Chemical, physical and microbial properties of casing materials used in the commercial production of white button mushrooms [*Agaricus bisporus* (Lange)].

by

LOUIS PIETER VAN JAARSVELD

Submitted in partial fulfillment of the requirements for the degree
M.Sc. (Agric) Plant Pathology
In the Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria

Supervisor: Prof. L. Korsten

October 2010

DECLARATION

I, Louis Pieter van Jaarsveld, declare that the thesis which I hereby submit for the Degree of Master of Science to the University of Pretoria is my own work and has not been submitted by me for a degree at this or any other tertiary institution.

Signed on _____ of _____ 2010.

_____

Louis Pieter van Jaarsveld
October 2010

Supervisor: Prof L. Korsten

ACKNOWLEDGEMENTS

I would like to acknowledge the following people mentioned below:

- Prof. Lise Korsten for her editing, expert advice, motivation and support.
- Mr. Morris Gilbert, Mr. Nichol Mulder and Mr. Hannes Sevenster of Country Mushrooms for showing me the various mushroom farming methods and cultivation techniques.
- Mr. Ross Richardson of Highveld Mushrooms and Mr. Roddy Cairns of Denny Mushrooms for their invaluable input.
- Dr. Martmari van Greuning of Sylvan South Africa for her enthusiastic support, technical input, comments and valued discussions.
- Ms. Amanda Redmond for assisting with the planning, design and building of the micro-mushroom growing unit and execution of experimental trials.
- Dr. Thierry Regnier for helping with the phenolic work during my studies.
- Prof. Terry Aveling for excellent and meaningful advice.
- Mrs. Karin Zeeman for her emotional support and for inspiring me.
- Mrs. Daleen Muller and Mrs. Amelita Lombaard for their organisational and general help in ordering equipment and supplies.
- My brother and his wife (Mr. Arthur and Ms. Sibyl van Jaarsveld) for their loyal support and encouragement.
- My father and mother (Mr. Koos and Ms. Babs) for all their daily prayers, love and financial support.
- Sincere appreciation to everyone who believed in me.
- Thanks to God who created me with big plans in mind for the future.

This work was partly supported by a grant from the South African Mushroom Farmers Association (SAMFA) and the Human Resource for Industry Programme (THRIP), a partnership programme funded by the Department of Trade and Industry and managed by the National Research Foundation (NRF). Editorial support was given by Ms. Amanda Redmond, Ms. Ané van Heerden and Dr. Linda Meyer (University of Pretoria). Statistical analysis was carried out by Ms. Marie Smith (Agricultural Research Council, Silverton, Pretoria).

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Chemical, physical and microbial properties of casing materials used in commercial production of white button mushrooms [*Agaricus bisporus* (Lange)].

Supervisor: Prof. L. Korsten

Abstract

Agaricus bisporus (Lange) Sing (white button mushroom), is the most common cultivated mushroom in South Africa and many other places in the world. *Agaricus bisporus* was first cultivated on a commercial scale in South Africa in 1940. Since then, approximately 35 commercial mushroom growing facilities have been established. These farms produce about 20 000 tons per annum. Peat is commonly used as casing material for mushroom production in South Africa. Natural indigenous peat sources are limited and expensive. Due to governmental legislation to prevent and restrict depletion of wetlands, mining thereof has been discontinued since 2006. Mushroom farmers were therefore forced to either use a local alternative waste product or imported peat. South African peat was previously pasteurised to eliminate pests and diseases. This treatment often resulted in the growth of *Chromelosporium fulvum* (Link) McGinty (*C. fulvum*) particularly when alternative casing materials such as wattle bark was used. Although the mushroom itself is not affected; this mould can significantly affect button mushroom production. A micro-mushroom growing unit was build and designed to evaluate the effect of *C. fulvum* on *A. bisporus* mycelium growth as well as to determine the yield of several casing materials due to pasteurisation.

The function of a casing material is well known and the physical and chemical parameters defined. Assessment of the influence of heat on these properties permits a broad estimation of the requirements needed for a suitable casing medium in order to produce mushrooms in a sustainable manner. This information can also provide some insight why weed moulds proliferate after pasteurization. In addition, the effect of heat on the total soluble phenols content may explain the growth of *C. fulvum* on certain materials. This investigation suggests that *C. fulvum* grows and sporulate at different rates on different casing materials. The presence of *C. fulvum* on pasteurized and unpasteurized casing

materials affect the growth of *A. bisporus* mycelium as well as the time of the 1st flush formation.

This study therefore provides some insight into the various parameters that can be used to profile an ideal casing media and the value of the system to predict whether a particular material has the potential to be used as an alternative to peat. An alternative to South African peat need to be found, however, partial substitution of peat with other materials is more promising than complete replacement. This was the first study that investigated specific heat capacities of casing materials, the effects of heat on the chemical properties as well as the use of a micro-mushroom unit to see the interaction of *C. fulvum* on *A. bisporus*. The determination of the sporulation rate of *C. fulvum* on several materials and mixes was a newly tested method to describe the preferred incidence of *C. fulvum* on certain materials.

Keywords: *Chromelosporium fulvum*, *Agaricus bisporus*, Physical, Chemical and Microbial properties/characteristics, Casing materials, Heat Capacities, Phenols

Contents

Chapter 1

1.1 Introduction 1
1.2 References 4

Chapter 2

2.1 The history of mushroom production 7
2.2 White button mushroom production 9
2.2.1 Compost 9
2.2.2 Casing 10
2.2.3 Environmental and growth conditions for mushroom production 13
2.3 Importance of peat 14
2.4 White button mushroom pests and diseases 15
2.5 Saprophytic fungi of white button mushroom 16
2.6 *Chromelosporium fulvum* 18
2.7 Control methods 19
2.8 Conclusion 19
2.9 References 19

Chapter 3

Physical characteristics of alternative casing materials used in the cultivation of *Agaricus bisporus*

3.1 Abstract 27
3.2 Introduction 27
3.3 Materials and Methods 27
3.3.1 Cultivation of *Agaricus bisporus* 29
3.3.2 Desorption curves 30
3.3.3 Physical characterisics 30
3.4 Results 32
3.4.1 Cultivation of *Agaricus bisporus* 32
3.4.2 Desorption curves 32

3.4.3 Physical characteristics 34
3.5 Discussion 36
3.6 References 39

Chapter 4

Chemical characteristics of several casing materials used for *Agaricus bisporus* production

4.1 Abstract 43
4.2 Introduction 43
4.3 Materials and methods 45
4.3.1 Elemental analysis of soil and plant materials 45
4.3.2 Measuring the pH of alternative casing materials at different heat treatments 46
4.3.3 Measuring the electrical conductivity of alternative casing materials at different heat treatments 46
4.3.4 Total soluble phenolic compounds 47
4.4 Results 48
4.4.1 Total elements 48
4.4.2 pH 50
4.4.3 Electrical conductivity 51
4.4.4 Total soluble phenols 52
4.5 Discussion 54
4.6 References 57

Chapter 5

Effect of pasteurisation on the role and presence of *Chromelosporium fulvum* on different casing materials used in the production of *Agaricus bisporus*

5.1 Abstract 61
5.3 Materials and methods 62
5.3.1 Heat capacity of casing material 62
5.3.2 Obtaining a pure *Chromelosporium fulvum* culture 63
5.3.3 Sporulation curve of *Chromelosporium fulvum* 64
5.3.4 Effect of *Chromelosporium fulvum* inoculum on casing material and *Agaricus bisporus* growth 64
5.4 Results 65
5.4.1 Heat capacities 65
5.4.3 Sporulation curves of *Chromelosporium fulvum* 67
5.4.4 Effect of *Chromelosporium fulvum* inoculum on casing material and *Agaricus bisporus* growth 69
5.5 Discussion 71
5.6 References 72

Chapter 6

6.1 General discussion 74
6.1 References 78

Appendix 1 81
Appendix 2 84
Appendix 3 86
Appendix 4 90
Appendix 5 94
Appendix 6 95
Appendix 7 97

Chapter 1

1.1 Introduction

Mushrooms are large reproductive structures of edible fungi belonging to either Ascomycotina or Basidiomycotina (Salunkhe & Desai, 1984; Arjunan & Dinakaran, 1999). Fungi are heterotrophic organisms (Mader, 2001) which are able to grow in complete darkness and without chlorophyll and are also incapable of producing their own carbohydrates (Chang & Miles, 2004). Therefore, the fungus need to accumulate carbohydrates, proteins, fats and nutrients from a suitable substrate (Atkins, 1955; Vedder, 1978). Mushrooms occur seasonally all over the world varying in colour, appearance and edibility (Arjunan & Dinakaran, 1999; Mattila *et al*., 2000; Cummins, 2006). From the 983 known edible and medicinal mushroom types described in China (Mao, 2001; Chen *et al*., 2008), more than 60 varieties of mushrooms are grown commercially (Chen *et al*., 2008). *Agaricus bisporus* (Lange) Sing., *Lentinus edodes* (Rerk.) Singer and *Pleurotus ostreatus* (Jaccquin ex Fries) are the three most commonly cultivated cultivars in China. Most of China's mushroom produce is destined for their domestic markets (Huang, 2005; Wang 2005). Consumption and production of mushrooms have also increased significantly in Europe, the United States of America (USA) and other parts of the world (Vedder, 1978; Eicker, 1993).

Although the cultivation of *Agaricus bisporus* has been known for centuries, it is only after the last half of the twentieth century that it has become commercially important (Vedder, 1978; Chang & Miles, 2004). This is mainly due to technical developments in terms of growing medium, production practices, growing rooms, selected cultivars, disease control, packaging technology and cold chain distribution. Commercial mushroom production in South Africa only became prominent in the 1940's. Currently, mostly white button mushrooms are produced for local consumption (Country Foods Prospectus, 2007).Major problems associated with commercial mushroom production in South Africa include pests, diseases and shortage of available and suitable casing materials.

Spoilage fungi associated with mushroom cultivation can be divided into two distinct groups according to the damage they cause (van Greuning, 1990). The first group

includes pathogens such as *Diehliomyces microsporus* (Preuss) Hassebrauk (dry bubble), *Trichoderma aggressivum* (green mould), *Cladobotryum dendroides* (Bull.) W. Gram & Hooz (cobweb) and *Mycogone perniciosa* Magnus (wet bubble) (Coles & Barber, 2002; Pieterse, 2005). These pathogens usually cause either malformation or death of the mycelium or inhibit mycelia growth in the mushroom beds (Van Greuning, 1990). The second group includes all saprophytic fungi (weed moulds) such as *Sporendonema purperascens* (Bonord.) E. W. Mason & S. Hughes, *Pythium oligandrum* Drechsler, *Corticium* mould and *Chromelosporium fulvum* (Link) McGinty (cinnamon brown mould) (Van Greuning, 1990; Beyer, 2002). These organisms are usually present in compost and casing material and live in competition with the mushroom mycelium for available nutrients. The presence of weed moulds in compost are usually an indication of poor quality compost (Van Greuning, 1990).

In order to produce a good quality white button mushroom, *Agaricus bisporus* mycelia need to be covered with a suitable casing layer (Verbeke & Overstyns, 1991). Originally, mushroom growers in Europe used their local soil as casing (Kinrus, 1971). Research carried out in the 1950's resulted in the discovery of sphagnum peat as casing soil (Flegg, 1951). This resulted in the commercial replacement of topsoils with sphagnum peat in mushroom production (Labuschagne, 1995; Bellmont, 2005).

Sphagnum peat is found predominantly in the Northern Hemisphere (Grundling, 2005). In the Southern Hemisphere spaghnum peat is scarce and mostly protected to prevent wetland depletion. Natural indigenous peat sources in South Africa are therefore limited (Eicker & Van Greuning, 1989) and no longer available to the South African mushroom industry as a casing soil, since being declared part of South Africa's protected wetlands. New government legislation (Section 31A of the Environment Conservation Act, No. 73 of 1989 and Section 28 of the National Environmental Management Act, No. 107 of 1998) to prevent and restrict depletion of wetlands (Wetland Legal Regime, 2001; Theron, 2005) and mining thereof, has stopped all commercial operations from supplying peat to South African mushroom growers at the end of 2006. Since then peat had to be imported for the South African mushroom grower at substantial higher costs from overseas sources (Van Greuning, 2007. Pers. comm.).

In addition, foreign exchange rates and labour costs increased the price of imported peat significantly. This had an enormous impact on the economic viability of mushroom production in South Africa (Cairns, 2007. Pers. comm.). Therefore, the South African mushroom industry was forced to either find alternative peat sources or develop alternative casing materials. Screening for alternative peat sources using waste products such as coir, wattle bark, bagasse and filtercake, has since become a critical requirement for the successful and sustainable production of mushrooms in South Africa. The scientific assessment of the substrate's chemical, physical and microbial properties are critical in the characterisation of suitable alternative casing material. These factors include water holding capacity, water retention, water release, pH, electrical conductivity, phenolic content, heat capacity and yield. The combination of these factors must be compared to standard peat soil in order to provide a potential alternative.

Techniques must be developed to assess peat quality since the material tested must comply with certain criteria in order to be regarded as a suitable casing material. Varying chemical and physical properties of the casing media and mixes to be used for mushroom production will have an influence on the yield and growth of commercial white button mushrooms. Similarly, it will influence the etiology and presence of weed moulds such as cinnamon brown mould (*C. fulvum*).

The aim of this study was therefore to determine the physical, chemical and microbial properties of peat and to establish a framework for comparative purposes to assess potential alternative casing products. This information is vital for the development of agricultural and industrial waste products to be used as alternative casing and casing blends for the South African mushroom industry. The growth and sporulation rate of *C. fuvum* was examined in this study on the different casing materials.

1.2 References

Arjunan, G. & Dinakaran, D. 1999. Commercial production of spawn and mushroom. Department of Crop Protection, ADAC & RI, Tiruchirappalli. 74pp.

Atkins, F. C. 1955. Mushroom Growing To-day. Faber & Faber. 24 Russell Square, London. 200pp.

Bellmont, B. 2005. Peat moss cultivates an earth-friendly reputation. Mushroom News 53(11):12-29.

Beyer, D. M. 2002. Pest Species Biology and Control. Weed and Indicator moulds. Mushroom Integrated Pest Management Handbook. The Pennsylvania State University. University Park. 91pp.

Cairns, R. 2007. Pers. comm.

Chang, S. T. & Miles, P. G. 2004. Edible Mushrooms and Their Cultivation. CRC Press, Inc. Boca Raton, Florida. 345pp.

Chen, L., Xie, B. & Wach, M. P. 2008. A general review of the Chinese mushroom industry. Proceedings of the 17th Congress of the International Society for Mushroom Science. 932pp.

Coles, P. S. & Barber, W. 2002. Pest Species Biology and Control. Fungal Pathogens. Mushroom Integrated Pest Management Handbook. The Pennsylvania State University. University Park. 91pp.

Country Foods Prospectus 2007. At: <http://www.countryfoodsholding.com/Docs/COUNTRY%20FOODS%20 combined.pdf>. accessed 1 October 2007.

Cummings, S. 2006. Unveiling Mushrooms' Nutritional Benefits Helps Generate Consumer Demand. Mushroom News 54(10):18-20.

Eicker, A. & Van Greuning, alternatives for topogenous peat as casing material in the cultivation of *Agaricus bisporus* in South Africa. South African Journal of plant and soil 6(2):129-135.

Eicker, A. 1993. Mushrooms as a source of protein for Africa? African Journal of Mycology and Biotechnology 1:12-23.

Flegg, P. B. 1951. Casing soil. Mushroom station, Yaxley, Annual report. pp 26-30

Grundling, P-L. 2005. Transcript from 50/50. Date: Sunday, February 06, 2005. (www.5050.co.za/inserts.asp?ID=6150.Cached - Similar pages) Producer: Rehana Dada.

Huang, N. L. 2005. Most promising medicinal mushrooms for development in China. Edible Fungi 27(1):3–4.

Kinrus, A. 1971. Some important facts about a casing soil. Mushroom News 19(3):3-6.

Labuschagne, P. M. 1995. Casing media for *Agaricus bisporus* cultivation in South Africa. MSc thesis. University of Pretoria. 55pp.

Mader, S. S. 2001. Biology 7th edition. McGraw-Hill, Inc. NY. 946pp.

Mao, X. L. 2001. About the proper names of edible and medicinal in China. Edible Fungi 23:1–8.

Mattila, P., Suonpa, K. & Piironen, V. 2000. Functional Properties of Edible Mushrooms. Nutrition 16(7/8).

Pieterse, Z. 2005. Mycogone perniciosa, a pathogen of Agaricus bisporus. MSc. Thesis, University of Pretoria. 104pp.

Salunkhe, D. K. & Desai, B. B. 1984. Postharvest Biotechnology of Vegetables 2:147-159.

Samp, R. 2006. Recent developments and future possibilities in the *Agaricus Spp*. (Button) mushroom industry. Mushroom News 54(3):12-23.

Theron, P. 2005. Transcript from 50/50. Date: Sunday, October 02, 2005. (http://5050.co.za/print.asp?table=AllInserts&ID=6619&referrer=/insert.asp?ID.. . 2006/09/07). Producer: Rehana Dada.

Tschierpe, H. J. 1981. Cultural practices from casing to cropping. Proceedings of the Eleventh International Congress on the Science and Cultivation of Edible Fungi. Australia. Mushroom Science XI:463-491.

U.S. Geological Survey, 1998

Van Greuning, M. 1990. A Mycological study of some pathogens and weed moulds of the cultivated mushroom *Agaricus bisporus*. PhD thesis. University of Pretoria.

Van Greuning, M. 2007. Pers. comm.

Vedder, P. J. C. 1978. Modern Mushroom Growing. Educaboek, Culemberg. The Netherlands. 420pp.

Verbeke, M. N. & Overstyns, A. 1991. Interrelationship between activated charcoal, carbon dioxide, oxalate and iron chemistry of *Agaricus bisporus*. Mushroom Science 13(2):737-746.

Vetter, J. 1994. Mineral elements in the important cultivated mushroom *Agaricus bisporus* and *Pleurotus ostreatus*. Food Chemistry 50(3):277-279.

Wang, Z. S. 2005. The status quo and developmental trend of internal and international mushroom industry. Mycosystema 24:1–6.

Wetland legal regime, 2001. The legal regime applicable to wetlands and wetland rehabilitation (March 2001).

LITERATURE REVIEW

2.1 The history of mushroom production

Mushrooms are thought to have originated 570 million years ago and since the early tertiary era, fossilised imprints of the lamellae of Basidiomycete have been found (Mader, 2001). The first known cultivation of mushrooms in the western world started on the outskirts of Paris in France (Atkins, 1974; Tschierpe, 1981; Chang & Miles, 2004). In 1650, Bonnefons gave the first account of *Agaricus* button mushroom cultivation and stated that mushrooms occurred naturally in horse manure, which was used as the inoculum source. The Frenchman, J. P. De Tournefort (1707) is regarded as the first person in the western world to provide a comprehensive description of commercial production of edible mushrooms. Some of the basic principles are still applicable in mushroom cultivation today (Salunkhe & Desai, 1984; Chang & Miles, 2004). In 1780, a French gardener named Chambry, cultivated button mushrooms in underground quarries in Paris (Vedder, 1978; Flegg *et al*., 1985). These environments provided favourable growing conditions and ensured all-year production.

The English were the first to commercially supply *Agaricus* spawn (in brick form) from their extensive natural wild supplies. However, it was of uncertain vigour and strain and was often contaminated by other moulds and insects (Chang & Miles, 2004). In 1894, pure spawn was successfully prepared from *Agaricus* mushroom spores in France. In America, this procedure was improved and commercialised in 1905. Since then, the *Agaricus* mushroom industry developed into a major commercial production sector (Chen *et al*., 2008). Even though mushrooms consist of a large diverse group of fungi, only five species have been commercially cultivated outside China during the 1980's (Salunkhe & Desai, 1984; Arjunan & Dinakaran, 1999). Currently the world mushroom production has increased to include more than 20 species commercially cultivated (Chang & Miles, 2004; USDA, 2009).

In China, more than 60 species of mushrooms are commercially cultivated of which *Pleurotis ostreatus* (3.9 million tons), *Lentinula edodes* (2.5 million tons) and *A.*

bisporus (1.6 million tons), ortant. *Agaricus bisporus* was not commercially cultivated in China until the 1930's and then only in small areas with yields as low as 3.3 kg/m^2 (Chen *et al*., 2008). Initially, fresh mushroom (edible and medicinal) production in China totalled only 60000 tons in 1978 (contributing 5.7% of the world's production), in 2002, 8.65 million tons (contributing 70.6% of the world's production) (Chen *et al*., 2008) and in 2007, 16 million tons (FAO, 2009). In terms of world production, China is the recognised leader in mushroom cultivation (Huang, 2005; Wang 2005), followed by the USA (3.9 million tons), Holland (2.4 million tons), Spain (1.6 million tons), Poland (1.3 million tons) and France (1.2 million tons) (FAO, 2009). In South Africa, *A. bisporus* was commercially cultivated for the first time by Mrs. Robertson in 1940. The business was bought by Monty Denny and he became the leading button mushroom producer in South Africa (Pieterse, 2005; Labuschagne, 1995). Since then, 35 commercial mushroom growing facilities have been established in South Africa. Currently, these farms produce about 380 tons of white button mushrooms per week and approximately 20 000 tons per annum (Country Foods Prospectus, 2007). Although considered small by world standards, the South African mushroom industry saturates the local market.

Agaricus bisporus is the most common cultivated mushroom in South Africa (Pieterse, 2005; Van Greuning, 2007. Pers. comm.). The two-spored form was first described by J. E. Lange as *Psalliota hortensis* Lange, but was renamed *Agaricus bisporus* (Lange) Sing. in I949 by Rolf Singer. The International Botanical Congress in Paris later confirmed this re-classification in 1954 (Atkins, 1974; Chang & Hayes, 1978). In this study, reference is made to the white button mushroom species as *Agaricus bisporus* (Lange) Sing. (Kendrick *et al*., 2003).

The first modern mushroom houses where temperature, humidity and CO_2 concentrations could easily be manipulated and controlled (Van Greuning, 1990; Noble, 1991) were developed in 1754. This provided favourable growing conditions for all year production (Atkins, 1974; Vedder, 1978).

Substrates used: Compost and casing

Compost made from conditioned wheat straw for mycelium growth and a suitable casing layer on top of the compost for the mushroom mycelium to fructify, was found to be ideal (Verbeke & Overstyns, 1991; Pardo *et al*. 2003; Sabeh *et al*., 2006).

2.2.1 Compost

Compost used for button mushroom cultivation in South Africa is available in large quantities. The compost consists of cereal wheat straw [*Triticum aestivum* L. subsp. *durum* (Desf.) Thell or *T. monococcum* L. subsp. *aegilopoides* (Link)], chicken manure and gypsum [United Kingdom (UK) uses lime] (Fletcher *et al*., 1989; Sevenster, 2007. Pers. comm.). Poultry manure is a cheap source of nitrogen and is exclusively added to raw compost materials in most countries (Samp, 2006). Too much nitrogen in the compost increases the amount of ammonia, which results in no yield or a very poor mushroom yield (Noble & Gaze, 1996). Before the wheat straw can be used as a suitable selective medium for mushroom production it must go through a composting or fermentation process. This procedure takes place within 14 days in two distinct phases; i.e. phase I and phase II (Fletcher *et al*., 1989; Székely *et al*., 2008).

- **Phase I compost**

The wheat straw is thoroughly hydrated when mixed with chicken manure and gypsum. It is placed into long narrow piles and turned several times with mechanical compost turners. During the fermentation process, which normally takes seven to ten days, the core is heated to 76℃, which is sufficient to kill most pest and pathogens. However, the outside layers never reach such high temperatures; therefore an extra phase is necessary (Fletcher *et al*., 1989; Jess *et al*., 2006).

- **Phase II compost**

Phase II compost is referred to as peak heating, pasteurisation or sweat-out, produced in environmental conditioned control rooms. The air temperature of the compost is increased to 60°C for eight hours after which the room temperature is maintained at 49°C for three days (UK use 52°C) until the compost is both suitable and selective for white button mushroom mycelium growth. Each farm uses different management practices in composting based on economical considerations and available facilities (Jess *et al*., 2006).

- **Spawn run**

Spawn is mushroom mycelium cultured on sterile rye (*Secale cereale* L. var. *vulgare* Körn. & H. Werner) or milled grain (*Pennisetum glaucum* (L.) R. Br.). In South Africa, Sylvan Africa Pty Ltd. produces commercial spawn (Van Greuning, 2006. Pers. comm.). Spawn running can be defined as the colonisation of the compost from the grain inoculums. After the completion of phases I and II composting, spawn is mixed v/v in an economically viable ratio into the compost. The compost temperature is maintained at 24°C with a high relative humidity to prevent compost from drying out and high CO_2 levels are maintained by recirculation of the air in the room to promote quick colonisation of the compost (Fletcher *et al*., 1989; Sabeh *et al*., 2006). Spawning and spawn run normally takes approximately 10-14 days depending on the mushroom strain used, the ratio whereby it is mixed with the compost, compost quality, compost conditioning and management practices applied on the farm (Fletcher *et al*., 1989). This procedure can be lengthened to 14-16 days.

- **Phase III compost**

Phase III compost is the compost after spawn has been introduced and the spawn run is completed. Phase III compost is then cased with a suitable casing medium (Sabeh *et al*., 2006).

2.2.2 Casing

Casing takes place on phase III compost after the spawn run is completed. The mycelia of *A. bisporus* do not produce sporophores, unless they are covered with a suitable casing layer (Hayes, 1973; Sinden, 1982; Masaphy & Levanon, 1989; Verbeke & Overstyns, 1991; Labuschagne, 1995; Pardo *et al*., 2003). When a casing material is placed on the mushroom compost's vegetative mycelium, a

profound, irreversible changing bodies form (Sinden, 1982). Casing material is usually applied as a 4-5 cm thick layer on top of the surface of the colonised compost (Kurtzman, 1995). Different countries use a variety of materials and mixes as casing material (Vedder, 1978). The casing plays an important role in the yield and quality of the mushrooms produced and the compost will be more productive if the casing is well chosen. It will also influence the size, production cost as well as the mushroom spread over a bed. When choosing a casing material it should be in constant supply and uniform in structure (Wuest & Beyer, 1996; Van Gerwen, 2006).

