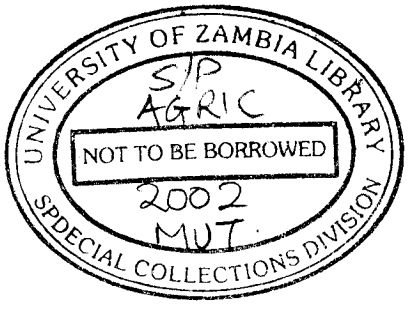
THE EFFECT OF CASING MATERIALS ON THE YIELD OF BUTTON MUSHROOM *(AGARICUS BISPORUS)*.



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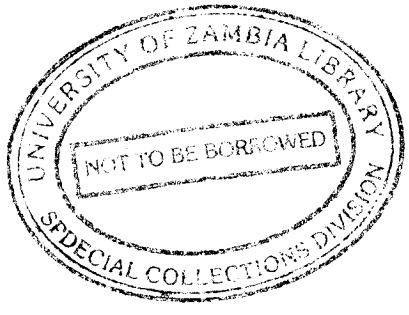

DEPARTMENT OF CROP SCIENCE.

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DEDICATION

To my mother and father (I owe them my existence), also to my sisters Stella and Anne for their unconditional love and sacrifices. God bless you abundantly, I love you all.

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I am highly indebted to Dr. C. Gwanama for providing research materials and for supervising the project up to its completion.

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ABSTRACT

Application of a casing on compost fully run with mycelia has been said to improve yields of button mushroom (*Agaricus bisporus*). A study was undertaken at the University of Zambia, School of Agricultural Sciences (Field Station) with the objective of determining the effect of using different casing materials on the yield of button mushroom. Two materials, black soil and pine bark were used as independent casing materials while the third casing comprised a mixture of 50% black soil and 50% pine bark (1:1 by volume).

Analysis revealed significant differences among the treatments for total yield. Of the three casing materials used in the study, the mixture produced the highest yields followed by black soil and lastly, pine bark. For mean yields per flush, there were significant differences in the first two flushes with mixture producing the highest yields and pine bark the least. Mean yields in the third flush were not significantly different. No significant differences were observed in both days to flushing and duration of each flush for all treatments.

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1.0 INTRODUCTION

The common commercial mushrooms, *Agaricus bisporus* (or White button mushroom) were first cultivated in France around 1700. They are achlorophyllous and thus incapable of carrying on photosynthesis. Nutritionally, they are absorptive heterophytes requiring preformed organic compounds for their energy and growth. The organic materials should be in solution to be taken into fungal cells.

Since mushrooms are fungi and do not photosynthesize, they are grown on a compost which when colonized with spawn is cased with a layer of soil to support the final stages in the life cycle involving formation of fruit bodies (Chang and Hayes, 1978).

According to Chang and Hayes (1978), fruiting of mushrooms does not usually occur in a medium best suited for mycelial growth such as well-prepared compost fully run by feeding hyphae. All fungi follow a quite general rule: fruiting initiation (formation of primordia) occurs only when the conditions of the medium present a sharp change, which is less suitable for the growth (elongation) of the mycelium.

The change described above is effected in *A. bisporus* by casing or capping spawned compost. Atkins (1972) defines casing as the operation of covering the spawned compost beds with a thin layer of sterile soil or peat. Stamets (2000) states that placing a casing layer of peat over compost grown through with mushroom mycelium greatly enhances yields. Casing fulfils the following functions;

- Maintaining the correct water-air relationship for the culture as a whole,
- Buffering climatic conditions in the growing room,
- Providing an environment suitable for the stimulation of fruit bodies and their development, and

- Providing mechanical support for the developing mushrooms, permitting the picking of mature mushrooms without harming the young ones (De Jonghe, 1997).

Important characteristics of casing materials include high water holding capacity and, at the same time, porosity to air so that aerobic respiration could be maintained in the compost.

Cultivation of edible mushrooms is a new crop diversification in Zambia, having been introduced recently from European Countries. As such, most of the technologies associated with it have not been adopted yet because they are too expensive to be implemented in Zambia. Therefore Zambian growers have to improvise using cheap and readily available local materials or resources. The common naturally occurring casing material used in Western countries is peat and its various modifications. It is regarded as being most convenient and suitable casing medium. It has a unique physical structure, chemical nature, water-holding capacity and microbiological status. This, however, is not easily obtainable in Zambia from the limited deposits in Western province. Stamets (2000) writes that mushroom cultivators in Western countries also use artificial materials and mixtures prepared from extensive research such as "water crystals" and Black Magic peat moss. Due to inaccessibility of such materials, Zambian growers should use a variety of materials and soils as casing. Such materials differ in their water holding capacity, physical, chemical and microbiological characteristics. Yield of mushrooms is related to the aforementioned attributes; therefore identification of casing materials giving high and stable yields is important in production.

