LABORATORY CULTURE OF SOME SPECIES OF **MUSHROOMS COLLECTED IN AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA**

BY

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DEDICATION

This work is dedicated to my grand aunt Mrs. Awele Egbuniwe, my departed grand mother Mrs Comfort Nwanze, my two dearly departed friends Tony Asoya and Perry Kigwana and all the Nwanze family members who stood by me during the course of the project.

DECLARATION

I hereby declare that this thesis is a record of my own research work. It has not been presented by me or any previous application for a higher degree.

Pnwanze

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November 15, 1986

Date

CERTIFICATION

This thesis titled: Laboratory culture of some species of mushrooms collected in Ahmadu Bello University, Zaria, Nigeria by Nwanze P.I. meets the regulations governing the award of the degree of Master of Sciences (Educational Biology) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Studies on spore germination of 15 mushroom species collected from various areas in Ahmadu Bello University, Zaria were carried out on potato dextrose, Sabouraud's dextrose, and yeast extract agar. Each of them was supplemented with peptone, malt extract, yeast extract, spawn, and charcoal in 32 different combinations. Out of 15 species only the spores of Lentinus squarrosulus (Mont.)Singer consistently germinated in all the media. Attempt was also made to culture the 15 mushroom species from the hyphae collected from the pileus on 4 different hyphal culture media. Only L. sauarrosulus grew in all different media while Termitomyces clypeatus Heim. only grew in the media which contained thiamine hydrochloride.

Carpogenesis was studied in L. squarrosulus using two composted media and five non-composted media. Carpogenesis successfully occurred in 4 out of the 5 non-composted media while carpogenesis failed to occur in any composted media.

Fruiting was induced by direct casing of spawn, casing of some non-composted media and traumatizaton of hyphae. Only the spawned sawdust media cased with sterilized soil to which activated charcoal had been added produced fruit while both the cased spawn and the traumatized hyphae produced negative results.

The study shows that it is possible to effectively culture L. squarrosulus for laboratory purposes within 25 days. It also confirmed that this can easily be done using locally sourced materials.

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CHAPTER ONE

INTRODUCTION

The order Agaricales is composed of 16 families and 197 genera. The gilled fungi or mushrooms belong to one of these families, viz Agaricaceae, which is composed of 31 genera (Thompson and Lim, 1965; Weber, 1970; Shaffer, 1975; Singer, 1975).

The study of the mushroom started in the dim past before the invention of the microscope by Leeuwenhoek in the 17th century (Ainsworth, 1976). They drew the attention of mycologists because of their big size. Now they occupy a very unique position because of their innumerable economic advantages. They are responsible for biodegradation of agricultural wastes (Durrant et al, 1991; Boominathan and Reddy, 1992; Bono et al, 1992). In addition they are also important for their medicinal and educational uses (Christensen, 1975; Gerard 1975; Vogel et al, 1975; Bossard, 1978; Flegler, 1979a; Ellis, 1991; Oei, 1991). Many species of mushrooms are eaten in many parts of the world because of their nutritional values (Zakia et al, 1971; Ammirati et al, 1977; Crisan and Sands, 1978; Smith, 1978; Annakkage and Ratnasiri, 1990). It is due to these facts that they are cultured in various parts of the world in glass houses, green houses, caves etc. in order to maintain their regular supply in off seasons as they are ephemeral and seasonal.

In Nigeria very scanty attempts have been made to culture the mushrooms (Alasoadura, 1967; Zoberi 1972; 1973; Oso, 1977; Nicholson, 1989; and Alabi, 1994). A project was therefore designed to find out the various ways to culture different mushrooms in the laboratory using different culture media and two different parts of mushrooms including spores, and mycelial fragments from the pileus with the sole aim to make them available throughout the year for educational purposes. In addition an attempt was also made to initiate carpophore production.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Procurement/Storage/Viability of Spores

Mushroom spores may be obtained from a spore print. All that is required is a mature carpophore with an open pileus, contrasting paper, and a bell jar (Anderson et al, 1973; Alexopoulos and Mims, 1979; Burge, 1979). The procedure may also be modified by placing the mushroom gills down on a glass slide and covering it with a Petri dish to prevent air currents and the resultant airborne contaminants (Thiers, 1975).

The spores may be stored at a low temperature in the refrigerator without affecting the viability of the spores for a long time. Spores stored at 4^0C and - 18^0C retained their viability for 2 to 4 months respectively (Fries, 1977).

2.2. Exclusion of Contaminants

During the culture of the spores of different mushrooms obtained in nature, culturing practices determine the extent of contamination. Aseptic methods were found to be adequate to exclude the contaminants (Watling, 1971; Chua and Ho, 1973; Epp, 1977; Murashige, 1979; Seabrook, 1980).

2.3. Culture Media

Different types of media have been used for culturing the spores of mushrooms. The most commonly used media include Potato dextrose agar (PDA)(Raabe, 1972; Stack et al, 1975; Flegler, 1979a), Potato-carrot agar (PCA) (Thakuri, 1976; Watling, 1981) malt/yeast extract agar (Heintz and Niederpruen, 1971; Oso, 1977; Roxon and Jong, 1977; Chapman and Barankovich, 1979), and Sabouraud's dextrose agar (SDA) (Cole and Samson, 1979; Mckane and Kandel, 1986). Watling (1981) however, reported PDA to be the best media.

Sometimes problems are encountered with sterilized media as a compound inhibiting the germination of spores has been reported to be formed by autoclaved agar (Fries, 1966). The work done on smuts indicated that the inhibitory effect could be removed through the addition of different concentrations of activated charcoal to the media (Klein and Bopp, 1971; Butler and Bolkan, 1973). With mushrooms the problem was solved by dusting half of the Petri dish with 7mg of activated charcoal (Fries, 1977; 1978; 1983a).

2.4 Inducing In Vitro Germination

2.4.1 *Media additives*

Many substances have been shown to induce germination. Partially processed natural products such as yeast and malt extracts and peptone are widely used in making media (Lilly, 1965). Peptone which is obtained from lean meat, heart muscle and soya bean flour (Bilgrami and Verma, 1978) is a good

source of nitrogen and is highly recommended for the cultivation of fungi. It has widely been used in the culturing of mushrooms (Leonard and Dick, 1973; Marvanova and Bandoni, 1987; Whitney and Arnott, 1987) and is known to increase germination rate in Laccaria laccata spores by up to 75% (Fries, 1983a). More importantly, however, it serves to stabilize the pH of the culture media (Child et al, 1973).

Malt extract is also a good germination inducer (Bistis, 1970; Stocks and Hess, 1970) and apparently provides a stimulatory substances in sufficient quantity to yield a high percentage of germination (Chapman and Barankovich, 1979).

Yeast extract which is an excellent source of vitamin B complex is known to increase germination (McDaniel and Hindal, 1982) as well as stimulating good mycelial growth (McTeague et al, 1959; Paranjpe et al, 1979).

Live yeast has also been reported to induce germination. Bulmer and Beneke (1961, 1962) used Rhodotarula mucilaginosa to germinate spores of Calvatia gigantea, while Fries (1978, 1983b) used Rhodotarula glutinis to germinate some mycorrhyiza - forming Hymenomycetes and some species of Bolitaceae. It has since been discovered that the active substance produced by the live red yeast is isovaleric acid (Rast et al, 1979; Weete, 1980). This same isovaleric acid is also produced by spores, vegetative mycelium, and sporophores of Agaricus bisporus (Rast and Stauble, 1970). Some spores have been successfully germinated using trace amounts of isovaleric acid

as well as other organic acids such as propionic and butyric acids (Losel, 1967).

2.4.2 *Use of mycelia*

According to Cayley (1936), Ferguson in 1902 made a very significant break through while working with Agaricus campestris. She discovered that germination could be stimulated by the introduction of small pieces of mycelium from a culture of same into the spore suspension. The work was later confirmed using Agaricus bisporus (McTeague et al, 1959; Losel, 1964).

2.5 Mycelial (Hyphal) Culture

When spores prove very difficult to germinate it is sometimes possible to obtain a pure culture by culturing hyphae which has been aseptically cut from the inner flesh of the upper part of the stipe and pileus (Mclaughlin, 1974; Stack et al, 1975). The mycelia may then be cultured on malt extract agar (Murashige, 1979), yeast extract agar (White, 1963) or acidic potato dextrose agar (McLean and Cook, 1958; Christensen, 1965) in culture tubes (Moscona et al, 1965) with steel caps for more uniform gas exchange (Murashige, 1973).

Antibiotics such as streptomycin/tetracycline sulphate (100mg/l) may be added in order to keep down bacterial contamination (Whitaker, 1972; Thomas and Davey, 1975). Antibiotics were however, found to have adverse effects on

mycelial cultures (Coriell, 1973; Schaffner, 1979). Supplementation with macronutrients in the form of salts such as $MgSo_4$. 7 H_2O, NH_4NO_3, KCl, K_2HPO_4 and KH_2PO_4 in conjunction with the vitamin thiamine hydrochloride followed by incubation in the dark between 20-28^{0}C has been reported to enhance mycelial growth (Street, 1966; Hudson, 1972; Butcher and Ingram, 1976; Chandra and Purkayastha, 1977).

2.6 Mycelial Preservation

There are many methods of preserving a pure culture. The most sophisticated is the process of lyophilization (freeze-drying) but it is very expensive in terms of both manpower and machinery (Hwang et al, 1976; Tan et al, 1992). Liquid nitrogen may also be used (Hwang, 1968) but in the tropics a relatively simple and inexpensive method is to use glass (Turner, 1971) or preferably disposable screw cap plastic tubes (Smith, 1971) to make agar slants. These slants are then covered with demineralised water or mineral oil such as liquid paraffin (Thompson and Lim, 1965; Hawksworth, 1974; Perrin, 1979). The cultures may remain viable in this form for 1 to 10 years respectively (Sathe and Dighe, 1987; Oei, 1991).

2.7 Spawn Production

Spawn may be defined as horse manure or grain which has been sterilized and impregnated with selected mushroom tissue

or mycelium under aseptic laboratory conditions (Atkins, 1974). The grains used include wheat, corn, rye, millet, birdseed, and brown rice (Bigwood, 1977; Fritsche, 1978; Zervakis and Balis, 1991; Cangy, 1994). Spawn has been reported to induce spore germination while the grains used in making it (rye, corn, and wheat) induce fruiting (Schisler et al, 1967, Royse and May, 1982).

2.8 Carpogenesis

Mushroom species such as Agaricus bisporus, Pleurotus ostreatus and Volvariella volvacea have been reported to prefer composted media (Delmas, 1978; Edwards, 1978; Kim, 1978). According to Hayes (1978) a good compost may be formulated by using different manures to provide nitrogen and carbohydrates, wet brewers grains and potato wastes to provide carbohydrates, urea or ammonium sulphate to provide nitrogen and supplements such as fertilizers (superphosphate, potash) and gypsum to rectify mineral deficiencies. Some work has also been done with phosphogypsum which in addition to increasing yield also induces a more rapid growth (Chen et al, 1988). There is need for composting because raw manure would heat up and kill the spawn. Wheat straw is favoured but rice, barley, rye, and oat straw may also be used to provide a reservoir of cellulose, hemicellulose and lignin.

Casing has been reported to induce fruiting (Genders, 1976; Delmas, 1978; Hayes, 1978). The process of casing or capping is simply the covering of compost beds or spawn with a thin layer of sand, gravel, peat, or medium loam soil (Hayes et al, 1969). Special care must be taken to sterilize the soil properly in order to kill pests and pathogens which may be present in the soil. The soil, however, should not be autoclaved since this kills certain bacteria (Pseudomonas putida) which trigger sporophore formation (Marx and Haasis, 1965; Manning and crossan, 1966; Hayes et al, 1969; Park and Agnihotri, 1969). If the soil is highly contaminated with pathogens, it must be autoclaved. The inhibitory effect may then be removed through the addition of activated charcoal to the casing soil (Long and Jacobs, 1974; Angeli- Couvy, 1975).

Soil may also be sterilized by the use of steam for 5 minutes at 100^0C or 2% formaldehyde spread over the soil. The formaldehyde treatment may be better since steam is also destructive to bacteria which are beneficial to the crop (Hayes, 1978; Vedder, 1978).

It has long been discovered that there is a relationship between the addition of lipids (vegetable oils) and the stimulation of fruiting (Schisler and Sinden, 1962; Wardle and Schisler,1969). Schisler and Sinden (1962) are of the opinion

that any enhancement in first-break yield must be due to the initiation of a greater number of sporophores. Highly proteinaceous materials such as ground pigeon pea (Cajanus cajan) (L.) Millsp. and soya bean (Glysin max) (L.) Merr. stimulate fruiting (Srivastava and Bano, 1962; Schisler, 1967). Sometimes the addition of natural extracts (yeast and malt) to mycelium colonized compost is enough to induce fruiting (Watling, 1981). Some species may be stimulated to fruit in Petri dishes when their mycelium are traumatized (Bigelow, 1970; Leonard and Dick, 1973) while others require cooked apple extract in the media (Chua and Ho, 1973). A faster and more direct method of fruit body production in the laboratory is the direct casing of grain spawn (San Antonio, 1971). In addition to the above Watling (1981) also suggested that relatively high humidity and a supply of rich moist well-aerated medium may also induce fruiting.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 COLLECTION, PROCUREMENT AND STORAGE OF SPORE PRINTS

Fifteen different mushrooms (Table 1) found growing in different residential areas of the main campus of Ahmadu Bello University, Zaria (Map) were collected, wrapped in newspapers and brought to the laboratory for further studies. The spores were obtained as described by Anderson et al, (1973), placed in envelopes and stored in a laboratory refrigerator (Watling, 1981).

3.2 MEDIA PREPARATION

The following three different categories of media were used in these studies.

(a) germination test media - to study the germination and culture of mushrooms from spores.
(b) hyphal culture media - to generate pure cultures from mushroom tissues.
(c) carpophore production media - to stimulate the production of carpophores.

3.2.1 *Germination Test Media*: Three basic media including potato dextrose agar (PDA), Sabouraud's dextrose agar (SDA) and yeast extract agar (YEA) were used to study the germination of spores and culture of mushroom from spores. In all the cases both fresh and ready made ones were prepared (Table 2a).

Table 1: Mushrooms collected from different residential areas of the main campus of Ahmadu Bello University, Zaria.

Sample No.	Name of Mushrooms	localities
1.	Bolbitius spp. A	Univ. chapel (U-4)
2.	Bolbitius spp. B	Biological sciences garden(Y-9)
3.	Bolbitius spp. C	Amina hall (V-4)
4.	Coprinus spp. A	Amina hall (V-4)
5.	Coprinus spp. B	Swimming pool (V-3)
6.	Coprinus spp. C	Dan-Fodio hall (O-5)
7.	Lentinus squarrosulus (Mont.)Singer	Univ. bookshop (W-5)
8.	Lepiota spp.	Senior staff club (V-9)
9.	Pholiota spp. A	Biological sciences garden (Y-9)
10.	Pholiota spp. B	Biological sciences garden (Y-9)
11.	Psathyrella spp.	Junior staff resid. areas (S-2)
12.	Psilocybe spp.	Akenzua hall (Z-2)
13.	Termitomyces spp.	V.C's residence (Z-8)
14.	Termitomyces clypeatus Heim.	Sen. staff residential areas (Area A, Y-5)
15.	Termitomyces microcarpus (Berk & Br.)Heim.	Sen. staff resid. areas (Area A, Y-5)

NB: Coordinate codes U-4, Y-9, etc. are to be used in conjuction with the map on the following page.