There are various causes of pinhead formation; Firstly, the difference in the CO_2 concentration between the beds and the air in the room as well as between the compost and the casing layer (Tschierpe, 1973; Vedder, 1978; Bechara *et al*., 2006); Secondly, the casing contains certain bacteria (*Pseudomonas putida* Trevisan & Migula) and volatile metabolites, which promotes and stimulates pinhead formation (Riber Rasmussen *et al*., 1971; Masaphy & Levanon, 1989; Pardo *et al*., 2003). Thirdly, it is considered that a link may exist between available nutrients and fructification (Vedder, 1978). Finally, factors such as moisture content, degree of evaporation and changes in climate could lead to fructification (Vedder, 1978; Pardo *et al*., 2003). Mycelium initiation to fruit bodies does not solely depend on genetic capacity but also on the physical, chemical, environmental, nutritional and microbial factors of the casing soil (Pardo *et al*., 2003). The potential to produce high quality mushrooms hugely depends therefore on the type of casing material used as well as the preparation thereof (Botha, 1984).

The casing material always consists of three layers. The bottom layer is in contact with the compost and must ensure strong, uniform mycelium growth. The middle layer acts as a reservoir for water while the top layer is where gases are exchanged (Hayes, 1993; Labuschagne, 1995). Casing provides sufficient physical support for emerging fruit bodies by maintaining a suitable, moist microclimate and containing adequate nutritional supplements to help feed the mycelium in a low osmotic environment (Pardo *et al*., 2003). It maintains a healthy bacterial micro flora, which includes mainly *Pseudomonas* species (Masaphy & Levanon, 1989). The casing provides enough water for growth and development of mushrooms (Samp, 2002; Pardo *et al*., 2003) as well as a suitable environment for gas interchange (Flegg,

1951; Kurtzman, 1995). A sidered to have low soluble ions and a low conductivity (Masaphy & Levanon, 1989). The optimal casing soil or material should not be nutritionally inert; it should rather have a lower nutrient value relative to the compost (Hayes, 1973; Pardo *et al.*, 2003). Ultimately, the casing also serves as a protective layer for the compost (Van Gerwen, 2006). Good casing soil requires that pin set should be easily controlled or manipulated and not stain or taint the mushroom sporocarps (Samp, 2002). The casing material must comply with certain physical, chemical and biological requirements to fulfil its function successfully (Masaphy & Levanon, 1989; Pardo *et al.*, 2003).

In the past farmers used local mineral soils or topsoils as a casing material. A mineral soil consists predominantly of inorganic materials and contains less than 20% organic matter (Van der Watt & Van Rooyen, 1995). A mineral soil is much denser than conventional casing and is difficult to mix and handle. It requires a different water regime and can obtain acceptable yields but unfortunately not on a consistent basis. In addition, a mineral soil dries out much more rapidly and thus needs lighter but more frequent irrigation. However, the mushrooms are much cleaner when harvested (Huerta *et al.*, 2001). Research carried out in the 1950's by the Mushroom Research Association in Yaxley, Peterborough (UK), led to the replacement of topsoils and loamy soils with sphagnum peat and limes (Labuschagne, 1995; Bellmont, 2005). Farmers using sphagnum peat moss reported a decrease in mushroom diseases and an increase in mushroom production (Bellmont, 2005). With its high success rate, it quickly developed into a well-established practice throughout the mushroom industry, especially in Europe.

There are four different types of peat depending on the source of origin (Brady & Weil, 2002; Moore, 1995). If the parent material was moss (sphagnum) it is classified as a moss peat. The residues of plants (reeds, sedges and cattails) are classified as herbaceous peat, the remains of trees and shrubs are classified as woody peat and lastly, the remains of algae and faecal material of aquatic animals are classified as sedimentary peat (Galvan, 1976; Brady & Weil, 2002). Unfortunately, South Africa has no indigenous peat moss, only reed sedge peat which is classified as a topogenous peat. This reed sedge peat is of an inferior quality with high clay content and often results in dirty mushrooms (Eicker & Van Greuning, 1989).

Peat moss is generally free from pests and diseases (Hayes, 1993). Almost all the viable alternatives to peat are known to carry some pest or disease of mushrooms. Therefore, these alternatives need to be pasteurised before use as a casing medium (Hayes, 1991; Kinrus, 1971). A very precise temperature treatment is thus required for optimum results (Hayes, 1991). The heat treatments can be applied either as dry or moist heat. Moist heat is more efficient and non-selective for micro-organisms (Schisler, 1978). However the casing soil should contain adequate moisture and contain no clods as this serves as a reservoir for pests (Schisler, 1978). Only a few Dutch mushroom growers steam their casing soil (Vedder, 1978). Steam is used in order to eradicate most of the pathogens, in particular *Mycogone perniciosa* (Magnus) Delacroix or wet bubble which is a problem in the South African industry where the practice is applied (Pieterse, 2005).

2.2.3 Environmental and growth conditions for mushroom production

Modern mushroom cultivation is focused on efficient production using less energy. Growers strive towards higher yields from the same cultivation area and decreased use of raw material with improved conversion into mushroom material (Samp, 2006). The most vital factor involved in high mushroom yields is the correct preparation of the casing and compost (Gibbons *et al*., 1991). In general, casing occurs approximately 14 days after spawn running. During case-running the casing itself is colonised by the mycelium for seven days. The environmental conditions are the same as for spawn-running (Fletcher *et al*., 1989; Chang & Miles, 2004). When the mycelium reaches the surface, the air temperature is lowered to between 16°C and 18°C. Also the CO_2 concentration is lowered by allowing fresh air to enter the room (Fletcher *et al*., 1989; Sabeh *et al*., 2006) which induces fruitbody development.

Fruitbody development or pinning commences between 11-14 days after casing in well defined flushes or breaks. Each break lasts for 7-10 days followed by the next break. Crops are usually terminated after three breaks due to disease development and economic considerations. With each successive break the yield is reduced (Fletcher *et al*., 1989; Royce *et al*., 2007) and the risk of disease increases. The casing is continuously watered at set time intervals (Kinrus, 1971; Chang & Miles, 2004).

2.3 Importance of peat

- **Peat availability and distribution in South Africa and around the world**

In 1991, peat lands covered over 400 million hectares of the earth's land surface (Robertson, 1993). Ten years later it was estimated at 340 million hectares (Brady & Weil, 2002). About one third of the world's soil carbon is stored in mires, mostly in the Northern Hemisphere (Grundling, 2005). Mires store water and releases it slowly as needed by the ecosystem (Grundling, 2005). Unfortunately, South Africa has limited natural indigenous peat (Eicker & Van Greuning, 1989). Most of the local wetlands fall under sensitive ecological sites (Wetland legal regime, 2001) and are now protected (www.polity.org.za/govdocs/white papers/diversity3.html). On the South African Highveld, peat grows less than a millimetre a year (Grundling, 2005).

- **Protection of wetlands**

There are 15 Ramsar Wetland Sites in South Africa (Ramsar Convention). However, the majority of wetlands in South Africa that needs to be protected, fall outside these protected areas (www.polity.org.za/govdocs/white papers/ diversity3.html). In the past about 45000 m^3 of peat have been mined annually from peat mines in the North-West Province (Grundling, 2005). Peat bog mining damage and deplete wetlands through digging, excavating and mining operations conducted (Border, 1993). However, peat farmers in South Africa used floating excavators to harvest peat moss to minimize damages to the wetlands. Despite this, government and environmentalists have since stopped all legal peat mining operations (www.polity.org.za/govdocs/white papers/diversity3.html) in South Africa and have thereby forced the mushroom industry to search for alternative casing materials (Grundling, 2005). Eicker and Van Greuning predicted the depletion of South African wetlands in 1989, urging industry to develop alternative source. Today, this is a reality and South African mushroom farmers are importing peat from Europe and Canada due to the lack of an effective alternative casing material (Eicker & Van Greuning, 1989; Van Greuning, 2007. Pers. comm.).

2.4 White button mushroom pests and diseases

There are many mushroom fungal and bacterial pathogens, but only a few of them affect production detrimentally (Salunkhe & Desai, 1984; Coles & Barber, 2002). Some of the most common white button mushroom diseases caused by fungi, bacteria and viruses are shown in Table 2.1.

Table 2.1: Common diseases of *Agaricus bisporus* (Coles & Barber, 2002; Wuest, 2002; Gaze & Fletcher, 2007)

General name	Scientific name
Fungal diseases	
Brown spot, Dry bubble	*Verticillium fungicola* (Preuss) Hassebrauk var. *fungicola*
Green Mould	*Trichoderma aggressivum* f. *aggessivum* Samuels & W. Gams
Cobweb mould, Soft decay, Soft mildew	*Cladobotryum dendroides* (Bulliard 1791) W.Gams et Hooz
Wet Bubble	*Mycogone perniciosa* (Magnus) Delacroix
Bacterial diseases	
Bacterial Blotch	*Pseudomonas tolaasii* (*P. fluorescens* biotype G)
Mummy and False Mummy	*Pseudomonas* spp. related to *P. tolaasii* but not the same
Bacterial Pit	*Pseudomonas* spp.
Viral diseases	
La France Mushroom virus X	

2.5 Saprophytic fungi of white button mushroom

White button mushroom production can be significantly affected by the presence of weed moulds by delaying pinhead formation as a result of spawn overgrowth (Howard *et al*., 1994). The presence of a weed mould may be due to certain nutritional, chemical or physical conditions of the compost or casing material. Weed moulds usually dominate when the compost is poorly prepared and serve as an indication that certain modifications are necessary in composting, hygiene or cultural practices (Van Greuning, 1990; Beyer, 2002). In most cases modifications to the compost process during phase I or phase II result in a reduction of their occurrence. Some weed moulds occur when the compost is under-composted, the C: N ratio is too high or where overheating during the spawn-run period occurred (MAFF Canada, 1993; Beyer, 2002). In addition, delayed or a decrease in the growth of the white button mushrooms will promote the growth of some weed moulds and *vice versa* (Coetzee, 1987). Some weed moulds reported in or on the compost and casing material are shown in Table 2.2.

Table 2.2: Weed moulds and casings used during the production of *Agaricus bisporus*.

General name	Scientific name	
	Weed moulds	
Ink cap, ink weed, wild mushrooms	*Coprinus fimetrius* Fr., C. *radiatus* (Bolton) Grey	(Wuest, 1990) (Beyer, 2002)
Olive green mould	*Chaetomium globosum, C. oliveaceum*	(Beyer, 2002)
Penicillium mould	*Penicillium* spp.	(Howard *et al*., 1994)
Black whisker mould	*Doratomyces microsporus (Sacc.) Morton*	(Fletcher *et al*., 1989)
	D. stemonitis, D. Purpureofuscus,	(Beyer, 2002)
Brown mould	*Oedocelphalum spp., O. fimetarium*	(Beyer, 2002) (Howard et al., 1994)
Lipstick mould, red lipstick	*Sporendonema purpurascens (Bonord.) Mason*	(Howard *et al*., 1994) (Beyer, 2002)
Brown Plaster mould and Flour moulds	*Papulaspora byssina, Scopulariopsis spp. Botryotrichum piluliferum, Trichothecium roseum*	(Wuest, 1982) (Beyer, 2002)
Cinnamon brown mould	*Chromelosporium fulvum* (Link)McGinty	(Van Griensven, 1988b)
	Chromelosporium fulva	(Beyer, 2002)

2.6 Chromelosporium fulvum

In order to control unwanted fungi successfully, early detection, correct identification and knowledge of their general biology are essential factors. Each fungus has its own optimal growth conditions and environmental requirements in order for it to grow and thrive (Stoller, 1969; Van Greuning, 1990).

Chromelosporium fulvum (peat mould, cinnamon brown mould or brown mould (Coetzee, 1987) is one of the most abundant fungi known in the mushroom industry and occurs mainly on the casing material (Van Griensven, 1988b; Beyer, 2002). It is opportunistic can grow on any surface, especially on too wet casings, where a strong formalin solution has been used or where the natural competition has been eliminated in some way (Stoller, 1969; MAFF Canada, 1993).

Cinnamon brown mould was first described in South Africa by Eicker and Coetzee in 1983 (Coetzee, 1987). Numerous cases of *C. fulvum* occurring in mushroom farms in the Cape and Gauteng have since been reported (Van Greuning, 2006. Pers. comm.). The mould is quickly renewed through vigorously growing mycelium and vast spore production and can rapidly spread from one infection point throughout the whole growing room (Vedder, 1978; Coetzee, 1987; Linfield, 2000). It retards mushroom growth and reduces yields (Stoller, 1969) and produces masses of spores that can cause respiratory allergies in workers during heavy outbreaks (Oei, 1996; Linfield, 2000; Moore *et al*., 2005; http://niaid.nih.gov/ publications/ allergens/ mould. htm). The first sign of *C. fulvum* infection on a mushroom bed is a round white mass of mycelium, which expands rapidly. Sporulation causes a change in colour from white to light yellow or golden brown. As the spores mature the colour turns to a darker golden brown or cinnamon colour with granular appearance (Wuest & Bengston, 1982; Van Greuning, 1990; Pieterse, 2005). It usually retains a white colour at the edges where it is still actively growing (Vedder, 1978; Pieterse, 2005). This mould grows fast and usually disappears within ten days (Wuest & Bengston, 1982). Severe outbreaks of this mould in casing trials conducted at the University of Pretoria prompted a need to investigate its role and presence in this study.

2.7 Control methods

The best approach in an effective disease control programme is a preventative strategy. However, once the disease has been established it is often crucial to eliminate the inoculum source by means of fungicides (Linfield, 2000). During the protection of a commercially cultivated crop that is also a fungus like *A. bisporus,* disease control becomes more complicated since some chemicals that control *C. fulvum* infection will also influence the crop (Coetzee, 1987; MAFF Canada, 1993). It is therefore a challenge to develop a selective fungicide that only targets the pathogen and not also negatively affect the crop (MAFF Canada, 1993; Staunton & Dunn, 1999; Coles & Barber, 2002). Control of the invading fungi is normally maintained by regulating the environmental conditions in such a way that the habitat is unsuitable for the germination of their spores and their growth (Van Greuning, 1990). The use of chemicals (fungicides, fumigants), the implementation of good hygiene practices and the adaptation of the growing environment are basic procedures that can be followed to effectively control weed fungi in mushroom beds (Vedder, 1978).

2.8 Conclusion

The initial concern for the sustainability and economic viability of our natural peat resources was brought forward from the South African mushroom industry. The protection of wetlands through legislation furthermore encouraged the search for alternatives. Studies have been conducted by the Mushroom Research Group at the University of Pretoria to find alternative materials to incorporate into peat to reduce peat usage. These materials incorporated with local peat were assessed for physical and chemical characteristics to determine their suitability as a casing material in mushroom production. The effect of heat during the pasteurisation process on the chemical and physical properties were also assessed to determine the influence of cinnamon brown mould on mushroom yield.

2.9 References

Arjunan, G. & Dinakaran, D. 1999. Commercial production of spawn and mushroom. Department of Crop Protection, ADAC & RI, Tiruchirappalli. 74pp.

Atkins, F. C. 1974. Guide to mushroom growing. Faber & Faber. 3 Queen Square, London. 122pp.

Bechara, M., Heinemann, P., Walker, P. & Romaine, C. P. 2006. Non-Composted Grain-Based Substrates For Mushroom Production (Agaricus bisporus). Transactions of the American Society of Agricultural and Biological Engineers. 49(3): 819-824.

Bellmont, B. 2005. Peat moss cultivates an earth-friendly reputation. Mushroom News 53(11):12-29.

Beyer, D. M. 2002. Pest Species Biology and Control. Weed and Indicator moulds. Mushroom Integrated Pest Management Handbook. The Pennsylvania State University. University Park. 91pp.

Border, D. 1993. Alternatives to peat. Mushroom Journal. July. 1993. 523:23-24.

Botha, W. J. 1984. Die fisiologiese en morfologiese studie van vier probleemfungusse in die sampioenbedryf. MSc thesis. University of Pretoria. 183pp.

Brady, N. C. & Weil, R. R. 2002. The nature and properties of soils. 13th edition. Prentice Hall. Upper Saddle River. New Jersey. 960pp.

Chang, S. T. & Hayes, W. A. 1978. The Biology and cultivation of edible mushrooms. Academic press, New York. 521pp.

Chang, S. T. & Miles, P. G. 2004. Mushrooms: Cultivation, Nutritional value, Medicinal effect and Environmental impact. 2nd Edition by CRC Press. 451pp.

Chen, L., Xie, B. & Wach, M. P. 2008. A general review of the Chinese mushroom industry. Proceedings of the 17th Congress of the International Society for Mushroom Science. 932pp.

Coetzee, J. C. 1987. Die biologie van enkele Fungussoorte wat by die Kommersiele verbouing van Agaricus bisporus in Suid-Afrika probleme skep. MSc thesis. Universiteit van Pretoria. 219pp.

Coles, P. 2002. Introduction to Mushroom IPM. Mushroom Integrated Pest Management Handbook. The Pennsylvania State University. University Park. 91p.

Coles, P. S. & Barber, W. 2002. Pest Species Biology and Control. Fungal Pathogens. Mushroom Integrated Pest Management Handbook. The Pennsylvania State University. University Park. 91pp.

Country Foods Prospectus 2007. At: <http://www.countryfoodsholding.com/Docs/COUNTRY%20FOODS%20 combined.pdf>. accessed 1 October 2007.

Eicker, A. & Van Greuning, M. 1989. Economical alternatives for topogenous peat as casing material in the cultivation of *Agaricus bisporus* in South Africa. South African Journal of plant and soil 6(2):129-135.

Flegg, P. B. 1951. Casing soil. Mushroom station, Yaxley, Annual report. pp 26-30.

Flegg, P. B., Spencer, D. M. & Wood, D. A. 1985. The Biology and Technology of the Cultivated Mushroom. John Wiley & Sons, Inc., New York. 347pp.

Fletcher, J. T., White, P. F. & Gaze, R. H. 1989. Mushrooms: Pests and Disease control. 2nd edition. Intercept, Andover, Hants, UK. 174pp.

Galvan, L. F. 1976. Physical Properties of Irish Peats. Irish Journal of Agricultural Research 15:207-221.

Gaze, R. H. & Fletcher, J. T. 2007. Mushroom Pest and Disease Control: A Color Handbook. Academic Press. 192pp.

Gibbons, W. R., Maher, A. A. & Todd, R. L. 1991. Button Mushroom Production in Synthetic Compost Derived from Agricultural wastes. Bioresource Technology 38:67-77.

Grundling, P-L. 2005. Tra e: Sunday, February 06, 2005. (www.5050.co.za/inserts.asp?ID=6150.Cached - Similar pages. Producer: Rehana Dada.

Hayes, W. A. 1973. The casing layer and its function in mushroom nutrition. Mushroom News 21(12):9-14.

Hayes, W. A. 1991. Some thoughts on peat and the quest for an alternative. The Mushroom Journal. July 1991. 223:25-27.

Hayes, W. A. 1993. An overview of the casing layer. The Mushroom Journal. July 1993. 523:26-29.

Howard, R. J., Garland, J. A. & Seaman, W. L. 1994. Mushroom. Chapter 26. Diseases and Pests of Vegetable Crops in Canada. The Canadian Phytopathological Society, Canada.

Huang, N. L. 2005. Most promising medicinal mushrooms for development in China. Edible Fungi 27(1):3–4.

Huerta, F., Spaulding, T. & Kelly, C. 2001. Investigations on casing soils with a high mineral content. Mushroom News 49(10):4-9.

Jess, S., Murchie, A. & Bingham, J. 2006. Potential sources of sciarid and phorid infestations and implications for centralised phases I and II mushroom compost production. Crop protection 26(4):455-464.

Kendrick, B. 2003. The Fifth Kingdom. Sidney-by-the-Sea .British Columbia, Canada, V8L 1M8 (electronic copy).

Kinrus, A. 1971. Some important facts about a casing soil. Mushroom News 19(3):3-6.

Kurtzman, R. H. 1995. Aga[illegible]). Casing Layer, II: Porosity, The most important character. International Journal of Mushroom Science 1(1):11-17.

Labuschagne, P. M. 1995. Casing media for *Agaricus bisporus* cultivation in South Africa. MSc thesis. University of Pretoria. 55pp.

Linfield, H. J. 2000. The 1999 Sinden award lecture. The Mushroom Journal, April 2000. 603:13-19.

Mader, S. S. 2001. Biology 7th edition. McGraw-Hill, Inc. NY. 946pp.

MAFF (Ministry of Agriculture and Food) Canada 1993. Ontario mushroom pesticide recommendations. MAFF Publication 367, revised January 1993.

Masaphy, S. & Levanon, D. 1989. Proceedings of the Twelfth International Congress on the Science and Cultivation of Edible Fungi. Braunschweig, Germany. Mushroom Science XII(1):417-426.

Moore, J. E., Rao, J. R., Millar, B. C. & Elborn, J. S. 2005. Hypersensitive Pneumonitis: An update on mushroom worker's lung in new exotic edible fungi: New insights into an old disease. Mushroom Journal. June 2005. pp 15-18.

Noble, R. 1991. Manipulation of temperature at controlled CO_2 level to syncronise the flushing pattern of the mushroom *Agaricus bisporus*. Scientia Horticulturae 46:177-184.

Noble, R. & Gaze, R. H. 1996. Preparation of mushroom (*Agaricus bisporus*) composts in controlled environments: Factors influencing compost bulk density and productivity**.** International Biodeterioration and Biodegradation 37(1-2):93-100.

Oei, P. 1996. Mushroom cultivation. Tool-CTA Publications, Leiden. 274pp.

Pardo, A., de Juan, A. & F cterisation of different substrates for possible use as casing in mushroom cultivation. Food, Agriculture and Environment 1(1):107-114.

Pieterse, Z. 2005. Mycogone perniciosa, a pathogen of Agaricus bisporus. MSc. Thesis, University of Pretoria. 104pp.

Riber Rasmussen, C., Slack, C. I. & Mitchell, R. 1971. Early casing of mushroom compost. Mushroom News. July 1971. pp 4-21.

Robertson, R. A. 1993. Peat, horticulture and environment. Biodiversity and Conservation 2:541-547.

Royse, D., Sanchez, J. & Beelman, R. 2007. Re-supplementing and re-casing mushroom (*Agaricus bisporus*) compost for a second crop. World Journal of Microbiology and Biotechnology. 24(3):319-325.

Sabeh, N., Wheeler, E. & Beyer D. 2006. Environmental Conditions within Small-Scale *Agaricus Bisporus* Mushroom Production Rooms. Applied Engineering in Agriculture. 22(6): 907-917.

Salunkhe, D. K. & Desai, B. B. 1984. Postharvest Biotechnology of Vegetables 2:147-159.

Samp, R. 2002. Obtaining the optimum casing soil. Mushroom News 50(6):22-23.

Samp, R. 2006. Recent developments and future possibilities in the *Agaricus Spp*. (Button) mushroom industry. Mushroom News 54(3):12-23.

Schisler, L. C. 1978. Casing soil treatment. Mushroom News. December 1978. pp 21-31.

Sevenster, H. 2007. Pers. comm.

Sinden, J. W. 1982. Th significance. Mushroom News 30(11):14-17.

Staunton, L. & Dunn, R. 1999. Intergrated disease and pest control in Irish mushroom tunnels. Kinsealy Research Centre. Teagasc. 20pp.

Stoller, B. B. 1969. The science and practice of mushroom growing. 1(1).

Székely, A. J., Sipos, R. & Berta, B. 2008. DGGE and T-RFLP Analysis of Bacterial Succession during Mushroom Compost Production and Sequence-aided T-RFLP Profile of Mature Compost. Microbial Ecology 57(3):522-533.

Tschierpe, H. J. 1981. Cultural practices from casing to cropping. Proceedings of the Eleventh International Congress on the Science and Cultivation of Edible Fungi. Australia. Mushroom Science XI:463-491.

Van der Watt, H. & Van Rooyen, T. 1995. A Glossary of Soil Science. 2nd Edition. The Soil Science Society of South Africa, Pretoria. 464pp.

Van Gerwen, H. 2006. Casing: Don't underestimate the value of casing soil. Mushroom business. June 2006.17:6 -7.

Van Greuning, M. 1990. A Mycological study of some pathogens and weed moulds of the cultivated mushroom *Agaricus bisporus*. PhD thesis. University of Pretoria.

Van Greuning, M. 2006; 2007. Pers. com.

Van Griensven, L. J. L. D. 1988. The Cultivation of Mushrooms. Darlington Mushroom Laboratories, Ltd., Rustington, Sussex, England. 515pp.

Van Griensven, L. J. L. D. 1988b. Wet bubble (*Mycogone perniciosa*). The Cultivation of Mushrooms. pp 401-402.

Vedder, P. J. C. 1978. M[illegible]ng. Educaboek, Culemberg. The Netherlands. 420pp.

Verbeke, M. N. & Overstyns, A. 1991. Interrelationship between activated charcoal, carbon dioxide, oxalate and iron chemistry of *Agaricus bisporus*. Mushroom Science 13(2):737-746.

Wang, Z. S. 2005. The status quo and developmental trend of internal and international mushroom industry. Mycosystema 24:1–6.

Wuest, P. J. & Bengston, G. D. 1982. Penn State Handbook for the Commercial Mushroom Growers. Pennsylvania Agric. Exp. Sta., University Park, Pennsylvania. 129pp.

Wuest, P. J. & Beyer, D. M. 1996. Manufactured and Recycled Materials Used as Casing in (*Agaricus bisporus*) Mushroom Production. 10pp.

Wuest, P. J. 1990. Sanitation and Hygiene at the Mushroom Farm. Mushroom News. April 1990. pp 23-25.

Wuest, P. J. 2002. Pest Species Biology and Control. Bacterial Diseases. Mushroom Integrated Pest Management Handbook. The Pennsylvania State University. University Park. 91pp.

Wetland legal regime, 2001: The legal regime applicable to wetlands and wetland rehabilitation (March 2001).

(www.au2002.gov.za/docs/key_oau/nepad3.htm) Accessed: June 2007.

(www.polity.org.za/govdocs/white_papers/diversity3.html) Accessed: June 2007.

(http://niaid.nih.gov/publications/allergens/mould.htm). Accessed: June 2007.