1.1 OBJECTIVE

The objective of this study was to determine the effect of different casing materials on the yield of white button mushroom.

2.0 LITERATURE REVIEW

Miles and Chang (1997) describe a mushroom as a macrofungus with a distinctive fruiting body, which can be either above the ground (epigeous) or below ground (hypogeous). They have been used by man from very early times as a source of food.

All edible mushrooms belong to the kingdom MYCETAE or fungi, a group very distinct from plants, animals and bacteria. Most fungi have plant-like cells but lack the important feature of plants: ability to use energy from the sun directly through the use of chlorophyll. Thus fungi depend on other organisms for food (Miles and Chang, 1997). The edible fungi fall into two major groups, which are Ascomycetes and Basidiomycetes. There are different types of fungi and they can be grouped according to their occurrence in nature. Some fungi such as *Coprinus* grow on little composted material; some grow on humus and soil; some, such as *Pleurotus, Lentinus*, and *Coprinus* on fresh or almost fresh plant residues; some mycorrhizal fungi are *Boletes* and *Amanita*; *Agaricus* fungi grow on very well composted material (Chang and Hayes, 1978). Species from the order Agaricales, among the Basidiomycetes, form the largest assemblage of fungi whose basidiocarps are grown commercially for food. *Agaricus* is the most important genus in the family Agaricaceae.

Stamets (2000) classifies the *Agaricus* mushrooms as secondary decomposers. These are mushrooms that rely on the previous activity of other fungi to break up a substrate to a state wherein they can thrive. Secondary decomposers typically grow from composted material. The action of other fungi, actinomycetes, bacteria, and yeasts all operate within compost. As plant residue is degraded by the microorganisms, the mass, structure, and composition of the compost is reduced, and proportionally available nitrogen is increased. Heat, Carbon dioxide, ammonia, and other gases are emitted as by products of the composting process. Once these microorganisms

(especially actinomycetes) have completed their life cycles, the compost is susceptible to invasion by a select secondary decomposer (Stamets, 2000).

Composts are of various types and include a variety of different substrates (Stamets, 2000). The purpose of composting is to take organic substrates and, through the metabolic activities of many microorganisms, make them preferentially suitable for the growth of a particular mushroom species and less suitable for other potentially contaminating microorganisms. Both the chemical composition and the physical qualities of the compost substrate are important (Miles and Chang, 1997). The substrate materials for *Agaricus* are wheat straw and horse manure. It is generally believed that the most productive composts are made from fresh manure from corn-fed and hard-worked horses, which have been bedded down on wheat straw (Atkins, 1972). The physical qualities in the compost must support aerobic conditions, hold waste materials without becoming waterlogged, have good drainage, and a proper pH (Miles and Chang, 1997).

Composting is done in two phases. In Phase I, the compost is mixed and arranged in long piles outdoors to decompose for 5-10 days (Baker and Cook, 1979). The compost heap has layers of different temperatures, different microorganisms, and different aeration. The metabolic activities of the microorganisms generate heat, so, in practice, the compost is turned over to permit more even temperatures and a more even breakdown of the substrate (Miles and Chang, 1997). The temperature and moisture are carefully controlled by repeated turning and by irrigation of the piles. Baker and Cook (1979) states that because of uneven thermogenesis and cool surfaces of the piles, this stage does not free the compost of weed moulds or pathogens, but it does produce a medium unfavourable to several weed moulds. When done on concrete areas, soil-borne weed moulds that commonly cause the truffle and mat disease, and that inhibit mycelial growth of the mushroom are eliminated. If the medium is over composted, two organisms, *Papulospora byssinia* and *Scopulariopsis fimicola* (brown and white plaster moulds) develop and may

subsequently inhibit mushroom mycelia. If the medium is kept too wet and partially anaerobic, *S. fimicola* is favoured, often giving a black gelatinous medium. Addition of gypsum at the beginning of composting improves porosity and aeration of the medium and reduces the incidence of this fungus (Baker and Cook, 1979). Vedder writes that addition of gypsum increases flocculation of certain chemicals in the compost, which adhere to the straw or hay rather than filling the pores between the straws. This allows for air, which is essential to the composting process, to permeate the pile more readily.