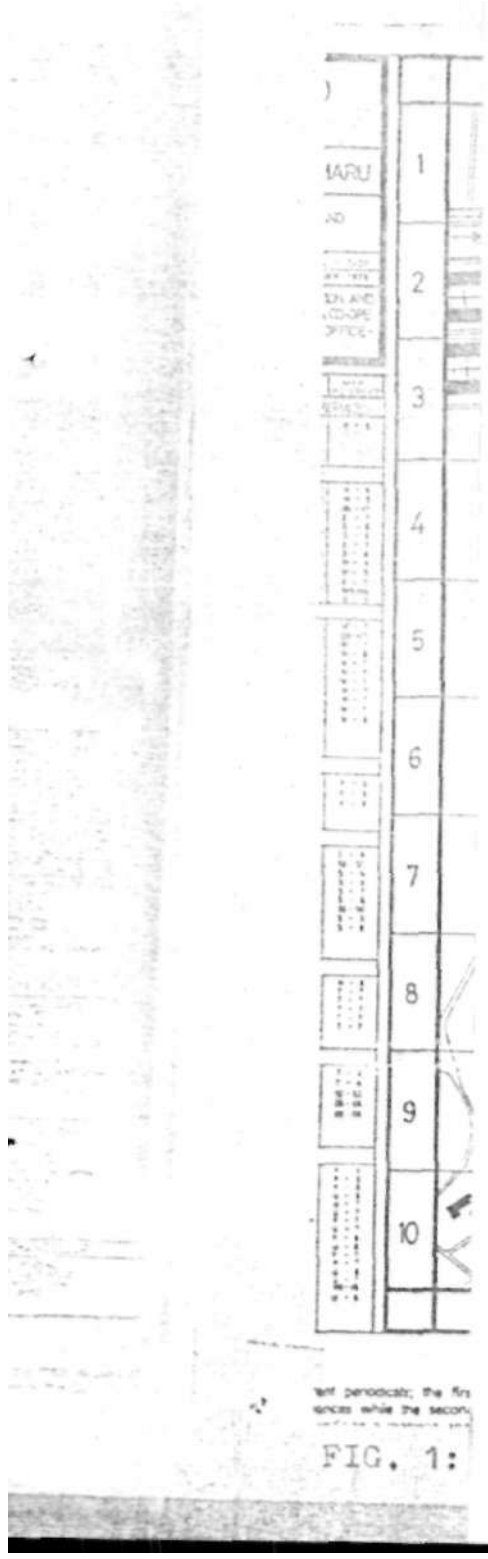


FIG. 1:

Table 2a: Basic media used in this study.

Media	Components	Method of preparation
Fresh potato dextrose agar (PDA)(Cheng Tu, 1978)	300g peeled Irish potato 20g agar 20 g glucose	the potatoes were boiled for 20 minutes and extract of it was mixed with the rest of the components and autoclaved at 121^{0}C for 15 minutes.
Ready made potato dextrose agar (PDA)	39g potato dextrose agar (OXOID)	the agar was dissolved in 1 litre distilled water and autoclaved for 15 minutes at 121^{0}C.
Fresh Sabouraud's dextrose agar(SDA) (Bilgrami and Verma, 1978).	40g glucose 10g peptone 15g agar	the components were suspended in 1 litre of distilled water and autoclaved at 121^{0}C for 15 minutes.
Ready made Sabouraud's dextrose(SDA)	65g Sabouraud's dextrose agar (OXOID)	the agar was dissolved in 1 litre of distilled water and autoclaved for 15 minutes at 121^{0}C.
Fresh yeast extract agar(YEA) (Watling, 1981)	10g glucose 3g yeast extract 0.2g $MgSO_4.7\ H_2O$ 2g K_2HPO_4 20g agar	all the components were suspended in 1 litre of distilled water and autoclaved at 121^{0}C for 15 minutes.
Ready made yeast extract agar(YEA)	23g yeast extract agar (OXOID)	the agar was dissolved in 1 litre of distilled water and autoclaved for 15 minutes at 121^{0}C.

All the above categories of media were incorporated with three different nutritional components viz peptone, malt extract, and yeast extract in different combinations. In addition they were also incorporated with activated charcoal and spawn (Table 2b).

All the three nutritional components were added prior to autoclaving. The activated charcoal was, however, dusted over half of each of the Petri dishes after autoclaving but prior to inoculation (Fries, 1983a) while the spawn was added after inoculation (Royse and May, 1982).

3.2.2 *Hyphal Culture Media*

For this purpose hyphal culture media (Batra and Batra, 1979; Whitney and Arnott, 1987) and hyphal culture media (Purkayastha and Chandra, 1975) were prepared and arbitrarily named as HCM1 and HCM2 respectively for the sake of convenience. In addition, acidic potato dextrose agar (Christensen, 1965) and streptomycin treated potato dextrose agar (Watling, 1981) were also used for these studies (Table 3).

3.2.3 *Carpophore Production Media*

Various non-composted media including rice straw (Jong and Peng, 1975), modified rice straw, sawdust (Carey, 1974), animal bedding and rice (Roxon and Jong, 1977), and sawdust and oatmeal (Pettipher, 1988) were used for these studies (Table 4a). In addition compost media (Kim, 1978) and Compost media (Hayes, 1978) were also used. These were arbitrarily named as CM1 and CM2 respecively (Table 4b).In addition both compost media CM1 and CM2 were supplemented with groundnut oil, ground pigeon pea, and malt extract (Table 5).

Table 2b: **Composition of three media with three different nutritional components, activated charcoal, and spawn.**

Category of media	Composition of media
A. Alone	all the three media without any nutritional supplements.
B. With nutritional components	a) 3g each of peptone, malt, and yeast extract mixed with one litre of each of the above three media separately. b) 3g of each of the above in different combinations with each other in one litre of each of the three media. i. 3g peptone + 3g yeast extract ii. 3g peptone + 3g malt extract iii. 3g yeast extract + 3g malt extract iv. 3g peptone + 3g yeast extract + 3g malt extract.
C. With activated charcoal	In addition to the different nutritional components in different components in different combinations as above, 7mg of activated charcoal was also added.
D. With spawn	In addition to the different nutritional components and activated charcoal, 2 spawn grains were also used in each case as described by Royse and May (1982).

Table 3: Different hyphal culture media.

media	Components	Method of preparation
Hyphal culture media (HCM1) (Batra and Batra,1979)	20g dextrose 2.0g mycological peptone 2.0g malt extract 2.0g yeast extract 1.0g K_2HPO_4 0.5g KH_2PO_4 0.5g $MgSo_4.7H_2O$ 0.5g NH_4Cl	all the above components were suspended in 1 litre of distilled water and autoclaved at 121 ^{0}C for 15 minutes.
Hyphal culture media (HCM2) (Purkayastha and Chandra, 1975)	the same as above plus the inclusion of 0.5g thiamine hydrochloride	same as above.
Acidic potato dextrose agar (christensen, 1965)	39g potato dextrose agar (OXOID) 5 drops of 50% lactic acid per 100 ml media	the media was prepared by adding 5 drops of 50% lactic acid per 100ml of sterilized media which has been cooled to 45^{0}C.
Streptomycin treated potato dextrose agar (Watling, 1981).	39g potato dextrose agar (OXOID) 0.3ml antibiotic solution per 100ml of agar	the antibiotic was prepared by adding 1g of streptomycin to 100ml of sterile water. The media was sterilized, cooled to 45^{0}C and 0.3ml of the antibiotic solution was added to each 100ml of agar.

Table 4a: Non-composted media.

media	components	method of preparation
Rice straw (Jong and Peng, 1975)	30g rice straw 23.4g hardwood sawdust 6.0g rice bran 0.6g corn meal	the rice straw was soaked for 4 days, mixed with the remaining components and autoclaved for 20 minutes.
Modified rice straw	30g rice straw 23.4g hardwood sawdust 6.0g rice bran 0.6g corn meal 30g oatmeal (Quaker oats)	same as above.
Sawdust (Carey, 1974)	20g hardwood sawdust 20g hardwood chips 40g brown rice	the components were wetted with 200ml of distilled water and autoclaved at 121^{0}C for 30 minutes.
Animal bedding and rice (Roxon and Jong, 1977)	13g softwood chips 13g brown rice	the materials were thoroughly moistened and autoclaved at 121^{0}C for 35 minutes.
Sawdust and oatmeal (pettipher, 1988)	105g hardwood sawdust 45g oatmeal (Quaker oats)	the material were thoroughly wetted and autoclaved at 121^{0}C for 20 minutes.

Table 4b: Composted media

media	components	method of preparation
Compost media (CM1) (Kim, 1978)	50g chicken manure 0.500kg chopped wheat straw (Siete cerros) 7.5g urea 5.0g gypsum	all the materials were wetted, thoroughly mixed and composted for 30 days.
Compost media (CM2) (Hayes, 1978)	100g horse manure 0.500kg chopped wheat straw (Siete cerros) 200g potato waste 10g ammonium sulphate 10g gypsum 10g superphosphate	all the materials were wetted, thoroughly mixed and composted for 30 days.

Table 5: Supplementation of compost media at spawning.

compost	supplement
50g CM1	none
50g CM1	2.86 ml groundnut oil
50g CM1	2.86g ground pigeon pea
50g CM1	2.86g malt extract
50g CM2	none
50g CM2	2.86 ml groundnut oil
50g CM2	2.86g ground pigeon pea
50g CM2	2.86g malt extract

All the media were inoculated with 2g of spawn.

3.3 SPAWN PRODUCTION AND TREATMENT

Spawn was produced by a method described by Fritsche (1978). 100g wheat grains were taken in 27 different jars, inoculated with mycelia obtained from pure cultures and incubated in darkness at 27^{0}C for 2 weeks. Out of these 9 spawn jars were cased with 1 and half inches of formaldehyde treated top soil loam while another 9 jars were cased with non-sterilized top soil loam. These were used for fruiting studies. The jars were grouped and incubated under 3 categories viz continuous light, intermittent light and continuous darkness at 26-27^{0}C to see fruiting. The rest of the 9 were used for germination and carpophore production tests.

3.4 CULTURE TECHNIQUES

3.4.1 *Culture of mushrooms from spores.*

All the test media were aseptically poured in Petri dishes and inoculated using both the diluted (Fries, 1977) and undiluted (Watling, 1981) methods of inoculation. The plates were grouped into three categories of 3 plates each. Group 1 was incubated in continuous darkness, group 2 in intermittent light while the last group was incubated in total darkness at room temperature ranging between 26-27^{0}C for a period of 1-2 months. Later the number of germinated and ungerminated spores were counted out of a total of one thousand. The data was subjected to analysis of variance (ANOVA) and compared by using Duncan multiple range test (Losel, 1967).

3.4.2 *Culturing of mushrooms from the hyphae from the pileus in solid media.*

The culture technique was based as described by Chua and Ho (1973), McLaughlin (1974), and Thomas and Davey (1975). It was performed by aseptically removing small bits of mycelium from the pileal context of 15 different mushrooms collected afresh from different localities and plating it on the desired agar. The plates were grouped and incubated under three different conditions viz, continuous light, intermittent light and continuous darkness at the room temperature of 26-27^{0}C for three weeks.

3.4.3 *Culturing of mushrooms in liquid media.*

The pure cultures obtained from the hyphal cultures were maintained on each of the 4 different hyphal culture media at a range between 26-27^{0}C under three conditions of incubation. The test media were prepared as reflected in table 3 but without the addition of agar as described by Jennison (1948), Hacskaylo et al, (1954), and Sugihara and Humefeld (1954). The culture of the mycelia in the test media was performed in 250ml Erlenmeyer flasks containing 100ml of each of the 4 different media at 27^{0}C on a shaker (90 rotations/min) (Jennison et al, 1955). Two pieces of 1cm^2 pieces of mycelium and agar cut from 2 week old cultures growing under the different conditions of incubation were used as inoculum. After 18 days of incubation all the flasks were autoclaved for 10 minutes. The mycelium was filtered, washed, dried, and

as described by Schisler and Volkoff (1977).

The experimental data were subjected to an analysis of variance and Duncan's multiple range test (Wardle and Schisler, 1969).

3.4.4: *Carpophore production in mushrooms cultured in the lab.*

50g each of supplemented and non-supplemented compost media were placed in plastic containers previously sterilized by boiling in water at 100^{0}C and inoculated with 2g spawn as described in table 5. Each of them was later cased with 1 and a half inches of top loam soil which had been previously sterilized as described by Vedder (1978) and incubated in continuous darkness for three weeks. The trays were then brought outside for aeration.

The non-composted media were inoculated as described in Table 6 and incubated for three weeks in continuous darkness and then brought outside for aeration and exposure to light.

The sudden development of many fruiting bodies at the same time is known as a flush and there is usually a resting period of a few days between flushes. The fruiting bodies from the different flushes in the different experiments were collected and the pileus diameter, stipe diameter, and stipe length measured and subjected to analysis of variance (ANOVA). The length of time for pinhead and carpophore production were also measured.

Table 6: **Treatment of non-composted media.**

	media	inoculation method	inoculation temperature	Casing
1.	Rice straw	mycelial plug	27^{0}C	-
2.	Modified rice straw	mycelial plug	27^{0}C	-
3.	Modified rice straw	2g spawn	26^{0}C	+ unsterilized soil
4.	Sawdust	mycelial plug	27^{0}C	-
5.	Sawdust	2g spawn	27^{0}C	-
6.	Sawdust	2g spawn	27^{0}C	+ autoclaved soil + 0.2g activated charcoal
7.	Sawdust	2g spawn	26^{0}C	+ unsterilized soil
8.	Animal bedding	mycelial plug	27^{0}C	-
9.	Sawdust and oatmeal	mycelial plug	27^{0}C	-

3.5 PRESERVATION OF PURE CULTURES

Pure cultures were preserved under liquid paraffin yellow as described by Perrin (1979) and Sathe and Dighe (1987).

3.6. TRAUMATIZATION OF HYPHAE

Pure cultures from the Lentinus squarrosulus were each transfered from stock slants to replicate Petri dishes containing freshly made supplemented PDA and replicate Petri dishes containing ready made supplemented PDA (both media were supplemented with 3g peptone, 3g malt extract, and 3g yeast extract). The hyphae were traumatized as described by Bigelow (1970), grouped and incubated under 3 categories viz continuous light, intermittent light, and continuous darkness at 26-27^{0}C.

3.7 SLIDE PREPARATION

Small pieces of hyphae were picked from the pure cultures with a sterile nichrome needle and mounted on a glass slide in methylene blue (Largent et al, 1977). The hyphae were then examined for presence or absence of clamp connections.

CHAPTER FOUR

4.0 RESULTS

4.1 COLLECTION AND IDENTIFICATION

Mushrooms were found growing in all the localities surveyed in Ahmadu Bello University, Zaria main campus during the rainy season in 1992. During the course of survey, 15 different mushroom spp. (Table 1) were collected from 11 different locations (map) and used for further studies. These were identified based on macroscopic features (Appendix 1, plate 1, 2, 3, 4 and 5) in conjunction with the key proposed by Largent (1986). Out of these 15 only 2 were identified to species level and the identification was confirmed by the Royal Botanic Garden, Kew. The remaining 13 were identified only to genus level.

