THE UNIVERSITY OF ASTON IN BIRMINGHAM

PHYSIOLOGICAL STUDIES ON THE GROWTH AND FRUCTIFICATION OF THE EDIBLE BASIDIOMYCETE SPECIES, *PLEUROTUS SAJOR-CAJU* (FR.) SING. AND *STROPHARIA RUGOSO-ANNULATA* FARL. AP. MURR.

by

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Being a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

June, 1981

PHYSIOLOGICAL STUDIES ON THE GROWTH AND FRUCTIFICATION OF THE EDIBLE BASIDIOMYCETE SPECIES, PLEUROTUS SAJOR-CAJU (FR.) SING. AND STROPHARIA RUGOSO-ANNULATA FARL. AP. MURR.

Cajetan Ikechukwu Stanley Okwu, B.Sc. Doctor of Philosophy 1981.

SUMMARY.

The influence of various cultural conditions on the growth of thirteen edible fungi was studied in preliminary investigations. Based on the results of these experiments, two basidiomycete species, Pleurotus sajor-caju (Fr.) Sing. and Stropharia rugoso-annulata Farl. ap. Murr. were selected for further studies on growth and fruit-body formation.

The two fungi proved to be good decomposers of cereal straw, but P. sajor-caju showed ability to degrade a wider range of plant waste materials than S. rugoso-annulata. Carbon : nitrogen ratios of 90 : 1 and 80 : 1 were optimal for P. sajor-caju and S. rugoso-annulata respectively.

Both species were found to be heterotrophic for thiamine.

Tolerance to high concentrations of carbon dioxide was exhibited by the two species during the vegetative phase, S. rugoso-annulata being more sensitive to changes in oxygen and carbon dioxide concentration. In the reproductive stage however, ventilation was necessary for both species.

While P. sajor-caju required light for primordium formation and fruit-body development, S. rugoso-annulata appeared to be indifferent to light.

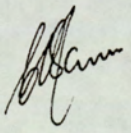
P. sajor-caju showed no need for a casing layer to fructify. In contrast, S. rugoso-annulata required casing soil for fruit-body formation.

The presence of micro-organisms was shown to be necessary for fruit-body formation of S. rugoso-annulata.

KEY WORDS : PHYSIOLOGY
PLEUROTUS SAJOR-CAJU
STROPHARIA RUGOSO-ANNULATA.

DECLARATION.

I declare that the work described in this thesis is the result of my own investigations, except where reference is made to published literature and where assistance is acknowledged, and that the work has not been submitted for any other award.



Candidate

June, 1981

DEDICATION

To the memory of my beloved father

Alfred Okwujiakọ Emeañụgwara.

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I owe deep gratitiude to my supervisor, Dr.W.A.Hayes not only for his guidance throughout the course of this work but also his kind hospitality. Many thanks go to Professor G.J.F.Pugh for supervising the first part of this work and also his hospitality.

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GENERAL INTRODUCTION

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Many fleshy fungi (mushrooms) are traditionally eaten in most parts of the world. Through the course of history man learnt, probably by trial and error, to identify the edible ones, and later cultivated mushrooms not only for eating but also for medicinal and ritualistic purposes (Hayes and Nair,1975). In recent years the cultivation of the species - Agaricus bisporus - has developed tremendously into a technology that compares favourably with any other fermentation-based industry like wine making. Spreading from France to most parts of Europe, the cultivation of A.bisporus is now a major industry in most countries of the developed world.

The situation is however, different in the less developed countries. In these areas edible fruit-bodies are still collected from forests and jungles. The mushrooms are commonly used as food and thus play an important role in the everyday life of the people. Unfortunately accidents do sometimes occur, a few of them fatal. For example, in 1977, twelve people died in a Nigerian village after eating a meal containing a poisonous mushroom (Ogundana,1979). There is therefore an urgent need to domesticate and cultivate edible species commercially so that people can eat their desired delicacy with maximum assurance of safety.

In the less developed countries also food production lags behind population growth, and because of inadequate supply of proteins and other essential nutrients, many people are undernourished. Mushrooms are a good source of proteins, vitamins and minerals (Hayes and Nair,1975; Chang,1972). Their large scale production can therefore help to improve the quality of food in these areas. The tropics abound with edible basidiomycetes, and Zoberi (1979) has identified 25 good-

quality edible species in Nigeria, the most prized being the Termitomyces, Pleurotus and Volvariella species.

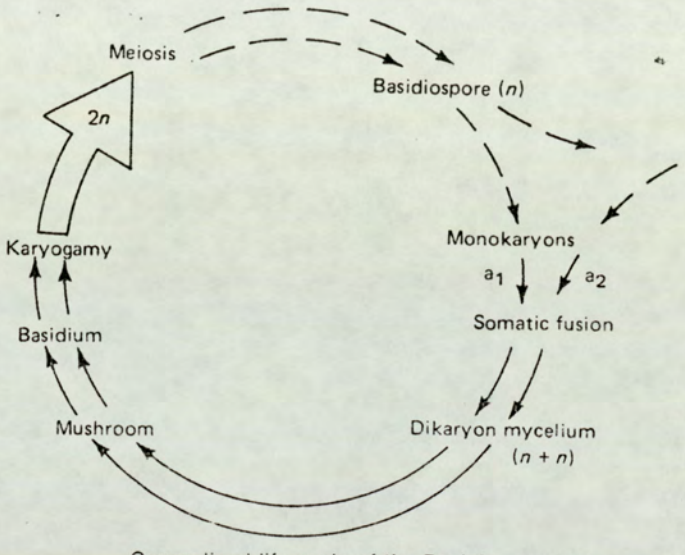
Some of these species are subject to some form of rather primitive cultivation in parts of Asia and Africa - mainly on logs of wood or other domestic waste products. The methods are based more on natural infection of the substrate than any scientifically controlled cultivation. While systematic cultivation is feasible for many of these fungi, the major obstacle to large-scale production is the difficulty of obtaining economically worthwhile yields.

Most of the edible fungi used in this work (and indeed most other edible mushrooms) belong to the family Agaricaceae, order Agaricales, class Hymenomycetes of the sub-division Basidiomycotina (basidiomycetes). Phallus impudicus is of the family Phallaceae, in the order Phallales and class Gasteromycetes. The morels (ie. the Morchella species) of the family Morchellaceae, order Pezizales and class Discomycetes are ascomycetes ie. sub-division Ascomycotina (Ainsworth, 1971).

The generalized life cycle of basidiomycetes is relatively simple. Haploid basidiospores germinate to give rise to haploid monokaryotic mycelia (dikaryotic in A. bisporus). Following the union of two vegetative mycelia (somatogamy) the dikaryon is produced and dominates the vegetative phase. The dikaryon, after some period of vegetative growth differentiates into the basidiocarp (the "mushroom" that is consumed) which forms the characteristic basidium that in turn produces the basidiospores again after karyogamy and meiotic division. The above is illustrated in Fig. (1). The capability (compatibility) of monokaryotic vegetative mycelia to join together and form the dikaryotic mycelium is regulated by a complicated genetic

system. Most basidiomycetes are heterothallic (Ross,1979), and with these species, the above genetic system is easier to manipulate by breeders to produce better strains from successful mating experiments, than with homothallic species.

Fig.(i)



Generalized life cycle of the Basidiomycotina.

Source : Ross (1979)

In addition to genetic studies, physiological studies on these edible species is also a sine qua non in the development of commercially viable cultivation methods. The observable characteristics of any organism (phenotype) are governed by both the constant, inherited factors (genotype) and the environmental factors including nutritional, physical and chemical factors.

The first section of the present work was aimed at investigating the influence of environmental factors affecting the growth of a range of selected edible fungi. From the results of the above investigation, two basidiomycetes, rather contrasting in their requirements for fructification, were selected for further physiological

studies with a view to establishing the optimum requirements for growth and fruit-body formation. The scope of this work does not cover genetic aspects of growth and reproduction.

SECTION 1

LITERATURE REVIEW.

1. LITERATURE REVIEW.

1.1. INTRODUCTION.

The literature on edible mushrooms is widely scattered, but recently it has been collated in a single text, *The Biology and Cultivation of Edible Mushrooms* (Chang & Hayes, 1978). It is estimated that of about 150 edible mushrooms, half are cultivated while the other half are collected wild in forests and meadows (Chang, 1972). Of the cultivated species, only 11 are extensively cultivated for commercial purposes (see Table 1).

Table 1

World Production of Cultivated Edible Mushrooms in 1975 According to Species.

Species	Common Name	Quantity (tonnes)
Agaricus (*bisporus* and *bitorquis*)	(French) Mushroom	670,000
Lentinus edodes	Shiitake	130,000
Volvariella volvacea	Straw Mushroom	42,000
Flammulina velutipes	Enokitake	38,000
Pholiota nameko	Nameko	15,000
Pleurotus (*ostreatus*, *abalonis*, *cornucopiae* & *florida*)	Oyster Mushroom Abalone	12,000
Tremella fuciformis	Tremella	1,800
Auricularia (*polytricha* & *auricula*)	Jew's ear	5,700
Stropharia rugoso-annulata	Strophaire	1,300
Tuber melanosporum	Black Truffle	200
Total		916,000

Source : Delcaire (1978) in *The Biology and Cultivation of Edible Mushrooms* Chang & Hayes (1978).

As can be seen from the above table, total world production of mushrooms in 1975 was 916,000 tonnes and it is now estimated to be well over one million tonnes. A. bisporus and A. bitorquis by far dominate the world market, accounting for nearly 75% of the total production. A. bisporus cultivation is said to have started a few centuries back in the underground caves of France (Hayes & Nair, 1975). Cultivation methods have developed through the years from an art into sophisticated technology. It is not therefore surprising that the existing literature on edible fungi is correspondingly dominated by studies on A. bisporus carried out by many European and American workers. Most of these works have been on production of fruit-bodies and only little attention has been given to its vegetative stage. However, a few workers including Treschow (1944), Bohus(1959) and Hayes (1972) studied also the environmental and nutritional requirements of the mycelium.

Many studies on A. bitorquis have been carried out by Fritsche (1974), Raper (1974) and Vedder (1975). These workers have shown the potential of A. bitorquis as a species which may have some superior characteristics to A. bisporus, and because of its ability to grow and fructify at higher temperatures, recommend its cultivation in the warmer countries of the world (Vedder, 1975).

Lentinus edodes amd Flammulina velutipes together make up 18% of the world production. Their cultivation on a large scale is done mostly in Japan, only a small quantity is produced in China and Korea. Japanese workers have carried out a lot of studies on these mushrooms, although the books published in the Japanese language on their cultivation have found only limited circulation.

Other mushrooms of commercial importance at the moment

include Volvariells volvacea, Pleurotus species and Stropharia rugoso-annulata. Although V.volvacea has been cultivated for centuries in the Orient, and some other places like Nigeria where it is cultivated traditionally on oil palm refuse for local consumption (Zoberi, 1972), literature on this fungus has been very scarce. However, recently Chang started his pioneering studies and his main text on this mushroom is "The Chinese Mushroom" (Chang, 1972). His studies and those of a few others have led to improved methods and now paddy straw mushroom (as it is popularly known) ranks third in world production.

Of the Pleurotus species, P. ostreatus, P. florida and P. cornucpiae have received the most attention. Different aspects of these species have been studied by Zadražil (1974) and others. Their method of cultivation is fast improving and production rapidly increasing. Another Pleurotus species that has recently attracted attention is P. sajor-caju. Most of the work has been done in India by Jandaik, Kapoor, Rangard and a few others.

The cultivation of Stropharia rugoso-annulata started in East Germany in 1969 and spread to Poland and the surrounding countries. It is now cultivated to some extent in Western Europe and U.S.S.R. (Szudyga, 1978).

Many recent workers are determined to see that new types of mushrooms are introduced into the market. Of particular interest to them are those mushrooms that are particularly suited for production in the developing tropical and sub-tropical regions. The characteristics being emphasized are :

(a) Simple and inexpensive techniques of cultivation eg. Stropharia rugoso-annulata and most species of Pleurotus (Jandaik and Kapoor, 1974; Szudyga, 1978; and Zadrazil, 1979).

(b) Ability to grow and fructify at higher temperatures eg Agaricus bitorquis, Agrocybe aegerita, V.volvacea and most species of Pleurotus except P.ostreatus (Vedder, 1975; Jaindaik and Kapoor, 1974; and Zadrazil, 1979).

1.2. EFFECTS OF PHYSICAL FACTORS ON THE VEGETATIVE GROWTH.

1.2.1. A.bisporus :

(a) Temperature

Treschow (1944) grew this fungus in liquid culture within the temperature range of 0°-33°C for 8, 20 and 30 days. He obtained the greatest yield following a 30-day incubation period. But irrespective of the time of incubation, optimum temperature was determined to be 24°C, the minimum and maximum temperature for growth being 3°C and 30°C respectively. He repeated the above experiment on composted horse manure and obtained the same results.

Litchfield (1967) recorded 25°C as the best temperature for mycelial growth and Hayes (1978) stated that the optimum was 24°C-25°C with a growth range of 3°-30°C.

(b) pH

Early workers who investigated the pH requirements of A.bisporus reported values for optimum pH between 6 and 9. However, Treschow thoroughly investigated the problem on a synthetic liquid medium, manure and on spruce litter, and concluded that both his brown and white strains had their best growth at 6.9 and could grow at any pH values between 4.0 and 9.0. He noticed a greater tolerance of alkaline conditions in manure than in his synthetic aqueous medium. Hayes (1978) reported the pH range to be 3.5 - 9.0 with optimum at 6.8 - 7.0.

(c) Light:

According to Lu (1974) A.bisporus is normally indifferent to light.

1.2.2. A.bitorquis

Fritsche (1974) determined the optimum temperature for mycelial growth to be 30°C. She grew it on compost in Petri-plates. Raper in the same year, noted that it grew best at 30°C during her breeding work on this fungus. Raper and Kaye (1978) recorded the same temperature optimum. They obtained their result from colony diameter measurements on a solid medium.

It should be noted however, that there has not been any intensive work done purposely to determine the environmental effects on the growth of A.bitorquis. Vedder (1975) observed that one of the major difficulties for potential growers of this mushroom is "insufficient knowledge of the desired cultural conditions" of this species which eventually keep average yield too low in a given growth period.

1.2.3. A.arvensis

Raper and Kaye (1978) observed that the biology of Agaricus species, except A.bisporus and A.bitorquis, was still obscure. They however, included A.arvensis in their work after which they recorded a description of its morphology and noted the temperature optimum for vegatative growth as 24°C for one strain and 30°C for another strain. Delmas (1978) reported its optimum to be 25°C.

1.2.4. Coprinus comatus

(a) Temperature:

Much detailed research on this fungus was done by Fries

(1955 and 1956). She found the optimum temperature for mycelial growth to be 25°C. Delmas (1978) citing Vaandrager (1975), stated that this fungus had the same temperature requirements as A. bisporus (24°-25°C opt.).

(b) pH :

Fries' work on its hydrogen ion concentration requirements (Fries, 1956) indicated the pH optimum to be 6.0 with a range of 3.5-7.5. However, precipitation of Fe, Zn, and Ca at pH greater than 7.0 occurred. In a repeat experiment she added the above micro-nutrients after sterilisation and overcame the precipitation problem. A new optimum of 6.8. was attained and the yield significantly increased.

1.2.5. Lepista nuda

(a) Temperature :

In his thorough physiological studies of Tricholoma species Norkrans (1950) obtained the best growth of Lepista nuda at 25°C. Votypka (1971) recorded that maximum growth occurred at 20°-24°C. Wright (1976) agreed with both workers however, in the sense that some of his isolates grew best at 20°C and others at 25°C. His temperature range was 4°-30°C.

(b) pH:

Norkrans (1950) recorded a pH range of 3.5-8.0 with maximum growth occurring at 6.0. This agreed with the pH (6.0)of the forest soils, covered with spruce needles, from where he obtained his isolates initially. Votypka (1971) also recorded a pH optimum of 6.0 for this mushroom. But Wright's (1976) figures differed from theirs : he determined the optimum to be 7.0 with a growth range of pH3.1 - 8.0.

1.2.6. Lepista saeva

(a) Temperature :

Wright's work (1976) is probably the only one with substantial information about the growth requirements of L. saeva. According to him this fungus grew at temperatures between 4°C and 27°(28°)C and best at 20°C. Its growth was rather slower than L. nuda, given the same conditions.

(b) pH :

Wright (1976) recorded its pH optimum as 6.0. Growth occurred at pH 4.0 and also in very alkaline situations.

1.2.7. Morchella species.

(a) Temperature :

Litchfield (1967) observed that the optimum growth temperature of 4 Morchella species was 25°C within a range of 10°C - 35°C; and 3° - 27°C for 3 other species.

(b) pH :

Grainger (1946) found Morchella esculenta growing on soils with pH 7.0 - 8.0. Brock (1951) apparently carried out the first detailed study of the physiology of Morchella esculenta. Among other useful results, he recorded a pH optimum of 6.93. His pH curve had two peaks with one peak at 6.93 and the other at 8.3.

Kaul (1975) determined the pH of soils where Morchella conica and Morchella esculenta (among several other Morchella species) were fruiting to be 7.0 - 7.9 for the former and 7.5 - 8.6 for the latter. He also germinated the spores of various species at different pH values; for M. conica and M. esculenta the optimum pH was 8.0.

Delmas (1978) said that Morchella vulgaris grew on soils

of pH 7.5 - 8.0 while M. conica preferred 6.0.

1.2.8. Phallus impudicus

Grainger (1946) recorded that this fungus grew on soils with pH range 3.7 - 7.0.

1.2.9. Pluteus cervinus

Like Phallus impudicus and other species mentioned above, this fungus has not attracted much attention.

Lloyd (1974) determined the temperature range to be 5°C-30°C with an optimum of 22°C. The pH was found to be 4.85 optimum.

Grainger (1946) observed that P. cervinus preferred wood with a pH of 5.5 as its substrate.

1.2.10. Pleurotus sajor-caju

Some work has been done on this mushroom in the past few years by a number of researchers. Most of it however, has been concerned with the production of fruit bodies and its cultivation especially in developing countries, both by local farmers and industrialists. Little or no work has been done to determine the physiological and ecological requirements of the vegetative mycelium. Jandaik and Kapoor (1974) however, indicated that the optimum temperature for spawn production was 25°C.

1.2.11. Stropharia rugoso-annulata

(a) Temperature :

Szudyga (1978) reported that the temperature for maximum development of this mushroom differed, in his experiment, according to variety. While the "Gartenriese" variety developed best at 28°C the "Winneton" variety favoured 25°C.

Using "Winnetou" and "Gelb" varieties of S.rugoso-annulata, Batazs and Szabo (1978) obtained a temperature optimum of 25°C. At 0°C development stopped and the mycelium died at -5°C. At the upper temperature limit development stopped at 35°C and the mycelium died at 40°C.

(b) pH:

Szudyga (1978) in the same experiment recommended pH 5.7 - 6.0 as the optimum. It should be noted however, that he used a natural substrate and not a liquid defined medium for his work.

Earlier Grainger (1946) said that Stropharia semiglobata, a closely related species, preferred a pH range of 4.0 - 6.0.

1.3. CELLULOSE, LIGNIN AND STARCH DECOMPOSITION.

Cellulose and lignin are the most common carbon sources for saprophytic fungi (Fries, 1955). In general, about 33% of the organic matter produced by green plants is cellulose. Mature wood tissues contain 40 - 60% of cellulose, 10 - 30% hemicellulose and 20 - 30% lignin; and cereal straw contains 40% cellulose (Hudson, 1972)

1.3.1. A.bisporus

Waksman et al (1932) showed that the cultivated mushroom used up lignins and organic nitrogenous complexes and to a less extent hemicelluloses and cellulose.

Treschow (1944) reviewed extensive literature on carbon nutrition of the cultivated mushroom including the works of Waksman, et al, (1931 & 1932). He found it reasonable to assume that Agaricus bisporus sought its carbon nutrition in xylan which was decomposed into xylose preferred by this fungus to the hexoses. It did not surprise him

therefore that wheat straw particularly rich in hemicelluloses (30 - 35% according to Bohus, 1959; and 36% according to Lynch, 1979) was considered to be the best substrate for mushroom growing. He, however, differed with Waksman's view that the purpose of composting was to accumulate lignin; Treschow argued that if this were so, then the period of composting should be longer. On the contrary the rapid composting methods guaranteed a greater amount of hemicelluloses and therefore higher yield. Concluding his work Treschow said that hemicelluloses (in particular xylan), cellulose and lignin were utilised by A. bisporus in that sequence.

Bohus (1959) agreed with Treschow.

Hayes (1978) citing Gerrits, et al (1965) and Turner (1977) disagreed with the above sequence of degradation and stated that lignin was mostly utilised during the vegetative phase, while cellulose and pentosan became the major carbon sources in the fruit-body stage.

O'Neil (1956) concluded from his work that though A. bisporus might utilise cellulose in nature, it apparently would not do so in submerged culture during the normal period of propagation.

1.3.2. Coprinus comatus

Fries (1955) tested the ability of C. comatus to decompose cellulose using Norkrans' method in which he measured the diameters of "clear zones" produced by the digestion of cellulose by cellulolytic enzymes in the test fungi. She found that C. comatus did not decompose cellulose.

She also tested the ability of this fungus to degrade lignin, employing Bavendamm's test and showed that C. comatus could

break down lignin.

Starch did not seem to be utilised to any extent in Fries' experiments.

1.3.3. Lepista nuda & Lepista saeva

According to Norkrans' experiments (Norkrans 1950) Lepista nuda was found to be a cellulose and lignin decomposer. He also discovered that this fungus very readily metabolised starch.

Reusser et al (1958 b) observed that L.nuda was able to breakdown cellulose, though slowly.

The work of Wright (1976) showed that L.nuda's cellulolytic ability was considerably weaker than that of L.saeva which decomposed cellulose to a great extent. After his Bavendamm's test with both gallic and tannic acids he noted that the oxidase activity of L.nuda was quite strong. L.saeva was also a lignin degrader, less so, however, than L.nuda. L.saeva, he observed, also utilised starch.

1.3.4. Morchella species

In his physiological work on carbon nutrition Kaul (unpubl.) observed that starch was the best carbon source for Morchella conica and M.esculenta. This was in accordance with the results of Brock (1951) In a previous experiment Brock obtained the best growth of M.esculenta on starch. Since it was a well known fact that starch contained a lot of impurities which might include some growth-promoting substances, Brock repeated his experiment with purified soluble starch and found that there was no decrease in growth as compared with the untreated starch.

There are no records of cellulose or lignin decomposition

by Morchella species.

1.3.5. Pleurotus sajor-caju.

Several authors have grown this mushroom on many different substrates including paddy straw, wheat straw, commercial mushroom compost and banana pseudostem (Jandaik, 1974; Jandaik & Kapoor, 1974; Rangaswami et al, 1975) but of all these substrates, banana pseudostem, gave not only the greatest yield but also the most rapid crops. Jandaik & Kapoor (1974) attributed this result to the presence of starch. Banana pseudostem contains 5% starch which is deficient in paddy straw (Jandaik & Kapoor, 1974). In their preliminary investigations on carbon nutrition in pure culture they observed the best yield on starch.

Zadražil (1979) described Pleurotus sajor-caju as a very good colonizer of straw with a high decomposition rate of the substrate. Since its ligno-cellulose decomposition is quite high, he argued that this mushroom would be useful in the production of animal feed by increasing the digestibility of the substrate.

1.3.6. Pluteus cervinus

Lloyd (1974) reported that in his work with this fungus cellulose and sucrose supported the most rapid growth, though with thin and sparse mycelium on the agar medium. This was followed by starch. However, he admitted that his work had "limited usefulness" as he employed agar media rather than defined liquid medium.

From his Bavendamm's test, he inferred that Pluteus cervinus degraded lignin and thus was a white-rot fungus, able to decompose both lignin and cellulose.