Chapter 3

Physical characteristics of alternative casing materials used in the cultivation of Agaricus bisporus

3.1 Abstract

The physical characteristics of a casing soil (i.e. peat) form a few of the many important factors to consider when deciding on a quality casing material. The use and incorporation of alternative materials to local peat could affect the sustainability and economic viability of button mushroom production. The different physical properties of these alternative materials need to be assessed and determined. Changes in the texture and structure could affect production. Numerous physical tests have been carried out on various casing materials and mixes in order to determine bulk density, natural unit weight and oven dried weight, water holding capacities, water uptake, water release and void ratios. In addition, an apparatus was build to determine the accumulated water loss in the tested material over a specific time period. From these measurements, the total potential water present, percentage water uptake as well as the potential water available for mushrooms development was calculated. The yield of these materials and mixes were determined by the Mushroom Research Group of the University of Pretoria and brought into context with the physical characteristics in this chapter to profile good casing soils. The use of less peat will have a positive influence on environmental and economical factors in the South African mushroom industry.

3.2 Introduction

Casing material provides physical support for mushrooms, ensuring adequate air ventilation and gas exchange (Pardo *et al*., 2003). The casing material regulates water absorption and temperature moderations (Kurtzman, 1995) whilst supplying essential nutrient elements to the mushroom mycelium and providing protection by decomposing or adsorbing organic toxins (Soeroes, 2005). Casing also suppresses toxin producing organisms and harbours beneficial microorganisms (Brady & Weil, 2002). Mushrooms utilise water throughout growth and fruiting body formation. Peat is commonly used as a casing material due to its porosity and high water holding capacity (Kinrus, 1971; Hayes, 1993; Kurtzman, 1995; Labuschagne, 1995;

Bellmont, 2005). Unfortunately, as a limited supply of indigenous peat and alternative casing materials had to be identified to ensure sustainability of the industry (Eicker & Van Greuning, 1989). Therefore, the different physical properties of these alternative materials need to be assessed and determined beforehand.

The physical properties of a soil will determine for which purpose it could best be used (Mcbrierty *et al*., 1996; Brady & Weil, 2002). The structure and texture of soil is dependant on pore space between soil particles. Each soil type also has a different architectural structure. This will determine the water holding capacity and efficiency of the soil. In addition it will determine how effective water and air can be conducted through the soil as well as the stability of the casing soil (Flegg, 1951; Brady & Weil, 2002). Peat structure is highly complex and cannot be characterised by a single parameter (Puustjarvi, 1992). Metabolic gases that influence primordium formation and mushroom growth are affected by the casing as well (Wuest & Beyer, 1996).

The water potential of casing hugely influences the uptake of water by the colonised mycelium (Noble *et al*., 1999; Noble & Dobrovin-Pennington, 2004; 2005). Depending on the growth stage of *A. bisporus* (Lange) Sing, fruiting bodies can obtain between 17-46% of water from the casing material (Kalberer, 1987). Water potential from casing material is in general much higher than that of the substrate. Although water is more readily available from the casing to the fruiting body it acquires more water from the substrate (Kalberer, 1987; Kurtzman, 1995). The weight of the fruit bodies is directly proportional to the amount of water extracted from the casing material (Kalberer, 1990). Mushrooms consist of 90-95% water (Kalberer, 1991). About 2 ℓ of water is required to produce 1kg of mushrooms (1 ℓ for evaporation and 1 ℓ is absorbed). Therefore, to produce a first flush of 17 kg/m^2, 34 ℓ of water is required (Van Gerwen, 2006b). White button mushroom production requires specific environmental conditions and is commercially grown where temperature, humidity and CO_2 concentrations can easily be manipulated and controlled (Van Greuning, 1990; Noble, 1991).

The objectives of this study were therefore to develop a standardised framework for physiochemical analysis of potential casing materials for commercial mushroom growing.

3.3.1 Cultivation of *Agaricus bisporus*

Several alternative casing materials were obtained from various sources and research was conducted at the University of Pretoria during 2003 till 2007 (Appendix 1). The mushroom growing unit and alternative casing materials used in experimental mushroom growing are described in Appendix 2. The mushroom growing unit was disinfected with Desogerm® solution (20 mℓ/10 ℓ) (Des-O-germ Pty. Ltd, Johannesburg) before and after use. Similarly, the trays were disinfected before placing them into polyethylene plastic bags to minimize contamination during the cropping cycle. Growing trays (42-49 trays depending on the amount of casing samples tested) were placed in double plastic bags prior to filling to avoid contact with the metal. Growing trays were filled with 10 kg two week old spawned compost (phase III) obtained from Country Mushroom farm. The phase III compost blocks were placed in the smaller mushroom trays (10 kg per basket), covered with polyethylene plastic and transported to the University of Pretoria. The filled baskets were placed inside the mushroom growing unit and left for 72 h for the mushroom mycelium to re-colonise. Compost was lightly watered to prevent it from excessive dehydration. Different casing materials and mixes were prepared and applied evenly over the surface of the compost (4-5 cm thick). Each material (Appendix 2) was allocated in a random block design to seven trays. Each trial was completed in triplicate and was carried out over a four year period at the University of Pretoria by the Mushroom Research Group (Appendix 7).

The growing cycle for mushrooms from the day of casing to the end of the second flush takes on average thirty days. General growth and management practices for commercial mushroom growing in South Africa were followed by monitoring compost temperature, air temperature, percentage relative humidity and percentage CO_2. General commercial watering regimes were followed as required and based on information provided by the South African mushroom growers. Mushrooms were harvested when the sporocarps reached an average diameter of 3-4 cm and were cut just above the ring on the stipe (vertically along the bottom of the cap to ensure uniformity). Harvested mushrooms from each of the seven trays per treatment for the first two flushes were counted and weighed. Yield was expressed in kg/m^2 after 30% of the weight was added to the final yield to account for the flush cut stalks.

The experiment was terminated after the second flush (4-5 weeks after casing) to prevent the build-up and risk of diseases as well as to save time.

3.3.2 Desorption curves

One of the functions of the casing material is to provide a water reservoir for the mushrooms. The casing must be able to retain water as well as release it gradually. A simple but effective method and apparatus was used to determine this property i.e. desorption. An apparatus and method described by Ralph Noble from the Horticultural Research International, University of Warwick, United Kingdom (UK), was further developed and used in this study (Appendix 4). Water uptake, water retention and water release was determined for each material (Appendix 4 and 5) and compared to South African reed-sedge peat (control).

3.3.3 Physical characterisics

In order to determine the physical characteristics of a casing material a clear distinction has to be made from materials used in their natural wet state, expressed as the unit weight (natural) or saturated unit weight and the natural dry state as the unit weight (dry) or bulk density (Galvin, 1976; Bowles, 1984). In addition, oven dried density was determined by drying these materials in an oven for 24 h at 50ºC. These three densities were expressed in g/cm^3 (Schimilewski & Gunter, 1988). Densities of these materials (Appendix 5) were determined by measuring the weight of material contained in 283.83 cm^3 plastic cylinders.

The water holding capacity was calculated by firstly desiccating the material contained in the plastic cylinders after saturation and secondly after leaching the water content. This was done in triplicate. The maximum water holding capacity was calculated using the following calculation: Weight of water present in soil when water-logged or saturated, divided by the weight of the oven-dried soil.

Due to the different bulk density variations, water retention curves of different materials was reported on a volumetric basis (Valat *et al*., 1991). The volume of water per 283.83 cm^3 sample was determined and transformed to 1 cm^3. The water holding capacity (WHC) as well as its ability to release available water (WA) gradually to the mushrooms is an important characteristic of a good casing layer (Beyer, 2002) and was therefore used as criteria in this study.

The void ratio was determined by completely saturating the casing samples with distilled water and measuring its moisture content at saturation. The volume of voids is equal to the volume of water at saturation, which in turn, equals the weight of water at saturation. The void ratio was calculated by multiplying the moisture content at saturation (% dry weight) with the specific gravity of the material (Galvin, 1976; Valat *et al*., 1991).

The water ratio is defined as the water volume ratio to the volume of the solid. The pore space is equal to the total volume of water present when soil is water-logged, divided by the volume of the cylinder (expressed in %)(Flegg, 1951). The water ratio and the pore space were calculated accordingly (Galvin, 1976).

The physical structure of the soil particles determines the volume of water retained in the soil. Matrix potential is a way to quantify and understand the relationship between the water and the surface it is attracted to (Metelerkamp, 2009). Desorption curves were determined over a period of time for the casing materials and mixes (Appendix 5) and were expressed as water loss over time.

Data was statistically analysed using Statistica 8.0 by Statsoft Inc. Analysis of variance was performed on the mean averages and differences between means were determined with Fisher's t-test at a 5% level of significance.

3.4 Results

3.4.1 Cultivation of *Agaricus bisporus*

Yields of different casing materials and their mixes: Mushroom yield from trials conducted on different casing materials and mixes (Appendix 3) in the mushroom growing unit (Appendix 2) by the Mushroom Research Group to attain comparable yield results is shown in (Appendix 7 and Appendix 3). Mushrooms were able to grow on several agricultural waste materials mixed with peat and this included a wide range of chemical and physical parameters (Appendix 3).

3.4.2 Desorption curves

Each alternative casing material tested on its own and their mixtures in a 70:30 v/v and 50:50 v/v ratio were individually compared to the South African peat. Results from this experiment are depicted in Figure 3.1. The bagasse and bagasse (70:30) treatments had a similar water loss trend. However, when peat was blended in a (50:50) ratio with the bagasse the water released in each interval was higher. Filtercake (70:30) had a similar water loss trend as the peat. Filtercake (50:50) had a higher water loss, while the filtercake had more water loss due to its high porosity. Coir (70:30) and coir (50:50) showed a similar water loss trend as the peat. However, the water release from the coir, coir (70:30) and coir (50:50) was much higher than that of the peat. The water released from coir (70:30) was slightly higher. The coir had a steep increase in water release as the intervals increased. This indicated that even though coir had a high water holding capacity it released water more easily. Wattle and its mixtures had a similar water release curve as the peat. Wattle (70:30) and wattle (50:50) had similar water release curves. However, the wattle released more water than the peat but less than the wattle (70:30) and wattle (50:50) mixes. Topterra released much more water than the peat. Spent compost had a similar water release curve as the peat whilst Soyafodder and dam soil released less water. The mine dump tailing released much less water.

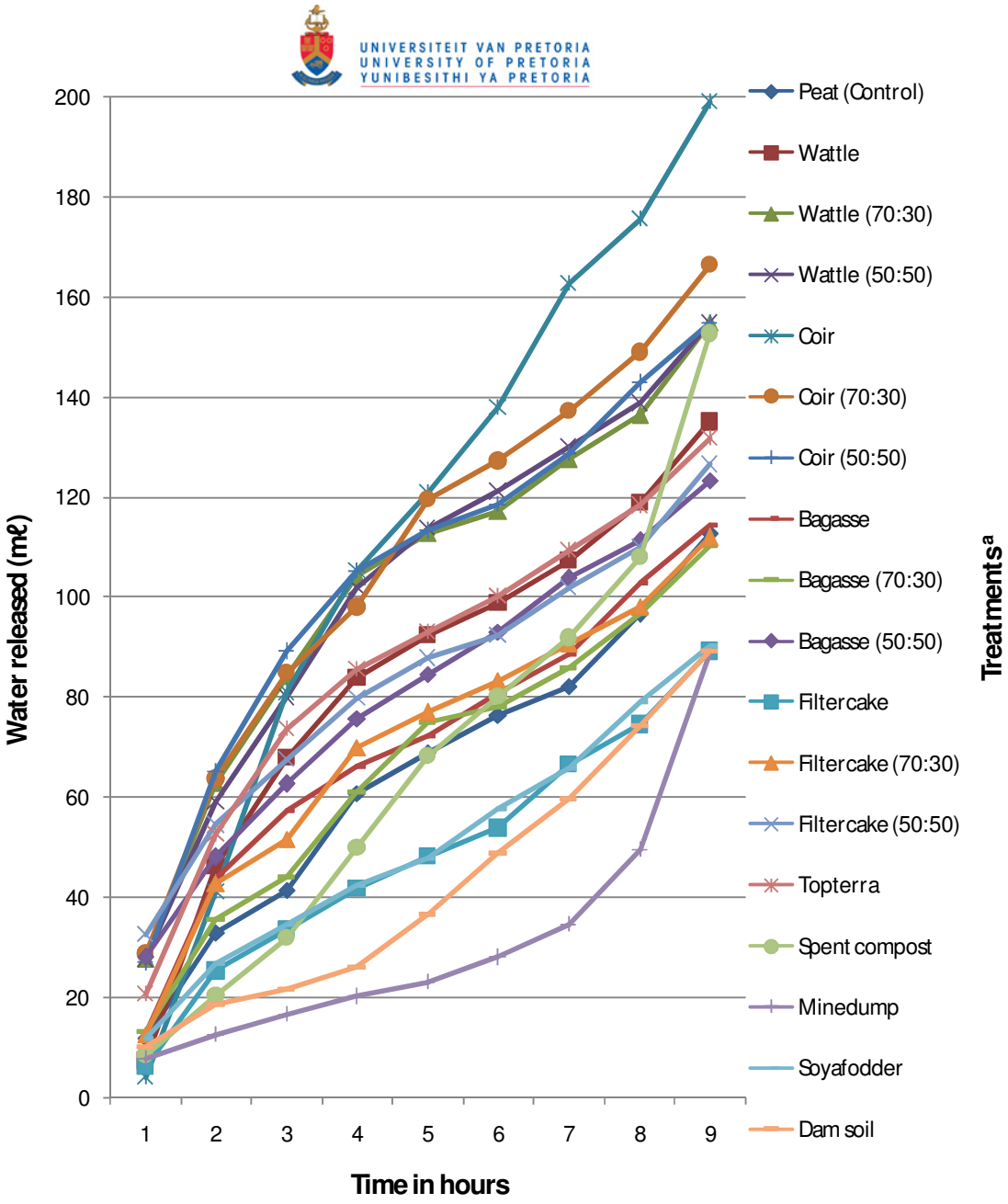

Figure 3.1: Accumulated water loss of several saturated casing materials and mixes over a time period of nine hours subjected to the modified desorption apparatus. [a] Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2 and 5.

3.4.3 Physical characteristics

Wattle bark had the smallest bulk density (0.37 g/cm^3), followed by coir (0.40 g/cm^3). Wattle bark had a water holding capacity (WHC) of 2.07 kg/kg and coir, 9.55 kg/kg. Topterra had a low bulk density of 0.73 g/cm^3, WHC of 1.97 kg/kg and water retention (WR) of 0.37 kg/kg. The South African control peat had a bulk density of 1.03 g/cm^3, WHC of 0.89 kg/kg and a WR of 1.16 kg/kg. The bulk density of wattle bark and coir increased when mixed with South African peat. Their WHC, water uptake and water release decreased while their WR increased (Appendix 3).

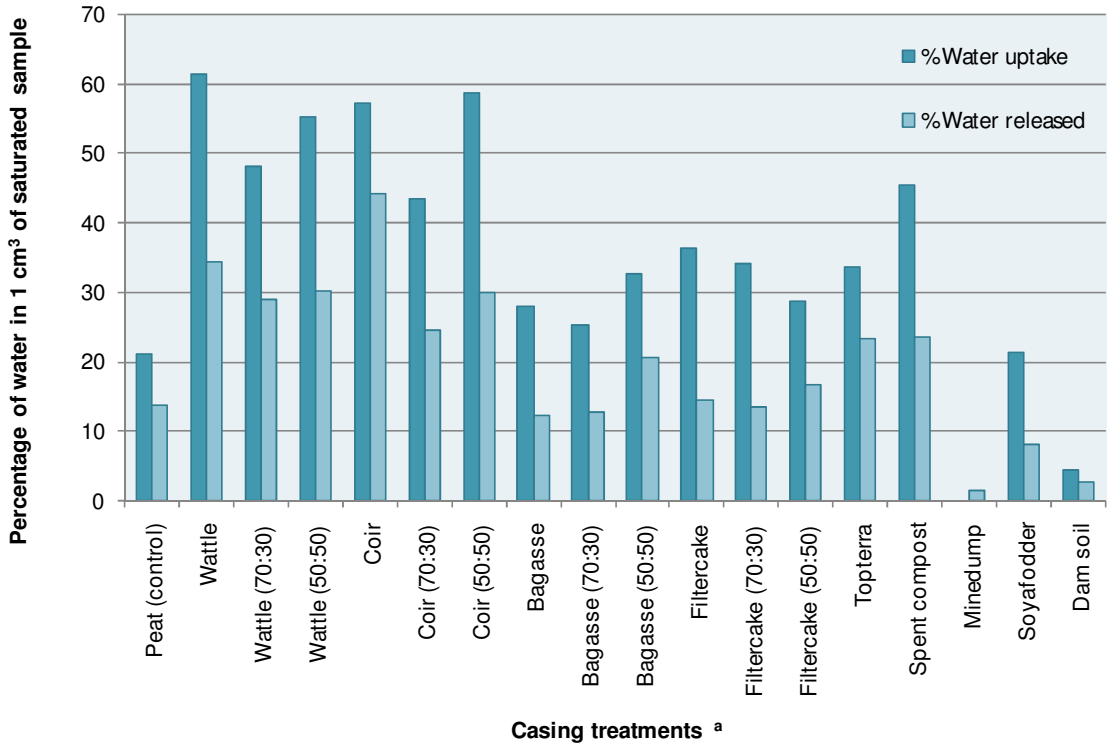

Figure 3.2: Water uptake and water release of several casing materials and their mixtures. The difference between the water uptake and water released depicts the amount of water remaining in the sample after it was leached in percentage. [a] Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2 and 5. [b]Mean values and statistical analysis is shown in Appendix 3.

When comparing the dry weight and oven dry weight of several casing materials it was observed that the mine dump, Topterra, peat, coir, soyafodder and dam soil contained the most water in the samples in their natural dry state (before drying)

than the filtercake (70:30), , wattle (70:30), wattle (50:50), coir (70:30), coir (50:50), bagasse (70:30), bagasse (50:50) which had less water in their natural dry state before drying. In comparing the dry weight to the saturated weight of several casing materials and their mixtures we found that the mine dump tailing had no uptake of water in its natural wet state (±0 %) after it was soaked in water for 24 hours. The dam soil had very little water uptake (±5 %). Peat, bagasse (70:30), bagasse and soyfodder had a moderate water uptake (±21-25 %). Filtercake (70:30), filtercake (50:50) and bagasse (50:50) had more than moderate water uptake (±29-36 %). Coir (70:30), coir (50:50), coir, wattle (70:30), wattle (50:50), wattle and spent compost had the most water uptake (±44-61 %) (Figure 3.2). However, when water is expressed as a percentage of weight of a specific volume (10 cm^3) of a saturated material it is easier to comprehend.

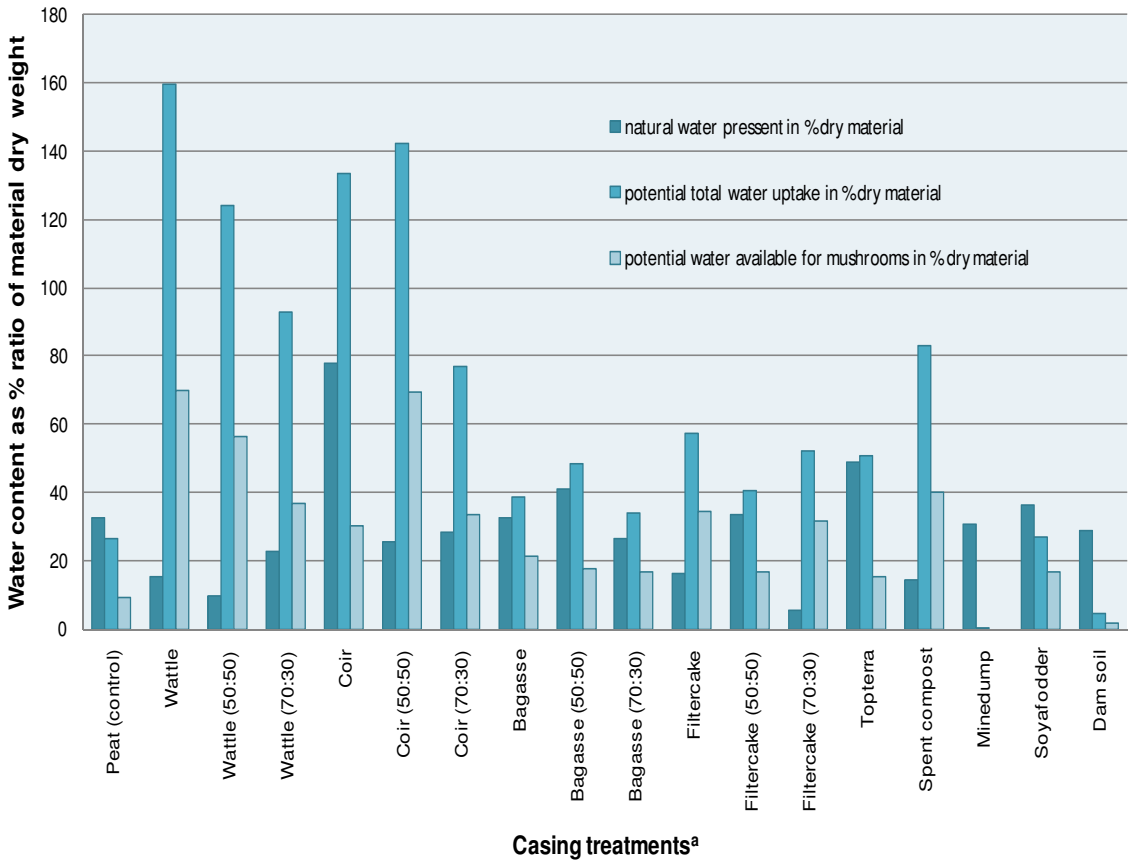

Figure 3.3: Potential water composition of several casing materials as % of their bulk density. [a]Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2 and 5. [b]Mean values and statistical analysis is shown in Appendix 3.

3.5 Discussion

The partial substitution of South African peat with other fibrous materials resulted in a slight to moderate change in desorption characteristics. This was found as a dependant factor on the rate of substitution and the characteristic of the material used as a source of substitution. When a material with similar desorption characteristics to peat is mixed with peat, the change will be to a lesser extent (Noble & Dobrovin-Pennington, 2004; 2005). However, pure filtercake had more water loss due to its high porosity. When filtercake was mixed with peat (50:50 v/v and 70:30 v/v ratios) the water retention characteristics improved. Although pure coir had a lower water retention rate than peat, at near saturation, under applied suction, it held much less water. The water retention of coir mixtures (50:50 v/v and 70:30 v/v ratios) were slightly higher, whilst the water release was more gradual, compared to the pure coir (with an increase of the matrix potential). Similar results were obtained by Labuschangne (1995) for pure coir and by Noble & Dobrovin-Pennington (2004) with coal mine dump tailings.

Results obtained in this study indicate that the water retention characteristics of materials with high water retention could be improved by adding a fibrous material with lower water retention characteristics and *vice versa*. The desorption curve characteristics of casing materials are important to determine the suitability of the material for optimal production of *A. bisporus* (Noble & Dobrovin-Pennington, 2004). Desorption curve characteristics of peat-based casing materials were previously described by Noble & Dobrovin-Pennington (2004; 2005). In this study the methodology was adopted and optimised for local use. However, the optimum matrix potentials were not determined as the results were expressed in the amount of water released over a specific time period, under increased suction.

Saturation of wattle bark and filtercake materials took longer compared to the coir, topterra, soyafodder and peat samples. In 2002, Samp indicated that mixing a casing mixture with warm or cold water, the duration of hydration time as well as the amount, type and quality of lime added, will influence the quality and productivity of the casing. It was found in this study that substrates with high organic content such as coir, especially wattle bark, was difficult to re-hydrate after being dried. Coir, wattle bark and filtercake needed to be soaked in water (heated to boiling point) for 24-48 hours before being used. Similar problems occurred in trials conducted by

Labuschagne (1995). When water is added onto dry filtercake, the water tend to run from the surface. A possible explanation for this is in the way water is bound and absorbed in the host matrix during the process of water loss during drying. Drying has different effects on different colloids (Puustjarvi, 1992; Misnikov, 2005). Excessive drying cause the characteristics natural capillary-porous soils such as peat to change into irreversible water-repelling colloids (Puustjarvi, 1992; Gamayunov *et al*., 1993; Misnikov, 2005). Similar observations were noted for filtercake and wattle bark. Also, an increase in temperature has an influence on the mobility of the different forms of water in the peat (Mcbrierty *et al*., 1996). This might explain why boiling water could re-hydrate the wattle bark and filtercake. Therefore, moisture content, particle size, porosity, fibrosity and organic fraction will have a marked effect on the water retention of the casing material.

The differences in water release/retention characteristics will result in casing materials requiring different watering regimes. Denser materials such as South African peat and bagasse, tend to pan the water on the surface whilst a porous material such as filtercake, needs to be watered in smaller but more frequent intervals. The type of casing and the irrigation methods used, volume of water and application times will influence mushroom production (Gibbons *et al*., 1991; Linfield, 2000; Huerta *et al*., 2001; Wuest, 2002; Samp, 2002; Samp, 2006).

The physical properties of the mixed materials improved when peat was blended in a 50:50 v/v ratio with alternative casing materials. Similar results were obtained with other materials (Levanon *et al*., 1986; Labuschagne, 1995; Noble & Gaze, 1996; Gulser & Peksen, 2003; Pardo *et al*., 2003; Noble & Dobrovin-Pennington, 2004; 2005). The local South African peat was found to have substantial buffering capacity and represented more typical soil characteristics than a typical peat. The bulk density of wattle bark and coir increased when mixed with South African peat. Their WHC, water uptake and water release decreased whilst their WR increased. No correlation between a single physical characteristic and yield could be found. Gulser & Peksen (2003) reported that a high WHC alone is not enough to determine high yields. They included porosity and organic mater content to have important effects on yield.

The difference between w ration and the amount of water released during the desorption test, is indicative of the water retention (WR) characteristic of the material. This is the amount of water which is gradually released and potentially available for the mushrooms. The pore space ultimately determines the amount of water absorbed (Bowles, 1984; Flegg, 1951).

Physical parameters are not influenced by only one but many interrelated factors such as the structure, organic composition, organic fraction, types of colloids, soluble substances and water characteristics of the material (Bowles, 1984; Brady & Weil, 2002). It was concluded that the physical parameters of a material ultimately determine the functionality and the use thereof in terms of commercial exploitation (Mcbrierty *et al*., 1996; Brady & Weil, 2002). Noble and Dobrovin-Pennington showed in 2004 that partial replacement of peat with an agricultural waste by-product from sugar beet as well as from the tailings from the coal mining industry, is possible. Partial substitution of peat with products similar to the ones used in this study have both environmental and economical benefits.