Out of the 15 species collected 4 were found to be edible, Lentinus squarrosulus and all three species of Termitomyces. They are popularly used in African countries such as Cameroon, Ivory Coast, Ghana, and Nigeria to make soup (Zoberi, 1972).

4.2 GERMINATION TESTS

Out of the 15 different samples of spores collected from different mushrooms in nature, only the spores from Lentinus squarrosulus consistently germinated in all the different media under the different incubation conditions. The hyphae produced from the pure culture were septate with clamp

connections (Plate 6).

4.2.1 *Effect of different treatments on germination rates*

Although the spores of L. squarrosulus germinated in all the media, there were still some differences in the rate of germination in the different media. The mean number of germinated spores was highest in PDA(601.78) and YEA(452.44) treated with all the nutrients and additives followed by those which were treated with all the nutrients and additives except charcoal. It was, however, just reversed in the case of SDA. The poorest germination was recorded in the control. It is also clear that the malt extract consistently produced a higher mean germination rate in all 6 different media than peptone or yeast extract alone and produced mean germination rates which were comparable to media which had been treated with spawn only. (Table 7a - 7f)

The rate of germinations were statistically different. (Appendix 2) The pooled results also confirmed that the media treatment with all the nutrients and additives produced the best results while the control produced the poorest results (Table 7g).

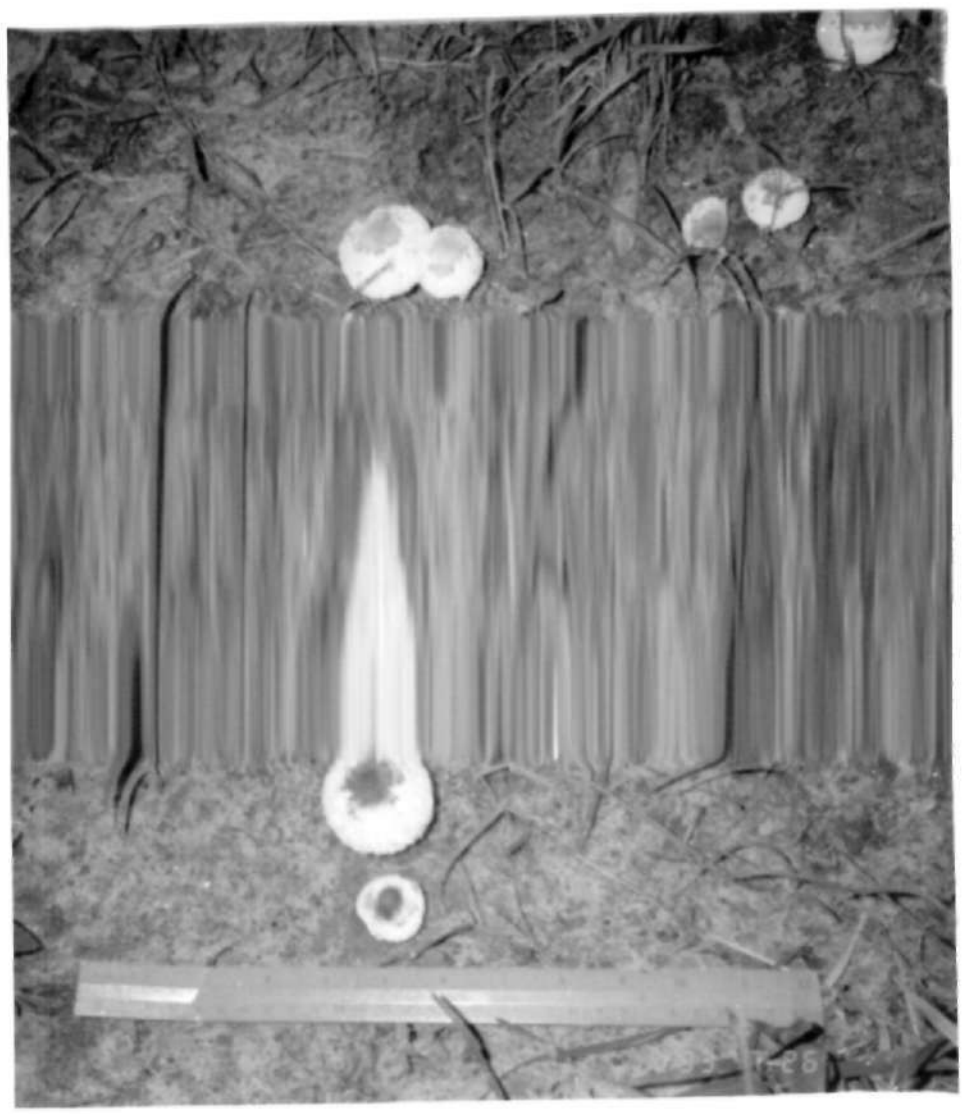


Plate 1. Bolbitius spp. A



Plate 2. Coprinus spp. A

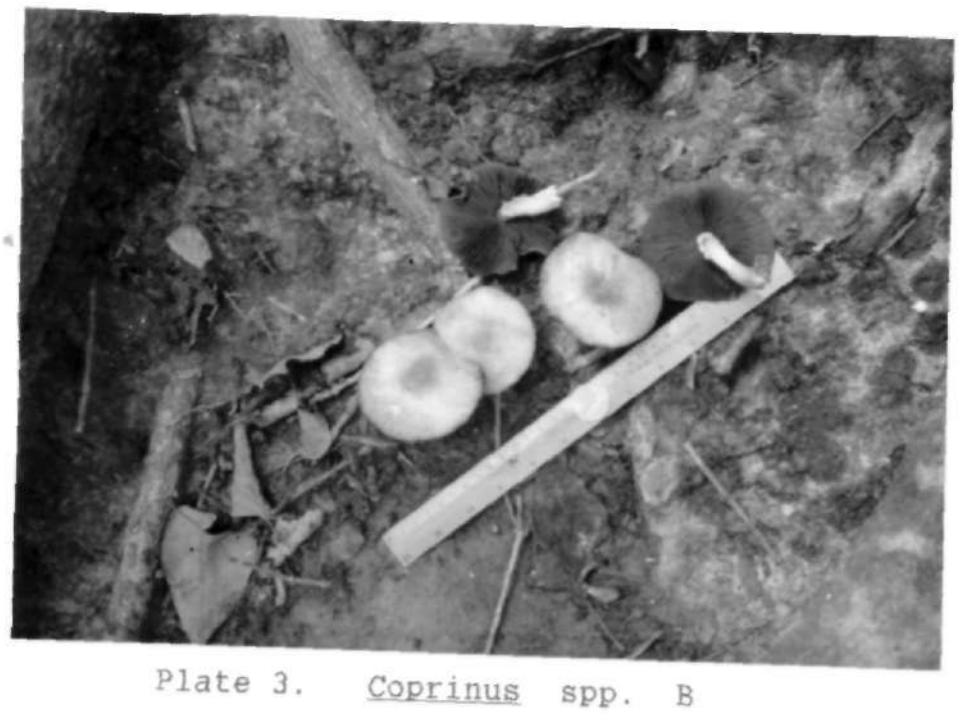


Plate 3. Coprinus spp. B



Plate 4. Lentinus squarrosulus



Plate 5. Lepiota spp.

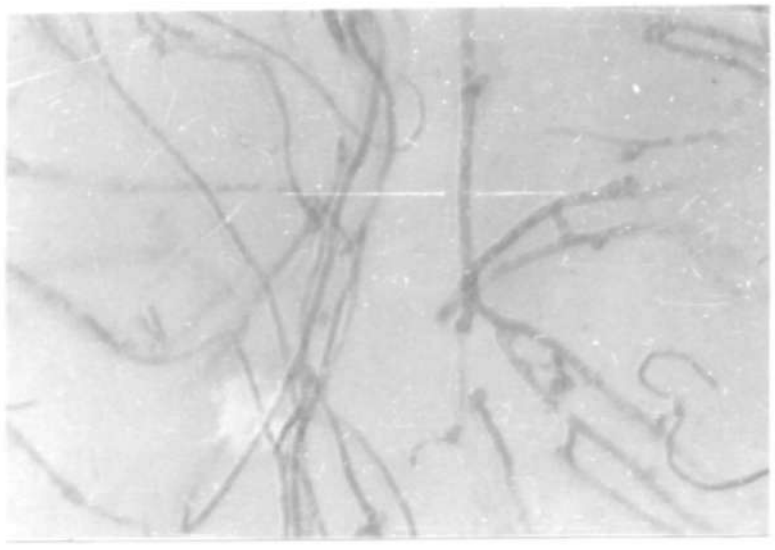


Plate 6. L. squarrosulus hyphae showing clamp connections.

Table 7a: Rate of germination of spores of Lentinus squarrosulus under different treatments in fresh potato dextrose agar (PDA1).

	Treatment	N	Mean
1.	PDA + 3g peptone, 3g malt ext.,3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	601.78^{a}
2.	PDA + 3g peptone, 3g malt ext., 3g yeast ext., 2 spawn grains.	9	588.67^{ab}
3.	PDA + 3g peptone, 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	561.33^{abc}
4.	PDA + 3g malt ext., 3g yeast ext., 2 spawn grains.	9	556.33^{abcd}
5.	PDA + 3g peptone, 3g malt ext., 2 spawn grains.	9	542.89^{bcde}
6.	PDA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	542.00^{bcde}
7.	PDA + 3g peptone, 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	525.33^{cde}
8.	PDA + 3g peptone, 3g malt extract, 7mg activ. charcoal.	9	520.44^{cdef}
9.	PDA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	512.89^{defg}
10.	PDA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	512.33^{defg}
11.	PDA + 3g peptone, 3g yeast ext., 2 spawn grains.	9	507.78^{efgh}
12.	PDA + 2g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	498.44^{efghi}
13.	PDA + 3g peptone, 3g malt ext., 3g yeast extract.	9	497.78^{efghi}
14.	PDA + 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	476.33^{fghij}
15.	PDA + 3g peptone, 3g yeast ext., 7mg activ. charcoal.	9	470.78^{ghijk}
16.	PDA + 3g malt ext. 3g yeast extract.	9	469.11^{hijkl}

17.	PDA + 3g malt ext., 2 spawn grains.	9	464.11^{hijkl}
18.	PDA + 3g peptone, 3g malt extract.	9	462.11^{hijkl}
19.	PDA + 3g peptone, 7mg activ. charcoal, 2 spawn grains.	9	460.56^{hijkl}
20.	PDA + 3g yeast extract, 2 spawn grains.	9	457.44^{ijkl}
21.	PDA + 3g malt extract, 7mg activ. charcoal.	9	447.56^{jklm}
22.	PDA + 3g peptone, 3g yeast extract.	9	443.44^{jklmn}
23.	PDA + 3g peptone, 2 spawn grains.	9	436.11^{jklmno}
24.	PDA + 3g malt extract.	9	433.89^{jklmno}
25.	PDA + 7mg activ. charcoal, 2 spawn grains.	9	427.89^{klmno}
26.	PDA + 2 spawn grains.	9	417.89^{lmno}
27.	PDA + 3g peptone, 7mg activ. charcoal.	9	406.00^{mno}
28.	PDA + 3g yeast ext., 7mg activ. charcoal.	9	402.11^{mno}
29.	PDA + 3g peptone.	9	398.44^{nop}
30.	PDA + 3g yeast extract.	9	394.11^{op}
31.	PDA + 7mg activ. charcoal.	9	390.78^{op}
32.	PDA + alone.	9	353.33^{p}

N = Number of Plates.
ANOVA (df 31,252) (0.05) = 12.84:- Significant.
means with the same letter are not significantly different.

Table 7b: Rate of germination of spores of Lentinus squarrosulus under different treatments in ready made potato dextrose agar (PDA2).

	Treatment	N	Mean
1.	PDA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	599.67^{a}
2.	PDA + 3g peptone, 3g malt ext., 3g yeast ext., 2 spawn grains.	9	588.44^{a}
3.	PDA + 3g peptone, 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	562.89^{ab}
4.	PDA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	562.56^{ab}
5.	PDA + 3g malt ext., 3g yeast ext., 2 spawn grains.	9	561.78^{ab}
6.	PDA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	546.67^{bc}
7.	PDA + 3g peptone, 3g malt ext., 2 spawn grains.	9	545.44^{bc}
8.	PDA + 3g peptone, 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	523.67^{bcd}
9.	PDA + 3g peptone, 3g malt ext., 7mg activ. Charcoal.	9	518.00^{cd}
10.	PDA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	515.00^{cde}
11.	PDA + 3g peptone, 3g yeast ext., 2 spawn grains.	9	503.33^{def}
12.	PDA + 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	497.89^{defg}
13.	PDA + 3g peptone, 3g malt ext., 3g yeast extract.	9	494.00^{defg}
14.	PDA + 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	475.22^{efgh}
15.	PDA + 3g peptone, 7mg activ. charcoal, 2 spawn grains.	9	470.00^{fgh}

16.	PDA + 3g peptone, 3g yeast extract, 7mg activ. charcoal.	9	469.89^{fgh}
17.	PDA + 3g peptone, 3g malt extract.	9	464.44^{fghi}
18.	PDA + 3g malt extract, 2 spawn grains.	9	463.56^{fghi}
19.	PDA + 3g malt extract, 3g yeast extract.	9	459.22^{ghi}
20.	PDA + 3g malt extract. 7mg activ. charcoal.	9	445.44^{hij}
21.	PDA + 3g peptone, 3g yeast extract.	9	438.11^{hijk}
22.	PDA + 3g yeast ext., 2 spawn grains.	9	435.78^{hikkl}
23.	PDA + malt extract.	9	428.11^{ijklm}
24.	PDA + 7mg activ. charcoal, 2 spawn grains.	9	434.78^{ijklm}
25.	PDA + 3g peptone, 2 spawn grains.	9	424.00^{ijklm}
26.	PDA + 2 spawn grains.	9	417.11^{jklm}
27.	PDA + 3g peptone, 7mg activ. charcoal.	9	403.89^{klm}
28.	PDA + 3g peptone.	9	402.33^{klm}
29.	PDA + 3g yeast ext., 7mg activ.charcoal.	9	296.67^{lmn}
30.	PDA + 7mg activ. charcoal.	9	395.78^{lmn}
31.	PDA + 3g yeast extract.	9	289.11^{mn}
32.	PDA + alone.	9	357.56^{n}

N = Number of plates.
ANOVA (df 31,252) (0.05) = 18.55 :- Significant.
means with the same letter are not significantly different.

Table 7c: Rate of germination of spores of Lentinus Squarrosulus under different treatments in fresh yeast extract agar (YEA1).

