

**THE EFFECT OF PASTEURIZATION AND STERILIZATION OF  
SUBSTRATES ON THE YIELD OF *PLEUROTUS CORNUCOPIAE*  
AND *PLEUROTUS SAJOR-CAJU* IN ZAMBIA**

**BY**

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## DECLARATION

I EDGAR REED hereby declare that this dissertation represents my own work and it has not previously been submitted for a degree at this or another university.

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## APPROVAL

This dissertation of **EDGAR REED** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy (Crop Science) by the University of Zambia.

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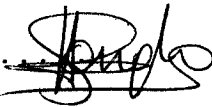
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## **DEDICATION**

This dissertation is dedicated to my wife, mother, father, brothers, sisters and children.

## ABSTRACT

A study was conducted to determine the effect of substrate pasteurization and sterilization on the yield of *Pleurotus cornucopiae* and *Pleurotus sajor-caju*, and on the suitability of sorghum, wheat and maize grains, and wooden sticks as mycelium carriers in spawn production. The *Pleurotus* species were inoculated on sterilized carriers and incubated at 25 °C for fourteen days.

Results revealed that wheat and sorghum grains were suitable for spawn production while maize grains and wooden sticks permitted only partial colonization. Because of the easy availability and low price of sorghum, spawn multiplication was done on sorghum grains.

The effect of different substrate materials and methods of substrate treatment on yield of *Pleurotus* species were studied by employing the split-split plot design.

Spawn raised on sorghum grain was seeded on maize stalks, wheat straw and mixed wood shavings. Prior to seeding, each substrate type was treated by pasteurization in water heated to 65 °C and by sterilization in an autoclave at 120 °C and 15 lb/in<sup>2</sup> pressure, both for one hour.

The results on fruiting body production of *Pleurotus* species showed that there were interactions among the factors studied.

There were no significant differences in the yield of the two *Pleurotus* species ( $p < 0.05$ ) on pasteurized wheat straw while there were differences in the yield between the two species on pasteurized maize stalks with *P.cornucopiae* yielding more than *P.sajor-caju*.

*P.sajor-caju* performed significantly better ( $p < 0.05$ ) on pasteurized wheat straw than on pasteurized maize stalks while the yield *P.cornucopiae* on the two pasteurized substrate was not significantly different. However, the opposite was true on sterilized substrate, the yield of *P.cornucopiae* was higher on wheat straw while that of *P.sajor-caju* was not significantly different.

A comparison of mushroom yields on pasteurized and sterilized wheat straw and maize stalks showed significant differences. The mushroom yield was higher on pasteurized than sterilized substrate. However yields were generally very poor on the mixed wood shavings and the results were not significantly different.

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## ABBREVIATIONS

|               |                              |
|---------------|------------------------------|
| °C            | degree centigrade            |
| cm            | centimeter                   |
| LSD           | least significant difference |
| ml            | milliliter                   |
| %             | percent                      |
| g             | gram                         |
| gms           | grammes                      |
| Kg            | kilogrammes                  |
| X             | by                           |
| Hrs           | hours                        |
| Etc           | et cetera                    |
| <i>P.corn</i> | <i>Pleurotus cornucopiae</i> |
| <i>P.s-c</i>  | <i>Pleurotus sajor-caju</i>  |

## APPENDIX

### 1. Temperature and Relative Humidity Data.

## 1.0 INTRODUCTION

From prehistoric times mushrooms gathered in the wild have been an important part of human diet (Addey, 1992). Mankind has savoured the desirable flavours, acknowledged the nutritional value, and recognised the medicinal and tonic properties of mushrooms (Chang and Bushwell, 1993). Mushrooms were gathered by the early civilizations of Egypt and Rome as a special delicacy and were perceived by the latter as 'The food of the gods' (Chang and Bushwell, 1993). Some people have regarded mushrooms as 'vegetable beef steak' (Crisan and Sands, 1978). In mushrooms there is a likeliness to meat, at least by those who prefer meat.

In Zambia, like in most other countries, many kinds of wild mushrooms are gathered and consumed in large quantities during each rainy season (Pearce, 1984). Thus, the consumption of fresh mushrooms in Zambia is restricted only to the rainy season although certain species are also dried and stored for later use (Ram, 1997).

It is reported that the first attempt to cultivate mushrooms took place around 600 A.D. in China (Poppe, 1978). Later the white button mushroom-*Agaricus bisporus* (Lange) Singer was cultivated in the vicinity of Paris in about 1850 (Atkins, 1972; Oei, 1991; Addey, 1992). Today, the

button mushroom is widely cultivated all over the world (Oei, 1991) and is marketed in most stores and supermarkets.

Experiments were conducted to develop a suitable technique for the cultivation of *Agaricus bisporus* (Lange) Singer in Lusaka in 1975-76 period (Alian and Musenge, 1978). However, despite good mycelial growth, the fruiting body development failed due to the prevailing high temperatures of 20-30 °C (Alian and Musenge, 1978). By 1979 the commercial production of *Agaricus bisporus* (Lange) Singer had started at a farm 23 Km east of Lusaka with an initial production of 513 Kg per year. Two years later production rose sharply to 19 tones per year.

Recent studies have demonstrated that *Pleurotus* species could also be grown successfully. Unlike *Agaricus*, *Pleurotus* forms fruiting bodies readily under the tropical conditions. It is also credited with the fact that it requires neither manipulation of the environment nor complicated composting procedures (Khonga, 1995). *Pleurotus* species are by far the easiest and least expensive to grow. They grow on a variety of forest and agricultural wastes. In addition, their nutrition and medicinal properties are excellent and have been reviewed in some detail by Bano and Rajarathnam (1988) and Breen (1990). *Pleurotus* specie are increasing in popularity and are cultivated worldwide. Their world production has

increased from 169,000 metric tones in 1987 to 909,000 by 1990 (Stamets, 1993).

It has been estimated that a hectare of land will produce 80 Kg beef or 660 Kg fish- but when the same land is used for mushroom cultivation the yield could be as high as 80,000Kg (Kendrick, 1992).

The pre-requisite for mushroom cultivation is the preparation of spawn, which is the 'seed' of the mushroom. Spawn is commonly produced on wheat grains (Oei, 1991). However the scarcity and high price of wheat in Zambia necessitates work on alternative materials.

Mushrooms offer an excellent alternative source of protein for the developing countries. They are cheap and easy to cultivate and thus provide a suitable option to combat malnutrition and offer a good source of income. Their protein value and income generating properties have been recognized by many workers ( Crisan and Sands, 1978; Bano and Rajarathnam, 1982; Griensven, 1987; Sohi, 1982; Oei, 1991; Fiordiponti, 1995).

Mushrooms are considered a healthy food though their nutritional value has been extensively debated. It is however generally accepted that mushrooms possess an excellent amino acid profile. Their fat content is

very low, often as low as 10%. Their carbohydrate and fiber content is 3-32 % (Birch, 1973), and they are regarded to be a good source of vitamins (Oei, 1991; Breen, 1990; Giacomini, 1957). Several medicinal properties of mushrooms have also been identified (Vogel *et al.*, 1974).

A study was therefore undertaken to investigate the possibility of oyster mushroom production in Zambia. The main objectives of the study were to:

1. Introduce *Pleurotus cornucopiae* (Paul. Ex Fr.) and *Pleurotus sajor-caju* (Fr.) for cultivation in Zambia,
2. Investigate the use of different types of carriers for spawn production,
3. Determine the suitability of pasteurization and sterilization for substrate treatment for *Pleurotus* production and
4. Determine the most suitable local substrate from among wheat straw, maize stalks and wood shavings.