1.3.7. Stropharia rugoso-annulata.

Zadražil (1980) carried out a detailed experiment using Stropharia rugoso-annulata and found that this fungus utilised 46.5% of the total lignin content of beech sawdust (Fagus silvatica L.) and 37.5% of the total lignin content of wheat straw substrate after 60 days of fermentation. When he supplemented the substrates with ammonium nitrate up to 56.7% of the lignin in the saw-dust was utilised during the same period. The presence of lignin impedes the digestion of cellulose by rumen micro-organisms (Pigden & Heaney 1969 cited by Zadražil 1980). He therefore reasoned that solid-state fermentation of beech saw-dust and wheat straw by Stropharia rugoso-annulata increased the digestibility of the substrate, making the spent material useful as feed for ruminants.

1.4. NITROGEN SOURCES

Treschow (1944) reviewed the literature on nitrogen nutrition to show that for most fungi organic sources were preferred to inorganic ones. Ammonium nitrogen was utilised more than other inorganic sources. Of the sources he tried asparagine gave the best results. Optimum requirement for A. bisporus was found to 0.0375mol (=0.105%N), concentrations up to 0.375mol gave considerable growth.

Working with P. ostreatus, Hashimoto and Takahashi (1974) also observed that organic nitrogen sources gave better yield than inorganic ones. Eger (1974) found that asparagine was the best of all the sources of nitrogen she tried for Pleurotus ostreatus.

For all his isolates of Lepista nuda and L. saeva, Wright (1976) obtained the greatest yield when he used casein hydrolysate as a nitrogen source. Asparagine, however, was the best single source of nitrogen for most of his isolates.

1.5. CARBON : NITROGEN RATIO AND NITROGEN FIXATION.

At 0.1% asparagine Wright (1976) determined the best C : N ratio for L.nuda to be 21 : 1. At higher nitrogen level, 0.5592% N (ie 0.3% asparagine) 21 : 1 was also optimal for L.nuda up to 3 weeks of incubation.

At 4 weeks of incubation the best C : N ratio for L.saeva was also 21 : 1. Overall, the L. saeva isolates demonstrated an ability to grow at sustained high rate in media becoming increasing deficient in carbon and nitrogen, including its ability to reduce its mycelial content of nitrogen below 5%.

He however, recommended a compromise nitrogen level of 0.3728% (ie 0.2% asparagine) at a ratio of 22 : 1, since at 0.5592% both nitrogen and carbon were in excess and drastic shifts in pH were difficult to control, and at 0.1864% N, carbon limitation occurred with also some limitation in nitrogen.

Giovannozzi-Sermanni et al (1979) after studying the biochemical changes occurring in the compost during growth and reproduction of Pleurotus ostreatus and A.bisporus said that C : N ratio of P.ostreatus compost remained constant indicating a low requirement of exogenous nitrogen, due probably to its capability to fix atmospheric nitrogen.

Pleurotus sajor-caju has also been reported to fix atmospheric nitrogen by Rangaswami et al (1975). When they grew it in a nitrogen-free synthetic medium they obtained good mycelial yield. After cultivating the fruit-bodies on a natural substrate and comparing the nitrogen content of the substrate at the beginning and at the end of the experiment and also in the sporophores they discovered that the nitrogen content of the spent substrate and the sporophores was

greater than that of the initial substrate.

A number of other workers have also claimed nitrogen fixation by higher fungi especially Pleurotus species. Kurtzman Jr. (1979) attempted to settle this controversial argument but could not reach any categorical solution. However, he was sure that the Pleurotus species he used required almost no supplementary nitrogen in its substrate.

Optimum C : N ratio for A. bisporus was determined to be 17 : 1 (Smith and Hayes, 1972).

1.6. REQUIREMENTS FOR VITAMINS

In the great majority of higher fungi, the capacity for synthesis of thiamine has been lost completely or partially (Treschow, 1944). In a minority of them the ability to synthesize biotin as well as thiamine has been lost. A few species have lost the capacity to synthesize thiamine, biotin and inositol, whereas only one species has been found to have lost the capacity for synthesis of only biotin, i.e. Marasmius androsaceus.

Treschow (1944) tried out various vitamins and found that either thiamine or biotin was required by A. bisporus to grow on a synthetic medium of sugar, asparagine and mineral salts. In the absence of both of these vitamins A. bisporus could not assimilate the nutrients present. One or both of thiamine and biotin, he suggested, was present in the composted manure.

Lilly and Barnett (1951) stated that only a few basidiomycetes have been reported to be able to synthesize thiamine. Almost all the fungi in the order Agaricales investigated required thiamine, replaceable for most by the pyrimidine moity. This is the

nearest approach to a correlation of a taxonomic group with a vitamin requirement (Cochrane, 1958). Naturally these fungi are found inhabiting wood, litter or habitats likely to contain thiamine.

Wright (1976) found that his isolates of L.nuda, L.saeva and Calocybe gambosum all exhibited a definite requirement for thiamine. He also recorded a partial requirement for biotin and possibly pyridoxine and folic acid for these species. Calocybe gambosum appeared to be suppressed by choline chloride and inositol in his experiments. This might possibly be due to the high concentrations used.

Volz (1972) claimed that for hyphal growth L.nuda and P.ostreatus showed no apparent requirement for most of the vitamins he used including thiamine, biotin and inositol. According to his report, Cantherellus cibarius appeared to be the only fungus he tried that required vitamins for mycelial growth.

Thiamine has been found to be required by Pleurotus ostreatus (Block, et al 1959) and Pleurotus sajor-caju (Jandaik et al 1975)

1.7. INFLUENCE OF MINERAL ELEMENTS

Foster (1939) did a thorough study of trace element nutrition of fungi. From his work he concluded that the need for trace amounts of various heavy metals such as zinc, iron, manganese, copper and possibly others, was a phenomenon widespread among fungi. He reviewed extensive literature on this subject and carried out his own detailed experiments after which he enumerated the possible functions of the various metals and their optimum concentrations.

Treschow (1944) showed that calcium was indispensable for A.bisporus, very small amounts having extraordinarily strong

physiological effect in increasing the dry matter weight of mycelium. He concluded that calcium antagonised potassium and magnesium. Phosphorus and iron were also shown to be required.

Fries (1956) recorded iron, zinc and calcium as essential requirements for Coprinus species.

Cochrane (1958) listed the macro-nutrients as potassium, phosphorus, sulphur and magnesium. These are essential, but equally essential are at least five micro-nutrients known so far to be iron, zinc, copper, manganese and molybdenum. Calcium requirement, where specific, is to be classified with the micro-nutrients.

Hayes (1972) recorded that iron stimulated fruiting of A. bisporus and suggested that the stimulatory effect of Pseudomonas putida and other micro-organisms in fruiting was possibly due to their ability to release iron. Manganese was shown to produce inhibitory effect

Jandaik (1976) showed iron and zinc to be essential for P. sajor-caju, among the heavy metals he tried.

Wright (1976) observed that the trace elements he used in his work did not significantly increase mycelial yield of L. nuda and L. saeva singly. However, when zinc and calcium were added together yield increased to a level almost equal to the yield harvested from the complete mineral addition. He suggested that the effect of calcium was that of removing the effects of high concentrations of metals antagonistic to the growth of the fungi or counteracting the effects of inhibitory products of metabolism, eg. some Krebs cycle organic acids.

The ashes of yeast extract, malt extract and manure had

growth-promoting effects.

From all evidence it seems that the essentiality of mineral elements is certain but the mechanism of their role in metabolism is still uncertain.

1.8. EFFECT OF FATTY ACIDS ON GROWTH

Wardle & Schisler (1969) obtained significant increases in the yield of A. bisporus when they supplemented their basal medium with various vegetable oils. They repeated this experiment with a mineral oil (a hydrocarbon exhibiting similar physical properties) but failed to achieve the same effect. They therefore ruled out attributing the increased growth yield to the physical characteristics of the lipids. After determining the carbon content of the oils and comparing it with the carbon content of mycelium, they found that the increased yield effect was not due to the carbon content of the oils. After further experiments they suggested that the increase was due to oleic and linoleic acids in the lipids.

Wright (1976) did not obtain any significant growth promotion with his medium supplements except oleic acid, linoleic acid, stearic acid and palmitic acid. There was some tissue development of L. saeva mycelium when supplemented with oleic and linoleic acids.

Kurtzman Jr. (1976) obtained a four-fold increase in the growth of Pleurotus sapidus upon addition of corn oil in soya soluble liquid medium. He also showed that all of the hydrolysis products of corn oil (fatty acids, glycerol and unsaponifiables) were all inhibiting. Certain esters did promote growth (especially esters of long chains). He attributed the promotory effect to either ester bonds or some physical characteristic.

It seems certain that lipids do play a part in the

metabolism in fungi, but it also seems that the mechanism is still unknown.

1.9. OXYGEN AND CARBON DIOXIDE TENSION AND GROWTH.

Griffin and Nair (1968) grew Scelerotium rolfsii in different atmospheres whose composition was controlled. The mycelial growth rate was constant within a range of oxygen concentrations from 3 - 21% but diminished steadily as CO_2 concentration increased from 0.03%. Sclerotium germination was also reduced by O_2 concentration less than 6% and by CO_2 concentrations greater than 10%. No sclerotium was formed if the concentration of O_2 fell below 15% or CO_2 passed 4%.

Wells and Uota (1970) determined the effects of low oxygen and high carbon dioxide concentrations on the growth and germination of 5 fungi :- Alternaria tenuis, Fusarium roseum, Botrytis cinera, Cladosporium herbarium and Rhizopus stolonifer. Mycelial growth of all these fungi linearly decreased with O_2 concentration below 4%. At 0% of O_2 there was some significant growth of R. stolonifer but not of the others. Higher than normal concentrations of CO_2 greatly inhibited mycelial growth when O_2 level was 21% except for F. roseum whose growth was stimulated at 10% CO_2. But when O_2 became limiting, concentrations of CO_2 higher than normal caused an increase in the growth responses of all the fungi tested except R. stolonifer; and increased germination of all except A. tenuis.

Cochrane (1958) attributed CO_2 stimulation of growth to CO_2 fixation.

Macauley & Griffin (1969) showed that in both soil and a liquid medium, with increasing level of CO_2, the activity of some soil fungi was suppressed in an alkaline medium to a greater extent than

in acid conditions. This they attributed to high bicarbonate formation in alkaline conditions.

Zadražil's work (Zadražil,1974) showed a markedly high stimulation of growth of Pleurotus species (P. ostreatus, P. florida and P. eryngii); P. ostreatus and P. florida by CO_2 up to the level of 28% while P. eryngii was stimulated to the concentration of 22%. At 37% CO_2 suppression of growth occurred by 40% as compared to growth at 0.03% CO_2.

This contrasts sharply with many of the fungi as shown above and with A. bisporus. A. bisporus growth is adversely affected by raising the concentration of CO_2. At 32% CO_2 growth is halted (Tschierpe, 1959). Many other basidiomycetes behave like A. bisporus (Zadrazil 1974). Inhibition of A. bisporus growth starts at 15% CO_2 (Tschierpe 1959). Nair (1972) even showed significant inhibition at 6.6% CO_2.

San Anthonio and Thomas indicated a stimulatory effect of CO_2 on A. bisporus at 0.3 - 0.6%. (San Anthonio and Thomas,1972)

Wright (1976) observed very low growth of L. nuda and L. saeva from 0 - 2% oxygen from where there was increase up to 5% O_2. Between 5 - 21% the growth rate remained more or less steady. CO_2 exhibited inhibitory effects on the growth of these two fungi even at 0.35% CO_2 in one isolate and 1% in others.

It is suggested that in the fungi where CO_2 can be fixed, CO_2 enters the lactic, fumanic, citric and other acids of the Krebs cycle which are later utilised for energy and growth (Cochrane, 1958).

The high tolerance of CO_2 by Pleurotus species has another advantage in their cultivation in that it serves as a shield for these species against other micro-organisms, which either cannot

grow or die off at higher CO_2 concentrations.

The influence of CO_2 in primordium formation will be reviewed in a later part of this section.

1.10. FRUIT-BODY PRODUCTION

In the fungi which form fleshy fruit-bodies, the formation of fruit-body initials is the visible start of sexual reproduction. The mechanism of this initiation of primordia and their subsequent development into sporophores have been the subject of numerous researches.

For reproduction to take place all the genetic and environmental (including nutritional) factors must be favourable (Cochrane, 1958). It is generally assumed that factors favouring profuse mycelial growth tend to suppress reproduction (Lilly & Barnett, 1951; Cochrane, 1958).

A lot of weight is placed on the role of nutritional factors, for, one of the important functions of the vegetative phase of growth is to build up protoplasm and store energy for reproduction. In most fungi exhaustion of nutrients by vigorous mycelium favours reproduction. Fruiting structures of different fungi have specific requirements for nutrient concentration. But generally disaccharides and polysaccharides support more fruiting than simple hexose sugars (Cochrane, 1958; Hashimoto and Takahashi, 1974). Wood (1976) also suggested that sugars like hexoses, xylose and cellobiose completely inhibited primordium formation in his experiments. Giovannozzi-Sermanni, et al (1979) agreeing with him, concluded that the production and role of oligosaccharides and monosaccharides, and the inhibition of laccase could suggest that, production of mushrooms was dependent on

inhibition of metabolism and not lack of nutrients.

Adequate concentration of nitrogen sources in the substrate is also very important for fructification, but if the optimal value is exceeded suppression of fruiting results (Cochrane 1958). Hashimoto and Takahashi (1974) found that P.ostreatus preferred organic sources of nitrogen to inorganic for fructification. In Eger's work (Eger, 1974) asparagine was superior to the other nitrogen sources she used in inducing primodium initiation. Zadrazil (1980) achieved a 50% increase and 300% increase in yield of Pleurotus sajor-caju when he supplemented wheat straw with ammonium nitrate and soya-bean respectively. The nitrogen content of the fruit-bodies also increased. Netzer (1979) obtained more primordium initiation with glutamine than with other metabolically closely related nitrogen sources :- asparagine, aspartic acid and glutamic acid.

In Psilocybe panaeoliformis the ratio of carbon to nitrogen must be right if fruit-body production is to be maximized. Using this fungus Urayama (1967) stressed the importance of C : N ratio in pinhead formation, stating that excess carbon prevented pinhead formation due to the production of acids and in excess nitrogen pinhead formation was prevented due to the production of an inhibitor. (Smith and Hayes, 1972) showed that there was a substantial decrease in yield if C : N ratio at the optimal level of 17 : 1 was not maintained for A.bisporus. The above is also true for V.volvacea which has an optimal C : N ratio of 75 : 1 (Chang-Ho & Ho, 1979).

Lipids have also been suggested as playing a role in fruit-body formation. Schisler (1967) reported increased yield of fruit-bodies when vegetable oils were applied to the substrates, and therefore suggested a relationship between lipid matabolism and initiation of

fruiting in A. bisporus. He saw no correlation between stimulation of fruiting and specific fatty acids but suggested that sterols were involved. Hayes (1972) suggested that "acetate building blocks" synthesized from probably fats and oils by micro-organisms played a major role in the fruiting of A. bisporus.

Vitamins which are available naturally in compost materials or synthesized by the active micro-organisms in the compost are also important for primordium formation (Hayes, 1972). The vitamins may accelerate sugar utilization and so hasten the onset of reproduction. Hormones and other unidentified growth factors have also been connected with fructification (Giovannozzi-Sermanni, et al, 1979)

It is now widely recognised that micro-organisms play a significant part in the formation of fruit-body primordia. Urayama (1961) achieved greater fruit-body production of Psilocybe panaeoliformis and A. bisporus as well as earlier initiation of their primordia by spraying his cultures with a suspension of the bacterium, Bacillus psilocybe. Coprinus macrorrhinus and Stropharia species did not form any primordia at all in the absence of bacteria. As mentioned earlier Urayama (1967) emphasized the importance of C : N ratio in fruiting. He suggested that the role of bacteria in primordium formation was to produce an anti-inhibitor that could counteract the effect of the inhibitor(s) produced as a result of excess nitrogen in the substrate.

O'Donoghue (1962) attributed fruiting to the activity of actinomycetes. Eger (1963) demonstrated the important role of micro-flora in the casing layer in the initiation of fruit-bodies. The work of Hayes et al (1969) confirmed Eger's observation and went further to identify the active bacteria as Pseudomonas putida and its

close relatives. Hume and Hayes (1972) using Petri-plates showed that by inoculating Ps.putida into pure cultures of A.bisporus much more primordia were formed than without it and therefore concluded that Pseudomonas bacteria played a major role in primordium induction.

Thomas et al (1964) had earlier supported the view that bacterial activity was responsible for fruit-body formation instead of carbon-dioxide gradient. Park and Agnihotri (1969) found that sporophore initiation was triggered off by metabolites of soil-inhabiting bacteria, particularly the nodule-forming isolates. Giovannozzi-Sermanni's explanation (Giovannozzi-Sermanni,1979) was that the inhibition of primordium formation by excess sugars produced in the compost could be removed via metabolization by bacteria.

The role of carbon dioxide concentration in fruiting of mushrooms has also been much studied. Lambert (1933) found that CO_2 accumulation inhibited fruit-body formation. In fungi generally sporulation is reduced in closed containers and submerged mycelium fails to develop spores (Cochrane, 1958). This is usually attributed to oxygen deficiency, but he suggested that accumulation of carbon dioxide was probably the critical factor. Tschierpe (1959) suggested that a gradient of CO_2 existed in the casing layer between the high concentration in the spawn-run compost and the low concentration in the atmosphere of the growing room; and that primordium initiation was a response to this gradient. Tschierpe and Sinden (1964) confirmed this suggestion and went further to give 0.03 - 0.1% as the optimal concentration of CO_2 for fruit-body formation. But Thomas, et al (1964) disagreed with the above view and laid more weight to the role of micro-flora. The work of Long and Jacob (1969) showed that CO_2 concentrations 340 - 1000 ppm played a role not only in fruit-body initiation but also in its development. Nair and Hayes (1974) adopted a new approach to explain

the role of CO_2 in the initiation of primordia via bicarbonate ion formation. Nair et al (1974) again stressed this view, suggesting that aeration of the casing layer brought about a decrease in bicarbonate ions, and an increase in the number of Pseudomonas cells which played a major role in the initiation of primordia. There was therefore a correlation in their works between the microbiological and aeration effects in fruit-body formation, hitherto treated separately by previous workers. Though CO_2 concentrations as high as 28% stimulates the vegetative growth of Pleurotus ostreatus and P.florida, in the reproductive stage this concentration has to be reduced by aeration to promote fruiting (Zadrazil, 1974).

Of other physical factors affecting the production of fruit-bodies temperature, pH and humidity are probably the most important and apply in all mushrooms.

The temperature range permitting reproduction is usually narrower than that permitting vegetative growth (Cochrane, 1958). Many mushrooms require lowering of temperature (temperature shock) to induce them to fruit. It appears that this immediate stimulus is a check on vegetative growth and induction of fruiting. Indeed mushrooms can be classified on the basis of whether or not they require temperature shock to fruit. The former include typically A.bisporus, Coprinus comatus, Flammulina velutipes, Kuehneromyces mutabilis, Lepista nuda, Pleurotus ostreatus and Stropharia rugoso-annulata. These do not usually fruit at temperatures above 18°C. A few examples of those which fruit at warmer conditions are :- Agaricus bitorquis (Vedder, 1975), Agrocybe aegerita, Lentinus edodes, many species of Pleurotus including P.sajor-caju (Jandaik, 1974) but excluding P.ostreatus, and Volvariella volvacea (Zadražil, 1979). The cultivation of the latter group should be highly encouraged in the developing tropical and sub-tropical countries.

Humidity and pH are also of vital importance. For most cultivated mushrooms a relative humidity of 80 - 95% is usually recommended (Flegg & Gandy, 1963; Chang, 1972). Below this range fruit-body formation is either delayed or prevented and above it there is too much condensation of water which is also detrimental to yield.

The pH at the end of composting should be 7.0 - 7.5 for A. bisporus (Hayes, 1978). This pH range should also be maintained in the casing layer. Szudyga (1978) recommended pH 5.7 - 6.0 as the optimal range for Stropharia rugoso-annulata casing soil. He revealed a drastic fall in yield where this range was not maintained. Kovacsne (1979) used casing soils of pH 7.2 and above. But he did not compare these with Szudyga's values. As colonization of the substrate progresses there is accummulation of metabolic products which lower the pH of the substrate. Therefore the choice of casing materials should be influenced by their buffer capacity necessary to stabilize pH and so maintain a reasonable level of productivity.

A mixture of Sphagnum peat and limestone of pH 7.0 - 7.5 has been traditionally used for A. bisporus cultivation. The same formulation has also been adopted for A. bitorquis. For S. rugoso-annulata a mixture of humus soil and peat is used (Szudyga, 1978). He also advised strongly against the addition of limestone. But Kovacsne (1979) preferred mixtures of peat, limestone with or without sawdust.

Good casing material should have high water-holding capacity, allow good gaseous and ion exchange, capable of supporting the beneficial microbes while at the same time free of known mushroom pathogens. Peat satisfies these conditions (Hayes, 1978), and has been mainly used in the U.K. and other countries. In India the use of decomposed farmyard manure has been encouraged (Hayes & Shandilya, 1978).

A new synthetic casing material has been developed from paper and pulp mill by-products by Yeo and Hayes (Yeo & Hayes, 1979). This is said to have the same effects as peat and equal to it in yield. Stoller's quinone theory showed that quinones resulting from the activity of laccases were the real inhibitors of fruiting. This postulate led him to formulate a new synthetic casing medium which has been said to be very poor in nutrients to discourage laccase production while retaining the qualities found in peat (Stoller, 1979).

In many basidiomycetes light plays a significant role in the fruiting process. Alasoadura (1963) found that Sphaerobolus stellatus required light not only for initiation of primordia but also for development of sporophores. Using the same fungus Ingold and Nawaz (1967) confirmed the above observation. At 20°C the fungus could not fructify in the absence of white light above 10 lux and as development continued light requirement gradually fell. Lu (1965) discovered that Cyathus stercoreus at 25°C needed approximately 17,200 ft-candle-hours to form fruit-bodies. Miller (1967) demonstrated light requirement both for primordium formation and sporophore development in Panus fragilis. Coprinus domesticus, according to Chapman and Fergus (1973) was unable to form primordia and basidiocarps in the dark but could do so in continuous light. The same observation was made for Coprinus lagopus (Madelin,(1956); Lu,(1974)). Lu found that exposure of cultures to light for as short as $\frac{1}{2}$minute (or less than 1ft-c-h) was sufficient. For A. bisporus, Lu said that light was not needed. The same observation was made by Treschow (1944) for A. bisporus. Light requirement has also been demonstrated for Pleurotus ostreatus (Eger, 1974; Zadražil, 1974; Cailleux & Diop. 1974). Eger showed that fruiting of Pleurotus ostreatus depended much on both the quality and quantity of light. The requirement of light for fruiting has also been demonstrated for

Volvariella volvacea (Chang 1972). Laccaria laccata has also been shown to require light for initiation and development of fertile sporophores (Davis & Jong, 1976). They observed that alternating periods of light and dark were necessary for normal development.

It is said that light acts by suppressing vegetative growth (Eger, 1974) and the stimulus produces the switch from vegetative stage to reproduction, though the mechanism of this "switch" is not yet known.

SECTION 2

MATERIALS AND METHODS.

2.1 LIST OF FUNGAL SPECIES INVESTIGATED AND SOURCES OF ISOLATION.

(With synonyms)

1. Agaricus arvensis Schaeff. ex Secr.

   Psalliota arvensis (Schaeff. ex Secr.) Kummer

   Edge of mixed woodland, Coleshill Pool, Birmingham.

2. Agaricus bisporus (Lang) Sing.

   Psalliota hortensis var bispora. Lange.

