswana, Botswana University of Agriculture and Natural Resources between January 2017 and December 2018. It is original except where references were made and it will not be submitted for award of any other degree or diploma of any other University.

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ACKNOWLEDGEMENT

Funding was received from Regional Universities Forum for Capacity Building in Agriculture (RUFORUM). I am forever grateful. My sincere gratitude goes to my supervisor Professor Elenimo B. Khonga for his guidance and assistance during the study, his patience and support kept me going. I would like to thank the co-supervisors Dr Thabsile V. Balole and Dr Davis S. Marumo for their support and Keletso Ntokome for his assistance during my research. I would also like to thank the Botswana University of Agriculture and Natural Resource staff more especially technicians who assisted me in the laboratory.

To the farmers who took part in the surveys your effort is highly recognised and appreciated. I extend much thanks to my mother who was always there to support me through my learning process. I am especially grateful to the Almighty GOD for keeping the breath of life in me and giving me strength and hope to persevere even when the going was tough. His grace and love was sufficient for me to overcome.

DEDICATION

This work is dedicated to my late grandfather Mr John R. Motlhalamme who passed away in 2006. You always believed in me and you are dearly missed. My lovely mother Ms Sabinah B. Motlhalamme who was always there for me. Thank you very much.

‘I will never leave you nor forsake you’.

Hebrews 13:5b (NIV)

ABSTRACT

The average cereal grain yields for Botswana small-scale farmers is 108kg/ha with gross income of P233.95/ha, respectively. The low yields are due to frequent droughts and pest outbreaks resulting in food and nutrition insecurity. Cereal crop residues are left in the fields to be grazed or harvested for feed but their income potential has not been assessed. The objectives of this study were to assess: current cereal residue use and farmers' perceptions on incorporation of oyster mushroom production in their cereal cropping system; the additional potential farmers' income when crop residues are used to grow mushrooms; and the nutritional composition of mushrooms grown on residues and their contribution to farmers' nutritional status. Sixty-three farmers from southern Botswana were interviewed and crop residue biomass was estimated from 10m x 10m plots in selected farms. A 3x2x3 split-split plot factorial design experiment was used to assess biological efficiency (BE%) of three *Pleurotus spp.* grown on steamed and hydrogen peroxide disinfested maize, millet and sorghum stalks. Potential income was estimated using mushroom yield of the best combination of substrate, disinfestation method and *Pleurotus* species and nutritional analysis of mushrooms was done using standard procedures. Farmers still leave crop residues to be grazed in the field and those who harvest them for feed do not weigh them. All farmers were receptive to incorporating mushroom production in their cropping system. Residue yields of maize, sorghum and millet were 1206.7kg/ha, 1213Kg/ha and 4530.0 Kg/ha, respectively. The highest (69.4%) and lowest (8.1%) BE were recorded for *P. ostreatus* HK35 grown on steamed millet and *P. floridanus* on hydrogen peroxide-treated sorghum. Average potential additional income from mushrooms grown on millet stalk was P102,695/ha compared to P233.95/ha from grain. The protein, fat and mineral content of the mushrooms was favourable and can contribute to improved diets of rural farmers. It is concluded that incorporation of oyster mushroom production in small-scale cereal production system has the potential of improving food and nutrition security among small-scale farmers and creation of jobs for unemployed youth in rural areas.

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LIST OF SYMBOLS AND ABBREVIATIONS

RUFORUM	Regional Universities Forum for Capacity Building in Agriculture
BAMB	Botswana Marketing Board
FAO	Food and Agriculture Organisation
H _a	Alternate hypothesis
H ₀	Null hypothesis
BE	Biological efficiency
SR	Spawn running
LSD	Least significant difference
SDM	Substrate disinfestation method
Po × Pf	Hybrid of <i>Pleurotus ostreatus</i> and <i>Pleurotus floridanus</i>
Pf	<i>Pleurotus floridanus</i>
Po HK35	<i>Pleurotus ostreatus</i> strain HK35
MZ	Maize stalk
MT	Millet stalk
SG	Sorghum stalk

CHAPTER ONE: INTRODUCTION

1.1 Agricultural production in Botswana

The agricultural sector is vital to the economic development of Botswana, but its contribution to the GDP has continued to decline, from 42.7% at independence in 1966 to 1.9% in 2008 (UNDP, 2012). By 2011 it contributed about 3%, of which crop production was only 0.4%; whereas livestock contributed 2.6%. However, agriculture employs more than 70% of the country's population (UNDP, 2012). Botswana's agricultural industry is largely driven by the international beef market which includes South Africa and the European Union. There is very little commercial crop production, and most crops are produced for subsistence, or for sale locally (Burgess, 2006). Crop production in Botswana is predominantly traditional dry land/rain-fed cropping of mainly cereal and vegetables. In the north, where there are ephemeral rivers which flow and dry up as flood waters recede, farmers plant grains and vegetables in the soils that can retain moisture at relatively shallow depths, within the root zones of the crops. The urban areas practice horticulture, focusing mainly on extensive vegetable and fruit farming, as well as intensive nursery plant production (Burgess, 2006).

1.2 Cereal crop production and income of small-scale farmers in Botswana

Despite diversified farming efforts in Botswana, the country is not self-sufficient in cereals and has always been a net importer of cereals especially from South Africa. About 95% of its grain needs are imported by the Botswana Agricultural Marketing Board (BAMB) from South Africa and the other 5% is produced locally mainly by commercial farmers (African Development Bank, 2008). Cereal crop production in Botswana is low due to erratic and poorly distributed rainfall, persistent droughts and pests resulting in low yields and income per hectare. Grain yield figures for 2012 for maize, sorghum and millet for small-scale and commercial farmers were 53 kg/ha, 144kg/ha and 153 kg/ha, and 580 kg/ha, 1476 kg/ha and 660 kg/ha, giving an

overall average yield of 117 kg/ha and 906 kg/ha respectively (Statistics Botswana 2014). Excess grain from small scale farmers is sold to BAMB. BAMB sets annual producer prices based on the regional grain market prices controlled by demand and supply. The grain producer prices for grade 1 sorghum, millet and white maize for the past 10 growing seasons are summarised in Table 1 below.

Table 1: Botswana Agricultural Marketing Board (BAMB) annual producer prices for the period 2008 to 2018

Cereal	Producer prices (BWPula) per 50kg bag for 2008/09 to 2017/18 growing seasons										Average/kg (BWP)
	08/09	09/10	10/11	11/12	12/13	13/14	14/15	15/16	16/17	17/18	
Sorghum	92.50	82.50	70.00	101.15	130.00	147.50	105.00	100.00	125.00	106.75	2.12
Millet	110.00	130.00	130.00	150	130.00	100.00	85.00	130.00	150.00	125.00	2.48
Maize	90.00	72.50	62.50	99.17	118.00	120.00	80.00	103.00	150.00	70.75	1.93
Average	97.50	95.00	87.50	116.77	126.00	122.50	90.00	111.00	141.67	100.83	2.18

Source: Botswana Agricultural Marketing Board (www.bamb.co.bw)

Estimated potential average gross incomes per hectare based on the average grain yields from sorghum, millet and maize grain sales to BAMB by small scale farmers for the 2008/09 to 2017/2018 were P305, P379 and P102, respectively. Using the average cereal grain yield of 117 kg/ha and the average producer price of P2.18/kg, the average income of the small holder farmer from grain sale is P256 (US\$26) per hectare. In Botswana, the average farm size for small scale farmers is about 5 hectares with most farmers owning only 2 hectares (African Development Bank, 1994). Hence, the annual gross income from grain sales for small scale farmers is about P1024. The production costs per hectare for small scale farmers are not fully estimated, since most depend on government subsidies for seed, herbicides and fertilizers but these are likely to be higher than the gross income.

1.3 Importance of small-scale farming and challenges faced by small- scale farmers

In many Sub-Saharan African countries small scale-farming is a very important aspect of the community livelihood because farmers focus on growing enough food to feed themselves and their families, hence the activity is very important in rural development and livelihood improvement (Govereh *et al.*, 1999). Smallholder agriculture is a farming system consisting of a farmer who practices both subsistence and selling surplus in which most family members provide labour in the farm however reducing production costs (Cornish *et al.*, 1999). Though the main focus of small-scale farmers is to provide for their families the little surplus is then sold. Small-scale farmers generate income, provide food for local markets and make important contributions to nutrition at household level and also represent a diversity and cultural richness that is of global significance (World Bank, 2013). The significance of small-scale farmers has been shown through its role of providing food at household level, generating income and also providing employment for unskilled individuals in rural areas.

Farming at small-scale has always played a vital role in rural areas in Botswana. Due to climate change and other factors such as insect pests, weeds and diseases yields have steadily declined over the past decade. Climate change has brought further setback in the agricultural industry in Botswana making the country to be persistently not food self-sufficient (Burgess, 2006). Agriculture is the backbone of rural poor people and it helps to combat poverty at household level and it provides food and to some extent generates income. Growth in agriculture delivers more poverty reduction than other sectors in lower-income countries (World Bank, 2013).

According to Frank and Buckley (2012), smallholder farmers are vulnerable to factors such as climate change, poverty and reliance on natural resources. This could be due to lack of knowledge, financial constraints hence failure to adapt to modern technologies (IFAD, 2012). Amongst the factors that affect small holder farmers, climate change is the most negative impacting factor. Climate change is likely to lead to decreasing crop yields in most tropical and

sub-tropical regions, negatively impacting agricultural sectors and reducing food security in developing countries (Frank and Buckley, 2012). Impacts of climate change on agriculture clearly suggest that in the future productivity and production stability will be reduced in areas which are already food insecure hence food shortage at household levels (FAO, 2010). Therefore, there is a need to identify approaches that strengthen the adaptive capacity of smallholder farmers and enhance their ability to respond to climate change.

Small scale farmers employ traditional practices in most cases and normally deal with unpredicted events through the use of past experience. In most cases traditional practices don't survive unpredicted changes such as climate change and drought leading to crop failure or low yield which leaves small-scale farmers with less to survive on (Cornish *et al.*, 1999). This has necessitated incorporation of modern technology and identification of approaches that will strengthen the adaptive capacity of smallholders to climate change.

In order to adapt to climate change, smallholder producers need new and improved technologies, skills and knowledge, or in many cases, to be linked to existing technologies which are currently inaccessible (Frank and Buckley, 2012). Small scale-farmers should be able to utilise the available farm materials to generate more income as evidenced by mushroom cultivation using crop residues (Mshigeni and Chang, 2000). As stated above, cereal crop production in Botswana is low due to erratic and poorly distributed rainfall, persistent droughts and pests resulting in low grain yields and income per hectare. According to UNDP (2012) the persistent low crop yields are contributing to high poverty levels in the rural areas of Botswana.

1.4 Value-addition of crop residues

Value- addition is the process of taking a raw commodity and changing its form to produce a high quality end product. Value-addition of farm outputs is vital for the improvement of

farmer's incomes and nutrition more especially smallholder farmers. This means embarking on strategies to emphasise or enhance the quality and value of agricultural products, thereby raising the product above the basic 'commodity' level and also making use of materials in order to acquire more returns. This can include projects to raise the quality of production to meet market needs and ensure consistency, the formation or development of collaborative groups to market quality products, consumer quality assurance schemes, production of speciality foods, establishing farmers' markets, regional or local branding of foodstuffs, and other similar strategies (Mshigeni and Chang, 2000). Diversification and value addition in agriculture is an important aspect for improvement of farmer's income and also for supply of food at household level.

In cereal production in Botswana crop residues are potential candidates for value-addition in order to improve the overall income per hectare. The average potential income from grain yields of about 117kg/ha is only about P256 (\$26/ha) so value addition of the crop residues could make significant improvement in the farmers' income. Cereal crop residue is the portion of the crop plant that normally remains in the field after the grain or marketable portion is harvested. These are often left in the field to be grazed by animals or burnt or ploughed under (Madibela and Lekgari, 2005). Unlike grain, yields of crop residues are normally not quantified or recorded by the farmers since they are considered as waste, hence they are not utilised to their full potential (Smil, 2009). However in developed countries the economic value of crop residues is now increasing (Hofstrand, 2009).

Cultivation of oyster mushrooms by small scale farmers using crop residues from their farms is a potential value- addition activity in Botswana. Oyster mushrooms are a high-value crop

which can sell for between P100-P300/kg (US\$10 -30). Assuming cereal crop residues yields of 2-5tons/ha and biological efficiency of 20%, estimated mushroom yields of 400 to 1000Kg can be realised resulting in additional gross incomes of up to P40,000/ha.

1.5 Justification of the study

In Botswana, cereal crop residues are normally left in the open farmlands for livestock to graze upon after harvesting the grain. As animals graze in the fields they can destroy soil structure rendering the soil unfertile in years to come and leading to low yields. However, mushrooms, which are a choice delicacy of global acceptability (Mshigeni and Chang, 2000) grow on cereal crop residues as substrates. Utilisation of these crop residues as substrates for production of mushrooms would add utility value to the crop residues through sale or consumption of mushrooms and use of the mushrooms spent substrates as livestock feed. Cultivation of *Pleurotus* spp., though not requiring much skills is labour intensive and may provide job opportunities for rural people, thus improving their livelihoods through income generated by working in mushroom production farms.

The diet of most rural people is heavily dependent on starch from grains, therefore oyster mushrooms can provide a supplementary protein to the diet at household level. In Botswana not much has been done on small-scale mushroom production, hence it is an unfamiliar crop. Current market prices of mushrooms in the shops in Botswana range from P80 to P350/kg for button and exotic mushrooms, which include oyster mushrooms, respectively. This signifies that mushroom production on crop residues can yield higher returns than the cereal grains alone. Therefore, there is a need to embark on popularisation of small-scale oyster mushroom production in Botswana. The current study is aimed at assessing the potential of oyster

mushroom production in value-addition of cereal crop residue for improved income and nutrition of small scale farmers.

1.6 Objectives

The main objective of the study is to evaluate the potential income from oyster mushroom (*Pleurotus* spp.) grown on maize (*Zea mays*), sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) crop residues from small-scale farmers using various production techniques.

Specific Objectives

1. To assess the current uses of cereal crop residues by small-scale farmers and estimate residue yields in southern part of Botswana.
2. To determine the effect of cereal residue type, substrate disinfestation method and oyster mushroom species on mushroom yields and the potential income from mushroom sales.
3. To evaluate the effect of substrate type, substrate disinfestation method and oyster mushroom species on mushroom nutritional composition and their potential in improving the nutrition of small-scale farmers.

1.7 Hypotheses

For objective 2 the null and alternate hypotheses are:

H₀: Substrate type, substrate disinfestation method and species type have no effect on mushroom yield and income thereof.

H_a: Substrate type, substrate disinfestation method and *Pleurotus* spp. have an effect on mushroom yield and income thereof.

For objective 3, the null and alternate hypotheses are:

H₀: Substrate type, substrate disinfestation method and *Pleurotus* spp. have no significant effect on nutritional composition of mushrooms and potential in improving the nutrition of small-scale farmers

H_a: Substrate type, substrate disinfestation method and *Pleurotus* spp. have a significant effect on nutritional composition of mushrooms and potential in improving the nutrition of small-scale farmers

CHAPTER TWO: LITERATURE REVIEW

2.1 Status of cereal crop production by small scale farmers in Botswana

Small-scale farmers in Botswana are dependent on rain-fed agriculture and yields have been declining due to climate change-induced droughts and poor rainfall distribution. Furthermore, poor management, poor soil fertility among other factors, have also contributed to the excessively low and stagnant declining yield trends over time. The termination of Accelerated Rain fed Arable Programme (ARAP) in 1995/1996 growing season led to a decline in cereal crop output (Seleka and Lekobane, 2014). ARAP was effective in improving rural household food security and welfare. Most small -scale farmers in Botswana are unable to meet their basic household food requirements. The annual national production and yields of grains (mainly sorghum and maize) vary considerably ranging from 8,200 MT to 175,000 MT with a mean annual production of 46,000 MT. Currently local farmers supply only 5% of the demand for grains hence thus making Botswana a net importer of cereal grains mainly from South Africa (African Development Bank, 2008). However importing food from other countries leads to high market prices which are beyond the reach of the rural poor who make almost 50 % of the population.

In Botswana a large percentage of large-scale and small-scale farmers sell to BAMB although they receive low prices as summarised in Table 1. The low incomes negatively impact small-scale farmers making them food insecure.

2.2 Uses of crop residues

Smil (1999) reviewed the uses of crop residues some of which will be highlighted below. Firstly, crop residues when ploughed into soil may serve as a source of nutrients and soil organic matter. According to Smil (1999) nitrogen and phosphorus incorporated annually into crop residues is equivalent to approximately 30% of each nutrient contained in synthetic fertilizers, while potassium approximately twice as much as is available in fertilizer compounds. Secondly, crop residues maybe used for making fibreboards and paper which are biodegradable and environmental friendly. Thirdly crop residues may be used in the building industry as building materials such as bricks from straw clay, ceiling boards and used directly for thatching houses. Fourthly, residues can be used in the generation of biofuels since the main residual chemical composition comprises of cellulose, hemicellulose, and lignin which can be fermented into ethanol for fuel (Hofstrand, 2009). In Nebraska hydrogen production capacity is expected to rise from 53.59 to 164.41 kilo tonnes with cereal crop residues such as wheat, barley, rice and corn being the main contributors (Nooshin *et al.*, 2016). Fifthly, the residues may be used as feed or part of the feed for livestock but they are generally low in protein and high in fibre (Owen, 1976). Lastly cereal crop residues may also be used for mushroom cultivation as a value addition activity since mushrooms are a high-value crop. Incorporation of oyster mushroom production may not necessarily conflict with uses as organic fertilizer or animal feed because the spent substrate can be fed to livestock or incorporated into soil. In this study, the use of oyster mushrooms for adding value to crop residues in order to improve income of small scale farmer's income will be explored.

2.2.1 Use of cereal crop residues for mushroom production

Introduction of oyster mushroom production in the cereal production system among small-scale farmers could play a vital role in improving their income and livelihoods. Oyster

mushrooms utilise cereal crop residues for their growth and reproduction (Oei, 1991). In a drought scenario where sorghum or millet grain filling failed or quileas attacked the grain resulting in total loss of grain, the remaining crop residues could be used to grow mushrooms, thus mitigating against total loss of income and food security (Khonga, Pers. comm). Assuming a biological efficiency (BE) of 50% and crop residue yield of 1000kg/ha (Kossila, 1988), and production cost/Kg of residues of BWP10/kg and mushroom selling price of BWP100/kg the farmer could realise gross profit of BWP40, 000 /ha ((500kg mushrooms x P100)-(1000 Kg residues x P10/kg = P40, 000) compared to zero income from grain sales.