This study suggests that alternative by-products from agricultural wastes (Levanon *et al*., 1986; Labuschagne, 1995; Noble & Gaze, 1996; Gulser & Peksen, 2003; Pardo *et al*., 2003; Noble & Dobrovin-Pennington, 2004; 2005) can be incorporated into South African peat to support and improve structural, physical, chemical and microbial characteristics, making it a more suitable casing material for white button mushroom production.

3.6 References

Bellmont, B. 2005. Peat moss cultivates an earth-friendly reputation. Mushroom News 53(11):12-29.

Beyer, D. M. 2002. Casing Management. Mushroom News. October 2002. 50(10):8-15.

Bowles, J. E. 1984. Physical and Geotechnical Properties of Soils. 2nd edition. McGraw-Hill international editions. Civil Engineering Series. 578pp.

Brady, N. C. & Weil, R. R. 2002. The nature and properties of soils. 13th edition. Prentice Hall. Upper Saddle River New Jersey. 960pp.

Eicker, A. & Van Greuning, M. 1989. Economical alternatives for topogenous peat as casing material in the cultivation of *Agaricus bisporus* in South Africa. South African Journal of plant and soil 6(2):129-135.

Flegg, P. B. 1951. Casing soil. Mushroom station, Yaxley, Annual report. pp 26-30.

Galvan, L. F. 1976. Physical Properties of Irish Peats. Irish Journal of Agricultural Research 15:207-221.

Gamayunov, N. I. Stotland, D. M. & Shekhab, K. 1993. Effects of the dehydration conditions on structurization of natural capillary-porous materials. Translated from Kolloidnyi Zhurnal 55(5):35-44.

Gibbons, W. R., Maher, A. A. & Todd, R. L. 1991. Button Mushroom Production in Synthetic Compost Derived from Agricultural wastes. Bioresource Technology 38:67-77.

Gulser, P. & Peksen, A. 2003. Using tea waste as a new casing material in mushroom (Agaricus bisporus (L.) Sing.) cultivation. Bioresource technology 88(2):153-156.

Hayes, W. A. 1993. An overview. The Mushroom Journal. July 1993. 523:26-29.

Huerta, F., Spaulding, T. & Kelly, C. 2001. Investigations on casing soils with a high mineral content. Mushroom News 49(10):4-9.

Kalberer, P. P. 1987. Water Potentials of Casing and substrates and Osmotic Potentials of Fruit Bodies of *Agaricus bisporus*. Scientia Horticulturae 32:175-182.

Kalberer, P. P. 1990. Water relation of the mushroom culture *Agaricus bisporus*: Study from a single break. Scientia Horticulturae 41(4):277-283.

Kalberer, P. P. 1991. Water relations of the mushroom culture (*Agaricus bisporus*): Influence on crop yield and on dry matter content of dry bodies. Science and Cultivation of Edible Fungi, Maher. Balkema Rotterdam. pp 269-274.

Kinrus, A. 1971. Some important facts about a casing soil. Mushroom News 19(3):3-6.

Kurtzman, R. H. 1995. Agaricus bisporus (Lge.)Imb. Casing Layer, II: Porosity, The most important character. International Journal of Mushroom Science 1(1):11-17.

Labuschagne, P. M. 1995. Casing media for *Agaricus bisporus* cultivation in South Africa. MSc thesis. University of Pretoria. 55pp.

Levanon, D., Hardar, Y. & Wuest, P. J. 1994. Nature and use of spent mushroom substrate. Computer Science Utilization 2:22-23.

Linfield, H. J. 2000. The 1999 Sinden award lecture. The Mushroom Journal, April 2000. 603:13-19.

Mcbrierty, V. J., Wardell, G. E. & Keely, C. M. 1996. The characteristics of water in peat. Journal of the Soil Science Society of America 60(4):991-1000.

Metelerkamp, B. 2009. Soil water research officer. Institute for Commercial Forestry Research. University of Natal. Host of Sowacs (Soil Water Cintent Sensor) Discussion List. http://www.icfrnet.unp.ac.za. (Accessed May, 2009).

Misnikov, O. S. 2005. Physicochemical principles of hydrophobization of mineral binders by additives produced from peat raw material. Theoretical Foundations of Chemical Engineering. 40(4): 423-430.

Noble, R. 1991. Manipulation of temperature at controlled CO_2 level to synchronise the flushing pattern of the mushroom *Agaricus bisporus*. Scientia Horticulturae 46:177-184.

Noble, R. & Dobrovin-Pennington, A., Evered, C. E. & Mead, A. 1999. Properties of peat based casing soils and their influence on the water relations and growth of the mushroom (Agaricus bisporus). Plant and Soil. 207:231–232.

Noble, R. & Gaze, R. H. 1996. Preparation of mushroom (*Agaricus bisporus*) composts in controlled environments: Factors influencing compost bulk density and productivity**.** International Biodeterioration and Biodegradation 37(1-2):93-100.

Noble, R. & Dobrovin-Pennington, A. 2004. Use of fine particle tailings in mushroom casing. Science and Cultivation of Edible and Medicinal Fungi. pp 335-342.

Noble, R. & Dobrovin-Pennington, A. 2005. Partial substitution of peat in mushroom casing with fine particle coal tailings. Scientia Horticulturae. 104(3):351-367.

Pardo, A., de Juan, A. & Pardo, J. E. 2003. Characterisation of different substrates for possible use as casing in mushroom cultivation. Food, Agriculture and Environment 1(1):107-114.

Puustjarvi, V. 1992. Problematic structure standards of horticultural peat. International Peat Journal 4:55-63.

Samp, R. 2002. Obtaining Mushroom News 50(6):22-23.

Samp, R. 2006. Recent developments and future possibilities in the *Agaricus Spp*. (Button) mushroom industry. Mushroom News 54(3):12-23.

Schimilewski, G. & Gunter, J. 1988. An International Comparative Study on the Physical and Chemical Analysis of Horticultural Substrates. Acta Horticulturae 221:425-442.

Soeroes, C. S. 2005. Arsenic uptake and arsenic compounds in cultivated *Agaricus bisporus*. Food control 6(5):459-464.

Stoller, B. B. 1969. The science and practice of mushroom growing. 1(1).

Valat, B. Jouany, C. & Riviere, L. M. 1991. Characterization of the Wetting Properties of Air-dried Peats and Composts. Journal of Soil Science 152(2):100-106.

Van Gerwen, H. 2006. Casing: Don't underestimate the value of casing soil. Mushroom business. June 2006.17:6 -7.

Van Greuning, M. 1990. A Mycological study of some pathogens and weed moulds of the cultivated mushroom *Agaricus bisporus*. PhD thesis. University of Pretoria.

Wuest, P. J. & Beyer, D. M. 1996. Manufactured and Recycled Materials Used as Casing in (*Agaricus bisporus*) Mushroom Production. 10pp.

Wuest, P. J. 2002. Pest Species Biology and Control. Bacterial Diseases. Mushroom Integrated Pest Management Handbook. The Pennsylvania State University. University Park. 91pp.

Chapter 4

Chemical characteristics of several casing materials used for *Agaricus bisporus* production

4.1 Abstract

In South Africa, casing material is pasteurised for commercial use. From previous yield trials conducted at the University of Pretoria, it was found that some casing materials promote the growth of the weed mould, *Chromelosporium fulvum* or Cinnamon brown mould when pasteurised. It is therefore essential to determine how heat affects the chemical characteristics of a casing material. Element analysis was performed on all casing materials tested. The effect of heat on pH, electrical conductivity and total soluble phenolics was investigated. Materials and mixes which responded similar to different heat treatments were grouped together. In this study it was concluded that it is possible to obtain adequate yields from various agricultural by-products with a wide range of pH's, ranging from pH level <5.7 (in bagasse) to >8.4 (in filtercake). In addition, it was possible to obtain adequate yields from various agricultural by-products with a wide range of electron conductivities (EC), ranging from EC level <159 dS/m (in bagasse) to >1105 dS/m (in wattle (50:50)). Casing materials with a high EC had low mushroom yields and casing materials with a very high EC produced almost no mushrooms. Materials from plant origin (bagasse, coir and wattle bark) contained significant higher concentrations of phenols.

4.2 Introduction

The chemical and physical characteristics of a casing material can have a major impact on the passage of nutrients through the mycelial duct to the growing mushrooms. The yield and quality can be influenced by what may seem like negligible differences in the casing layer (Sinden, 1982). Nutrient depletion and the accumulation of toxic metabolites in compost are thought to be the limiting factors and responsible for lowering mushroom yield as the crop ages (Beyer & Muthersbaugh, 1996; Beyer, 1998). However during primordium (pinhead) formation button mushrooms receive the most nutrition from the casing layer (Hayes, 1973).

A casing material must ha (pH 7.0-7.5 (H_2O). Since the pH level decreases during the mushroom growth cycle, lime is therefore normally added. It serves as a buffer and promotes the structure of the casing material (Allison & Kneebone, 1962; Vedder, 1978). Pasteurised materials either increase in soluble salts and manganese or accumulate ammonia in amounts that might be toxic to mushrooms and ultimately affect yield negatively (Schisler, 1978). Therefore pH is regularly used in mushroom growing as an important parameter (Allison & Kneebone, 1962; Vedder, 1978). Furthermore *Agaricus bisporus* is moderately salt-sensitive (Awad & Nair, 1989). Therefore, salinity is measured as electrical conductivity (EC) or as total dissolved solids (TDS) which gives an indirect measurement of the salt content in the casing material.

By manipulating and controlling environmental conditions, undesirable pathogens and pests can be minimised. One such method used is pasteurisation (Stoller, 1969; Coetzee, 1987; Van Greuning, 1990). Pasteurisation prevents the formation of a biological vacuum or void niche, in the casing material. This prevents unfavourable microorganisms from recolonising the medium prior to mushroom production (Schisler, 1978; Vedder, 1978; Van Greuning, 2006. Pers. comm.). When alternative casing materials are used as a mixture or on its own, the potential risk of introducing pest and pathogens into the mushroom growing medium exist (Hayes, 1991). Production practices must therefore be adapted to ensure sustainable growth and optimum yields (Kinrus, 1971; Linfield, 2000; Huerta *et al*., 2001; Samp, 2006). The source (imported vs. local) and origin (plant, soil, waste by-product from agriculture or industry) of the initial peat material as well as the preparation procedures used (leaching, filtration, milling, drying and heating) could have an effect on the physiochemical and microbiological properties.

In South Africa, alternative casing is pasteurised for commercial use (Vedder, 1978; Eicker & Van Greuning, 1989; Labuschagne, 1995). The alternative casing materials are treated with steam in order to eradicate most of the pathogens, in particular *Mycogone perniciosa* (Magnus) Delacroix which was a major problem in the industry in 2005 (Pieterse, 2005). Previous yield trials conducted at the University of Pretoria indicated that some alternative casing materials promote the growth of the weed mould, *C. fulvum* when pasteurised (Labuschagne *et al.,* 2004). It is therefore

essential to determine how cal characteristics of the casing material.

One of the main objectives of this study was to accumulate as much chemical characteristic information on the different alternative casing materials as possible. The effect of heat on the pH, electrical conductivity and total soluble phenolics was therefore investigated. The total elements for each casing material were also determined.

4.3 Materials and methods

4.3.1 Elemental analysis of soil and plant materials

Small single representative sample of each casing material (Appendix 1) were taken and sent to an ISO 17025 accredited soil laboratory at the University of Pretoria (Department of Soil Science) for element analysis. Due to the high cost of the element analysis only pure samples were analysed and only one replicate of each sample was tested to obtain an indication of the elemental profile differences. No statistical analysis was performed on this data.

Determining Nitrogen and Phosphorus content

Single aliquots of 0.25 g were weighed out into 100 mℓ glass beakers for each alternative casing material analysed as mentioned in Appendix 1. Next, 5 mℓ of a H_2SO_4 and H_2O_2 mixture was added to each sample. The sample was then left to dissolve at 400℃ until it became translucent. The sample volume was made up with distilled water to 75 mℓ after which the % N and % P was determined, using the flow-system (Auto Analyzer II, Varian). Results were expressed as a percentage per sample analysed. No statistical analysis was performed on this data.

Determining various compounds and percentage ash content

Single aliquots of 0.5 g were weighed out into 100 mℓ glass beakers for each alternative casing material analysed as mentioned in Appendix 1. Next, 5 mℓ of $HClO_4$ and NaO_3 mixtures were added to each sample. Samples for various elements were dissolved at different temperatures (ranging between 0℃ to 220℃) for approximately 80 min (Moore & Johnson, 1967). The sample volumes were made up with dH_2O to 75 mℓ. The concentration of K, Ca, Mg, Na, Zn, Cu, Fe, Mn

and SO_4 were determined on on a Spectra AA 200, Varian. The ash content was determined on dry matter basis of a weight sample. Samples were dried at 550ºC for 3-5h and expressed in % dry matter (Schimilewski & Gunter, 1988). No statistical analysis was performed on this data.

4.3.2 Measuring the pH of alternative casing materials at different heat treatments

The pH levels of different alternative casing materials (Appendix 5) when heated and during pasteurisation were investigated as well as the pH changes when alternative casing materials were mixed with local peat. Three replicates of 17 g per alternative casing material were weighed out. Next, 100 mℓ dH_2O was added into 200 mℓ glass beakers. For each temperature treatment, 100 mℓ dH_2O was pre-heated to the specific temperature sample (20℃, 40℃, 60℃ or 80℃), stirred and left for 1 h. The pH was determined using a Jenway 3020 pH meter. An analysis of variance was performed on mean averages of three replicates and differences between means were determined with Fisher's t-test at a 5% level of significance, using Statistica 8.0 by Statsoft Inc.

4.3.3 Measuring the electrical conductivity of alternative casing materials at different heat treatments

The EC of different alternative casing materials (Appendix 5) when heated in the pasteurisation process as well as how it changes when mixed with local peat were measured. Three replicates of 17 g per alternative casing material were weighed, placed in 200 mℓ glass beakers and treated as discussed in 4.3.2. The EC probes were inserted into the glass beakers (containing the sample solution). The EC was determined using a Jenway 4010 conductivity meter. Pure water is a poor conductor of electricity. Therefore, the probes were rinsed with distilled water between each reading. Salinity is primarily measured in total dissolved solids (TDS) or as electrical conductivity (EC). The EC increases with an increase of salts in the water. The EC gives an indirect measurement of the salt content of the alternative casing material and is measured in decisiemens per meter (dS/m). An analysis of variance was performed on mean averages of three replicates and differences between means were determined with Fisher's t-test at a 5% level of significance, using Statistica 8.0 by Statsoft Inc.

4.3.4 Total soluble phe

The changes and amount of total soluble phenolics in different alternative casing materials (Appendix 5) when heated during pasteurisation as well as the amount of total soluble phenolics that change when it is mixed with local South African reed-sedge peat was determined.

Phenolic extraction from alternative casing materials

Three replicates of 17 g per alternative casing material were grounded into a fine powder with a mortar and pestle. From each sample, 0,05 g was placed in separate Eppendorf tubes and 1 mℓ of methanol/ acetone/ water (7:7:1) was added to each tube. Samples were vortexed for 5 s and centrifuged at 10000 rpm for 5 min before heat treatments were administered. Heat treatments (at 20, 40, 60 and 80℃), were performed by placing the Eppendorf tubes in a calibrated Eppendorf heating bath for 1 h after which samples were vortexed for 5 s and centrifuged at 100 rpm for 5 min. The supernatants were transferred into new Eppendorf tubes. One ml of the methanol/ acetone/ water solvent was placed in the Eppendorf tube with the remaining pellet and vortexed for 5 s, centrifuged at 10000 rpm for 5 min and added to the initial extract. The samples were left open to evaporate to 1 mℓ. Phenolic extraction were performed in triplicate for each sample and used for quantification thereof. Results are expressed in mℓ Gallic acid per mg casing material. An analysis of variance was performed on mean averages of three replicates and differences between means were determined with Fisher's t-test at a 5% level of significance, using Statistica 8.0 by Statsoft Inc.

Quantification of the soluble phenolic concentration in alternative casing materials

The Folin-Ciocalteau reagent method was adapted for measuring the soluble phenol extractions from each sample (Ikawa & Scharper, 1988; T. Regnier, 2007. Pers. comm.). Four ELISA plates (96 wells) were used for each heat treatment analysis (20℃, 40℃, 60℃ and 80℃). Analysis of the three heat treated sub-samples were repeated four times. The four negative controls were four wells containing 200 μℓ dH_20. In each well, 175 μℓ dH_20 was dispensed, followed by 10 μℓ of the phenolic extraction and 25 μℓ of the Folin-Ciocalteau reagent (Sigma, S.A.). The suspensions were lightly stirred on an orbital shaker for 2 min. Finally 50 μℓ of sodium carbonate ($NaCO_3$) was added to each well. The plates were incubated for 30 min at 40℃.

The plates were read at a[illegible]m with a multiscan ELISA plate reader. Results were expressed as mℓ Gallic acid per mg sample. An analysis of variance was performed on mean averages of three replicates and differences between means were determined with Fisher's t-test at a 5% level of significance, using Statistica 8.0 by Statsoft Inc.

4.4 Results

4.4.1 Total elements

Each sample analysed differed in element composition. The total elements could not be compared directly to yield due to physical differences in samples. However, it does give a broad view of the range of elements present in alternative casing materials compared to peat. The results are summarised in Table 4.1.

Concentration of total elements: Wattle bark contained the highest percentage nitrogen (2.6 %) compared to Topterra (1 %) and the South African peat only contained (0.4 %) (Table 4.1). Phosphorus percentages ranged between 0.1 and 0.2 % except for spent compost (1.3 %) and filtercake (3.1 %). Calcium levels were very low in coir (0.26 %) and bagasse (0.62 %) and potassium levels were very low in the South African peat (0.06 %), Topterra (0.07 %) and wattle bark (0.05 %). Soyafodder and filtercake were the only alternative casing materials with a magnesium content higher than 0.6 % (7.7 % and 1.3 % respectively). Coir and soyafodder both contained 0.23 % sodium. Sodium ranged between 0 and 0.07 % for all the other alternative casing materials. South African peat contained 1.66 % phosphate compared to spent compost (5.61 %). Coir had a low iron content (957 mg/kg) whilst filtercake (15089 mg Fe/kg), spent compost (12824 mg Fe/kg), dam soil (25800 mg Fe/kg) and minedump tailings (39750 mg Fe/kg) had an extremely high iron content. Filtercake had an excessive amount of manganese (6000 mg Mn/kg) compared to the other alternative casing materials (varied between 44-644 mg Mn/kg). Filtercake also had a higher zinc content (245 mg Zn/kg) compared to the minedump tailing (236 mg Zn/kg). Coir had the highest percentage organic material (92.7 %), followed by wattle bark (88.5 %), Topterra (62 %) and South African peat (26.6 %) (Table 4.1).

Table 4.1: Concentration of total elements in the pure samples

Casing materials[a]	N	P	Ca	K	Mg	Na	SO_4	Cu	Fe	Mn	Zn	Org.	Ash
	%	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	%	%	%
Control (Peat)	0.4	0.1	18.59	0.06	0.58	0.01	1.66	33	8159	554	6	26.6	73.4
Wattle bark	2.6	0.1	3.06	0.05	0.1	0	0.17	41	3119	102	18	88.5	11.5
Coir	0.4	0.1	0.26	0.82	0.15	0.23	0	38	957	44	12	92.7	7.3
Filtercake	1	3.1	11.43	0.14	1.28	0.01	0.16	120	15089	5999	245	33.9	66.1
Bagasse	0.8	0.2	0.62	0.12	0.16	0.01	0.03	30	9419	173	122	73.8	26.2
Topterra	1	0.2	9.48	0.07	0.31	0.02	0.96	27	4214	207	41	62	38
Spent compost	1.8	1.3	7.92	0.25	0.25	0.02	5.61	138	12824	644	60	39.6	60.4
Soyafodder	0.7	0.2	11.28	0.99	7.68	0.24	0.45	131	5594	251	105	46.3	53.7
Mine dump	0.05	0.09	0.97	0.6	0.31	0.07	0	108	39750	183	236	0	100
Dam soil	0.22	0.14	0.59	0.24	0.33	0.03	0	51	25800	254	84	0	100

Control (Peat): South African reed-sedge peat; N: Nitrogen; P: Phosphorus; Ca: Calcium; K: Potasssium; Mg: Magnesium; Na: Sodium; SO_4: Sulfates; Cu: Copper; Fe: Iron; Mn: Manganese; Zn: Zinc; Org: Organic material; Ash: Ash content

[a]Casing materials as described in Appendix 1.

4.4.2 pH

The pH results of the different alternative casing materials and mixes were used to create three groups, based on the pH change after different heat treatments. In group one, increased temperatures resulted in increased pH values. This group consisted of wattle bark, coir, bagasse (50:50), bagasse (70:30), soyafodder (50:50), spent compost, dam soil, Topterra and the South African reed-sedge peat (Figure 4.1.). Group two consists of wattle bark (50:50), wattle bark (70:30), coir (50:50), coir (70:30), filtercake (50:50) and filtercake (70:30) (Figure 4.1.). Here the pH increased from room temperature to 40ºC and the pH steadily decreased when the temperature of 60ºC to 80ºC were used.

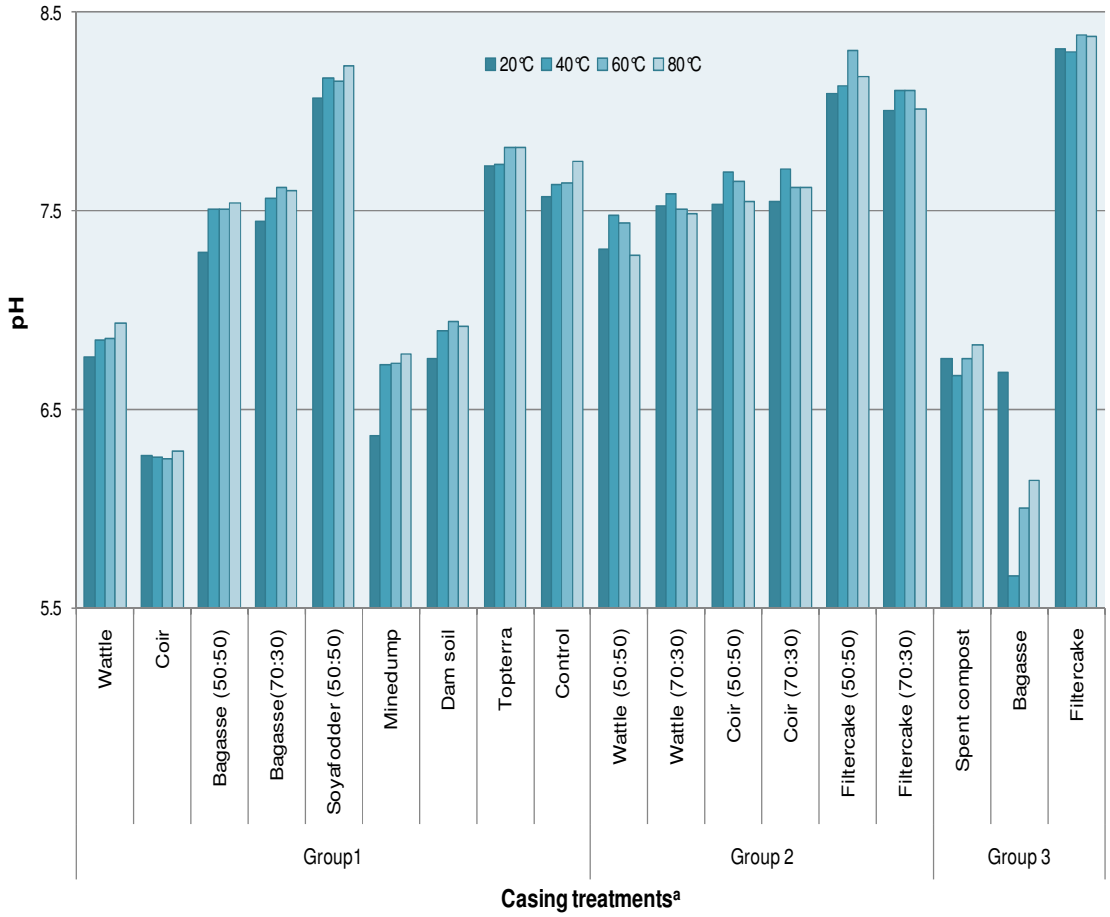

Figure 4.1. pH response of several casing materials and its mixtures after being exposed to four different temperatures for one hour. Materials with similar response in pH to the different heat treatments were grouped together. [a]Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2 and 5. [b]Mean values and statistical analysis is shown in Appendix 3.

Group three represented s ecreased at 40ºC and increased with higher temperatures [bagasse and filtercake]. Heat treatments did not change the pH dramatically, however, small changes did occur and materials which had similar changes were grouped together.

4.4.3 Electrical conductivity

From the results obtained, the materials were divided into five groups according to increased or decreased EC values after different heat treatments. The first group consisted of materials which had a gradual increase in the EC as the temperature increased [wattle bark (70:30), coir (50:50), filtercake and dam soil] (Figure 4.2.). The second group consisted of materials with similar EC fluctuations between different heat treatments. These materials were coir (70:30), soyafodder (50:50), spent compost, wattle bark (50:50) and coir (Figure 4.2.). The third group indicated an increase in EC at 40ºC and 60ºC but a decrease at 80ºC [spent compost (50:50), spent compost (30:70), Topterra, bagasse and wattle bark]. Group four shared tendencies of increased EC up to 40ºC where after the EC decreased at 60ºC. This group consisted of bagasse (50:50), bagasse (70:30), filtercake (50:50), filtercake (70:30) and South African reed-sedge peat. Mine dump tailing and soyafodder (with added lime) make up the fifth group, where the EC decreased when exposed to temperatures up to 40ºC and increased when temperatures exceeded 60ºC.

Only four materials, [coir (70:30), soyafodder (50:50), spent compost and spent compost (50:50)] had a lower EC value at 80ºC than at 20ºC. The EC value decreased when South African reed-sedge peat was incorporated into wattle bark, filtercake, soyafodder and spent compost. It was also found that there was an increase in the EC value when South African reed-sedge peat was incorporated into coir and bagasse. In general, it is concluded that a good casing material should have a low EC. Coir, bagasse, bagasse (50:50), bagasse (70:30), filtercake, filtercake (50:50), filter cake (70:30), Topterra and the South African reed-sedge peat had a low conductivity (EC<500 dS/m). Wattle bark, wattle bark (50:50), wattle bark (70:30), coir (50:50), coir (70:30) and dam soil had a medium conductivity (EC > 500 dS/m < 1000 dS/m). Soyafodder, soyafodder (50:50), mine dump tailing, spent compost and spent compost (50:50) had a very high EC (EC < 1000 dS/m).