## 2.0 LITERATURE REVIEW

### 2.1 *PLEUROTUS* CLASSIFICATION

*Pleurotus* species are generally called oyster mushroom because the pileus or cap is shell-like or spatulate or tongue shaped and the stipe is lateral. According to Alexopoulos, Mims and Blackwell (1996) the genus *Pleurotus* has the following taxonomy:

Kingdom: Myceteae

Division: Mastigomycota

Subdivision: Basidiomycotina

Class: Basidiomycetes

Subclass: Halobasidiomycetidae

Order: Agaricales

Family: Tricholomataceae

Genus: *Pleurous*

## 2.2 *PLEUROTUS* SPECIES

*Pleurotus* species are widely cultivated edible fungi and are popularly known as 'oyster mushroom'. This type of mushroom is cosmopolitan and occurs on dead wood as a saprophyte and are primary decomposers (Sun and Yu, 1981). It has been described as celestial flower mushroom in ancient China and regularly used in palace recipes during Tang dynasty (Sun and Yu, 1989). Now a days oyster mushrooms are widely cultivated in many countries in subtropical and temperate regions (Leong, 1982).

Several species of *Pleurotus* have been investigated by Bano and Srivastava (1962), Herzig *et al.* (1968), Kalberer (1974), Stanek and Rysava (1971), Eger *et al.* (1974), Jandaik and Kapoor (1974) and Rangaswami *et al.* (1975) as shown in Table 1.

**Table 1: Cultivated species of *Pleurotus***

| <b><i>PLEUROTUS</i> SPECIES</b> | <b>COUNTRY OF CULTIVATION</b>                         |
|---------------------------------|-------------------------------------------------------|
| <i>P.calyptratus</i>            | China (Change and Hayes,1978)                         |
| <i>P.citrinopileatus</i>        | Malaysia (Change and Hayes,1978; Oei, 1991)           |
| <i>P.columbinus</i>             | France (Oei, 1991)                                    |
| <i>P.cornucopiae</i>            | Belgium, Germany (Oei, 1991)                          |
| <i>P.cystidiosus</i>            | Malaysia, Taiwan (Oei, 1991)                          |
| <i>P.drynus</i>                 | Norway (Quimio <i>et al.</i> , 1990)                  |
| <i>P.eryngii</i>                | India (Change and Hayes,1978)                         |
| <i>P.flabellatus</i>            | India, Indonesia, Malasia (Oei, 1991)                 |
| <i>P.floridanus</i>             | America, Belgium, Spain (Quimio <i>et al.</i> , 1990) |
| <i>P.ostreatus</i>              | Belgium, India (Change and Hayes,1978)                |
| <i>P.pulmonarius</i>            | France (Change and Hayes,1978)                        |
| <i>P.sajor-caju</i>             | India (Change and Hayes,1978)                         |
| <i>P.sapidus</i>                | China (Change and Hayes,1978; Oei,1991)               |

The special feature of *Pleurotus* species is that it transforms a wide variety of agricultural waste rich in lignocellulosic biomass into food of very high quality which is rich in flavour and nutritive value (Quimio, 1978; Zadrazil, 1978; Bano and Rajarathnam, 1982).

The nutritive value of *Pleurotus* species has been studied by Sun and Yu (1989). They reported that this mushroom contains 22-28% crude protein, 1-2% fat, 40-43% carbohydrate, 1-3% fibre and some minerals like phosphorus, calcium and several amino acids. Crisan and Sands (1978) have described it as fragrant and of delicious nature. The oyster mushrooms are among some of the most commonly encountered agarics and are choice edible species (Alexopoulos, Mims and Blackwell, 1996). The biological characteristics of the two species of *Pleurotus* used in this study are as follows:

### **2.2.1 *PLEUROTUS CORNUCOPIAE***

In nature *Pleurotus cornucopiae* (Paul. Ex Fr.) grows on wood of oak (*Quercus*), beech (*Fagus*), and elm (*Ulmus*) (Zadrazil, 1978). It is an excellent species suitable for cultivation in the tropical and subtropical environment because it fruits at temperatures between 22-27 °C for optimum mycelial growth and fruits between 15-25 °C (oei, 1991). The cap is 5-12 cm across, convex at first, later depressed to funnel shaped and becoming wavy or cracked at the margin. It is at first cream coloured and covered in a whitish bloom and then becomes smooth, ochraceous and ochre-brown. The stem is 20-50 X 10-25 mm long and several stems are fused into a common whitish base becoming tinged with ochraceous caps. It has white flesh which smells pleasantly of flour or ammonia. The gills

are deeply decurrent and white to pale and produce a pale lilac spore print (Phillips, 1981).

### 2.2.2 *PLEUROTUS SAJOR-CAJU*

*Pleurotus sajor-caju* (Fr.) Singer is a saprophyte and grows on the stumps of *Euphorbia royleana*. Jandaik and Kapoor (1974) worked on its suitability to be cultivated in India while Bano (1976) reported that this species could be grown under temperature range of 21-28 °C and relative humidity of 55 to 75%. Oei (1991) reported that the species requires temperatures of between 22-27 °C for optimum growth of mycelium and fruits at temperatures between 18-30 °C. Kandaswamy and Ramasamy (1978) recorded an average yield of 625 g per 3 Kg dry rice straw whereas Chakraborty and Sarkar (1978) obtained about 1 Kg per one kilogram paddy straw. *Pleurotus sajor-caju* must be the most popularly cultivated saprophytic mushroom since much literature has accumulated on its production technology (Jandaik and Kapoor, 1974; Rangaswamy *et al.*, 1975).

## 2.3 SPAWN PRODUCTION FOR *PLEUROTUS* MUSHROOM

The strains of *Pleurotus* fungi can occur in many forms viz. fruiting body, spores, on agar culture and spawn. However, in the production of

mushrooms, spawn, which consist of a suitable medium impregnated with the mycelium of the desired strain is mostly used. Spawn is prepared in a laboratory under sterile conditions on different types of carriers. *Pleurotus* spawn can be raised on manure, grain, or perlite types (Chang and Hayes, 1978).

The use of grain spawn has been extensively utilized in mushroom cultivation throughout the world. The technique was developed by Lemke as cited by Stoller (1962). Kligman (1950) reported that the use of grain was introduced way back by Sinden in 1931. Now a days wheat, rye, sorghum and probably other millets are used as mycelium carrier. It is important to remember that grain spawn must include the use of gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) and lime ( $\text{CaCO}_3$ ) which prevent grains from sticking together.

Although grain spawn is common and quite popular it is not the only type which has been used by mushroom growers. Wood plugs, wooden sticks and sawdust have also been attempted and exploited in commercial mushroom production. This has been explained by Oei (1991). The comparative merits and demerits of diverse spawn types have been described by various investigators but Fritsche, as cited by Stoller (1962) and Oei (1991), have demonstrated the relative advantages of grain spawn. This type of spawn provides better mycelial development around

each grain and ensures thorough distribution of inoculum throughout the mushroom growing medium. While a good source of fungal nutrition, it must be used with care because it is prone to rapid contamination by moulds and bacteria.

## **2.4 CHOICE OF SUBSTRATUM FOR *PLEUROTUS* CULTIVATION**

Although modern agriculture has made significant progress towards abundant food production, a serious consequence of this is that more than half of the total agricultural plant production always goes to waste. This agricultural waste comprises straw, leaves and other materials of various types. While imaginative and resourceful farmers do find varying uses of agricultural waste as fuel, fodder and compost, much of this can be transformed into useful material for the cultivation of edible fungi.

The agricultural wastes are rich in cellulose and lignin and such fungi as species of *Pleurotus* are able to break them down and use carbohydrates for their nutrition. Consequently a large variety of carbohydrate containing materials have been tested and tried in the commercial production of mushrooms (Quimio *et al.*, 1990; Oei, 1991). Thus paddy rice, wheat straw, maize stalks and cobs, cotton waste, water hyacinth, banana leaves, coffee husks are among the many substrates that can be used (Poppe and Hofte, 1995). These and other similar agricultural wastes

can be profitably exploited, depending upon their abundance and availability.

Maize is the staple food of people in Zambia. It is grown during the rainy season (which is November to April). After harvest, maize provides a good source of stalks, cobs and other materials on which *Pleurotus* species will grow successfully.