With such income, most small-scale farmers would be food secure as they would afford to buy food from the income generated from mushroom production. Consumed directly as part of the diet, mushrooms would also improve the nutritional and health status of farmers since mushrooms are nutritious and are known to contain compounds which are anti-cancer, blood pressure lowering and immunity boosting (Chang and Miles, 2004). Mushroom production may offer jobs to unskilled personnel in rural areas as some of activities during production do not require special skills. This would help to alleviate poverty at small-scale farm level.

2.3 History of mushroom consumption and cultivation

Wild mushrooms have been used by humans as food, medicine and in worship from time immemorial. Archaeological records show mushroom consumption dating as far back as 1300 years ago in Chile (Chang and Miles, 2004). Historical data reveal that mushroom cultivation and consumption occurred in ancient civilizations of China, Rome, Greece, Egypt and Central America. In ancient Greek and Roman times edible fungi were highly valued and were food for royals only. Asian civilizations have been cultivating edible mushrooms for almost 1400 years, since the first mushroom, *Auricularia auricula* (wood ear), was cultivated in China around 600 A.D. Soon to follow were *Flammulina velutipes* (enokitake) around 800-900 A.D., *Lentinula edodes* (shiitake) around 1000-1100, *Agaricus bisporus* (button) around 1600, *Volvariella volvacea* (paddy straw) around 1700, *Tremella fuciformis* (white jelly) around 1800, and *Pleurotus ostreatus* (oyster) around 1900. Of the leading mushrooms today that were cultivated before 1900, *Agaricus* is the only one that was not first grown in China (Chang, 1991). The cultivation of *Pleurotus* spp. is an economically important food industry worldwide which has expanded in the past few years. *P. ostreatus* is the third most important cultivated mushroom for food purposes (Cohen *et al.*, 2002). In nature mushrooms can be found growing during the rainy season in fields, pasture land, on decaying plant materials and wood logs as saprotrophs or mycorrhizal (Zadrazil, 1978). Mushrooms are increasingly becoming an important new non-traditional food and cash crop in the world (Mshigeni and Chang, 2000).

The importance of mushrooms in Africa dates back to when wild edible fungi (truffles, chanterelles, termite mushrooms and morels) were very common and played a significant role in the nutrition and economic wellbeing of most African communities (Boa, 2004). Mushrooms were collected from forests mainly by women whose knowledge of edible mushrooms was passed through indigenous knowledge system from generation to generation. In some countries

wild mycorrhizal mushrooms such as chanterelles were exported to Europe, thus generating foreign exchange. However, with environmental degradation and climate change most communities are no longer collecting mushrooms because the forests are destroyed or termite mounds affected by application of pesticides. Mushrooms are a potential source of valuable metabolites some of which have been commercialised as nutraceuticals and drugs for treatment of various human ailments.

2.4 Description of oyster mushrooms

Oyster mushrooms belong to the Division Basidiomycota, Class Agaricomycetes, Order Agaricales, Family Pleurotaceae and Genus '*Pleurotus*'. The genus name "*Pleurotus*" originates from the Greek word "Pleuro" which means laterally or in a side-ways position, referring to the lateral position of the stipe in relation to the pileus (Jandaik and Kapoor 1974). It is commonly termed oyster as it forms an oyster like shape. The fruiting bodies of oyster mushrooms are distinctly shell, fan or spatula shaped with different shades of white, cream, grey, yellow, pink or light brown depending upon the species. Oyster mushrooms are characterised by edible fruit bodies with eccentric stalk attached to the pileus that opens like an oyster shell during morphogenesis. *Pleurotus* species are described as food delicacies because of their texture and flavour (Chang and Miles, 2004). The oyster mushroom is one of the most suitable fungal organism for producing protein rich food from various agro wastes without composting. Table 2 shows the commonly cultivated *Pleurotus* spp.

Fungi include eukaryotic, spore-bearing, achlorophyllous organisms with either a typically cell-walled thallus with absorptive nutrition or with no walled thallus with phagotropic nutrition (Alexopoulos and Mims, 1979). Fungi are usually filamentous and multicellular while a few (yeasts) are unicellular. The cell wall of true fungi consist of glucan and chitin as opposed to plant cells which are made of cellulose (Alexopoulos and Mims, 1979). Fungi consist of branched filaments or hyphae which form the thallus or mycelium. The hyphae can be vegetative (for absorption of nutrients) or reproductive. Fungi reproduce by means of asexual or sexual spores some of which are formed in microscopic and macroscopic specialised reproductive structures. Some fungi in the division Ascomycota and Basidiomycota form large fleshy sexual fruit bodies some of which are edible and others are poisonous to humans.

According to Chang and Miles (1991) “A mushroom is a macro fungus with a distinctive fruiting body which can either be epigeous (growing on or close to the ground) or hypogeous that is growing under the ground” Alexopoulos and Mims (1979) define a mushroom as a fleshy, umbrella- like sporophore that bears their basidia on the surface of gills. However, mushrooms can also mean the group of fungi mainly in the Basidiomycota which form large fleshy or woody fruiting bodies.

Table 2: Morphological characteristics of commonly cultivated *Pleurotus* species and their common names

<i>Pleurotus</i> spp.	Common name	Characteristics
<i>P. ostreatus</i>	Oyster mushroom	-Variable colours ranging from steel to mouse grey and almost white. -Fine texture and strong taste.
<i>P. sajor-caju</i>	-	-Thin flesh -Pale grey colour when mature
<i>P. florida</i>	Florida oyster mushroom	-Funnel shaped -Colours vary from light – beige to greyish
<i>P. cornucopiae</i>	Branched oyster mushroom	-Funnel shaped -Brittle structure and yellow in colour
<i>P. eryngii</i>	King oyster	-Funnel shaped and strong in texture
<i>P. pulmonarius</i>	Lung oyster	-Sepal grey to buff colour -Tough stipe
<i>P. flabellatus</i>	-	-Cream to brown colour, -Thin caps -Beautiful rose appearance
<i>P. djamor</i>	Pink oyster	-Oyster shape and pink in colour

Source: Oei (1991)

2.5 Cultivation of oyster mushrooms on different substrates

Oyster mushrooms are saprotrophic or primary decomposers but some are weak pathogens of trees where they attack the lignocellulose rich heart of the stem, hence their cultivation using lignocellulose rich substrates such as logs, cereal crop residues and other agro-wastes. In nature oyster mushrooms are associated with tree logs or diseased living trees (Stamets, 1993).

Several agricultural crop residues which are rich in cellulose and lignocellulose have been used to produce oyster mushrooms. These include saw dust, sugarcane bagasse, maize stalks, maize cobs, sorghum stalks, millet stalks, rice straw, rice bran, wheat straw, water hyacinth, banana leaves, paper wastes, cotton waste, poultry droppings (Bano *et al.*, 1993; Cohen *et al.*, 2002, Fasidi and Kadiri, 1993;, Shah *et al.*, 2004). This makes oyster mushroom cultivation a useful method of environmental waste management and waste disposal as it makes use of substances usually referred to as waste. Many agricultural and industrial by-products can be used in mushroom production (Smil, 2009). Different experiments have been conducted to determine the yields of *Pleurotus* spp. using locally available substrate materials in various parts of the world. In Bangladesh, sawdust and rice straw are widely used as the main substrate for oyster mushroom cultivation (Moonmoon, 2010). According to Sharma and Jandaik (1981) good quality paddy straw could give high yields as it contains less weeds and moulds which would be killed at time of sterilisation.

Crop residues are readily available even when grain yields are low and their utilization in mushroom production can help farmers in additional generation of income or improving food at household level. Table 3 shows different substrates used in growing different species of oyster mushrooms.

Table 3: Some Pleurotus species and substrates used in their cultivation

Species	Substrate	Source
<i>P. sajor-caju</i>	Cotton stalks Sorghum stover Paddy straw	Ragunathan and Swaminathan (2003)
<i>P. florida</i>	Soya bean	Ahmed <i>et al.</i> (2009)
<i>P. ostreatus</i> & <i>P. pulmonarius</i>	French been straw	Rusuku (1989)
<i>P. ostreatus</i> and <i>P. cystidiosus</i>	Sawdust, sugarcane, corn cobs and bagasse	Hoa <i>et al.</i> (2015)
<i>P. ostreatus</i>	Rice straw	Bonatti <i>et al.</i> (2004), Obodai <i>et al.</i> (2003)
<i>P. pulmonarius</i>	Corn cobs	Oei (1991)
<i>P. pulmonarius</i> , <i>P. floridanus</i> , <i>P. ostreatus</i> , <i>P. eous</i> , & <i>P. sajor-caju</i> .	Saw dust, maize husks, Maize stalks and millet stalks	Khonga (2001)
<i>P. ostreatus</i> and <i>P. sajor-caju</i> and <i>P. columbinus</i>	Chopped office papers, sawdust, plant fiber	Mandeel <i>et al.</i> (2005)

2.6 Nutritional status of oyster and other mushrooms

Oyster mushrooms are considered as food because of their high nutritional value, flavour and taste. Generally, mushrooms possess most of the attributes of a nutritious food as they contain many essential nutrients in good quantity (Chang and Mshigeni, 2001). Mushrooms have quantities of proteins, carbohydrate, fibre, fats and minerals required by the human body if incorporated in the human diet, especially of the rural poor in developing countries where cases of malnutrition are high. Table 4 summarises the proximate analysis of some common cultivated mushrooms.

Table 4: Nutritional profile for some cultivated mushroom

Species	Crude protein (%)	Fat (%)	Total carbohydrate (%)	Fibre (%)	Ash (%)	Energy (Kcal/100g dry material)
<i>Agaricus bisporus</i>	24-34	1.7	51.3-62.5	8.0-10.4	7.7-12	328-368
<i>Lentinus edodes</i>	13-17	4.9	67.5-78.0	7.3-8.0	3.7	387-392
<i>Pleurotus ostreatus</i>	10.5-30.4	1.6-2.2	57.6—81.8	7.5-8.7	6.1-9.8	345-367
<i>Pleurotus sajor-caju</i>	26.6	2.0	50.7	13.3	6.5	276
<i>Pleurotus ostreatus</i> (var <i>florida</i>)	27.0	1.6	58.0	11.5	9.3	265
<i>Volvariella volvacea</i>	25.9	2.4	—	9.3	8.8	276

Adapted from Crisan and Sands (1978) in Hayes and Chang, (1978) and Bano *et al.*, (1981)

Malnutrition is common in developing countries more especially in Africa due to intake of nutritionally poor quality diets low in protein (Chang and Mshigeni, 2001). In order to meet the deficit most developing countries tend to import essential protein sources of food such as legume and meat products, spending large sums of their meagre foreign exchange reserves. Though these products may be available, poor people cannot afford to buy them. In order to mitigate this existing challenge of food supply nutritionists have thought of unconventional alternative sources of protein such as mushrooms whose production at small scale level is cheap and easy.

Pleurotus ostreatus has a high nutritional value due to its high level of vitamins and proteins and its non-saturated fatty acids. Among cultivated mushrooms such as *Agaricus bisporus*, *Lentinus edodes*, *Volvariella volvacea* and *Volvariella diplasia*, *Pleurotus ostreatus* has higher levels of amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, valine,

tryptophan and histidine (Chang *et al.*, 1993). Protein tends to be present in an easily digested form on a dry weight basis. In general, cultivated mushroom protein content normally ranges between 20 and 40% which is better than many legume sources like soybeans and peanuts, and protein-yielding vegetable foods (Chang and Buswell, 1996; Chang and Mshigeni, 2001). However, crude protein values vary among strains and according to fructification (Chang *et al.*, 1993). Moreover, mushroom proteins contain all the essential amino acids needed in the human diet and are especially rich in lysine and leucine which are lacking in most staple cereal foods (Chang and Buswell, 1996). The availability of amino acids may be altered by the composition of growth substrate though having no effect in the crude protein content. In addition to their high-quality protein, mushrooms are a relatively good source of the following individual nutrients: fat, phosphorus, iron, and vitamins including thiamine, riboflavin, ascorbic acid, ergosterol, and niacin (Khatun *et al.*, 2014).

Furthermore, mushrooms are low in total fat content and have a high proportion of polyunsaturated fatty acids (72 to 85%) relative to total fat content, mainly due to linoleic acid. The high content of linoleic acid is one of the reasons why mushrooms are considered a health food (Chang and Mshigeni, 2001; Sadler, 2003). Mushrooms compared to other foods contain significant carbohydrate components which include compounds such as pentose, hexose, disaccharides, amino sugars, sugar alcohols and sugar acids (Crisan and Sands, 1978). The antioxidants present in foods, especially vegetables, are phenolic compounds (phenolic acids and flavonoids), carotenoids, tocopherol and ascorbic acid which are important protective agents for human health which are also present in mushrooms.

2.7 Medicinal uses of wild and cultivated mushrooms

Based upon ancient literature the nutritional and medicinal uses of mushrooms were known as early as 1500 BC (Chang and Miles, 2004; Patel *et al.*, 2014). In China diseases such as

hypertension, cancer, viral diseases and blood platelets aggregation were cured by mushrooms such as *Ganoderma lucidum*, *Lentinus edodes* and *Tremella fuciformis* (Hang *et al.*, 2008). There are about 15000 species of mushrooms in the world and of these about 700 have known medicinal properties and about 1800 species have potential medicinal properties (Chang and Miles, 2004). Some mushrooms have both nutritional and medicinal properties and are used in formulation of nutraceuticals and functional foods. Functional foods are foods which contain an ingredient that gives that food health-promoting properties over and above its usual nutritional value, while nutraceuticals are whole foods packaged in the form of a tablet or capsule (Patel *et al.*, 2014).

Ganoderma lucidum is number one medicinal mushroom in the world and is as considered as king of medicinal mushrooms followed by *Lentinula edodes* and others including *Pleurotus* (Patel *et al.*, 2014). *Ganoderma lucidum* has been used by many companies to produce food supplements and functional foods hence making it the most significant medicinal mushroom in the world. *Ganoderma lucidum* is useful in management of different human infectious diseases including HIV. *Lentinula edodes* is useful in the treatment of various human ailments such as cancer, high cholesterol level and blood pressure. Recent studies have shown that water and methanol crude extracts of various *Pleurotus* spp. had therapeutic activities, such as antitumor, immuno-modulatory, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antiplatelet-aggregating, anti-hyperglycaemic, antimicrobial and antiviral activities (Gregori *et al.*, 2007). Due to an increase in cancers proper selection of food is an important aspect of human health in combating such diseases. These diseases in most cases are attributed to consumption of processed and refined foods commonly available in the shops. However, mushrooms are an important functional food and a source of drugs and nutraceuticals and should be part of the daily diet.

2.8 Oyster mushrooms as a source of income

Oyster mushroom production has a great potential in generation of income and job creation for rural people or small scale farmers in order to alleviate poverty. Furthermore, it can also be an additional income to farmers taking into account value added product and a way to supplement farm income while making use of by products or co-products of other crops (Celik and Peker, 2009). In a survey conducted by Celik and Peker (2009) in Kenya, it was shown that mushrooms were considered a leading cash crop. Due to the increase in demand of mushrooms because of their nutritive value it has a good opportunity in finding foreign market hence earning foreign exchange (Zadrazil, 1982).

2.9 Role of oyster mushroom cultivation in bioconversion of agricultural wastes

Large amounts of agro wastes are generated after harvesting and processing crops and their disposal is a problem. Citrus fruit processing plants produce 50,000 tons per year of citrus bagasse which is adequate for feeding ruminants, however it has palatability problems due to contamination by mycotoxins (Alborés *et al.*, 2008). Rice straw on the other hand is also produced in large quantities of 2000 tons per hectare, though it is fed to ruminants it has low protein and low digestibility. However, *Pleurotus* has the ability to upgrade the residues to cattle feed by colonising different types of crop wastes thereby increasing their digestibility through delignification (Salmones *et al.*, 2005). The ability of *Pleurotus* species to bio-convert agro-waste to valuable feed is due to the presence of non-specific oxygenases. However, *Pleurotus sajor-caju* produces biodegrading enzymes such as lignases, hemicellulases and xylanases which play an important role in biodegradation of agro-wastes. Millions of tons of spent mushroom substrates which possess important chemical, physical and biological properties are generated world-wide annually (Smil, 1999). The spent substrates are potential

resource for various value-added end uses such as soil conditioner, organic fertilizer or livestock feed (Singh *et al.*, 2007).

2.10 Factors affecting growth of *Pleurotus* species

Physical factors such as temperature, substrate moisture, relative humidity, and carbon dioxide and oxygen concentrations affect the mycelial growth of oyster mushrooms. *Pleurotus* spp. mycelium can grow at a temperature range of 20 to 30°C which is a fair range for spawn running and 22 to 25°C for optimum fructification. *Pleurotus sajor-caju*, *P. eous* and *P. pulmonarius* are the most adaptive species with wide growing temperature range of 15-30°C whereas strains of *P. ostreatus* require colder temperature of 12 - 20°C while species such as *P. florida*, *P. columbinus*, *P. djamor* and *P. flabellatus* exhibit an intermediate range (Rajarathnam *et al.*, 1992). Humidity range of 80 to 90% was found to be ideal for fruit body formation (Chang and Miles, 1989). Low moisture content will result in the death of fruiting body while high moisture makes development of diseases possible. Different species have different optimal pH range for development. However the optimal pH for mycelia growth and sequent fruiting body development is obtained at between 6.5 - 7.0 (Kalmis *et al.*, 2007). Oxygen is a vital factor during the growth of mushrooms for aeration. Inadequate aeration and enrichment of carbon dioxide in the atmosphere results in abnormal fruiting and also the fruit bodies developed are long and slender (Oei, 1991). Levels of spawn running affect the colonization time, increasing spawning rate shortens mycelial colonization time, primordial formation and first mushroom crop (Singh *et al.*, 2007).