According to Baker and Cook (1979), Phase II or compost conditioning is essentially a pasteurization process called 'peak heating'. Live steam is introduced to provide a temperature of the compost in the beds in the mushroom house that will eradicate insects and pests surviving Phase I, including vegetative spores of contaminating microorganisms. Temperatures are maintained in Phase II for periods of time that will promote further decomposition of the substrate by thermophilic microorganisms (Miles and Chang, 1997). This phase is very important as a control measure of weed moulds and parasites, but also provides continued decomposition to free the medium of ammonia and amines, and to produce the microbial protein to support mushroom mycelial growth. This phase is continued until the ammonia has been eliminated and the temperature drops, indicating reduced thermogenesis. At the end of decomposition, the medium should have 2.0-2.5% nitrogen, with less than 0.05% ammonia (dry weight). Since *Coprinus fimetarius* (ink cap), *Oedocephalum* spp. (brown mould), and *Thielavia thermophile* are able to use ammonia and amines, which are toxic to mushroom mycelia, the balance swings to favour the weeds if this process is incomplete. *C. fimaterius* is favoured by compost with a pH above 8.2 from excessive ammonia (Baker and Cook, 1979).

Compost supports the vegetative phase of *A. bisporus* growth but the establishment of the reproductive stage in the commercial system of culture is influenced by the microorganisms

colonising the casing layer (Stamets and Chilton, 1983). Eger, in 1961, first noted that the fruit body formation was dependent on the activity of microorganisms in the casing layer, which include bacteria, actinomycetes and algae (Eger, 1962). The growth of *A. bisporus* in compost and casing soil is dependent on other microorganisms which must be taken into account when considering those factors that optimise growth conditions in the commercial techniques of culture, all of which employ mixed culture methods (Chang and Hayes, 1978).

According to Stamets (2000), the casing serves several functions. Foremost, the casing layer acts as a moisture bank where water reserves can be replenished through the course of each crop. The casing layer also limits damage to the mycelium from fluctuations in relative humidity. Besides moisture, the casing provides stimulatory microorganisms and essential salts and minerals. The combined properties make casing a perfect environment for the formation and development of primordia. Atkins (1972) lists theoretical reasons for casing as being:

- Mushrooms tend to form on the surface of the compost, but they are heavy, and if there be nothing to support them they may fall over and break the delicate 'roots' through which they derive their sustenance.
- The surface of the compost dries out very readily, and it is extremely tricky to replace that moisture without killing the spawn at that level. Therefore, the presence of the casing layer prevents drying out.
- Vegetative mycelium is encouraged to fruit when it enters a medium deficient in food; it attempts to ensure its survival by producing fruit containing spores. A suitable casing medium provides this.
- Sudden reduction of temperature may stimulate fruiting. The casing loses moisture by evaporation, and after each watering a cool layer is provided which appears to shock the warmth-loving mycelium into activity.

The work of Eger (Baker and Snyder, 1965) in Germany and of Hayes *et al.* (1969) in England indicates that bacteria in the casing mixture stimulate cap formation, and are in turn stimulated by volatiles given off by the mycelia.

An ideal casing, according to Atkins (1972), is one that possesses the following characteristics:

- It absorbs water quickly and releases it slowly (by evaporation). If the material permits the applied water to run straight through it (as will sand), the surface of the compost will become waterlogged and useless.
- Its moisture-holding capacity is such that it can be watered without sealing off the compost. Carbon dioxide (and other gases), is formed in the compost during spawn running and fruiting, and these must be able to escape through the casing. Effects of high Carbon dioxide concentration include production of small caps and elongation of stipe.
- Watering does not noticeably alter its texture. Some silt that can be applied to the beds in lumpy form break down at once under the impact of water and in due course cakes and cracks.
- It is neutral in reaction. A slightly alkaline reaction (up to pH 8) is preferable to one that is slightly acid, for the pH falls during cropping.
- It is free from disease organisms and insects.

The casing layer should thus be considered as a substrate which supports not only the mushroom but also an assorted microflora which apparently benefits and supports its growth through the transition from mycelial growth to a fruit (Chang and Hayes, 1978). The choice of materials for advanced culture methods, together with required pasteurisation treatments, necessitates an appreciation not only of the materials and water-holding capacity but also the physical, chemical, and microbiological characteristics of the materials.