#### **2.4.1 ORGANIC COMPOSITION AND TREATMENT OF SUBSTRATE**

Organic materials differ in their composition from place to place (Dalzell *et al.*, 1987; Oei, 1991). For instance, wheat straw in Southern Europe is different from that of the North. The weather during the growing period of the crop also affects the composition of the substrate (Oei, 1991).

A high Carbon-Nitrogen (C:N) ratio may result into a shorter spawn run period resulting in poor mycelium development and therefore, low mushroom production. Also, the carbon compounds that remain after incomplete fermentation of a too high C:N material become food for competitors which may in turn attract mites (Oei, 1991).

Sawdust contains high C:N ratio than straw. For example in douglas fir sawdust has a C:N ratio of 620:1 whereas straw has 373:1. Thus sawdust decompose at a much slower rate than straw (Verdonck, 1991).

The use of either a single substrate or a combination of substrates with additives has also received extensive attention of researchers (Chalraborty and Sarkar, 1978).

In order to render the substrate suitable for the cultivation of edible mushrooms, variously differing procedures have been applied. It is important, however to remember that the substrate should not provide a chance to the contaminating microbes and insects to invade it. It must therefore be treated to get rid of the potential, and always present, contaminants (Fletcher *et al.*, 1989).

Stanek and Rysava (1971), as cited by Stoller (1962), fermented paddy straw for 24 hours at 55 °C and thus provided a way for the production of *Pleurotus* species. The substrate can be fermented but it has been shown that there is a loss of organic matter of the substrate (Zadrazil, 1978).

Substrate used for the cultivation of *Pleurotus* species have also been subjected to pasteurization. This can either be applied by using steam or by immersing the substratum into hot water (65 °C) for one to two hours

(Quimio *et al.*,1990; Oei, 1991). Another extensively used procedure for the treatment of substratum has been sterilization under pressure at 120 °C. Quimio *et al.*, (1990) and Oei (1991) have discussed this procedure in detail.

In the early years of *Pleurotus* cultivation the substrate was sterilized. Later it was found that heat treatment at relatively lower temperature was also sufficient (Oei, 1991). The processes of pasteurization and sterilization are explained below:

#### **2.4.1.1 SUBSTRATE PASTEURIZATION**

Pasteurization of the substrate is done by either passing steam through the substrate or by immersing it in hot water. Both pasteurization by steam and pasteurization by immersion in hot water share the benefit of treatment at relatively low temperature in comparison with sterilization. Steam or water is used to keep the substrate at 65 °C for sometime. At this temperature most competitors of *Pleurotus* are killed, but favourable microorganisms will survive (Quimio *et al.*,1990; Oei, 1991).

#### 2.4.1.2 SUBSTRATE STERILIZATION

The use of sterilization of the substrate is not recommended as it kills both beneficial and harmful microorganisms in the substrate. In addition, intense heat breaks down compost nutrients and favours the growth and development of competing microorganisms (FAO, 1983 cited by Quimio *et al.*, 1990; Dalzell *et al.*, 1987). Thus, sterilized substrate is easily contaminated unless spawning is done under very aseptic conditions, such as using agar media for spawn production (Quimio *et al.*, 1990; Oei, 1991).

#### 2.4.2 MOISTURE CONTENT OF SUBSTRATE

The substrate must contain reasonable amount of moisture. Too high water content clogs air flow whereas too low water content prevents mycelial growth. Under anaerobic conditions, microbes develop a foul smell and may also develop toxic metabolites during their growth (Oei, 1991).

In Malawi, water is added to the substrate to bring the moisture content to between 65-75% (Khonga, 1995). Oei (1991) suggested that the moisture content of a substrate can be determined by getting a handful of substrate and squeezing it, if it produces only a few water drops it is considered to be a good substrate. However, Atkins (1972) stated that a good substrate must contain sufficient water to moisten the palm when a handful is squeezed, without any trickling through the fingers.

## 2.5 SPAWNING METHODS AND SPAWN RUNNING

Spawning refers to the inoculation of the culture into the substrate whereas spawn run is the stage where the spawn of the mycelium is allowed to grow through the substrate (Quimio *et al.*, 1990; Oei, 1991).

In South-East Asia, the amount of grain spawn used in oyster cultivation on steam pasteurized wheat straw substrate is about 30 to 35 liters per ton of wet substrate (2.2-2.3 %) whereas 250 g is used per 10 Kg (7-10 %) on substrate that has been immersed in hot water (Oei, 1991). In Mexico about 25 Kg spawn is used per ton (2.5 %) for the cultivation of *Pleurotus sajor-caju* (Fr.) Singer on coffee pulp immersed in hot water (Quimio *et al.*, 1990). In the cultivation of oyster mushrooms in Malawi, 40-50 g per Kg of substrate (4-5 %) is used (Khonga, 1995).

Quimio *et al.* (1990) reported that in the cultivation of oyster mushrooms in plastic bags of about 15cm X 30 cm, about 1-2 teaspoonful are added into the substrate bag. Thus one bottle of about 500 ml is sufficient to inoculate 40-50 bags (Quimio *et al.*, (1990).

Spawned bags are kept in the dark room (Oei, 1991). During spawn-run, the mycelium produces enzymes that break down the macromolecules of

the substrate and absorbs simple molecules into the mycelium for further growth and development (Quimio *et al.*, 1990).

Spawn-run period depends on strain, temperature of substrate, nature of substrate and the carbondioxide concentration in the substrate (Oei, 1991). Generally the spawn-run for *Pleurotus* species is 2-4 weeks (Quimio *et al.*, 1990). When the mycelium has fully penetrated the substrate, it appears white due to its growth; however generally they are kept for an additional week before they are opened to allow the mycelium to mature enough (Quimio *et al.*, 1990). In oyster mushroom production in Malawi the substrate was seen to be fully colonized by the mycelium after about 14 days (Khonga, 1995).

## **2.6 DEVELOPMENT OF FRUITING BODY**

Fruiting bodies as described here, are the edible mass of the mushroom for which these fungi are cultivated. The genetic factors and the composition of substratum can influence the fruiting body development in *Pleurotus* species (Zadrazil, 1978). However, the key factors for the fruiting body development and mushroom yield depend on environmental factors (Oei, 1991). Zadrazil and Schneidereit, as cited by Zadrazil (1978) demonstrated the effect of light and insufficient ventilation on the development of fruiting bodies of *Pleurotus ostreatus*. It has been shown

that greater exchange of air reduces the amount of fruiting bodies per surface unit. Zadrazil (1978) reported that light is very important in the development of mushroom primordia. According to Zadrazil (1978) the duration and *intensetity* of light will increase the number of mushroom primordia in *Pleurotus florida*. The effect of light on *Pleurous* development has also been reported by Poppe (Zadrazil, 1978). Zadrazil (1978) and Eger *et al.*, (1974) have estimated light intensity of 10,000 lux per hour to be adequate for basidiocarp development in *Pleurotus ostreatus*. Oei (1991) has also proved that *Pleurotus* species need light for proper cap development. The effect of temperature of fruit development is variable and *Pleurotus* species differ in this respect from each other. Samajpati (1978) reported that *Pleurotus ostreatus* can be cultivated at about 25 °C.

Kandaswamy and Ramasamy (1978) showed that *Pleurotus sajor-caju* could be grown at a temperature range of 21-28 °C. Krishna (1978) has also shown that *Pleurotus ostreatus* is rather flexible in its temperature requirements, as it would do well at the temperature range of 15-25 °C. It would therefore appear that *Pleurotus* species are well tolerant to light and temperature and can be successfully grown under variable climatic conditions.