   Supplied by Darlington-Darmisel Company, Sussex.

3. Agaricus bitorquis (Quel.) Sacc.

   Agaricus edulis (Vitt.) Moller & Schaeff.

   Agaricus rodmanii Peck.

   Psalliota rodmanii (Peck.) Kaffin.

   Psalliota subedulis Heinemann.

   Supplied by Darlington-Darmisel Company, Sussex.

4. Agrocybe aggregatum

   Pholiota aggregata Beeli

   Italy

5. Coprinus comatus (Mull. ex Fr.) Gray

   Coprinus ovatus (Schaeff. ex Fr.) Fr.

   Grass lawn, University of Aston, Gosta Green, Birmingham.

6. Lepista nuda (Bull. ex Fr.) Cooke

   Tricholoma nudum (Bull. ex Fr.) Kummer

   Rhodopaxillus nudus (Bull. ex Fr.) Maire

   Tricholoma personatum (Fr. ex Fr.) Kummer

   An old flower bed in Sheffield.

7. Lepista saeva (Fr.) Orton

   Agaricus personatus Fr.

   Tricholoma saevum (Fr.) Gillet

   Rhodopaxillus saevus (Fr.) Maire

Tricholoma personatum var anserinum (Fr. ex Fr.) Bres.

Lepista personata (Fr. ex Fr.) Cook

Rhodopaxillus personatus (Fr. ex Fr.) Sing.

Pasture in Burton-on-Trent.

8. Morchella conica Pers. ex Fr.

Brandon Wood, Coventry.

9. Morchella vulgaris

India.

10. Phallus impudicus (Linn.) Pers.

Beechwood (among litter and mosses) Sutton Park, Birmingham.

11. Pleurotus sajor-caju (Fr.) Sing.

Lentinus sajor-caju Fr.

India

12. Pluteus cervinus (Schaeff. ex Fr.) Kummer

Pluteus curtisii (Berk. & Br.) Sacc.

Decaying tree stump in Warwickshire.

13. Stropharia rugoso-annulata Farlow apud Murrill

Stropharia imaina Bendix

Nematoloma ferii (Bres.) Sing.

Stropharia ferii Bres.

West Germany.

2.2. CULTURE MAINTENANCE.

Cultures were maintained in a 10 ml 2% malt extract agar slopes in universal bottles at 5°C. These were subcultured every three months.

2.3. PETRI-PLATE STUDIES.

(a) SINGLE-VENT PETRI-PLATES :

8.5 cm sterile disposable polystyrene Petri-plates were used. Except where otherwise stated, 20 ml of an agar medium was used in each Petri-plate.

All inoculations were carried out under sterile conditions. Mycelial agar discs, 6 mm in diameter were employed as inocula. These were taken from the peripheral zone of young colonies where the cells were most active to ensure rapid establishment on the new substrate. The plates were kept in incubators maintained at appropriate temperatures or in other places satisfying specific experimental conditions. Period of incubation varied with the fungus involved but such experiments were terminated when the mycelium was about to touch the vertical edge of the plate, unless otherwise stated.

(b) DIVIDED PETRI-PLATES :

The method adopted was that of Hume & Hayes (1972). Sterile disposable polystyrene Petri-plates each with a single central vertical partition were used. The vegetative medium was 2% malt extract agar while the reproductive medium was either 2% water agar or 2% agar with various other substances as required by the particular experiment.

Both the vegetative and reproductive media were added in volumes of 20 ml per compartment.

The malt extract agar was introduced into the first half, inoculated and incubated at 25°C until it had been completely covered by the mycelium. Then the reproductive medium was introduced into the second half under sterile conditions. The plates were then transferred into a room where the temperature was maintained at 14° - 18°C. This phase of incubation lasted up to 2 months.

2.4. LIQUID CULTURES :

100 ml or 150 ml pyrex Erlenmeyer flasks were employed. These contained 30 - 50 mls of the liquid medium as indicated and stoppered with non-absorbent cotton wool.

In most of the experiments involving liquid media, except where otherwise stated, the basal medium used was that employed by Fries (1956) for Coprinus species and was composed of as follows :

Glucose	10.0 g
Asparagine	1.0 g
KH_2PO_4	0.35 g
K_2HPO_4	0.15 g
$Mg\ SO_4.7H_2O$	0.50 g
$Ca\ SO_4.2H_2O$	0.10 g
$FeCl_3$	1.0 mg
$ZnSO_4$	1.0 mg
NaCl	0.5 g
Thiamine	50.0 µg
Distilled water	400.0 ml

Buffer solutions used were Sorensen's phosphate buffer, using appropriate volume combinations of $\frac{M}{15}$ KH_2PO_4, $\frac{M}{15}$ Na_2HPO_4, $\frac{M}{15}$ K_3PO_4 and $\frac{N}{10}$ HCl according to the table of Fries (1956) who recommended it because of the following :-

(a) It is quite effective.

(b) It covers the main part of the pH range within which most fungi can grow.

(c) The phosphates are not toxic in the concentrations used.

(d) The phosphates take part in metabolism in small quantities.

The basal medium and the buffer solutions were separately autoclaved at 1 Kg / cm^2 and mixed aseptically when cool at the ratio of 3 volumes of the buffer solution : 2 volumes of the basal medium to give 30 ml or 50 ml of buffered standard solution. pH was measured after autoclaving.

Inoculations were done as described earlier, with the following modification :- Six-millimetre inocula were placed on fresh malt extract agar plates or water agar as appropriate and incubated for 24 - 48 hours at 25° or 30°C before being transferred into the flasks. This process facilitated buoyancy (Norkrans, 1950) necessary for better aeration and also helped to minimise the initial lag period in the stationary culture. Four or five replicates were employed.

Time of incubation varied according to the growth rate of the fungus investigated. At the end of the incubation time the mycelial mats were each drained over a 7 cm diameter filter paper in a Buchner funnel and flask connected to a suction pump. The filter paper discs were previously dried to constant weight. The mycelial mats were then washed with deionized water, dried to constant weight at 80°C in an oven and weighed on a Mettler balance to the nearest

tenth of a milligram. The culture filtrates from all the replicate flasks for each treatment were pooled together and the final mean pH measured.

2.5. CELLULOLYTIC ACTIVITY :

The method employed was that of Reutella and Cowling (1966) using cellulose columns since it was found (from earlier trials) to give more distinct results than the Petri-plate technique. It was more convenient to use 72-hour ball-milled cellulose medium of Eggins and Pugh (1962) than the method adopted by Reutella and Cowling.

Composition of Eggins & Pugh cellulose agar :

L-asparagine	0.5 g
$(NH_4)_2SO_4$	0.5 g
KH_2PO_4	1.0 g
KCl	0.5 g
$MgSO_4.7H_2O$	0.2 g
Agar	20.0 g
Cellulose suspension	250.0 ml

1% cellulose concentration was used.Test tubes with metal caps formed the columns. The cellulose agar medium, after autoclaving at 1 Kg / cm^2 (121°C), was continually agitated and after cooling to about 45°C, was quickly dispensed into sterile test-tubes. These were immediately capped amd plunged into a bucket of ice for instant solidification. This was necessary in order to obtain a homogeneous dispersion of the cellulose particles throughout the resulting cellulose-agar columns.

These columns were inoculated and incubated at appropriate temperatures. Measurement of the cleared (transparent) top zone

under the mycelial mat (clearly visible against the original opaque milk-white cellulose column) was done.

One inherent problem encountered was that of evaporation of water from the columns which caused them to shrink over prolonged incubation, and thus tended to exaggerate the result. However, this problem was overcome by the use of control columns which were given exactly the same treatment as the experimental ones except that they were not inoculated. As the shrinkage was common to all the columns, the mean depth of shrinkage in the controls was subtracted from the average depth of clearing obtained for the experimental tubes. Also the controls served to give a colour contrast to the clearing in the experimental tubes.

2.6. STARCH HYDROLYSIS :

Starch agar was made up of the following ingredients (Kuthubutheen, 1977) :-

Soluble starch	10.0	g
Casein	1.0	g
L-proline	0.5	g
L-asparagine	0.5	g
$MgSO_4 . 7H_2O$	0.5	g
NaCl	0.5	g
$FeSO_4 . 7H_2O$	0.01	g
K_2HPO_4	2.0	g
Agar	15.0	g
Distilled water to	1.0	l

Iodine solution used as an indicator had the following composition :-

Iodine (resublimed)	0.127 g
Potassium iodide (KI)	0.300 g
Distilled water	100.000

Preparation of starch agar was done as described earlier. The plates were incubated at 25°C or 30°C as appropriate. At regular intervals dilute KI / iodine solution was poured into a set of five plates. The zone in which the starch had been digested (usually under and around the mycelial mat) was clear while the rest of the agar still containing starch turned blue-black.

2.7. STUDIES WITH GAS DIFFUSION COLUMN

The technique of Griffin et al (1967) was adopted. The column consisted of a sand-filled brass cylinder 180 cm long and 12.5 cm in diameter with side arms along its length. Both ends had tubes for connection to external sources of gas. At both ends therefore specific gas concentrations could be introduced and as they passed through the sand-filled column maintained a concentration gradient obeying the physical laws of diffusion. The concentration at any point x along its length could therefore be calculated using the formula :-

$$Cx = CM - \frac{x}{l}(CM - CN)$$ at steady state conditions of flow,

where - l is the length of the cylinder and

CM and CN are the individual gas concentrations at the two ends M and N respectively.

Flat-bottomed test-tubes (15cm X 2.5cm) were used as the specimen tubes and the medium employed was composed of :-

2% malt extraxt

2% Agar (for additional rigidity)

Distilled water to 1 litre.

The tubes each containing 20 ml of the above medium were autoclaved at 1kg / cm^2 for 15 minutes and thereafter laid on their sides so that the medium formed a flat layer along their length. The tubes were then inoculated in the centre with 6 mm mycelial discs from malt extract agar colonies and connected to the side arms of the column. Mycelial extension was measured longitudinally at regular intervals of time. Four replicate tubes were attached at each concentration. Temperature fluctuated about $23^\circ \pm 2^\circ C$

Commercial cylinders of oxygen, nitrogen and carbon-dioxide were used. Atmospheric air was also employed. If CO_2 required removal, this was done by passing air through a 10% KOH solution and then through deionized water for washing. Standard gap meters and also calibration by displacement of water were used to regulate the gas volumes flowing into the column.

2.8. VEGETATIVE GROWTH ON NATURAL SOLID SUBSTRATES. :

Various leaf litters, rice and wheat straw, mushroom compost and old newspaper were collected and all (except mushroom compost) were cut up separately into 1 cm pieces. These were soaked overnight except wheat straw which was soaked for 48 hours, in tap water. They were then packed as evenly as possible into glass tubes, 25cm X 3.1cm, and allowed to drain.

They were plugged at both ends with non-absorbent cotton wool and autoclaved at 1kg / cm^2 for 1 hour for three consecutive days. Inoculation was done with 1.8cm agar (mycelial) discs under sterile conditions and kept upright in incubators at $15^\circ C$. Depth of mycelial growth was measured at regular intervals.

2.9. GRAIN SPAWN :

Cereal grains (rye or barley) were washed in a continuous stream of tap water for one hour to remove chaff and dust, and left in a bucket of tap water to soak overnight. After draining the grains were distributed into one-litre wide-mouthed pyrex flasks up to half-a-litre level and autoclaved at 1kg / cm^2 for 1 hour for 3 consecutive days. The final moisture level was about 50 - 55% and pH was 5 - 6.

The flasks were then inoculated with ten 6mm mycelial discs taken from malt extract agar colonies and incubated at 25°- 30°C. Shaking of the flasks at regular intervals of 3 - 4 days was found necessary to disperse colonized grains and obtain a homogeneous spawn. without much tissue formation.

2.10. CULTIVATION IN CABINETS :

Clean dry straw (wheat or rice) was cut up into pieces less than 10 cm long and soaked for 48 hours (wheat straw) or 24 hours (rice straw) in a barrel of water. The water was then poured away and the wet straw packed into 18.5cm x 18.5cm x 14cm or 13cm x 13cm x 12cm polypropylene pots with holes at the base, and allowed to drain until straw squeezed in the hand, allowed drops of water to form between the fingers (Szudyga, 1978). The water content of straw so treated was 80 - 85%. Usually 500g or 1kg of the prepared substrate per pot was used (in small and big pots respectively).

These pots were covered with aluminium foil and autoclaved at 1kg / cm^2 for one hour for 3 days consecutively. Inoculation was aseptically done with grain spawn and incubation was achieved in specially constructed cabinets.

Each cabinet had a rectangular tray filled with sand on which a wooden frame was kept to support the cultivation pots. The tray was covered with a rectangular perspex hood 125cm x 65cm x 63cm. Both the perspex structure and the sand-filled tray were fitted with electrical heating cables with thermostats which heated the air and the sand respectively to constant (desired) temperature. The sand was watered as needed to maintain the required humidity of 85 - 90%.

The aluminium foil covering the pots, apart from reducing contamination of the cultures also helped to maintain the right water balance and also trap CO_2 which "helped" to stimulate vegetative growth (Zadražil, 1974). These coverings were removed after seven days (for Pleurotus sajor-caju) or 14 - 21 days (for Stropharia rugoso annulata), when the mycelium had completely permeated the substrate.

In the cultivation of S.rugoso-annulata, the substrate was then covered with various casing formulations to a thickness of 2cm, and maintained at 25°C, until the mycelium had grown into the casing layer. The temperature of the cabinet was then adjusted to 16°- 18°C and ventilation with pre-cooled air commenced. Relative humidity would then fall to 75 - 80%.

5-10% of grain spawn was usually used as inoculum.

2.11. CULTIVATION IN FLASKS (For S. rugoso-annulata)

228g of wheat straw was prepared as described earlier and introduced into each of 16 wide-mouthed one-litre flasks. These were plugged with non-absorbent cotton wool, covered with aluminium foil (to prevent excessive soaking of the cotton wool) and autoclaved as above for 3 days. On cooling the aluminium foil was removed and

Plate 2.1 The apparatus used in the experiment to demonstrate the necessity of micro-flora in the casing layer, for fruit-body formation of S.rugoso-annulata .

the flasks after inoculation under aseptic conditions with five 1.8cm mycelial discs, were incubated at 25°+ 1° C for 4 weeks until the substrate was thoroughly permeated by the mycelium.

Thereafter, the experiment was set up as shown in Plate 2.1. The flasks were connected by previously sterilized glass tubes and rubber connecting tubes to sterile water contained in another one-litre flask. This in turn was connected to sterile water in a 5-litre conical flask to which was attached an air-filter. The whole apparatus therefore enabled air from the compressor to reach the culture flasks as humid sterile air.

Under aseptic conditions the mycelium in 8 of the flasks was covered with sterile humus soil-peat casing mixture to a depth of 1cm and incubation continued at the same temperature. The other 8 flasks received the same treatment except that the casing material was not sterile.

After a further 6-days incubation when the mycelium in all the flasks had grown into the casing layer, the flasks were transferred into a room where the temperature fluctuated between 14° and 18°C throughout the remainder of the experimental period. Four of the flasks with sterile casing and four with non-sterile casing were aerated while the others (i.e. also 4 with sterile and 4 with non-sterile casing) remained as controls.

2.12. ELECTRICAL CONDUCTIVITY :

5g fresh casing soil was put into a pre-washed 100ml beaker and 50ml de-ionized water added, stirred and allowed to stand in a water bath at 20°C for one hour. The conductivity of the soil suspension was then measured, with a portable conductivity meter,

type MC 3 of Electronic Instruments Limited.

The reading gave a measure of the salt content of the soil.

The same sample was also used to measure the soil pH.

2.13. ESTIMATION OF BACTERIAL POPULATIONS :

The method of Hayes (1968) was adopted. Into 100 ml 0.1% sterile peptone water in a clean flask 10g of fresh casing soil was added and shaken in a gyratory shaker for 5 minutes.

Serial dilutions (ten-fold) were prepared from this suspension. 10 ml of each dilution was added into 90 mls of nutrient agar before pouring into Petri-plates for gelling. The nutrient agar used had the following composition :-

Agar		15 g
Peptone		5 g
Lab-Lemco beef extract		2 g
NaCl		5 g
Deionized water	to	1 l

pH = 6.0

The plates were incubated at 25°C for about 48 hours before colonies were counted. Dilutions bearing more than 200 colonies per plate were not counted.

2.14 STATISTICAL ANALYSIS OF RESULTS.

Results, where appropriate, were subjected to Analysis of variance for comparison of many sets of experimental data. It showed whether any significant difference existed among the treatment means.

The variance ratio (F) was calculated by means of the following formula :

$$F = \frac{\text{between treatments mean square}}{\text{within treatment mean square (Error mean square)}}$$

Confidence limits at 95% or 99% were calculated thus :

$$\bar{x} \pm t_{0.05 \text{ or } 0.01} \sqrt{\frac{\text{Error mean square}}{\text{No. of replicates}}}$$

SECTION 3

EXPERIMENTAL AND RESULTS.

Section 3 EXPERIMENTAL & RESULTS

PART 1.

3.1.1. PRELIMINARY INVESTIGATIONS.

These investigations were carried out to determine the effects of physical factors viz: temperature and pH on the vegetative growth of the fungi listed in Section 2.1, and also to determine their ability to utilize various carbon sources - cellulose, lignin and starch - which are abundant in their natural habitat.

3.1.1.(a) TEMPERATURE :

The procedure describedin Section 2.4 was used for all isolates. This gravimetric method which measures the total biomass gives a more accurate estimate of growth than colony diameter measurement which measures growth in only one plane (horizontal). The basal medium was adjusted to pH 6 and incubated at 5°- 35°C at intervals of 5°C. Four or five replicates were prepared. Incubation time varied from 3 days for Morchella vulgaris to 5 weeks for Pluteus cervinus.

All the species investigated had an optimum temperature, 25°C with a range of 5°- 35°C except A.bitorquis and P.sajor-caju whose optimum was 30°C (Fig.3.1 & 3.2; Appendix 1.1(a)).

3.1.1.(b) pH :

The method described in Section 2.3. was adopted. With the aid of buffer, pH values between 3.0 and 8.5 were fixed. Incubation was at 25°C or 30°C (for A.bitorquis and P.sajor-caju). Four or five replicate flasks were used. Incubation time also varied.

The majority of the species studied preferred acid

Fig.3.1 : EFFECT OF TEMPERATURE ON THE VEGETATIVE GROWTH OF THE FOLLOWING FUNGI IN A LIQUID DEFINED MEDIUM.

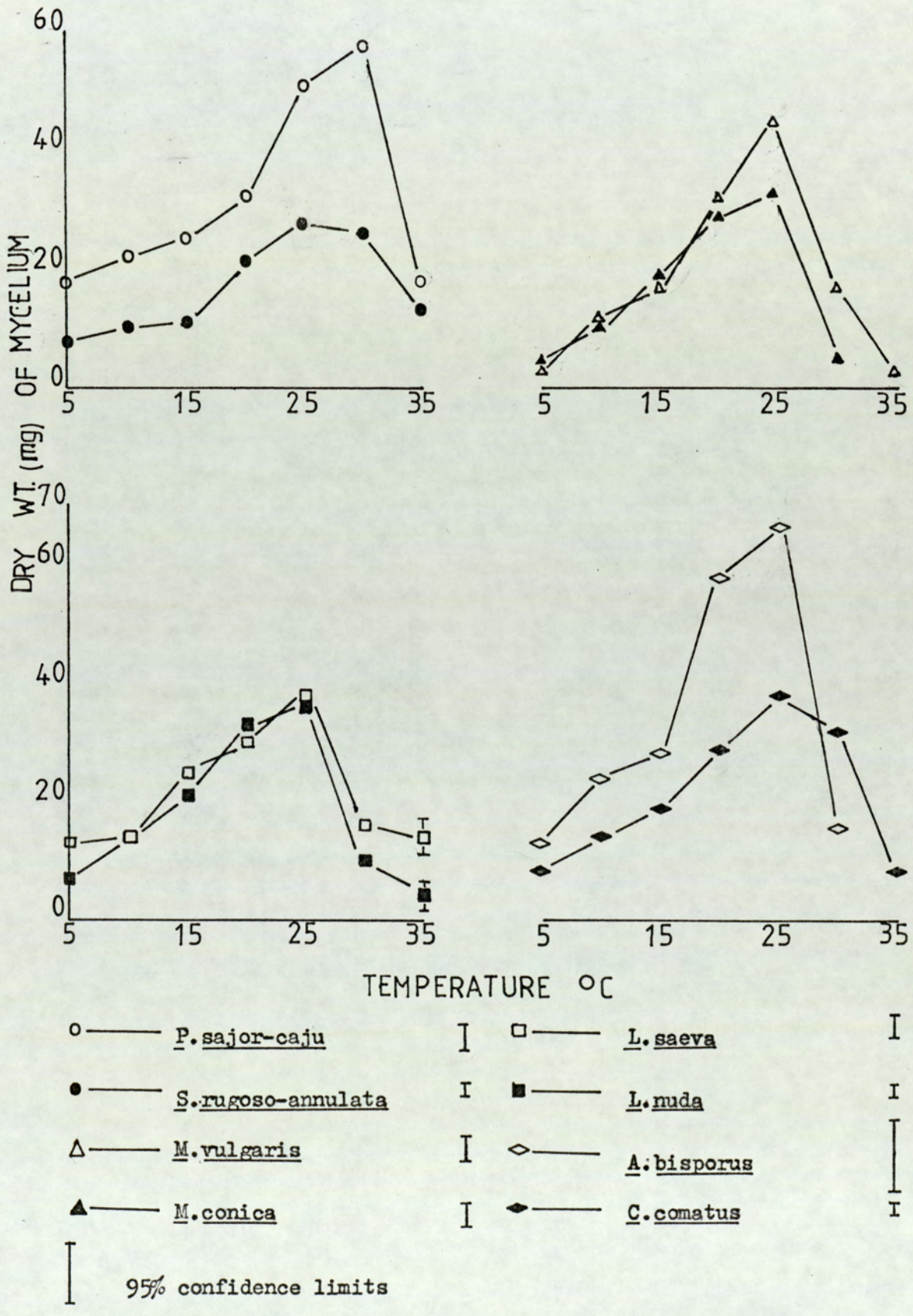


Fig.3.2 : EFFECT OF TEMPERATURE ON THE VEGETATIVE GROWTH OF THE FOLLOWING FUNGI IN A LIQUID DEFINED MEDIUM.