A dozen or so casing soils have been used successfully in the commercial cultivation of mushrooms (Stamets, 2000). The type and nature of the soil influences many of the management practices, particularly in relation to watering and ventilation. In its excavation and preparation for use as a casing layer, particular care is required to maintain structure. Soils with a good proportion of clay and not much sand have the best physical properties (Chang and Hayes, 1978). According to Atkins (1972), growers can use topsoil or subsoil dug from at least 40 cm depth, where it is practically free from disease organisms (unless cultivation has gone deeper than this). Where topsoil is used, or if the subsoil has been contaminated by the tunnelling of worms and/or by deep rooted vegetation, some means of killing the potential enemies must be resorted to. The most popular way to sterilize is undoubtedly with steam, but some growers prefer baking, others use electricity or chemicals. Temperatures approaching 100°C are required. Although steam sterilization is held to be the cheapest and most effective system, its capital cost is high. Also, uniformity of treatment is practically impossible and often times the soil becomes muddy (Atkins, 1972). Overheating of casing soil may reduce yield and mushroom size (Baker and Cook, 1979).

Peat is used in Europe and generally regarded as being the most suitable casing medium. It holds more water than most soils. It is acid; therefore, limestone or chalk is needed to neutralize (Chang and Hayes, 1978). Brady (1984) describes peat as unconsolidated soil materials consisting largely of undecomposed or slightly decomposed organic matter accumulated under excessive moisture. Peat soils on the other hand are described as organic soils containing more than 50 % organic matter. Organic soils have high water holding capacity on a dry weight basis.

Based on the writtings of Chang and Hayes (1978), the time for casing depends on the kind and quantity of spawn used, quality of compost, and conditions prevalent.

It is usually done 10 to 14 days after spawning. It can also be done immediately after spawning. Stamets (2000) outlines the benefits of immediate casing as; to assure dominance, prevent contamination, and set primordia evenly. The dry components are mixed together in a clean bucket or wheelbarrow. Water is added slowly and evenly. When water can be squeezed out to form rivulets, then proper moisture has probably been achieved. 75 % moisture content is ideal and can be tested by measuring the amount of moisture lost from s sample dried in a hot oven. Once wetted, the casing is applied on the top of a substrate. Typically, 2.5 to 5 cm layer of casing is placed onto 10 to 25 cm of colonized substrate (Stamets, 2000).

3.0 MATERIALS AND METHODS

3.1 Preparation of casing materials

Pine bark, a by-product of many wood processing industries, was obtained from Match Corporation. It was milled to diameters of 1 cm and less using a small hammer mill. This was followed by pre-wetting for a week to remove dirt and most importantly, for it to absorb moisture. Black soil was also obtained from within the field station. This is dark montmorillonitic clay prevalent over large areas of the University. The soil was dug from below a depth of 50 cm to avoid problems associated with antagonistic soil microorganisms. Since the soil was excavated at such a depth, there was no need for sterilization/pasteurization. Large aggregates present in the soil were broken down to a consistent size.

In one treatment of casing materials, the same quantities by volume (1:1) of pre-wetted pine bark and black soil were thoroughly mixed by hand. This was done on clean 220 litre drum lids. Dry black soil was also slowly and evely wetted to comprise a separate treatment. The third treatment consisted of pre-wetted pine on its own.

3.2 Casing

After full spawn (the strain used was D 649) run or full mycelial colonization of the compost, the casing materials were applied on top of the compost at a uniform thickness of 3 cm. Each of the casing materials was applied to three uniform boxes (replicates) containing 15 cm deep of spawned compost, the dimensions of the boxes being 20 cm by 75 cm by 100 cm. The nine boxes were arranged in a Completely Randomized Design in a converted mushroom growing room. Each box received 1.0 to 1.5 l of water as irrigation once every other day, the rate being based on the relative dryness of the casing materials.

3.3 Characteristics measured

(i) Days to the first, second and third flushes.

(ii) Duration of each of the three flushes.

(iii) Mean yields of mushroom (fresh weight in grams) for each of the first three flushes.

(iv) Total yield of mushroom (fresh weight in grams) per treatment from the first three flushes

3.4 Statistical Analysis

The three treatments were subjected to the F-Test using M-STAT C Programme (Fischer, 1986). The null hypothesis was that there was no difference in the performance among the three casing materials.