Fruiting body formation in *Pleurotus* species is characterized by rhythmic cycles called flushes or breaks (Quimio *et al.*, 1990). Studies on the cultivation of these species by Bano (1971), Chackrorty and Sarkar (1978), Jandaik and Kapoor (1974), Kaul and Kachroo (1975) and Samajpati (1978) reveal that the economic life of substrate is between 45 to 80 days during which three to four flushes can be harvested. However these flushes are not of the same magnitude. The first flush is by far the best which yields nearly 60% mushroom biomass in relation to the substrate weight whereas the subsequent flushes yield between 20-22% of the substrate weight (Quimio *et al.*, 1990; Oei, 1991).

*Pleurotus* species are harvested as soon as the fruiting body has opened (Quimio *et al.*, 1990). The time of harvest has been found to depend on mushroom species, cultivation method, temperature and the type of container.

There are different methods of determining yield. The most accurate method in a scientific sense is dry weight of mushrooms versus dry weight of substrate (Oei, 1991). More convenient for growers is the biological efficiency method, which considers fresh weight of mushrooms versus air-dry weight of the substrate (Kulkarni, 1989; Oei, 1991). It has the advantage that the grower can easily determine the expected yield from a given amount of waste (Oei, 1991). Another commonly used measure is

the fresh weight of the mushroom versus the moist weight of the substrate. This method benefit growers who buy already prepared substrate (Oei, 1991).

The yield of *Pleurotus ostreatus* (Fr.) Kummer in Taiwan is limited to about 300 grams per kilogram while *P. florida* may yield one kilogram per kilogram substrate (Quimio *et al.*, 1990).

Mushroom yields range from about 100-200% of the dry weight of the substrate and depend on the substrate condition as well as the way in which the substrate has been managed during the fruiting season (Quimio *et al.*, 1990). Kulkarni (1989) reported 145% biological efficiency of *Pleurotus sajor-caju* grown on cotton waste in plastic bags at 15 °C and 80-90% humidity over a period of 70 days of harvesting.

### 3.0 MATERIALS AND METHODS

#### 3.1 CULTIVATION OF *PLEUROTUS* SPECIES IN ZAMBIA

*Pleurotus cornucopiae* and *Pleurotus sajor-caju* cultures were obtained from the International Bank of Edible Mushrooms, Rome, Italy with the courtesy of Dr. Umberto Tomati. The cultures were flown into the country in test tubes containing potato dextrose agar (PDA) as a carrier in 1995. Upon arrival the cultures were cleared by the phytosanitary section of the Ministry of Agriculture Food and Fisheries.

#### 3.2 DETERMINATION OF THE MOST SUITABLE LOCAL MYCELIUM CARRIERS FOR *PLEUROTUS* SPAWN PRODUCTION

Grains of maize, sorghum, wheat and wooden sticks and two mushroom species, *Pleurotus cornucopiae* and *Pleurotus sajor-caju* were used (Table 2). The carriers were bought in Zambian Kwacha from the market at the following price per Kg; 1000, 350, 300 and 400 respectively (Table 4). Wheat and sorghum cultivars were Loerie II and MMSH 373 respectively while the maize was of an unknown variety. The wooden sticks were made from pinewood (*Pinus kesiya*). They were of 10 cm length and 0.5 cm

thickness (slightly smaller than a pencil in length and thickness) and are used for making skewered meat.

The substrate types and the two mushroom species resulted into eight treatments (4X2). These were replicated five times resulting to 40 treatments (Table 2).

**Table 2: The spawn carrier type and *Pleurotus* species used in spawn production**

| SPAWN CARRIER                         | MUSHROOM SPECIES             |
|---------------------------------------|------------------------------|
| Maize (undetermined type) grain       | <i>Pleurotus cornucopiae</i> |
| Sorghum (MMSH 373) grain              | <i>Pleurotus sajor-caju</i>  |
| Wheat (Loerie II) grain               |                              |
| Wooden sticks ( <i>Pinus kesiya</i> ) |                              |

Seven kilograms each of wheat, sorghum and maize grains were put in three separate plastic dishes and thoroughly washed. Following the procedure described by Oei (1991), the grains were then soaked for two hours after which excess water was drained off. The grains were put into a 5 liter pyrex beaker and boiled for 30 minutes. Thereafter the grains were put back into the dishes. Few grains were seen to have cracked and were removed. This procedure was carried out for all grain types.

To each grain type, 140g calcium sulphate ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) and 42 g calcium carbonate ( $\text{CaCO}_3$ ), (2 % and 0.6 % respectively on weight basis), were added. The grains were partially dried for 15 minutes to reduce sticking. Six hundred grams of each grain type was transferred into ten 750 ml Mazoe glass bottles (a local juice). This brought the total number of bottles to 30, with each grain type occupying 10 bottles each.

The wooden sticks were boiled for 30 minutes and later submerged in a tray of sterile water overnight (Oei 1991). Fifty skewer sticks together with 10 ml sterile water were put in each of the ten bottles. Thus, forty bottles were used for this purpose. Ten per each of the substrate carrier.

The bottles were plugged with cotton wool and covered with aluminum foil to prevent the cotton plugs from being soaked by steam during sterilization. They were autoclaved at  $120^\circ\text{C}$  and  $15\text{ lb/in}^2$  pressure for 20 minutes. Under aseptic conditions of a laminar flow chamber,  $1\text{ cm}^2$  agar blocks of actively growing fungus were cut out with a flame-sterilized scapel and inoculated into the bottles containing autoclaved grain. To inoculate skewer sticks, 50 g of wheat grain spawn was used. The bottles were laid horizontally to achieve maximum contact between the inoculum and the sticks.

Of the 10 bottles of each substrate, 5 were inoculated with agar blocks of *Pleurotus cornucopiae* and the remaining five with *Pleurotus sajor-caju*. In the case of wooden sticks, five bottles were inoculated with wheat grain spawn of *Pleurotus cornucopiae* and the remaining five with *Pleurotus sajor-caju*. The date, the grain type and the name of the mushroom species were labeled on each bottle with a permanent marker pen. The bottles were incubated at 25 °C until abundant colonization was visible. The bottles were shaken thoroughly to break the clumps of colonized grains.

### 3.2.1 EXPERIMENTAL DESIGN

The 40 bottles containing the four different spawn carriers such as grains of wheat, sorghum, maize and wooden sticks were inoculated by the two *Pleurotus* species. Five of the ten bottles were inoculated with *Pleurotus cornucopiae* and the other five with *Pleurotus sajor-caju*.

The rate of colonization of the *Pleurotus* mycelium was the desirable data to be collected. However, the Researcher was unable to come up with an appropriate scientific method of collecting data for this experimentation due to varying shapes and sizes of the substratum. It was, however, agreed to simplify the findings to a mere visual score. This score was to be observed after 14 days of incubating the experimental units in an

incubator at 25 °C (Oei, 1991). At the end of 14 days the visual score for substrate colonization by *Pleurotus* mycelium was considered 'good' when it was completely colonized with fluffy looking mycelium and 'poor' when it was partially colonized with oppressed mycelium.

The lay out of the experiment was as shown in Figure 1.

**Figure 1:** The experimental layout for determination of the most suitable local mycelium carriers for *Pleurotus* spawn production.

| Wheat                                                                              |                                                                                     | Maize                                                                               |                                                                                     | Sorghum                                                                             |                                                                                     | Wooden Sticks                                                                        |                                                                                       |
|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| <i>P.c</i>                                                                         | <i>P.s-c</i>                                                                        | <i>P.c</i>                                                                          | <i>P.s-c</i>                                                                        | <i>P.c</i>                                                                          | <i>P.s-c</i>                                                                        | <i>P.c</i>                                                                           | <i>P.s-c</i>                                                                          |
|    |    |    |    |    |    |    |    |
|    |    |    |    |    |    |    |    |
|    |    |    |    |    |    |    |    |
|   |   |   |   |   |   |   |   |
|  |  |  |  |  |  |  |  |

**Key:**

 : - 750 ml Mazoe glass bottle

*P.c*: - *Pleurotus cornucopiae*

*P.s-c*: - *Pleurotus sajor-caju*

### **3.3 DETERMINATION OF THE MOST SUITABLE SUBSTRATES AND SUBSTRATE HEAT TREATMENT FOR OPTIMUM GROWTH OF *PLEUROTUS* SPECIES**

Three substrate materials viz. wheat straw, maize stalks and mixed wood shavings were used in the experiment. Wheat straw was collected from the nearby Zamseed farm after the rainfed wheat had been harvested. The maize stalks were taken from the fields on the eastern side of the University of Zambia Campus. They were of an open pollinated local variety grown by the residents of Kalingalinga Township. The mixed wood shavings were collected from a carpenter's shop and consisted of pine wood (*Pinus kesiya*) and mukwa wood (*Pterocarpus angolensis*). Half of each substrate type was pasteurized and the other half sterilized before it was spawned.