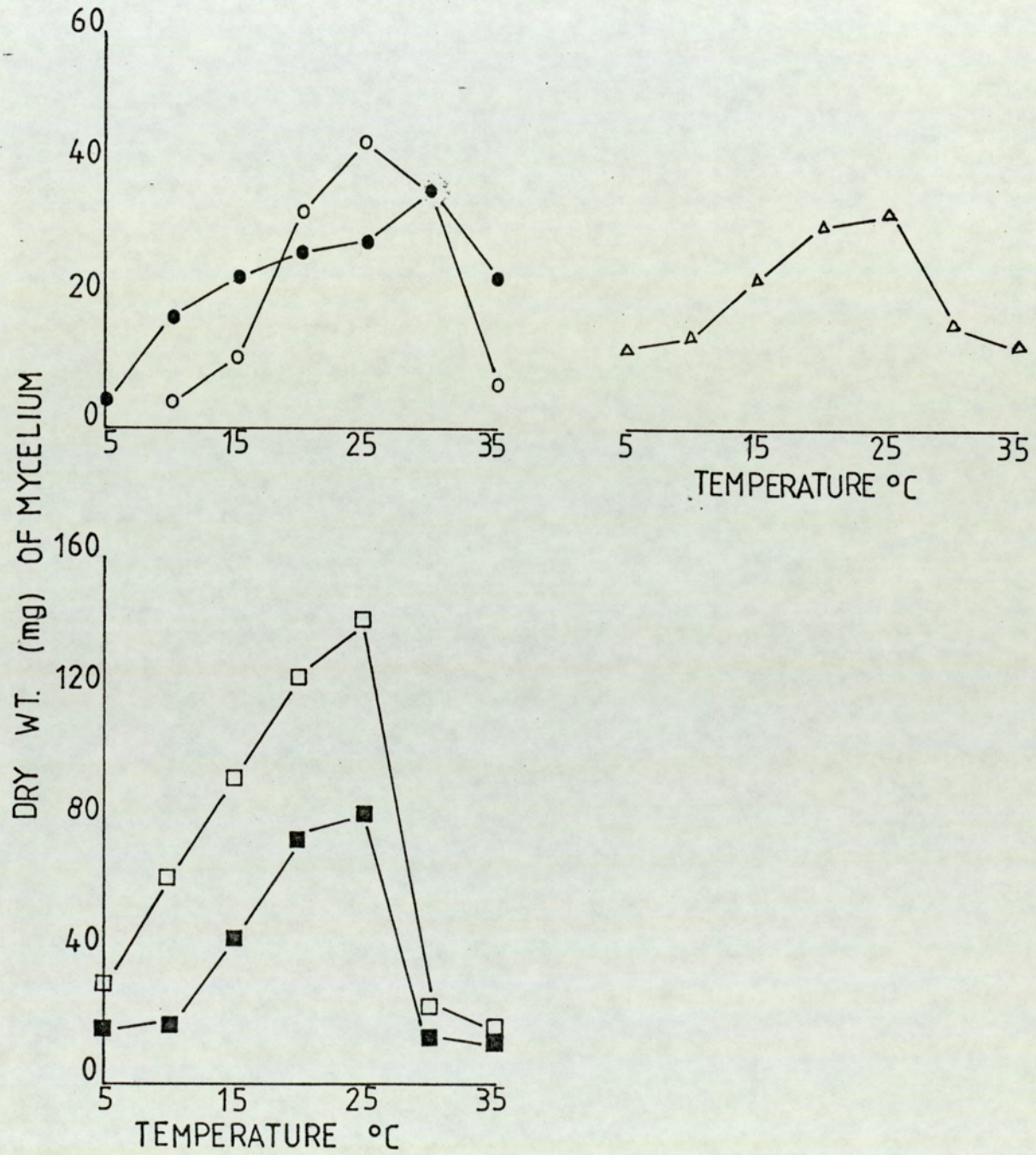
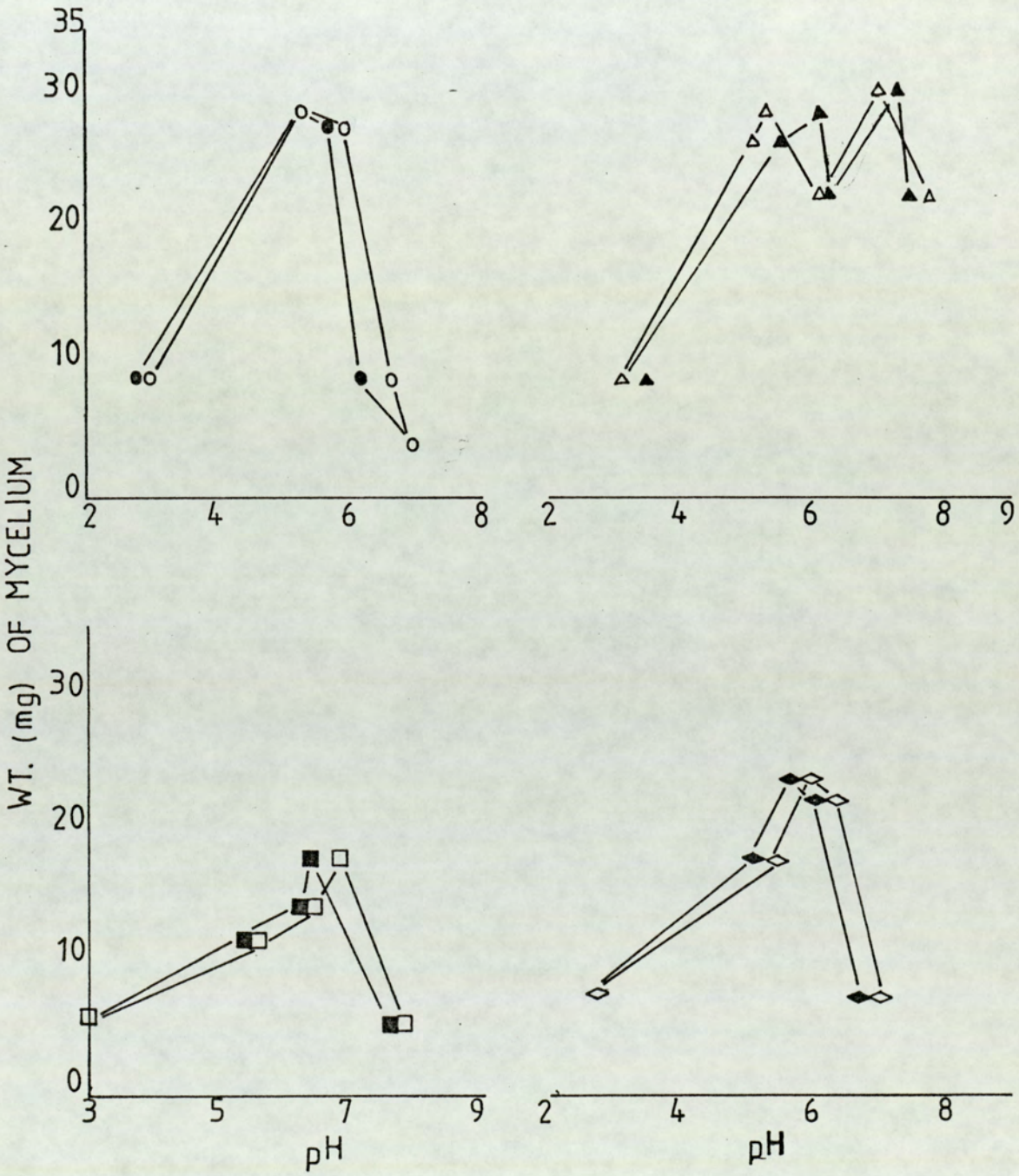


Fig.3.3 : EFFECT OF pH ON THE VEGETATIVE GROWTH OF THE FOLLOWING FUNGI IN A LIQUID DEFINED MEDIUM



o —— Ph.impudicus initial pH I

● —— Ph.impudicus final pH

△ —— A.aggregatum initial pH I

▲ —— A.aggregatum final pH

□ —— A.bisporus initial pH I

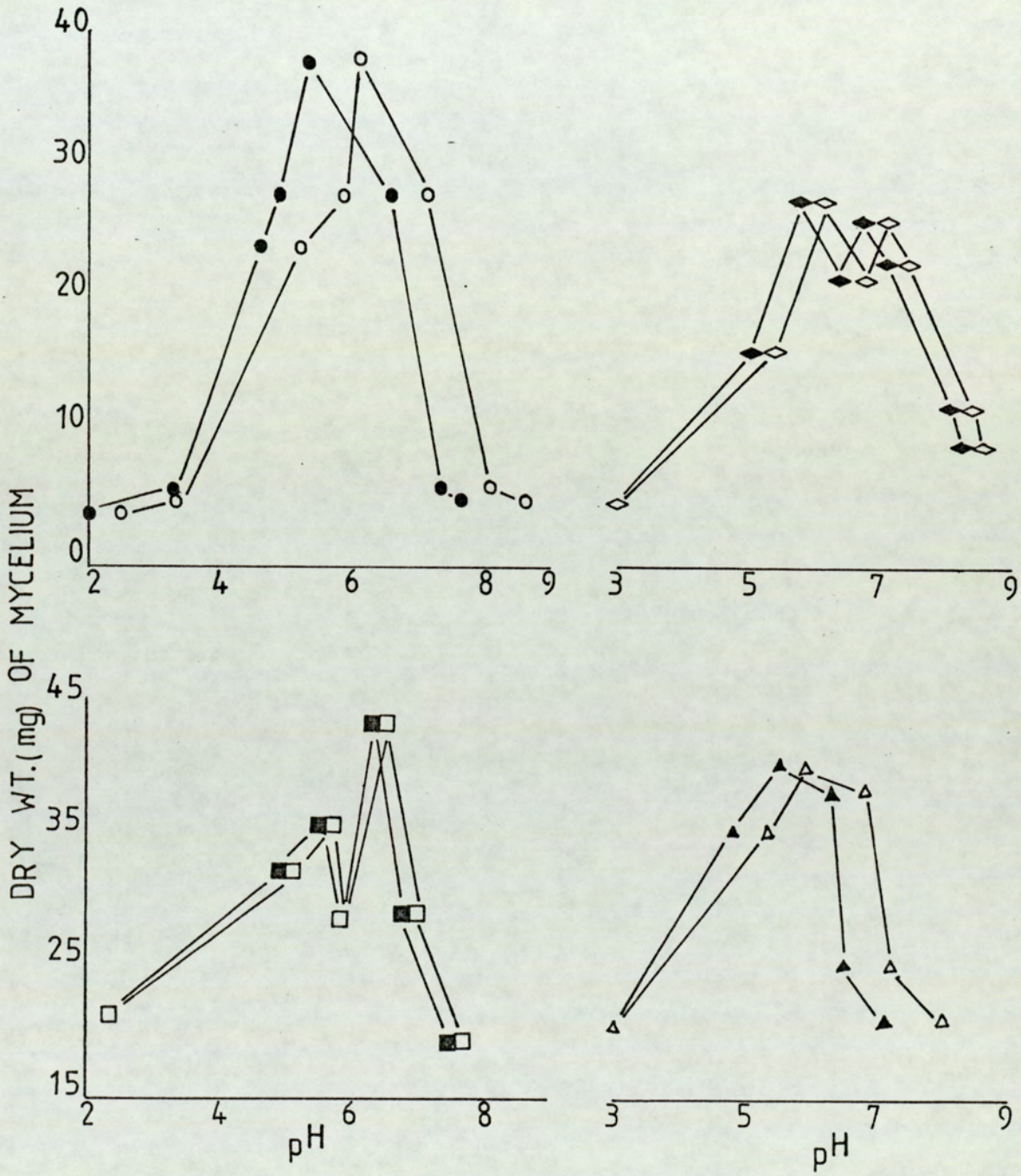
■ —— A.bisporus final pH

◇ —— A.arvensis initial pH I

◆ —— A.arvensis final pH

I 95% confidence limits

Fig.3.4 : EFFECT OF pH ON THE VEGETATIVE GROWTH OF THE FOLLOWING FUNGI IN A LIQUID DEFINED MEDIUM.



o—— A. bitorquis initial pH

●—— A. bitorquis final pH

◇—— L. saeva initial pH

◆—— L. saeva final pH

□—— L. nuda initial pH

■—— L. nuda final pH

△—— C. comatus initial pH

▲—— C. comatus final pH

95% confidence limits

Fig.3.5 : EFFECT OF pH ON THE VEGETATIVE GROWTH OF THE FOLLOWING FUNGI IN A LIQUID DEFINED MEDIUM.

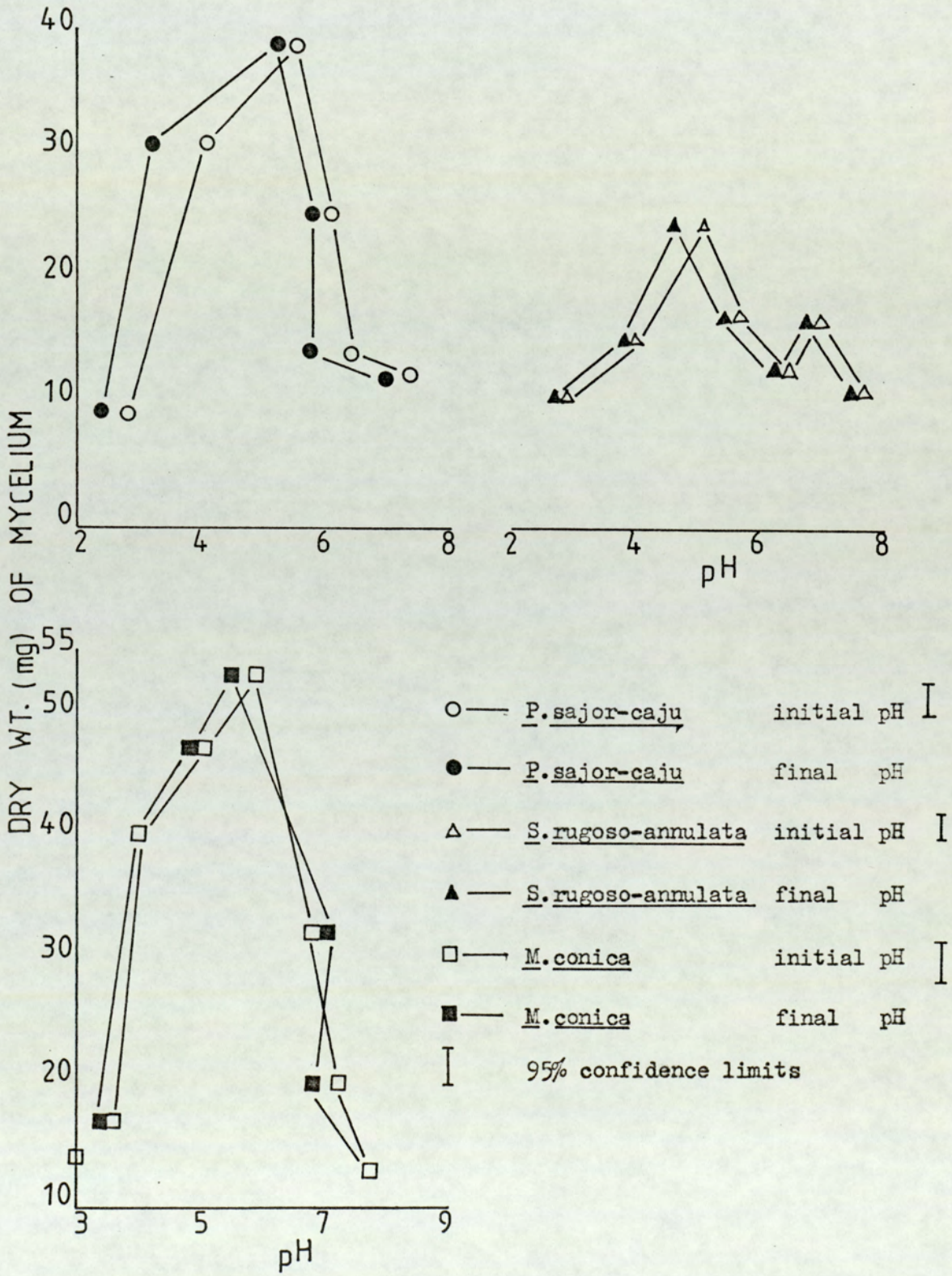
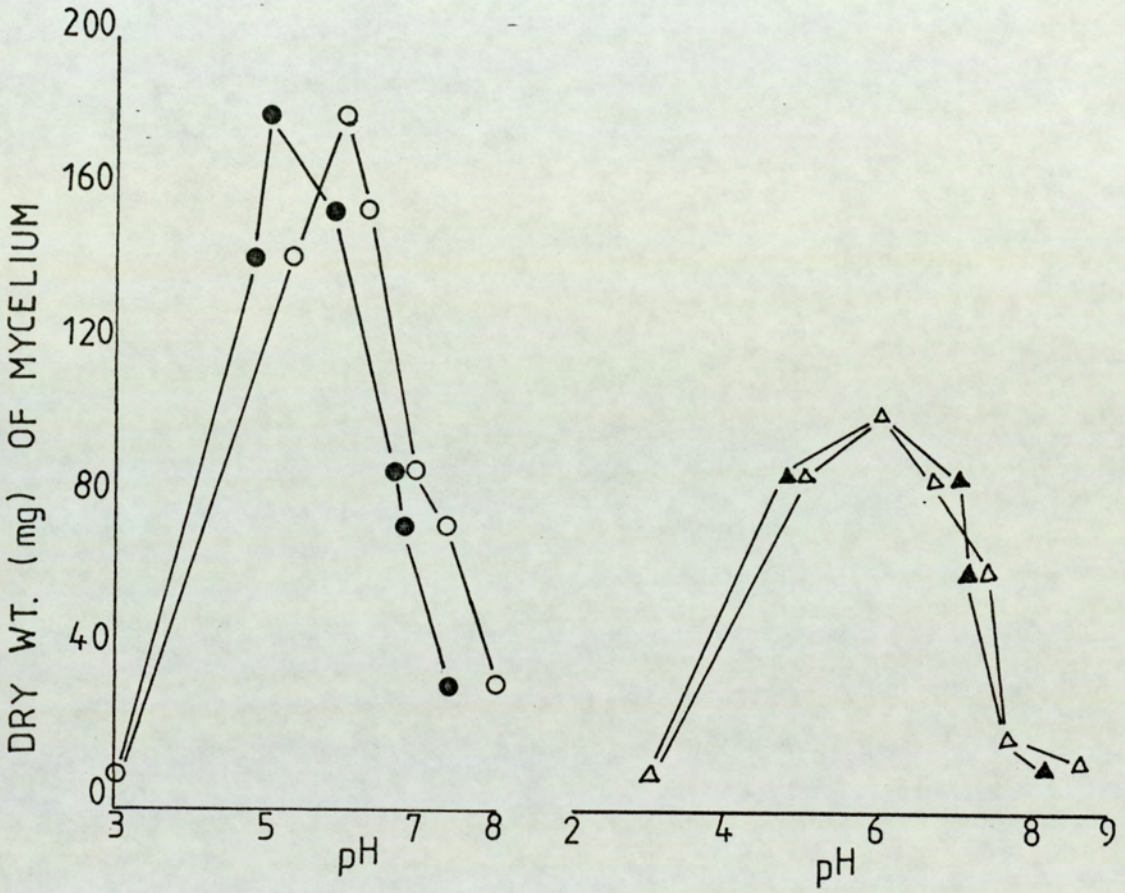


Fig.3.6 : EFFECT OF pH ON THE VEGETATIVE GROWTH OF P.CERVINUS AND M.VULGARIS IN A LIQUID DEFINED MEDIUM.



P.cervinus initial pH

P.cervinus final pH

M.vulgaris initial pH

M.vulgaris final pH

95% confidence limits

conditions to alkaline. Morchella vulgaris , M.conica, A.bitorquis, A.arvesis, P.cervinus, L.saeva and C.comatus had an optimum at about pH 6. L.nuda grew best at 6.5, P.sajor-caju 5.5 and Phallus impudicus 5.3. The most acidophilic was S.rugoso-annulata with maximum growth at pH 5.0. A.bisporus and Agrocybe aggregatum preferred a neutral pH.

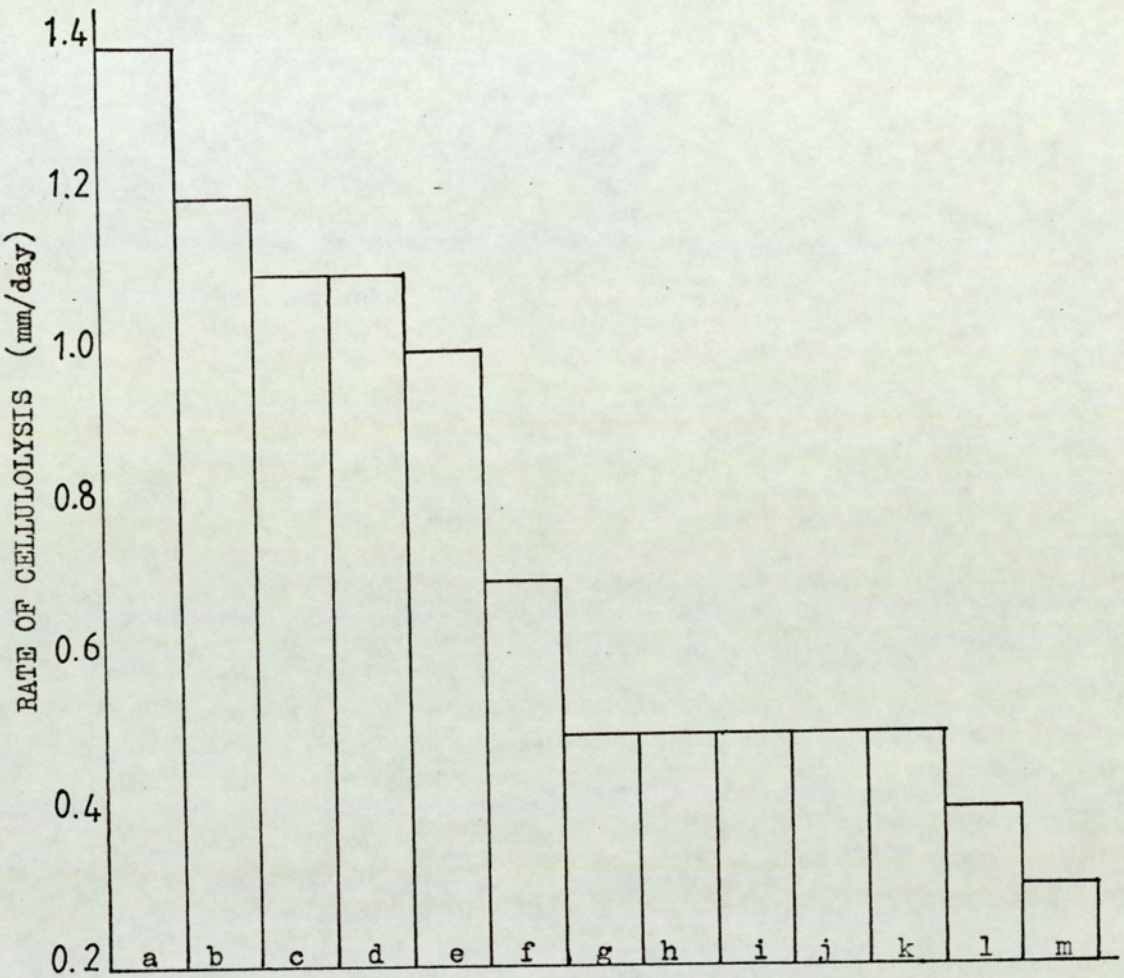
S.rugoso-annulata, L.saeva and A.aggregatum had double peaks. Generally there was a shift in pH towards the more acid region but A.aggregatum caused a shift towards an alkaline pH. (Fig.3.3 - 3.6; Appendix 1.1a).There was a distinct effect of pH variation on the morphology of Phallus impudicus mycelium. At low pH, about 3.0, there was relatively more extensive development of rhizomorphs and less hyphae than at higher pH values. This gradation continued until pH 7.0 where there were only fine hyphae and no rhizomorphs. Growth virtually stopped at pH 7.3.

3.1.1.(c) CELLULOLYSIS :

The technique of Reutella and Cowling (1966) described in Section 2.4. was adopted. This was based on the principle that dissolution of cellulose, an insoluble substrate, is an essential aspect of its degradation. As the organisms grew they secreted cellulolytic enzymes that lysed the cellulose particles and created a sharply defined clear zone in the milky white opaque medium.

