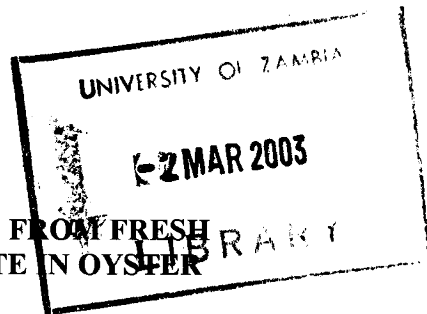

COMPARISON OF MUSHROOM YIELDS OBTAINED FROM FRESH GRAIN SPAWN AND SPENT MUSHROOM SUBTRATE IN OYSTER MUSHROOMS *PLEUROTUS HK 35*

BY

MATANDA MUSUKWA

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SUPERVISOR

DR. C. GWANAMA

DEDICATION

This thesis is dedicated to my mother Florence Nkungamina, my Auntie Dora Nkungamina, my special friend Ethel, brothers and friends for their encouragement and support towards my academic work at the University of Zambia.

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ABSTRACT

This study investigated the possible use of spent mushroom substrate as spawn. It was conducted at the University of Zambia in May 2002 using *Pleurotus HK 35* strain and giant star grass (*Cynodon Plectostarhycum*) as substrate. The substrate was pasteurised by immersing in hot water at 70° C for 2 hours. Fresh grain spawn and spent substrate post third flush (55 days) were inoculated into the substrate and incubated.

In this study the mean time to full colonization for fresh grain spawn and spent substrate was 23 and 33 days while the mean time to first flush was 31 and 50 days, respectively.

The mean yields per flush in fresh grain spawn were 417.70 g, 334.0 g and 90.42 g of mushrooms, respectively. In spent substrate, there was one flush with a mean yield of 97.35 g mushrooms, with only one substrate out of 10 producing a second flush with a yield of 288.81 g. There was no third flush in spent substrate. The mean flushing intervals in fresh grain spawn 9.7 and 10 days respectively, whereas in spent substrate there was mean interval of 16 days between full colonisation and first flush. The total mean yield for fresh grain spawn was 910.10 g mushrooms, that of spent substrate was 126.20 g of mushrooms. The contamination rate in fresh grain spawn 10 % as opposed to 100 % in spent substrate, while were responsible for the reduction in yields. It was thus concluded that spent mushroom substrate cannot be used as an alternative to fresh grain spawn.

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1.0 INTRODUCTION

Edible mushrooms are fast becoming an important food crop in many countries around the world; such that a lot of research is going on to improve cultivation techniques and technologies.

This should eventually increase the yields being obtained from conventional cultivation practices. Besides being a source of food, mushrooms have many uses, in the carbon cycle of nature, production of antibiotic drugs with mushroom synthesized biochemical compounds. The breaking down of substances in waste cleaning programs since certain types of mushrooms posses the ability to decompose or break down waste materials.

In developing countries, such as Zambia, mushroom production is increasing because of the high nutritional value and ease of cultivation of mushrooms. There is however a number of problems that limit production.

The factors hampering mushroom production in Zambia include the lack of market information, specialist extension services and the cost of equipment to use in mushroom production as well as the limited capital investment for constructing growing houses.

Mushroom seed, or spawn as it is known is another limiting factor in that it is not readily available in Zambia. It is clear that the mushroom grower faces a number of problems, however efforts are being made to find solutions to these problems. This lead to the decision to investigate the possibility of using spent substrate as spawn in order find a solution to unavailability of spawn. In this study *Pleurotus HK 35* was the mushroom strain used.

1.1 Objectives

To compare production parameters of oyster mushroom obtained from fresh grain spawn and recycled mushroom substrate.

2.0 LITERATURE REVIEW

There are many mushroom types and these are grouped according to their occurrence in nature. Edible mushrooms are basically epigeous and hypogeous fruiting bodies of macroscopic fungi (Chang and Hayes, 1978).

Fungi are organisms that cause decay, and their environments in nature are usually heterogenous. Fungi are organism that colonise substrate by using their hyphae in spreading within the substrate, using energy as they spread, from substrate and nutrients in first patch of substrate for hyphae growth to the next (Jennings and Lysek, 1996).