	Treatment	N	Mean
1.	YEA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	452.44^{a}
2.	YEA + 3g peptone, 3g malt ext.,3g yeast ext., 2 spawn grains.	9	452.33^{a}
3.	YEA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	439.56^{ab}
4.	YEA + 3g peptone, 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	437.78^{ab}
5.	YEA + 3g malt ext., 3g yeast ext., 2 spawn grains.	9	$416.89.^{bc}$
6.	YEA + 3g malt ext., 3g yeast ext., 7mg activated charcoal.	9	415.22^{bc}
7.	YEA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	414.67^{bc}
8.	YEA + 3g peptone, 3g malt ext., 2 spawn grains.	9	409.22^{cd}
9.	YEA + 3g peptone, 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	408.67^{cd}
10.	YEA + 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	408.44^{cd}
11.	YEA + 3g peptone, 3g malt ext., 3g yeast extract.	9	399.67^{cde}
12.	YEA + 3g peptone, 3g malt extract, 7mg activ. charcoal.	9	398.22^{cdef}
13.	YEA + yeast extract, 7mg activ. charcoal, 2 spawn grains.	9	394.78^{cdef}
14.	YEA + 3g malt ext., 3g yeast ext.	9	383.89^{defg}
15.	YEA + 3g peptone, 3g malt ext.	9	381.11^{efgh}

16.	YEA + 3g peptone, 3g yeast ext., 7mg activ. charcoal.	9	374.44^{efgh}
17.	YEA + 3g peptone, 3g yeast ext., 2 spawn grains.	9	373.00^{fgh}
18.	YEA + 3g peptone, 7mg activ. charcoal, 2 spawn grains.	9	365.67^{ghi}
19.	YEA + 3g malt extract, 2 spawn grains.	9	358.22^{ghij}
20.	YEA + 3g yeast extract, 2 spawn grains.	9	355.56^{hij}
21.	YEA + 3g malt extract, 7mg activ. charcoal.	9.	344.89^{ijk}
22.	YEA + 3g peptone, 3g yeast extract.	9	344.11^{ijk}
23.	YEA + 3g peptone, 2 spawn grains.	9	335.11^{jkl}.
24.	YEA + 7mg activ. charcoal, 2 spawn grains.	9	334.00^{jkl}
25.	YEA + 3g yeast ext., 7mg activ. charcoal.	9	322.44^{klm}
26.	YEA + 2 spawn grains.	9	316.44^{lm}
27.	YEA + 3g malt extract.	9	314.33^{lmn}
28.	YEA + 3g peptone, 7mg activ. charcoal.	9	305.10^{mno}
29.	YEA + 3g yeast extract.	9	290.00^{no}
30.	YEA + 3g peptone.	9	287.11^{o}
31.	YEA + 7mg activ. Charcoal.	9	282.56^{o}
32.	YEA + alone.	9	250.78^{p}

N = Number of plates.
ANOVA (df 31, 252) (0.05) = 32.78:- Significant.
means with the same letter are not significantly different.

Table 7d: Rate of germination of spores of Lentinus squarrosulus under different treatments in ready made yeast extract agar (YEA2).

	Treatment	N	Mean
1.	YEA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	445.78^{d}
2.	YEA + 3g peptone, 3g malt ext., 3g yeast ext., 2 spawn grains.	9	442.78^{ab}
3.	YEA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	436.22^{abc}
4.	YEA + 3g peptone, 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	431.33^{abcd}
5.	YEA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	422.11^{bcde}
6.	YEA + 3g malt ext., 3g yeast ext., 2 spawn grains.	9	418.00^{cdef}
7.	YEA + 3g peptone, 3g malt ext., 3g yeast ext.	9	416.89^{cdef}
8.	YEA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	411.33^{defg}
9.	YEA + 3g peptone, 3g malt ext., 2 spawn grains.	9	407.67^{efg}
10.	YEA + 3g peptone, 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	404.78^{efg}
11.	YEA + 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	397.78^{fgh}
12.	YEA + 3g peptone, 3g malt ext., 7mg activ. charcoal.	9	393.78^{ghi}
13.	YEA + 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	381.56^{hij}
14.	YEA + 3g malt ext., 3g yeast extract.	9	379.67^{hij}
15.	YEA + 3g peptone, 3g yeast ext., 2 spawn grains.	9	373.00^{ijk}

16.	YEA + 3g peptone, 3g yeast ext., 7mg activ. charcoal.	9	368.67^{jk}
17.	YEA + 3g peptone, 3g malt extract.	9	364.00^{jkl}
18.	YEA + 3g malt extract, 2 spawn grains.	9	360.22^{jklm}
19.	YEA + 3g yeast extract, 2 spawn grains.	9	352.67^{klmn}
20.	YEA + 3g peptone, 7mg activ. charcoal, 2 spawn grains.	9	345.33^{lmno}
21.	YEA + 3g malt extract, 7mg activ. charcoal.	9	341.89^{lmno}
22.	YEA + 3g peptone, 3g yeast extract.	9	339.56^{mno}
23.	YEA + 7mg activ. charcoal, 2 spawn grains.	9	334.33^{nop}
24.	YEA + 3g peptone, 2 spawn grains.	9	332.33^{nop}
25.	YEA + 2 spawn grains.	9	326.22^{opq}
26.	YEA + 3g malt extract.	9	314.33^{pqr}
27.	YEA + 3g yeast ext., 7mg activ. charcoal.	9	307.00^{qrs}
28.	YEA + 3g peptone, 7mg activated charcoal.	9	300.78^{rs}
29.	YEA + 3g yeast extract.	9	290.78^{st}
30.	YEA + 3g peptone.	9	288.33^{st}
31.	YEA + 7mg activated charcoal.	9	273.33^{tu}
32.	YEA + alone.	9	251.44^{u}

N = Number of plates.
ANOVA (df 31,252) (0.05) = 41.77 :- Significant.
means with the same letter are not significantly different.

Table 7e: Rate of germination of spores of Lentinus squarrosulus under different treatments in fresh Sabouraud's dextrose agar (SDA1).

	Treatment	N	mean
1.	SDA + 3g peptone, 3g malt ext., 3g yeast ext., 2 spawn grains.	9	571.56^{a}
2.	SDA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	567.89^{a}
3.	SDA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	558.22^{a}
4.	SDA + 3g peptone, 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	552.11^{ab}
5.	SDA + 3g malt extract, 3g yeast ext., 2 spawn grains.	9	549.33^{abc}
6.	SDA + 3g peptone, 3g malt ext., 2 spawn grains.	9	544.78^{abcd}
7.	SDA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	532.22^{abcde}
8.	SDA + 3g peptone, 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	514.33^{bcde}
9.	SDA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	508.89^{cdef}
10.	SDA + 3g peptone, 3g malt ext., 7mg activ. charcoal.	9	506.67^{def}
11.	SDA + 3g peptone, 3g yeast ext., 2 spawn grains.	9	492.67^{efg}
12.	SDA + 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	471.33^{fgh}
13.	SDA + 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	464.11^{gh}
14.	SDA + 3g peptone, 3g yeast ext., 7mg activ. charcoal.	9	453.78^{ghi}
15.	SDA + 3g peptone, 3g malt ext., 3g yeast extract.	9	452.00^{ghi}

16.	SDA + 3g malt ext., 2 spawn grains.	9	447.67^{hi}
17.	SDA + 3g malt ext., 3g yeast extract.	9	445.89^{hi}
18.	SDA + 3g peptone, 7mg activ. charcoal, 2 spawn grains.	9	437.56^{hij}
19.	SDA + 3g peptone, 3g malt extract.	9	436.78^{hij}
20.	SDA + 3g malt ext., 7mg activ. charcoal.	9	421.44^{ijk}
21.	SDA + 3g yeast ext., 2 spawn grains.	9	418.88^{ijkl}
22.	SDA + 3g peptone, 2 spawn grains.	9	413.22^{ijkl}
23.	SDA + 7mg activ. charcoal, 2 spawn grains.	9	404.00^{jkl}
24.	SDA + 3g yeast ext., 7mg activ. charcoal.	9	389.89^{klm}
25.	SDA + 2 spawn grains.	9	385.67^{klmn}
26.	SDA + 3g malt extract.	9	378.67^{lmn}
27.	SDA + 3g peptone, 7mg activ. charcoal.	9	377.89^{lmn}
28.	SDA + 3g peptone.	9	359.33^{mn}
29.	SDA + 3g yeast extract.	9	356.44^{mn}
30.	SDA + 3g peptone, 3g yeast extract.	9	350.22^{mn}
31.	SDA + 7mg activated charcoal.	9	347.67^{n}
32.	SDA + alone.	9	296.78^{o}

N = Number of plates.
ANOVA (df 31,252) (0.05) = 26.34:- Significant.
means with the same letter are not significantly different.

Table 7f: Rate of germination of spores of Lentinus squarrosulus under different treatments in ready made Sabouraud's dextrose agar (SDA2).

	Treatment	N	Mean
1.	SDA + 3g peptone, 3g malt ext., 3g yeast ext., 2 spawn grains.	9	570.22^{a}
2.	SDA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	558.67^{a}
3.	SDA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	554.67^{a}
4.	SDA + 3g peptone, 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	547.89^{ab}
5.	SDA + 3g malt ext., 3g yeast ext., 2 spawn grains.	9	540.22^{ab}
6.	SDA + 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	531.56^{abc}
7.	SDA + 3g peptone, 3g malt ext., 2 spawn grains.	9	530.67^{abc}
8.	SDA + 3g peptone, 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	508.67^{bcd}
9.	SDA + 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	9	507.11^{bcde}
10.	SDA + 3g peptone, 3g malt ext., 7mg activ. charcoal.	9	497.56^{cde}
11.	SDA + 3g peptone, 3g yeast ext., 2 spawn grains.	9	490.33^{cdef}
12.	SDA + 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	9	469.11^{defg}
13.	SDA + 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	9	465.67^{efg}
14.	SDA + 3g peptone, 3g malt ext., 3g yeast extract.	9	454.78^{fgh}
15.	SDA + 3g peptone, 3g yeast ext., 7mg activ. charcoal.	9	454.11^{fgh}

16.	SDA + 3g malt ext., 2 spawn grains.	9	445.78^{gh}
17.	SDA + 3g malt ext., 3g yeast extract.	9	443.78^{gh}
18.	SDA + 3g peptone, 3g malt extract.	9	436.67^{gh}
19.	SDA + 3g peptone, 7mg activ. charcoal, 2 spawn grains.	9	434.78^{gh}
20.	SDA + 7gm activ. charcoal, 2 spawn grains.	9	418.56^{hi}
21.	SDA + 3g yeast ext., 2 spawn grains.	9	416.56^{hi}
22.	SDA + 3g malt ext., 7mg activ. charcoal.	9	416.33^{hi}
23.	SDA + 3g peptone, 2 spawn grains.	9	413.44^{hij}
24.	SDA + 2 spawn grains.	9	392.33^{ijk}
25.	SDA + 3g yeast ext., 7mg activ. charcoal.	9	385.22^{ijkl}
26.	SDA + 3g peptone, 3g yeast extract.	9	381.89^{ijkl}
27.	SDA + 3g peptone, 7mg activ. charcoal.	9	374.44^{jkl}
28.	SDA + 3g malt extract.	9	373.67^{jkl}
29.	SDA + 3g yeast extract.	9	395.44^{kl}
30.	SDA + 3g peptone.	9	353.44^{kl}
31.	SDA + 7mg activated charcoal.	9	350.56^{l}
32.	SDA + alone.	9	297.78^{m}

N = Number of plates.
ANOVA (df 31,252) (0.05) = 22.80:- Significant
means with the same letter are not significantly different.

Table 7g: Rate of germination of spores of Lentinus squarrosulus under different treatments (pooled data).

	Treatment	N	Mean
1.	media, 3g peptone, 3g malt ext., 3g yeast ext., 7mg activated charcoal, 2 spawn grains.	54	537.70^{a}
2.	media, 3g peptone, 3g malt ext., 3g yeast ext., 2 spawn grains.	54	535.67^{ab}
3.	media, 3g peptone, 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	54	515.56^{abc}
4.	media, 3g malt ext., 3g yeast ext.,7mg activ. charcoal, 2 spawn grains.	54	510.69^{bc}
5.	media, 3g malt ext., 3g yeast ext., 2 spawn grains.	54	507.09^{cd}
6.	media, 3g peptone, 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	54	498.20^{cde}
7.	media, 3g peptone, 3g malt ext., 2 spawn grains.	54	496.78^{cde}
8.	media, 3g peptone, 3g yeast ext., 7mg activ. charcoal, 2 spawn grains.	54	480.91^{def}
9.	media, 3g malt ext., 3g yeast ext., 7mg activ. charcoal.	54	478.31^{efg}
10.	media, 3g peptone, 3g malt ext., 7mg activ. charcoal.	54	472.44^{efg}
11.	media, 3g malt ext., 7mg activ. charcoal, 2 spawn grains.	54	457.17^{fgh}
12.	media, 3g peptone, 3g yeast ext., 2 spawn grains.	54	456.69^{fgh}
13.	media, 3g peptone, 3g malt extract, 3g yeast extract.	54	452.52^{ghi}
14.	media, 3g yeast ext., 7mg activated charcoal, 2 spawn grains.	54	442.94^{hij}

15.	media, 3g peptone, 3g yeast ext., 7mg activated charcoal.	54	431.94^{hijk}
16.	media, 3g malt ext., 3g yeast extract.	54	430.26^{ijk}
17.	media, 3g peptone, 3g malt extract.	54	424.19^{jkl}
18.	media, 3g malt extract, 2 spawn grains.	54	423.26^{jkl}
19.	media, 3g peptone, 7mg activ. charcoal, 2 spawn grains.	54	418.98^{jkl}
20.	media, 3g yeast ext., 2 spawn grains.	54	406.07^{klm}
21.	media, 3g malt ext., 7mg activ. charcoal.	54	402.93^{lm}
22.	media, 3g peptone, 2 spawn grains.	54	392.37^{mn}
23.	media, 7mg activ. charcoal, 2 spawn grains.	54	390.59^{mn}
24.	media, 3g peptone, 3g yeast extract.	54	382.89^{nno}
25.	media, 2 spawn grains.	54	375.94^{no}
26.	media, 3g malt extract.	54	373.83^{nop}
27.	media, 3g yeast extract, 7mg activ. charcoal.	54	367.56^{nopq}
28.	media, 3g peptone, 7mg activ. charcoal	54	361.33^{opqr}
29.	media, 3g peptone.	54	348.17^{pqr}
30.	media, 3g yeast extract.	54	346.65^{qr}
31.	media, activated charcoal.	54	340.11^{r}
32.	media alone.	54	301.28^{s}

N = Number of plates.
ANOVA (df 31,1658)(0.05) = 43.18 :- Significant.
means with the same letter are not significantly different.

4.2.2 *Effect of media on germination rate irrespective of different treatment and incubation method.*

The results show that there is a significant difference in the germination rate in the different media. The best germination rate was produced in potato dextrose followed by Sabouraud's dextrose and yeast extract agars respectively (Table 8a). There was however, no significant difference in the mean germination rate in reference to the mode of media preparation viz. fresh or ready made, within the same media categories. There was, however, significant differences in the rate of germination between three different categories of media with the lowest number of germinated spores occuring on synthetic yeast extract agar (Table 8b, Plate 7).

4.2.3 *Effect of different incubation methods on germination rate.*

There was a significant difference in the germination rates under different incubation methods. The highest germination rate was recorded in continuous darkness in four of the six media including ready made yeast extract, fresh potato dextrose, ready made Sabouraud's dextrose and fresh Sabouraud's dextrose while in fresh yeast extract and ready made sabouraud's dextrose the best germination was found under intermittent light (Table 9a). The overall pooled results showed that continuous darkness was the best followed by intermittent and continuous light (Table 9b).

Table 8a: Rate of germination of spores of Lentinus squarrosulus on three different agar media irrespective of different treatment and incubation method (pooled data).

media	N	mean
Potato dextrose	576	474.411[a]
Sabouraud's dextrose	576	449.740[b]
Yeast extract	576	365.944[c]

N = Number of plates.
ANOVA (df 2,1685)0.05) = 384.07:- Significant.
means with the same letter are not significantly different.

Table 8b: Rate of germination of spores of Lentinus squarrosulus in six different agar media.