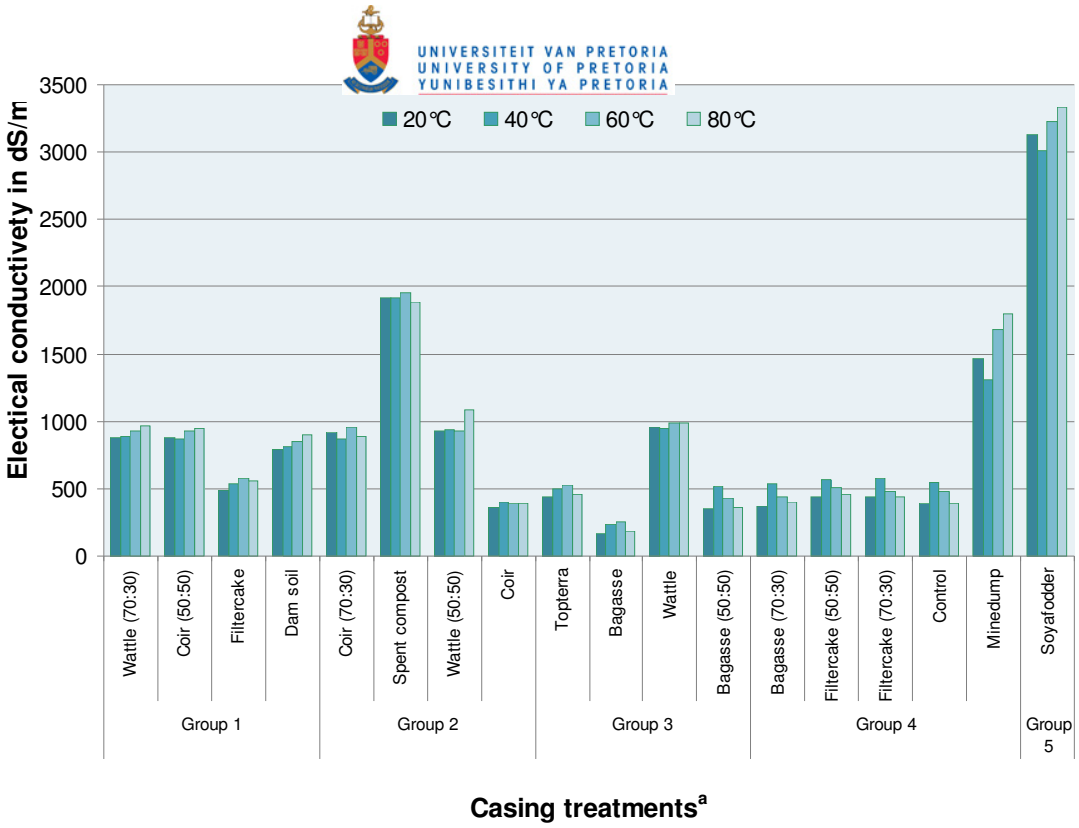

Figure 4.2. EC response of several casing and mixtures after subdued at four different temperatures for 1 hour. Materials with similar response in EC to the different heat treatments were grouped together. [a]Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2 and 5. [b]Mean values and statistical analysis is shown in Appendix 3.

4.4.4 Total soluble phenols

The TSP concentration of the various alternative casing materials compared to reaction to different temperatures was grouped. In Group 1, the phenols in the material decreased at 40ºC, increased at 60ºC and decreased again at 80ºC. This group consisted of wattle (50:50), wattle (70:30), filtercake (50:50), filtercake (70:30), Topterra, wattle and spent compost (Figure 4.3.). The second group consisted of materials that showed a gradual increase in total soluble phenols [coir, coir (50:50), coir (70:30), soyafodder, bagasse and spent compost (50:50)]. Materials with a miscellaneous effect towards heat treatments were placed in group tree. Bagasse contained a large amount of soluble phenols compared to filtercake that contained fewer phenols that were removed after bagasse was processed. The total soluble phenols decreased dramatically in these alternative casing materials

when South African reed orated as a mix into the pure materials.

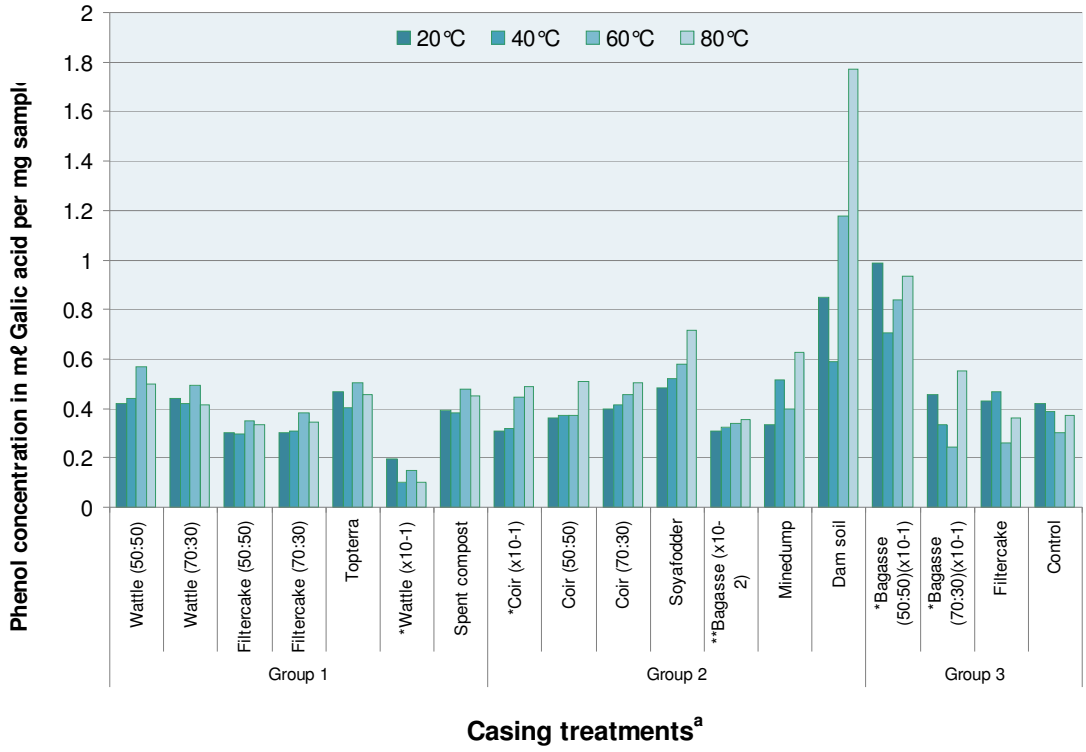

Figure 4.3: Total soluble phenolic (TSP) changes in mℓ Gallic acid per mg of different mushroom casing materials as an alternative for peat when treated with different temperatures for one hour. Materials with similar response in amount of TSP to the different heat treatments were grouped together. [a]Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2 and 5. [b]Mean values and statistical analysis is shown in Appendix 3.

*= $x10^{-1}$ Wattle, Coir, Bagasse (50:50), Bagasse (70:30)

**= $x10^{-2}$ Bagasse

4.5 Discussion

Most of the nutrition for white button mushroom production is provided by the compost, the casing is considered to be low in nutrients (Hayes, 1973; Botha, 1984; Eicker & Van Greuning, 1989; Hayes, 1993; Kurtzman, 1995; Van Gerwen, 2006). The effect of the compost on mushroom yield can be excluded since the compost originated from one source and was conditioned similarly in phase III. In addition, the same weight of compost was used in all the mushroom yield trials. The only uncommon denominator was the different casing materials used. Though the concentrations of elements alone could not solely be responsible for the yield results obtained (Appendix 7), it may provide some information of the elemental parameters which mushrooms can tolerate. Since elemental composition could not be quantified for all the different ratio-mixed casing materials in this study, no direct conclusion could be made between mushroom yield and casing material element composition. Future work could focus on correlating casing material element concentration with mushroom yield.

Organic matter is frequently the limiting factor for heterotrophic microorganisms where specific organic substrates often favour the growth of a particular population (Atlas & Bartha, 1998). Organic matter tends to bind and chelate heavy metals thereby reducing their toxicity (Atlas & Bartha, 1998). Many inorganic substances (e.g. elements in Table 4.1.) are essential nutrients for microorganisms (Stotzky & Norman, 1964).

Results from previous studies indicated that some elements present in growth medium and irrigation water (Pb, Hg, Cs, Se, Cd, As) are able to accumulate in high concentrations in cultivated mushrooms (Kurtzman, 1995; Pardo *et al*., 2003; Soeroes, 2005). These accumulated elements, in high dosages, might pose serious health risks to consumers (Border, 1993; Bellmont, 2005; Soeroes, 2005). Minedump tailings used in this study have been re-mined and most of the toxic elements have been removed. However, traces of hazardous elements could still be present in the raw waste material (Soeroes, 2005) and should therefore be evaluated for heavy metal content in future studies. The different types of heavy metals and contaminants as well as their quantities, were not determined in this

study. Their presence is s further investigation. Minedump tailing contain low or no nutrients and has a high clay content.

It was concluded that minedump tailings produce almost no mushroom yield and is not suitable (on its own or as part of a mixture with peat) as a casing material. Re-mined (gold) minedump tailing release much less water due to very high water retention. Although this could be corrected by adding porous and fibrous materials, it is not advisable to pursue this mixture further due to the possibility of heavy metal contamination.

The pH of the soil solution is a crucial factor to determine the growth of organisms in the soil (Brady & Weil, 2002). In general microorganisms cannot tolerate extreme pH values (Atlas & Bartha, 1998). Mushrooms prefer a casing material with a pH level between 6.5 and 7.7 (Pardo *et al*., 2003). However, in this study it was possible to obtain a reasonable mushroom yield (Appendix 7) from various agricultural by-products with a wide pH range [pH level <5.7 (in bagasse) to >8.4 (in filtercake)]. Most alternative casing material mixes tested, fell within a favourable pH range to sustain a healthy microbial population. The pH also affects the type of microorganisms present, microbial enzymes and the dissociation and solubility of many molecules which indirectly influence microorganisms (Atlas & Bartha, 1998).

A good casing material should have a low EC (Pardo *et al*., 2003). However, in this study it was possible to obtain reasonable mushroom yields (Appendix 7) from various agricultural by-products with a wide range of EC's [EC level <159 dS/m (in bagasse) to >1105 dS/m (in wattle (50:50))]. Materials with a high EC [spent compost (50:50) (EC>1408-1891 dS/m), soyafodder (50:50) (EC>1880-2390 dS/m) and soyafodder EC (2650-3670 dS/m)] had low mushroom yields or none. Processes applied to sugar cane in the processing of bagasse resulted in an increase in EC when processed into filtercake. The difference in EC in fresh coir and wattle material, and leached/weathered coir and wattle material, indicates that the time of leaching and sun exposure increased the EC. This observation was in direct contrast with the amount of total soluble phenols found in wattle and coir.

Various phenolic compou[illegible]ncentrations are known to be present in plant material (Sharma, Furlan & Lyons, 1999; Agrios, 1998; Sharma, 1996; Baker & Snyder 1965; Eger, 1965).

The processing technology used to produce sugar cane with its resultant waste product, bagasse, resulted in a decrease in TSP. A further decrease in TSP occurred when filtercake was processed into bagasse. The difference between TSP in fresh material and leached/ weathered material, could therefore indicate that the length of leaching and sun exposure resulted in a TSP decrease. Results obtained using the Folin-Ciocalteau method for total soluble phenols indicate that temperature definitely plays a role in the amount of soluble phenols extracted from the casing materials. Plant materials (bagasse, coir and wattle bark) contained significant higher concentrations of phenols. This might explain why fungi are more dominant in plant materials whereas bacteria favour clayey soils. However, further studies and identification of these phenolic compounds from the different casing materials are necessary to quantify this.

4.6 References

Allison, W. H. & Kneebone, L. R. 1962. Influence of Compost pH and Casing pH on Mushroom Production. Mushroom Science 5:81-89.

Arios, G. N. 1998. Plant Pathology 4th Edition. Academic press, London. 803pp.

Atlas, R. M. & Bartha, R. 1998. Microbial ecology: Fundamentals and application. 4th edition. Benjamin/Cummings Science Publishing, California. 639pp.

Awad, A. S. & Nair, N. G. 1989. Salt tolerance of Agaricus bisporus in relation to water stress and toxicity of sodium ions. Annals of applied Biology 115(2):215-220.

Baker, K. F. & Snyder, W. C. 1965. Ecology of soil-borne pathogens. Prelude to Biological Control. An international symposium on the factors determining the behaviour of plant pathogens in soil. Held at the University of California, Berkeley, April 7-13, 1963.

Bellmont, B. 2005. Peat moss cultivates an earth-friendly reputation. Mushroom News 53(11):12-29.

Beyer, D. M. & Muthersbaugh, H. 1996. Nutrient supplements that influence later break yield of *Agaricus bisporus*. Canadian Journal of Plant Science 76:835–840.

Beyer, D. M. 1998. The use of ion exchange resins to assess the changes in mineral element availability during the production of the cultivated mushroom *Agaricus bisporus*. Canadian Journal of Botany 76(12):2084-2092.

Border, D. 1993. Alternatives to peat. Mushroom Journal. July. 1993. 523:23-24.

Botha, W. J. 1984. Die fisiologiese en morfologiese studie van vier probleemfungusse in die sampioenbedryf. MSc thesis. University of Pretoria. 183pp.

Brady, N. C. & Weil, R. R. 2002. The nature and properties of soils. 13th edition. Prentice Hall. Upper saddle river. New Jersey. 960pp.

Coetzee, J. C. 1987. Die biologie van enkele Fungussoorte wat by die Kommersiele verbouing van Agaricus bisporus in Suid-Afrika probleme skep. MSc thesis. Universiteit van Pretoria. 219pp.

Eger, G. 1965. Antibiosis and fungistasis of soil microorganisms. In: The Ecology of soil-borne pathogens. Prelude to Biological Control. An international symposium on the factors determining the behaviour of plant pathogens in soil. Held at the University of California, Berkeley, April 7-13, 1963. Edited by Baker, K. F. and Snyder, W. C. 1965.

Eicker, A. & Van Greuning, M. 1989. Economical alternatives for topogenous peat as casing material in the cultivation of *Agaricus bisporus* in South Africa. South African Journal of plant and soil 6(2):129-135.

Hayes, W. A. 1973. The casing layer and its function in mushroom nutrition. Mushroom News 21(12):9-14.

Hayes, W. A. 1991. Some thoughts on peat and the quest for an alternative. The Mushroom Journal. July 1991. 223:25-27.

Hayes, W. A. 1993. An overview of the casing layer. The Mushroom Journal. July 1993. 523:26-29.

Huerta, F., Spaulding, T. & Kelly, C. 2001. Investigations on casing soils with a high mineral content. Mushroom News 49(10):4-9.

Ikawa, M. and Schaper, D. 1988. Reaction of Folin-Ciocalteau phenol reagent with purines, pyrimidines, and pteridines and its relationship to structure. Journal of Agricultural and Food Chemistry. *36*(2):309–311.

Kinrus, A. 1971. Some imp ng soil. Mushroom News 19(3):3-6.

Kurtzman, R. H. 1995. Agaricus bisporus (Lge.)Imb. Casing Layer, II: Porosity, The most important character. International Journal of Mushroom Science 1(1):11-17.

Labuschagne, P. M. 1995. Casing media for *Agaricus bisporus* cultivation in South Africa. MSc thesis. University of Pretoria. 55pp.

Labuschagne, P. M., Aveling, T. A. S. & Korsten, L. 2004. Mushroom casing research. The Spawn Run, 2004. pp 15-20.

Linfield, H. J. 2000. The 1999 Sinden award lecture. The Mushroom Journal, April 2000. 603:13-19.

Moore, W. & D. Johnson. 1967. *Procedures for the Chemical Analysis of Wood and Wood Products*. Madison, WI: U.S. Forest Products Laboratory, U.S. Department of Agriculture.

Pardo, A., de Juan, A. & Pardo, J. E. 2003. Characterisation of different substrates for possible use as casing in mushroom cultivation. Food, Agriculture and Environment 1(1):107-114.

Pieterse, Z. 2005. Mycogone perniciosa, a pathogen of Agaricus bisporus. MSc. Thesis, University of Pretoria. 104pp.

Regnier, T. 2007. Pers. comm.

Samp, R. 2006. Recent developments and future possibilities in the *Agaricus Spp*. (Button) mushroom industry. Mushroom News 54(3):12-23.

Schimilewski, G. & Gunter, J. 1988. An International Comparrative Study on the Physical and Chemical Analysis of Horticultural Substrates. Acta Horticulturae 221:425-442.

Schisler, L. C. 1978. Casing soil treatment. Mushroom News. December 1978. pp 21-31.

Sharma, H. S. S. 1996. Compositional analysis of neutral detergent, acid detergent, lignin and humus fractions of mushroom compost. Thermochimica Acta 285:211-220.

Sharma, H. S. S., Furlan, A. & Lyons, G. 1999. Comparative assessment of chelated spent mushroom substrates as casing material for the production of *Agaricus bisporus*. Applied Microbiology Biotechnology 52(3):366-372.

Sinden, J. W. 1982. The casing layer and its significance. Mushroom News 30(11):14-17.

Soeroes, C. S. 2005. Arsenic uptake and arsenic compounds in cultivated *Agaricus bisporus*. Food control 6(5):459-464.

Stoller, B. B. 1969. The science and practice of mushroom growing. 1(1).

Stotzky, G. & Norman, A. G. 1964. Factors limiting microbial activity in soil. Part III. Suplimentary substrate additions. Canadian Journal of Microbiology 10:143-149.

Van Gerwen, H. 2006. Casing: Don't underestimate the value of casing soil. Mushroom business. June 2006.17:6 -7.

Van Greuning, M. 1990. A Mycological study of some pathogens and weed moulds of the cultivated mushroom *Agaricus bisporus*. PhD thesis. University of Pretoria.

Van Greuning, M. 2006. Pers. comm.

Vedder, P. J. C. 1978. Modern Mushroom Growing. Educaboek, Culemberg. The Netherlands. 420pp.

Effect of pasteurisation on the role and presence of *Chromelosporium fulvum* on different casing materials used in the production of *Agaricus bisporus*

5.1 Abstract

Understanding why a particular species is found in a certain habitat at a specific time could provide some insight into the complex nature of microbial ecosystems of casing materials. It has been known that by adding organic amendments to the soil, the microbial equilibrium is changed. Dominant fungi and bacteria were isolated and identified from alternative casing materials used in a preliminary study. Fungi were found to be more prevalent in materials with a high organic content. Pasteurisation is another method used to change the microbial equilibrium in casing materials. Heat capacities were determined to provide a better understanding of why some casing materials can be overpasteurised. Overpasteurisation creates a microbial void which can be re-colonised by pathogens and opportunistic microorganisms. It was found that, both the water content and the percentage organic matter influenced the specific heat capacity of the casing material. *In vitro* sporulation curves of *Chromelosporium fulvum* (*C. fulvum*) were determined on alternative and mixed peat casing materials. In this study it was found that the sporulation rate of *C. fulvum* increased as the organic content increased. A micro-mushroom growing unit was designed and build to do a microcosm study to determine the *in vivo* effect of pasteurisation on *Agaricus bisporus* yield when casing materials re-inoculated with *C. fulvum.* By inoculating South African peat casing material with *C. fulvum* the growth and cover of the mushroom mycelium was greater than with uninocculated South African peat (control). Pots inoculated with *C. fulvum* also showed a slight delay in the formation of the first flush mushrooms. It was concluded that the growth rate at which mushroom mycelium colonise the surface of the casing material may result in r-strategists such as *C. fulvum* to appear.

5.2 Introduction

Soil contains billions of micoorganisms of different species; some of which may be producers, consumers, predators, prey, parasites or even opportunistic organisms (Atlas & Bartha, 1998; Brady & Weil, 2002). Soil provides an ordered and complex environment which acts as a template for chemical reactions (Brady & Weil, 2002). Small changes to the microbial environment of soil could, however, alter the habitat in such a way that it would support the growth of selected species and decrease the occurrence of undesirable ones (Baker & Snyder, 1965). Microbial processes are also influenced by soil temperature changes. In addition, increasing soil temperatures can be used as a method of pasteurisation to exclude certain pathogens (MacDonald *et al*., 1995; Brady & Weil, 2002).

In terms of population dynamics, organisms follow either a r- or K-selection strategy (Pianka, 1970). The r-selected species are poor competitors (opportunistic organisms). In general these organisms exploit and pre-dominate in a void microbial niece (unstable environments) by quickly producing and dispersing large quantities of spores and *vice versa* for K-selected species (Weinbauer & Höfle, 1998). In this study *C. fulvum* which is seen as a oppertunistic weed mould, typically represents the characteristics of a r-strategistic organism.

The improvement in management practices to achieve more successful disease control, will only be obtained when more extensive knowledge concerning the interactions and interferences of microorganisms within their habitats in the soil are better understood and comprehended (Baker & Snyder, 1965; Van Greuning, 1990). This chapter focuses on the effect of pasteurisation on casing materials, the growth of *C. fulvum* (r-strategist) and its effect on *A. bisporus* production on a micro scale.

5.3 Materials and methods

5.3.1 Heat capacity of casing material

In order to determine the effect of heat during the pasteurisation process on the casing materials, the following trial was performed in triplicate for each casing material and mix as described in Appendix 1 and Appendix 5. Blue plastic rings (8.5 cm diameter × 5 cm high) were used to measure the same volume of casing materials (Appendix 5). A sieved cloth was placed around the bottom edge of each

ring and secured with a rul[illegible]t of each sample was measured and placed in a container filled $^{2}/_{3}$ with tap water for 24 h to allow the samples to saturate and reach field capacity. The saturated samples were weighed, placed and sealed in autoclave bags in order to preserve water in the saturated sample. Samples were then autoclaved at 121ºC for 20 min. The bags were punctured once with a knife (5 mm). Thermometers were inserted and the temperature was measured in 10 min intervals. A complete randomised block design in triplicate was used during the temperature measurements. The temperature data was incorporated into the following formula to obtain a specific heat capacity for each casing material tested: $Q = c \times m \times \Delta t$

Where: Q = The amount of heat

C = Specific heat capacity

Δt = Change in temperature (T2-T1)*

m = Mass in kg

t = time in seconds

*T2 = initial temprerature

*T2 = end temperature

(Van Staden *et al*., 1992; Giancoli, 1998)

An analysis of variance was performed on mean averages of three replicates and differences between means were determined with Fisher's t-test at a 5% level of significance, using Statistica 8.0 by Statsoft Inc.

5.3.2 Obtaining a pure *Chromelosporium fulvum* culture

A single sample from infected casing material containing *C. fulvum* was collected from a growing room on a commercial mushroom farm in Gauteng. The collected sample was placed in a sealed, sterile container and transported to the University of Pretoria laboratories for isolation. A small sporulating fungal sample was plated out on potato dextrose agar (PDA) with 0,25 g/dm^3 Novobiocin (Merck). A young mycelium strand of a 14 day-old culture and a single fruiting body of a month-old culture were examined with a light microscope (40X magnification) and lactofuchin (Astrolab, Johannesburg) as staining solution. This was done to confirm identity of *C. fulvum*. The purified cultures were stored on PDA slants for further *in vitro* and *in vivo* experiments and submitted to the Plant Protection Research Institute (PPRI) in Pretoria for positive identification. For experimental purposes, cultures were revived from slant on PDA plates.

5.3.3 Sporulation curve fulvum

In order to determine the sporulation rate of *C. fulvum* on different casing materials (Appendix 5) the following *in vitro* study was conducted. Each casing material (Appendix 5) was grinded with a Tobacco leaf grinder/shredding/cutting machine (SS-4, Anyang Sino-Shon Co., Ltd. China) to obtain similar particle sizes. Due to density differences, samples were measured out in 500 mℓ glass beakers, placed in separate autoclavable bags and sealed. The bags were autoclaved at 121℃ for 20 min and left overnight at room temperature. The samples were mixed in a 50:50 (v/v) ratio with sterile PDA + Chloramphenicol at 50℃ to obtain a homogenous mixture and poured into 90 mm Petri dishes. Inoculations were done by placing a single 4 mm plug of the pure *C. fulvum* culture in the centre of each Petri plate. Plates were incubated at 25℃ for 9-21 days. Three replicates of each casing material and mix were used in a complete randomised block design, for every day enumeration. Since *C. fulvum* spores tend to disseminate over the whole surface of the fungal plate, it was impossible to measure the diameter of a central colony. Therefore, it was decided to capture the total spores of the *C. fulvum* culture with sterile quarter strength Ringer's solution (Merck). A phase contrast microscope (Zeiss, Germany) was used to count the spores in a Haemocytometer counting chamber (Neuwbauer Impruved, Germany). The total spore concentration was determined and the final concentration was expressed as $1x10^6$ spores/mℓ. The number of spores produced on each material for 8-21days after inoculation were determined in order to establish which casing material favours *C. fulvum* the most.

5.3.4 Effect of *Chromelosporium fulvum* inoculum on casing material and *Agaricus bisporus* growth

Mushrooms require an environment which can easily be altered to obtain optimum conditions needed for production. The unavailability of mushroom growing facilities for *in vivo* tests created an intuitive initiative. A one cubic meter micro-mushroom growing unit was designed and build from perspex to grow mushrooms on small a scale (Appendix 6). Casing materials used are described in Appendix 5. Phase III compost was weighed out (500 g) and filled $^3/_4$ way in (15 cm) horticultural pots (Calibre Plastic, RJE Distributors). The pots were placed in the micro-mushroom unit for two days prior to casing. The casing materials were measured per volume to ensure that each treatment received the same amount of casing. In one treatment the samples were pasteurised while the other treatment was left unpasteurised.

Each sample in each treatment was replicated. Both the pasteurised and unpasteurised treatments were inoculated with one mℓ of a high concentrated suspension of *C. fulvum* spores ($6x10^7$ spores per mℓ) directly after casing and placed in the micro-mushroom growing unit. A general growing schedule as for commercial mushroom production was followed. After the first flush, mushroom mycelium growth and cover were rated on a scale from zero to ten (0= no mycelium; 1-3=poor; 4-6=moderate; 7-9=good; 10=over grown). The mushroom sporocarps were harvested and weighed. Mycelium growth and cover were used to compare the effect of pasteurisation on inoculated casing materials. Uninoculated pots with South African peat were used as a control in both treatments. An analysis of variance was performed on mean averages of three replicates and differences between means were determined with Fisher's t-test at a 5% level of significance, using Statistica 8.0 by Statsoft Inc.