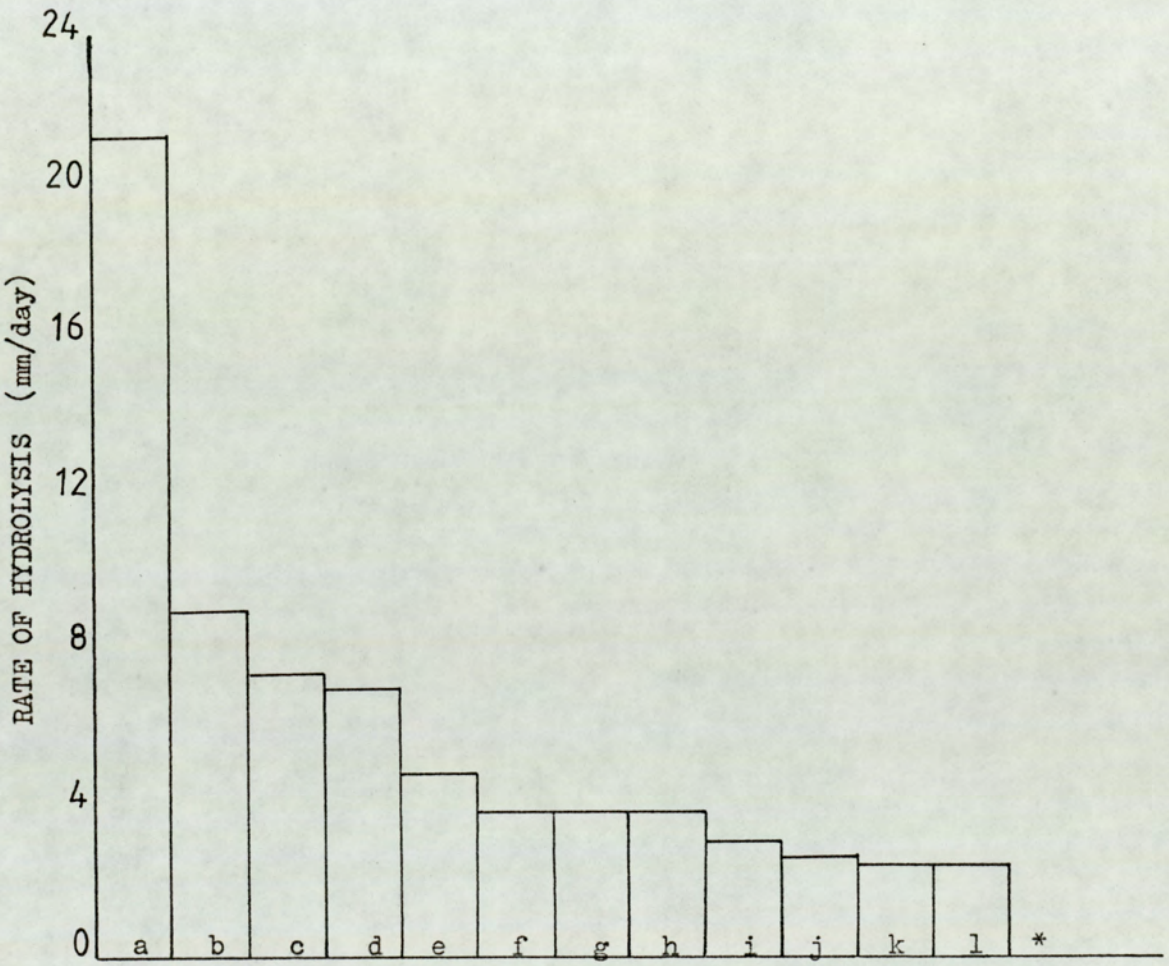
The columns were set up in five replicates and incubated at 25°C and 30°C (for A.bitorquis and P.sajor caju). The activity of the various fungi was determined on a continuous, cumulative basis by visually measuring the depth of clearing in the columns at regular intervals for 28 days.

Fig.3.7 : CELLULOLYTIC ACTIVITY OF A RANGE OF EDIBLE FUNGI IN CELLULOSE COLUMNS.



a. M.vulgaris
b. P.cervinus
c. A.bisporus
d. A.bitorquis
e. L.saeva
f. P.sajor-caju
g. A.arvensis
h. A.aggregatum
i. L.nuda
j. M.conica
k. S.rugoso-annulata
l. Ph.impudicus
m. C.comatus

Fig.3.8 : RELATIVE STARCH HYDROLYSIS BY A RANGE OF EDIBLE FUNGI.



a = Morchella vulgaris

b = Agaricus bitorquis

c = Pleurotus sajor-caju

d = Agrocybe aggregatum

e = Stropharia rugoso-annulata

f = Morchella conica

g = Coprinus comatus

h = Lepista nuda

i = Agaricus bisporus

j = Lepista saeva

k = Pluteus cervinus

l = Agaricus arvensis

* Hydrolysis by Phallus impudicus was too patchy to be measured.

The fungi studied varied in their ability to clear cellulose. M.vulgaris and P.cervinus followed closely by A.bisporus, A.bitorquis and L.saeva showed the greatest cellulolytic ability. Others showed about equal and moderate activity except C.comatus and Phallus impudicus which were relatively poor or slow utilizers of cellulose (Fig.37).

3.1.1.(d) STARCH HYDROLYSIS

The method employed was described in Section 2.5. Five replicate plates were used for each test. All the species were able to hydrolyse starch but the rate of hydrolysis differed considerably from 2.4mm/day by P.cervinus to 21.3mm/day by M.vulgaris. For some, eg. A.bitorquis the clear zone was surrounded by a distinct halo 3 - 5mm across before the blue-black (starch) zone. For some others, such as L.nuda there was no distinguishable halo separating the two zones. In the case of the remaining fungi eg. L.saeva there were blue-black patches within the clear zone, indicating incomplete hydrolysis (Fig 3.8).

3.1.1.(e) LIGNIN DEGRADATION

Ability of the fungi to degrade lignin was tested by assessing laccase (p - diphenol - oxidase) activity. Bavendamm's method (Lindeberg, 1948; Fries, 1955 and Walker, 1975) was adopted. The principle is based on the oxidation of phenolic compounds like gallic and tannic acids by the extra-cellular enzyme laccase which gives rise to quinone-containing products with a characteristic brown colour. Lignin is a complex polymer of phenolic compounds, the breakdown of which plays a major role in the formation of humus.

0.1% of tannic acid was added aseptically into 2% malt extract agar after autoclaving and the mixture thoroughly agitated.

TABLE 3.1. P - DIPHENOL OXIDASE REACTION

Species	Reaction with tannic acid
Morchella vulgaris	+ + +
Pleurotus sajor-caju	+ + +
Agaricus bitorquis	+ + +
Agrocybe aggregatum	+ + +
Coprinus comatus	+ + +
Stropharia rugoso-annulata	+ + +
Morchella conica	+ +
Agaricus bisporus	+ +
Lepista nuda	+ +
Pluteus cervinus	+ +
Lepista saeva	+ +
Agaricus arvensis	+
Phallus impudicus	+

KEY

+	Discolouration of medium
+ +	Strong discolouration of medium
+ + +	Very strong discolouration

The agar plates, after gelling, were incubated at 25°C or 30°C as appropriate. As the fungi grew, brown zones were produced on the agar. The intensity of browning was visually estimated and the result summarized in Table 3.1.

The speed of discolouration followed the order in the above table. Discolouration was most rapid and pronounced with M.vulgaris. It was also dark-brown in the case of C.comatus and P.cervinus. Natural pigments produced by these fungi might have contributed to colour intensity. The intensity of colour was about the same in all the remaining fungi. Tannic acid inhibited the growth of all the fungi.

3.1.1.(f) FRUIT-BODY FORMATION ON PETRI-PLATES

An attempt was made to induce the fungi to form fruit-bodies on agar plates. The medium used was 2% malt extract agar with pH adjusted to the optimum for each fungus. After the agar had been well colonized, either one or a combination of the following treatments were given :

(i) The plates were left in the incubator for a maximum period of 4 months at 25°C or 30°C as appropriate.

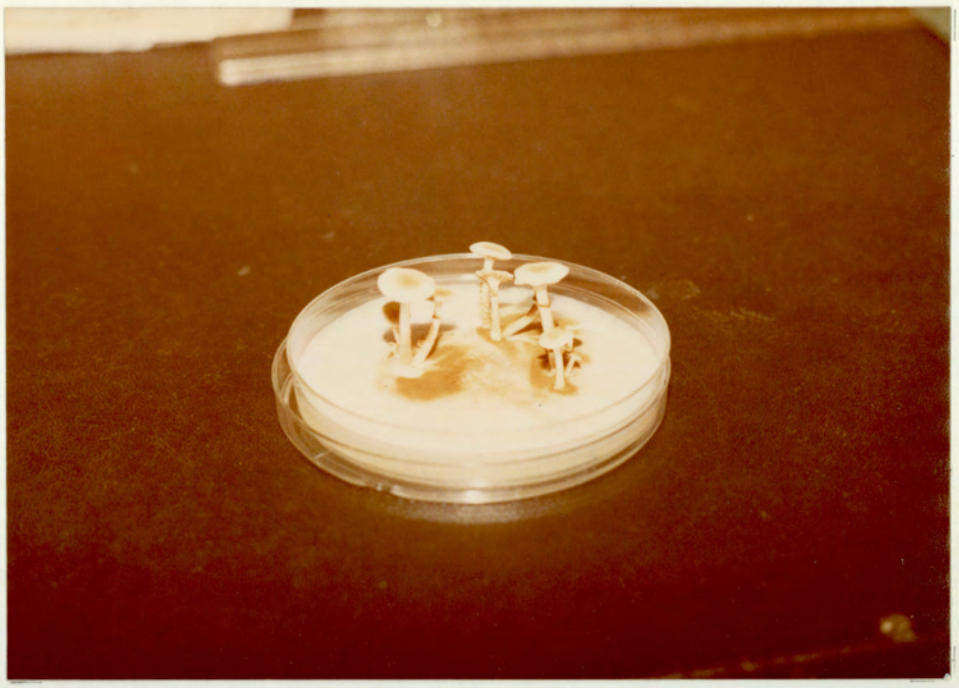
(ii) The plates were treated to a temperature shock by lowering the incubation temperature to 15°C ± 2°C

(iii) The cultures hitherto incubated in the dark, were illuminated to about 1500 lux continuously or alternating light and dark periods.

(iv) Treatments (ii) and (iii) above were applied simultaneously.

(v) Biochemical stimulants e.g. hormones (indole-3-acetic acid, gibberellic acid and cytokinins) and also exudates of fresh fruit-

Plate 3.1 Fruit-body formation on agar plates.



(a)Agrocybe aggregatum

(b) Pleurotus sajor-caju

bodies of the same or other species were introduced with treatments (i) and (ii).

Of the thirteen fungi tested, only P. sajor-caju and Agrocybe aggregatum could produce mature fruit-bodies (see Plate 3.1 a & b) in agar plates after treatment (iii). Agaricus bitorquis produced fruit-body primordia which failed to develop further.

3.1.1.(g) FRUIT-BODY FORMATION ON STRAW

A preliminary test was done for all the fungi on uncomposted rice straw. The procedure adopted was as described in Section 2.9 except that mycelial discs were used as inocula instead of grain spawn. For each fungus 5 replicate pots were covered with a casing layer as done in A. bisporus cultivation and 5 pots were left uncovered.

P. sajor-caju produced fruit-bodies in both treatments in two weeks. S. rugoso-annulata produced fruit bodies after 8 weeks in covered pots only. All the others failed to fructify. In many of them like the Lepista species, Agaricus species and Phallus impudicus, the mycelia died from competition with contaminating micro-organisms.

3.1.2. CONCLUSIONS

3.1.2.(a) TEMPERATURE

The results of the temperature experiments clearly indicate that all the fungi investigated are mesophiles, most grow within the same range, 5°- 35°C with an optimum of 25°C. The optimum obtained for A. arvensis agrees with the records of Raper and Kaye (1978); and Delmas (1978). The results for A. bisporus also corresponds with the data recorded by Treschow (1944); Litchfield (1967); and Hayes (1978). That A. bitorquis grew best at 30°C is in agreement with the data of

Fritsch (1974); and Raper and Kaye (1978). Similarly the data obtained for the other fungi correspond to earlier records. No records are available for the growth requirements of A.aggregatum and Phallus impudicus.

The relation between temperature and habitat is not clear, for many fungi are cosmopolitan despite temperature differences among various parts of the world (Cochrane,1958). Apparently the growth of such fungi is limited by factors other than temperature. But in general fungi inhabiting warmer areas tend to have higher temperature optima than those in the temperate zones. All the fungi involved in this study except P.sajor-caju (which inhabits the tropical and sub-tropical areas), are natives of the temperate regions. P.sajor-caju and A.bitorquis grew best at 30°C.

3.1.2.(b) pH

All the fungi studied, except A.bisporus and A.aggregatum favoured acidic media. The pH value 6.9 determined as optimal for A.bisporus compares favourably with 6.8 - 6.9 (Treschow,1944); and 6.8 - 7.0 of Hayes (1978). Fries' optimal values for C.comatus were 6.0 in one medium and 6.7 in another. In this work,6.0 was obtained as the optimum for the same fungus. For L.nuda 6.0 was recorded as its optimum pH value (Norkrans,1950 ; Votypka,1971; Wright,1976). Wright also recorded 7.0 for some isolates of L.nuda. The optimum pH value obtained for this fungus was 6.4. For L.saeva, 6.0 was obtained as its optimum, Wright had 6.0 for some isolates and 5.0 for some others. There are no records in literature of the optimum pH requirements for the growth of the remaining fungi investigated.

In contrast with bacteria and actinomycetes,fungi are generally more able to invade acid environments, though not entirely

restricted to them. In culture the larger basidiomycetes are generally unable to grow at initial pH of 7.0 (Cochrane,1958). However, coprophilic species and a few soil-inhabiting species are basiphilic or at least prefer neutral pH.

Shifts in pH occurred in all cases during incubation as a result of metabolic activities. But in most cases there was lowering of pH rather than increase. Increase in acidity is usually caused by the formation of organic acids or the utilization of cations, while increase in alkalinity is caused by production of basic metabolites, most commonly ammonia or by utilization of anions.

3.1.2.(c) CELLULOLYSIS :

The results on cellulolysis experiment suggest that all the fungi investigated could utilize cellulose when available as a sole source of carbon. Ability to break down ball-milled cellulose which has been reduced to finely divided particles may not be equated to ability of an organism to directly degrade native cellulose. Other contents of the artificial cellulose medium especially organic nitrogen sources like asparagine may also reduce the level of cellulase production (Wright,1976) by the fungi.

However, Reutella and Cowling (1966) also determined the loss in weight of native cellulose caused by the activity of the same test fungi and their results correlated (Correlation co-efficient more than 0.6) well with those obtained by clearing method. They therefore, recommended this column clearing method as a simple and reliable cultural assessment of cellulolytic activity closely approximating degradation of native cellulose.

Several authors such as Waksman and Nissen (1932),

Bohus, (1959) and Hayes (1978), have recorded the utilization of cellulose by A.bisporus. L.nuda and L.saeva are capable of degrading cellulose (Lindeberg,1946; Norkrans,1950 and Wright,1976). In Wright's work L.saeva isolates exhibited more cellulolytic activity than L.nuda. Fries (1955) showed that C.comatus was incapable of hydrolysing cellulose in her work but in this investigation C.comatus proved capable of doing so, though poorly.

3.1.2.(d) LIGNIN DEGRADATION

Lindeberg (1948) and Walker (1975) considered the production of brown zones by fungi on a medium containing polyphenolic compounds like gallic acid, tannic acid or catechol as an indication of the production of laccase. The discolouration is said to be caused by ortho- and para-quinones (and/or their protein complexes) which are breakdown products of polyphenols.

However, Norkrans (1950) found that a positive result did not always prove the ability to decompose lignin in natural substances.

Polyphenoxidases consist of tyrosinase and laccase enzymes. Tyrosinase oxidises monophenols (i.e. tyrosine, p-cresol) and ortho-diphenols (i.e. catechol, pyrogallol). Oxidation of tyrosine leads to the formation of black melanin pigments. Laccase oxidises ortho-diphenols and para-diphenols (i.e. para-phenylamine diamine, guiacol).

The activities of the two enzyme systems therefore interact to some extent. Discolouration of gallic or tannic acid or catechol does not therefore clearly distinguish between the activities of the two enzyme systems. Nevertheless, tyrosinase is intra-cellular while laccase is extra-cellular. Bavendamm's test shows the presence of extra-cellular laccase.

Laccase activity has been demonstrated for a number of fungi including A. bisporus. It has been shown that while most of its tyrosinase is found in the sporophore, most of the laccase is in the mycelium and both occur in the strands (Lindeberg, 1950; Turner,1968, 1974). Thus while laccase plays a major role in the vegetative growth, tyrosinase plays its part during fructification (Turner,1974). Norkrans (1950) showed that L. nuda could weakly decompose lignin. Wright (1976) also showed that both L. nuda and L. saeva are ligninolytic. C. comatus is also a lignin decomposer (Fries,1955)

3.1.2.(e) STARCH HYDROLYSIS:

From the above results it may be concluded that all the species under investigation could produce amylases and thus could utilize starch as a sole carbon source. Like cellulose, starch is a polymer of glucose molecules. It is known that there are two types of amylases :

(a) β - amylase which hydrolyses starch directly into maltose and finally glucose.

(b) α - amylase which digests starch, via dextrins and maltose into glucose.

The latter is more common in fungi than the former (Lilly and Barnett,1951).

Most fungi are able to utilize starch, sometimes as better substrate than glucose. Brock (1951) obtained the greatest yield of M. esculenta mycelium on starch. Coprinus species including C. comatus grew very well on starch (Fries,1955). She could not attribute the high yield obtained to the effect of growth-promoting substances contained as impurities in starch, but rather Fries speculated that it might be due to the jelly-like consistency of starch. She also suggested that it might be due to the direct breakdown of starch to glucose compounds by

phosphorylation and not via maltose. Starch also supported good growth in all Morchella species used by Kaul (unpublished) and was the best carbon source for M. esculenta and M. conica. Jandaik and Kapoor (1974) obtained the greatest growth on starch for P. sajor-caju. L. nuda and L. saeva also utilized starch well in Wright's work (Wright, 1976).

Like other extra-cellular enzymes, amylases perform their function outside the cell. They degrade the complex polysaccharide, starch, into low-molecular-weight glucose which is able to enter the cell. The appearance of a halo in some cultures like A. bitorquis, could thus indicate the high production of extra-cellular amylases which diffused in advance to hydrolyse starch into simple sugars, for ready utilization by the advancing hyphae. The species that did not produce haloes were generally slower in growth and would suggest poor amylase production and therefore slow starch digestion.

3.1.2.(f) FRUITBODY FORMATION :

It has been observed in 3.1.1. that only Pleurotus sajor-caju and Stropharia rugoso-annulata were able to form mature fruit-bodies on straw substrate within 8 weeks. Their requirements for fruit-body formation differed. For example, while S. rugoso-annulata required the casing layer and lowering of the temperature, P. sajor-caju required neither of these conditions. In view of the above, these two fungi were selected for further studies.

PART 2 PLEUROTUS SAJOR-CAJU.

3.2.1. VEGETATIVE GROWTH.

3.2.1.(a) EFFECT OF VITAMINS :

Generally fungi must obtain from their substrates, vitamins for growth, reproduction and other vital functions. A few fungi like Aspergillus niger are, however, said to be self-sufficient with respect to vitamins, in that, they are able to synthesize all the necessary vitamins they require for growth (Lilly and Barnett,1951). Although there is no correlation between vitamin deficiency and taxonomic relationship, basidiomycetes in particular the Hymenomycetes, are partially or totally deficient in thiamine or its components (Jennison et al,1955). Volz (1972) claimed that some fungi including P.ostreatus and L.nuda showed no definite requirement for any of the vitamins he tried,including thiamine.

The following experiment was therefore carried out to determine the vitamin requirements of P.sajor-caju.

The medium used was as follows :-

Glucose	25.0 g
Asparagine	1.0 g
$MgSO_4 . 7H_2O$	0.5 g
KH_2PO_4	1.0 g
Fumaric Acid	1.32g
Na_2CO_3	1.12g
$Fe_2(SO_4)3$	0.2mg
$ZnSO_4$	0.2mg
$MnSO_4$	0.1mg
Distilled water	to 1.0 l

This medium was a modification of that suggested by Lilly and Barnett (1951) for the study of vitamin deficiencies. They used casein hydrolysate as the nitrogen source. In this investigation asparagine was substituted for casein hydrolysate since the latter contains traces of vitamins. The above medium was autoclaved but the vitamins and yeast extract used were sterilized by filtration. Riboflavin was handled only in dim light as this compound is destroyed by strong light. Glucose and asparagine were also filter - sterilized.

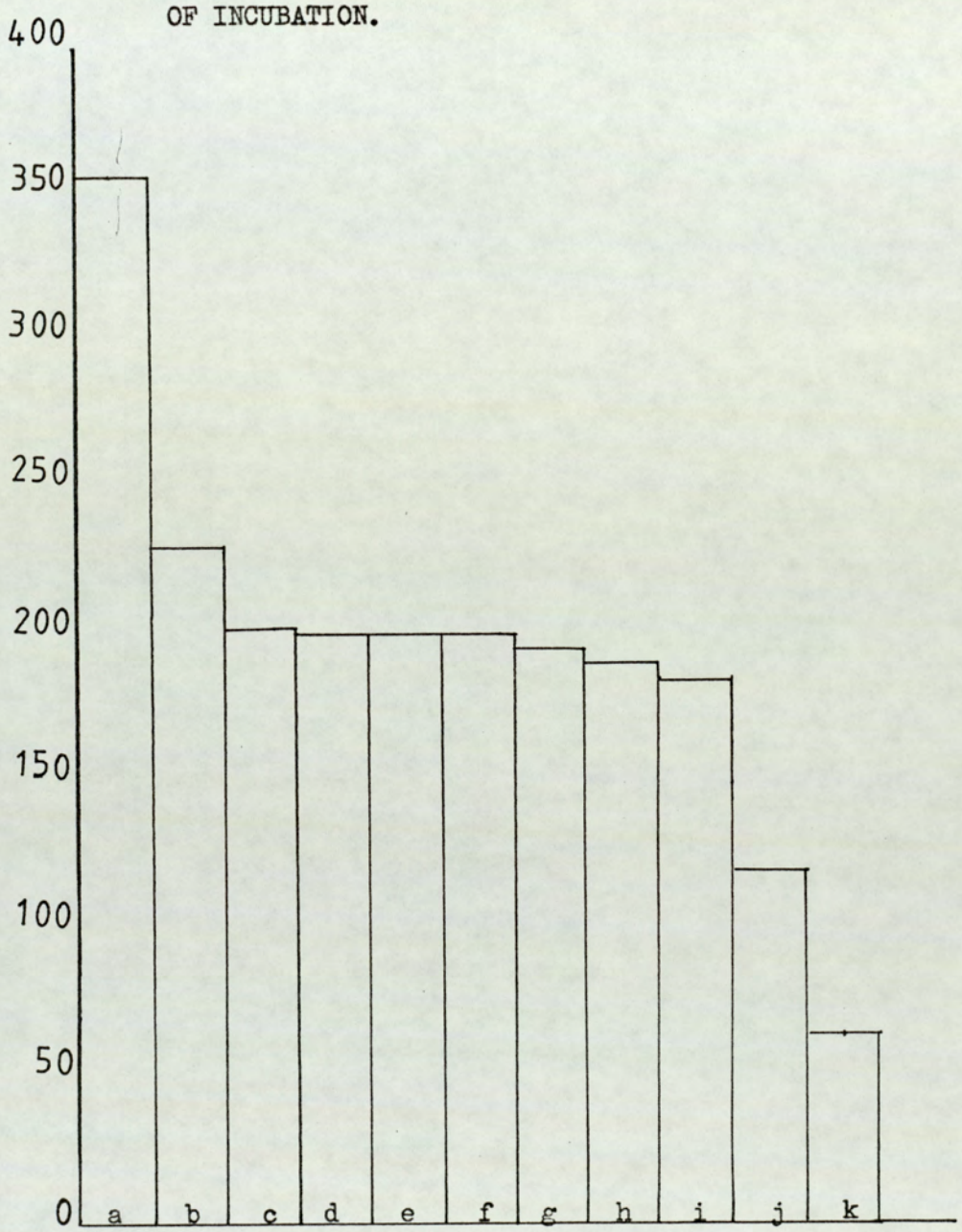
The innocula were cut out from 2nd-transfer water agar colonies, in order to reduce vitamin carry-over from malt agar innocula.