	N	Mean	Standard Deviation	Sum	Min	Max
Fresh yeast extract	288	367.70486[c]	121.84516	105899	152.0000	612.0000
Ready made yeast extract	288	364.18403[c]	119.85867	104885	140.0000	591.0000
Ready made potato dextrose	288	474.44792[d]	181.25562	136641	198.0000	876.0000
Fresh potato dextrose	288	474.37500[d]	180.19536	136620	198.0000	865.0000
Ready made sabouraud's dextrose	288	449.24653[b]	184.41541	129383	162.0000	841.0000
Fresh sabouraud's dextrose	288	450.2364[b]	184.99983	129667	162.0000	835.0000

N = number of plate.
ANOVA(df 5, 1685)(0.05) = 384.07 :- Significant.
means with the same letter are not significantly different.

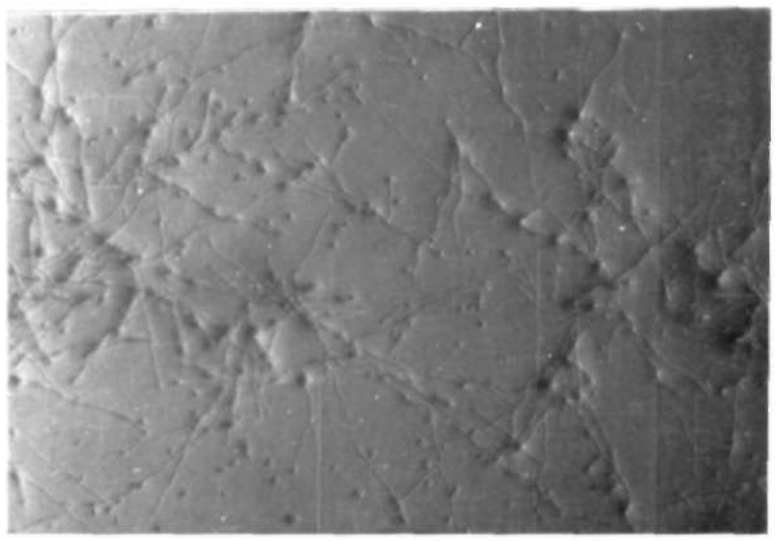


Plate 7. L. squarrosulus spores germinating on non-supplemented yeast extract agar incubated in intermittent light.

Table 9a: Effect of incubation methods on the germination rate of Lentinus squarrosulus spores in 6 different media.

Media	Incubation method	N	Mean
Fresh yeast extract	intermittent light continuous darkness continuous light	96 96 96	500.740[a] 362.542[b] 239.833[c]
Ready made yeast extract	continuous darkness intermittent light continuous light	96 96 96	496.969[a] 355.958[b] 239.625[c]
Ready made potato dextrose	intermittent light continuous darkness continuous light	96 96 96	671.906[a] 481.845[b] 269.562[c]
Fresh potato dextrose	continuous darkness intermittent light continuous light	96 96 96	668.281[a] 483.875[b] 270.969[c]
Ready made Sabouraud's dextrose	continuous darkness intermittent light continuous light	96 96 96	652.646[a] 446.135[b] 248.958[c]
Fresh Sabouraud's dextrose	continuous darkness intermittent lights continuous light	96 96 96	653.229[a] 446.521[b] 250.948[c]

N = number of plates
fresh yeast extract - ANOVA (df 2,252)(0.05)= 2099.15:- sign.
ready made yeast extract - ANOVA (df 2,252)(0.05) = 2642.45:- sign.
ready made potato dextrose - ANOVA (df 2,252)(0.05) = 1948.75:- sign.
fresh potato dextrose - ANOVA (df 2,252)(0.05) = 1430.06 :- sign.
ready made Sabouraud's dextrose - ANOVA (df 2,252)(0.05) = 1936.68:- sign.
fresh Sabouraud's dextrose - ANOVA (df(2,252)(0.05) = 2022.60:- sign.
means with the same letter are not significantly different.

Table 9b: Results for the effect of incubation methods on the germination rate of Lentinus squarrosulus spores (pooled data).

Incubation method	N	Means
Continuous darkness	576	552.590[a]
Intermittent light	576	484.189[b]
Continuous light	576	153.316[c]

N = Number of plates.
ANOVA (df 2,1685)0.05) = 2921.71:- Significant
means with the same letter are not significantly different

4.3 SPAWN EXPERIMENT

The casing experiments conducted with the spawn of Lentinus squarrosulus produced negative results. Neither the one cased with sterilized soil nor the one cased with non-sterilized soil produced fruiting bodies under any of the incubation conditions.

4.4 HYPHAL CULTURE

Out of the 15 mushroom samples only L. squarrosulus (sample No.2) and T. clypeatus (sample No.9) were found to grow. Lentinus squarrosulus grew on all the different media under the three conditions of incubation while the Termitomyces clypeatus only grew in formulated media HCM2 which had been incubated in continuous darkness. Microscopic examination of the hyphae revealed that it was septate without clamp connections.

There was a significant difference in mycelia growth on the different media. The best growth was exhibited under formulated media HCM2 while the poorest growth was found in the streptomycin treated PDA. This same trend was noticed in the pooled data (10 and 11a-c). The method of incubation had a significant effect on mycelial growth. Both species of mushroom produced their best mycelial growth under continuous darkness and their poorest under continuous light. The pooled data also confirmed the same general trend (11d - f).

It was evident from the data that there exists a significant difference in the rate of mycelial production from the two mushroom species. The L. squarrosulus produced a much higher mean value of mycelial growth than the T. clypeatus (Table 11g).

Table 10: The mycelial growth of T. clypeatus and L. squarrosulus in liquid cultures under different conditions of incubation.

media	Incubation Method	Mushroom Species					
		T. clypeatus			L. squarrosulus		
Formulated media HCM1	continuous light	11*	18	15	90	96	89
	intermittent light	32	39	41	125	131	121
	continuous darkness	63	64	75	164	161	167
Formulated media HCM2	continuous light	74	77	81	109	104	115
	intermittent light	142	138	146	140	137	149
	continuous darkness	184	192	179	172	166	181
Acidic PDA	continuous light	25	19	23	65	77	62
	intermittent light	30	35	31	109	102	112
	continuous darkness	45	50	53	134	129	126
Streptomycin treated PDA	continuous light	08	12	10	45	52	36
	intermittent light	20	18	17	70	65	52
	continuous darkness	32	21	26	92	96	87

*mg dry weight mycelium per 100ml culture medium.

Table 11a: Effect of different hyphal culture media on mycelial growth of L. squarrosulus.

Media treatment	N	Mean
Formulated media HCM2	9	141.444[a]
Formulated media HCM1	9	127.111[b]
Acidic PDA	9	101.778[c]
Streptomycin treated PDA	9	60.556[d]

N = Number of plates.
ANOVA (df 3,30) = 88.40:- Significant.

Table 11b: Effect of different hyphal culture media on mycelial growth of T. clypeatus.

Media treatment	N	Mean
Formulated media HCM2	9	134.778[a]
Formulated media HCM1	9	39.778[b]
Acidic PDA	9	34.556[b]
Streptomycin treated PDA	9	18.222[c]

N = Number of plates.
ANOVA(df 3,30) = 91.55:- Significant.

Table 11c: Effect of different hyphal culture media on mycelial growth of L. squarrosulus and T. clypeatus (pooled result).

Media treatment	N	Mean
Formulated media HCM2	18	138.111[a]
Formulated media HCM1	18	83.444[b]
Acidic PDA	18	68.167[c]
Streptomycin treated PDA	18	39.389[d]

N = Number of plates.
ANOVA(df 3,65) = 69.92:- Significant.

Table 11d: Effect of different incubation methods on mycelial growth of L. squarrosulus.

Incubation method	N	Mean
Continuous darkness	12	139.583[a]
Intermittent light	12	105.250[b]
Continuous light	12	78 333[c]

N = Number of plates.
ANOVA(df 2,30) = 88.32:- Significant.

Table 11e: Effect of incubation method on mycelial growth of *T. clypeatus*.

Incubation method	N	Mean
Continuous darkness	12	82.000[a]
Intermittent light	12	57.417[b]
Continuous light	12	31.083[c]

N = Number of plates.
ANOVA(df 2,30) = 28.42:- Significant.

Table 11f: Effect of incubation method on mycelial growth of *L. squarrosulus* and *T. clypeatus*(pooled results).

Incubation method	N	Mean
Continuous darkness	24	110.792[a]
Intermittent light	24	81.333[b]
Continuous light	24	54.708[c]

N = Number of plates.
ANOVA(df 2,65) = 42.68:- significant

Table 11g: The mycelial growth of *L. squarrosulus* and *T. clypeatus* under different treatments and methods of incubation (pooled data).

Mushroom species	N	Mean
Lentinus squarrosulus	36	107.722[a]
Termitomyces clypeatus	36	56.833[b]

N = Number of plates.
ANOVA (df 6, 65) = 105.34 :- Significant.

4.5 CARPOPHORE PRODUCTION

Carpophore was not produced in compost media 1, compost media 2, rice straw, cased modified rice straw, and spawned sawdust cased with unsterilized soil while fruiting bodies developed in the rest of the media (Table 12). Time required for carpogenesis was also found varying. It took much shorter time in modified rice straw and longest in cased spawned sawdust (Table 13, Plate 8). It is also clear from table 13 that two flushes of carpophore were produced in each of the three media viz. modified rice straw, spawned sawdust, and animal bedding and rice, while only one flush of carpophores was produced in each of the rest of the two media.

Measurement of the carpophores produced in different media were recorded. Pileus of the carpophores from different media apparently showed variations in their mean diameter but they proved to be statistically insignificant (Table 14a). Similar results were obtained with the measurements of stipes (Table 14b and c).

4.6 TRAUMATIZED HYPHAE

The traumatized hyphae of L. squarrosulus produced negative results. The hyphae in both the synthetic and fresh potato dextrose failed to produce fruit under all conditions of incubation.

Table 12: Studies on the development of fruiting bodies of Lentinus squarrosulus in different media.

media	fruit production
Compost 1	-
Compost 2	-
Rice straw	-
Modified rice straw	+
Cased modified rice straw (unsterilised soil)	-
Spawned sawdust	+
Cased spawned sawdust (sterilized Soil)	+
Cased spawned sawdust (unsterilized soil)	-
Animal bedding and rice	+
Sawdust and oatmeal	+

Key - = no fruit production.
+ = successful production of fruit.

Table 13: Time period for carpogenesis of Lentinus squarrosulus in different media.

	first flush		second flush		third flush	
media	pinhead formation	opening of pileus	pinhead formation	opening of pileus	pinhead formation	opening pileus
Modified rice straw	22-23days	25-28days	40-41days	44-45days	-	-
Spawned sawdust	37-44days	47-51days	53-57days	61-62days	-	-
Animal bedding and rice	38-39days	43-44days	53-54days	59-60days	-	-
Sawdust and oatmeal	37-38days	42-43days	-	-	-	-
Cased spawned sawdust	74-75days	77-78days	-	-	-	-

Plate 8. L. squarrosulus fruiting on spawned sawdust media cased with sterilized soil.



Table 14a: Pileus diameter of Lentinus squarrosulus in different media.

media	pileus diameter range	mean	standard deviation	pooled standard deviation	F
Modified rice straw	1.1-1.4cm	1.27cm	0.126	1.188	2.03
Spawned sawdust	1.2-5.2cm	3.00cm	1.661		
Cased spawned sawdust	0.8-1.5cm	1.13cm	0.351		
Animal bedding and rice	1.5-1.9cm	1.70cm	0.283		
Sawdust and oatmeal	2.1cm	2.10cm	0.000		

df (4, 12) (0.05) = 3.25 :- Not significant.

Table 14b: Stipe diameter of Lentinus squarrosulus in different media.

media	Stipe diameter range	mean	standard deviation	pooled standard deviation	F
Modified rice straw	0.3-0.3cm	0.300cm	0.000	0.07918	1.08
Spawned sawdust	0.3-0.5cm	0.386cm	0.899		
Cased spawned sawdust	0.3-0.4cm	0.333cm	0.577		
Animal bedding and rice	0.3-0.5cm	0.400cm	0.141		
Sawdust and oatmeal	0.3cm	0.300cm	0.000		

df (4, 12) (0.05) = 3.25 :- Not significant.

Table 14c: Stipe length of Lentinus squarrosulus in different media.

Stipe length					
media	range	mean	standard deviation	pooled standard deviation	F
Modified rice straw	2.9-3.2cm	3.07cm	0.126	1.091	1.27
Spawned sawdust	2.8-7.2cm	4.07cm	1.493		
Cased spawned sawdust	4.2-5.4cm	4.90cm	0.624		
Animal bedding and rice	3.4-5.0cm	4.20cm	1.131		
Sawdust and oatmeal	3.2	3.2	0.000		

df (4, 12) (0.05) = 3.25 :- Not significant.

CHAPTER FIVE

DISCUSSION

Fungi have been used as an educational medium for long. Herbivore dung, for example has been used to demonstrate the principles of succession and competition in fungi (Hudson, 1972). More recently gilled fungi have been cultured in the laboratory for demonstration purposes. (Flegler, 1979a, 1979b).

The primary problem in mushroom culture is the germination of basidiospores. The present investigations showed that some Basidiomycetes do not germinate even when the media were supplemented with natural extracts such as yeast extract, malt extract and peptone. The use of activated charcoal to remove any inhibitory affect which may be present in the media produced negative results for 14 out of the 15 specimens (Table 1) although it produced positive results for Butler and Bolkan (1973) and Fries (1978;1979;1983a). The attempt made to use mycelium of L. squarrosulus to induce germination of the other 14 species failed, probably because the Lentinus spp. was not taxonomically closely related to the other mushroom species as confirmed by earlier findings (Losel,1964; Fries 1979; 1983b).

The only mushroom species which consistently germinated and developed in all the different media was L. squarrosulus, which is a lignicolous variety. This tallied with the findings

by Fries (1966) that lignicolous spp. produced spores which readily germinate due to the fact that they live on ephemeral substrates. The hyphae produced was found to be septate with clamp connections.

The germination rate of L. squarrosulus was closely examined under 6 different media, 3 incubation methods, and 32 treatments(Tables 7a - 7g, 8b,9a, and 9b). The media treatment which produced the highest germination rate in most of the different media was the one with all the supplements and additives with the control having the poorest germination rate. In most cases the mean germination rate increased by up to one-third after supplementation with malt extract which produced the highest mean germination rate of all the supplements when added alone. These results on L. squarrosulus are in agreement with earlier findings that supplements such as yeast extract, malt extract, and peptone aid germination (Bistis, 1970; Stocks and Hess, 1970; Chapman and Baranchovich, 1979; McDaniel and Hindal, 1982; Fries,1983a; 1983b).

Spawn was also found to be effective in increasing germination rates and produced effects which were comparable to media which had been supplemented with malt extract. Scrutiny of the results support findings by Schisler et al, (1967) and Royse and May (1982).

Lentinus squarrosulus produced the second poorest germination rate in 5 out of the 6 different media which were

only supplemented with activated charcoal. Much better germination rates were obtained when charcoal was added in conjunction with other supplements as also reported by Fries (1977;1978;1983a) (Table 7a - 7g).

There was a significant difference in the germination rates in different media. The results showed that potato dextrose agar was the best media for the germination of spores (Table 8a) which confirmed earlier findings by Watling (1981). There was, however, no significant difference in the germination rate in media made fresh from laboratory components or ready made (Oxoid) (Table 8b).