Pleurotus mushrooms, the type which is of interest in our case, are commonly known as oyster mushrooms due to the shape of the pileus which is shaped like the shell of an oyster. They belong to a category of organisms of the Kingdom Myceteae. Their division is Mastigomycota, class Basidiomycete, order Agaricales and the family Tricholomatacea. The edible type fall into two major groups—the Ascomycetes and Basidiomycetes. Oyster mushrooms have many advantages over most domesticated mushrooms. These mushrooms are competitive, highly productive and adaptable. This explains their wide cultivation across the world (Miles and Chang, 1997).

The *Pleurotus* species are generally wood decomposers. They thrive on a variety of forest and agricultural by-products. They will grow on most liginocellulocis materials. Among these are cereal straws, maize cobs, saw-dust, sledge coffee residues and many others. This attribute is unique to only a few mushroom species. Besides these advantages *Pleurotus* species have biological efficiencies of about 100%, in some cases as high as 125%. Their nutritional value is also an advantage, protein content in *Pleurotus* species ranges from15 – 35% on a dry weight basis. They contain significant quantities of free amino acids and a variety of vitamins. Some of the vitamins include vitamin C (30 –144 mg per 100g), vitamin B and Niacin (109 mg per 100g) (Stamets, 1993). There are however variations in the nutritional status in

mushrooms which are attributed to the spawning media, rates and type of substrate. Finally *Pleurotus* species are the easiest and least expensive to grow, for that reason they make the best choice for small mushroom growers.

Pleurotus species, like any other crop have their disadvantages with regard to production. The first of the disadvantages is that they may be a potential health hazard to the worker because the spores generated can cause allergies. Secondly, they spoil very quickly. This makes it difficult for producers to transport and sell oyster mushrooms in for away markets. Thirdly *Pleurotus* species attract a lot of sciorid and phorid flies (*Megaselia tamilnaduensis*) which spread contamination. The phorid flies not only spread contamination but their Larvae also feed on mycelium (Zadrazil and Kurtsman, 1985).

2.1 GROWTH PARAMETERS FOR PLEUROTUS MUSHROOM

2.1.1 Spawn run

Temperature affects the rate of spawn run. In *Pleurotus* species growing in commercial conditions, the optimum temperature range is between 25 and 28° C (Poppe, 1985). Oyster mushroom require high moisture environments and so they grow well at relative humidity of 90 to 100% during spawn run. At this stage of development oyster mushrooms do not need any light (Stamets, 1993). The Carbon dioxide requirement at spawn run for *Pleurotus* species is 5000 ppm (Stamets, 1993). High Carbon dioxide concentration inhibits growth of other microorganisms (Zadrazil, 1975).

2.1.2 Primordia formation

The temperature requirement at this stage ranges between 15 – 30 °C for most oyster mushrooms (Stamets, 1993). At this stage of growth the relative humidity is between 95 to 100% while the Carbon dioxide requirement is about 1000 ppm (Stamets, 1993). 1000 to 1500 lux of light is required at this stage of growth for *Pleurotus* species (Stamets, 1993).

2.1.3 Fruitbody Development

The optimal temperature range for fruitbody formation is from 20 to 30 °C while the relative humidity should be about 85 to 90% for Oyster mushroom. The carbon dioxide and light requirements at this stage are 1000 ppm and 500 – 15000 lux of light for most *Pleurotus* species (Stamets, 1993). At all stages of growth, air exchange should be allowed to ensure that oxygen is also available for the developing mushrooms, but at lower concentrations than Carbon dioxide.

2.1.4 pH

Pleurotus species require pH that is between 4.0 and 6.0 (Miles and Chang, 1997). The substrate pH however, reduces as the mycelium grows. This is because of the acidic nature of compounds produces by mycelium (Höfta, 1985). A very acidic substrate inhibits the growth of *Pleurotus* mushroom.

2.2 Spawn

Spawn is a substrate that has been impregnated with mushroom mycelium and can be used for propagation purposes (Miles and Chang, 1997). When developing spawn a fruiting culture that has the capacity to form fruiting bodies under suitable conditions is used. Before the culture is used it is tested on a substrate selected under suitable conditions. The spawn, which is developed, must be a pure culture of known origin and free from contaminants. Spawn carries the genetic and cultural characteristics of mushrooms. Spawn is very important because of the genetic characteristic of the fruiting culture it carries. The quality and quantity of mushroom produced depend upon these genetic characteristics. Therefore for spawn to produce high quality and quantity yields it must posses desirable genetic characteristics otherwise the crop will be poor regardless of the good cultivation practices. Besides good genetic characteristics, spawn should exhibit vigorous and aggressive mycelial growth in spawn substrate and fruiting containers in growing houses (Miles and Chang, 1997). Spawn must be fresh have a good shelf life and free of contaminating microorganisms.