2.11 Effect of substrate type on growth and yield of *Pleurotus* species

The constituents of a substrate are very important as they influence yield. Nutritious substances for oyster mushroom can be categorized into two: staples, which are the lignocellulose-rich

substrates, and additives, which are protein and nitrogen sources (Gabriel, 2004; Sharma and Jandaik, 1981). Zadrazil (1982) observed that cellulose and lignin content of the substrate have direct impact on growth and development of oyster mushrooms (Zadrazil, 1982). Cellulose rich organic substrate are good for mushroom cultivation as they enhance cellulase production which is positively correlated to yield. Cellulose is actively used during mycelial growth and its content reduces after mushroom harvest. Oyster mushrooms need substrates abundant in polysaccharides (cellulose and hemicelluloses) and lignin for their growth. The mycelial growth of oyster mushrooms makes use of soluble carbohydrates, glucose, organic nitrogen sources like wheat bran, barley, oat, maize, soybean crust and sunflowers, as well as mineral sources such as ammonium sulphate (Gabriel, 2004). Carbon to nitrogen ratio is important during the growth of mushrooms. *Pleurotus* species use more nitrogen during fruit body formation, hence adequate supply of nitrogen increases mushroom yield.

2.12 Importance of spawn quality in mushroom production

Spawn is defined as a living ramified mycelium of a mushroom, multiplied on a suitable sterile base material or carrier under aseptic techniques. Some of the carriers that can be used for spawn production are chopped rice, sawdust, tea leaves, coffee hull, cotton waste and cereal grains (Oei, 1991). Cereal grain, colonized with mycelia on the surface, may readily be mixed with various substrate formulations, thus providing many points of inoculum. In most cases spawn is used on weight basis hence small grains such as millet or sorghum give a greater number of inoculation points per kg than large grains such as rye though large grains have a greater food reserve that can sustain the mycelium for longer periods of time (Fritsche and Sonnenberg, 1988). However, this makes small grain a good material in making spawn. The quality of spawn is a key element in the production of high yields of mushroom. In an experiment conducted by Gabriel (2004) it was concluded that in order to achieve fast and high

yields the grain spawn should have a nice scent and the grain should be thoroughly colonized (Gabriel, 2004).

2.13 Biological efficiency

Biological efficiency (BE %) is the yield of fresh mushrooms as a percentage of to the dry or wet weight of substrate or compost spawned. Biological efficiency was found to be influenced by the carbon and nitrogen content of the substrate (Zadrazil, 1978). The yield of oyster mushroom would be maximum when the C: N ratio of the substrate is 61:1 (Bano *et al*, 1993). On the other hand Torres-Lopez and Hepperly (1987) accentuate that C: N ratio of about 60:1 could stimulate growth and yield of oyster mushroom. As the mushroom grows the overall carbon content of substrate reduces due to its utilization in the growth and yield of oyster mushrooms. Hence yield reduces in between flushes during harvest. *Pleurotus* species are popular and widely cultivated throughout the world mostly in Asia, America and Europe because of their simple, low cost production technology and high biological efficiency (Hoa *et al.*, 2015).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Assessment of the current uses of cereal crop residues by small-scale farmers in Southern part of Botswana

3.1.1 Farmer Survey

A farmer survey was carried in the Kweneng, Southern and Kgatleng districts of Botswana to assess the current uses of cereal crop residues by small-scale farmers. Areas targeted in the Kweneng district were Mmopane, Gabane, Lentsweletau, Kopong and Medie. In the Southern District the areas were Mmathethe and Goodhope while in Kgatleng District Oodi, Dikgonnye and Malotwane were surveyed. A total of 63 farmers which were selected by the district extension officer were interviewed face to face using a structured questionnaire (refer to Appendix). The survey was carried out by visiting the farmers at their homes or farms (Fig. 1) with the help of extension officers of the three districts.

3.1.2 Quantification of cereal crop residues in farmers' fields

The yield of maize, sorghum and millet residues was estimated from three selected farmers in order to estimate gross income from grains and the potential extra income from sale of mushrooms if the residues were used in mushroom production. Quantifying of the residues was done by measuring 10 m by 10 m plots in which the crop residues were hand harvested with a panga knife, tied in bundles and weighed (Fig. 2). Three replicate plots were harvested from each field of maize, sorghum and millet.

3.1.2 Data collection and analysis

The answers to the survey questions were coded and analysed using Statistical Package of the Social Sciences (SPSS, version 22) and means and standard deviations were calculated.



a)



b)

Figure 1: Farmers being interviewed at home (a) and the farm (b) during the Farmer' survey in the Southern part of Botswana



a)



b)

Figure 2. Estimating residue yield for sorghum (a) and millet (b) in 10m x 10m plots

3.2 Evaluation of the effect of residue type, substrate disinfestation method and mushroom species on yield and potential income from oyster mushroom production.

3.2.1 Study site

The study was conducted at the Botswana University of Agriculture and Natural Resources (BUAN), Sebele, Gaborone (Latitude 24° 33' S, Longitude 25° 54' E, Altitude 994 m above sea level) (Mojeremane *et al.*, 2014). Preparation of spawn was carried out in the pathology laboratory in the Crop Science and Production Department while mushroom production was carried out in low technology mushroom house constructed by Professor Khonga (Fig 3). The house was 6m long, 4m wide and 2.5m high. The walls and the roof were made of gum poles and timber branderings covered with black plastic for maintenance of high humidity and thatched with grass for insulation. For ventilation, the houses were fitted with wooden windows and a door (Khonga, 2001). The experiment was conducted from August to December 2017.



Figure 3: Low technology production house

3.2.2 Sourcing of cereal crop residues and *Pleurotus* species

Maize, millet and sorghum residues used for mushroom cultivation were sourced from farmers who were interviewed during the surveys and from BUAN farm. Two of the *Pleurotus* cultures used in the study were sourced from Mycotheque de L'Universite Catholique de Louvain (MUCL) in Belgium. MUCL access numbers were MUCL 38055 (*P. floridanus*), MUCL 30494 (*P. ostreatus x floridanus*, a hybrid of *P. ostreatus* and *P. floridanus*) and the third one was sourced from a local farmer (*P. ostreatus* HK35)



Figure 4: Harvesting and weighing of stalk from the BUAN Notwane farm

3.2.3 Spawn Preparation

Heat resistant or autoclavable glass bottles (750 ml) were washed with soap and stored in a clean place. Sorghum grains (*Sorghum bicolor L*) were soaked overnight and the grains were washed with tap water to remove any germinated microorganisms. The grains were boiled for 15-30 minutes until soft but taking note not to allow them to reach the cracking stage. The hot water was drained using a colander and grains were transferred into a plastic tub to cool down. Grains (500ml) were packed in the 750 ml bottles whose mouths were thereafter plugged with cotton wool. The cotton wool plugs were covered with aluminium foil to minimize microbial contamination. The grains were sterilised twice in an autoclave for 60 minutes at 115 kg/cm² pressure. Once the grains had cooled they were inoculated with 30 grams of mother spawn of *Pleurotus floridanus*, *P. ostreatus* HK35 and *P. ostreatus* × *Pleurotus floridanus*. The spawned sorghum bottles were incubated at 25° C for mycelium colonisation for 8-12 days.

3.2.4 Chopping of substrates

Maize, pearl millet and sorghum stalks were chopped into pieces about 3 -5 cm long using a chaff cutter (Trapp[®] TRF 300G Super Metalurgica Trapp Ltda, Caixa Postal 106, CEP 89256-506, Jaragua do Sul SC, Brazil, Supplied by Mechanized Farming Botswana, Gaborone) (Fig 5). These were stored in 50kg bags in a shade until used.



Figure 5: Chaff cutter/hammer mill used in chopping cereal crop residues.

3.2.5 Substrate spawning and spawn running

Disinfested substrate was packed in clear polyethylene plastic bags (250mm x 450mm and 40µm thick). The weight of the bags of substrates were 1.5 - 2.0 Kg. The substrates were spawned at 4% by mixing the top third of the substrate with 60-80g of spawn. The top of the plastic bag was fitted with a plastic collar of 20mm diameter and a rubber band used to tie the plastic onto the collar and the mouth of the bag plugged with cotton wool (Figure 6). Weight of the bags was recorded. The spawned bags were placed upright on a bench and covered with black plastic for spawn to run in the dark for 3-4 weeks at 27-28°C in the mushroom house. The temperature in the house was maintained using a conditioner if necessary.



Figure 6: Plastic bags containing spawned substrate

3.2.6 Agronomic practices

The inside of the mushroom house was sprayed daily with water using a hose pipe to avoid substrate or mushrooms from drying. Also to keep dust from the mushroom house as it brings about contamination to the growing mushrooms. Minimum and maximum temperature inside the house were recorded daily at 1400hrs during the spawn running and production using a minimum and maximum thermometer. Weather data for Sir Seretse Khama International

Airport (SSKIA) during the study period were obtained from the Department of Meteorological Services in Gaborone

3.2.7 Substrate Treatments and Experimental Design

Maize, sorghum and millet stalks were soaked in water for 24 hours and then disinfected using two methods.

1. Steaming for 3-4 hours and
2. Soaking in 0.04% hydrogen peroxide for 24 hours

The following *Pleurotus* species were used to spawn the substrates:

1. *P. ostreatus x floridanus* (Po x Pf)
2. *P. floridanus* (Pf) and
3. *P. ostreatus strain HK 35* (Po HK35)

The experiment was a 3 x 2 x 3 split-split-plot factorial with residue type as main plot (Factor A), disinfestation method as sub-plot (Factor B) and mushroom species as sub-subplot (Factor C) with 5 replicate bags per treatment. In the mushroom house the bags were arranged in a completely randomised design (CRD). Table 5 shows a summary of the 18 treatments for the experiment. The steaming and hydrogen peroxide disinfestation treatments are described below.

Table 5: Summary of the treatments for the experiment to determine the effect of substrate type, substrate disinfestation method and mushroom species on mushroom yield

Treatment	Substrate type	Disinfestation method	<i>Pleurotus</i> spp.
1	Maize stalk	Steam	Po x Pf
2			<i>Pf</i>
3			<i>Po</i> HK35
4		H ₂ O ₂	Po x Pf
5			<i>Pf</i>
6			<i>Po</i> HK35
7	Millet stalks	Steam	Po x Pf
8			<i>Pf</i>
9			<i>Po</i> HK35
10		H ₂ O ₂	Po x Pf
11			<i>Pf</i>
12			<i>Po</i> HK35
13	Sorghum stalks	Steam	Po x Pf
14			<i>Pf</i>
15			<i>Po</i> HK35
16		H ₂ O ₂	Po x Pf
17			<i>Pf</i>
18			<i>Po</i> HK35

3.2.8 Substrate Steaming

Each dry chopped substrate (12.5 kg) was soaked in water in 200 L drums for 24-hrs. To ensure that the substrates are fully immersed in water, a black plastic was placed on top of the substrates and a 10 kg concrete block placed on top of the plastic. After soaking, water was drained from the substrate and rinsed in two changes of clean water and allowed to drain. A 200 L drum was placed above a fire place and 30-40 L of water was added. A mesh wire cage was placed into the drum (Fig 7) and the substrates were transferred into the wire cage and covered with black plastic and a metal lid. When the water in the drum started boiling, the substrates were steamed for 3-4 hours and the fire was extinguished. The substrates were allowed to cool overnight.



Figure 7: Mesh wire cage and drums used for steaming substrate

3.2.9 Hydrogen Peroxide Treatment of Substrate

Substrate was soaked for 24 hours and rinsed with clean water as described above. A 50% Hydrogen peroxide solution was diluted to 0.04% (Wayne, 2000) by adding 80ml of hydrogen peroxide into 100 L of water in a 200L steel drum. Clean substrate was transferred into the

hydrogen peroxide solution and allowed to soak for 24hrs. The drums were covered with black plastic. The hydrogen peroxide solution was drained and the substrates rinsed twice with clean water. The substrate was allowed to drain in a mesh wire cage and transferred to the mushroom house for packing, spawning and spawn running as described above.

3.2.10 Mushroom harvesting

After 4 weeks of spawn running, the bags were transferred to a production house whose temperature was maintained at 23-26°C and 75-90% relative humidity. Horizontal slits on the plastic bags were made using an alcohol-disinfested scalpel blade and the bags were suspended on wooden branderings. The relative humidity inside house was maintained high (75-90%) by spraying water on the floor and walls in the morning and evening daily. Once mushrooms were mature they were harvested all at once by gently twisting them out of the bag and the weights recorded. The mushroom harvest period was 45 days before the spent substrate was discarded.

3.2.11 Data collection

The following yield parameters were recorded:

1. Percentage spawn running
2. Number of days from bag opening to the harvest of each mushroom flush
3. Total number of flushes per treatment
4. Yield (g) of mushrooms per flush –An electric weighing balance was used.
5. Total mushroom yield (g) and Biological Efficiency (BE %)

The total mushroom yield per bag was the total yields of all flushes.

Biological efficiency (BE (%)) was calculated as follows:

$$BE = (FW_m / DW_s) \times 100$$

Where FW_m is total fresh weight of mushroom (g) for all flushes/bag, and DW_s is substrate dry weight (g)/bag.

3.3. Evaluation of potential income from oyster mushroom production

Mushroom yield data (BE) for the substrate by disinfestation method interaction and the best two *Pleurotus* spp. yields will be used to conduct a profit analysis in order to determine the most profitable substrate, disinfestation method and species combination. The relative profitability (RP) of mushroom production was calculated as: $RP = [(Yield \times Price) - (Fixed\ costs + variable\ costs)]$. Three yields were used in RP, namely: optimistic yield (OY), realistic yield (RY) and pessimistic yield (PY) where OP is the experimental yield obtained in this study, RY is 80% and PY 60% of OP, respectively (Kelly *et al.*, 1995). The fixed costs used in the study were: 1. Mushroom house rental with drums and utilities included; 2. Chaff cutter rental; 3. Spawn; 4. Substrate; 5. Consumables and 6. Labour. The variable costs were firewood and hydrogen peroxide.

3.4 Evaluation of the nutritional composition of oyster mushrooms grown on maize, sorghum and millet residues from small-scale farmer's field and their potential in improving the nutrition of small-scale farmers

3.4.1 Nutritional analysis

Mushrooms harvested from the experiment above were analysed for their nutritional content and the following parameters were analysed using selected standard procedures from AOAC (2000) and Bultosa (2015).

3.4.1.1 Proximate analysis

Proximate analysis involved the determination of the crude protein, total ash and crude fat.

3.4.1.2 Sample drying

Fresh mushroom sample was placed in moisture dishes and heated at 130 °C for one hour in a forced air draught oven. After drying the samples was removed from the oven and placed in a desiccator and allowed to cool. The sample was then ground and digested.

3.4.1.3 Crude Protein (CP)

Samples of oyster mushrooms were dried at 50 °C for 90 hours and ground in a mill to pass a 1-mm sieve screen. Then 2.5 g of dried samples were digested in 10 ml of concentrated sulphuric acid (95-97%) and 2 ml of 15-45% hydrogen peroxide in a digestion block heater for 8 hours. The temperature of the digester was kept at 350°C. After digestion was complete, the samples were allowed to cool and then transferred into 200 ml volumetric flasks and filled with distilled water to the mark. For crude protein analysis, the digested samples were titrated by a standard acid (0.1N HCl). The end product of the titration changed from green to steel blue

when half a drop of acid was added. Taking the volume of acid consumed from the burette reading, percentages of nitrogen and protein were calculated using the equations below:

$$\text{Nitrogen (\%)} = \frac{(V \text{ HCl in L for Sample} - V \text{ HCl in L for blank}) \times N \text{ HCl (ca.0.1)} \times 14.00}{\text{Sample weight in g on dry matter basis}} \times 100$$

$$\% \text{ Protein} = \text{protein measured} \times \frac{100 - M}{100 - \% \text{ moisture measured}}$$

Where v is volume, M is mass

3.4.1.4 Ash

The amount of ash was determined by completely burning to ash a weighed amount in a muffle furnace (Labcon muffle furnace) at 550 °C until free from carbon and residue appears grayish (approximately for 8 hours). The percentage ash content was expressed by the following equation;

$$\% \text{ Ash} = \frac{m_3 - m_1}{m_2 - m_1} \times 100$$

Where; m_2 , m_1 =sample mass in grams before ashing and $m_3 - m_1$ =mass of ash in grams

3.4.1.5 Crude fat

Five grams of dry mushroom sample was weighed into a thimble lined with a circle of filter paper. The thimble and its contents were placed into a 50 ml beaker and dried in an oven for 2 hours at 110 °C. Thimble and contents were then transferred into the extraction apparatus. The sample contained in the thimble was extracted with solvent in a Soxhlet apparatus for 6-8 hours at a condensation rate of 3-6 drops per second. At the completion of extraction, the fat extracts were transferred from the extraction flask into a pre-weighed (m_i) small evaporating beaker (150-250 ml) with several rinsing with the solvent. The evaporating beaker was then placed in

a fume hood and solvent was evaporated off on a steam bath until no odour of the solvent was detectable. The beaker and its contents were dried in an oven for 30 minutes at 100 °C. The sample was removed from the oven and cooled in a desiccator and the beaker plus contents were weighed (m_f) and the percentage of crude fat was calculated by the formula:

$$\text{Lipid (\%)} = \frac{M_f - M_i}{\text{Sample mass dry basis}} \times 100$$

Where M_f is the final mass and M_i is the initial mass.

3.4.1.6 Selected minerals

The digested samples from the CP analysis were used for analysis of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe) and zinc (Zn) using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

3.4.2 Data analysis

Data collected were subjected to three factor analysis of variance (ANOVA) and if the f-value was significant ($P \leq 0.05$), the treatment means were separated using the Least Significant Difference (LSD) test at $P \leq 0.05$. MSTATC Statistical Package (Michigan State University) was used.

CHAPTER FOUR: RESULTS

4.1 Assessment of the current uses of cereal crop residues by small-scale farmers in Southern part of Botswana

A total of 63 farmers from Kweneng (25), Kgatleng (22) and Southern (16) were orally interviewed and the results of their biographic data, farming status, farming system and their perception and attitudes toward mushrooms and mushroom production are presented below.

4.1.1 Biographic data of farmers

4.1.1.1 Age, gender, marital status and level of education of heads of farming households

The majority (58.7%) of farming households in the survey area were male headed (Figure 8) and most of the farmers (42.9%) were over 65 years old (Figure 9).