The different substrate types were gathered in three separate heaps. Wheat straw and maize stalks were chopped down to about 2-3 cm length whereas the mixed wood shavings were used as they were. Water was sprinkled on each heap to soak the substrate materials. Each pile was covered with a plastic sheet overnight to prevent it from drying.

### **3.3.1 PASTEURIZATION OF SUBSTRATE**

The materials to be pasteurized were collected from each heap and transferred into 75 cm X 50 cm polypropylene bags used locally for packing of 25 Kg maize meal (mealy meal). The bags containing substrate materials were immersed into a 210 liter drum of hot water (65 °C) for one hour. The water temperature was maintained by wood fire burning under the drum. The bags were taken to a room to cool and drain. The substrate's moisture content was checked until few drops of water could be squeezed out of it.

### **3.3.2 STERILIZATION OF SUBSTRATE**

Polypropylene bags of 50 cm X 30 cm size were filled with 2 Kg substrate and autoclaved at 120 °C and 15 lb/in<sup>2</sup> pressure for one hour. After this, the bag with its substrate was pushed into another heavy duty plastic bag previously sterilized by immersing into a water bath at 96 °C for one hour in order to get rid of potential contaminants.

### **3.3.3 SPAWNING OF SUBSTRATE**

#### **3.3.3.1 SPAWNING OF PASTEURIZED SUBSTRATE**

Two kilograms substrate material was filled into each heavy duty plastic bag of 50 cm X 30 cm. While the substrate was filled in each bag, substrate layers were alternated with evenly distributed sorghum spawn such that each bag contained 4 layers of the substrate and 40 g spawn. Before tying up the bag, a piece of cotton wool was placed at the mouth of the bag to keep out potential contaminants and for adequate gas exchange.

#### **3.3.3.2 SPAWNING OF STERILIZED SUBSTRATE**

Each unit of the sterilized substrate had two plastic covering one porous heavy duty polypropylene and the other an ordinary plastic bag (similar to the one used in pasteurized treatment) to maintain the same environment as the pasteurized substrate. Like in the pasteurized substrate, each bag was inoculated with 40 g of sorghum grain spawn in layers of four. The inoculation was done under aseptic conditions.

3.3.4 SPAW-RUN AND FRUITING BODY FORMATION

The bags were kept on the floor of a dark growth room at the School of Agriculture Sciences. When the bags were thoroughly colonized, the mycelium showed signs of pinhead formation. At this point the bags were opened from the top and natural light was allowed into the room by removing the black plastic covers from the windows.

3.3.5 EXPERIMENTAL DESIGN

The experimental design for the determination of the most suitable substrates and substrate heat treatment for optimum growth of *Pleurotus* species comprised three factors viz. substrate material, substrate treatment and the *Pleurotus* species used (Table 3)

Table 3: Substrate type, its method of treatment and the species of *Pleurotus* used.

| SUBSTRATE MATERIAL | SUBSTRATE TREATMENT | PLEUROTUS SPECIES            |
|--------------------|---------------------|------------------------------|
| Maize stalks       | Pasteurized         | <i>Pleurotus cornucopiae</i> |
| Wheat straw        | Sterilized          | <i>Pleurotus sajor-caju</i>  |
| Wood shavings      |                     |                              |

The substrate types, the method of substrate treatment and the species of *Pleurotus* gave 12 treatments (3 X 2 X 2) and each treatment was replicated 10 times resulting to 120 experimental units.

All bags were arranged on the floor of a room following a split-split plot design with substrate type as main plot, heat treatment as sub-plot and mushroom species as sub-sub plot. The bags were arranged in three groups of 40, depending on the substrate. In each group, 20 bags contained pasteurized substrate while the other 20 had sterilized substrate. In both types of bags containing pasteurized and sterilized substrate, half of the bags were inoculated with *Pleurotus cornucopiae* and the other half with *Pleurotus sajor-caju* (Figure 2).

**Figure 2: The experimental layout for the determination of the most suitable substrates and substrate heat treatment for optimum growth of *Pleurotus* species**

| Maize stalks |              |            |              | Wheat straw |              |            |              | Wood Shavings |              |            |              |
|--------------|--------------|------------|--------------|-------------|--------------|------------|--------------|---------------|--------------|------------|--------------|
| Pasteurized  |              | Sterilized |              | Pasteurized |              | Sterilized |              | Pasteurized   |              | Sterilized |              |
| <i>P.c</i>   | <i>P.s-c</i> | <i>P.c</i> | <i>P.s-c</i> | <i>P.c</i>  | <i>P.s-c</i> | <i>P.c</i> | <i>P.s-c</i> | <i>P.c</i>    | <i>P.s-c</i> | <i>P.c</i> | <i>P.s-c</i> |
| ○            | ○            | ○          | ○            | ○           | ○            | ○          | ○            | ○             | ○            | ○          | ○            |
| ○            | ○            | ○          | ○            | ○           | ○            | ○          | ○            | ○             | ○            | ○          | ○            |
| ○            | ○            | ○          | ○            | ○           | ○            | ○          | ○            | ○             | ○            | ○          | ○            |
| ○            | ○            | ○          | ○            | ○           | ○            | ○          | ○            | ○             | ○            | ○          | ○            |
| ○            | ○            | ○          | ○            | ○           | ○            | ○          | ○            | ○             | ○            | ○          | ○            |
| ○            | ○            | ○          | ○            | ○           | ○            | ○          | ○            | ○             | ○            | ○          | ○            |
| ○            | ○            | ○          | ○            | ○           | ○            | ○          | ○            | ○             | ○            | ○          | ○            |
| ○            | ○            | ○          | ○            | ○           | ○            | ○          | ○            | ○             | ○            | ○          | ○            |
| ○            | ○            | ○          | ○            | ○           | ○            | ○          | ○            | ○             | ○            | ○          | ○            |
| ○            | ○            | ○          | ○            | ○           | ○            | ○          | ○            | ○             | ○            | ○          | ○            |

**Key:**

- : - 2 kg substrate in a bag
- P.c*: - *Pleurotus cornucopiae*
- P.s-c*: - *Pleurotus sajor-caju*

Data collected was subjected to analysis of variance (ANOVA) and when the f-value was significant (p=0.05), the mushroom mean yields were

compared using Tukeys test (t-test) to determine differences among different substrates, between two methods of heat treatment and between the two *Pleurotus* species, and the interactions.

## 4.0 RESULTS

### 4.1 CULTIVATION OF *PLEUROTUS* SPECIES IN ZAMBIA

*P. cornucopiae* and *P. sajor-caju* can be cultivated in Zambia. These species can be propagated successfully on wheat and sorghum grain for spawn production and on pasteurized wheat straw and maize stalks for fruiting body production with an average yield of 263g/kg substrate.

### 4.2 DETERMINATION OF THE MOST SUITABLE LOCAL

#### MYCELIUM CARRIERS FOR *PLEUROTUS* SPAWN PRODUCTION

The mycelial colonization of both *Pleurotus cornucopiae* and *Pleurotus sajor-caju* after 14 days incubation at 25 °C was generally better on wheat and sorghum grains than on maize grain and wooden sticks (Table 4, Figures 1, 2 and 3).