Spawn consists of a carrier material, which has been completely colonised by mushroom mycelia. There are different types of carrier each type differs according to mushroom species being cultivated. The carriers are generally cereal grains. They include sorghum, wheat, rice millet, rye grass and wheat grass seed, and rye grain among others (Stamets 1993). The carrier material ensures even distribution of mycelium and serve as a nutritional supplement. The material therefore affects spawn quality by influencing the rate and thoroughness of mycelium growth in the spawn container and fruiting container.

Pasteurisation process of the spawn carrier material will affect the quality of spawn if not carried out properly. The steam produced must sufficiently penetrate the structural cavities in the grain, which harbour the contaminating micro- organisms in order to eliminate them. These micro- organisms will compete with mycelia growth and eventually reduce the quality of spawn if they are not eliminated. Spawn quality can also be affected by the rate of mycelia growth. If spawn run continues beyond a certain point, valuable nutrients for fruit body formations will be used in vegetative growth (Stamets, 1993).

2.3 Recycling of spawn

This is the use of spent or partially spent substrate as spawn for inoculating fresh prepared substrate. Recycling spawn can be done very easily, however it has a big disadvantage in that the spent substrate is exposed to a lot of contamination before it can be used for spawning. The contaminations are of various types. These will show once the fresh substrate has been spawned with spent substrate. Some of the likely contaminations would be *Trichoderma* spp and larvae of phorid and sciorid insects among others. Therefore recycled spawn should be derived from substrate under very high standards of hygiene. Otherwise there you will be transferring contamination to the fresh substrate and consequently achieve very low yields of mushroom.

3.0 MATERIALS AND METHODS

Giant star grass (*Cynodon Plectostachyum)* was used as substrate and pasteurised by immersion in water in 220L drum kept at a temperature of 60 – 70 °C for 2 hours. The substrate was subsequently removed, drained of excess water and cooled.

The experiment was set up with two treatments. Fresh grain spawn and spent substrate at 55 days from spawning. The spent substrate was also post 3 flush. In the fresh grain spawn treatment, 10 bags were spawned with fresh grain spawn at a rate of 1 – 2%. In the recycled spawn treatment, 10 bags were spawned with spent substrate. All the bags were then incubated in the growing room, where relative humidity was kept high by watering.

Each treatment had 10 single-bag replications in a completely randomised design (CRD) in the growing room.

3.1 Characteristics measured

1. Days from spawning to full colonisation.
2. Days from spawning to first flush.
3. Yield per flush (g).
4. Number of flushes per mould.
5. Days between flushes or flushing interval.
6. Total yield per mould (yield from all flushes).
7. Days from spawning to contamination.

3.2 Statistical analysis

The two treatments were compared using the student t-Test by Genstat Statistical programme.

4.0 RESULTS

4.1 Days from spawning from to full colonisation

The average number of days to attain full colonisation was 23 days for fresh grain spawn. On the other hand, spent substrate took 33 days to fully colonise. Colonisation in spent substrate occurred in only 50% of the 10 substrates. The other 50% did not colonise because their mycelia was out-competed by the contaminating micro-organisms which lengthened the time required for full colonisation to be attained. The two treatments were not significantly different with regard to full colonisation at $p = 0.05$ (Table 1).

4.2 Days from spawning to first flush

The first flush in fresh grain spawn occurred on average 31 days from spawning while in spent substrate it took 50 days. The calculation in spent substrate was based on 50% of the substrate that colonised. In the remaining 50% of the substrates, which did not colonise there was no flushing. The two treatments were not significantly different with regard to days to first flush at $p = 0.05$ (Table 1).

4.3 Yield of mushrooms per flush

At first flush, fresh grain spawn yielded significantly higher than spent substrate averaging 417.70 g mushrooms per substrate while spent substrate produced 97.35 g mushrooms per substrate $p = 0.05$ (Table 1).

At second flush, fresh grain spawn produced on average 334.00 g mushrooms per substrate whereas spent substrate gave a yield of 288.81 g of mushrooms from one substrate only. When expressed as an average it was 28.88 g of

mushrooms per substrate. Hence the two treatments were significantly different at $p = 0.05$ (Table 1).