The condition of incubation had a significant effect on the germination rate of L. squarrosulus. The results showed that continuous darkness was the best followed by intermittent light and continuous light (Table 9a and 9b). This confirmed earlier findings that excess light reduces spore germination (Singer, 1961; Gottlieb, 1978).

The casing of L. squarrosulus spawn produced negative results unlike the findings of San Antonio (1971) and Bigwood (1977). Evidently L. squarrosulus behaves differently from Agaricus bisporus and certain species of Psilocybe which readily produce carpophores when their spawn is cased (San Antonio, 1971; Bigwood, 1977).

Because of the difficulty of spore germination, hyphal culture was attempted. Of the 15 samples, only L. squarrosulus and T. clypeatus could be cultured. The T. clypeatus only

grew in media which had been supplemented with thiamine hydrochloride, supporting earlier findings that most Termitomyces are heterotrophic for thiamine (Purkayastha and Chandra, 1975; Chandra and Purkayastha, 1977; Batra and Batra, 1979). The hyphal culture was found to be septate without clamp connections as earlier reported by Zoberi (1972).

The rate of hyphal growth in the 4 different media was examined quantitatively (Table 10). The results suggested that formulated media HCM2 produced the best hyphal growth in both L. squarrosulus and T. clypeatus(Table 11a and 11b). The T. clypeatus did poorly in the other three media because they did not contain thiamine hydrochloride. Both mushroom species produced better hyphal growth in formulated media HCM 1 than in the acidic or streptomycin treated PDA because it contained supplements. This is supported by the finding that supplements such as yeast and malt extract stimulate good mycelial growth (Burkholder and Sinnot, 1945; Jennison et al, 1955; McTeague et al, 1959; Paranjpe et al, 1979). The poorest results for both species was produced in the streptomycin treated PDA (Table 10b, 11a - 11c). The results are supported by findings that streptomycin tends to inhibit mycelial growth (Coriell, 1973; Schaffner, 1979).

The incubation method had a significant effect on mycelial growth. Both species produced their highest rate of mycelial growth when incubated under continuous darkness followed by intermittent light and lastly continuous light

(Table 11d-11f). The results support the earlier findings of Burkholder and Sinnott (1945), Hacskaylo et al (1954),and Cangy (1994).

The pooled data suggested that L. squarrosulus had a much higher rate of mycelial growth than the T. clypeatus (Table 11g). This may have been due in part to the poor growth of T. clypeatus on media without thiamine hydrochloride. The results support findings of Jennison (1948), Sugihara and Humefeld (1954), Jennison et al (1955), and Zadrazil (1978) that lignicolous species such as Armillaria, Pleurotus and Lentinus grow well and rapidly in submerged cultures.

Lentinus squarrosulus failed to produce fruiting bodies in the two composted media (Table 12). This may have been due to the preference of most lignicolous species for pasteurized or sterilized media (Oei, 1991).

The period for carpogenesis of L. squarrosulus was shortest under the modified rice straw media and longest under the spawned sawdust media which had been cased with sterilized soil (Table 13).

L. squarrosulus produced the best results with 7 basidiocarps on sawdust media. The replicates which were inoculated with mycelial plugs did not fruit while the ones inoculated with spawn produced the 7 fruiting bodies. This finding was similar to the observation by schisler (1967) and Zervakis and Balis (1991) that grains such as wheat used in the manufacture of spawn stimulate fruiting. L. squarrosulus

produced the 2nd best result of 4 basidiocarps on modified rice straw followed by cased spawned sawdust with 3 basidiocarps. Carpogenesis occurred on the cased spawned sawdust because charcoal was added to the sterilized casing soil. These findings are in agreement with those of Long and Jacob (1974) and Angeli - couvy (1975). The fourth best results of 2 fruiting bodies was observed on the animal bedding and rice media while the poorest result of 1 fruiting body was observed on the sawdust and oatmeal media. The Lentinus produced stipe extensions which never reached above the soil level in the spawned sawdust media cased with non-sterilized soil but it totally failed to fruit on the cased modified rice straw because the media became contaminated upon contact with the unsterilized soil.

There was no statistical difference in the measurement of the basidiocarps but there was an obvious difference in the number of fruiting bodies produced in each media (Table 14a-14c). All the basidiocarps produced were sterile or sporeless, a character which is quite common in Pleurotid species or wood-rotting agarics such as the Lentinus spp. (Eger, 1978; Watling, 1981). It may be due to misbalance in nutrients, lack of light, or the atmosphere above the culture being too dry or stagnant (Watling, 1981).

The traumatized hyphae of L. squarrosulus failed to produce fruit although positive results have been obtained with Clitocybe tenuissima and Schizophyllum commune by Bigelow (1970) and Leonard and Dick (1973).

CHAPTER SIX

CONCLUSION

The present studies show that it is possible to easily culture certain species of mushroom in the laboratory around the year so that they may be available for educational purposes. Further work is required to be done so that all the other species may be grown in the laboratory.

There is a need for more work on the higher Basidiomycetes in Nigeria, not only for educational purposes but for nutritional as well. Since spores are generally difficult to germinate, emphasis should be placed on the generation of pure cultures through the culturing of hyphae from the pileus. Once this is achieved then work can progress to the more pressing issue of fruit induction.

REFERENCES

Ainsworth, G.C. (1976). Introduction To The History of Mycology . Cambridge University Press, London. 82-196.

Alabi, R. O. (1994). Mycology and Nigerian Culture: Past Present and Future. In: Aspects of African Mycology. Proceedings of The First Regional Conference on Mycology in Africa. (Ed. Hennebert, G.L) Mauritius 13-15 June 1990. 43-52.

Alasoadura, S.O. (1967). Studies in the Higher Fungi of Nigeria. I. The Genus Termitomyces Heim. J.W. African Sci. Assoc. 12: 139-140.

Alexopoulos, C.J. and Mims, C.W. (1979). Introductory Mycology . Ed.3. John Wiley and sons, New york. 456.

Ammirati, J.E., Thiers, H.D., and Horgen, P.A. (1977). Amatoxin - containing mushrooms: Amanita ocreata and A. phalloides in California. Mycologia 69: 1095-1108.

Anderson, N.A., Wang, S.S., and Schwandt, J.W. (1973). The Pleurotus ostreatus - sapidus species complex. Mycologia 65: 28-35.

Angeli - Couvy, J. (1975). La Fructification de L' Agaricus bisporus (Lange) Sing. Sur Milieu Aseptique En Presence de Charbon Actif. C.R. Hebd. Seances Acad. Sci. 280: 2101-2103.

Annakkage, S. and Ratnasiri, A.J. (1990). Production of straw mushroom using rice straw. The Nigerian Farmers Weekly 1(4),14-15.

Atkins, F.C. (1974). Guide to Mushroom Growing. Faber and Faber Ltd., Oxford. 1-122.

Batra, L.R. and Batra, S.W.T. (1979), Termite- fungus mutualism In: Insect-Fungus Symbiosis. Proceedings of the 2nd. International Mycological Congress. Tampa, Florida. August 27 - September 3, 1977. 117-163.

Bigelow, H.E. (1970). Basidiocarp production by Clitocybe tenuissima in culture. Mycologia 62: 203-205.

Bigwood, J.(1977). Psilocybian mushroom cultivation. In: TEONANCATYL: Hallucinogenic Mushrooms of North America. (Eds., Ott, J. and Bigwood, J.) Madrona Publishers, Inc., Seattle. 117-147.

Bilgrami, K. S. and Verma, R.N. (1978). Physiology of Fungi. Vikas Publishing House PVT Ltd., New Delhi. 272-275.

Bistis, G.N. (1970). Dikaryotization in Clitocybe truncicola. Mycologia 62: 911-924.

Bono, J.J., Chalaux, N. and Chabbert, B. (1992). Benchscale composting of two agricultural wastes. Bioresource Technology 40 (2), 119-124.

Boominathan, K., and Reddy, C.A. (1992). Fungal degradation of lignin: biotechnological applications. In: Handbook of Applied Mycology, Vol.4: Fungal Biotechnology.(Eds. Arora, D.K., Elander, R.P., and Mukerji, K.G.) Marcel Dekker Inc., New York. 763 - 822.

Bossard, E. (1978). Lexicon of the healing. In: Healing Plants: A Modern Herbal (Eds., William, A.R., and Thomas, M.O).Macmillan London Ltd., London. 33-112.

Bulmer, G.S. and Beneke, E.S. (1961). Studies on Calvatia gigantea. I. Germination of the basidiospores. Mycologia 53: 123-136.

Bulmer, G.S. and Beneke, E.S. (1962). Studies on Calvatia gigantea. II. Germination of the basidiospores. Mycologia 54: 34-43.

Burge, H.A. (1979). Basidiospore structure and development in the genus Russula. Mycologia 79: 977-995.

Burkholder, P.R. and Sinnott, E.W. (1945). Morphogenesis of fungus colonies in submerged shaken cultures. Amer. J. Bot. 32: 424-431.

Butcher, D.N. and Ingram, D.S. (1976). Plant Tissue Culture. Edward Arnold (Publishers) Ltd., London. 1-65.

Butler, E.E. and Bolkan, H. (1973). A medium for heterokaryon formation in Rhizoctonia solani Phytopathology 63: 542-543.

Caley, M.D. (1936). Spores and spore germination in wild and cultivated mushrooms (Psalliota spp.) Trans. Br. Mycol. Soc. 20: 225-241.

Cangy, C.L. (1994). The cultivation of Pleurotus in Mauritius. In: Aspects of African Mycology. Proceedings of the First Regional Conference on Mycology in Africa. (Ed. Hennebert, G.L.) Mauritius. 13-15 June, 1990. 95-109.

Carey, S.T. (1974). Clitocybe illudens: Its cultivation, chemistry, and classification. Mycologia 66: 951-968.

Chandra, A. and Purkayastha, R.P. (1977). Physiological studies on Indian edible mushrooms. Trans. Br. Mycol. Soc. 69 (1): 63-70.

Chapman, E.S. and Barankovich, V. (1979). Germination of basidiospores and oidia of Coprinus domesticus. Mycologia 71: 870-873.

Chen, Z.B., Zou, M., Shen, J., Zhu, P.J., Wang, W.C., Zhang, H.L., and Chen, G.S. (1988). Preliminary report on the addition of phosphogysum to compost for Agaricus bisporus and Pleurotus sapidus. Edible Fungi of China No.4 22-23.

Cheng, S. and Tu, C.C. (1978). Auricularia spp. In. The Biology and Cultivation of Edible Mushrooms. (Eds. Chang, S.T. and Hayes, W.A.) Academic Press. New York. 606-625.

Child, J.J., Knapp, C., and Eveleigh, D.E. (1973). Imroved pH control of fungal culture media. Mycologia 65: 1078-1086.

Christensen, C.M. (1965). The Moulds and Man, an Introduction to the Fungi. Ed.3. University of Minnesota Press, Minneapolis. 242-243.

Christensen, C.M. (1975). Molds, Mushrooms, and Mycotoxins. University of Minnesota Press, Minneapolis. 4-33.

Chua, S.E. and Ho, S.Y. (1973). Fruiting on sterile agar and cultivation of straw mushrooms on padi straw, banana leaves, and sawdust. World Crops 25: 90-91.

Cole, C.T. and Samson, R.A. (1979). Patterns of Development in Conidial fungi. Pitman Publishing Ltd., California. 127.

Coriell, L.L. (1973). Detection, elimination and prevention of bacteria and fungi in tissue cultures. In: Tissue Culture Methods and Applications. (Eds. Kruse, P.F. and Patterson, M.K.) Academic Press, New York. 718-720.

Crisan E.V. and Sands, A. (1978). Nutritional value. In: The Biology and Cultivation of Edible Mushrooms. (Eds. chang, S.T. and Hayes, W.A.) Academic Press, New York. 137-168.

Delmas, J. (1978). Cultivation in western countries: Growing in caves. In: The Biology and Cultivation of Edible Mushrooms (Eds., chang, S.T. and Hayes, W.A.) Academic Press, New York. 251-298.

Durrant, A.J., Wood, D.A. and Cain, R.B. (1991). Lignocellulose biodegradation by Agaricus bisporus during solid substrate fermentation. Journal of General Microbiology 137(4), 751-755.

Edwards, R.L. (1978). Cultivation in western countries: Growing in houses. In: The Biology and Cultivation of Edible Mushrooms (Eds., chang, S.T. and Hayes, W.A.) Academic Press, New York. 299-336.

Eger, G. (1978). Biology and breeding of Pleurotus. In: The Biology and Cultivation of Edible Mushrooms. (Eds., chang, S.T. and Hayes, W.A.) Academic Press, New York. 497-557.

Ellis, W.S. (1991). California's harvest of change. National Geographic. 179 (2), 49-73.

Epp, B.D. (1977). A rapid method for the isolation of tetrads in higher Basidiomycetes. Mycologia 69: 210-211.

Flegler, S.L. (1979a). An improved method for production of fruit bodies of Nidularia pulvinata in culture. Mycologia 71: 875-876.

Flegler, S. L. (1979b). An improved method for production of Cyathus stercoreus fruit bodies in culture. Mycologia 71: 625-627.

Fries, N. (1966). Chemical factors in the germination of spores of Basidiomycetes. In: The Fungus spore.(Ed., Madelin, M.F.) J.W. Arrowsmith Ltd., London. 189-199.

Fries, N. (1977). Germination of Laccaria laccata spores in vitro. Mycologia 69: 848-850.

Fries, N. (1978). Basidiospore germination in some mycorrhiza - forming Hymenomycetes. Trans. Brit. Mycol. Soc. 70: 319-324.

Fries, N. (1979). Germination of spores of Cantharellus cibarius. Mycologia 71: 216-219.

Fries, N. (1983a). Spore germination, homing reaction, and intersterility groups in Laccaria laccata (Agaricales). Mycologia 75: 221-227.

Fries, N. (1983b). Basidiospore germination in species of Boletaceae. Mycotaxon 18(2), 345-354.

Fritsche, G. (1978). Breeding work. In: The Biology and Cultivation of Edible Mushrooms. (Eds., Chang, S.T. and Hayes, W.A.) Academic Press, New York. 239-250.

Genders, R. (1976). Growing Mushrooms. Pelham Books Ltd., London. 50-75.

Gerard, J. (1975). The Herbal or General History of Plants. Revised and Enlarged. Dover Publications Inc., New York. 1365-1367.

Gottlieb, D. (1978). The Germination of Fungus spores. Meadowfield Press Ltd., England. 9-49.

Hacskaylo, J., Lilly, V.G. and Barnett, H.L. (1954). Growth of fungi on three sources of nitrogen. Mycologia 46: 691-701.

Hawksworth, D.L. (1974). Mycologists Handbook. Commonwealth Mycological Institute, England. 36.

Hayes, W.A. (1978). Nutrition, substrates, and principles of disease control. In: The Biology and Cultivation of Edible Mushrooms. (Eds., chang, S.T. and Hayes W.A.) Academic Press, New York. 219-237.

Hayes, W.A., Randle, P.E., and Last, F.T. (1969). The nature of the microbial stimulus affecting sporophore formation in Agaricus bisporus (Lange) Sing. Ann. Biol. 64: 177-187.

Heintz, C.E. and Niederpruem, D.J. (1971). Ultrastructure of quiescent and germinated basidiospores and oidia of Coprinus lagopus. Mycologia 63: 745-766.