Once the casing mixes (Appendix 5) were prepared it was placed in autoclaved bags and steam pasteurised at 60-65°C for 6-8 h at the mushroom farm in Babsfontein. After pasteurisation, the materials were left for 24 h before casing. A volume of 5 ℓ of each sample from the different materials and mixes were kept in sealed plastic bags at 5ºC for physical, chemical and element analysis at the University of Pretoria.

5.4 Results

5.4.1 Heat capacities

Results from the specific heat capacities (SHC) experiment are depicted in Figure 5.1. All the casing materials followed a hyperbolic curve. Each casing material reached a different maximum temperature after the same amount of heat energy had been applied during a similar time period. The initial heat loss in temperature after 10 min was the greatest, thereafter it decreased every 10 min time interval that followed. The drop in temperature continued until room temperature was reached.

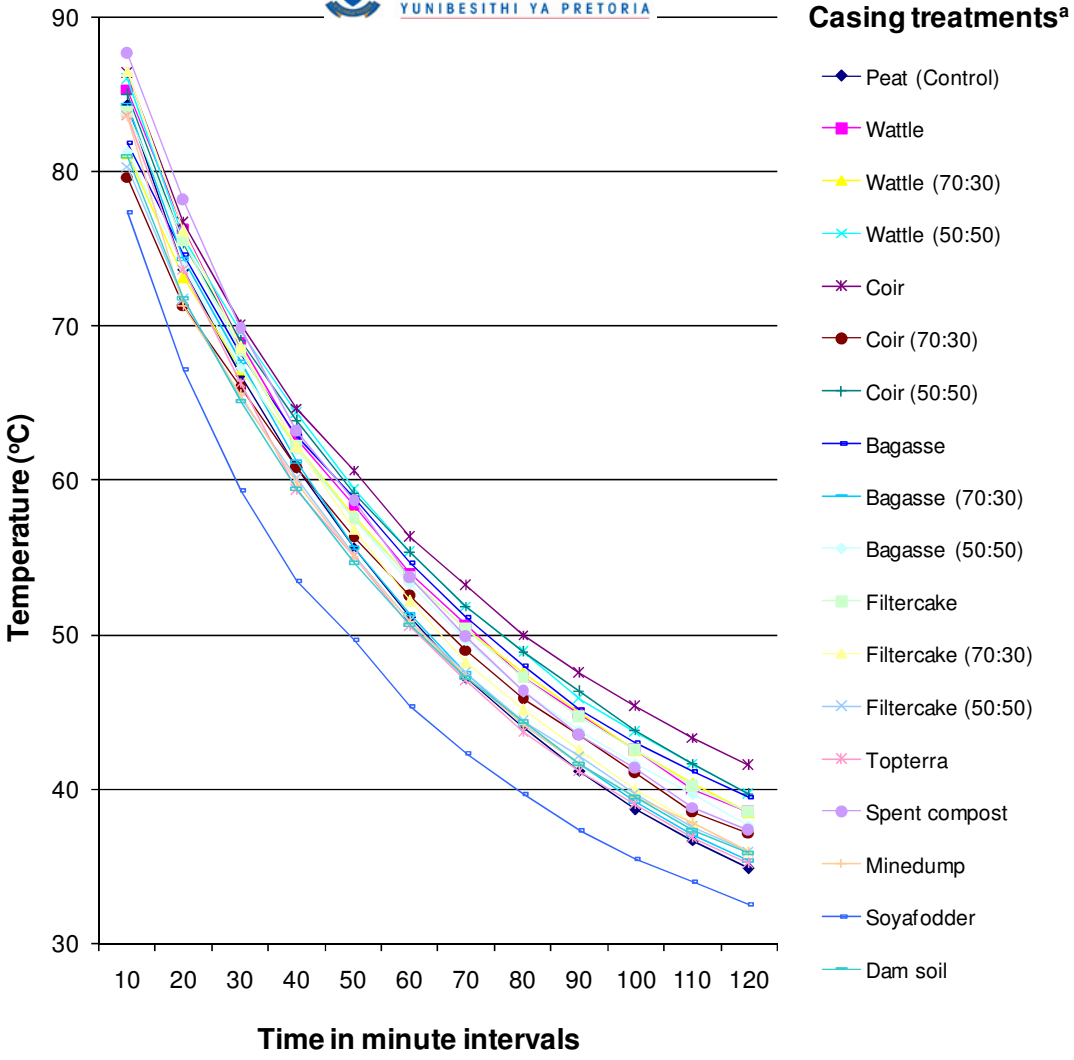

Figure 5.1: Temperature drop of several casing materials and their mixtures measured in ten minute intervals. [a]Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2 and 5.

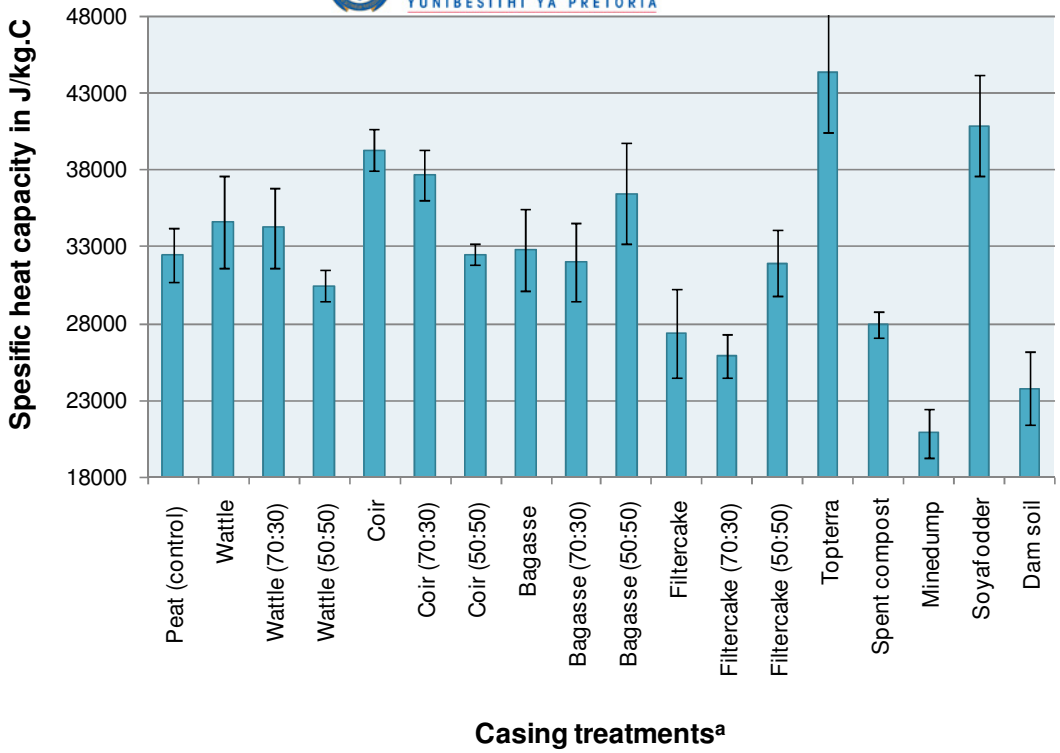

Figure 5.2: Specific heat capacities of several casing materials and their mixtures in Joule/kg.°C. [a]Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2 and 5. [b]Mean values and statistical analysis is shown in Appendix 3.

5.4.3 Sporulation curves of *Chromelosporium fulvum*

The spores of *C. fulvum* are easily distinguished from other foreign particles in the suspension. Bagasse showed the highest sporulation rate after 12-14 days for *C. fulvum* followed by wattle bark, spent compost, Topterra and coir (Figure 5.3A). The casing material mixed with peat had a lower sporulation rate than when the casing material was used on its own without any peat (Figure 5.3B). The sporulation rate of *C. fulvum* can be reduced by mixing casing materials with a high organic content with materials with a lower organic matter content such as South African peat. Wattlebark and bagasse tend to favour the growth of *C. fulvum* especially when pasteurised.

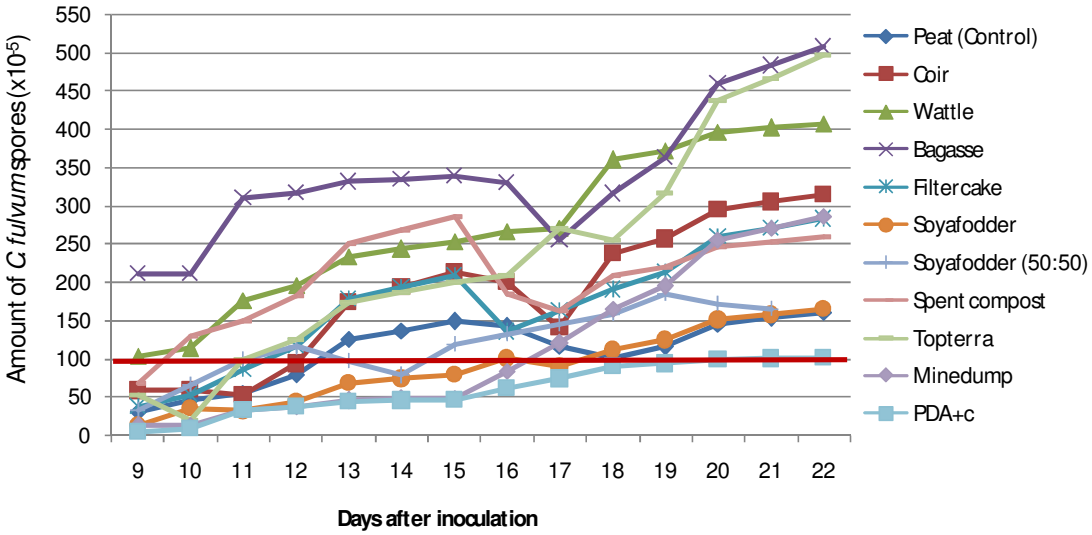

Figure 5.3A

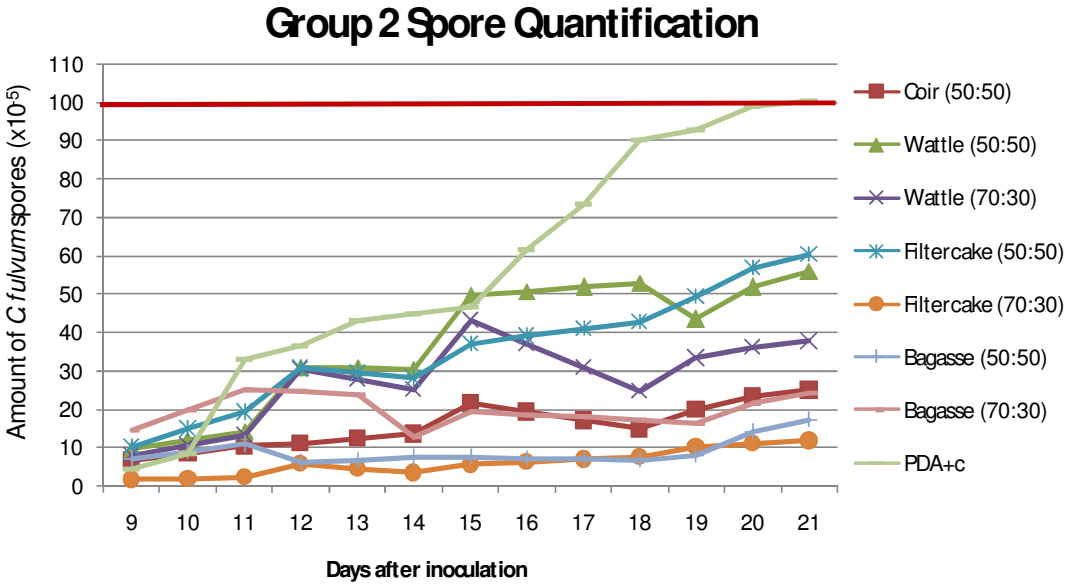

Figure 5.3B

Figure 5.3. Growth and sporulation of *Chromelosporium fulvum* on several alternative casing mediums. A) Materials with a greater sporulation rate of *C. fulvum* than the sporulation rate of *C. fulvum* grown on the potato dextrose agar and chloramphenicol (PDA + C) medium. B) Materials with a sporulation rate smaller or less than the sporulation rate of *C. fulvum* grown on the PDA + C medium.
(Note: The Y-axis is five times smaller in figure 5.3B than in figure 5.3A. Used (PDA + C) as reference).

5.4.4 Effect of *Chromel*[illegible]*ulum* on casing material and *Agaricus bisporus* growth

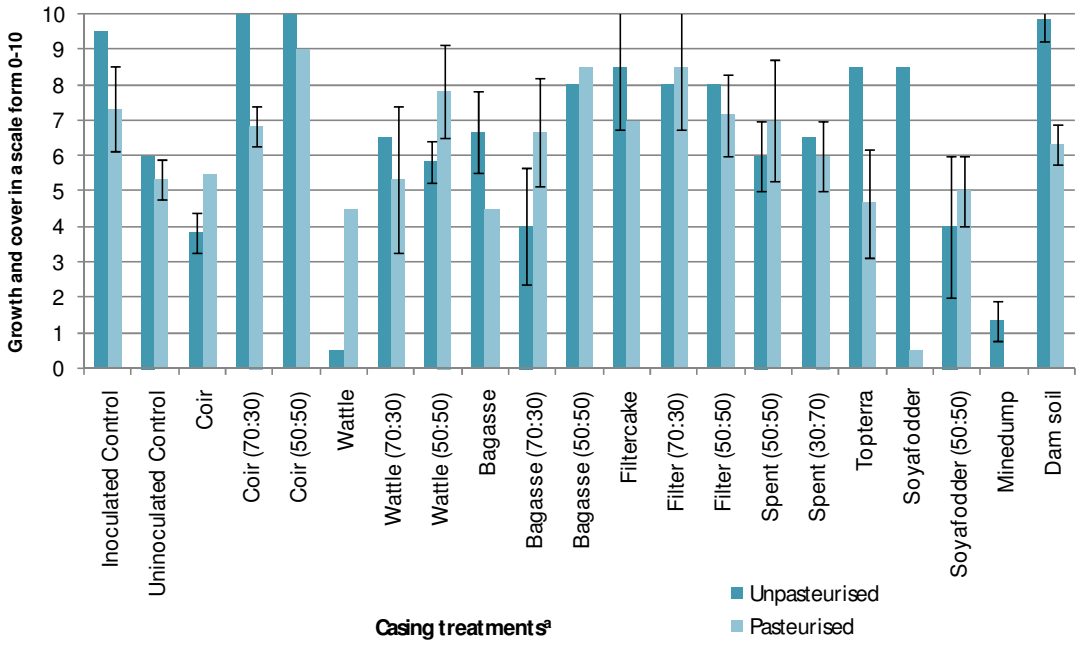

Figure 5.4: Mycelium growth on different casing materials in the micro-mushroom growing unit in a scale from zero to ten (0= no mycelium; 1-3=poor; 4-6=moderate; 7-9=good; 10=over grown). [a]Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2 and 5. [b]Mean values and statistical analysis is shown in Appendix 3.

Although all the pots used in the pasteurised and unpasteurised trials were inoculated with a (6 x10^6 spores/ mℓ) spore suspension of *C. fulvum,* no visual symptoms or onset of the mould were visible on the surface of the casing material. However, there were clear differences between mycelium growth and cover on the different casing materials as well as between the pasteurised and unpasteurised trials. Mushroom yield results and mycelium growth are shown in Figure 5.4 and Figure 5.5.

Both the inoculated South African peat (control) as well as the uninoculated South African peat had better mycelium growth and cover when the casing material was left unpasteurised (Figure 5.5). This was found to be similar for the South African (control) peat (inoculated and uninoculated), filtercake (50:50), spent compost (30:70), wattle (70:30), bagasse, filtercake, Topterra, soyafodder, minedump

tailings, dam soil, coir (70:30), bagasse (70:30), spent compost (50:50), filtercake (70:30), bagasse (50:50), spent compost (50:50), soyafodder (50:50), wattle (50:50), wattle and coir had better mycelium growth and cover when it was pasteurised. The dam soil, coir (70:30) and coir (50:50) had excessive mycelium growth and cover when it was left unpasteurised whilst wattle presented very poor mycelia cover in similar conditions (Figure 5.4).

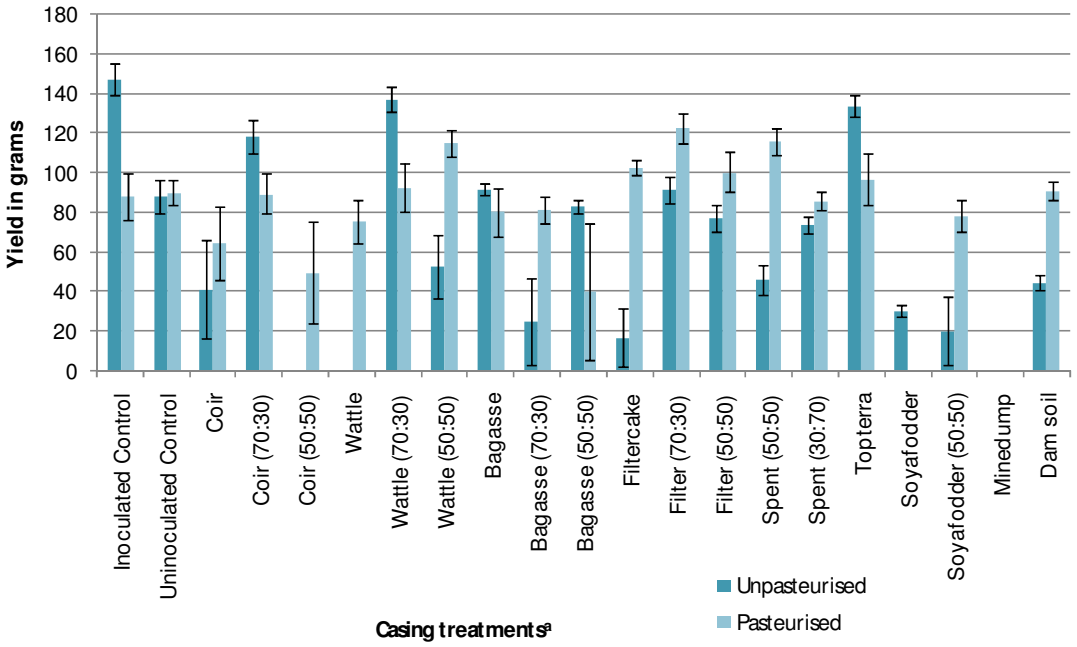

Figure 5.5: Yield of several different casing materials in the micro-mushroom growing unit. Yield is expressed in grams. [a]Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2 and 5. [b]Mean values and statistical analysis is shown in Appendix 3.

Mushroom yield results obtained from the micro-mushroom unit, indicated that the inoculated peat, bagasse (50:50), wattle (70:30), bagasse, Topterra, soyafodder and coir (70:30) had higher mushroom yields when unpasteurised. In contrast, Bagasse (70:30), filtercake (70:30), filtercake (50:50), spent compost (50:50), soyafodder (50:50), wattle (50:50), filtercake, wattle, coir, dam soil and coir (50:50) had higher mushroom yields when pasteurised. There were no differences in mushroom yield between unpasteurised uninoculated peat or pasteurised uninoculated peat. Wattle and coir (50:50) had no mushroom yield in the unpasteurised trial whist only soyafodder had no mushroom yield when pasteurised. Minedump tailings had no

mushroom yield in both pasteurised trials which indicated that it is unsuitable as a casing material for mushrooms (Figure 5.5).

5.5 Discussion

In South Africa it was a common practice to pasteurise peat in order to eradicate pests and diseases (Van Greuning, 1990; Pieterse, 2005). An optimum and precise temperature for a certain amount of time is necessary to achieve this (Fletcher *et al*., 1989; Hayes, 1991). Over pasteurising sometimes occurs, creating an ecological vacuum which could be colonised by undesirable pathogens and weed moulds (Stoller, 1969; Schisler, 1978; Van Greuning, 2006 Pers. comm.). It was found that the casing materials under the same water content and weight had a wide range of SHC (<46000 to>1800 Joule/kg.℃). Although different casing materials have different SHC, the HC of a specific material with lower moisture content will have a higher HC. The higher the HC, the more energy or heat it will require to heat up one kg of the material with one degree Centigrade and *vice versa* (Bowles, 1984; Giancoli, 1998). A dry soil is more easily heated than a wet one (Brady & Weil, 2002). This might explain why the same pasteurising process followed for the same casing material from different batches were either under-/overpasteurised.

From previous findings, *C. fulvum* was found to be a major problem in the mushroom growing unit at the University of Pretoria (unpublished data) on pasteurised wattle bark and bagasse. Although *C. fulvum* is not considered a major problem in the commercial mushroom industry, it caused the termination of several experimental trials. During preliminary experiments the incidence and severity of *C. fulvum*'s presence differed depending on the substrate used for mushroom cultivation. The sporulation curve was used in assessing the potential risk of *C. fulvum* contamination and severity thereof. In this study it was found that casing materials with a high organic content stimulated the growth and sporulation of *C. fulvum*. By mixing South African peat (low organic content) with these casing materials with a high organic content the sporulation rate of *C. fulvum* decreased significantly.

In the micro-mushroom growing unit, the inoculation of the South African peat with *C. fulvum* stimulated the growth and cover of the mushroom mycelium compared to the uninocculated South African peat (control). The rate of *A. bisporus* mycelium

growth was faster in the ur ots. Interestingly the *A. bisporus* mycelium growth rate from the pasteurised pots were faster during the first flush (16-18days) compared to the unpasteurised pots (18-20days). This suggests that overgrowth of *A. bisporus* mycelium on the surface of the casing material might result in crop delay. The reason for this needs to be further investigated; however, the delay in crop development with the presence of *C. fulvum* is confirmed by various authors (Van Greuning, 1990; Coetzee, 1987; Vedder, 1978).

5.6 References

Atlas, R. M. & Bartha, R. 1998. Microbial ecology: Fundamentals and application. 4th edition. Benjamin/Cummings Science Publishing, California. 639pp.

Baker, K. F. & Snyder, W. C. 1965. Ecology of soil-borne pathogens. Prelude to Biological Control. An international symposium on the factors determining the behaviour of plant pathogens in soil. Held at the University of California, Berkeley, April 7-13, 1963.

Brady, N. C. & Weil, R. R. 2002. The nature and properties of soils. 13th edition. Prentice Hall. Upper saddle river. New Jersey. 960pp.

Bowles, J. E. 1984. Physical and Geotechnical Properties of Soils. 2nd edition. McGraw-Hill international editions. Civil Engineering Series. 578pp.

Coetzee, J. C. 1987. Die biologie van enkele Fungussoorte wat by die Kommersiele verbouing van Agaricus bisporus in Suid-Afrika probleme skep. MSc thesis. Universiteit van Pretoria. 219pp.

Fletcher, J. T., White, P. F. & Gaze, R. H. 1989. Mushrooms: Pests and Disease control. 2nd edition. Intercept, Andover, Hants, UK. 174pp.

Giancoli, D. C. 1998. Physics, Principles with Applications. 5th edition. Prentice Hall, Upper Saddle River, New Jersey. 1096pp.

Hayes, W. A. 1991. Some thoughts on peat and the quest for an alternative. The Mushroom Journal. July 1991. 223:25-27.

MacDonald, N. W. D., Zac, D. R. & Pregitzer, K. S. 1995. Temperature effects on kinetics of microbial respiration and net nitrogen and sulphur mineralization. Soil Science Society American Journal 59:233-240.

Pianka, E.R. 1970. On r and K selection. American Naturalist 104: 592-597.

Pieterse, Z. 2005. Mycogone perniciosa, a pathogen of Agaricus bisporus. MSc. Thesis, University of Pretoria. 104pp.

Schisler, L. C. 1978. Casing soil treatment. Mushroom News. December 1978. pp 21-31.

Sharma, H. S. S., Furlan, A. & Lyons, G. 1999. Comparative assessment of chelated spent mushroom substrates as casing material for the production of *Agaricus bisporus*. Applied Microbiology Biotechnology 52(3):366-372.

Stoller, B. B. 1969. The science and practice of mushroom growing. 1(1).

Van Greuning, M. 1990. A Mycological study of some pathogens and weed moulds of the cultivated mushroom *Agaricus bisporus*. PhD thesis. University of Pretoria.

Van Greuning, M. 2006. Pers. comm.

Van Staden, J. C., Swanepoel, R. & Koen, J. W. 1992. Fisika. 1ste Uitgawe. Die Nasionale Boekdrukkery, Goodwood, Kaap. 1046pp.

Vedder, P. J. C. 1978. Modern Mushroom Growing. Educaboek, Culemberg. The Netherlands. 420pp.

Weinbauer, M. G. & Höfle, M. G. 1998. Distribution and Life Strategies of Two Bacterial Populations in a Eutrophic Lake. Appllied Environmental Microbiology 64 (10): 3776–3783.

Chapter 6

6.1 General discussion

Agaricus bisporus (Lange) Sing, is the most common cultivated mushroom in South Africa and in many other major mushroom producing countries in the word (Pieterse, 2005; Vetter, 1994). Cultivation of mushrooms has been known to man for centuries. However, the most important developments in this field have mainly taken place during the last half of the 20th century (Atkins, 1974; Fletcher *et al*., 1989; Chang & Miles, 2004). Mushroom growing has changed significantly since 1973. Today it is less profitable and internationally, more emphasis is placed on quality. Hybrid strains have replaced white strains and an obvious tendency emerged with growers that no longer harvest the 3rd, 4th and 5th cropping cycles (Hayes, 1993). This implies that higher yields have to be obtained over a shorter period of time with less labour and energy to increase profit margins. These practices reduce (or optimise) raw material usage and provides for a better conversion of input cost into mushroom production (Samp, 2006). The average yield per growing cycle therefore increased while the number of production units decreased (Vedder, 1978; Vetter, 1994; Samp, 2006).

Agaricus bisporus requires two different substrates for growth i.e. compost and a casing material (Bellmont, 2005; Pardo *et al*., 2003; Labuschagne, 1995). The type of casing, its composition and properties greatly affects the yield and quality of the mushroom (Van Gerwen, 2006; Samp, 2002). A major challenge associated with commercial mushroom production is the availability of suitable casing materials (Eicker & Van Greuning, 1989; Border, 1993). Due to the lack of local quality peat, the South African mushroom industry is forced to import peat at a high cost. This forces the industry to find alternative cheaper casing media for sustainable commercial production of *A. bisporus* (Van Greuning, 1990). Finding alternative waste products may therefore provide a solution to industry even if used as a mix to add to imported peat to reduce production costs and “stretch” the use of the imported peat without compromising on yield and quality.