In one treatment all the seven vitamins were added to make a "complete" vitamin solution (CV), in other treatments single vitamins were depleted in sequence. There were three control treatments : a vitamin-free treatment (negative control), a yeast extract treatment and the "complete" vitamin mix (positive controls). Biotin was added at a concentration of 5μg/l; inositol 5mg/l and the remaining vitamins at 100μg/l concentration. Yeast extract addition was made at 0.4g/l.

Harvesting was done twice : after 2 weeks and three weeks. The extended incubation was necessary to see if the fungus could overcome its deficiency for any particular vitamin(s).

Fig. 3.9 summarizes the result. The dry weight of the mycelium after 2 and 3 weeks followed a similar pattern among the treatments. The dry weight obtained from thiamine-free flasks was significantly ($P = 0.01$) small, closest only to the vitamin-free control. The flasks treated with yeast extract gave by far, the greatest yield, followed by those with complete vitamin solution. All the single vitamin depletion treatments (except CV-thiamine) gave slightly poorer yields than CV and were all nearly equal to one another.

Fig.3.9 : EFFECT OF VARIOUS VITAMINS AND YEAST EXTRACT ON THE VEGETATIVE GROWTH OF P.SAJOR-CAJU AFTER 3 WEEKS OF INCUBATION.



a. yeast extract
b. 'Complete' vitamin solution (CV)
c. CV - inositol
d. CV - folic acid
e. CV - pyridoxine
f. CV - nicotinic acid
g. CV - pantothenic acid
h. CV - riboflavin
i. CV - biotin
j. CV - thiamine
k. vitamin-free control

| 95% confidence limits.

3.2.1.(b) EFFECT OF VARIOUS NITROGEN SOURCES :

Fungi can be broadly classified into four main groups, based on the type of nitrogen sources utilised (Lilly & Barnett,1951) :

(i) Fungi capable of fixing atmospheric nitrogen; utilizing nitrate, ammonium and organic nitrogen.

(ii) Those which can utilize nitrate, ammonium and organic nitrogen but not atmospheric nitrogen.

(iii) Those which are able to utilize ammonium and organic nitrogen but incapable of making use of either atmospheric nitrogen or nitrates.

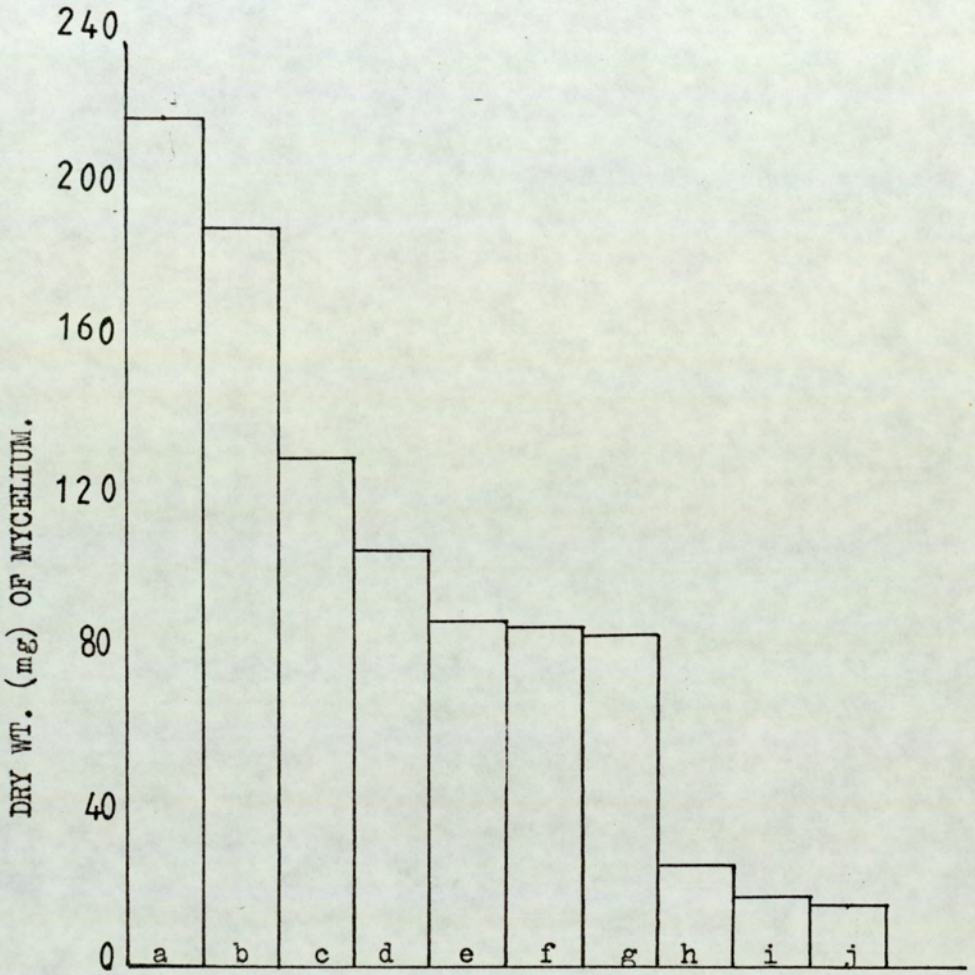
(iv) Fungi which can only use organic nitrogen.

Cochrane (1958) said that many basidiomycetes belonged to the third group.

The following experiment was therefore set up to find what types of substances could serve as suitable sources of nitrogen for P. sajor-caju. A range of organic nitrogen sources, ammonium compounds, nitrates and a nitrite were selected. The method described in Section 2.3 was adopted. the nitrogen compounds were added at the concentration of 0.1863gN/l (=1.0g asparagine), and they as well as glucose were filter-sterilized. The pH was adjusted to 6.0.

As can be seen from Fig.3.10, the organic sources- casein hydrolysate, urea, peptone and ammonium tartrate, in that order, gave the greatest yields. Ammonium bicarbonate, asparagine and ammonium nitrate supported moderate growth. Growth in sodium nitrate and potassium nitrite was much suppressed. The buffer was effective, the pH did not change beyond $\pm$ 0.5.

Fig.3.10 : EFFECT OF VARIOUS NITROGEN SOURCES ON THE VEGETATIVE GROWTH OF P.SAJOR-CAJU IN A LIQUID DEFINED MEDIUM AFTER 2 WEEKS OF INCUBATION.



a. Casein hyd.

b. Urea

c. Peptone

d. Amm. Tartrate

e. Amm. bicarbonate

f. Asparagine

g. Amm. nitrate

h. Sodium nitrate

i. Potassium nitrite

j. N-free control

I 95% confidence limits.

3.2.1.(c) CARBON : NITROGEN RATIO :

For optimum growth there must be strict proportionality between carbon and nitrogen supply. The demand for nitrogen depends on the carbon supply (Cochrane,1958) and vice versa. The following experiment was carried out to determine the optimum C : N ratio for this fungus.

Asparagine which served as the nitrogen source was used at 1g/l (0.1864gN/l). In an initial experiment in which 0.5g/l, 1g/l, 2g/l and 3g/l levels of asparagine were used, the most consistent results were obtained with 1g/l level. Glucose was the carbon source. C : N ratios ranging from 10 : 1 to 100 : 1 at intervals of 10 units were taken by altering the level of glucose. Glucose and asparagine were separately autoclaved. The basal medium described in Section 2.3 was used and its pH was maintained at 6.0 by means of a phosphate buffer. Final pH was measured. Five replicate flasks were used for each ratio.

The optimum C : N ratio was 90 : 1 (see Fig.3.11) after two weeks of incubation. Slight shifts in pH towards the more acid range were observed.

3.2.1.(d) EFFECT OF FATTY ACIDS AND THEIR ESTERS :

A number of workers have indicated increased growth effect of fatty acids and oils on certain fungi (Wardle and Schisler,1969; Kurtzman Jr.,1976; Wright,1976). But their effect on P.sajor-caju has not been studied. It was the aim of this experiment therefore, to investigate the effect of some fatty acids and their esters on the growth of P.sajor-caju.

The medium used was formulated by Hashimoto and Takahashi (1974) and made up of the following ingredients :

Fig.3.11 : OPTIMUM CARBON : NITROGEN RATIO FOR VEGETATIVE GROWTH OF P.SAJOR-CAJU AT 1g/LITRE LEVEL OF ASPARAGINE (0.19gN/L) AFTER 2 WEEKS OF INCUBATION.

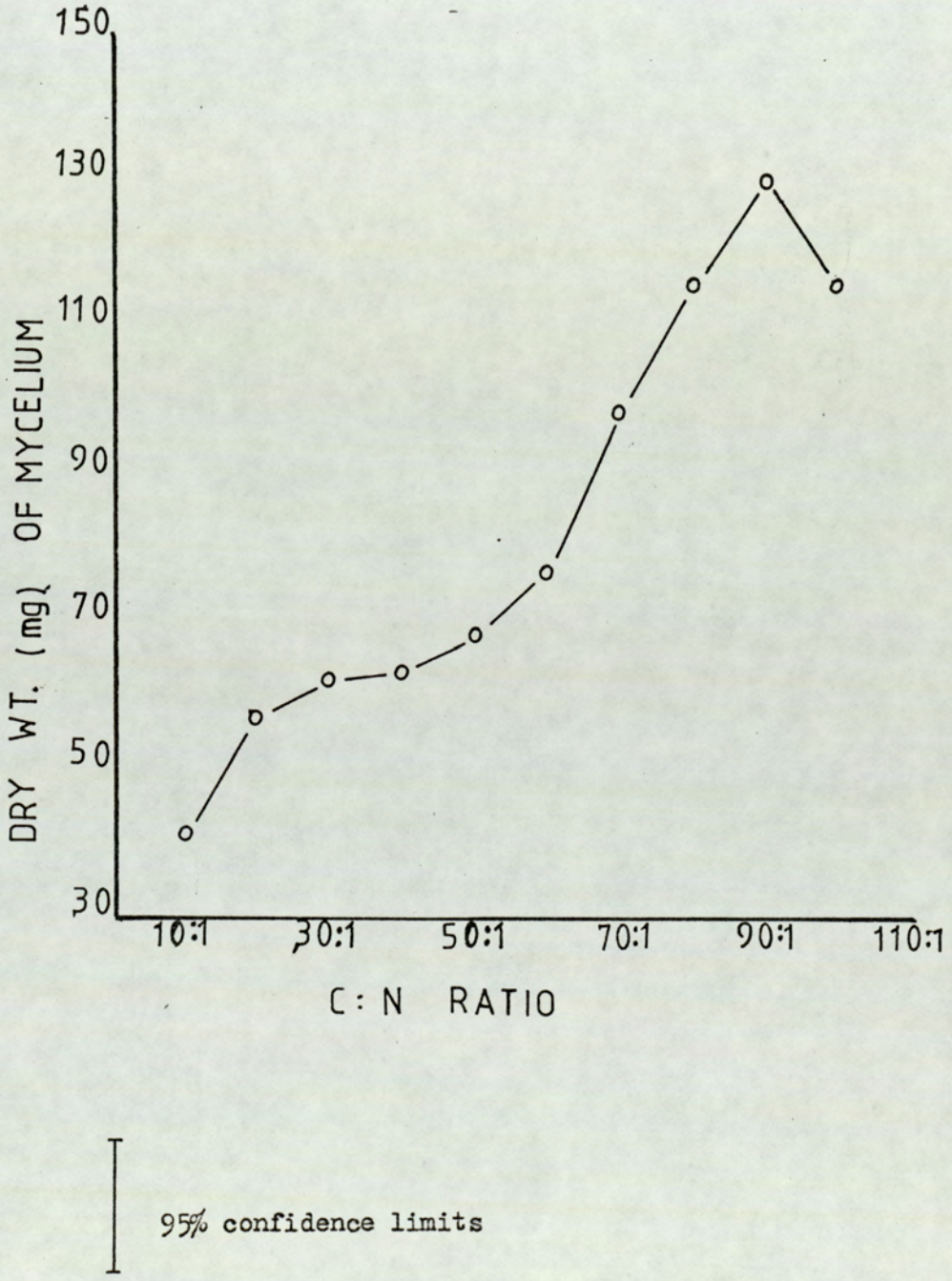
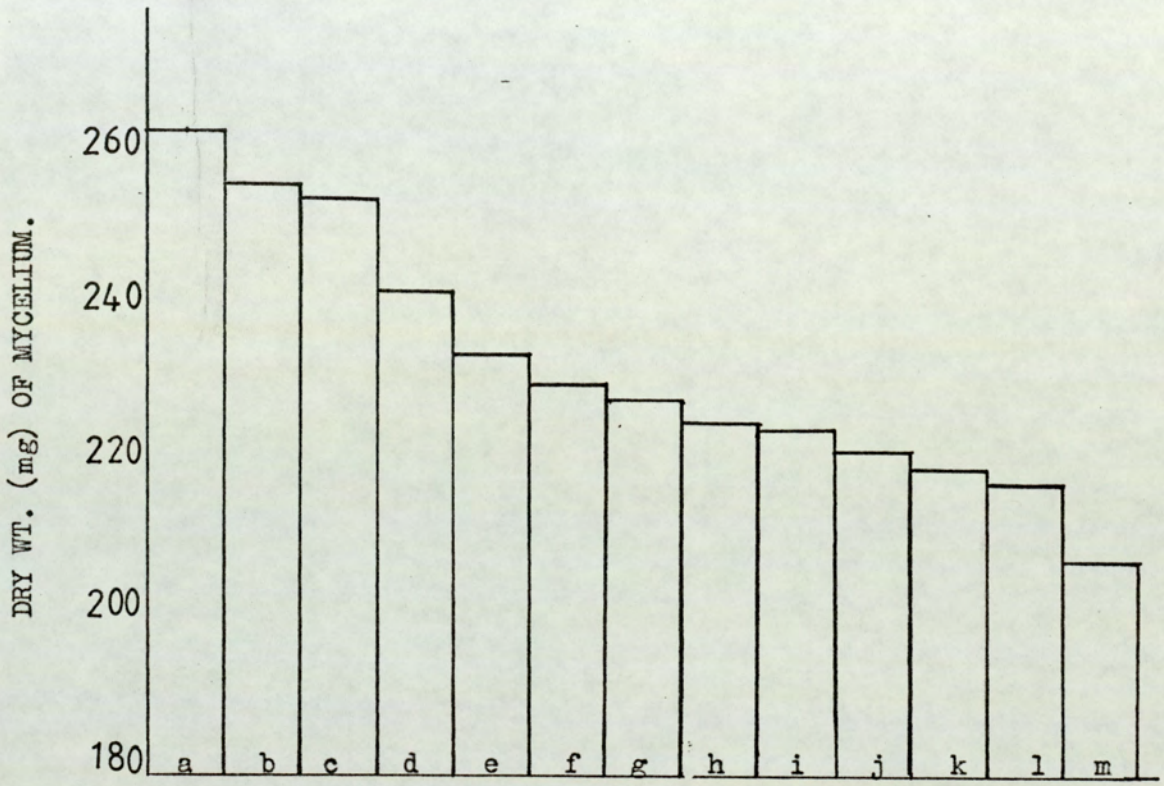


Fig.3.12 : EFFECT OF VARIOUS FATTY ACIDS AND ESTERS ON THE VEGETATIVE GROWTH OF P.SAJOR-CAJU.



a. Oleic acid
b. Ethyl acetate
c. Methyl stearate
d. Methyl oleate
e. Methyl palmitate
f. Linoleic acid
g. Ethyl oleate
h. Lipid-free control
i. Stearic acid
j. Myristic acid
k. Methyl myristate
l. Palmitic acid
m. Ethyl linoleate

95% confidence limits.

D - glucose	20.0 g
Peptone	2.0 g
KH_2PO_4	0.5 g
$MgSO_4 . 7H_2O$	0.5 g
$CaCl_2$	0.1 g
Thiamine	100. μ g
Distilled water	1.0 l

The fatty acids and esters were added separately at the concentration of 100 mg/l after being dissolved in 1ml of diethyl ether. All the ingredients were autoclaved together.

There was no significant (P = 0.05) effect of the lipid supplements. The greatest growth, however, was obtained from oleic acid (Fig.3.12). The yield from ethyl acetate, methyl stearate, methyl oleate, methyl palmitate, linoleic acid and ethyl oleate were just slightly greater than that of the lipid-free control. The yields from the remaining treatments were less than the control.

3.2.1.(e) TRACE ELEMENT REQUIREMENTS :

The trace element nutrition of A. bisporus has been studied (Treschow,1944; Hayes,1972). However, there is scanty information on the trace element requirements of other edible fungi. Jandaik (1976) studied iron, zinc, manganese, boron and molybdenum in connection with P. sajor-caju and found that iron and zinc increased mycelial growth.

In this experiment additional micro-elements have been investigated and compared with the effect of ashes from various natural substances on this edible species. The basal medium (BM) used was formulated by Coscarelli and Pramer (1962) with the following composition :-

Glucose	30.0 g
Asparagine	1.0 g
KCl	0.1 g
$MgSO_4 . 7H_2O$	0.5 g
Deionised water	1.0 l

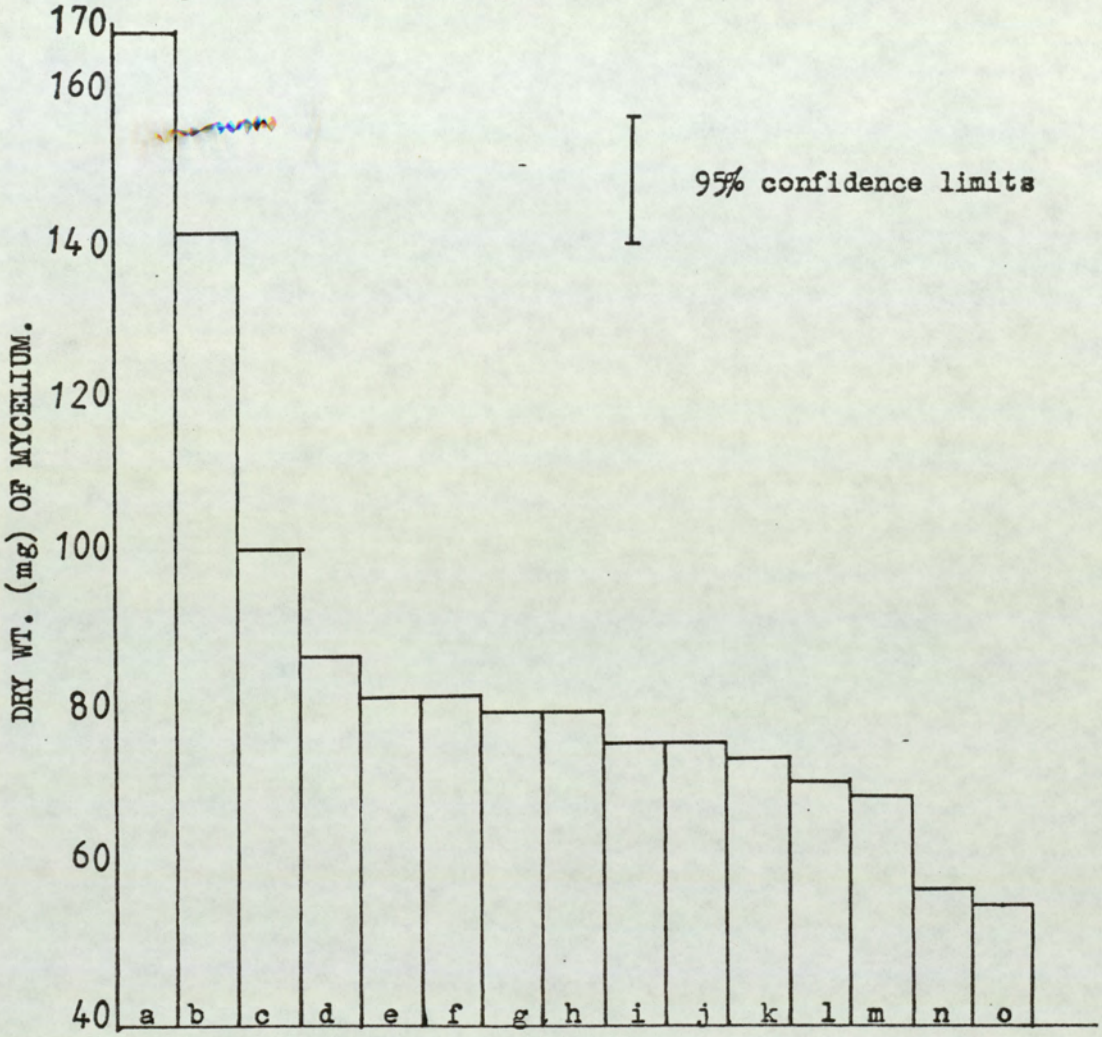
There were some modifications eg. adjusting the weights to make use of sulphates instead of oxides of zinc and manganese. A synthetic "complete" mineral solution (CM) was formulated thus, per litre :

$CaCl_2 . 2H_2O$	0.74 mg
$(NH_4)6Mo_7O_{24}$	0.37 mg
$CuSO_4 . 7H_2O$	0.80 mg
$MnSO_4 . 7H_2O$	0.60 mg
$ZnSO_4$	0.88 mg
$FeCl_3$	0.88 mg
LiCl	0.60 mg

These compounds were then eliminated in sequential single depletion so that the resulting solutions contained all but the metal investigated. Since the basal medium did not contain any vitamin, in addition to the two controls (BM + CM, positive control and BM-CM, negative control) two supplementary controls were introduced :
BM + thiamine (100μg) and BM + biotin (5μg).
pH was maintained at 6.0 with buffer.

Ash supplements were made at 0.4g/l. malt extract ash and yeast extract ash were prepared by incinerating their powders at 450°C in a muffle furnace for 18 hours. Rice and wheat straw ashes were prepared by first burning oven-dried straw in air before incineration at 450°C. The ashes were solubilised in small amounts of concentrated HCl (Coscarelli and Pramer,1962).

Fig.3.13 : EFFECT OF TRACE ELEMENTS AND ASH ON THE VEGETATIVE GROWTH OF P.SAJOR-CAJU IN A DEFINED LIQUID MEDIUM AFTER 3 WEEKS OF INCUBATION.



Supplements to the basal medium (BM)

a. Thiamine
b. Yeast extract ash
c. Biotin
d. Malt extract ash
e. Complete mineral soln. (CM) - Cu
f. CM - Fe
g. CM - Mo
h. Rice straw ash
i. CM - Li
j. CM - Ca
k. CM - Zn
l. Wheat straw ash
m. CM - Mn
n. CM
o. BM only

All the metals tried exerted approximately equal effects (Fig. 3.13). The dry weight obtained from the "complete" mineral solution was the second lowest, the lowest being that from "trace-element-free" control. By far the greatest yield was obtained from the flasks containing thiamine (replacing the trace elements) followed by those with yeast extract ash and biotin in that order. Generally there was a drastic fall in pH in all except the flasks containing the various ashes.

3.2.1.(f) EFFECT OF OXYGEN AND CARBON DIOXIDE TENSION.