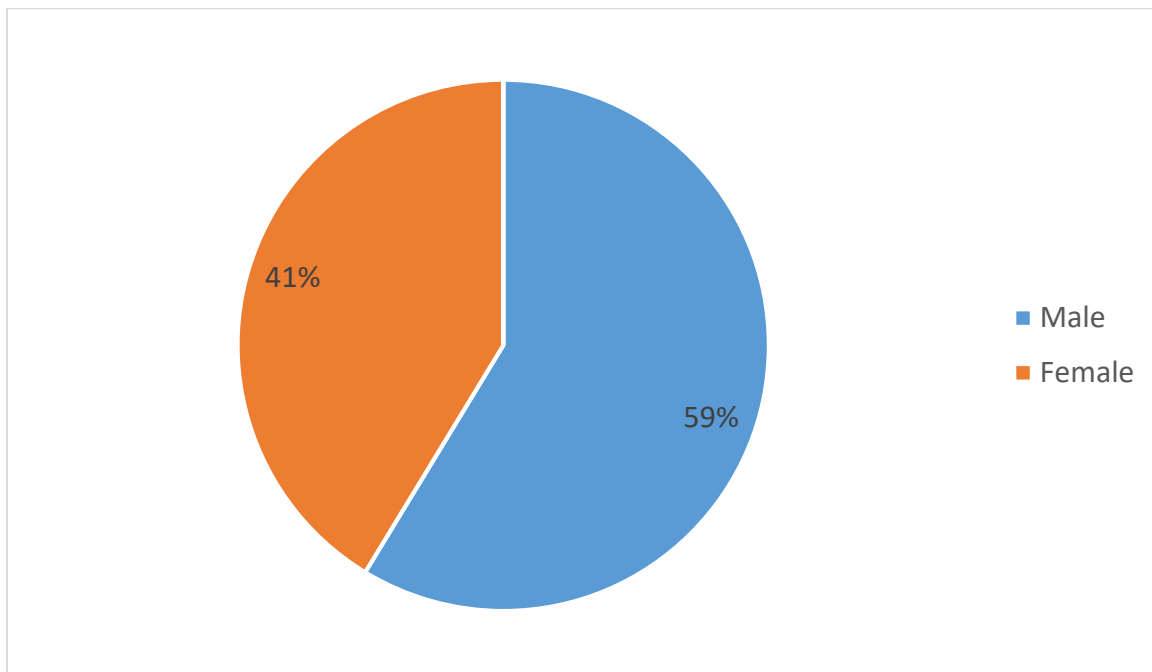


Figure 8: Gender of heads of farming household in Southern Botswana

The proportion of young farmers (≤ 35 years) constituted only 4.8% of the surveyed farmers.

The majority of the farming households were headed by individuals aged above 65, followed by those aged 61-65, then those aged 46-50 and 56-60 years. Few households were headed by individuals aged below 41 (Figure 9).

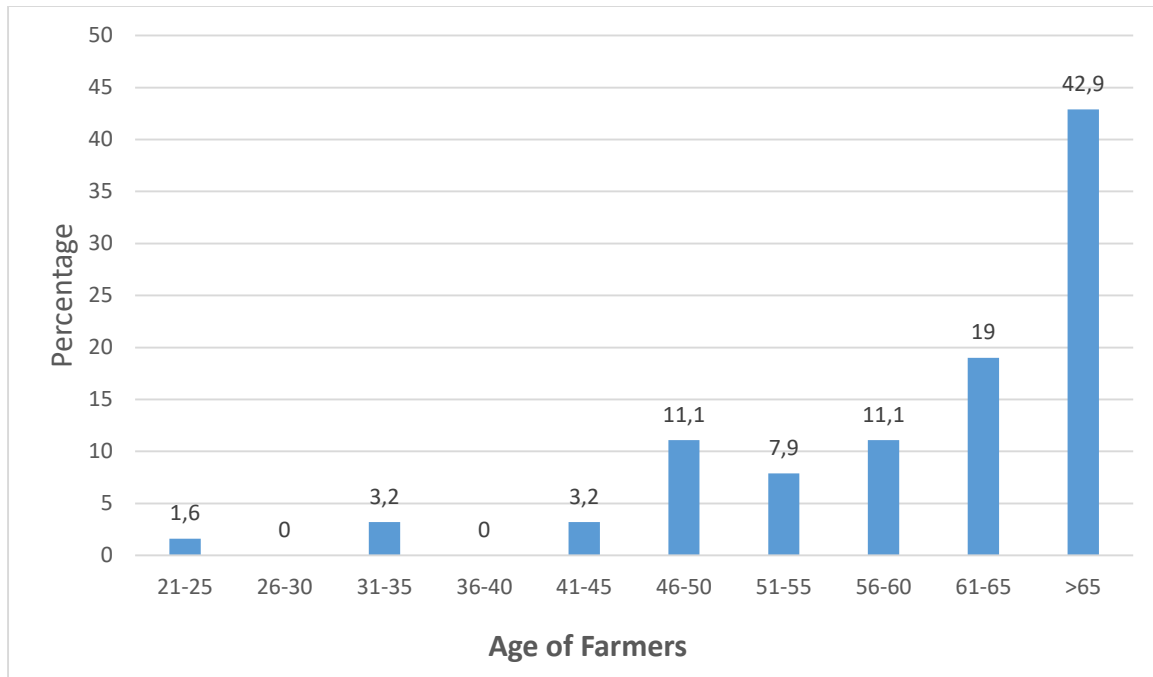


Figure 9: Age of heads of farming households in Southern Botswana

Among the interviewed individuals most of them were married (65.1%) followed by single and widowed (10%) and divorced and cohabiting (1.6%) (Table 6).

Table 6: Marital Status of the head of farming household

Marital Status	Frequency	Percent	Cumulative Percent
Single	10	15.9	15.9
Married	41	65.1	81.0
Divorced	1	1.6	82.5
Cohabiting	1	1.6	84.1
Widowed	10	15.9	100.0
Total	63	100.0	

Most heads of farming household (57.1%) had the highest educational level of primary school followed by junior secondary (11.1%) and tertiary (3.2-7.9%).The other educational levels had percentages ranging from 3.2 to 7.9 (Table 7).

Most farmers (95.2% owned the farms while few 3.2% and 1.6% rented and leased, respectively (Figure 10).

Table 7: Highest educational level attained by farming household head

Educational level	Frequency	Percent	Cumulative Percent
Primary School	36	57.1	57.1
Secondary (Junior)	7	11.1	68.3
Secondary (Senior)	2	3.2	71.4
College Certificate	2	3.2	74.6
College Diploma	4	6.3	81.0
College Degree	2	3.2	84.1
Masters	2	3.2	87.3
Doctorate	3	4.8	92.1
Other	5	7.9	100.0
Total	63	100.0	

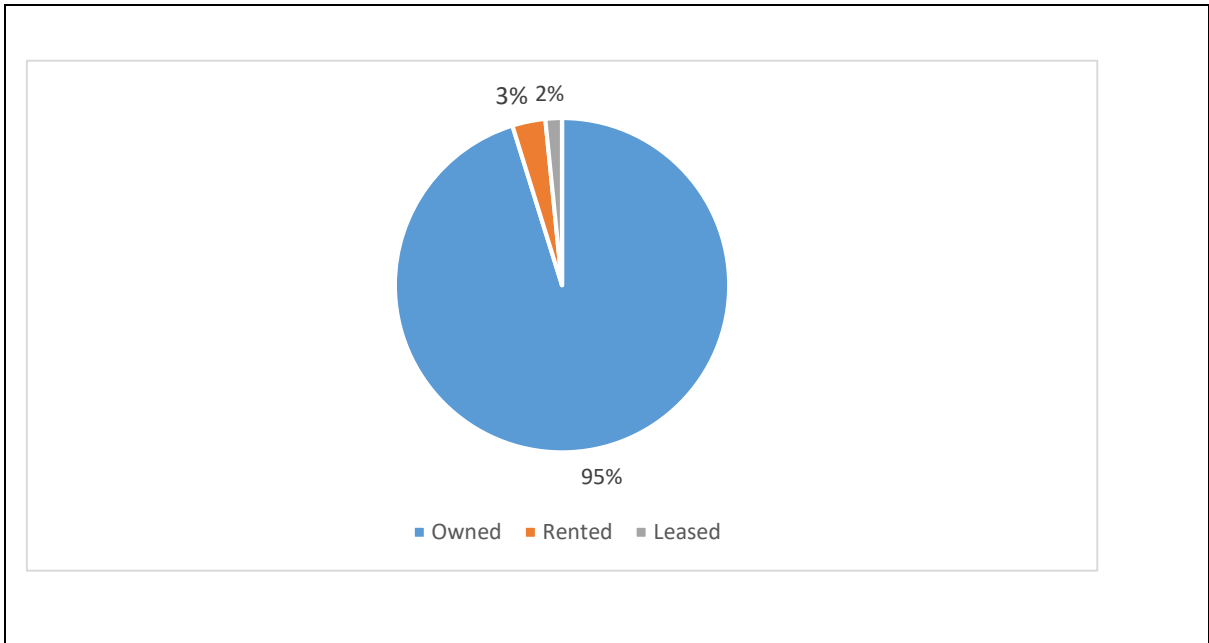


Figure 10: Percentage of ownership status of the farm

4.1.2 Farming status

The majority of farmers (87%) practiced farming on full time basis while a few (13%) were part time farmers as indicated in Figure 11.

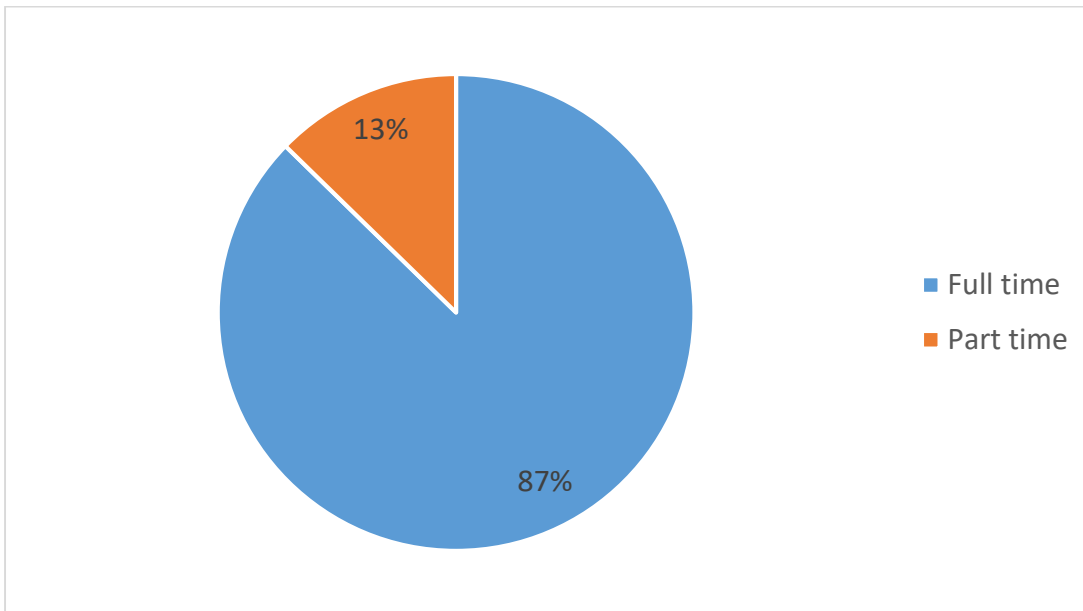


Figure 11: Percentage of farmer`s commitment to farming

For individuals who practiced farming on part time basis 52.4% had paid employment while 23.8% were not economically active, 17.5% were involved in other businesses and 6.3% were in unpaid family businesses (Table 8).

Table 8: Full Time Economic Activity of surveyed farmers

Economic Activity	Frequency	Percent	Cumulative Percent
Not Economically Active	15	23.8	23.8
Non-Agriculture Own Business	11	17.5	41.3
Paid Employment	33	52.4	93.7
Unpaid Family Employment	4	6.3	100.0
Total	63	100.0	

4.1.3 Farming systems and use of crop residues by farmers in Southern Botswana

4. 1.3.1 Farming system

The majority (90%) of the farmers surveyed used row planting of cereals and other crops while only 3% used the traditional method of broadcasting and 7% combined both row planting and broadcasting (Figure 12). Row planting is considered the best method of planting while broadcasting is performed by a few farmers as indicated below.

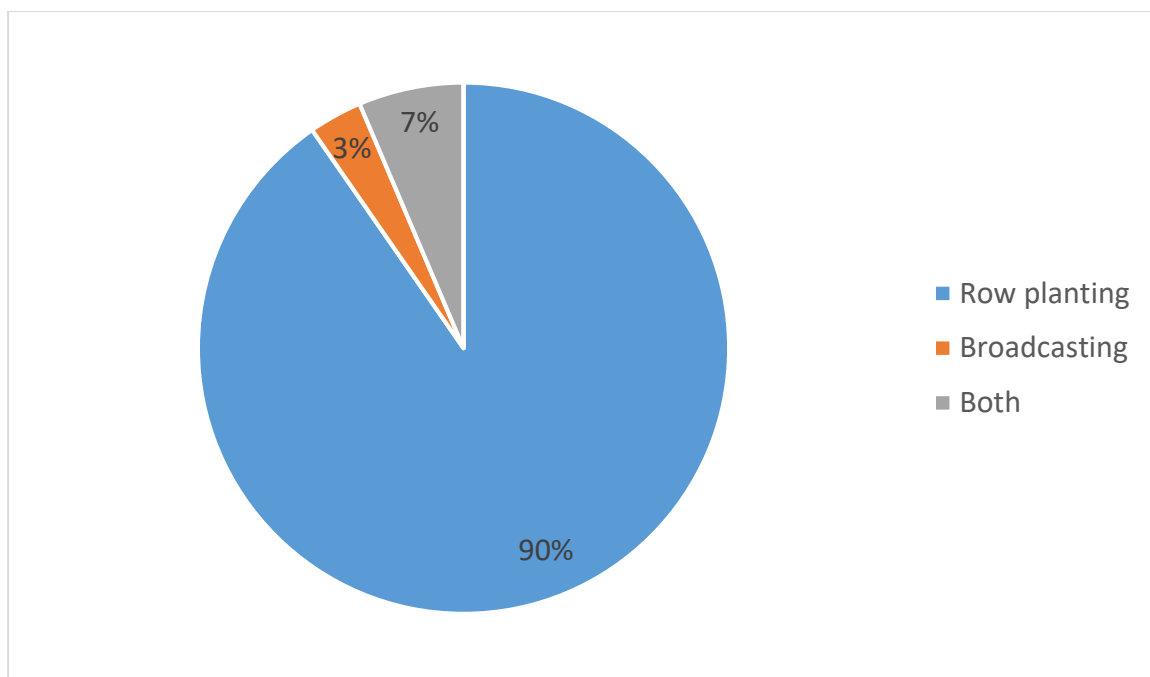


Figure 12: Methods of crop planting used by surveyed farmers in southern Botswana

Most farmers (66.7%) planted both maize and sorghum followed by maize alone (23.8%), then maize, sorghum and millet (7.9%) and lastly maize and millet (1.6%) (Table 9).

Table 9: Cereal crop combinations grown by farmers in southern Botswana

Crop (s) grown	Frequency	Percent	Cumulative Percent
Maize	15	23.8	23.8
Maize and sorghum	42	66.7	90.5
Maize and millet	1	1.6	92.1
Maize, sorghum and millet	5	7.9	100.0
Total	63	100.0	

4.1.3.2 Crop residue yield and disposal

After harvesting the grains, the residues were either harvested and fed to animals or left in the field for animals to graze upon. Most farmers (66.7%) harvested and stored the residues for

animal feeding while 33.3% left them in the field for animals to graze upon (Table 10). Even though these were harvested and stored none of the farmers weighed the residues.

Table 10: Disposal of cereal crop after harvest

Method of Disposal	Frequency	Percent	Cumulative Percent
Harvested and stored for feeding animals	42	66.7	66.7
Left in field to be grazed by animals	21	33.3	100.0
Total	63	100.0	

Though farmers used the residues to feed to animals they indicated that the quality is low hence a need to supplement with food supplements such as salt, molasses, beef finisher and lablab as indicated in Table 11.

Table 11: Measures used by farmers to improve nutritional status of cereal residues before feeding livestock in southern Botswana

Supplements added to cereal residues	Frequency	Percent	Cumulative Percent
Salt	10	15.9	15.9
Molasses	3	4.8	20.6
Molasses and salt	6	9.5	30.2
Molasses and beef finisher	1	1.6	31.7
Molasses, wheat bran, and salt	7	11.1	42.9
Molasses, wheat bran, beef grower and salt	1	1.6	44.4
Molasses, Di Calcium and Salt	5	7.9	52.4
Molasses, lablab and salt	1	1.6	54.0
Lablab	2	3.2	57.1
Lablab and salt	4	6.3	63.5
Cowpeas stover and molasses	4	6.3	69.8
wheat bran and salt	1	1.6	71.4
Calf and goat meal	1	1.6	73.0
Nothing done	17	27.0	100.0
Total	63	100.0	

4.1.4 Farmer's perceptions of mushrooms and attitude towards mushroom production

The majority of the farmers 88.9% knew and ate wild mushroom while 1.6% did not know mushrooms (Table 12). On consumption of mushrooms, 85.7% indicated that they eat mushrooms and for those who do not eat mushrooms, the reasons for not eating mushrooms included lack of knowledge of mushrooms, mushroom allergies, dislike of mushroom taste and lack of knowledge of how to cook the mushrooms. The majority of farmers (63.8%) did not know mushrooms can be cultivated.

Table 12: Mushroom consumption and reasons for not consuming mushrooms by farmers in southern Botswana

Mushroom consumption and reasons for non-consumption	Frequency	Percent	Cumulative Percent
Eat mushrooms	54	85.7	85.7
Makes me sick	2	3.2	88.9
Does not know mushrooms	4	6.3	95.2
Does not know how to cook mushrooms	1	1.6	96.8
Does not enjoy taste of mushrooms	2	3.2	100

Most farmers showed interest in growing mushrooms to improve family's nutritional status (93.7%), livestock feed's nutrition (95.2%), improve soil fertility (82.5%) and generate income (98.4%) while a few showed no interest in growing mushrooms (Table 13).

Table 13: Farmers' reasons for taking up mushroom production in southern Botswana

Reasons	Farmers' Responses			
	Yes		No	
	Frequency	Percentage	Frequency	Percentage
To improve family's nutritional status	59	93.7	4	6.3
To improve livestock feed's Nutritional status	60	95.2	3	4.8
To improve soil fertility in the field	53	82.5	11	17.5
To generate extra income for the household	62	98.4	1	1.6

The majority of farmers (85.7%) showed interest in participating in on-farm mushroom production research trials while only 3.2 % were undecided and 11.1 were not interesting in the study (Figure 13).