It can be seen from Table 4 that the colonization of *Pleurotus cornucopiae* and *Pleurotus sajor-caju* was good on wheat and sorghum grains and poor on maize grain and wooden sticks. No contamination by bacteria or fungus was observed. The wheat and sorghum grains appeared 'white' due to the excessive colonization with *Pleurotus* mycelium. In contrast, maize grain and wooden sticks were poorly colonized. It can be further noted from

Table 4 that the ability of wheat, sorghum, maize and wooden sticks to loosen up upon shaking after mycelial colonization was generally good.

Figure 3 shows substrate used for spawn production before inoculation of *Pleurotus* mycelium. Figure 4 shows the same materials but after mycelial colonization note that the substrate appear whitish due to the colour of the mycelium. From Figure 4, we can observe that maize grain and wooden sticks were poorly colonized. Figure 5 shows the complete colonization of *Pleurotus sajor-caju* of sorghum in a bottle.

**Table 4: Substrate material and its unit price, species of *Pleurotus* and their ability to colonize the substrate 14 days after inoculation at 25 °C, and the substrate's ability to loosen up upon shaking after mycelial colonization.**

| SUBSTRATE MATERIAL | PRICE PER KG | <i>PLEUROTUS SPECIES</i> | SUBSTRATE COLONIZATION | SUBSTRATE ABILITY TO LOOSEN UP |
|--------------------|--------------|--------------------------|------------------------|--------------------------------|
| Maize              | K300         | <i>P. cornucopiae</i>    | Poor                   | Good                           |
| c.v                |              | <i>P. sajor-caju</i>     | Poor                   | Good                           |
| Unknown            |              |                          |                        |                                |
| Sorghum            | K350         | <i>P. cornucopiae</i>    | Good                   | Good                           |
| c.v                |              | <i>P. sajor-caju</i>     | Good                   | Good                           |
| MMSH 373           |              |                          |                        |                                |
| Wheat              | K1000        | <i>P. cornucopiae</i>    | Good                   | Good                           |
| c.v                |              | <i>P. sajor-caju</i>     | Good                   | Good                           |
| Loerie             |              |                          |                        |                                |
| Wooden             | K4000        | <i>P. cornucopiae</i>    | Poor                   | Good                           |
| Sticks             |              | <i>P. sajor-caju</i>     | Poor                   | Good                           |



Fig. 3: Grains and wooden sticks before inoculation.  
From left to right: sorghum, maize, wheat and wooden sticks.



Fig. 4: Grains and wooden sticks carrying *Pleurotus* mycelium 14 days after inoculation at 25 °C.  
From left to right: maize, sorghum, wheat and wooden sticks.



Fig. 5: Sorghum (cultivar MMSH) grain completely colonized with *Pleurotus sajor-caju* 14 days after inoculation at 25 °C.

### 4.3 DETERMINATION OF THE MOST SUITABLE SUBSTRATE AND SUBSTRATE HEAT TREATMENT FOR OPTIMUM GROWTH AND YIELD OF *PLEUROTUS* SPECIES

*Pleurotus cornucopiae* and *Pleurotus sajor-caju* successfully produced fruiting bodies on wheat straw and maize stalks whereas production on wood shavings was very poor. While mushroom production was successful for both methods of substrate treatment, pasteurization proved to be a better method of treatment than sterilization (Table 5). It can be observed from Table 5 that there were interactions among the factors studied. The interactions are as described below.

There were no significant differences in the yield of the two *Pleurotus* species ( $p < 0.05$ ) on pasteurized wheat straw while there were differences in the yield between the two species on pasteurized maize stalks with *Pleurotus cornucopiae* yielding more than *Pleurotus sajor-caju*.

*Pleurotus sajor-caju* performed significantly better ( $p < 0.05$ ) on pasteurized wheat straw than on pasteurized maize stalks while the performance of *Pleurotus cornucopiae* on these two pasteurized substrate was not significantly different.

*Pleurotus cornucopiae* performed better on sterilized wheat straw than on sterilized maize stalks while the performance of *Pleurotus sajor-caju* on both substrates was not significantly different ( $p < 0.05$ ).

While there were significant differences ( $p < 0.05$ ) in the performance between pasteurization and sterilization in wheat straw and maize stalks with pasteurized substrates performing better, there were no significant differences in the two methods of substrate treatment on mixed wood shavings. Production on mixed wood shavings was too poor to warrant recognition.

**Table 5: Average production of *Pleurotus cornucopiae* and *Pleurotus sajor-caju* on pasteurized and sterilized wheat straw, maize stalks and wood shavings per 2Kg substrate over a period of 80 days at 24°C and 64% relative humidity. (n=10 per treatment).**

|               |                | Yield of mushrooms (g/2Kg bag) |                      |
|---------------|----------------|--------------------------------|----------------------|
| Substrate     | Heat Treatment | <i>P. cornucopiae</i>          | <i>P. sajor caju</i> |
| Wheat straw   | Pasteurized    | 657 <sub>a</sub>               | 537 <sub>a</sub>     |
|               | Sterilized     | 366 <sub>b</sub>               | 268 <sub>bc</sub>    |
| Maize stalks  | Pasteurized    | 570 <sub>a</sub>               | 341 <sub>b</sub>     |
|               | Sterilized     | 220 <sub>c</sub>               | 209 <sub>c</sub>     |
| Wood shavings | Pasteurized    | 58 <sub>d</sub>                | 80 <sub>d</sub>      |
|               | Sterilized     | 121 <sub>d</sub>               | 159 <sub>cd</sub>    |

\* Means in columns and rows followed by the same letter are not significantly different ( $p=0.05$ , LSD)

Mean separation by LSD at 5% level.  $LSD_{0.05}$  for comparing mean mushroom yield is 130 g/2 kg substrate.

#### 4.3.1 YIELD PER FLUSH AND BIOLOGICAL EFFICIENCY OF *PLEUROTUS SPECIES*

The production of *Pleurotus* species was characterized by flushes (Table 6). Table 6 shows mushroom yield in g/2 Kg substrate per flush. The yields were obtained in four flushes. The frequency of each flush noted in this experiment was between 15 and 20 days. Fruiting bodies were harvested for a period of 80 days after which production declined considerably. There were, however, some mushrooms sprouting in smaller quantities in between major flushes.

It can be further observed from Table 6 that the flushes were not of the same magnitude. The first flushes were by far the best and yielded between 50% and 60% of the mushroom biomass in relation to the total weight. The percentage yield of the first to the fourth flush of *Pleurotus cornucopiae* were about 60%, 21%, 12% and 6% respectively whereas those for *Pleurotus sajor-caju* were about 50%, 28%, 20% and 2% of the overall total yields. The values of the yield of *Pleurotus* species in relation to the total yield were true for both substrates.

The first flushes were by far the best. The second flushes were about half of the first while the sum of the third and fourth flushes were far below 25% of the total mushroom yield.

The Biological Efficiency (Table 6) was determined by following the formula stated by Kulkarni (1989), Quimio *et al.* (1990) and Oei (1991). This was by dividing mushroom fresh weight with the substrate dry weight and the ratio expressed as a percentage. Table 6 also shows that the biological efficiency of *Pleurotus* species on wheat straw and maize stalks ranged from 57 to 110%.

The biological efficiency of *Pleurotus* species on wood shavings have been ignored due to very poor yield obtained during the experimentation.