4.0 RESULTS

4.1 Yields Of Button Mushrooms

There were significant differences in the total yields of button mushroom obtained from the three treatments.

The casing comprising the mixture produced superior yields, followed by that of black soil and lastly, that of pine bark. In this study, the casing comprising the mixture gave total yields of 1754.3 g; that comprising black soil gave 1001.9 g; and that comprising pine bark gave 485.1g per treatment. The average yields per box are the same as those for total yields per treatment (Table 1).

For all treatments, mean yields per flush were significantly different in the first two flushes while the third flush revealed no significant differences.

Table 1: Mean yields per flush and total yields (g) of button mushroom per treatment.

Casing material	No. of flushes	First flush	Second flush	Third flush	Mean total yield (g)
Mixture	3	464.9a	1022.7a	266.7a	1754.3a
Black soil	3	269.0b	578.0b	155.0a	1001.9b
Pine bark	3	96.2c	232.4c	156.5a	485.1c
LSD		143.8	272.4	127.1	334.3
C.V (%)		45.1	38.6	57.2	26.8

LSD: Least Significant Difference at $p = 0.05$

C.V: Coefficient of Variation.

Means in a column followed by the same letter(s) are not significantly different from each other at $p = 0.05$

The mixture produced higher total yields than the other two treatments due to improvement of aeration by addition of pine bark to black soil and black soil' s high water holding capacity. The low yields recorded in pine bark casing may be attributed not only to its inability to hold sufficient moisture but also the rapid rate at which it loses it by infiltration and evaporation. Water instead of just wetting the casing, may have been draining down into the compost thereby causing sogginess, which interferes with myceial development and performance.

4.2 Flushing Intervals

Three distinct flushes were observed during which yields of button mushrooms were obtained. The results are shown in Table 2.

4.2.1 Days to Flushes

Mean days to flushes showed that there were no significant differences in the days to first, second and third flushes for all treatments.

The casing comprising the mixture was colonized earlier than the rest (15 days after casing) leading to earlier pinning and picking of mushrooms.

4.2.2 Duration of each flush

Results obtained for the mean duration (days) showed no significant differences for the treatments concerned. Duration of each of the three flushes were longer in the casing comprising the mixture. The first flush lasted five days for the mixture, three days for black soil and two days for pine bark. This trend was also shown for the second and third flushes. In the second flush, the mixture had twelve days, black soil had eight days while the least, pine bark had six days. The duration of the third flushes were shorter probably as a result of increased temperatures and corresponding reduction in relative humidity in the growing room. This was experienced during the last days of September.

Table 2: Mean duration and days to each flush of button mushroom per treatment.

Casing material	No. of flushes	First flush		Second flush		Third flush	
		Days to	Duration	Days to	Duration	Days to	Duration
Mixture	3	14.7b	4.7a	24.0a	12.3a	42.3a	4.3a
Black soil	3	19.7a	3.0ab	24.3a	8.3ab	38.7a	2.0a
Pine bark	3	20.7a	1.7b	29.0a	6.0b	39.7a	1.7a
LSD		4.8	2.3	6.6	5.4	3.6	2.7
C.V (%)		22.5	63.4	22.3	52.6	7.8	88.4

LSD: Least Significant Difference at p = 0.05

C.V: Coefficient of Variation.

Means in a column followed by the same letter(s) are not significantly different from each ther at p = 0.05.

The experiment showed significant differences in total yields and mean yields per flush among the treatments. No significant differences were observed in both days to flushes and duration of each of the three flushes.

The generally low yields obtained in the study can be attributed to the delay in casing which encouraged the occurrence of pests such as mushroom flies, mites and competitive weeds belonging to the *Coprinus* genus. Low yields encountered can also be attributed to inability to adequately mitigate (control) environmental conditions such as temperature and humidity in the growing room.

5.0 CONCLUSION

Comparing total yields per treatment, the casing mixture comprising 50% black soil and 50% pine bark was found to produce the highest yields of mushroom amounting to 1754.3 g, followed by black soil with 1001.9 g. The least yields were obtained with pine bark casing, which gave 485.1 g. The low yields obtained in pine bark casing disqualify it for use as an independent casing material. Due to the high performance of the mixture, pine bark may be recommended as a soil conditioner. Total yields per treatment were found to be significantly different while mean yields per flush were significantly different in the first two flushes only. However duration and days to flushes were not significantly different for all the three treatments.

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