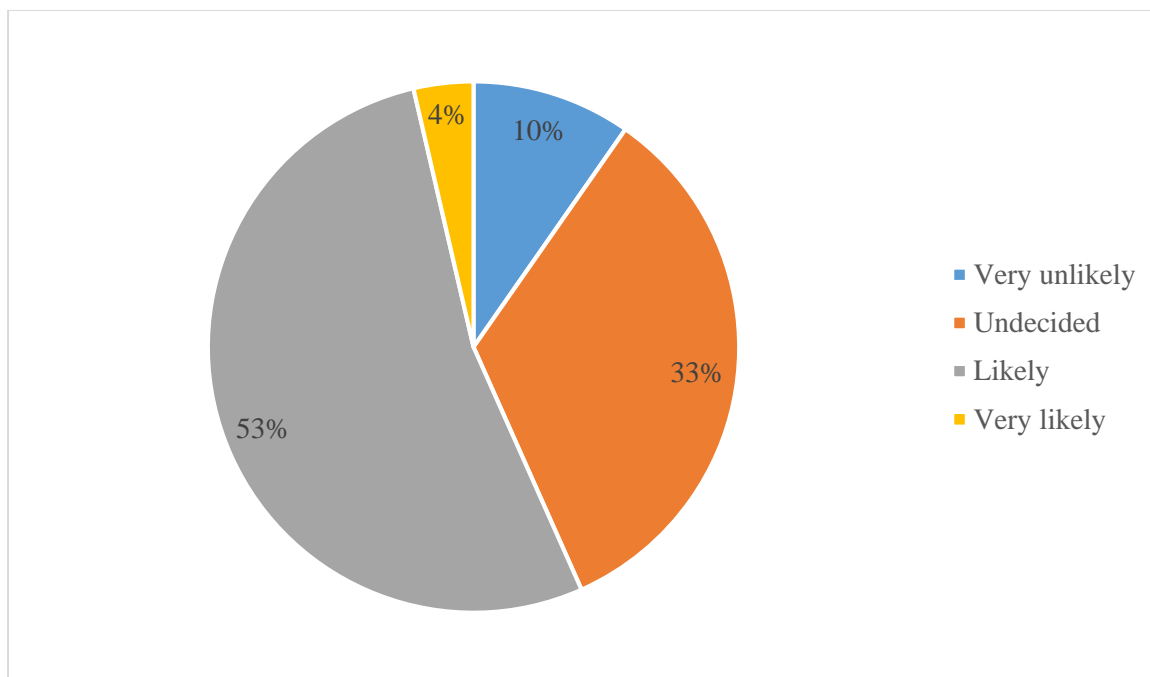


Figure 13: Percentage of farmer`s likelihood in participating in on-farm mushroom production study.

4.1.5 Quantification of cereal crop residues on farmers' fields

The estimated average yields of maize, sorghum and millet residues were 1206.7kg/ha, 1213Kg/ha and 4530.0 Kg/ha, respectively.

4.2 Effect of cereal residue type, substrate disinfestation method and oyster mushroom species on mushroom yields and the potential income from mushroom sale.

4.2.1 Environmental factors during the study period

The mean weekly temperatures inside the spawned substrates ranged from 20.9 to 26.9°C with an overall mean of 24.3°C while the ranges for minimum and maximum air temperatures in the mushroom house were 13.3-27.9 and 23-37.7°C with overall means of 22.9 and 31.7, respectively (Table 14). In general the temperatures were increasing from spawn running to fruiting of mushrooms due to increasing outside temperatures as recorded in Tale 15

Table 14: Mean weekly temperature in spawned bags and minimum and maximum temperatures in mushroom house during the study period.

Weeks	Bag Temp (°C)	Mushroom house Temperatures (°C)		
		Minimum	Maximum	Average
1	20.9	13.3	23	18.2
2	23	21.7	30.4	26.1
3	26.5	23.1	31.9	27.5
4	23	24.3	31.9	28.1
5	25.7	27.9	38.1	33.0
6	23	26.3	37.7	32
7	26.9	22.7	34.6	28.6
8	25.3	23.6	32.3	28.0
9	22.9	24.6	31	27.8
10	26	21	36	28.8
Overall Means	24.3	22.9	31.7	27.3

Table 15: A summary of weather data recorded at SSKIA during the study period

Week	Temperature (°C)			Total rainfall (mm)	Relative humidity (%)		
	Min	Max	Ave.		0800hrs	1400hrs	Ave
1	9.9	27.6	18.8	42.2	67	34.6	50.8
2	13.0	33.8	23.4	10.4	57	27.1	42.1
3	15.3	31.8	23.6	0	48.7	13.7	31.2
4	13.5	31.5	22.5	0	49	27.3	38.2
5	15.6	29.6	22.6	29.3	40.6	24.1	32.4
6	13.0	28.2	20.6	3.7	54.6	15.9	35.3
7	13.2	32.6	22.9	0.5	31	16.7	23.9
8	19.8	33.4	26.6	43.9	42	13.3	34.2
9	15.8	29.0	22.4	0	46.3	26.3	36.3
Overall	14.3	30.8	20.0	25.6	48.5	22.1	35.1
Means							

4.2.2 Effect of substrate type on spawn running, yield per flush and total yield of oyster mushrooms

There were significant differences in spawn running of *Pleurotus* spp. among the substrates with millet having the highest percentage of 77% followed by maize 74% and lastly sorghum (64%) (Table 16). Among the three substrate there were no significant differences in the mean number of days from spawning to the first mushroom flush (mean 44.2 days) but for the second and third flushes, mushrooms took significantly shorter period on millet stalks (53 and 59 days, respectively) than on maize (57 and 60 days, respectively) and sorghum stalks (57 and 60 days, respectively).

The average mushroom yields for flush 1 (100.9g), 2 (78.2g) and 3 (29.6g) were significantly higher on millet than on maize (74.3g, 47.6g and 3.5g, respectively) and sorghum stalks (53.3g, 26.5g and 0.0g, respectively). The mushroom yields for flushes 1, 2 and 3 were generally higher on maize stalks than on sorghum but the means were not significantly different except for flush

2. Within the 45 day harvesting period of the mushrooms, *Pleurotus* spp. had a higher number of mushroom flushes on millet (2.3) than on maize (1.7) and sorghum (1.5) stalks. The average total mushroom yield per bag and BE (%) was significantly higher on millet (211.3g and 62.3%) than on maize (126.3g and 27%) but not significantly higher than on sorghum stalk (81.6g and 16.8%).

Table 16: Effect of substrate type (Factor A) on percentage spawn running and number of days from spawn running to flush 1, 2 and 3, yield of flush 1 to 3, total number of flushes and biological efficiency.

Substrate [§]	Mushroom yield factors [#]								TY ^{&} (g)	BE% [@]
	% SR	DASF1	DASF2	DAS3	YF1 (g)	YF2 (g)	YF3 (g)	TF		
MZ	74.5	44.3	57.1a [¥]	59.9a	74.3b	47.3b	3.5b	1.7b	126.3b	29.4b
MT	77.0	42.4	53.0b	59.1b	101.0a	78.2a	29.6a	2.2a	211.3a	49.1a
SG	64.0	45.9	57.8a	60.0a	53.3b	26.5c	0.0b	1.5b	81.6c	19.0b
LSD value	NS	NS	2.24	0.58	21.9	17.2	11.53	0.35	36.59	8.5

[§] MT: Millet stalks, SG: Sorghum stalks, MZ: Maize stalks

[#] % SR: Percentage spawn running; DASF1: Days from spawning to flush 1; DASF2: Days from spawning to flush 2; DASF3: Days from spawning to flush 3; YF1: Yield of mushroom of flush1; YF2: Yield of mushrooms flush2; Yield of mushroom of flush 3; TF: Total number of flushes.

[&] TY: Total yield of mushroom per 430g of dry substrate

[@] BE: Biological efficiency (fresh weight of mushrooms/dry weight of substrate *100)

[¥] Means in a column followed by the same letter are not significantly different, $p \leq 0.05$, LSD test, NS= Not Significant

4.2.3. Effect of substrate disinfestation method (Factor B) on spawn running, yield per flush and total yield of Oyster mushrooms

Percentage spawn running of *Pleurotus* spp. on substrates disinfested with hydrogen peroxide (88.7%) was significantly higher than those disinfested by steam (55.0%) (Table 17). There

were no significant differences in the number of days taken by *Pleurotus* spp. from spawning to first to third flush between substrates disinfested by steam and hydrogen peroxide. Mushroom yields for flushes 1 and 2 were significantly higher on substrates treated with hydrogen peroxide (91.4g and 67.4g, respectively) than those treated by steam (61.0g and 34.2g, respectively) while yields for third flush were not significantly different. There were more mushroom flushes on hydrogen peroxide (2.1) than on steamed substrates (1.4). The total mushroom yield and BE per bag were also higher on hydrogen peroxide (170.3g and 36.4% respectively) than steam disinfested (109.2g and 34.3%, respectively) substrates.

4.2.4 Effect of *Pleurotus* species (Factor C) on spawn running, yield per flush and total yield of Oyster mushrooms

There were no significant differences in percentage spawn running among the three *Pleurotus* spp. with a mean of 71.9% and a range of 67.2 to 74.5% (Table 18). No significant differences were also observed for the number of days *Pleurotus* spp. took from spawning to produce first flush of mushrooms and the period was 42 days. Among the three mushroom species Po × Pf took lower number of days from spawning to flush 2 (54.4) and 3 (59.3) than Po HK35 (56.7 and 59.7, respectively) and PF (56.7 and 60.0, respectively) which were similar. There were no significant differences among the *Pleurotus* spp. in mushroom yield for flush 1 (63.8 -87.7g) and 2 (44.1 -55.5 g) while for flush 3 Pf had no mushrooms compared to Po x Pf (18.8g) and Po HK35 (14.3g). There were no differences in number of flushes among the three species with means ranging from 1.6 to 2.0 to a grand mean of 1.8 (Table 18). Po x Pf had significantly higher total mushroom yield and BE than *P. floridanus* while Po HK35 was not significantly lower than Po x Pf and not significantly higher than *P. floridanus*. Po HK35 had the highest biological efficiency followed by Po x Pf and lastly PF (Table18).

Table 17: Effect of substrate disinfestation method (Factor B) on percentage spawn running and number of days from spawn running to flush 1, 2 and 3, yield per flush, total number of flushes, total yield and biological efficiency.

SDM [§]	Mushroom yield factors [#]								TY(g) ^{&}	BE (%) [@]
	%SR	DASF1	DASF2	DASF3	YF1(g)	YF2(g)	YF3(g)	TF		
H ₂ O ₂	88.7a [¥]	41.9b	55.6b	59.7a	91.4a	67.4a	11.8a	2.2a	170.3a	39.6a
ST	55.0b	46.5a	56.3a	59.6b	61.0b	34.2b	10.2b	1.4b	109.2b	25.4b

[§]SDM: substrate disinfestation method; H₂O₂: Hydrogen peroxide, ST: Steaming

[#] % SR: Percentage spawn running; DASF1: Days from spawning to flush 1; DASF2: Days from spawning to flush 2; DASF3: Days from spawning to flush 3. YF1: Yield of mushroom of flush1; YF2: Yield of mushrooms flush2; Yield of mushroom of flush 3; TF: Total number of flushes.

[&] TY: Total yield of mushroom per 430g of dry substrate

[@] BE: Biological efficiency (fresh weight of mushrooms/dry weight of substrate *100)

[¥] Means in a column followed by the same letter are not significantly different, $p \leq 0.05$, ANOVA, NS = Not Significant

Table 18: Effect of mushroom species (Factor C) on percentage spawn running and number of days from spawn running to flush 1, 2 and 3, yield per flush, total number of flushes, total yield and biological efficiency

Species ^{\$}	Mushroom yield factors [#]								TY(g) ^{&}	BE% [@]
	% SR	DASF1	DASF2	DAS3	YF1 (g)	YF2 (g)	YF3 (g)	TF		
Po x Pf	73.8	44.0	54.4	59.3	77.1	52.7	18.8a	2.0	153.2a	35.6a
Pf	67.2	44.1	56.7	60.0	63.8	44.1	0.0b	1.6	108.5b	25.2b
Po HK35	74.5	44.5	56.7	59.7	87.7	55.5	14.3a	1.8	157.5a	36.6a
LSD	NS	NS	NS	NS	NS	NS	11.53	NS	36.59	8.5

^{\$}Po xPf: hybrid of *P. ostreatus* and *P. floridanus*, Pf: *P. floridanus*, Po HK35: *P. ostreatus* strain HK35

[#] % SR: Percentage spawn running; DASF1: Days from spawning to flush 1; DASF2: Days from spawning to flush 2; DASF3: Days from spawning to flush 3; YF1: Yield of mushroom of flush1; YF2: Yield of mushrooms flush2; Yield of mushroom of flush 3; TF: Total number of flushes.

[&] TY: Total yield of mushroom per 430g of dry substrate

[@] BE: Biological efficiency (fresh weight of mushrooms/dry weight of substrate *100) [¥] Means in a column followed by the same letter are not significantly different, $p \leq 0.05$, LSD test, NS= Not Significant

4.2.5 Effect of substrate and disinfestation method (A x B interaction) on spawn running, yield per flush and total yield of oyster mushrooms.

The substrate by disinfestation method interaction had no significant effect on all parameters except number of days from spawning to flush 2 (DASF2) where mushrooms took the shortest period of 51.7 days on steamed millet and longest (58.7) on steamed sorghum (Table 19). Percentage spawn running ranged from 45.3 on steamed sorghum to 96.0 on hydrogen peroxide treated maize stalks. Oyster mushrooms took 41.2 to 48.7 days and 59.3-60 days to flushes 1 and 3, respectively and yields for flushes 1, and 3 were 46-117.7g, 10.5-83.3 g and 0-30.7g. The total mushroom yield and BE%, respectively ranged from 60.4g (steamed sorghum stalks) to 228.5g (steamed millet stalks) and from 14.0% (steamed sorghum) to 53.1% (steamed millet).

4.2.6 Effect of substrate type and *Pleurotus* spp. (A x C interaction) on spawn running, yield per flush and total yield of Oyster mushrooms.

The substrate by *Pleurotus* spp. interaction had significant effects on DASF2, YF3, TF and TY and no significant effect on %SR, DASF1, DASF2, YF1 And YF2 (Table 20). Percentage spawn running ranged from 62.0% (Po HK35 on SG) to 86.0% (Po HK35 on MT); DASF1 ranged from 41.0 days (Po HK35 on MT) to 45.2 days (Po x Pf on MZ); DASF3 ranged from 58.1 to 60 days, YF1 and YF2 ranged from 48.9g to 101.6g and 18.2g to 102.0g. For DASF2, Po HK35 on MT took significantly fewer number of days (52.1) than the other treatment and for YF3, Po x Pf and HK35 on MT had significantly higher yields (45.9g and 42.8g respectively) than on the other treatments. Similarly, Po x Pf and Po HK35 on MT had significantly higher number of flushes than the other treatments. The total yield and BE for Po HK35 (274.2g, 63.8%) and Po x Pf (232.2g, 54.0%) on MT were similar and significantly higher than the other treatments and the lowest was HK35 on sorghum (69.5g, 16.2%), respectively.

Table 19: Effect of substrate type and substrate disinfection method (A×B interaction) on percentage spawn running and number of days from spawn running to flush 1, 2 and 3, yield per flush, total number of flushes ,total yield and biological efficiency.

Substrate ^{\$}	SDM*	Mushroom yield factors #								TY (g) ^{&}	BE(%) [@]
		%SR	DASF1	DASF2	DASF3	YF1(g)	YF2(g)	YF3(g)	TF		
MZ	H ₂ O ₂	96.0	41.2	55.6ab	59.9	96.3	76.5	6.9	2.1	179.5	41.7
MZ	ST	53.0	47.3	58.5a	60.0	52.3	18.8	0.0	1.3	73.1	17.0
MT	H ₂ O ₂	87.3	41.2	54.3bc	59.3	117.7	83.3	28.5	2.4	228.5	53.1
MT	ST	66.7	43.5	51.7c	58.9	84.3	73.1	30.7	2.1	194.1	45.1
SG	H ₂ O ₂	82.7	43.2	56.9ab	60.0	60.3	42.5	0.0	2.0	102.7	23.9
SG	ST	45.3	48.7	58.7a	60.0	46.4	10.5	0.0	0.9	60.4	14.0
LSD		NS	NS	3.169	NS	NS	NS	NS	NS	NS	NS

^{\$}MZ: Maize stalk, MT: Millet stalk, SG: Sorghum stalk

*SDM: substrate disinfestation method, ST: Steaming, H₂O₂: Hydrogen peroxide

% SR: Percentage spawn running; DASF1: Days from spawning to flush 1; DASF2: Days from spawning to flush 2; DASF3: Days from spawning to flush 3. YF1: Yield of mushroom of flush1; YF2: Yield of mushrooms flush2; Yield of mushroom of flush 3; TF: Total number of flushes.

& Total yield of mushroom per 430g of dry substrate

@ BE: Biological efficiency (fresh weight of mushrooms/dry weight of substrate *100)[¥] Means in a column followed by the same letter are not significantly different, p≤ 0.05, LSD test, NS= Not Significant

Table 20: Effect of substrate type and *Pleurotus* species (A x C interaction) on percentage spawn running and number of days from spawn running to flush 1, 2 and 3, yield per flush, total number of flushes, total yield and biological efficiency.