Hudson, H.J. (1972). Fungal Saprophytism: The Institute of Biology's Study in Biology No.32. Edward Arnold (Publishers) Ltd., London. 4-66.

Hwang, S-W. (1968). Investigation of ultralow temperature for fungal cultures. I. An evaluation of liquid-nitrogen storage for preservation of selected fungal cultures. Mycologia 60: 613-621.

Hwang, S-W., Kwolek, W.F. and Haynes, W.C. (1976). Investigation of ultralow temperatures for fungal cultures.Ill. Viability and growth rate of mycelial cultures following cryogenic storage. Mycologia 68: 377-387.

Jennison, M.W. (1948). The growth of wood-rotting fungi in submerged culture. (Abstract). Amer. J. Bot. 35: 801.

Jennison, M.W., Newcomb, M.D. and Henderson, R. (1955). Physiology of the wood-rotting Basidiomycetes. I. Growth and nutrition in submerged culture in synthetic media. Mycologia 47: 275-304.

Jong, S.C. and Peng, J.T. (1975). Identity and cultivation of a new commercial mushroom in Taiwan. Mycologia 67: 1235-1238.

Kim, D.S. (1978). Cultivation in Asian countries: Growing in temporate zones. In: The Biology and Cultivation of Edible Mushrooms. (Eds., Chang, S.T. and Hayes, W.A.) Academic Press, New York. 345-363.

Klein, B. and Bopp, B. (1971). Effect of activated charcoal in agar on the culture of lower plants. Nature 230: 474.

Largent, D. (1986). How to identify mushrooms to genus I: macroscopic features. Mad River Press Inc., California. 1-166.

Largent, D., Johnson, D., and Watling, R. (1977). How to identify mushrooms to genus III:microscopic features. Mad River Press Inc., California. 1-148.

Leonard, T.J. and Dick, S. (1973). Induction of haploid fruiting by mechanical injury in Schizophyllum. commune. Mycologia 65: 809-822.

Lilly, V.G. (1965). The chemical environment for fungal growth: media, macro, and micronutrients. In: The Fungi. (Eds., Ainsworth, G.C. and Sussman, A.S.) Academic Press, New York and London. 465-478.

Long, P.E. and Jacob, L. (1974). Aseptic fruiting of the cultivated mushroom, Agaricus bisporus. Trans. Br. Mycol. Soc. 63: 99-107.

Losel, D.M. (1964). The stimulation of spore germination in Agaricus bisporus by living mycelium. Ann. Bot. N.S. 28: 54.

Losel, D.M. (1967). The stimulation of spore germination in Agaricus bisporus by organic acids. Ann. Bot. N.S. 31: 417-425.

Manning, W.J. and Crossan, D.F. (1966). Effects of a particular soil bacterium on sporangial production in Phytophthora cinnamomi in liquid culture. Phytopathology 56: 235.

Marvanova, L. and Bandoni, R. J. (1987). Naidella fluitans Gen. Et. Sp. Nov:a conedial Basidiomycete. Mycologia 79:578-586.

Marx, D. H., and Haasis, F.A. (1965). The induction of aseptic sporangial formation in Phytophthora cinnamomi by metabolic diffusates of soil micro-organisms. Nature 206: 673.

McDaniel, L.L. and Hindal, D.F. (1982). Spore swelling, germination, and germ tube formation in axenic culture among four species of Piptocephalis. Mycologia 74: 271-274.

Mckane, L. and Kandel, J. (1986). Microbiology: Essentials and Applications. International Edition. McGraw Hill Book company, New York. 143.

McLaughlin, D.J.(1974). Fruitbody formation by Tylopilus felleus in axenic culture. Mycologia 66: 197-202.

McLean, R.C. and cook, I. (1958). Plant Science Formulae. Macmillan and Co. Ltd., London. 102-113.

McTeague, O.M., Hutchinson, S.A., and Reed, R.J. (1959). Spore germination in Agaricus campestris L. ex. Fr. Nature. Lond. 183: 1736.

Moscona, A., Trowell, O.A., and Willmer, E. N. (1965). Methods. In: Cell and Tissues in Culture. Vol I, methods, biology, and physiology. (Ed. Willmer, E.N.) Academic Press, London and New York. 19-98.

Murashige, T. (1973). Sample preparation of media: plant cultures. In: Tissue Culture Methods and Applications. (Eds., kruse, P.F. and Patterson, M.K.) Academic Press, New York. 698-703.

Murashige, T. (1979). Plant tissue culture and its importance to agriculture. In: Practical Tissue Culture Applications. (Eds., Maramorosch, K. and Hirumi, H). Academic Press, New York. 27-44.

Nicholson, R.A. (1989). Common mushrooms found in Akwa Ibom state. The Nigerian Field 54: 19-32.

Oei, P. (1991). Manual on Mushroom Cultivation: Techniques, Species and Opportunities For Commercial Applications in Developing Countries. Tool Publications, Amsterdam. 1-222.

Oso, B.A. (1977) Pleurotus tuber-regium from Nigeria. mycologia 69: 271-279.

Paranjpe, M.S., Chen, P.K. and Jong, S. C. (1979). Morphogenesis of Agaricus bisporus: changes in protein and enzyme activity. Mycologia 71: 469-478.

Park, J.Y. and Agnihotri, V.P. (1969). Bacterial metabolites trigger sporophore formation in Agaricus bisporus. Nature 222: 984.

Perrin, P.W. (1979). Long-term storage of cultures of wood-inhabiting fungi under mineral oil. Mycologia 71: 867-869.

Pettipher, G.C. (1988). Cultivation of the shiitake mushroom(Lentinus edodes) on lignocellulosic waste. Journal of the science of Food and Agriculture 42(3), 195-198.

Purkayastha, R.P. and Chandra, A. (1975). Termitomyces eurhizus, new Indian edible mushroom. Trans. Br. Mycol. Soc. 64: 168-170.

Raabe, R.O. (1972). Variation in pathogenicity and virulence in single-spore isolates of Armillaria mellea. Mycologia 64: 1154-1159.

Rast, D. and Stauble, E. J. (1970). On the mode of action of isovaleric acid in stimulating the germination of Agaricus bisporus spores. New Phytol. 69: 557-566.

Rast, D., Stussi, H., and Zobrist, P. (1979). Self-inhibition of the Agaricus bisporus spore by CO_2 and/or glutaminyl -4- hydroxybenzene and glutaminyl - 3-4 benzoquinone: a biochemical analysis. Physiol. Pl. 46: 227-234.

Roxon, J.E. and Jong S.C. (1977). Sexuality of an edible mushroom,Pleurotus sajor-caju. Mycologia 69: 203-205.

Royse, D. J. and May, B. (1982). Use of isozyme variation to identify genotypic classes of Agaricus brunnescens. Mycologia 74: 93-102.

San Antonio, J.P. (1971). A laboratory method to obtain fruit from cased grain spawn of the cultivated mushroom, Agaricus bisporus. Mycologia 63: 16-21.

Sathe, A.V. and Dighe, S. (1987). A simple and economic method for long-term preservation of mushroom culture.Current Science 56 (10) 485.

Schaffner, C.P. (1979). Animal and plant tissue culture decontamination. In: Practical Tissue Culture Applications . (Eds., Maramorosch, K. and Hirumi, H.) Academic press, New York. 203-214.

Schisler, L.C. (1967). Stimulation of yield in the cultivated mushroom by vegetable oils. Appli Microbiol. 15: 844-850.

Schisler, L.C. and sinden, J.W (1962). Nutrient supplementation of mushroom compost at casing - vegetable oils. Canadian Journal of Botany 44: 1063-1069.

Schisler, L.C., Sinden, J.W., and Sigel, E.M. (1967). Etiology, symtomatology, and epidemiology of a virus disease of cultivated mushrooms. Phytopathology 57: 519-526.

Schisler, L.C. and Volkoff, O. (1977). The effect of safflower oil on mycelial growth of Boletaceae in submerged liquid cultures. Mycologia 69: 118-125.

Seabrook, J.E. (1980). Laboratory culture. In: Plant Tissue Culture as a Source of Biochemicals (Ed. staba, J.) CRC Press, Florida. 1-20.

Shaffer, L.R. (1975). The major groups of Basidiomycetes. Mycologia 67: 1-18.

Singer, R. (1961). Mushrooms and Truffles: botany cultivation, and utilization. Interscience Publishers Inc., New York. 42-139.

Singer, R. (1975). The Agaricales in Modern Taxonomy. Ed.3. J. Cramer, New York. 141.

Smith, A.H. (1978). Morphology and classification. In: The Biology and Cultivation of Edible Mushrooms (Eds., chang, S.T. and Hayes, H.A.) Academic Press, New York. 3-34.

Smith, R.S. (1971). Maintenance of fungal culture in presterilized disposable screw cap plastic tubes. Mycologia 63: 1218-1221.

Srivastava, H.C. and Bano, Z (1962).Studies on cultivation of Pleurotus spp. on paddy straw. Food Science 11,12: 363-365.

Stack, R.W., Sinclair, W.A. and Larsen, A.O. (1975). Preservation of basidiospores of Laccaria laccata for use as mycorrhizal inoculum. Mycologia 67: 167-170.

Stocks, D.L. and Hess, W.M. (1970). Ultrastructure of dormant and germinated basidiospore of a species of Psilocybe. Mycologia 62: 176-191.

Street, H.E. (1966). The nutrition and metabolism of plant tissue and organ culture. In: Cell and Tissues in Culture. Vol III, Methods, Biology and Physiology.(Ed., Willmer, E.N.) Academic Press, London and New York. 533-629.

Sugihara, T.F. and Humefeld, H. (1954). Submerged culture of various species of mushrooms. Appl. Microbiol. 2: 170-172.

Tan, C.S., Ingen, C.W. Van, and Stalpers, J.A. (1992). Freeze drying of fungal hyphae and stability of the product. In: Genetics and Breeding of Agaricus. Proceedings of the First International Seminar on Mushroom Science. (Ed. Griensven, L.J.L.D. Van) Horst, Netherlands. 14-17 May 1991. 25-30.

Thakuri, S.B. (1976). Report of Burrillia (Ustilaginales) in pure culture. Mycologia 68: 166-167.

Thiers, H.D. (1975). California Mushrooms: a field guide to the Boletes. Hafner Press, New York. 18-21.

Thomas, E. and Davey, M.R. (1975). From Single Cells to Plants. Wykeham Publications Ltd., London. 25-80.

Thompson, A. and Lim, G. (1965). Laboratory Manual of Tropical Mycology and Elementary Bacteriology. University of Malaya Press, Kuala Lumpur. 10.

Turner, W. B. (1971). Fungal Metabolites. Academic Press, London and New York. 1-60.

Vedder, P.J.C. (1978). Cultivation. In: The Biology and Cultivation of Edible Mushrooms. (Eds., Chang, S.T. and Hayes, W.A.) Academic Press, New York. 377-392.

Vogel, F.S., Kemper, L.A., McGarry, S.J. and Graham, D.G. (1975). Cytostatic, cytocidal and potential anti-tumor properties of a class of quinoid compounds, initiator of dormant state in the spores of Agaricus bisporus. Amer. J. Pathol. 78: 33-48.

Watling, R. (1971). Basidiomycetes: Homobasidiomycetidae. In: Methods in Microbiology, Vol. iv (Ed., Booth,C.) Academic Press, London and New York. 219-236.

Watling, R. (1981). How to Identify Mushrooms to Genus V: Cultural and Developmental Features. Mad River Press, California. 1-167.

Wardle, K.S. and Schisler, L.C. (1969). The effects of various lipids on growth of mycelium of Agaricus bisporus. Mycologia 61: 305-314.

Weber, J. (1970). Introduction to Fungi. Cambridge University Press, London. 1-135.

Weete, R. (1980). Lipid Biochemisty of Fungi and Other Organisms. Plenum Press, New York. 50-150.

Whitaker, A.M. (1972). Tissue and Cell Culture. Williams and Wilkins Company, Baltimore. 8-17.

White, P.R. (1963). The Cultivation of Animal and Plant Cells Ed. 2. Ronald Press Company, New York. 52-62.

Whitney, K.D. and Arnott, H.J. (1987). Calcium oxalate crystal morphology and development in Agaricus bisporus. Mycologia 79: 180-187.

Zadrazil, F. (1978). Cultivation of Pleurotus In: The Biology and Cultivation of Edible Mushrooms (Eds., Chang, S.T. and Hayes, W.A.) Academic Press, New York. 521-557.

Zakia, B., Srinivasan, K.S. and Singh, N.S. (1971). Essential amino acid composition of the proteins of a mushroom (Volvariella diplasia) J. Food Sci. Technol. 8: 180-182.

Zervakis, G. and Balis, C. (1991). Pleurotus species of Greece: an evaluation of their morphological and physiological characteristics. In: Mushroom Science XIII. Volume 2.Proceedings of the 13th International Congress on the Science and Cultivation of Edible Fungi. (Ed., Maher, M.J.) Dublin, Irish Republic. 1-6 September, 1991.

Zoberi, M.H. (1972). Tropical Macrofungi: some common species. Macmillian Press Ltd., London. 57-92.

Zoberi, M.H. (1973). Some edible mushrooms from Nigeria. Nigerian Field 38: 81-90.

APPENDIX I

Macroscopic features of mushroom species used in the study.

Bolbitius spp. A

A Characters of the pileus.

1. Size (width) 6.2cm.
2. Shape of pileus.
a Side view - plane.
b Top view - orbicular.
3. Colour of pileus - white with tan disc.
4 Margin of pileus.
a Shape of margin (cross section) - plane.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface) - downy - fibrillose.

B. Characters of the Lamellae.

1 Attachment - free.
2 Spacing of gills - crowded.
3 Margin of gills - smooth.

C Characters of the stipe.

1. Size of stipe.
a Length - 4.4cm.
b Width - 1.2cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - equal.
4 Consistency of stipe - fibrous, outside white and cottony.
5 Flesh of stipe - stuffed.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - absent.

D Growth habit- gregarious.
E Type of fruiting body attachment - stipitate.
F Spore colour - date brown

Bolbitius spp. B

A Characters of the pileus.

1. Size (width) 4.0cm.
2. Shape of pileus.
a Side view - mammilate
b top view - orbicular.
3. Colour of pileus - white.
4 Margin of pileus.
a Shape of margin (cross section) - inrolled.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface) - pruinose.

B. Characters of the Lamellae.

1 Attachment - free.
2 Spacing of gills - crowded.
3 Margin of gills - crisped.

C Characters of the stipe.

1. Size of stipe.
a Length - 4.2cm.
b Width - 0.3cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - tapered from base to apex.
4 Consistency of stipe - fibrous.
5 Flesh of stipe - hollow.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - absent.

D Growth habit- scattered.
E Type of fruiting body attachment - stipitate.
F Spore colour - date brown.

Bolbitius spp. C

A Characters of the pileus.

1. Size (width) 2.8cm
2. Shape of pileus.
a Side view - broadly umbonate.
b top view - orbicular.
3. Colour of pileus - cartridge buff pileus with vinaceous buff disc.
4 Margin of pileus.
a Shape of margin (cross section) - incurved.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface) - glabrous.

B. Characters of the Lamellae

1 Attachment - free.
2 Spacing of gills - close.
3 Margin of gills - smooth.

C Characters of the stipe.