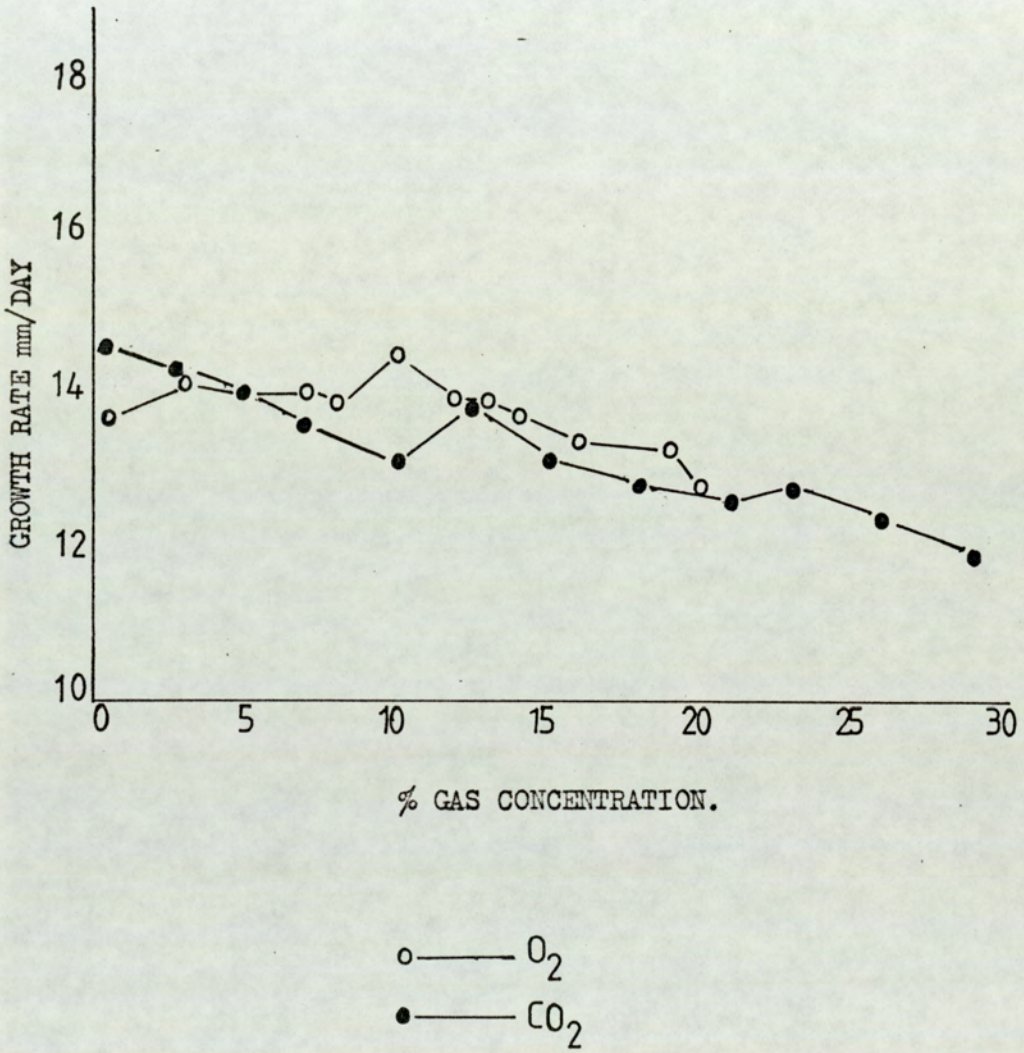
These experiments were carried out to determine the direct influence of oxygen and carbon-dioxide concentrations on the growth of mycelium. The technique of Griffin, et al (1967) described earlier (Section 2.6) was used. This involved the use of a sand-filled gas diffusion column through which known concentrations of gases introduced at both ends created predictable concentration gradient.

Growth response in oxygen range of 0.3 - 21.0% and carbon-dioxide range of 0 - 30.0% were measured. However, the practicable ranges that could be used were 0.48 - 20.4% for oxygen and 0.48 - 29.4% for carbon-dioxide. After introducing the gases at the beginning of each experiment the column was allowed to stabilize for 24 hours.

OXYGEN : When the influence of oxygen was to be assessed, atmospheric air (containing 21% O_2) was introduced at one end and nitrogen gas was introduced at the other end to flow against air. Nitrogen was acting as an inactive displacement gas to replace oxygen as its concentration fell along the length of the column. 0.3% represented its maximum level as an impurity in the commercial nitrogen used.

As Fig. 3.14 reveals, there seemed to be only very minor effect on mycelial growth by oxygen within the range of concentration

Fig.3.14 : EFFECT OF OXYGEN AND CARBON DIOXIDE CONCENTRATION ON THE VEGETATIVE GROWTH OF P.SAJOR-CAJU.



tested. The least growth was obtained at the highest concentration 20.4% but the yield (growth rate 12.8mm/day) was not significantly (P = 0.05) lower than the highest yield (14.5mm/day) at 10.2% oxygen. Growth remained fairly constant within this range. The critical concentration of oxygen, i.e. that at which growth could be said to be effectively constrained in nature, was considered to be the point where growth was half the maximum. This critical point was not reached.

CARBON DIOXIDE : The procedure was the same as above. Commercial Carbon-dioxide was introduced at one end of the column, carbon dioxide-free air was introduced at the other end. Period of incubation, like above, was 16 days.

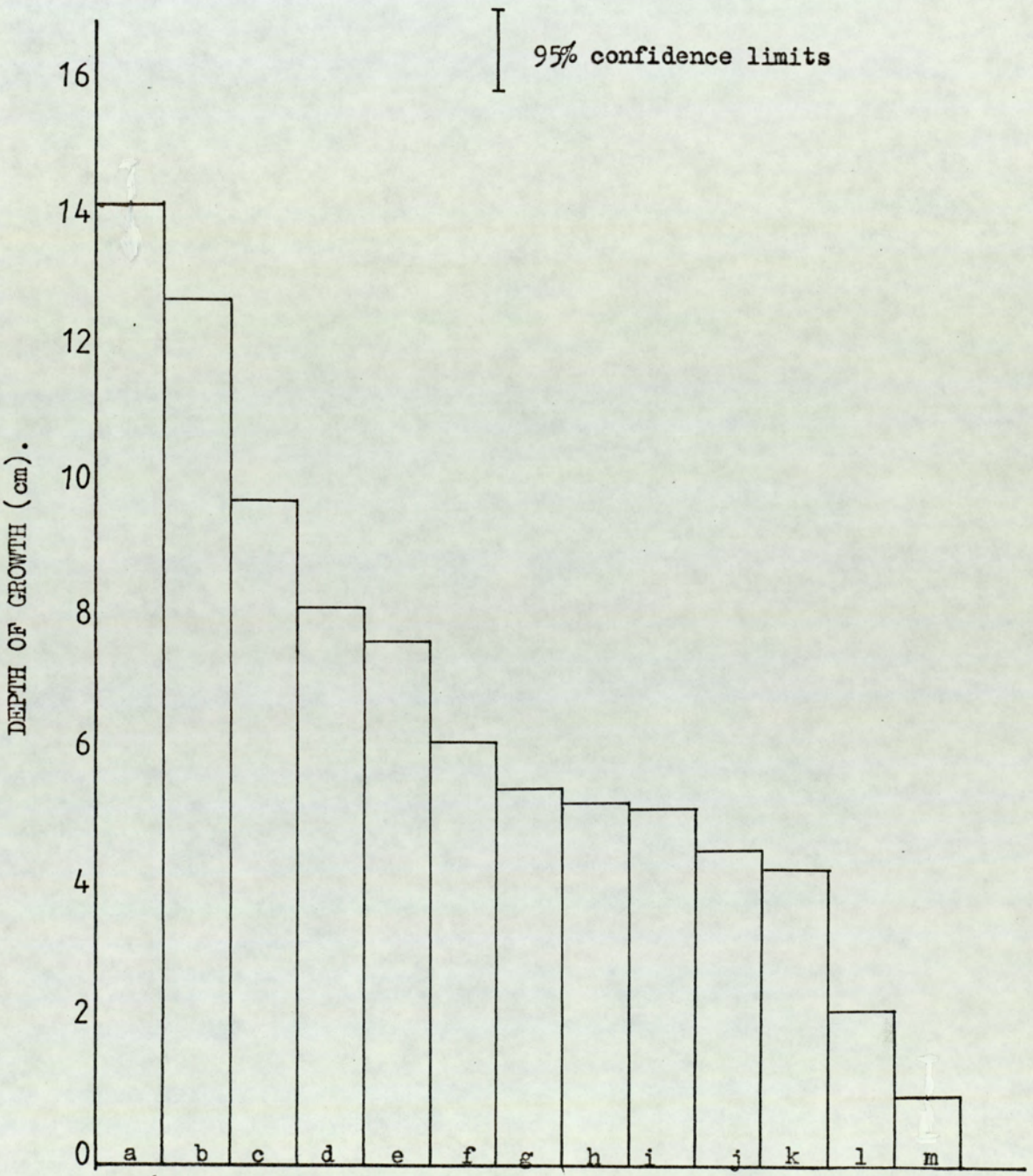
As can be seen from Fig. 3.14 , although no actual increase in growth occurred with increased carbon dioxide concentration, P. sajor-caju could tolerate much higher concentrations than atmospheric level of 0.03%. The lowest growth rate, 12.4mm/day, occurred at the highest carbon dioxide concentration 29.4% but this was not very significantly (P = 0.05) lower than the maximum 14.6mm/day at the lowest concentration 0.48%.

3.2.1.(9) GROWTH ON SOLID NATURAL SUBSTRATES.:

Up to this point of the work, most of the substrates employed have been laboratory media of purely synthetic or semi-synthetic nature or at least industrially treated substances. P. sajor-caju is a saprophyte on soft plant material (Jandaik,1974). It was therefore considered relevant to the possibilities of cultivating this species, to assess its vegetative growth on mostly untreated plant wastes.

The method has already been described in 2.7. The wastes used were rice straw (Oryza sativa), wheat straw (Triticum aestivum),

Fig.3.15 : VEGETATIVE GROWTH OF P.SAJOR-CAJU ON VARIOUS SOLID NATURAL SUBSTRATES IN GLASS TUBES FOR 12 DAYS.



a. Rice straw
b. Wheat straw
c. Mushroom compost (sterile)
d. Lawson's cypress litter
e. Sycamore litter
f. Mushroom compost (non-sterile)
g. Fir litter
h. Larch litter (fresh)
i. Old newspaper
j. Oak litter
k. Chestnut litter
l. Larch litter (1-yr. old)
m. Pine litter

composted wheat straw (mushroom compost), old newspaper, pine letter (Pinus sylvestris), one-year old larch litter (Latrix decidua) fresh litters from larch, Chestnut (Aesculus hippeastanum), sycamore (Acer pseudoplatarus) and Lawsons Cypress (Cupressus species)

Fig. 3.15 shows that the greatest growth occurred on rice straw, wheat straw and mushroom compost in that order. Growth on Lawsons cypress litter, sycamore litter and unsterile mushroom compost followed. The poorest growth was obtained from pine litter.

3.2.2. REPRODUCTIVE STAGE

3.2.2.(a) EFFECT OF LIGHT ON FRUCTIFICATION

In the preliminary investigations cultures of P.sajor-caju either failed to fruit or produced initials which could not develop further in dark incubators. On the contrary they produced better developed fruit-bodies when exposed to light. In view of this observation the following experiments were designed to study the influence of light on its fructification.

(i) 35 Petri-plates of 2% malt extract agar were prepared and inoculated :

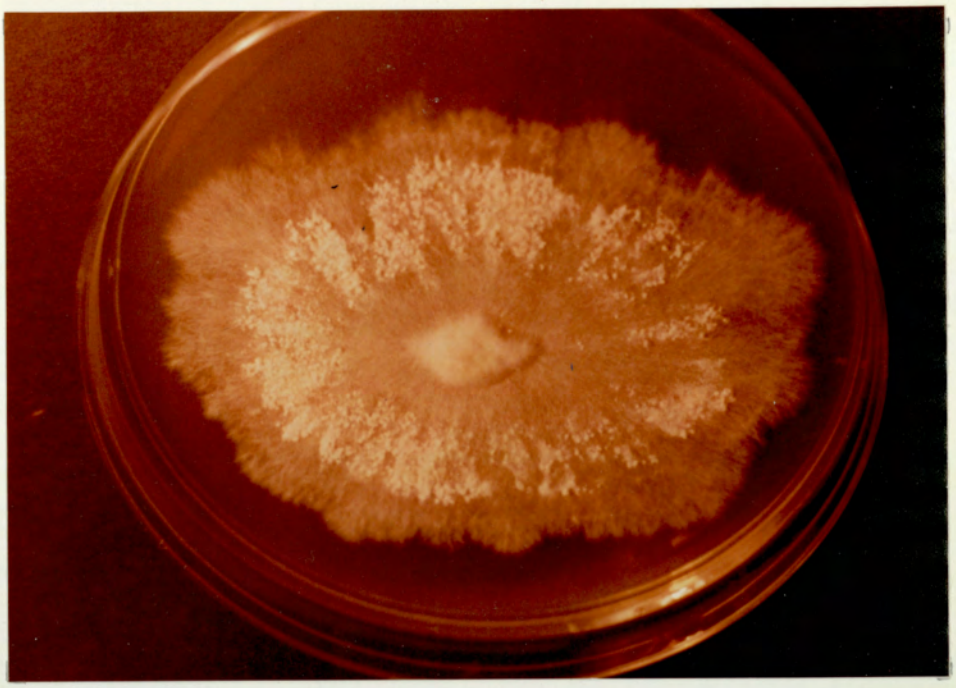
A) 25 of them were incubated in continuous darkness

B) 5 were incubated in alternating light (12 hours of 460 lux) and dark periods.

C) 5 in continuous light (460 lux).

After five days, 5 plates were withdrawn from A, examined and discarded after examination. Subsequent examinations were carried out at7-day intervals. The plates in B and C were examined on the 5th and 7th days and left for further development

Plate 3.2 Effect of light on the growth and fruit-body formation of P.sajor-caju.



(a) In continuous light :poor mycelial growth,numerous primordia.

(b) In alternating light and dark conditions:more mycelial growth,less primordia.

Plate 3.2(continued)

(c) In continuous darkness:profuse mycelial growth, no primordia.

The results are given in Table 3.2.

Table 3.2. Effect of Light on Fruit-body Formation of Pleurotus sajor-caju grown on Malt Extract Agar.

	Continuous dark.	Alt. light & dark.	Continuous light.
Mean growth rate in 5 days (mm/day)	15.8	12.3	7.0
Mean no. of primordia after 7 days	0	55	330

As evident in the above table, vegetative growth after 5 days in A (continuous darkness) was the greatest and growth in C (continuous light) was the least. The effect on primordium formation was in reverse order to that on vegetative growth. After 7 days no primordia were formed in A while 55 and 330 primordia were produced in B and C respectively. (Plate 3.2)

(ii) The second experiment was similar to (i) above except that the substrate in this case was rice straw treated as in Section 2.9 instead of malt agar in Petri-plates.

A) In continuous darkness the mycelium had completely permeated and covered the substrate before the 12th day, with a fluffy over-growth covering the top edges of the pots to 2cm on the outside (see Plate 3.3). But up to the 28th day of incubation there were no primordia.

B) The pots in alternating light and dark periods had less vegetative growth but primordia were observed on the 15th day. These developed in mature fruit-bodies.

C) The pots in continuous light showed the least vegetative growth, the mycelium was scarcely visible on the top layers but numerous primordia

Plate 3.3 Effect of light on the vegetative growth and fruit-body formation of P.sajor-caju on rice straw .

i. In continuous darkness: profuse mycelial growth but no fruit-body.

ii. In alternating light and dark conditions:less mycelial growth ,few fruit-bodies.

iii. In continuous light :least mycelial growth ,many fruit-bodies.

had developed on the 12th day. These matured into fruit-bodies.

At the end of the experiment, 28 days, the yield was as in Table 3.3.

Table 3.3. Effect of Light on Fruit-body Formation of Pleurotus sajor-caju grown on Rice Straw.

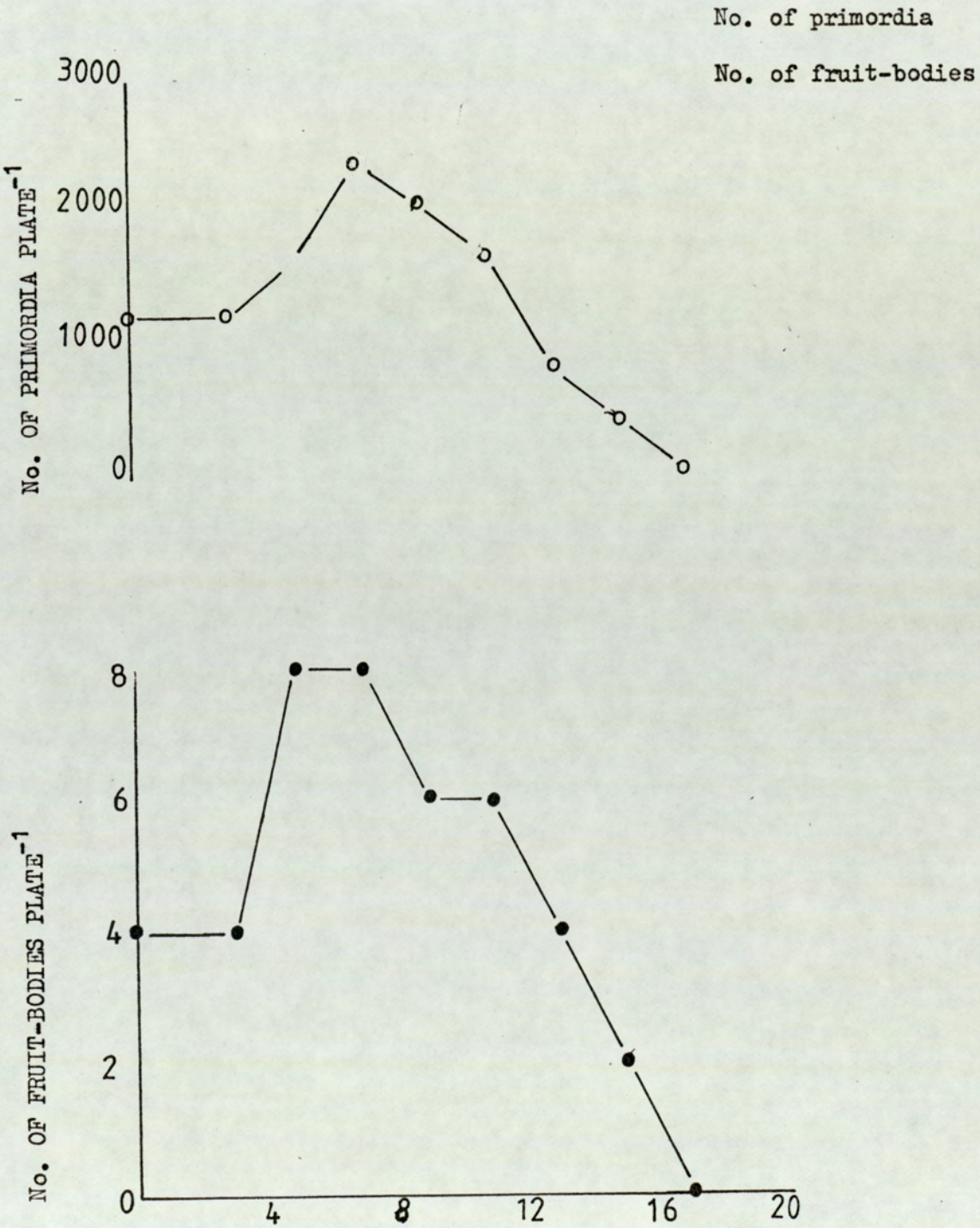
Replicates	Weight (g) of fresh mushrooms.		
	Continuous light C.	Alt.light & dark B.	Cont. dark A.
(i)	50.3	39.4	0
(ii)	70.0	25.7	0
(iii)	45.2	32.5	0
(iv)	55.9	42.8	0
(v)	48.1	20.3	0
Mean Weight	53.9	32.4	0
Yield / Kg	107.8	64.28	0
Time taken to produce primordia	12 days	15 days	0

(iii) The aim of this third experiment was to determine at what stage in the incubation of P. sajor-caju, was the mycelium most sensitive to light for fruit-body formation.

45 Petri-plates of 2% malt extract agar were prepared and inoculated. Five plates were exposed to continuous light from the start. The remaining 40 plates were left in continuous darkness from which 5 plates each were, at two days intervals, successively exposed to continuous light. The first set was exposed after 3 days to allow for the lag phase.

At the exposure of the first set, the mean colony diameter was 2.6cm. After two days numerous fruit-body initials appeared. The set in continuous light from the start had not yet initiated fruiting

Fig.3.16 : RESPONSE OF P.SAJOR-CAJU TO DIFFERENT DURATIONS OF CONTINUOUS DARK AND LIGHT CONDITIONS FOR FRUIT-BODY FORMATION.



and just 1.4cm in colony diameter.

The second set followed after 2 days with mean colony diameter of 6.26cm. All subsequent sets were completely covered with mycelium before exposure.

The results are summarized in Fig. 3.6 and indicate that the greatest number of initials and fruitbodies was produced in the set exposed seven days after incubation in continuous darkness. This also corresponded to the time when the mycelium was just touching the vertical edge of the plate. The yield of the plates in continuous light from the start was relatively low, due perhaps to poor mycelial growth.

3.2.2.(b) COMPARISON OF YIELD ON "CASED" AND "UNCASED" CULTURES :

P. sajor-caju does not normally require casing for fruitbody production. However, an experiment was performed to see whether covering the colonized substrate with a casing layer would improve the yield.

Mushroom compost (as used for A. bisporus cultivation) was employed as the substrate and the experiment was carried out as described in Section 2.9. Five pots were covered with a casing layer as for A. bisporus except that the pH was adjusted to 6.0. The other five pots were left uncovered.

Primordia appeared on the covered pots from the 12th day and on the uncovered ones from the 14th day. As shown in Table 3.4, the yield in the covered pots were insignificantly greater than the uncovered ones.

Table 3.4. COMPARISON OF YIELD OF PLEUROTUS SAJOR-CAJU ON 'CASED' AND 'UNCASED' COMPOST.

REPLICATES	FRESH WT (g)	
	CASED	UNCASED
i	59.88	51.82
ii	38.50	40.45
iii	49.65	45.67
iv	40.73	54.98
v	51.16	41.56
Total	239.92	234.48
Mean	47.98	46.90
Yield Fresh Wt/Kg Compost	95.96	93.80
Time taken to produce primordia	12 days	14 days

t value not significant at P = 0.05.

3.2.2.(c) EFFECT OF VITAMINS ON FRUITING :

The procedure employed here is similar to that used to determine the influence of the same vitamins on the vegetative growth (Section 3.2.1.(a)). The same medium was used. However, in this case 1.5% agar was added to solidify the medium and Petri-plates were employed instead of Erlenmeyer flasks.

The result illustrated on Plate 3.4, shows the same trend as the effect on vegetative growth. After 6 days of incubation yeast extract produced abundant primordia, a few of which developed into small fruit-bodies. Most of the other treatments yielded numerous primordia after 8 days, though less than in yeast extract. The only treatment without primordia was CV - T (i.e. without thiamine) as was the vitamin-free control.