Substrate [§]	Species [*]	Mushroom yield factors [#]								TY (g) ^{&}	BE(%) [@]
		%SR	DASF1	DASF2	DASF3	YF1(g)	YF2(g)	YF3(g)	TF		
MZ	Po × Pf	72.0	45.2	56.6a	59.8	69.8	52.7	10.4b	1.9b	133.9b	31.1b
	Pf	76.0	42.3	55.5ab	60.0	70.6	43.8	0.0b	1.8b	116.4bc	20.1c
	Po HK35	75.5	45.3	59.1a	60.0	82.5	46.4	0.0b	1.4b	128.7bc	29.7bc
MT	Po × Pf	82.5	41.8	49.0a	58.1	101.6	77.0	45.9a	2.6a	232.2a	54.0a
	Pf	62.5	44.3	57.8a	60.0	71.9	55.6	0.0b	1.5b	127.5bc	29.7bc
	Po HK35	86.0	41.0	52.1b	59.1	129.4	102.0	42.8a	2.6a	274.2a	63.8a
SG	Po × Pf	67.0	45.0	57.6a	60.0	59.8	28.5	0.0b	1.4b	93.5bc	21.7bc
	Pf	63.0	45.7	56.8a	60.0	48.9	32.8	0.0b	1.6b	81.7bc	19bc
	Po HK35	62.0	47.0	59.0a	60.0	51.3	18.2	0.0b	1.4b	69.5c	16.2c
LSD		NS	NS	3.88	NS	NS	NS	19.97	0.607	63.37	14.7

[§]MZ: Maize stalks, MT: Millet stalks, SG: Sorghum stalks

[€]ST: Steaming, H₂O₂: Hydrogen peroxide

^{*}PO ×PF: hybrid of *Pleurotus ostreatus* and *Pleurotus floridanus*, PF: *P. floridanus*, PO HK35: *P. ostreatus* strain HK35

[#]% SR: Percentage spawn running; DASF1: Days from spawning to flush 1; DASF2: Days from spawning to flush 2; DASF3: Days from spawning to flush 3. YF1: Yield of mushroom of flush1; YF2: Yield of mushrooms flush2; Yield of mushroom of flush 3; TF: Total number of flushes.

[&] TY: Total yield of mushroom per 430g of dry substrate

[@] BE: Biological efficiency (fresh weight of mushrooms/dry weight of substrate *100) [¥] Means in a column followed by the same letter are not significantly different, p≤ 0.05, LSD test, NS= Not Significant

4.2.7 Effect disinfection method and *Pleurotus* spp. (B x C interaction) on spawn running, yield per flush and total yield of Oyster mushrooms

The substrate disinfection method by *Pleurotus* species interaction had no significant effect on all parameters assessed. Percentage spawn running ranged from 43.3 for Pf grown on hydrogen peroxide treated substrates to 93.3 for Po x Pf on steamed substrates and the BE ranged from 15.3 to 42.4% for Pf and Po HK35 on hydrogen peroxide treated substrates (Table 21).

4.2.8. Effect of substrate type, disinfection method and *Pleurotus* species (A x B x C interaction) on spawn running, yield per flush and total yield of Oyster mushrooms.

There were no significant differences in all mushroom factors recorded except that of yield for second flush in which Po HK35 grown on millet disinfested by steam or hydrogen peroxide had the highest yields (101.4g-102.6g) (Table 22). Biological efficiency ranged from a low of 8.1% for PF on hydrogen peroxide treated sorghum stalk to a high of 69.4% for Po HK35 on steamed millet substrate.

Table 21: Effect of mushroom species and substrate disinfection method (B × C interaction) on percentage spawn running and number of days from spawn running to flush 1, 2 and 3.

SDM [§]	Species [*]	Mushroom yield factors [#]								TY(g) ^{&}	BE(%) [@]
		%SR	DASF1	DASF2	DASF3	YF1(g)	YF2(g)	YF3(g)	TF		
ST	Po × Pf	93.3	42.3	53.9	59.2	92.7	70.9	19.5	2.4	182.3	42.4
	Pf	91.0	40.9	56.1	60.0	86.4	64.7	0.0	1.9	151.1	35.1
	Po HK35	81.7	42.4	56.9	59.9	95.1	66.6	15.9	2.2	177.4	39.7
H ₂ O ₂	Po × Pf	54.3	45.7	54.9	59.4	61.4	34.5	18.0	1.5	124.1	41.3
	Pf	43.3	47.3	57.3	60.0	41.2	23.5	0.0	1.3	66.0	15.3
	Po HK35	67.3	46.5	56.6	59.5	80.4	44.5	12.7	1.4	137.5	32.0
LSD		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[§] SDM: Substrate disinfestation method: ST: Steaming; H₂O₂: Hydrogen peroxide

^{*} PO HK35: *Pleurotus ostreatus* strain HK35, PF: *Pleurotus floridanus*, PO × PF: hybrid of *Pleurotus ostreatus* and *Pleurotus floridanus*.

[#] % SR: Percentage spawn running; DASF1: Days from spawning to flush 1; DASF2: Days from spawning to flush 2; DASF3: Days from spawning to flush 3. YF1: Yield of mushroom of flush 1; YF2: Yield of mushrooms flush 2; Yield of mushroom of flush 3; TF: Total number of flushes.

[&] TY: Total yield of mushroom per 430g of dry substrate

[@] BE: Biological efficiency (fresh weight of mushrooms/dry weight of substrate *100)

[¥] Means in a column followed by the same letter are not significantly different, p ≤ 0.05, LSD test, NS= Not Significant

Table 22: Effect of substrate type, mushroom species and substrate disinfestation method (A x B x C interaction) on mushroom yield of flush 1, 2 and 3, yield per flush, total number of flushes, total yield and biological efficiency.

Substrate [§]	SDM*	Species [€]	Mushroom yield factors [#]								TY(g) ^{&}	BE(%) [@]
			%SR	DASF1	DASF2	DASF3	YF1(g)	YF2(g)	YF3(g)	TF		
MZ	ST	Po × Pf	100.0	42.6	53.6	59.6	96.0	91.4ab [¥]	20.8	2.4	208.2	48.4
		Pf	96.0	39.6	55.0	60.0	91.2	58.8bcd	0.0	2.0	150.0	34.9
		Po HK35	92.0	41.4	58.2	60.0	101.6	79.2ab	0.0	2.0	180.4	42.0
	H ₂ O ₂	Po × Pf	44.0	47.8	59.6	60.0	43.6	14.0ef	0.0	1.4	59.6	13.9
		Pf	56.0	45.0	56.0	60.0	50.0	28.8cdef	0.0	1.6	82.8	19.3
		Po HK35	59.0	49.2	60.0	60.0	63.4	13.6ef	0.0	0.8	77.0	17.9
MT	ST	Po × Pf	90.0	41.8	50.4	58.0	112.6	64.4abc	37.8	2.8	212.2	49.3
		Pf	83.0	41.6	59.6	60.0	90.8	84.0ab	0.0	1.8	174.8	40.7
		Po HK35	89.0	40.2	52.8	59.8	149.6	101.4a	47.6	2.6	298.6	69.4
	H ₂ O ₂	Po × Pf	75.0	41.8	47.6	58.2	90.6	89.6ab	54.0	2.4	252.2	58.7
		Pf	42.0	47.0	56.0	60.0	53.0	27.2cdef	0.0	1.2	80.2	18.7
		Po HK35	83.0	41.8	51.4	58.4	109.2	102.6a	38.0	2.6	249.8	58.1
SG	ST	Po × Pf	90.0	42.4	57.6	60.0	69.6	57.0bcd	0.0	2.0	126.6	29.4
		Pf	94.0	41.6	53.6	60.0	77.2	51.2bcde	0.0	2.0	128.4	29.9
		Po HK35	64.0	45.6	59.6	60.0	34.0	19.2def	0.0	2.0	53.2	12.4
	H ₂ O ₂	Po × Pf	44.0	47.6	57.6	60.0	50.0	0.0f	0.0	0.8	60.4	14.0
		Pf	32.0	49.8	60.0	60.0	20.6	14.4ef	0.0	1.2	35.0	8.1
		Po HK35	60.0	48.6	58.4	60.0	68.6	17.2def	0.0	0.8	85.8	20.0
LSD			NS	NS	NS	NS	NS	42.14	NS	NS	NS	NS

[§]MZ: Maize stalks, MT: Millet stalks, SG: Sorghum stalks

^{*}SDM: Substrate disinfestation method: ST: Steaming, H₂O₂: Hydrogen peroxide

[€]PO HK35: *Pleurotus ostreatus* strain HK35, PF: *P. floridanus*, PO ×PF: hybrid of *P. ostreatus* and *P. floridanus*.

% SR: Percentage spawn running; DASF1: Days from spawning to flush 1; DASF2: Days from spawning to flush 2; DASF3: Days from spawning to flush 3. YF1: Yield of mushroom of flush1; YF2: Yield of mushrooms flush2; Yield of mushroom of flush 3; TF: Total number of flushes.

& TY: Total yield of mushroom per 430g of dry substrate

@ BE: Biological efficiency (fresh weight of mushrooms/dry weight of substrate *100)

¥ Means in a column followed by the same letter are not significantly different, $p=0.05$, LSD test, NS= Not Significant

4.3. Estimation of potential income using the best *Pleurotus* sp., substrate and disinfestation method.

The fixed costs for processing 12.5Kg of the three substrates were estimated at P182.25 and variable costs for steaming and hydrogen peroxide treatments were P7.50 and P2.24 giving total production costs of P189.75 for steaming and P184.49 for hydrogen peroxide, respectively. (Table 23).

When the best four BEs (96.4%, 58.7%, 58.1% and 49.3%) in Table 23 were used in the profit analysis the highest optimist profit of 166% was realised when Po HK35 was grown on steamed millet, followed by 118% for Po x Pf on hydrogen peroxide-treated millet, 115% for Po HK35 on hydrogen peroxide- treated millet and 60% for Po x Pf on steamed millet (Table 24). Similar but lower profit trend as above was observed when the expected yield was used with expected profits of 91%, 55%, 52% and 8%, respectively. However, when the pessimistic yield was used only Po HK35 had 18% pessimistic profit while the others had losses ranging from -44% to -9%.

Using the estimated millet residue yield of 4530Kg/ha for surveyed farmers the potential additional incomes per hectare is P182876 (4530Kg x P40.37/Kg) using optimistic BE, P131,551 (4530Kg x P29.04/Kg) and P81404 (4530Kg x P17.97/Kg) using pessimistic BE for Po HK35 is grown on steamed millet.

Table 23: Fixed and variable costs of mushroom production using 12.5kg of substrate

Costs	Item description	Unit	Number	Unit cost (BWP)	Total cost (BWP)
Fixed	House rental	Month	2	25	50
	Chaff cutter rental	Hour	1	4.50	4.50
	Spawn	500ml	1500ml	10	30
	Substrate	25kg	0.5	62.50	31.25
	Consumables	Bag	30	0.50	15
	Labour	Hour	6	8.58	51.50
	Total Fixed Costs				
Variable	Firewood	1 bundle	0.5	15	7.50
	Hydrogen Peroxide	ml	80ml	0.028	2.24
	Total production cost with firewood				189.75
	Total Production cost with peroxide				184.49

Table 24: Returns for mushroom sales at P80/Kg during a six-week production period using 12.5 Kg of millet stalks spawned with Po HK35 and Po x Pf using BEs form Substrate by disinfestation method interaction

Item description	Millet disinfestation method and <i>Pleurotus</i> spp. Grown			
	Steam		H ₂ O ₂	
	Po HK35 (69.4%)	Po x Pf (49.3%)	Po HK35 (58.1%)	Po x Pf (58.7%)
Optimistic yield[§]				
Mushroom yield(g)	8.68	6.16	7.26	7.34
Gross returns(BWP)	694.40	492.80	580.8	587.20
Production costs(BWP)	189.75	189.75	184.49	184.49
Returns(BWP)	504.65	303.05	396.31	402.71
Cost/Kg of mushrooms(BWP)	21.86	30.80	25.41	25.13
Return/Kg of mushroom(BWP)	58.14	49.20	54.59	54.87
Return/Kg of substrate (BWP)	40.37	24.24	31.70	32.22
%Profit	166	60	115	118
Expected yield[#]				
Mushroom yield(g)	6.91	4.93	5.81	5.88
Gross returns(BWP)	552.80	394.4	464.80	470.4
Production costs (BWP)	189.75	189.75	184.49	184.49
Returns(BWP)	363.05	204.65	280.31	285.91
Cost/Kg of mushrooms(BWP)	27.46	38.49	31.75	31.38
Return/Kg of mushroom(BWP)	52.54	41.51	48.25	48.62
Return/Kg of substrate (BWP)	29.04	16.37	22.42	22.87
%Profit	91	8	52	55
Pessimistic Yield^{&}				
Mushroom yield(g)	5.18	3.70	4.36	4.4
Gross returns(BWP)	414.40	296.00	348.80	352.00
Production costs(BWP)	189.75	189.75	184.49	184.49
Returns(BWP)	224.65	106.25	164.31	167.51
Cost/Kg of mushrooms(BWP)	36.63	51.28	42.31	41.93
Return/Kg of mushroom(BWP)	43.37	28.72	37.69	38.07
Return/Kg of substrate (BWP)	17.97	8.5	13.14	13.40
%Profit	18	-44	-11	-9

[§] Optimal yield estimated using the experimental BE

[#] Expected yield estimated using 80% of the experimental BE

[&] Pessimistic yield estimated using 60% of the experimental BE

4.4 The effect of substrate types, substrate disinfestation method and *Pleurotus* spp. on mushroom nutritional composition and their potential in improving the nutrition of small-scale farmers.

4.4.1 Effect of substrate type (Factor A) on the chemical composition of *Pleurotus* spp.

Mushrooms grown on sorghum (28.5%) and maize (28.5%) had similar Crude Protein (CP) levels which were significantly higher than those grown on of millet (24.1%). There were no significant differences in ash and crude fat contents among mushrooms grown on the three substrates and these ranged from 7.4 to 9.29 and 5.47 to 6.66% respectively (Table 25). For mushroom mineral contents, there were no significant differences in contents of zinc, calcium and sodium in mushrooms grown on the three substrates. However, manganese content of millet (35.19%) was higher than that of maize (33.2%) and sorghum (33.12%) which were similar. Mushrooms planted on stalks of maize (14.26%) contained highest level of Cu, followed by sorghum (12.58%) while millet and the lowest (10.73%). Iron, magnesium and P levels of mushrooms grown on maize and sorghum were statistically similar but higher than those on millet. Mushrooms grown on millet and sorghum had higher potassium content than those planted in maize (Table 25).

4.4.2 Effect of substrate disinfestation method (Factor B) on the chemical composition of *Pleurotus* spp.

Pleurotus spp. grown on substrates disinfested using H₂O₂ had higher levels of CP, Mn, CF, Zn, Cu, Fe, Ca, Mg, Na and K compared to mushrooms which were planted on substrate that was steamed. For ash and P mushrooms planted in substrate disinfested by steaming had higher level than those grown on substrates disinfested by hydrogen peroxide (Table 26).

Table 25: Effect of substrate type on nutritional composition of *Pleurotus* spp.

Substrate	Nutritional composition of mushrooms [#]											
	Crude Protein(%)	Ash(%)	Crude fat (%)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	P (mg/kg)
Maize	28.5a	9.29	5.99	33.20b	97.00	12.58b	106.23a	340.71	1694.14a	143.92	3327.56b	5059.12a
Millet	24.1b	8.73	5.47	35.19a	91.39	10.73c	84.00b	341.40	1183.00b	156.16	3261.44a	3470.61b
Sorghum	28.6a	7.40	6.66	32.12b	106.20	14.26a	106.93a	342.30	1786.70a	113.82	4368.56a	5199.44a
LSD	2.51	NS	NS	1.104	NS	1.269	18.54	NS	112.2	NS	862.2	251.8

[#]Mn: Manganese, Zn: Zinc, Cu: Copper, Fe: Iron, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium, P: Phosphorus

[¥] Means in a column followed by the same letter are not significantly different, $p \leq 0.05$, LSD test, NS= Not Significant

Table 26: Effect of substrate disinfestation method on nutritional composition of *Pleurotus* spp.

SDM [§]	Nutritional composition of mushrooms [#]											
	Crude Protein(%)	Ash(%)	Crude fat(%)	Mn(mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	P (mg/kg)
H ₂ O ₂	27.6a	8.14b	5.73a	32.73a	106.86a	12.85a	100.92a	348.88a	1643.05a	143.64a	3684.07a	4532.70b
ST	26.5b	8.80a	6.35b	34.27b	89.53b	12.20b	97.19b	334.06b	1466.17b	132.29b	3620.96b	4620.08a

[§]SDM: Substrate disinfestation method: ST: Steaming; H₂O₂: Hydrogen peroxide

[#]Mn: Manganese, Zn: Zinc, Cu: Copper, Fe: Iron, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium, P: Phosphorus

[¥] Means in a column followed by the same letter are not significantly different, p≤0.05, ANOVA, NS= Not Significant.

4.4.3 Effect of *Pleurotus* species (Factor C) on nutritional composition of mushrooms

Crude Protein contents of Po × Pf (35.2%) and Po HK35 (32.7%) were similar but higher than that of Pf (13.2%), while the difference in ash content was not significant (Table 30). Pf had the highest level of CF, Ca and Na, followed by Po × Pf, while Po HK35 had the lowest of the respective nutrients. Po × Pf had the highest concentration of Mn, followed by Po HK35 which was also higher than Pf. Concentration of Zn (126.16) and Fe (179.64) was higher in Pf than Po × Pf and Po HK35 which had similar levels of these two minerals. The concentration levels of Cu, Mg, K and P were high in Po HK35, followed by Po × Pf which also had higher levels of respective minerals than Pf (Table 27).

4.4.4 Effect of substrate and disinfestation method (A x B Interaction) on nutritional composition of mushrooms

There were no significant differences in CP, ash, CF, Mn, Zn, Cu, Fe and Ca contents among the three *Pleurotus* spp. grown on steam and hydrogen peroxide disinfested substrates while significant differences were recorded for Mg, Na, K and P (Table 28). Magnesium content in mushrooms grown on millet disinfested by steam (901.4mg/kg) and hydrogen peroxide (1464.6mg/kg) were significantly different but they were significantly lower than those grown on the other substrate and disinfestation method combinations which ranged from 1692.99 to 1800.81. Sodium content (71.91mg/kg) was significantly lower in mushrooms grown on steamed sorghum (166.57mg/kg) than in the others which ranged from 129.45mg/l to 166.57mg/kg). Potassium was high in mushrooms that were grown on sorghum disinfested by steaming (4859.67mg/kg), followed by millet (3877.44mg/kg) and sorghum (3915.11mg/kg) treated with hydrogen peroxide. Mushrooms grown on steamed millet had the lowest potassium content (2607.78 mg/kg). Phosphorus was highest in mushrooms grown on steamed maize (5435.59mg/kg) and sorghum (5380.83mg/kg), followed by sorghum (5018.06mg/kg) and

maize (4682.64mg/kg) and treated with hydrogen peroxide and hydrogen peroxide disinfested millet (3897.39mg/kg) and lastly steamed millet (3043.82mg/kg).