Cinnamon brown mould caused by *Chromelosporium fulvum* (Link) McGinty is known as an opportunistic fungus found on overpasteurised casing material (MAFF

Canada, 1993). The pres d can significantly affect button mushroom production; however, the mushroom itself is not affected (Howard *et al*., 1994). Little is known regarding the use of this organism as an indicator mould to reflect a disturbed microbial ecosystem. In addition, little information is available regarding the impact of physical and chemical properties of casing materials and pasteurisation on the sporulation rate and growth of the organism.

South African mushroom growers pasteurise their casing material before using it mushroom production (Pieterse, 2005). It was necessary to determine the effect of heat on the physical and chemical characteristics of casing materials. This study was therefore aimed at determining the heat capacities and physiochemical impact of several casing materials. In order to better understand the multidimensional interactions between the physiochemical and biological properties of casing materials, a micro-mushroom unit was build to use in a microcosm study to determine the effect of pasteurisation on the yield and growth of *A. bisporus* when inoculated with *C. fulvum*. It was also the first study to determine the sporulation rate of *C. fulvum* on several materials over a 40 day time period.

From the physical and the desorption study we concluded that excessively dry material's properties to re-hydrate and absorb water, is severely altered. Materials differ in their capacity to hold water and to release it gradually. This will influence the rate of water applied in mushroom production (Kalberer, 1991). Depending on the growth stage of *A. bisporus* the fruit bodies obtain 17-46% of water from the casing (Kalberer, 1987). Results from this study indicate that the role of water-holding, water-retention and water-release characteristics of casing materials for the formation and development of mushrooms are underestimated. The partial substitution of local peat with other fibrous materials will result in a slight to moderate change in desorption and physical characteristics. The change will depend on the characteristics of the material to be used as substitute, as well as the rate of substitution (Noble & Dobrovin-Pennington, 2004).

Furthermore the surface structure of the casing material will influence the growth of the mushroom mycelia on the surface which in turn will influence the pin set, formation and spread of mushrooms on the surface. Casings with high clay content will result in dirty mushrooms, while fibrous casing materials provide cleaner

mushrooms when harvested that by-products from agricultural wastes can be incorporated into local peat to support and improve physiochemical and microbial characteristics. The reduction of local peat usage and incorporating cheaper agricultural waist products into casing materials will have both environmental and economical benefits for the South African mushroom industry (Noble & Dobrovin-Pennington, 2004).

In the heat capacity study we concluded that the thermal conductivity and specific heat capacity (SHC) is influenced by the moisture content and degree of compaction (air and water ratio in soil). The thermal conductivity also influences the air temperature above the soil (Brady & Weil, 2002). The study of SHC of casing materials was necessary to determine how these materials were influenced by the heating process during pasteurisation. However this effect of casing as a temperature buffering medium between air and compost has not been investigated and needs further investigation.

Casing should not contain any nutrients; nutrients should come from the compost (Van Gerwen, 2006). Traditionally, the nutrition required for the establishment, growth and development of the mushroom crop has been regarded by scientists and farmers to be a function of the compost (Hayes, 1973). In contrast to the compost, the casing layer is a dilute medium for growth and received little attention from researchers in terms of its function and relation to nutrition in the crop (Hayes, 1973). In this study we could not make any assumptions towards the function of nutrients in the casing. No direct conclusions could be made between yield and element composition. We assumed that this study provide information on the elemental parameters in the casing materials which mushrooms can tolerate. The heat treatments indicate that temperature have slight effects on the EC, pH and phenolic content. Although the phenolics were not identified in this study we suggest that materials will contain different types of phenols. In general the materials from plant origin had a higher phenolic content. The results obtained from the sporulation curves indicated that casing materials with a high organic content will favour the growth and sporulation of *C. fulvum*. This suggests that the total soluble phenols play an important role in the microbial characteristics of a casing material.

It has been known that by nents to the soil we change the microbial equilibrium. Normally this is done to favour the antagonistic organisms in the soil (Jackson, 1965). High concentrations of organic matter favoured the growth of heterotrophic microorganisms such as *Pseudomonas*, *Bacillus, Penicillium* and *Aspergillus* that are typical zymogenous (opportunistic) soil organisms. The sporulation rate of *C. fulvum* can be reduced by mixing the pure material with South African peat to reduce the percentage organic material in the original material.

Trials from the micro-mushroom growing unit supports that *C. fulvum* would promote the overgrowth of the mushroom mycelium if it is introduced at the time of casing as mentioned by Salunkhe & Desai in 1984 and Stoller in 1969. The inoculation of the South African peat casing with *C. fulvum* resulted in a greater growth and cover of the mushroom mycelium than on the uninocculated South African peat. However the inoculated unpasteurised South African peat had the best average yield. All the pots inoculated with *C. fulvum* had a slight delay in the formation of the first flush compared with the uninocculated control. The mushroom mycelium in the pasteurised pots was quicker to form the first flush compared to the unpasteurised pots.

The physical, chemical and microbial characteristics should be integrated and interpretated as a whole. Only then can this information be used for the altering of the casing material physical and chemical characteristics to improve *A. bisporus* mushroom production potential. A summary of results are given in Appendix 3. This information could also be used to disrupt the growth and development of certain pathogens and weed moulds (Sinden, 1982; Jackson, 1965). Future studies should focus on the identification of phenols extracted from different casing materials in relation to the microbial organisms found in the particular medium.

6.1 References

Atkins, F. C. 1974. Guide to mushroom growing. Faber & Faber. 3 Queen square, London. 122pp.

Bellmont, B. 2005. Peat moss cultivates an earth-friendly reputation. Mushroom News 53(11):12-29.

Beyer, D. M. 2002. Casing Management. Mushroom News. October 2002. 50(10):8-15.

Border, D. 1993. Alternatives to peat. Mushroom Journal. July. 1993. 523:23-24.

Brady, N. C. & Weil, R. R. 2002. The nature and properties of soils. 13th edition. Prentice Hall. Upper saddle river. New Jersey. 960pp.

Chang, S. T. & Miles, P. G. 1989. Edible Mushrooms and Their Cultivation. CRC Press, Inc. Boca Raton, Florida. 345pp.

Eicker, A. & Van Greuning, M. 1989. Economical alternatives for topogenous peat as casing material in the cultivation of *Agaricus bisporus* in South Africa. South African Journal of plant and soil 6(2):129-135.

Fletcher, J. T., White, P. F. & Gaze, R. H. 1989. Mushrooms: Pests and Disease control. 2nd edition. Intercept, Andover, Hants, UK. 174pp.

Hayes, W. A. 1973. The casing layer and its function in mushroom nutrition. Mushroom News 21(12):9-14.

Hayes, W. A. 1993. An overview of the casing layer. The Mushroom Journal. July 1993. 523:26-29.

Howard, R. J., Garland, J. A. & Seaman, W. L. 1994. Mushroom. Chapter 26. Diseases and Pests of Vegetable Crops in Canada. The Canadian Phytopathological Society, Canada.

Jackson, R. M. 1965. Antibiosis and fungistasis of soil microorganisms. In: The Ecology of soil-borne pathogens. Prelude to Biological Control. An international symposium on the factors determining the behaviour of plant pathogens in soil. Held at the University of California, Berkeley, April 7-13, 1963. Edited by Baker, K. F. and Snyder, W. C. 1965.

Kalberer, P. P. 1987. Water Potentials of Casing and substrates and Osmotic Potentials of Fruit Bodies of *Agaricus bisporus*. Scientia Horticulturae 32:175-182.

Kalberer, P. P. 1991. Water relations of the mushroom culture (*Agaricus bisporus*): Influence on crop yield and on dry matter content of dry bodies. Science and Cultivation of Edible Fungi, Maher. Balkema Rotterdam. pp 269-274.

Labuschagne, P. M. 1995. Casing media for *Agaricus bisporus* cultivation in South Africa. MSc thesis. University of Pretoria. 55pp.

MAFF (Ministry of Agriculture and Food) Canada 1993. Ontario mushroom pesticide recommendations. MAFF Publication 367, revised January 1993.

Noble, R. & Dobrovin-Pennington, A. 2004. Use of fine particle tailings in mushroom casing. Science and Cultivation of Edible and Medicinal Fungi. pp335-342.

Pardo, A., de Juan, A. & Pardo, J. E. 2003. Characterisation of different substrates for possible use as casing in mushroom cultivation. Food, Agriculture and Environment 1(1):107-114.

Pieterse, Z. 2005. Mycogone perniciosa, a pathogen of Agaricus bisporus. MSc. Thesis, University of Pretoria. 104pp.

Puustjarvi, V. 1992. Problematic structure standards of horticultural peat. International Peat Journal 4:55-63.

Salunkhe, D. K. & Desai st Biotechnology of Vegetables 2:147-159.

Samp, R. 2002. Obtaining the optimum casing soil. Mushroom News 50(6):22-23.

Samp, R. 2006. Recent developments and future possibilities in the *Agaricus Spp*. (Button) mushroom industry. Mushroom News 54(3):12-23.

Sinden, J. W. 1982. The casing layer and its significance. Mushroom News 30(11):14-17.

Stoller, B. B. 1969. The science and practice of mushroom growing.1(1).

Van Gerwen, H. 2006b. Casing: Don't underestimate the value of casing soil. Mushroom business. June 2006.17:6 -7.

Van Greuning, M. 1990. A Mycological study of some pathogens and weed moulds of the cultivated mushroom *Agaricus bisporus*. PhD thesis. University of Pretoria.

Vedder, P. J. C. 1978. Modern Mushroom Growing. Educaboek, Culemberg. The Netherlands. 420pp.

Vetter, J. 1994. Mineral elements in the important cultivated mushroom *Agaricus bisporus* and *Pleurotus ostreatus*. Food Chemistry 50(3):277-279.

Appendix 1

Different casing materials used in experimental trials in this dissertation

Stocks of raw materials were established at the University of Pretoria between 2002 and 2007 for use in various casing trials. These materials and others (directly obtained from various sources) were used in experimental trials reported on in this dissertation and included the following:

- **Compost:** The Phase III compost used in the trials was obtained from a mushroom farm near Babsfontein, Gauteng Province, South Africa. Compost was prepared from hydrated wheat straw; chicken manure and added gypsum (Fletcher et al., 1989; Sevenster, 2007. Pers. comm.). The A15 strain of *A. bisporus* from Sylvan Africa (Pty) Ltd, Pretoria, South Africa, was used for spawning (Van Greuning, 2008. Pers. comm.).

- **Control (South African reed sedge peat):** Control casing medium used in all experiments. South African peat, an indigenous reed-sedge peat excavated in the vicinity of Potchefstroom, South Africa. Preparation of the casing material was carried out by hydrating and mixing it with lime followed by steam pasteurisation for six to eight hours at 60-65°C.

- **Wattle:** The Black Wattle tree (*Acacia mearnsii* de Wild) in South Africa is classified as an invader plant and one of its main uses is charcoal. When the trees reach a desirable age or size they are cut down, de-barked and transported to the extraction plant. The trees are cut up into smaller pieces and placed in large steam units to extract tannins in water under high temperatures. The wood fibres are then composted (Labuschagne, 1995) and marketed as Fibregro®. The composted wattle bark used in the experiments was obtained from Fibregro (Pty) Ltd in Paulpietersburg, South Africa.

- **Coir:** Coir is a fibrous fibre that is removed from the seed of the coconut (*Cocos nucifera* L. Hughes C. Harries). It is a by-product of the coconut industry (Meerow, 1994; Labuschagne, 1995). Over fifty million cubic meters of the coir pith have accumulated in Sri Lanka whilst one million cubic meters are produced each year. Studies performed on coir have shown that it is

suitable as a casing of a mix (Kemp, 1990; Border, 1993). Coir samples for experiments were obtained from Galukuafrica, Sri-Lanka and shipped in a compressed brick form to Port Elizabeth, South Africa. One brick (30cm×30cm×15cm) was placed in a container and hydrated with water in order to reconstitute the product.

- **Bagasse:** Sugarcane (*Saccharum officinarum*) is produced on a commercial scale in the eastern sub-tropical regions of KwaZulu-Natal, South Africa. Bagasse refers to the raw waste material of sugar cane and it is mainly used as a supplement in animal feed (Gilbert, 2007. Pers. comm.). Bagasse was obtained from Sezela Sugar Mill in KwaZulu-Natal, South Africa for testing as an alternative casing material by the University of Pretoria (UP), South Africa (Labuschagne) in 2002/2003. It was leached for three years on an open concrete floor on the experimental farm at UP and annually turned.

- **Filtercake:** Filtercake is the raw by-product of sugar cane after the juice is extracted and the bagasse has been removed. Lime and soluble phosphates are added in the clarifying process followed by the filtration of the raw juice. The filtrate is known as filtercake (Redmond, 2005. Pers comm.). The filtercake was also obtained from Sezela Sugar Mill in KwaZulu-Natal, and exposed to a three year leaching process on an open concrete floor also at UP before being used in any experiments.

- **Topterra:** Topterra is a company situated in the Netherlands which exports peat to South-Africa. Topterra prepacks in 25 kg sealed, ready for use plastic bags.

- **Spent mushroom compost:** Mushroom compost consists of cereal wheat straw amended with animal manure and gypsum. Before it can be used for button mushroom production it must first undergo composting and conditioning procedures. Mushroom spawn is incorporated with the compost and after a few days (after spawn running) it is covered with a casing material and used for mushroom production. After this medium has been used for production (usually three to four weeks) the substrate is considered spent (Ntougias *et al.,* 2004). Spent mushroom compost (SMC) is the remaining by-

product of commer ation (Soler-Rivas *et al.*, 2004). Spent compost has been obtained from Gordon Bleuw mushroom farm, Babsfontein, South Africa and leached for thee years at UP's experimental farm as described previously.

- **Soyafodder:** Soyafodder is a raw waste product obtained from soybean fodder (*Glycine max* (L.) Merr) and sheep manure. The sheep manure and remains of the fodder mixed together is collectively called soyafodder (Redmond, 2005. Pers comm.). The mix was left outside for six months to be composted on a commercial sheep farm near Greylingstad, Mpumalanga Province, South Africa.

- **Mine dump tailing:** Mine dump tailing was obtained from re-mined goldmine dumps near Springs, Gauteng Province, South Africa, from the Ergo Mining Company, where some elements such as arsenic were removed (Redmond, 2005. Pers comm.).

- **Vertisol soil (Dam soil):** The soil was obtained from a dam made by the Springfield Collieries Coal Mining Company near Grootvlei, Mpumalanga Province, South Africa. The dam was used to pump out water and small coal particles which accumulated in the mine shafts. The Springfield Coal Reserve near Grootvlei is South Africa's third largest coal reserve. Mining stopped in the early 80's due to the low grade quality of the coal. The soil collected was a black swelling clay soil with traces of low grade coal (Van Jaarsveld, 2006. Pers comm.).

Appendix 2

Mushroom growing unit

A small scale mushroom growing unit was developed at the University of Pretoria by the Mushroom Research Group using a 6,06m x 2.44m shipping container (Figure 2.1). The mushroom growing unit was especially designed and modified to control the environmental conditions suitable for mushroom production. The container was modified with a misting unit for humidity and temperature control. The unit also has an automatic cooling and heating unit. The unit can seal completely and has ventilation holes which can be opened or closed to control the CO_2 and air ratios. The container is divided into two sections with a plastic curtain: a small red zone at the entrance and a green zone at the rear for mushroom production. In the production section two steel shelves are fitted to hold seven iron trays or baskets (39 cm×36 cm×17.5 cm) in a row and 6-7 rows on top of each other with adequate space between each row to ease casing, watering and harvesting. Temperature and humidity levels are manually controlled to support mushroom growing. This unit was used in the experimental trials described in this dissertation.

Figure 2.1 The small scale mushroom growing unit at the University of Pretoria.

Treatments used by the UNIVERSITEIT VAN PRETORIA UNIVERSITY OF PRETORIA YUNIBESITHI YA PRETORIA **Mushroom Research Group in their casing trials done between 2002 -2007**

Collected materials (Appendix 1) were mixed using a cement mixer.

The following treatments were prepared and used in this dissertation:

- A control casing medium (indigenous reed-sedge peat) was prepared by hydrating it for 24 hours and mixed with lime in a 40:10 (v/v) ratio before steam pasteurization for 6-8 hours at 60-65℃.
- Wattle bark on its own
- Wattle and peat mixture (50:50 v/v & 70:30 v/v)
- Coir on its own
- Coir and peat mixture (50:50 v/v & 70:30 v/v)
- Bagasse on its own
- Bagasse and peat mixture (50:50 v/v & 70:30 v/v)
- Filtercake on its own
- Filtercake and peat mixture (50:50 v/v & 70:30 v/v)

Different casing materials were mixed in a 50:50 or 70:30 ratio with South African peat to obtain a final volume of seven litres per sample (39 cm×36 cm×5 cm) for each of the seven trays per trial. Each trial was repeated thee times and data was statistically analysed on mean averages for significance between yields with the Fisher's t-test at a 5%, using Statistica 8.0 by Statsoft Inc. Yields are summarized in Appendix 3 and Appendix 7. Once the casing mixes were prepared they were placed in autoclaved bags for steam pasteurisation for 6-8 hours at 60-65℃ at a commercial mushroom farm near Babsfontein. After pasteurisation, the materials were left to cool down for 24 hours before casing.

Appendix 3

Table 1: Overall summary of results obtained in Chapter 3

	Appendix 7	Chapter 3						
Casing treatments*	**Yield from mushroom unit in Kg/m2**	**% Water uptake**	**% Water released**	**Water holding capacity**	**WR (kg/kg)**	**natural water pressent in % dry material**	**potential total water uptake in % dry material**	**potential water available for mushrooms in % dry material**
Peat (Control)	21.6	21.02 c	13.67 d	0.89 ab	1.16 bc	32.84 hi	26.62 b	9.31 b
Wattle	14.6	61.43 i	34.47 hi	2.07 d	0.18 a	15.46 d	159.25 j	69.88 ij
Wattle (70:30)	21.2	48.07 h	29.01 ghi	1.49 c	0.38 ab	9.86 b	123.86 gh	56.33 h
Wattle (50:50)	19.9	55.33 i	30.17 hi	1.48 c	0.32 ab	22.62 efg	92.58 f	36.71 ef
Coir	na	57.17 h	44.27 j	9.55 g	0.04 a	77.88 k	133.47 i	30.12 de
Coir (70:30)	22.3	43.54 h	24.66 fghi	1.48 c	0.45 ab	25.54 fg	142.03 i	69.49 ij
Coir (50:50)	21.6	58.68 j	29.97 i	2.25 e	0.21 ab	28.58 gh	77.11 e	33.44 def
Bagasse	12.5	27.91 de	12.31 cd	1.06 b	0.8 b	32.78 hi	38.72 bc	21.65 cd
Bagasse (70:30)	22.3	25.24 d	12.78 cd	0.82 ab	1.16 bc	41.1ij	48.62 bcd	17.8 c
Bagasse (50:50)	20.8	32.71 f	20.74 ef	1.52 c	0.53 ab	26.37 fg	33.76 bc	16.67 c
Filtercake	17.9	36.41 g	14.43 d	0.87 ab	0.88 b	16.11 d	57.27 d	34.57 def
Filtercake (70:30)	22.9	34.22 g	13.40 d	0.61 a	1.4 c	33.5 hi	40.34 bc	16.91 c
Filtercake (50:50)	20.7	28.74 ef	16.69 de	1.11 b	0.8 b	5.74 a	52.03 cd	31.66 de
Topterra	18.5	33.70 f	23.37 efg	0.44 a	3.72 e	49.16 j	50.83 cd	15.58 c
Spent compost	na	45.40 h	23.59 efgh	1.97 d	0.37 ab	14.37 cd	83.15 e	39.95 efg
Soyafodder	11.4	21.36 c	8.10 bc	1b	1.04 bc	36.29 i	27.15 b	16.86 c
Minedump	6.9	0 a	1.49 a	1.14 bc	0.54 ab	30.75 gh	0.35 bc	0.0 a
Dam soil	14.7	4.53 b	2.72 ab	0.47 a	3.07 d	28.82 gh	4.75 a	1.9 a

*Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2.
Values in columns followed by the same alphabetical letter do not differ significantly according Fisher's t-test (p = 0.05).

Appendix 3

Table 2: Overall summary of results obtained in Chapter 3.

Casing treatment*	Bulk density (g/cm^3)	Natural unit weight (g/cm^3)	Oven dried density (g/cm^3)	Maximum WHC (kg/kg)	WR (kg/kg)	Leach weight (g/l)	Water uptake (g/l)	Water released (g/l)	Total Water content (g/l)	Leached Water Content (g/l)	WHC of leached soil (%/vol)	Pore space (water vol/solid vol) $x10^{-3}$
Peat	1.03 n	1.30 hi	0.69 ij	0.89 ab	1.16 bc	1.12 h	272.92 c	177.45 d	609.62bcd	432.17 cde	0.63	0.48 c
Wattle	0.37 a	0.97 b	0.32 b	2.07 d	0.18 a	0.63 b	594.12 i	333.40 hi	651.78cde	318.37 a	1.01	1.05 i
Wattle (70:30)	0.57 e	1.10 bc	0.44 de	1.49 c	0.38 ab	0.78 c	529.29 h	319.43 ghi	658.59 de	339.16 ab	0.77	0.93 h
Wattle (50:50)	0.48 d	1.07 b	0.43 d	1.48 c	0.32 ab	0.75 c	593.18 i	323.41 hi	640.39cd	316.98 a	0.73	1.04 i
Coir	0.40 b	0.94 a	0.09 a	9.55 g	0.04 a	0.52 a	535.98 h	415.02 j	848.72 h	433.70 cde	4.88	0.94 h
Coir (70:30)	0.66 g	1.17 de	0.47 e	1.48 c	0.45 ab	0.88 e	508.27 h	287.84 fghi	696.64 ef	408.80 cd	0.87	0.90 h
Coir (50:50)	0.47 c	1.13 c	0.35 bc	2.25 e	0.21 ab	0.79 cd	660.82 j	337.51 i	779.67 g	442.15 cde	1.28	1.16 j
Bagasse	0.85 k	1.18 ef	0.57 g	1.06 b	0.80 b	1.03 g	328.94de	145.04 cd	607.39 bc	462.35 de	0.81	0.58 de
Bagasse (70:30)	0.95 m	1.27 gh	0.70 j	0.82 ab	1.16 bc	1.11 h	319.90 d	161.95 cd	569.81 b	407.86 cd	0.58	0.56 d
Bagasse (50:50)	0.81 j	1.20 ef	0.48 e	1.52 c	0.53 ab	0.95 f	393.30 f	249.32 ef	725.76 f	476.44 e	1.00	0.69 f
Filtercake	0.77 i	1.21 f	0.65 h	0.87 ab	0.88 b	1.04 g	440.86 g	174.75 d	564.87 b	390.13 bc	0.60	0.78 g
Filtercake (70:30)	0.86 k	1.30 hi	0.81 k	0.61 a	1.40 c	1.13 h	445.20 g	174.28 d	494.29 a	320.02 a	0.40	0.78 g
Filtercake (50:50)	0.89 l	1.25 g	0.59 g	1.11 b	0.80 b	1.04 g	360.18 ef	209.16 de	659.29 de	450.14 cde	0.76	0.63 ef
Topterra	0.73 h	1.10 bc	0.37 c	1.97 d	0.37 ab	0.84 de	371.57 f	257.66 efg	730.93 fg	473.27 de	1.27	0.65 f
Spent compost	0.62 f	1.14 cd	0.53 f	1.14 bc	0.54 ab	0.87 e	515.78 h	267.99efgh	604.92 bc	336.93 ab	0.63	0.91 h
Soyafodder	1.03 n	1.31 i	0.66 hi	1.00 b	1.04 bc	1.21 i	280.21 c	106.28 bc	654.71cde	548.43 f	0.83	0.49 c
Minedump	1.63 p	1.63 k	1.13 m	0.44 a	3.72 e	1.60 k	5.75 a	24.19 a	496.06 a	471.86 de	0.42	0.00 a
Dam soil	1.45 o	1.51 j	1.03 l	0.47 a	3.07 d	1.47 j	68.70 b	41.22 ab	485.49 a	444.27 cde	0.43	0.12 b

*Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2.
Values in columns followed by the same alphabetical letter do not differ significantly according Fisher's t-test (p = 0.05).