**Table 6: Yield (g/2 Kg substrate) per flush and the Biological Efficiency (BE) of *Pleurotus* species in pasteurized wheat straw and maize stalks.**

| Substrate    | Flush No.    | Yield and Biological efficiency (shown in brackets) of Mushrooms (g/2kg bag) |                                |
|--------------|--------------|------------------------------------------------------------------------------|--------------------------------|
|              |              | <i>P. cornucopiae</i>                                                        | <i>P. sajor-caju</i>           |
| Wheat straw  | 1            | 394 <sub>a</sub> *                                                           | 269 <sub>a</sub>               |
|              | 2            | 138 <sub>b</sub>                                                             | 150 <sub>ab</sub>              |
|              | 3            | 82 <sub>b</sub>                                                              | 107 <sub>b</sub>               |
|              | 4            | 43 <sub>b</sub>                                                              | 11 <sub>b</sub>                |
|              | <b>Total</b> | <b>657<sub>a</sub> (BE=110)</b>                                              | <b>537<sub>a</sub> (BE=90)</b> |
| Maize stalks | 1            | 342 <sub>a</sub>                                                             | 171 <sub>b</sub>               |
|              | 2            | 123 <sub>b</sub>                                                             | 96 <sub>b</sub>                |
|              | 3            | 71 <sub>b</sub>                                                              | 68 <sub>b</sub>                |
|              | 4            | 34 <sub>b</sub>                                                              | 6 <sub>b</sub>                 |
|              | <b>Total</b> | <b>570<sub>a</sub> (BE=95)</b>                                               | <b>341<sub>a</sub> (BE=57)</b> |

\* Means in columns and rows followed by the same letter are not significantly different ( $p=0.05$ , LSD).

## 5.0 DISCUSSION

It is evident that *Pleurotus* species can be grown in Zambia. This is because these species are able to fruit at the prevailing Zambian conditions. In addition, these species have an advantage of growing on a wide array of agricultural waste and require neither manipulation of the environment nor complicated composting procedures.

Results of spawn carrier colonization by *Pleurotus* species showed good mycelial colonization on wheat and sorghum grains and poor colonization on maize and wooden sticks after 14 days of inoculation at 25 °C. Previous reports have shown that mushroom spawn can be produced on different grain types such as wheat, rye, millet, rice and sorghum and wooden materials like wood plugs, wooden sticks and sawdust (Stoller, 1962; Quimio *et al.*, 1990; Oei, 1991). This study has considered the probable use of maize grain for spawn production of *Pleurotus* species.

The mycelial growth of *Pleurotus* on maize grain and wooden sticks was generally poor and therefore the use of these materials for spawn production can not be recommended. The poor growth of *Pleurotus* mycelium on maize grains was probably due to the insufficient sterilization of grains.

Oei (1991) in his book entitled 'Manual on Mushroom Cultivation' reported that the period of sterilization depends on the size of the container. While 2 hours may be sufficient for a 500g container, a 3 Kg container may require about 4 hours to sterilize. Similarly, it will require longer time to allow sufficient heat to sterilize the inner part of a relatively large grain. Thus heat may not have been sufficient to completely sterilize the bulky substrate, thus allowing bacteria to survive in the relatively cooler inner region of the grain (Dalzell *et al.*, 1987). It was therefore possible that during the fifteen minutes of sterilization, bacteria were eliminated in the smaller grains of wheat and sorghum while bacteria survived in maize. The bacteria of greenish appearance multiplied and oppressed the growth of fungi. Similar observation has also been made by Oei (1991) who discovered that spawn contaminated with bacteria ceases to grow.

It has been suggested that the pericarp of maize has wax on its surface (Watson and Moore, 1924). Wax is a very resistant compound for fungal attack and in addition presents hydrophobic properties (Jennings and Lysek, 1996). Thus moisture, the necessary requisite for mycelial propagation, is removed from the surface resulting in poor or no fungal colonization (Jennings and Lysek, 1996). Both factors explain the poor mycelial colonization of maize grain. This fact, however, recognizes the

fact that cooking removes the waxes but we do not know how much is removed and how much has remained on the surface.

The *Pleurotus* mycelial colonization of pine wood sticks was poor. The poor mycelial growth can be attributed to the fact that the wooden sticks were made from fresh wood. It has been reported by Oei (1991) and Quimio *et al.*, (1990) that in the cultivation of shiitake mushrooms, freshly cut trees normally show some resistance against mycelial colonization. Jennings and Lysek (1996) and Tarr (1992) pointed out that many plants contain substances which inhibit colonization by fungi, bacteria and virus. They also stated that these substances phase out after the death of the plant and make it vulnerable to fungal attack. Jennings and Lysek (1996) reported that resistant compounds of a plant like lignins are attacked only after several months.

Since the wooden sticks were made from freshly cut pine trees, it was likely that they had substances like lignin that inhibit fungal infection. It is interesting to note that the production of *Pleurotus* mushrooms on sawdust in Ghana is done by composting the sawdust for three to four weeks before spawning (Addey, 1992). This may explain why the *Pleurotus* mycelial colonization on the wooden sticks was poor.

The two *Pleurotus* species viz. *P.cornucopiae* and *P.sajor-caju* produced fruiting bodies on wheat straw and maize stalks at a temperature range of 15-26 °C and 55-90% relative humidity. Both pasteurized and sterilized substrate yielded fruiting bodies. The successful production of fruiting bodies on both wheat straw and maize stalks concurs with findings of Bano and Rajarathnam (1978), Breen (1990) and Stamets (1993). These authors reported that *Pleurotus* species grow on a wide array of plant straw and other agricultural wastes. The temperature and relative humidity at which the fruiting bodies were obtained also agree with the findings of Oei (1991) and Bano (1976). Oei reported that the temperature range for the fruiting of *P.cornucopiae* and *P.sajor-caju* was between 15-25°C and 18-30°C respectively while Bano (1971) reported that *Pleurotus sajor-caju* can be grown at a temperature range of 21-28°C and 55-75% relative humidity. These reports are in agreement with the results obtained in this study. The temperature range of 15-26°C and the relative humidity range of 55-90% recorded in the growing room during the present study were similar to those reported by Bano (1976).

During this study it was observed that fruiting bodies developed on both pasteurized and sterilized substrates. This was also reported by Quimio *et al.* (1990) and Oei (1991) who found that *Pleurotus* species can be produced on similarly treated substrates. This means that *Pleurotus* species can form fruiting bodies on both pasteurized and sterilized

substrates. However, pasteurization by immersion in hot water at 65°C for one hour proved to be a better method of substrate treatment than sterilization at 120°C and 15 lb/in<sup>2</sup> pressure for one hour for both wheat straw and maize stalks. Zadrazil (1978) reported that intense or prolonged heating of substrate resulted in a loss of organic matter and gave reduced mushroom yield. It has also been reported that intense heat breaks down compost nutrients which favour the growth and development of competing micro-organisms (Quimio *et al.* 1990).

The effects of pasteurization and sterilization were not significant ( $p < 0.05$ ) on wood shavings. The fresh wood shavings must have had substances that inhibited fungal growth. Jennings and Lysek (1996) and Tarr (1972) stated that plants contain substances which inhibit fungal colonization. Thus *Pleurotus* species were unable to colonize successfully the fresh wood shavings in this study. It must be mentioned here that well composted wood shavings can permit colonization by the fungus as known from Ghana (Addey, 1992).

The yield of *P.sajor-caju* was more on pasteurized wheat straw than on pasteurized maize stalks (Table 5) while that of *P.cornucopiae* was relatively less ( $p < 0.05$ ) on the two substrates. The opposite was however true on the sterilized substrate; the yield of *P.cornucopiae* was more on wheat straw while that of *P.sajor-caju* was somewhat smaller on the two

substrates. These and other interactions can be seen in Table 5 and confirms the finding of Poppe and Hofte (1995) who observed what substrate colonization of *Pleurotus* species varied greatly on differently treated substrates.