1. Size of stipe.
a Length - 2.5cm.
b Width - 0.3cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete
b Longitudinal view - equal.
4 Consistency of stipe - cartilaginous.
5 Flesh of stipe - stuffed.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - absent.

D Growth habit - caespitose.
E Type of fruiting body attachment - stipitate
F Spore colour - cigar brown.

Coprinus spp. A

A Characters of the pileus.

1. Size (width) 11.3 cm.
2. Shape of pileus.
a Side view - plane
b top view - orbicular.
3. Colour of pileus - light brown disc with the colour radiating and fading.
4 Margin of pileus.
a Shape of margin (cross section) - plane.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface)- glabrous.

B. Characters of the Lamellae.

1 Attachment - free.
2 Spacing of gills - crowded.
3 Margin of gills - smooth.

C Characters of the stipe.

1. Size of stipe.
a Length - 7.3 cm
b Width - 1.3 cm
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - equal.
4 Consistency of stipe - fibrous.
5 Flesh of stipe - solid.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - present, superior.

D Growth habit- Scattered.
E Type of fruiting body attachment - stipitate.
F Spore colour - fuscous black.

Coprinus spp. B

A Characters of the pileus.

1. Size (width) - 7.1 cm.
2. Shape of pileus.
a Side view - plane with a shallow and narrow depression 1 cm wide at the centre of the pileus.
b top view - orbicular.
3. Colour of pileus - white.
4 Margin of pileus.
a Shape of margin (cross section) - plane.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface)- glabrous.

B. Characters of the Lamellae.

1 Attachment - free.
2 Spacing of gills - crowded.
3 Margin of gills - smooth.

C Characters of the stipe.

1. Size of stipe.
a Length - 5cm.
b Width - 1cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - tapered from base to apex.

4 Consistency of stipe - cartilaginous.
5 Flesh of stipe - solid.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - present.

D Growth habit- scattered.
E Type of fruiting body attachment - stipitate.
F Spore colour - fuscous black.

Coprinus spp. C

A Characters of the pileus.

1. Size (width) - 8.8cm.
2. Shape of pileus.
a Side view - plane.
b top view - orbicular.
3. Colour of pileus - dull white with light brown disc 2.2cm wide.
4 Margin of pileus.
a Shape of margin (cross section) - incurved.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface) - glabrous.

B. Characters of the Lamellae.

1 Attachment - free.
2 Spacing of gills - crowded.
3 Margin of gills - smooth.

C Characters of the stipe.

1. Size of stipe.
a Length - 5.5cm.
b Width - 1.2cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - equal.
4 Consistency of stipe - cartilaginous.
5 Flesh of stipe - stuffed.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - present, superior.

D Growth habit - gregarious.
E Type of fruiting body attachment - stipitate.
F Spore colour - fuscous black.

Lentinus squarrosulus

A Characters of the pileus.

1. Size (width) - 5.4cm.
2. Shape of pileus.
a Side view - shallowly depressed.
b top view - orbicular.
3. Colour of pileus - White with light cream colour at the centre of the indentation. The colour not always present.
4 Margin of pileus.
a Shape of margin (cross section) - incurved.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface) - glabrous.

B. Characters of the Lamellae

1 Attachment - subdecurrent.
2 Spacing of gills - crowded.
3 Margin of gills - smooth.

C Characters of the stipe.

1. Size of stipe.
a Length - 4.0cm.
b Width - 0.8cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - equal.
4 Consistency of stipe - leathery - fibrous.
5 Flesh of stipe - solid.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - absent.

D Growth habit - caespitose.
E Type of fruiting body attachment - stipitate.
F Spore colour - cartridge buff.

Lepiota spp.

A Characters of the pileus.

1. Size (width) - 20.5cm.
2. Shape of pileus.
a Side view - plane.
b top view - orbicular.
3. Colour of pileus - beige.
4 Margin of pileus.
a Shape of margin (cross section) - plane.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - viscid.
c Texture of pileal surface (degree of hyphal association on pileal surface)- pruinose.

B. Characters of the Lamellae

1 Attachment - free.
2 Spacing of gills - close.
3 Margin of gills - wavy.

C Characters of the stipe.

1. Size of stipe.
a Length - 18cm.
b Width - 1.4cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - equal,slightly tapered at both the apex and the base.
4 Consistency of stipe - cartilaginous.
5 Flesh of stipe - stuffed.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - present, superior.

D Growth habit- scattered.
E Type of fruiting body attachment - stipitate.
F Spore colour - white.

Pholiota spp. A

A Characters of the pileus.

1. Size (width) - 8.7cm.
2. Shape of pileus.
a Side view - plane.
b top view - orbicular.
3. Colour of pileus - disc is light brown with colour radiating in waves toward the circumference, rest of pileus white.
4 Margin of pileus.
a Shape of margin (cross section) - inrolled.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface)- squamose.

B. Characters of the Lamellae.

1 Attachment - free.
2 Spacing of gills - crowded.
3 Margin of gills - wavy.

C Characters of the stipe.

1. Size of stipe.
a Length - 8.5 cm.
b Width - 0.5 cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - equal.
4 Consistency of stipe - fibrous.
5 Flesh of stipe - stuffed.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - present, large, dark brown (superior).

D Growth habit- scattered.
E Type of fruiting body attachment - stipitate.
F Spore colour - date brown.

Pholiota spp. B

A Characters of the pileus.

1. Size (width) - 8.9cm.
2. Shape of pileus.
a Side view - plane.
b top view - orbicular.
3. Colour of pileus - shallowly depressed tan disc, rest of pileus is white.
4 Margin of pileus.
a Shape of margin (cross section) - incurved.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface) - glabrous.

B. Characters of the Lamellae

1 Attachment - free.
2 Spacing of gills - crowded.
3 Margin of gills - smooth.

C Characters of the stipe.

1. Size of stipe.
a Length - 10.5 cm.
b Width - 1.1 cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - tapered from base to apex.
4 Consistency of stipe - fibrous.
5 Flesh of stipe - stuffed.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - present, superior, large, dark brown.

D Growth habit- gregarious.
E Type of fruiting body attachment - stipitate.
F Spore colour - date brown.

Psathyrella spp.

A Characters of the pileus.

1. Size (width) - 6.2 cm.
2. Shape of pileus.
a Side view - conic.
b top view - orbicular.
3. Colour of pileus - dark brown with light brown disc.
4 Margin of pileus.
a Shape of margin (cross section) - plane.
b Shape of margin (surface view) - entire
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist, viscid.
c Texture of pileal surface (degree of hyphal association on pileal surface)- granulose .

B. Characters of the Lamellae.

1 Attachment - subdecurrent.
2 Spacing of gills - crowded.
3 Margin of gills - smooth.

C Characters of the stipe.

1. Size of stipe.
a Length - 7.8 cm.
b Width - 0.3 cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - tapered from base to apex.
4 Consistency of stipe - fibrous.
5 Flesh of stipe - fistulose.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - present, superior.

D Growth habit - gregarious.
E Type of fruiting body attachment - stipitate.
F Spore colour - fuscous black.

Psilocybe spp.

A Characters of the pileus.

1. Size (width) - 2.5 cm.
2. Shape of pileus.
a Side view - plane, mammilate.
b top view - orbicular.
3. Colour of pileus - light brown with dark brown disc.
4 Margin of pileus.
a Shape of margin (cross section) - plane.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface)- glabrous.

B. Characters of the Lamellae.

1 Attachment - adnexed.
2 Spacing of gills - close.
3 Margin of gills - smooth.

C Characters of the stipe.

1. Size of stipe.
a Length -11.5 cm.
b Width - 0.4 cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - scarce.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - equal.
4 Consistency of stipe - fibrous.
5 Flesh of stipe - fistulose.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - absent.

D Growth habit- gregarious.
E Type of fruiting body attachment - stipitate.
F Spore colour - fuscous black.

Termitomyces spp.

A Characters of the pileus.

1. Size (width) - 8.3 cm.
2. Shape of pileus.
a Side view - acutely umbonate, plane.
b top view - orbicular.
3. Colour of pileus - White with light brown umbo.
4 Margin of pileus.
a Shape of margin (cross section) - inrolled.
b Shape of margin (surface view) - rimose.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - (degree of hyphal association on pileal surface) - glabrous.

B. Characters of the Lamellae.

1 Attachment - free.
2 Spacing of gills - crowded.
3 Margin of gills - wavy.

C Characters of the stipe.

1. Size of stipe.
a Length -3.2cm above ground extending 25.8cm down and ending in a circular disc 2.7cm wide with a hole in the middle.
b Width - 1 cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - equal.
4 Consistency of stipe - fibrous.
5 Flesh of stipe - fistulose.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - absent.

D Growth habit - gregarious.
E Type of fruiting body attachment - stipitate.
F Spore colour - cartridge buff.

Termitomyces clypeatus

A Characters of the pileus.

1. Size (width) - 7.3cm
2. Shape of pileus.
a Side view - umbonate.
b top view - orbicular.
3. Colour of pileus - Light tan with dark tan disc.
4 Margin of pileus.
a Shape of margin (cross section) - plane.
b Shape of margin (surface view) - rimose.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - dry.
c Texture of pileal surface (degree of hyphal association on pileal surface) - glabrous.

B. Characters of the Lamellae.

1 Attachment - free.
2 Spacing of gills - crowded.
3 Margin of gills - crenate.

C Character of the stipe.

1. Size of stipe.
a Length -7.3 cm.
b Width - 0.8 cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - radicated stipe.
3 Shape of stipe.
a Cross section - terete.
b Longitudinal view.
4 Consistency of stipe - fibrous
5 Flesh of stipe - fistulose.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - absent.

D Growth habit- scattered.
E Type of fruiting body attachment - stipitate.
F Spore colour - clay pink.

Termitomyces microcarpus

A Characters of the pileus.

1. Size (width) - 2.1cm.
2. Shape of pileus.
a Side view - plane.
b top view - orbicular.
3. Colour of pileus - cinnamon coloured disc with the rest of the pileus white.
4 Margin of pileus.
a Shape of margin (cross section) - plane.
b Shape of margin (surface view) - entire.
5 Surface of pileus.
a Shininess of pileal surface - dull.
b Wetness of pileal surface - moist.
c Texture of pileal surface (degree of hyphal association on pileal surface) - glabrous.

B. Characters of the Lamellae.

1 Attachment - free.
2 Spacing of gills - close.
3 Margin of gills - smooth.

C Characters of the stipe.

1. Size of stipe.
a Length - 3.4 cm.
b Width - 0.3 cm.
2 Attachment of stipe.
a Attachment to pileus - central.
b Attachment to substrate and basal tomentum - moderate.

3 Shape of stipe.
a Cross section - terete.
b Longitudinal view - equal.
4 Consistency of stipe - cartilaginous.
5 Flesh of stipe - fistulose.
6 Presence or absence of veils on stipe.
a Volva - absent.
b Annulus - present.

D Growth habit- caespitose.
E Type of fruiting body attachment - stipitate.
F Spore colour - olivaceous.

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APPENDIX II

STATISTICAL ANALYSIS

1A Analysis of variance (ANOVA) for the rate of germination of spores of L. squarrosulus under different treatments and incubation methods in fresh potato dextrose agar (PDA1)

Sources of variation	DF	Sum of squares	Mean of squares	F
replicates	2	3680.771	1840.385	0.69
treatment	31	1056416.389	34077.948	12.84
incubation	2	7590142.688	3795071.344	1430.06
error	252	668755.653	2653.792	
Total	287	9318995.500		

1B Analysis of variance (ANOVA) for the rate of germination of spores of L. squarrosulus under different treatments and incubation methods in ready made potato dextrose agar (PDA2)

Sources of variation	DF	Sum of squares	Mean of squares	F
replicates	2	141.083	70.542	0.04
treatment	31	1147721.219	37023.265	18.55
incubation	2	7778206.938	3889103.469	1948.75
error	252	502913.979	1995.690	
Total	287	9428983.219		

1C Analysis of variance (ANOVA) for the rate of germination of spores of L. squarrosulus under different treatments and incubation methods in fresh yeast extract agar (YEA1)

Sources of variation	DF	Sum of squares	Mean of squares	F
replicates	2	1309.465	654.733	0.84
treatment	31	791907.024	25545.388	32.78
incubation	2	3271298.257	1635649.128	2099.15
error	252	196357.167	779.195	
Total	287	4260871.913		

1D Analysis of variance (ANOVA) for the rate of germination of spores of L. squarrosulus under different treatments and incubation methods in ready made yeast extract agar (YEA2)

Sources of variation	DF	Sum of squares	Mean of squares	F
replicates	2	1219.694	609.847	1.01
treatment	31	781228.358	25200.915	41.77
incubation	2	3188582.007	1594291.003	2642.45
error	252	152041.188	603.335	
Total	287	4123071.247		

1E Analysis of variance (ANOVA) for the rate of germination of spores of L. squarrosulus under different treatments and incubation methods in fresh sabouraud's dextrose agar (SDA1)

Sources of variation	DF	Sum of squares	Mean of squares	F
replicates	2	171.361	85.681	0.04
treatment	31	1568537.635	50597.988	26.34
incubation	2	7769833.757	3884916.878	2022.66
error	252	484014.660	1920.693	
Total	287	9822557.413		

1F Analysis of variance (ANOVA) for the rate of germination of spores of L. squarrosulus under different treatments and incubation methods in ready made Sabouraud's dextrose agar (SDA2)

Sources of variation	DF	Sum of squares	Mean of squares	F
replicates	2	140.361	70.181	0.03
treatment	31	1427804.608	46058.213	22.80
incubation	2	7823646.465	3911823.233	1936.68
error	287	509004.063	2019.857	
Total	287	9760595.497		

1G Analysis of variance (ANOVA) for pileus width.

Sources of variation	DF	Sum of squares	Mean of squares	F
media	4	11.49	2.87	2.03
error	12	16.93	1.41	
Total	16	28.42		

1H Analysis of variance (ANOVA) for stipe length.

Sources of variation	DF	Sum of squares	Mean of squares	F
media	4	17.20	4.30	3.61
error	12	14.28	1.19	
Total	16	31.48		

1I Analysis of variance (ANOVA) for stipe width.

Sources of variation	DF	Sum of squares	Mean of squares	F
media	4	0.02711	0.00678	1.08
error	12	0.07524	0.00627	
Total	16	0.10235		

1J Analysis of variance (ANOVA) for the effect of different liquid culture media and incubation methods on the hyphal growth of T. clypeatus.

Sources of variation	DF	Sum of squares	Mean of squares	F
media	3	75180.11111	25060.03704	91.55
incubation	2	15561.16667	7780.58333	28.42
error	30	8211.72222	273.72407	
Total	35	98953.00000		

1K Analysis of variance (ANOVA) for the effect of different liquid culture media and incubation methods on the hyphal growth of L. squarrosulus.

Sources of variation	DF	Sum of squares	Mean of squares	F
media	3	33958.33333	11319.44444	88.40
incubation	2	22619.38889	11309.69444	88.32
error	30	3841.50000	128.05000	
Total	35	60419.22222		

1L Analysis of variance (ANOVA) for the effect of different liquid culture media and incubation methods on the hyphal growth of L. squarrosulus and T. clyeatus (pooled data)

Sources of variation	DF	Sum of squares	Mean of squares	F
media	3	92831.44444	30943.81481	69.92
incubation	2	37776.19444	18888.09722	42.68
species	1	46614.22222	46614.22222	105.34
error	65	28764.5833	442.5321	
Total	71	205986.4444		