Appendix 3

Table 3: Overall summary of results obtained in Chapter 4

Casing treatment*	pH (20º C)	pH (40º C)	pH (60º C)	pH (80º C)	EC (20º C)	EC (40º C)	EC (60º C)	EC (80º C)	Phenols (20º C)	Phenols (40º C)	Phenols (60º C)	Phenols (80º C)
Peat (Control)	7.57 ef	7.63 ef	7.64 ef	7.75 de	393 b	545 b	476 b	394 b	0.42 a	0.39 a	0.30 a	0.37 a
Wattle	6.76 c	6.85 c	6.86 b	6.94 b	955 d	953 c	991 c	987 c	1.95 ab	1.02 a	1.50 ab	1.00 a
Wattle (70:30)	7.53 def	7.59 ef	7.51 cde	7.49 cd	883 cd	891 c	928 c	970 c	0.44 a	0.42 a	0.49 a	0.41 a
Wattle (50:50)	7.31 de	7.48 de	7.44 cde	7.27 c	933 cd	936 c	925 c	1081 c	0.42 a	0.44 a	0.57 a	0.50 a
Coir	6.27 a	6.26 b	6.25 a	6.29 a	365 b	400 ab	388ab	389 ab	3.06 ab	3.21 ab	4.45 b	4.88 b
Coir (70:30)	7.55 ef	7.71 f	7.62 def	7.62 de	922 cd	869 c	961 c	888 c	0.40 a	0.41 a	0.46 a	0.51 a
Coir (50:50)	7.53 def	7.70 ef	7.65 ef	7.55 de	877 cd	868 c	932 c	953 c	0.36 a	0.37 a	0.37 a	0.51 a
Bagasse	6.69 bc	5.67 a	6.01 a	6.14 a	171 a	230 a	255 a	185 a	30.88 d	32.35 c	33.74 d	35.44 d
Bagasse (70:30)	7.45 def	7.57 ef	7.62 def	7.60 de	373 b	539 a	443 ab	404 ab	4.58 b	3.33 ab	2.42 ab	5.52 b
Bagasse (50:50)	7.29 de	7.51 ef	7.51 cde	7.54 cde	355 b	521 a	427 ab	361 ab	9.84 c	7.03 b	8.41 c	9.31 c
Filtercake	8.32 h	8.30 g	8.39 h	8.38 g	489 b	538 b	574 b	556 b	0.43 a	0.47 a	0.26 a	0.36 a
Filtercake (70:30)	8.01 gh	8.10 g	8.11 g	8.02 fg	438 b	572 b	482 b	439 b	0.30 a	0.31 a	0.38 a	0.35 a
Filtercake (50:50)	8.09 gh	8.13 g	8.31 gh	8.17 g	442 b	570 b	508 b	457 b	0.30 a	0.30 a	0.35 a	0.33 a
Topterra	7.73 fg	7.74 f	7.82 f	7.82 ef	438 b	503 b	529 b	464 b	0.47 a	0.40 a	0.51 a	0.46 a
Spent compost	6.75 c	6.67 c	6.76 b	6.82 b	1915 g	1914 f	1954 e	1883 ef	0.39 a	0.38 a	0.48 a	0.45 a
Soyafodder	8.07 h	8.17 g	8.15 gh	8.23 g	3133 i	3007 g	3223 g	3333 g	0.48 a	0.52 a	0.58 a	0.72 a
Minedump	6.37 ab	6.73 c	6.74 b	6.78 b	1469 e	1313 d	1680 d	1800 e	0.33 a	0.51 a	0.40 a	0.63 a
Dam soil	6.76 c	6.90 c	6.95 b	6.92 b	795 c	810 c	851 c	903 c	0.85 ab	0.59 a	1.18 a	1.77 a

*Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2.
Values in columns followed by the same alphabetical letter do not differ significantly according Fisher's t-test (p = 0.05).

Appendix 3

Table 4: Overall summary of results obtained in Chapter 5

Casing treatment*	Heat capacity	Sporulation curve of *C. fulvum*	Unpasteurised (yield in gram)	Pasteurised (yield in gram)	Unpasteurised (Mycelium growth rating)	Pasteurised (Mycelium growth rating)
Inoculated Control	-	-	147.33 i	88.03 efg	9.50 f	7.33 efgh
Peat (Control)	32411.73 ef	Med	87.93 fg	89.93 efg	6.00 bc	5.33 bc
Wattle	34586.11 fgh	High	0.00 a	75.53 de	0.50 a	4.50 b
Wattle (70:30)	34207.98 efgh	Low	137.03 hi	92.50 efg	6.50 cd	5.33 bc
Wattle (50:50)	30443.59 de	Low	52.97 e	114.87 gh	5.83 bc	7.83 fgh
Coir	39270.50 ij	High	41.10 cde	64.50 cd	3.83 b	5.50 bcd
Coir (70:30)	37703.45 hij	Low	118.33 h	89.53 efg	10.00 f	6.83 cdef
Coir (50:50)	32499.90 ef	Low	0.00 a	49.63 bc	10.00 f	9.00 h
Bagasse	32808.40 efg	High	92.10 g	80.33 def	6.67 cd	4.50 b
Bagasse (70:30)	31944.14 ef	Low	25.27 bc	81.27 defg	4.00 b	6.67 cdef
Bagasse (50:50)	36463.23 ghi	Low	83.33 f	40.13 b	8.00 def	8.50 gh
Filtercake	27356.44 bcd	High	17.00 ab	102.97 ghi	8.50 ef	7.00 cdefg
Filtercake (70:30)	25867.32 bc	Low	91.33 g	122.93 i	8.00 def	8.50 gh
Filtercake (50:50)	31923.37 ef	Low	77.30 fg	100.37 fgh	8.00 def	7.17 defg
Topterra	44339.09 k	High	133.70 hi	115.97 efgh	8.50 ef	7.00 cdefg
Spent compost	27906.17 cd	High	74.00 f	86.10 defg	6.50 cd	6.00 b
Soyafodder	40871.11 jk	Med	30.47 bcd	0.00 a	8.50 ef	0.50 a
Minedump	20840.09 a	High	0.00 a	0.00 a	1.33 a	0.00 a
Dam soil	23738.26 ab	Low	44.60 de	90.90 efg	6.83 cd	5.70 a

*Casings used as described in Appendix 1, using treatment mixes as described in Appendix 2.
Values in columns followed by the same alphabetical letter do not differ significantly according Fisher's t-test (p = 0.05).

Appendix 4

Desorption measuring unit

A desorption measuring unit was build (Figure 4.1) at the University of Pretoria, Mushroom Research Group during 2002 which was based on the design used by Dr. Ralph Noble, Horticultural Research International, University of Warwick, UK. Certain adjustments were made to ensure "*fit for purpose*" and the equipment described below was used in this dissertation to establish desorption curves for experimental purposes.

Set-up of desorption equipment: A two meter high retort stand was build to hold the Buchner funnels level and steady. Conical graduated test tubes were attached in such a manner so that it could be lowered at the required time intervals (Figure 4.2).

Calibration of equipment: Distilled water was used to moisten ten 90 mm Whatman filter papers (#40) which were placed in each of the ten Buchner funnels. A total of 20g Silica powder (Kieselgel Gnach Stahl (Typ 60), Merck) was placed on top of each filter inside the funnel and gently spread out over the surface. The tubes which connect the Buchner funnels to the conical graduated test tubes were completely filled with water to ensure that no air bubbles formed inside or any air pockets remained inside the funnels. Excess water was removed from all the conical graduated test tubes. Ten measuring cups were used to measure out 100ml of water. The 100 mℓ of water was added to each funnel consecutively and as quickly as possible. The water which ended up in the conical graduated test tube in the specified time intervals was then measured (Figure 4.3).

Optimising the method: Once similar volumes of the different materials were obtained, the dry weight was measured, and, the samples were placed in a container filled with water ($^{2}/_{3}^{rd}$ submerged). After 24 hours the samples were weighed and placed unto the moist filter paper inside the Buchner funnel. Readings were taken in 10 minute intervals after which the whole line of centrifuge tubes was dropped to a pre-determined height, to increase the matrix potential (Figure 4.3 & 4.4).

Lowering: Conical graduated and tightened to an iron bar. The bar was held horizontally with two clamps at both edges on two vertical bars. The vertical bars on both sides have several heights. As the bar was lowered, the clamps holding the tubes were loosened and moved downwards to the lower point (Figure 4.3 & 4.4).

Set-up of samples: Blue plastic rings were clearly marked and weighed after a sieved cloth was secured around the bottom edge of the rings with a rubber band. The blue plastic rings (8.5 cm diameter × 5 cm length) were used to measure out 283.83 cm^3 of each material tested.

Figure 4.1: Angle view of the desorption measuring unit used to determine the desorption curves of several alternative casing materials and mixes. The desorption apparatus were based on system developed by Dr. Ralph Noble, from the Horticultural Research Institute, University of Warwick, UK. Notice that the bar is dropped and is not level with the Buchner funnel. This is to create a vacuum in the conical graduated test tubes for water to be removed from the saturated materials.

Measuring: Excess water was removed using a syringe. After each reading, the wet conical graduated test tube was replaced with a dry one.

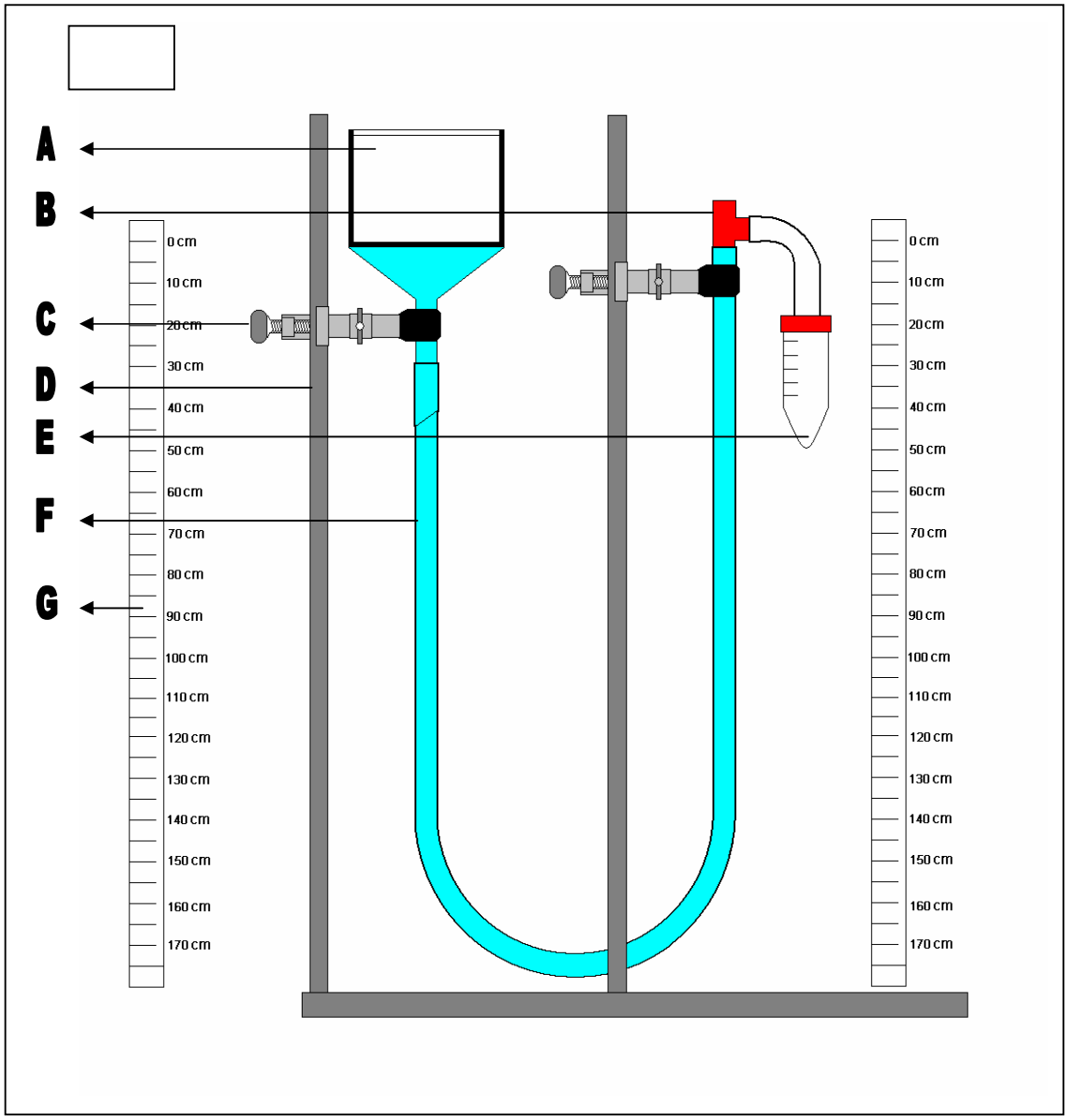

Figure 4.2: Diagram of the desorption measuring unit. Index to figure 4.2: A) Buchner funnel; B) "T" piece; C) Clamp; D) Stand; E) Conical graduated test tube; F) Rubber hose; G) Ruler; H) Base of stand.

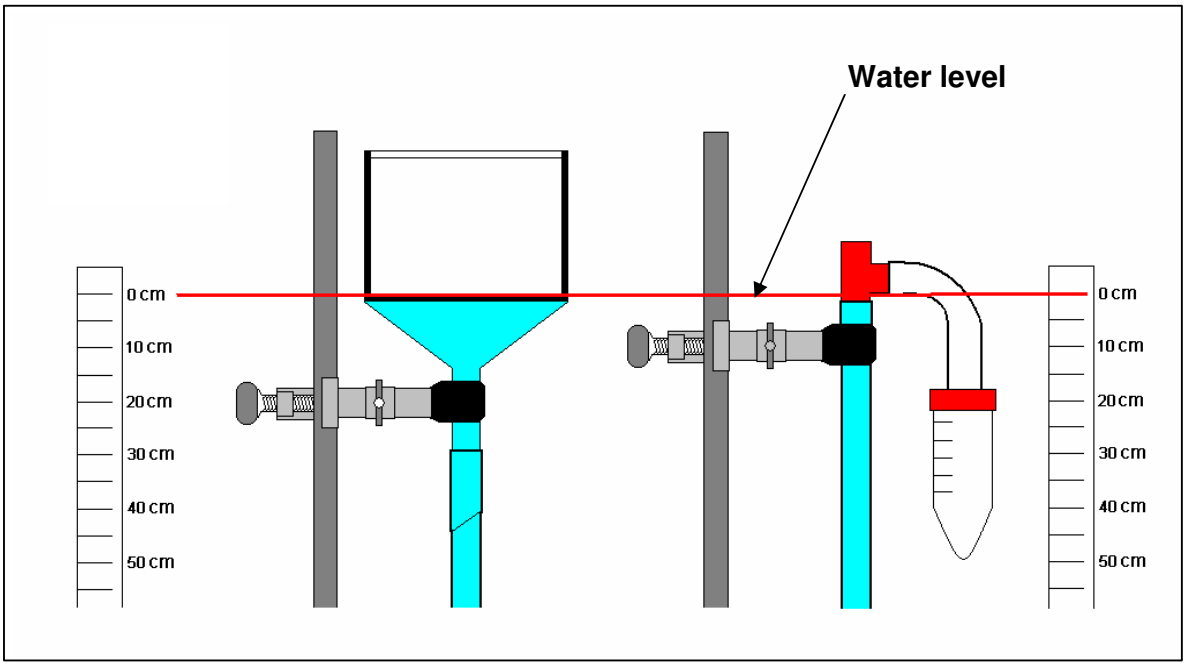

Figure 4.3: Diagrammatic illustration of the water levelling procedure.

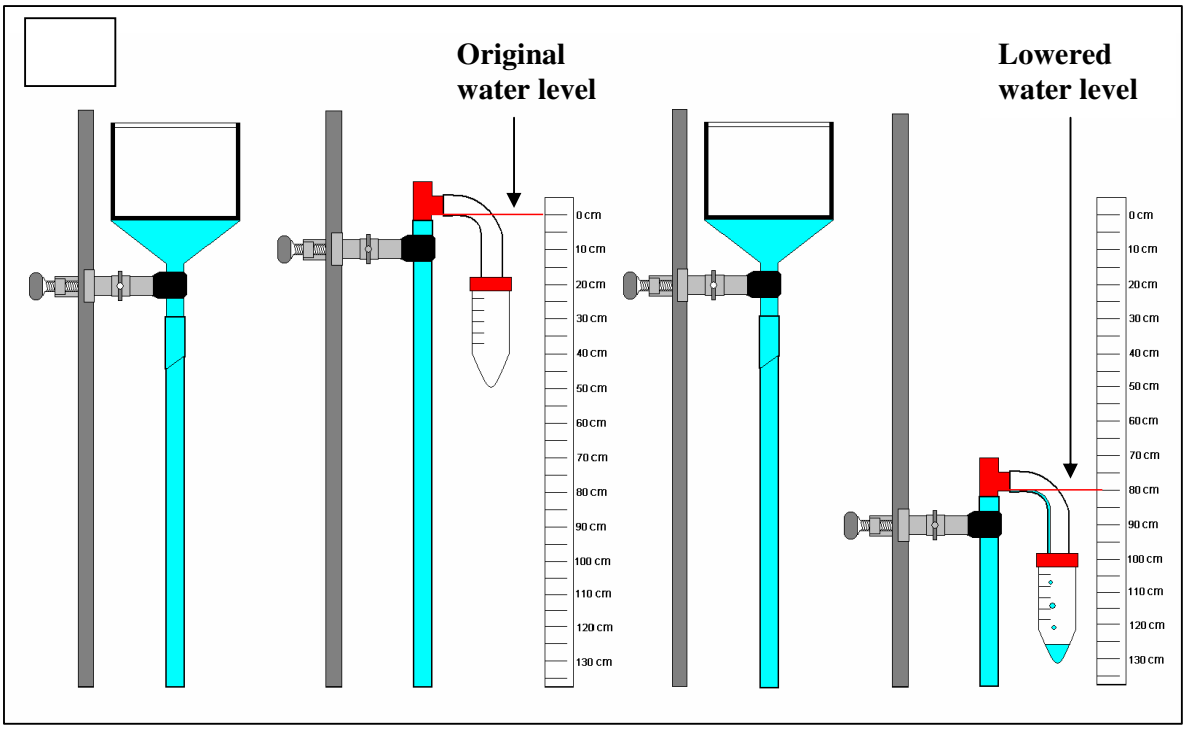

Figure 4.4: Diagrammatic illustration of the lowering of the bar from the same height as the Buchner funnel to 80 cm from the top. Excess water moves from the funnel into the tube due to the vacuum created in the conical graduated test tubes by the lowering procedure.

Appendix 5

Treatment combinations and mixtures used for pH, EC and phenolic evaluations

Table 5: The pure casing materials and their mixes that were used in the pH, EC, phenolic and other evaluations included the following:

Casing material mix or ratio	
South African reed-sedge peat obtained from Potchefstroom as the control	
wattle bark only;	
wattle bark mixed with peat	(50:50, v/v);
wattle bark and peat mixture	(70:30, v/v);
coir on its own;	
coir and peat mixture	(50:50, v/v);
coir and peat mixture	(70:30, v/v);
bagasse on its own;	
bagasse and peat mixture	(50:50, v/v);
bagasse and peat mixture	(70:30, v/v);
filtercake on its own;	
filtercake and peat mixture	(50:50, v/v);
filtercake and peat mixture	(70:30, v/v);
soyafodder and lime	(40:10, v/v);
soyafodder and peat mixture	(50:50, v/v);
spent compost only;	
spent compost and peat mixture	(50:50, v/v);
spent compost and peat mixture	(30:70, v/v);
Topterra on its own;	
mine dump tailings on their own;	
dam soil on its own;	

For a detailed description of the various casing materials see Appendix 1 and for mix preparations see Appendix 2.

Appendix 6

Design and development of a micro-mushroom growing unit

A micro-mushroom growing unit was designed and built for experimental purposes at the University of Pretoria during 2006. The corners of the unit were fortified with Aluminium angle-iron bars. The environment was easily manipulated to simulate the growing conditions of a normal mushroom growing room. The unit (Figure 6.1-6.3) was modified in such a way that it could seal so that the CO_2: O_2 ratio could be adjusted as required and is equipped with a heat gun with an automatic switch to regulate temperature. An ultrasonic humidifier (Salton, SUH20, Johannesburg) was included to supply a constant fine mist that blows into the mini unit through a see-through duct at the top of the unit. Two fans (LaboTec, Johannesburg) were incorporated into the lower and upper air-ducts of the unit. The functioning of the unit includes sucking air in by a fan from the lower area of the unit, pushed through a see-through plastic-bag pipe to the upper fan. The unit was also fitted with a sealable door on the one side for easy access.

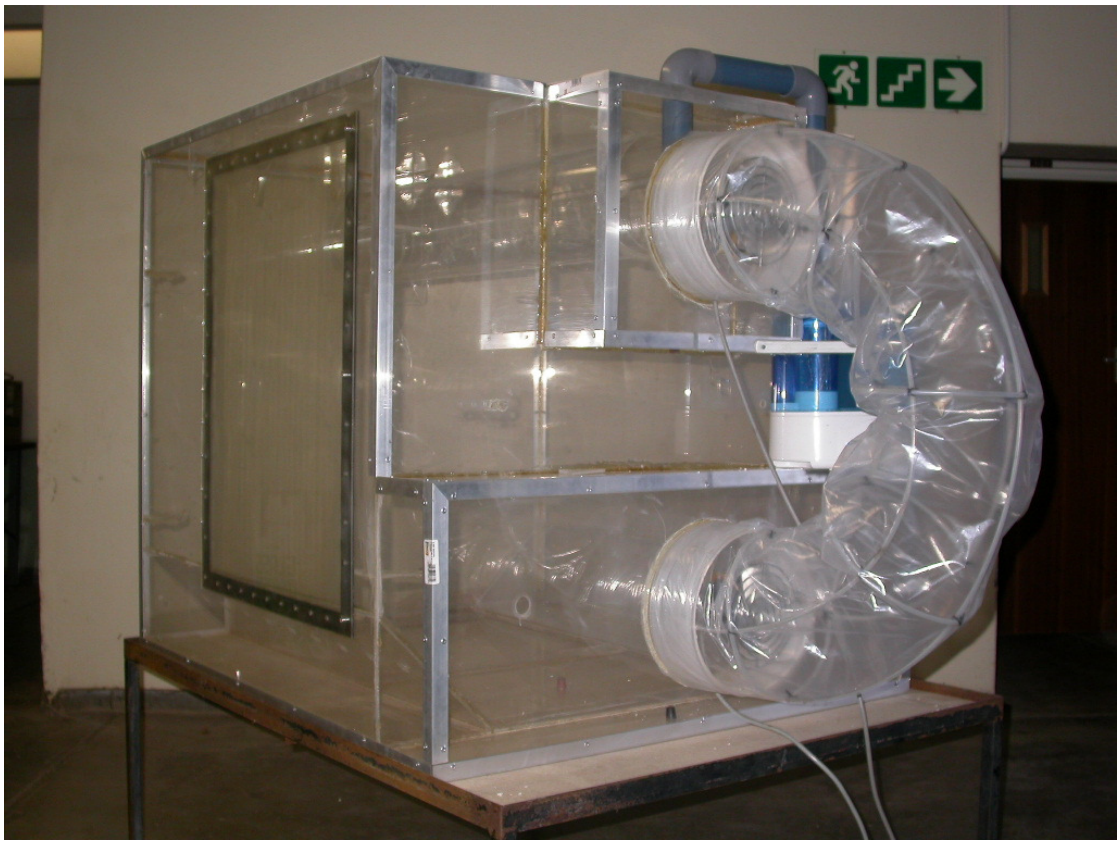

Figure 6.1: One cubic meter Perspex micro-mushroom growing unit.

Figure 6.2: Plastic duct removed, exposing fans of the micro-mushroom growing unit.

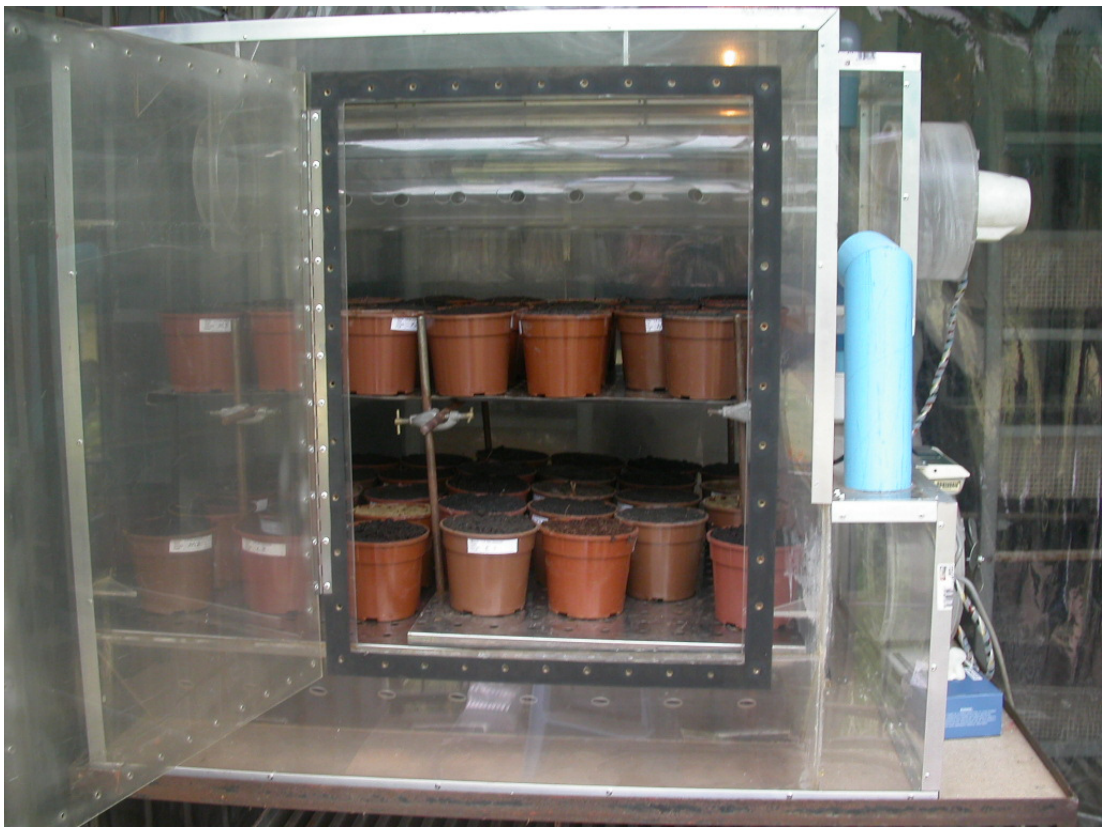

Figure 6.3: Side view of the micro-mushroom growing unit after pots was cased.

Appendix 7

Yield obtained on different casing materials conducted in the mushroom growing unit by the Mushroom Research Group of the University of Pretoria

Table 7: Compiled yield data from the Mushroom Research Group's casing trials on different casing materials and mixes in the mushroom unit between 2002 and 2007.

Experiment	Casing material	Mean yield in kg/m^2
1	Control [a]	21 0
	Coir [a]	14.6
	Wattle [a]	na
	Bagasse [a]	12.5
	Filtercake [a]	17.9
	Topterra [a]	18.5
2	Control (Peat) [b]	21.6
	Coir (70:30) [b]	21.2
	Coir (50:50) [b]	19.9
	Wattle (70:30) [b]	22.3
	Wattle (50:50) [b]	21.6
3	Control (Peat) [y]	22.4
	Bagasse (70:30) [y]	22.3
	Bagasse (50:50) [y]	20.8
	Filtercake (70:30) [y]	22.9
	Filtercake (50:50) [y]	20.7
4	Control (Peat) [z]	18.6
	Soyafodder [z]	na
	Soyafodder (50:50) [z]	11.4
	Minedump tailing [z]	6.9
	Dam soil [z]	14.7

[a and y] = yield data by Amanda Redmond and LP van Jaarsveld (Mushroom Research Group) [b] = yield data obtained from Petra Labuschagne and Amanda Redmond (Mushroom Research Group) [z] = preliminary yield data obtained in this study (Mushroom Research Group)