In this study significant fruiting body formation was noticed after every 15 to 20 days and a total of four flushes of varying magnitude were collected. This was also observed by Quimio *et al.* (1990) who reported that the fruiting body formation in *Pleurotus* species was characterized by rhythmic cycles called flushes. The period of time between flushes conforms with the work of Samajpati and Bandyopadhyay (1982) who reported that period between flushes of *Pleurotus ostreatus* is between 15 and 20 days. The economic period of 80 days for fruiting body formation and the fact that the first flush was by far the best has also been reported by Chackroborty and Sarkar (1978), Jandaik and Kapoor (1974), Kaul and Kachroo (1975) and Samajpati (1978). These workers reported that the economic life of substrate is between 45 to 80 days during which three to four flushes can be harvested. They further found that each flush is not of the same magnitude. The present study showed that the first flush yielded nearly 60% of mushroom biomass in relation to the total weight. The first flush of *Pleurotus cornucopiae* was exactly 60% of the total yield while that of *Pleurotus sajor-caju* was 50%.

In the cultivation of *Pleurotus* species on cotton waste Kulkarni (1989) provided a harvesting period of 70 days in order to evaluate the maximum production capability of the species. Similarly in this study a period of 80 days was the total length of time when *Pleurotus* species exhausted their yield potential.

Generally the yield of *Pleurotus* species ranged between 341g and 657g per 2kg moist pasteurized substrate. The moisture content of the substrate was 70% hence the substrate air dry weight was 600g.

The biological efficiency of *Pleurotus* species, *P.cornucopiae* and *P.sajor-caju* on pasteurized wheat straw and maize stalks was between 57% and 110%. This range is close to that determined by Kulkarni (1989) who reported the biological efficiency of *Pleurotus* species on cotton waste to be between 70% and 170%. Samajpati and Bandyopadhyay (1982) also reported the biological efficiency of *Pleurotus ostreatus* on paddy straw to be 110.4% while Quimio *et al.* (1990) reported biological efficiency of *Pleurotus florida* to be 100%. These values are similar to those obtained with *P.cornucopiae* on pasteurized wheat straw in this study.

However, the biological efficiency of *Pleurotus* species falling far below the values reflected above have also been recorded. Quimio *et al.* (1990)

reported the yield of *Pleurotus ostreatus* (Fr.) Kummer to be limited to about 300g per kilogramme waste while Kandaswamy and Ramasamy (1978) recorded an average yield of *Pleurotus cornucopiae* of 625g per 3kg of dry straw and Kristna (1978) reported a yield of 1.5kg of fresh mushrooms per 18kg of paddy straw. These yield indicate biological efficiencies of 30%, 21% and 8% respectively.

The wide range of biological efficiency agree with Kulkarni (1989) and Quimio *et al.* (1990) who stated that the yield and consequently the biological efficiency depends on the substrate, its condition and its management during the fruiting season.

## 6.0 CONCLUSION

*P.cornucopiae* and *P.sajor-caju* can be cultivated in Zambia. These two species have been propagated successfully on wheat and sorghum grain for spawn production and on wheat straw and maize stalks for fruiting body production. Production of spawn on maize grain and production of fruiting bodies on wood shavings was unsuccessful.

Although spawn was successfully produced on wheat and sorghum grains, the price of wheat being higher discourages its use. Since sorghum is more economical in comparison to wheat, it is suggested that its use as spawn carrier should be preferred.

Pasteurization of substrate was found to be a more economical method than sterilization. Pasteurization requires simple equipment and does not need high energy input and therefore its use should be adopted.

The yield of *P.cornucopiae* was approximately the same on both pasteurized wheat straw and maize stalks. However, the yield of this species on maize stalks was relatively better than that of *P.sajor-caju*. *P.cornucopiae* was in production for a period of 80 days while *P.sajor-caju* continued producing for a period of 60 days. The biological

efficiency of *P.cornucopiae* on both substrates was about 100%, a value that suggests the economic benefit of the technology.

The fruiting body formation of *Pleurotus* species was characterized by flushes. The period between flushes ranged from 15 to 20 days. The first flush was the best in *Pleurotus cornucopiae* and *Pleurotus sajor-caju* and both species produced about 60% and 50% of the total mushroom yield respectively. The consecutive flushes, especially the fourth one, were much smaller and therefore uneconomical.

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## 8.0 APPENDIX

**8.1 Temperature (°C) and percentage relative humidity data of the growth room during the period of spawn-run and harvesting of mushrooms, 1996. (Figures in brackets are minimum temperatures).**

|             | JUNE   |    | JULY   |    | AUGUST |    | SEPTEMBER |    |
|-------------|--------|----|--------|----|--------|----|-----------|----|
| DAY         | Temp   | RH | Temp   | RH | Temp   | RH | Temp      | RH |
| 1           |        |    | 22(17) |    | 24(20) | 75 | 24(20)    | 63 |
| 2           |        |    | 23(18) |    | 24(21) | 63 | 23(20)    | 73 |
| 3           |        |    | 23(16) | 58 | 23(19) | 63 | 23(19)    | 73 |
| 4           |        |    | 24(18) | 59 | 24(20) | 59 | 21(17)    | 63 |
| 5           |        |    | 25(19) | 70 | 22(18) | 68 | 22(18)    | 64 |
| 6           | 24(19) |    | 25(17) | 76 | 19(17) | 59 | 24(20)    | 67 |
| 7           | 24(21) |    | 24(19) | 80 | 21(17) | 62 | 21(19)    | 60 |
| 8           | 24(20) |    | 25(19) | 60 | 22(18) | 59 | 24(21)    | 59 |
| 9           | 25(19) |    | 24(19) | 66 | 24(20) | 73 | 25(22)    | 59 |
| 10          | 25(20) |    | 25(19) | 75 | 22(19) | 61 | 22(20)    | 81 |
| 11          | 24(20) |    | 20(18) | 75 | 24(20) | 55 | 22(19)    | 58 |
| 12          | 26(20) |    | 21(17) | 75 | 22(19) | 56 | 20(18)    | 64 |
| 13          | 26(21) |    | 21(17) | 80 | 23(19) | 57 | 24(19)    | 62 |
| 14          | 25(20) |    | 18(16) | 68 | 22(18) | 52 | 24(19)    | 67 |
| 15          | 24(21) |    | 24(20) | 90 | 21(16) | 63 | 23(19)    | 74 |
| 16          | 26(24) |    | 22(20) | 62 | 21(17) | 63 | 26(22)    | 57 |
| 17          | 25(19) |    | 23(19) | 66 | 20(16) | 76 | 25(22)    | 68 |
| 18          | 25(20) |    | 22(18) | 70 | 19(17) | 62 | 25(20)    | 68 |
| 19          | 24(20) |    | 23(19) | 78 | 21(16) | 61 | 26(23)    | 59 |
| 20          | 25(19) |    | 20(16) | 69 | 21(17) | 62 | 24(20)    | 57 |
| 21          | 23(19) |    | 21(16) | 78 | 20(17) | 63 | 25(20)    | 68 |
| 22          | 24(19) |    | 23(17) | 69 | 18(16) | 76 | 24(19)    | 68 |
| 23          | 24(19) |    | 21(15) | 64 | 20(18) | 76 | 26(22)    | 59 |
| 24          | 24(18) |    | 22(19) | 69 | 18(15) | 79 | 24(21)    | 57 |
| 25          | 23(19) |    | 22(21) | 75 | 21(17) | 67 | 22(19)    | 66 |
| 26          | 22(17) |    | 22(19) | 75 | 20(17) | 64 | 21(19)    | 55 |
| 27          | 23(16) |    | 20(19) | 70 | 23(18) | 57 | 25(23)    | 59 |
| 28          | 24(18) |    | 21(18) | 59 | 22(19) | 61 | 24(20)    | 58 |
| 29          | 23(18) |    | 20(19) | 69 | 23(19) | 60 | 22(19)    | 62 |
| 30          | 24(17) |    | 20(17) | 60 | 20(17) | 61 | 21(19)    | 77 |
| 31          |        |    | 21(18) | 57 | 23(16) | 65 |           |    |
| Mean        | 24(19) |    | 22(18) | 69 | 22(17) | 64 | 23(20)    | 64 |
| Temp. range | 16-24  |    | 15-25  |    | 15-24  |    | 17-26     |    |
| RH range    |        |    | 57-90  |    | 55-79  |    | 55-81     |    |