Table 27: Effect of *Pleurotus* spp. on nutritional composition of mushrooms

Species [§]	Nutritional composition of mushrooms [#]											
	Crude Protein(%)	Ash(%)	Crude fat(%)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	P (mg/kg)
Po × Pf	35.2a	7.50	4.98b	39.88a	73.11b	14.77b	65.07b	154.85b	1721.99b	131.50b	3989.94b	5022.44b
Pf	13.2b	9.34	7.03a	23.09c	126.16a	6.75c	179.64a	815.89a	753.68c	198.84a	2089.11c	2662.70c
Po HK35	32.7a	8.58	6.11b	37.53b	95.31b	16.05a	52.44b	53.67c	2188.16a	83.55c	4878.50a	6044.00a
LSD	2.51	NS	1.478	1.104	26.71	1.269	18.54	60.41	112.2	29.71	862.2	251.8

*PO HK35: *Pleurotus ostreatus* strain HK35, PF: *Pleurotus floridanus*, PO ×PF: hybrid of *Pleurotus ostreatus* and *Pleurotus floridanus*.

[#]Mn: Manganese, Zn: Zinc, Cu: Copper, Fe: Iron, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium, P: Phosphorus

[¥] Means in a column followed by the same letter are not significantly different, $p \leq 0.05$, LSD test, NS= Not Significant.

Table 28: Effect of substrate type and disinfestation method on nutritional composition of mushrooms

Substrate	SDM [§]	Nutritional composition of mushrooms [#]											
		Crude Protein(%)	Ash(%)	Crude fat(%)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	P (mg/kg)
Maize	H ₂ O ₂	28.9	7.86	5.71	32.71	104.09	12.89	109.09	344.25	1692.99a	129.45a	3259.66bc	4682.64b
	ST	28.1	10.73	6.27	33.68	89.06	12.27	103.37	337.17	1696.3a	158.39a	3395.44bc	5435.59a
Millet	H ₂ O ₂	25.6	8.98	5.12	33.49	109.92	10.85	81.38	314.27	1464.60b	145.75a	3915.11ab	3897.39c
	ST	22.5	8.48	5.82	36.89	72.86	10.62	86.61	368.53	901.40c	166.57a	2607.78c	3043.82d
Sorghum	H ₂ O ₂	28.2	7.60	6.37	31.99	105.72	14.81	112.28	388.14	1772.59a	155.72a	3877.44ab	5018.06b
	ST	28.9	7.20	6.96	32.24	106.68	13.70	101.58	294.47	1800.81a	71.91b	4859.67a	5380.83a
LSD		NS	NS	NS	NS	NS	NS	NS	NS	158.6	158.6	1219	356.1

[§]SDM: Substrate disinfestation method: ST: Steaming, H₂O₂: Hydrogen peroxide

[#]Mn: Manganese, Zn: Zinc, Cu: Copper, Fe: Iron, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium, P: Phosphorus

[¥] Means in a column followed by the same letter are not significantly different, p≤0.05, LSD test, NS= Not Significant

4.4.5 Effect of substrate type and *Pleurotus* species (A x C interaction) on the nutritional composition of oyster mushrooms

Averaged across the disinfestation methods, there were no significant differences in contents of CP, Zn, Fe, Na and K of the three *Pleurotus* species grown on the three substrates but significant differences were recorded for ash, CF, Mn, Cu, Ca and Mg (Table 29). The highest concentration of ash was in Pf grown on maize (11.62) while Po × Pf grown on millet and sorghum and Pf grown on sorghum had the lowest ash. Crude fat of Pf (8.1) and HK35 (8.0) were similar but significantly higher than that of HK35 on maize (5.27) Pf on maize (5.4), HK35 on millet (5.07) and Po x Pf on sorghum. Po × Pf grown on millet (43.11) had the highest level of Mn, followed by Po HK35 on millet (39.95) and Po x Pf on maize (39.46) which were similar but higher than Po x Pf (37.1) and Po HK35 (36.02) on sorghum. The lowest concentrations of Mn were in Pf grown on sorghum (23.25), maize (23.1) and millet (22.51), respectively. Highest level of Cu was obtained in Po HK35 (19.06) grown on sorghum substrate, followed by Po HK35 on maize (16.69), Po × Pf on sorghum (16.55) and Po× Pf on maize (15.0) which are similar, followed by Po x Pf on millet (12.76), Po HK35 on millet (12.4) and lowest in Pf grown on sorghum (7.160) and maize (6.05), respectively. Iron content of Pf grown on maize (200.24) was highest but similar to one on sorghum (171.32), both of which were significantly higher than the other substrate by species combinations. The lowest Fe content was in Po HK35 on maize (21.55) which was similar to that of Po x Pf on maize (47.16). *Pleurotus floridanus* grown on sorghum (869.2) and millet (842.52) had the highest ($P<0.05$) level of Ca followed by on maize (735.83) and Po x Pf on maize (2 33.92). The lowest ($P<0.05$) but similar levels were observed in Po HK35 grown on millet (87.75), maize (52.36) and sorghum (20.92). Concentration of Mg was highest in Po HK35 grown on sorghum substrate (2493.67) followed by the significantly lower but similar ones for Po HK35 on maize (2222.83), Po × Pf on sorghum (2043.83) followed by Po HK35 on millet (1848.0), Po x Pf on

millet (996.78) and the lowest concentrations in Pf on maize (815.77), sorghum (738.76) and millet (706.52). Table 29 also shows that different substrates also affected level of P in varieties of mushrooms. It shows that Po HK35 grown in sorghum (7726.83) substrate had the highest level of P. It was followed by Po HK35 on maize (6524.67) which was higher than Po × Pf on maize substrate (5572.7) and Po × Pf grown on sorghum (5374.3), which were similar but higher than other varieties. Po HK35 and Po × Pf grown on millet substrate had similar concentration of P, but were higher than Pf grown on maize (3080.55), sorghum (2497.17) and millet (2410.65).

Table 29: Effect of substrate type and *Pleurotus* spp. on nutritional composition of mushrooms

Substrate	Species*	Nutritional composition of mushrooms [#]											
		Crude Protein (%)	Ash (%)	Crude fat (%)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	P (mg/kg)
Maize	Po × Pf	37.3	8.38b	7.3ab	39.46b	76.4	15.0b	47.16de	233.9c	2043.8b	162.13	4302.2	5572.3c
	Pf	12.4	11.62a	5.4bc	23.51d	106.0	6.05d	200.24a	735.8b	815.8c	187.39	1791.0	3080.4f
	Po HK35	35.8	7.88bc	5.27bc	36.62c	108.5	16.69b	71.28cd	52.4de	2222.8b	82.23	3889.5	6524.7b
Millet	Po × Pf	31.1	7.17c	3.77c	43.11a	53.1	12.76c	63.07cd	93.9de	994.5d	92.03	3414.0	4120.7d
	Pf	11.4	10.13a	7.58ab	22.51d	151.0	7.04d	167.37b	842.5a	706.5e	222.08	1882.0	2410.7f
	Po HK35	29.7	8.88ab	5.07bc	39.95b	70.1	12.4c	21.55e	87.8de	1848.0c	154.36	4488.0	3880.5d
Sorghum	Po × Pf	37.2	6.95c	3.88c	37.1c	89.9	16.55b	85.00c	136.7cd	2127.7b	140.34	4253.33	5374.3c
	Pf	15.8	6.28c	8.1a	23.25d	121.5	7.16d	171.32ab	869.3a	738.86e	187.05	2594.3	2497.2f
	Po HK35	32.7	8.97ac	8.0a	36.02c	102.3	19.06a	64.49cd	20.9fe	2493.7a	14.07	6258.0	7726.8a
LSD		NS	2.737	2.56	1.91	NS	2.20	32.12	104.6	194.3	NS	NS	436.2

*PO HK35: *Pleurotus ostreatus* strain HK35, PF: *Pleurotus floridanus*, PO ×PF: hybrid of *Pleurotus ostreatus* and *Pleurotus floridanus*.

[#]Mn: Manganese, ZN: Zinc, Cu: Copper, Fe: Iron, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium, P: Phosphorus

[¥] Means in a column followed by the same letter are not significantly different, p=0.05, LSD test, NS= Not Significant.

4.4.6 Effects of substrate disinfection method and *Pleurotus* spp. (B x C Interaction) on mushroom nutritional composition mushrooms

Except for Mg and P, there were no significant differences among the *Pleurotus* species grown on substrates disinfested by hydrogen peroxide and steam in the concentrations of the other nutrients assessed (Table 30). *Pleurotus ostreatus* Po HK35 had the highest Mg content (2219.0mg/kg) when grown on substrates disinfested with hydrogen peroxide but was not significantly higher than when grown on steamed substrates (2157.3 mg/kg), and was followed by Po x Pf on hydrogen treated (2009.6 mg/kg), Po x Pf on steamed (1434.4 mg/kg), Pf on steamed and lastly on Po x Pf on hydrogen peroxide treated substrates. Phosphorus content was highest in Po HK35 on peroxide (5810.7 mg/kg) and steamed (6196.3 mg/kg) substrates followed by Po x Pf on steamed, (5077.9 mg/kg), Po x Pf on peroxide treated (4967.0 mg/kg) and lowest on Pf on peroxidized treated (2434.8 mg/kg) substrates.

4.4.7 Effect of substrate type, disinfection method and *Pleurotus* spp. (A x B x C Interaction) on nutritional composition of mushrooms

There were no significant differences in contents of CP, CF, Zn, Cu, Fe, Ca and K in *Pleurotus* spp. grown on the three substrates disinfested by steam and hydrogen peroxide and these ranged from 0.87 to 8.67; 3.27 to 179.74; 29.65 to 198.5; 8.81 to 959.72; and 1279.67 to 6418.67, respectively (Table 31).

As indicated in the table below Pf grown on steamed maize stalk (14.03) and Pf on steamed sorghum (3.87) had the highest and lowest ash content. Manganese was highest in Po x Pf grown steamed millet (47.53 mg/kg) and lowest in Pf on peroxide treated millet. Magnesium was highest in Po HK35 grown on peroxide treated sorghum (2598.0 mg/kg) and lowest in Pf on peroxide treated sorghum (699.77 mg/kg) while Na was highest in Po x Pf grown on peroxide treated sorghum and lowest in Po HK35 grown on peroxide sorghum. Phosphorus

was highest in Po HK35 grown on hydrogen peroxide treated sorghum (7997.67 mg/kg) and lowest in Pf on steamed millet (2263.8 mg/kg).

Table30: Effect of substrate disinfestation method and *Pleurotus* spp. on nutritional composition of mushrooms

SDM [§]	Species*	Nutritional composition of mushrooms [#]											
		Crude Protein(%)	Ash(%)	Crude fat(%)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	P (mg/kg)
H ₂ O ₂	PO×PF	35.8	7.63	4.3	38.7	79.37	15.02	63.28	132.12	2009.56b	149.07	4269.89	4967b
	PF	14.1	9.41	7.2	22.81	132.55	6.82	192.54	852.69	700.6d	200.14	1709.3	2434.76d
	HK35	32.2	7.39	5.7	36.68	108.64	16.72	46.93	61.85	2219.0a	81.72	5073	6196.33a
ST	PO×PF	34.5	7.38	5.7	41.07	66.84	14.52	66.87	177.59	1434.43c	113.94	3710	5077.89b
	PF	11.7	9.28	6.8	23.37	119.78	6.68	166.7	779.1	804.74d	197.54	2468.89	2890.69c
	HK35	33.3	9.77	6.5	38.38	81.98	15.39	57.95	45.50	2157.3ab	85.39	4684	5891.67a
LSD		NS	NS	NS	NS	NS	NS	NS	NS	158.6	NS	NS	356.1

[§]ST: Steaming, H₂O₂: Hydrogen peroxide

*PO HK35: *Pleurotus ostreatus* strain HK35, PF: *Pleurotus floridanus*, PO ×PF: hybrid of *Pleurotus ostreatus* and *Pleurotus floridanus*.

[#]Mn: Manganese, Zn: Zinc, Cu: Copper, Fe: Iron, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium, P: Phosphorus

[¥] Means in a column followed by the same letter are not significantly different, p=0.05, LSD test, NS= Not Significant.

Table 31: Effect of substrate type, disinfestation method and *Pleurotus* spp. on nutritional composition of mushrooms

Substrate	SDM [§]	Species*	Nutritional composition of mushrooms [#]											
			Crude protein(%)	Ash(%)	Crude fat(%)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	K (mg/kg)	P (mg/kg)
Maize	H ₂ O ₂	Po × Pf	38.5	7.67bcd	5.93	38.8bcde	84.96	14.67	64.03	193.3	2116.7bcde	162.13bcd	4057.67	5407.67d
		Pf	13.2	9.2bc	6.557	23.15h	113.35	6.84	212.17	751.97	791.63g	174.12bcd	1721.33	2432.93g
		Po HK35	34.8	6.7bcd	4.63	36.15egf	116.47	17.16	51.06	87.48	2167.67bcd	52.09ef	4000	6207.33c
	ST	Po × Pf	36.1	9.1bc	8.67	40.09bc	67.91	15.33	30.29	274.53	1971def	162.13bcd	4546.67	5737cd
		Pf	11.5	14.03a	4.23	23.87h	98.67	5.26	188.32	719.73	839.9g	200.67ab	1860.67	3727.77f
		Po HK35	36.8	9.07bc	5.9	37.09defg	100.61	16.22	91.50	17.24	2278bc	112.37de	3779	6842b
Millet	H ₂ O ₂	Po × Pf	31.4	8.87bc	3.27	38.69bcde	70.82	13.85	47.54	8.81	1892ef	21.40f	4818	4750.67e
		Pf	15.0	10.33ab	7.3	22.32h	179.74	6.56	166.95	846.38	610.47g	230.63ab	2127	2557.5g
		Po HK35	30.4	7.73bcd	4.8	39.46bcde	79.19	12.14	29.65	87.60	1891.33ef	185.22bc	4800	4384e
	ST	Po × Pf	30.7	5.47bcd	4.27	47.53a	35.27	11.68	78.59	179.07	96.96h	162.67bcd	2010.67	3490.67f
		Pf	7.8	9.93bc	7.87	22.7h	122.31	7.52	167.78	838.65	802.57g	213.53ab	1637	2263.8g
		Po HK35	29.0	10.03bc	5.33	40.44b	60.98	12.66	13.46	87.88	1804.67f	123.5cde	4175.67	3377f
Sorghum	H ₂ O ₂	Po × Pf	37.6	6.37cd	3.7	38.58bcde	82.34	16.55	78.26	194.23	2020.0cdef	263.67a	3934	4742.67e
		Pf	15.7	8.7bc	7.8	22.97h	104.56	7.04	198.5	959.72	699.77	195.67abc	1279.67	2313.83g
		Po HK35	31.4	7.73bcd	7.6	34.42g	130.27	20.85	60.1	10.46	2598.0a	7.84f	6418.67	7997.67a
	ST	Po × Pf	36.8	7.53bcd	4.07	35.58fg	97.35	16.55	91.74	79.17	2235.33	17.01f	4572.67	6006cd
		Pf	15.8	3.87d	8.4	23.53h	138.35	7.27	144.13	778.87	777.75g	178.43bcd	3909	2680.50g
		Po HK35	34.1	10.2abc	8.4	37.62cdef	84.35	17.28	68.88	31.37	2389.33ab	20.30f	6097.33	7456ab
LSD			NS	3.87	NS	2.705	NS	NS	NS	NS	274.7	72.77	NS	616.8

[§]ST: Steaming, H₂O₂: Hydrogen peroxide

*Po HK35: *Pleurotus ostreatus* strain HK35, PF: *P. floridanus*, Po ×Pf: hybrid of *P. ostreatus* and *P. floridanus*.

#Mn: Manganese, Zn: Zinc, Cu: Copper, Fe: Iron, Ca: Calcium, Mg: Magnesium, Na: Sodium, K: Potassium, P: Phosphorus

¥ Means in a column followed by the same letter are not significantly different ($p \leq 0.05$), LSD test, NS= Not Significant

CHAPTER FIVE: DISCUSSION

5.1 Assessment of current uses of cereal crop residues by small scale farmers in the Southern part of Botswana

5.1.1 Biographical data

Biographically, the majority of surveyed farmers in southern Botswana were male (58.7%), married (65.1%), aged over 65 years (42.9%) and with only primary school education (57.1%) (Fig 8 and 9 and Tables 6 and 7). The gender proportions of 58.7% male and 41.3% females is in agreement with Doss (2018) who reported 40% for women and has argued that the claim in literature of 60 to 80% for women in agricultural production in Africa and the world is not evidence-based. As a norm in a traditional setup males were household heads and breadwinners. However, with urbanisation, socio-economic changes such as breakdowns in family relationships female-headed households even in patriarchal societies are increasing rapidly in the world, more especially in developing countries (Dungumaro, 2008). In general, there is an increase in female headed households in developing countries mainly due to poverty (Habib, 2010).

The age and educational status of the farmers were in agreement with FAO (2014) who reported that the majority of small-scale farmers in Africa are elderly and not highly educated, thus less likely to adopt new technologies in agriculture. Even though the youth involvement in agriculture is low they are the future of food security. The compromised youth involvement in agriculture is due to challenges such as lack of arable land which makes it hard to start a farm, lack of access to credit and other productive resources for agriculture (FAO, 2014). Most farmers who were interviewed owned the land, an indication that most land is owned by old people and land ownership by the youth is minimal or non-existent. Hence lack of land is a challenge which limits youth involvement in agriculture. Land issues are of main concern in

most parts of the world. Embarking on mushroom production may be of great relevance as production does not need large sector of land (FAO, 2014; Mutamba and Ajayi, 2018). Mushroom cultivation requires no extra land and mushrooms are grown in mushroom houses using locally available cereal crop residues as substrates. Though cultivation of mushrooms has great potential it has received less attention from a lot of people especially in the developing countries (Easin *et al.*, 2017).

According to the survey the majority of the interviewees were full-time farmers and they all indicated that they realised very low yields in the 2016/2017 cropping season due to severe drought. Most of the farmers adopted row planting as opposed to the traditional broadcasting because they were beneficiaries of subsidised inputs through the Integrated Support Programme for Arable Agricultural Development (ISPAAD). In Africa average cereal grain yields are 600kg/ha, 650 kg/ha and 1100kg/ha in traditional varieties, improved varieties and improved variety together with management, respectively. However, with low productivity farmers need higher prices to break even when they fail to meet volume and quality thresholds (Mutamba and Ajayi, 2018).