The third flush in fresh grain spawn produced a yield of 90.42g of mushroom per substrate. In spent substrate spawn there was no third flush.

4.4 Number of flushes per substrate

Fresh grain spawn produced three distinct flushes while spent substrate produced one flush with only one substrate mould giving a second flush.

4.5 Flushing interval

The flushing interval in the two treatments could not be compared since spent substrate spawn flushed only once with the exception of just one substrate giving a second flush. However, the fresh grain spawn yielded three flushes. The first flushing interval, from full colonisation to first flush averaged nine days. The second and third flushes averaged seven and ten days, respectively.

4.6 Total mushroom yield

Total mushroom yields were significantly different at $p = 0.05$ between the two treatments. Fresh grain spawn produced 916.10 g mushrooms whereas spent substrate produced 126.20 g mushrooms (Table 1).

4.7 Days to contamination

Contaminations were observed in all substrate moulds of spent substrate spawn, nine days after spawning. In fresh grain spawn there was only one substrate in which contamination was observed also on the ninth day from spawning.

TABLE 1: Time to full colonisation, Time to first flush, Yield 1st flush, Yield 2nd flush and total yield of mushrooms from fresh grain spawn and spent substrate.

Treatment	Time to full colonisation		Time to first flush		Yield 1st flush		Yield 2nd flush		Total Yield	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Fresh grain Spawn	23	5.96%	31	7.04%	471.70	14.35%	334.00	16.88%	916.10	6.60%
Spent substrate	33	6.02%	50	6.88%	97.35	140.75%	28.88	316.26%	126.20	163.63%
Significance	t =1.86ns		t = 2.01ns		t= 8.16*		t =9.01*		t =11.61*	

5.0 DISCUSSION

Full colonisation in moulds of spent substrate was patchy and non-uniform. In fresh grain spawn, it was uniform and copious in all substrate moulds. The time to full colonisation in spent substrate was longer than fresh grain spawn because of the presence of contamination. The presence of contaminating organisms also lengthened the time to first flush in spent substrate while in fresh grain spawn the period to first flush was short. The pattern of colonisation and flushing observed in spent substrate moulds was caused by the insect flies and contaminating organisms passed on from the spent mushroom substrate used as spawn.

In the contaminated substrates the main competitor fungi was *Trichoderma* spp, which produces toxins that render the substrate unsuitable for mushroom growth (Stamets, 1993). Other forms of contamination were observed by yellowish brown discolourations. Healthy *Pleurotus HK 35* mycelia have a white colour. These were, however less problematic than the *Trichoderma* spp, which were responsible for reduced yields in spent substrate moulds. The other reason for reduced yields in spent substrate was due to infestations by insect pests, in spent substrate being higher than in fresh grain spawn because the inoculant substrate spawn carried with it eggs and Larvae of the insects, mainly phorid and sciorid flies which developed into adults that were partly responsible for spreading contamination and reducing yield of mushroom.

In spent substrate spawn, besides the contaminations of *Trichoderma,* another type of weed fungi called ink-caps was observed in four of the moulds. Again this was attributed to the inoculant from spent mushroom substrate carrying with it weed fungi spores. The ink-caps contributed to lower yields that were recorded in spent substrate while the yields in fresh grain spawn were normal with a biological efficiency of 91%. The number of flushes in fresh grain spawn were more than in spent substrate. There were three flushes in grain spawn while spent substrate had one flush except for one substrate, which produced a second flush. The higher number of flushes can be attributed to the vigorous nature of fresh grain spawn. The spent substrate had poor vigour and a lot of contamination, which reduced the flushes in moulds of spent substrate.

For the same reasons the flushing intervals in fresh grain spawn were shorter compared to that of spent substrate, which had only one flush.

6.0. CONCLUSION

Fresh grain spawn was found to perform better than spent substrate spawn as expected; it produced normal yields as opposed to the low yield in spent substrate spawn. This experiment, however indicated that it is possible to grow mushroom (*Pleurotus HK 35*) from spent substrate after three flushes. They yields from spent substrate showed a high level of inconsistence and generally low yields when compared fresh grain spawn. The cultivation of mushrooms from spent substrate spawn would not be economical considering the low yields due to contaminations.

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