5.1.2 Crop residue yields, current utilisation and potential use in mushroom production

In Botswana the average grain yields for maize, millet and sorghum are 255kg/ha, 192kg/ha and 192kg/ha kg/ha, bought at of P1.45, P1.82 and P1.40 per Kg by the Botswana Marketing Board, resulting in farmer gross incomes of P369.75, P349.44 and P268.80 per hectare, respectively. From the survey, estimated crop residue yields were 1213.3, 1206.7 and 4530kg/ha for maize, sorghum and millet, respectively. These residues can increase the farm income if they are sold or used for oyster mushroom cultivation. Currently, the crop residues are often harvested and fed to animals but these are not weighed while some farmers leave the

residues in the field to be grazed by animals. This agrees with findings of Madibela and Lekgari (2005) who reported that crop residues are less valued and normally fed to livestock in situ or as part of feed formulation or ploughed under or burnt. Farmers who reported selling crop residues, used volume rather weight. One farmer in Malotwane sold residues packed in a 50Kg fertiliser bags at P25/bag and when weighed, the weight ranged from 3.5 to 4.5kg per bag, translating to P5.56 to 7.14/kg.

Most farmers who used crop residues as feed reported that the cereal residues were generally nutritionally poor and required supplementation (Table 11). Maize was rated as being poorer than sorghum while most farmers could not rate the quality of millet stalks as feed. Farmers were ready to adopt mushroom production in order to improve the nutritional quality of cereal residues when mushroom spent substrate was fed to animals and to improve farm income and their nutrition through sale and consumption of oyster mushrooms. In general farmers' knowledge on mushroom cultivation was rather limited but they were willing to adopt the new technology if they were trained. Most farmers were not familiar with cultivated mushrooms but most knew the wild mushrooms that grow ant hills (*Termitomyces* spp.). In general, most farmers had mycophobia since they indicated they did not eat mushrooms for fear of being poisoned. This mycophobia in Botswana could be attributed to limited knowledge of identification of edible and poisonous mushrooms and to the fact that the most common mushroom after heavy is the poisonous *Chlorophyllum molybdites* (Khonga Personal communication, 2018). However, farmers showed high interest in acquiring knowledge and skills in order to incorporate oyster mushroom production in their cereal cropping system for increased income as well as improved human and livestock nutrition.

5.2 Effect of cereal residue type, substrate disinfestation method and oyster mushroom species on mushroom yields and the potential income from mushroom sale.

5.2.1 Main effects of substrate, disinfestation method and *Pleurotus* species on mushroom yield

Among the three locally available cereal crop residues, millet stalk was the best (BE:49.1%) followed by maize (BE:29.4%) and sorghum (BE:19%) as substrates for oyster mushroom production (Table 16). However, BEs on maize and sorghum were far less than the range of 47% to 134.5% stated by Mandeel *et al.*, (2005) for *Pleurotus* spp. *Pleurotus ostreatus* cultivated in wheat stalk, millet stalk, cotton stalk and soya bean stalk yielded the following BEs: 17.9%, 22.7%, 14.3% and 31.5% (Dundar *et al.*, 2009). This showed that millet was the second best and was suitable substrate for oyster mushroom production. Millet has a carbon to nitrogen ratio of 77.38 (Dundar *et al.*, 2009). Furthermore, Delpech and Olivier (1991) stated that high yields can be achieved from substrates which contain 0.7 to 0.9 % nitrogen hence millet is within the range. Khonga (2005) found maize stalks to have a lower biological efficiency (71.7%) than millet (103.7%) which are much higher than those found in this study. Furthermore, Malele (2018) recorded a biological efficiency of 23.4% which was higher than 19.0% recorded for sorghum stalks in this study. The poor yields for sorghum stalks could have been due to the chemical components of sorghum which may have negatively affected the formation of mushrooms once the substrate was colonised. The difference in yield could also be due to differences in moisture holding capacity of different substrates, high susceptibility to weed fungi and improper aeration (Tupatker and Jadhao, 2006). Among the three substrates, maize had the highest while millet had the lowest water holding capacity due to the presence of pith tissues which hold a lot water in maize. The yield of the first mushroom flush was highest and the yields declined in subsequent flushes on in all the three substrates. This is caused by depletion of substrate nutrients by the fungus as it produces fruiting bodies as reported other studies (Malele 2018, Khonga 2003).

Substrates disinfested with hydrogen peroxide gave higher biological efficiency (39.6%) than those steamed (25.4%) (Table 19). The results are not in agreement with Malele (2018) who found steaming to be a better disinfestation method with BE of 32.1% than hydrogen peroxide with BE 26.1%. The variation could be due to poor preparation after steaming. However, using hydrogen peroxide gave a high BE than that of Malele (2018) hence this clearly indicates high potential BE when using hydrogen peroxide. The majority of the bags with steamed substrates had 55% *Pleurotus* mycelium colonization compared to 88.7% in hydrogen disinfested substrate. The common contaminant was *Trichoderma sp.*

Pleurotus ostreatus Po HK35 (36.6% BE) and Po x Pf (35.6% BE) had similar yields but higher than *P. floridanus* (25.2%). In general the yields above were lower than those reported in the literature (Khonga, 2001, Jongman *et al.*, 2010) because no supplements were added and the poor temperature control in the mushroom house resulting in temperatures higher than those required for pinning since temperatures outside were naturally increasing during the experiment. Philippoussis (2009) accentuates that fructification of *Pleurotus ostreatus* is triggered by lowering the air temperature from about 28°C to 12 to 15°C.

5.2.2 Effect of substrate, disinfestation method and *Pleurotus* spp. on mushroom yield

The best five combinations were steamed millet inoculated with Po HK35 (69.4% BE), millet treated with hydrogen peroxide inoculated with Po × Pf (58.7% BE), millet treated with hydrogen peroxide and inoculated with Po HK35 (58.1% BE), steamed millet inoculated with Po × Pf (49.3% BE) and lastly steamed maize inoculated with Po × Pf (40.3% BE) (Table 23). This shows that millet is the best substrate and Po × Pf can thrive better under high temperature. Furthermore, other researchers have conducted experiments in which other species performed better though differences in growing condition and substrates are not emphasized. However,

when *P. floridanus* was cultivated in soybean straw a BE of 87.56% was realised (Ahmed *et al.*, 2009). This study was conducted during the hottest months in Botswana (September to December) with maximum temperatures of over 38°C which were unfavourable for fructification of the *Pleurotus* species resulting in relatively low yields.

5.3 Potential additional income using best *Pleurotus* sp., substrate and disinfestation method.

Growing oyster mushrooms on millet residues has the potential of bringing additional farm income per hectare ranging from P81,404 to P182,876 depending on level of expertise in mushroom cultivation by farmers. Using the estimated millet residue yield of 4530Kg/ha for surveyed farmers the potential additional incomes per hectare is P182876 (4530Kg x P40.37/Kg) using optimistic BE, P131,551 (4530Kg x P29.04/Kg) and P81404 (4530Kg x P17.97/Kg) using pessimistic BE for Po HK35 is grown on steamed millet. These figures represent profits ranging from 18 to 166%. Lower potential incomes were realised when millet was treated with hydrogen peroxide and spawned with Po x Pf Malele (2018) reported potential profits 212% and 391% when *Pleurotus* spp. were grown on steamed and hydrogen peroxide treated maize cobs, respectively. The results show that incorporating oyster mushrooms in the cereal cropping system in Botswana can improved household income and food security. In the Kilimanjaro highlands of Tanzania where bananas and coffee are produced, introduction of oyster mushroom cultivation resulted in surplus income among farmers who were adversely affected by unreliable rainfall and this later led to a blooming business for small scale farmers (Marshall and Nair, 2009). Easin *et al.*, (2017) reported that mushroom production is a secondary source of income for young Bangladesh entrepreneurs with monthly profits against investments ranging from US\$30-256.

5.4 The effect of substrate types, substrate disinfestation method and *Pleurotus* spp. on mushroom nutritional composition and their potential in improving the nutrition of small-scale farmers.

Nutritional analysis of the oyster mushrooms produced in this study showed good levels of crude protein (7.8-38.5%), ash (3.87 -14.03%), crude fat (3.7-8.67%), Mn (22.7-47.5mg/kg), Zn (35.5-178.7mg/kg), Cu (5.26-17.28mg/kg), Fe (13.46-188.32mg/kg), Ca (8.8-959.7mg/kg), Mg (97.0-2598.0 mg/kg), Na (7.8-263.7mg/kg), K (1279.7-6418.7mg/kg) and P (2263.8-7997.7mg/kg) (Table 33) which can complement the dietary intakes of rural farmers in Botswana. The nutritional composition of the mushrooms varied between and within species depending on the substrate and the substrate disinfestation method and were generally lower than those reported in the literature (Table 4). The crude protein range of 0.78-3.85% was much lower than 14.06% for *P. ostreatus* grown on millet stalks reported by Dundar *et al.*, (2009).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Crop residues of millet, sorghum and maize commonly grown in southern Botswana which are currently being fed to livestock or left in the field can be used to grow oyster mushrooms in order to supplement farmers' income and diets. Among the three substrates, millet had the highest field biomass of 4530kg/ha and was the best substrate when steamed and spawned with Po HK35 resulting in additional farm income of between P81,404 and P182,876/ha compared to the income from grain sales of between P300 - 600/ha. Maize and sorghum residues and the other two *Pleurotus* spp. can also be grown after disinfestation with either steam or hydrogen peroxide.

The nutritional profile of the oyster mushrooms in terms of crude protein, crude fat and mineral composition is relatively high and can complement the diet of farmers in addition to improving their incomes.

It is concluded from the study that incorporation of oyster mushrooms in the cereal farming system has great potential of improving farmers' incomes and household nutritional status. Mushroom cultivation also has the potential of creating additional employment for rural youth as they are engaged in the harvesting and processing of residues and cultivation and selling of mushrooms.

The mushroom production experiment was limited by lack of strict temperature control in the mushroom house and the fact that no supplements were added to the substrates resulting in lower mushroom yields than expected. More work on suitable locally available supplements such as legumes grains and residues for improving mushroom yield should be carried out in the future. There is also need to assess different concentrations of hydrogen peroxide in order to determine the most effective concentration for reducing contamination.

6.2 Recommendations and suggestions for further study

From this study, the following recommendations are made:

1. Farmers should be advised to harvest and weigh their cereal crop residues instead of leaving them in the field to be grazed upon by animals. This will enable fair trading when residues are sold per kg instead of the current system of selling residues by volume. If residues are used for mushroom production, residue weight will be used in estimating biological efficiency.
2. Incorporation of oyster mushroom production in the cereal production system has potential of increasing farm income since mushrooms are a high value crop than cereals with projected additional of up to P180, 000/ha if the farmers are supported with setting up mushroom houses, readily available mushroom spawn and training. The spent mushroom substrate can be used as livestock or incorporated back into the soil to improve organic matter content.
3. Farmers interested in growing oyster mushrooms should be encouraged to plant some millet as it is a better substrate for oyster mushrooms than maize and sorghum. For maize, the maize cobs are a better substrate than the stalks
4. Farmers should be trained on using hydrogen peroxide as a substrate disinfectant since it cheaper and more environmental friendly than use of firewood to steam the substrates. However, steam can still be used where firewood is readily available.

The main limitations of this study were:

1. The poor state of the mushroom houses where it was difficult to maintain ideal temperature and humidity levels since the study was carried out from September to December when outside temperatures were increasing and relative humidity decreasing.
2. The use of one concentration of hydrogen peroxide.
3. Lack of supplementation of bulk substrates. These contributed to the low mushroom yields obtained in this study.

For future research, the effects of different concentrations of hydrogen peroxide and locally available supplements for the substrates on mushroom yield should be conducted under well controlled conditions.

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5. College Diploma [] 6. College Degree [] 7. Masters []
 8. Doctorate [] 9. Other (specify)
-

SECTION B

FARM / FARMING STATUS

10. What is the size of the field / farm (hectares)? _____ hectares.
11. Describe possession status of the field / farm. 1. Owned [] 2. Rented [] 3. Leased []
12. How would you describe your farming practice? 1. Full Time [] 2. Part Time []
13. If you do farming on part time basis, what economic activity are you involved in on full time basis?
1. Not economically active [] 2. Non-agriculture own business (state type _____) []
 3. Paid employment [] 4. Unpaid family employment [] 5. Other (Specify): _____
14. How long have you practiced farming (years)?
 Less than 1 year [] 1 [] 2 [] 3 [] 4 [] 5 []
 More than 5 years []
15. What is the main economic activity you were doing before you started farming?
 1. Not economically active [] 2. Non-agriculture own business (state type _____) []
 3. Paid employment [] 4. Unpaid family employment [] 5. Other (Specify): _____

SECTION C

FARMING SYSTEM

16. Which of the following planting methods do you practice? (Please indicate ONLY ONE choice).
1. Row planting only [] 2. Broadcasting only [] 3. Both row planting and broadcasting []

If your answer is [1], skip to question 18.

If your answer is [3], proceed with question 17.

17. Specify how much area of land was under each planting method:
- Area row planted _____ hectares. Area Broadcasted _____ hectares.

18. What cereal crops do you grow?

1. Maize [] 2. Sorghum [] 3. Millet []

19. What grain legumes do you grow?

1. Cowpeas [] 2. Groundnuts [] 3. Bambara groundnuts []

SECTION D

GRAIN YIELD AND ITS DISPOSAL

20. Please provide a few details below on production, consumption and marketing of grain crops you grow.

Crop	Area Planted (Ha)	Yield/Ha (No. of 50kg bags per ha)	Proportion of grain consumed at home (No. of 50kg bags)	Proportion of grain sold (No. of 50kg bags)	Unit Price (Average price per 50kg bag)
Maize					
Sorghum					
Millet					
Cowpeas					
Groundnuts					
Bambara groundnuts					

SECTION E

CEREAL CROP RESIDUES AND THEIR DISPOSAL

21. What happens to cereal crop residues (stalks / stover) after harvest of grain?

1. Harvested and stored for feeding animals [] 2. Harvested and sold as animal feed []
3. Left in field to be grazed by animals [] 4. Ploughed back into the soil as manure []
5. Left on surface as part of conservation tillage []
6. Just cleared and burnt in field []

22. If your answer to Question 21 is [1], please specify the species and class of animals you feed with the cereal crop residues (stalks / stover):

Species of animals	Class of animals

23. If harvested, do you weigh the cereal crop residues? 1. Yes [] 2. No []

24. If residues are weighed, please provide a few details below on production, use and marketing of the cereal crop residues from your field.

Crop	Crop residue /Ha (No. of 50kg bags per hectare)	Proportion of crop residue used at home (No. of 50kg bags)	Proportion of crop residue sold (No. of 50kg bags)	Unit Price (Average price per 50kg bag of crop residue)
Maize				
Sorghum				
Millet				

25. How would you rate the nutritional value (quality) of the cereal crop residues from your field? Please place only one tick (✓) under each “crop residue column” to indicate your perceived quality score.

Perceived Quality Score	Maize residue	Sorghum residue	Millet residue
1. Very low quality			
2. Low quality			
3. I don't know			
4. High quality			
5. Very high quality			

26. If your answer to Question 25 is [1] or [2], please specify what you do (or you may do) to improve the quality of cereal crop residues if there are of low quality.

27. How likely would it be for you to participate in a study that aims at improving the nutritional value of cereal crop residues by growing mushrooms in your field?

1. Very Unlikely [] 2. Unlikely [] 3. Undecided [] 4. Likely []
5. Very Likely []

SECTION F

LEGUME CROP RESIDUES AND THEIR DISPOSAL

28. What happens to legume crop residues / stalks after harvest of grain?

1. Harvested and stored for feeding animals [] 2. Harvested and sold as animal feed []
3. Left in field to be grazed by animals [] 4. Ploughed back into the soil as manure []
5. Left on surface as part of conservation tillage []
6. Just cleared and burnt in field []

29. If your answer to Question 28 is [1], please specify the species and class of animals you feed with the legume crop residues / stalks:

Species of animals	Class of animals

30. If harvested, do you weigh the legume crop residues? 1. Yes [] 2. No []

31. If residues are weighed, please provide a few details below on production, use and marketing of the legume crop residues from your field.

Crop	Crop residue /Ha (No. of 50kg bags per hectare)	Proportion of crop residue used at home (No. of 50kg bags)	Proportion of crop residue sold (No. of 50kg bags)	Unit Price (Average price per 50kg bag of crop residue)
Cowpeas				
Groundnuts				
Bambara groundnuts				

32. How would you rate the nutritional value (quality) of the legume crop residues from your field?

Please place only one tick (√) under each “crop residue column” to indicate your perceived quality score.

Perceived Quality Score	Cowpeas residue	Groundnuts residue	Bambara groundnuts residue
1. Very low quality			
2. Low quality			
3. I don't know			
4. High quality			
5. Very high quality			

33. If your answer to Question 32 is [1] or [2], please specify what you do (or you may do) to improve the quality of legume crop residues if there are of low quality.

34. How likely would it be for you to participate in a study that aims at improving the nutritional value of legume crop residues by growing mushrooms in your field?

1. Very Unlikely [] 2. Unlikely [] 3. Undecided [] 4. Likely []

5. Very Likely []

SECTION G

FARMERS' PERCEPTIONS OF MUSHROOMS AND ATTITUDE TOWARDS MUSHROOM PRODUCTION

35. Do you know mushrooms? 1. Yes [] 2. No []

If your answer to Question 35 is NO, skip to question 41. Otherwise proceed to question 36.

36. If your answer to Question 35 is YES, please indicate which mushrooms you know:

I know mushrooms that grow naturally in the bush / wild 1. Yes [] 2. No []

I know mushrooms that are grown/cultivated in the fields 1. Yes [] 2. No []

37. Do you eat mushrooms? 1. Yes [] 2. No []

If your answer to Question 37 is YES, skip to question 39. Otherwise proceed to question 38.

38. If your answer to Question 37 is NO, please state reason why you do not eat mushrooms

39. If your answer to Question 37 is YES, which type of mushrooms do you eat?

40. If your answer to Question 37 is YES, where do you find the mushrooms that you eat?

41. Would you be interested in growing mushrooms for the following reasons:

To improve your family's nutritional status 1. Yes [] 2. No []

To improve your livestock feed's nutritional status 1. Yes [] 2. No []

To improve soil fertility in your field 1. Yes [] 2. No []

To generate extra income for your household 1. Yes [] 2. No []

**END OF QUESTIONNAIRE. THANK YOU FOR YOUR CONTRIBUTION AND
WILLINGNESS TO PARTICIPATE**