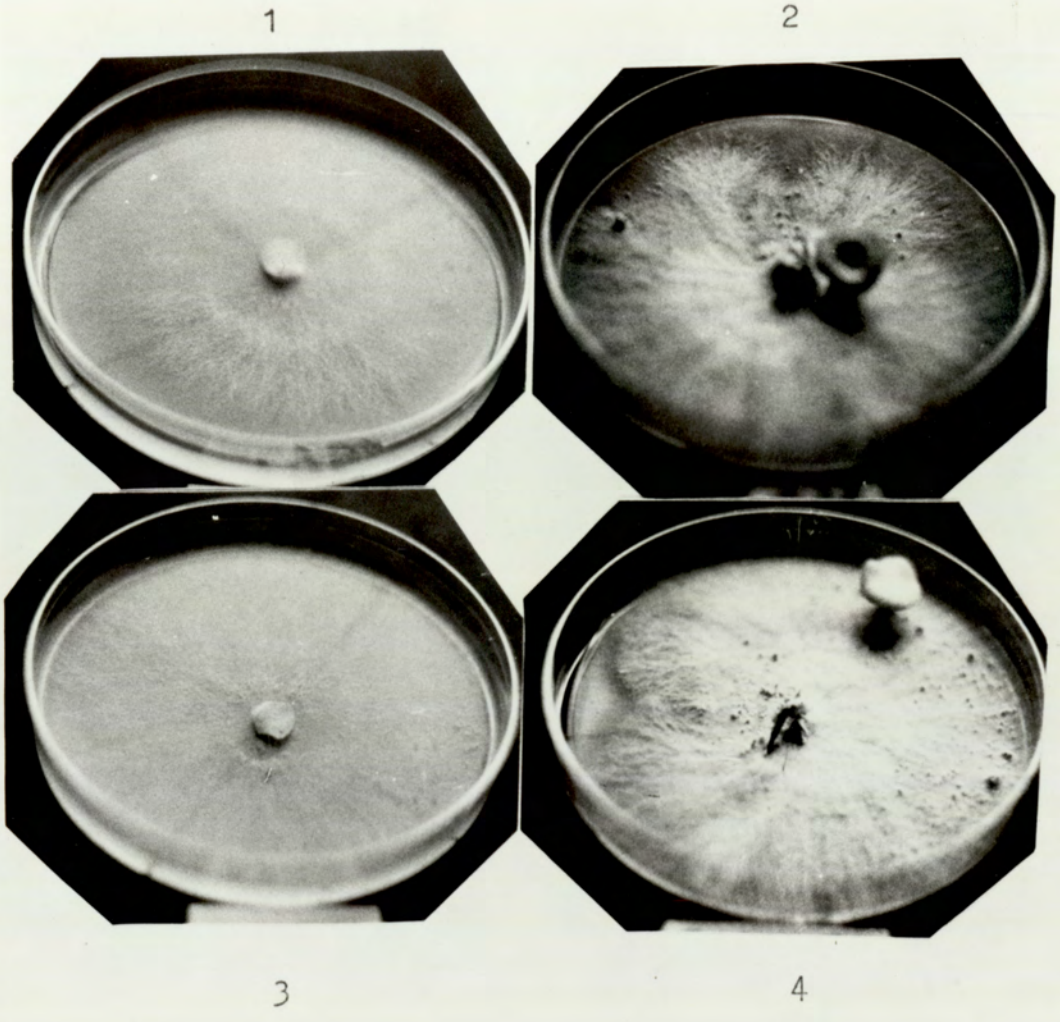
The mycelial growth also showed the same pattern. It is important to note that there was no significant difference between CV - T and the vitamin-free control. The yield on CV (complete vitamin treatment) and all the other treatments were similar.

3.2.2.(d) EFFECT OF FATTY ACIDS AND THEIR ESTERS ON FRUIT-BODY FORMATION

The fatty acids and esters used in this experiment were the same as those used in Section 3.2.1(d). The procedure was also the same except that while the medium in this experiment was solidified, the one used in Section 3.2.1(d) was liquid.

All treatments including the lipid-free control produced primordia from 15 days. Some primordia in each plate developed into small fruit-bodies.

Plate 3.4 Effect of vitamins on fruit-body formation of Pleurotus sajor-caju .



1:Basal medium (without vitamins) .

2:Basal medium + complete vitamin solution .

3:Basal medium with vitamins -thiamine .

4:Basal medium with vitamins - pantothenic acid .

3.2.3. CONCLUSIONS.

EFFECT OF VITAMINS :

From the results, it is clear that among the various vitamins tested, only thiamine was indispensable to the growth of P. sajor-caju, both for vegetative growth and fruit-body formation. This is in agreement with earlier investigations referred to in this text (Section 1.7).

In both experiments, growth and primordia formation and development were almost equal in the "complete" vitamin solution and those treatments with single vitamin depletion (except thiamine), implying that no other vitamin was necessary for normal growth and reproduction. Except Marasmius androsaceus and Collybia dryophila (Lindeberg,1946a), no known Hymenomycete fungus has been shown to be biotin deficient. Treshow (1944) stated that A. bisporus required an exogenous supply of either thiamine or biotin. Norkrans (1950) asserted that no other vitamins had been shown to be required by any other naturally occurring Hymenomycetes.

Although Volz (1972) indicated that some of his test fungi including L. nuda and P. ostreatus did not require thiamine for vegetative growth, evidence of other workers like Norkrans (1950); Lindeberg (1946a) and Wright (1976) for L. nuda; and Block et al (1963), Hashimoto and Takahashi (1974) for P. ostreatus, cast doubt to the authenticity of his assertion. It has been suggested that it is the pyrimidine moity of thiamine that is the active component (Norkrans,1950; Lilly & Barnett,1951). The yield in yeast extract treatment was much greater than the complete vitamin solution. This suggests that yeast extract contains some other growth substances not included in the vitamin mix even though analysis of "Oxoid" yeast extract does not indicate so

(Oxoid Manual 3rd Edition). It could also be that it was serving as an additional carbon and nitrogen source. Concentration effect might also have played a part.

NITROGEN SOURCES:

Organic sources of nitrogen were best utilized by P.sajor-caju.Ammonium nitrogen and asparagine supported moderate growth. This agrees with the findings of previous investigators. Cochrane(1958) stated that fungi in general were active utilizers of proteins and other organic sources of nitrogen.He also said that the enzyme urease was produced by most fleshy basidiomycetes that had been studied.

Ammonium tartrate was found to be better than other ammonium sources. Norkrans (1950) observed that of the ammonium souces,the tartrate and phosphate were more suitable than the chloride and sulphate, probably because the latter two caused a more marked shift in pH values, too low to support growth.This is so with other ammonium salts of strong acids. The slightly better growth in ammonium bicarbonate than ammonium nitrate was in accordance with this observation.

Nitrate sources supported varied amounts of growth. While ammonium nitrate supported moderate growth, sodium nitrate was very poorly metabolized. Nitrate users are uncommon among basidiomycetes (Lindeberg, 1946a, 1970; Norkrans, 1950; and Fries, 1955). Lilly and Barnett (1951) published a list of nitrate users, but it contained only a few basidiomycetes. L.nuda, however, utilized nitrates well,Norkrans (1950). Regarding the moderate utilization of by P.sajor-caju in this investigation, it may be asked whether there was preferential utilization of the ammonium ion or the nitrate ion. However, evidence supporting the preferential/
metabolization of ammonium ion in a medium containing ammonium nitrate exists (Lilly & Barnett, 1951; Fries, 1955; and Cochrane, (1958).

Ammonia suppresses nitrate assimilation (Fries,1955).

The nitrite nitrogen source employed in this investigation was potassium nitrite which proved to be quite toxic to P.sajor-caju. Some Coprinus species utilized nitrites in Fries' work (1955) although many could not. Only one nitrite source was employed in this work and therefore generalisations cannot be made. However, failure to utilize nitrite nitrogen is a common situation in fungi (Cochraine,1958) because of its toxicity.

The concentration of a nitrogen source in the medium is important for fruiting. There is no general optimum concentration for nitrogen sources. Optimum concentrations depend on specific conditions including the source and concentration of the carbon sources. In general high concentrations of nitrogen suppress fruiting.

The near absence of growth in the nitrogen-free control was surprising in view of the report of Rangaswami et al (1975) that P.sajor-caju fixed atmospheric nitrogen. Other Pleurotus species have also been said to be capable of nitrogen fixation (Rangad & Jandaik,1977; Giovannozzi-Sermanni, et al(1979). This claim cannot apply,for, if P.sajor-caju could fix atmospheric nitrogen, why could it not grow in a nitrogen-free medium in which other factors were not limiting.

CARBON : NITROGEN RATIO.:

The optimum C : N ratio for P.sajor-caju was determined to be 90 : 1. This is quite high compared with the optimum for A.bisporus 17 : 1 (Smith & Hayes,1972), and 21 : 1 for L.nuda and L.saeva (Wright,1976). The optimum C : N ratio is defined as, "that balance of carbon and nitrogen, at a specific concentration and in relation to other components of the medium, which places no restriction on metabolism and cell synthesis

required for maximal rates of growth" (Wright,1976). C : N ratio assessment in natural substrates is difficult because of the heterogenous nature of these substances. The values obtained from in vitrio determinations still depend on the nature of the substances used, the physical conditions as well as the duration of incubation.

FATTY ACIDS :

In this investigation fatty acids did not exert any significant influence on the growth and reproduction of P.sajor-caju. Some investigators have described positive effects on other fungi like A.bisporus (Wardle & Schisler, 1969) and P.sapidus (Kurtzman Jr.,1976). Growth was quite good in all treatments including the lipid-free control, and the number of primordia produced in the solid media was not significantly different. One limitation of this experiment was that only one concentration was tried. However, growth on oleic acid was the greatest. Although this fatty acid promoted growth in Wardle and Schisler's experiment and Wright's investigation (Wright,1976) no generalisation could be made.

TRACE ELEMENTS :

The yields obtained by sequential elimination of various metals from the "complete" mineral mix were approximately equal and poor. This made interpretation difficult. However, growth in the control (without any of the metals investigated) was significantly lower, suggesting that the metals did exert influence. The poor yields might have been due to the absence of vitamins in the media (compare with the treatment where thiamine replaced the minerals). The drastic fall in pH in most of the flasks could also account for the poor yields. The yield in the "complete" mineral solution was also poor. This might be attributed to toxicity due to high concentrations of the metals.

Ash from yeast extract supported good growth, but other ashes from rice straw, wheat straw and malt extract were not as effective in supporting growth.

EFFECT OF OXYGEN AND CARBON DIOXIDE TENSION ON VEGETATIVE GROWTH :

In this investigation Pleurotus sajor-caju has shown high tolerance of carbon dioxide. There was no apparent positive influence on growth but it was not inhibited up to a concentration of 29.3%. The maximum growth rate was 14.6mm/day and the least growth rate was 12.4mm/day. Carbon dioxide concentration therefore is unlikely to be a growth suppressant either in the natural habitat or in growing houses.

Zadražil (1974) found that carbon dioxide concentration up to 28% stimulated the growth of P.ostreatus and P.florida and up to 22% stimulated the growth of P.eryngii. The highest concentration used in his work was 37.5% and this reduced growth by 40% while 32% concentration of carbon dioxide completely halted the growth of A.bisporus. He thus reasoned that high carbon dioxide concentration in the substrate could serve as a shield for Pleurotus species against other micro-organisms, which either could not grow or die off at such high concentrations.

In Wright's work (Wright,1976) as low as 5% carbon dioxide was toxic to L.nuda, L.saeva and Calocybe gambosa. A.bisporus growth is stimulated at concentrations of 0.3 - 0.6% (San Anthonio & Thomas 1972) but is significantly inhibited at 6.6% (Nair,1972). The influence of carbon dioxide on primordium formation in A.bisporus has been shown by Lambert (1933); Tschierpe and Sinden (1964); Long & Jacob (1968) and Nair & Hayes (1974) as reviewed earlier (section 1.10).

As regards oxygen concentration effect, within the range 0.5 - 20.4% employed in this work oxygen concentration had no significant

effect on the vegetative growth of P. sajor-caju. This species therefore can grow well at semi-anaerobic as well as aerobic conditions, an advantage in commercial cultivation as many common contaminating micro-organisms cannot grow in semi-anaerobic situations. It could not be determined in this experiment if P. sajor-caju could grow in completely anaerobic conditions. Zadražil (1974), however, stated that the beneficial effect of carbon dioxide on the mycelial growth of Pleurotus species did not preclude the importance of oxygen. He obtained no growth in complete absence of oxygen. Nevertheless, oxygen deficiency is quite unlikely to influence the vegetative growth of this fungus either in its natural habitat or in growing houses.

In the reproductive stage the need for oxygen seems to rise. For good yield of normal fruit-bodies, ventilation is mandatory. Although no experiment was specifically designed to measure this effect, insufficient illumination and lack of ventilation had a combined effect of regeneration of fruit-bodies (from fruit-bodies) (see Plate 3.6), reducing both the quality and yield of fruit-bodies. This observation was also made by Zadražil (1974) for P. ostreatus.

GROWTH ON SOLID NATURAL SUBSTRATES :

The result summarized in Fig. 3.15 indicates that straw, uncomposted as well as composted, was preferred as a substrate by P. sajor-caju to other plant waste products tried in this investigation. The reason was not investigated but it may be related to the composition of the various substances. The various leaf litters might have contained certain toxic plant products that inhibited the growth of this fungus. They might have lacked certain nutrients required by P. sajor-caju. Old newspaper is mainly cellulose and such a substance was definitely poorer than other substrates containing various carbon sources (hemicelluloses,

lignin, etc., in addition to cellulose), nitrogen and other nutrients.

Cereal straw has traditionally been used as a substrate in the mushroom industry. The major components cellulose, hemicelluloses and lignin account for over 80% of the dry matter of the straw (Morrison, 1979; and Lynch, 1979). All these fractions are known to be utilized by A. bisporus. Treschow (1944) and Bohus (1959) stated that hemicelluloses particularly xylan, were utilized first, followed by cellulose and then lignin. But according to more recent researches, lignin is utilized mostly in the vegetative phase while in the fruit-body formation stage cellulose and pentosan make up the major carbon sources (Turner, 1975; Hayes, 1978 and Wood & Goodenough, 1977). According to Evans (1979), cellulose microfibrils are physically encapsulated by lignin in a hydrophobic matrix, thus preventing wetting and access to cellulose enzyme complexes. It therefore follows that in straw degradation, before cellulose decomposition could be achieved, lignin has to be digested first.

The difference in yield produced on uncomposted and composted wheat straw was caused mainly by the fermentation process in composted straw and the nitrogenous and other additives contained therein. The composting process is usually done in such a way that the finished product has a C : N ratio of about 17 : 1 which is the optimum for A. bisporus. Straw has a high C : N ratio of 100 : 1 (Lynch, 1979) which is close to 90 : 1, the optimum determined for P. sajor-caju. It is therefore not surprising that this species preferred uncomposted straw to the conventional mushroom compost as its substrate. The concentration of nitrogen sources in the compost might have reached toxic levels for P. sajor-caju.

Another reason for this preference might be the high initial pH of compost, 7.0 - 8.0, vis-a-vis 5.0 - 6.0, the optimum pH of P. sajor-caju.

COVERED AND UNCOVERED SUBSTRATE :

As can be seen from Table 3.4,there was no significant difference in the yield obtained from covered and uncovered pots. This therefore shows that casing is not a requirement in the cultivation of P.sajor-caju. However, casing the substrate had the advantage of maintaining a good water balance. The uncovered pots were frequently drying out and therefore had to be watered occasionally, a problem not encountered in the covered pots. On the other hand, fruit-bodies produced on cased pots were 'dirty'. Particles of the casing material which stuck in between the gills were difficult to remove.

LIGHT REQUIREMENTS :

The results clearly indicate that light inhibits vegetative growth but is absolutely required for the formation of primordia. Development of fruit-bodies from the primordial stage also requires a certain amount of light. For, when a plate containing developing primordia was put in the dark, the primordia gradually reverted to mycelium. This observation was also made by Eger (1974) for Pleurotus ostreatus. The sensitivity of the mycelium to light was not uniform throughout the life of the mycelium. In plate cultures the mycelium was found to be most sensitive when it was just touching the vertical wall of the plate.

Since vegetative growth must necessarily precede reproduction, there is a threshold amount of nutrient accumulation before the advent of fruit-body formation. Cultures of P.sajor-caju should therefore be incubated in the dark (or semi-dark) conditions for some days for good vegetative growth before exposure to light conditions for fruit-body formation.

The above observations have been made by earlier workers

for some other fleshy fungi as reviewed in Section 1.10, but not for P. sajor-caju. On the contrary Jandaik and Kapoor (1974) claimed that light did not affect sporophore production of P. sajor-caju.

PART 3. STROPHARIA RUGOSO-ANNULATA.

The contrasting basidiomycete chosen for special study - S. rugoso-annulata - was investigated in a series of experiments which when appropriate were done in parallel with those for P. sajor-caju. This fungus however, did not form primordia or fruit-bodies in Petri-plate culture in the preliminary work reported in Section 3.1. and also when grown on straw a casing soil was required to induce fructification. In order to study reproductive growth therefore experimental procedure differed from those adopted for P. sajor-caju.

3.3.1. VEGETATIVE GROWTH

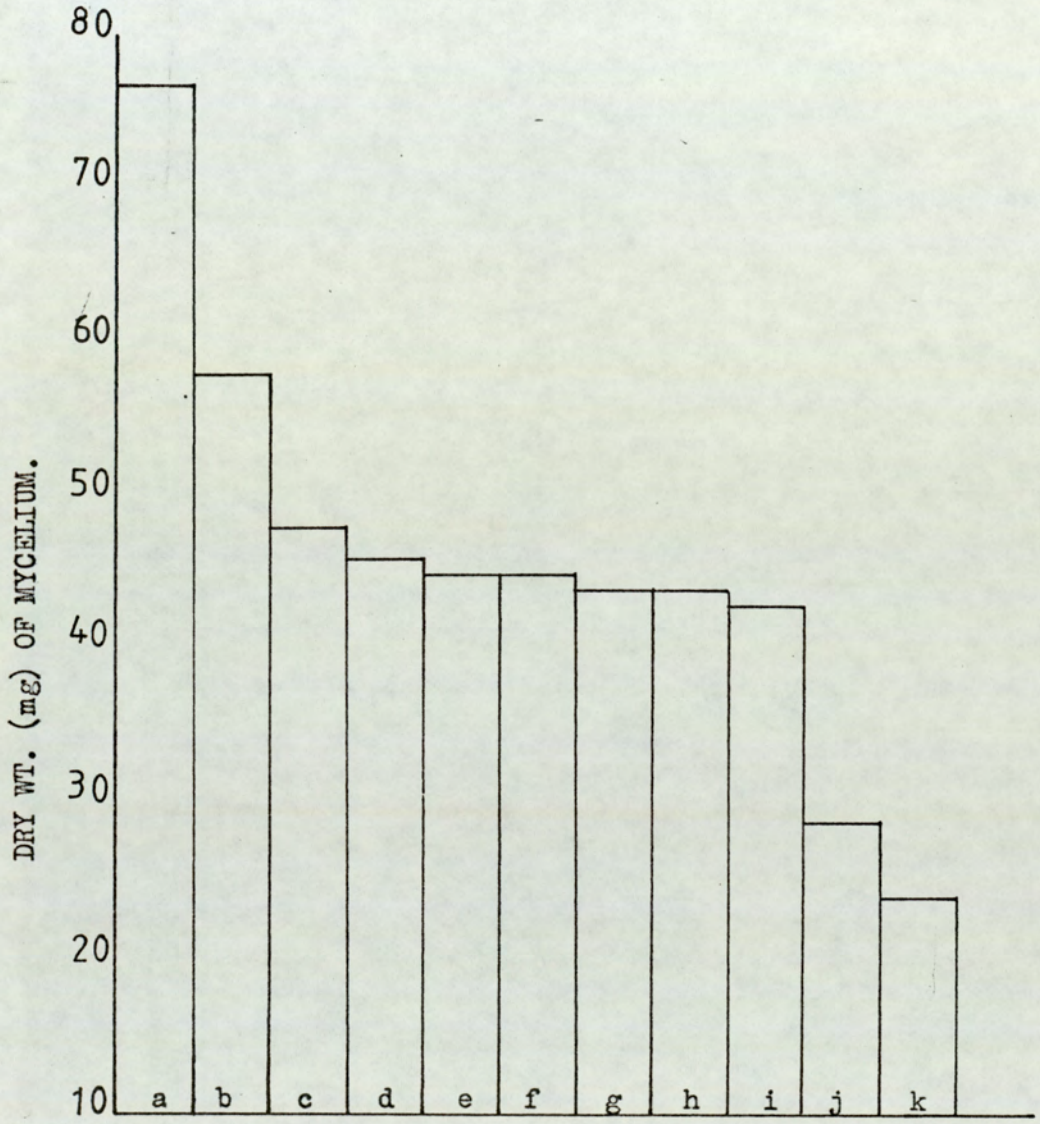
(a) EFFECT OF VITAMINS AND YEAST EXTRACT :

Vitamins exerted similar effects to those on P. sajor-caju. As summarized in Fig. 3.17, the greatest growth occurred on yeast extract and complete vitamin solution. Elimination of single vitamins, except thiamine, did not affect yield significantly. On the contrary there was drastic reduction of growth when thiamine was eliminated and also in the complete absence of vitamins.

(b) EFFECT OF VARIOUS NITROGEN SOURCES :

The best growth was obtained on organic and ammonium sources of nitrogen. Ammonium nitrate supported, rather surprisingly, the greatest growth of all ammonium sources. Sodium nitrate and potassium nitrite proved to be toxic to growth. These observations were generally similar to those made for P. sajor-caju. However, casein hydrolysate which supported the greatest growth of P. sajor-caju was in this case only moderately utilized. Ammonium nitrate was a more suitable nitrogen source for S. rugoso-annulata than for P. sajor-caju Fig. (3.18)

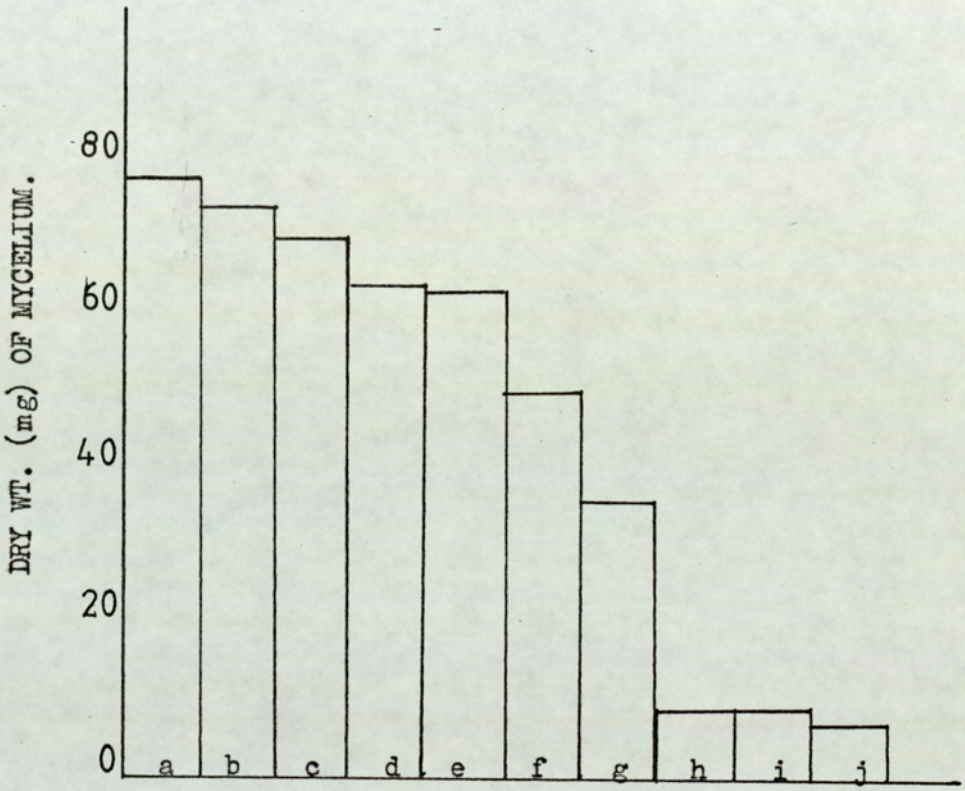
Fig.3.17 : EFFECT OF VARIOUS VITAMINS AND YEAST EXTRACT ON THE VEGETATIVE GROWTH OF S.RUGOSO-ANNULATA AFTER 5 WEEKS OF INCUBATION.



a. Yeast extract
b. Complete vitamin soln. (CV)
c. CV - inositol
d. CV - pantothenic acid
e. CV - nicotinic acid
f. CV - folic acid
g. CV - pyridoxine
h. CV - biotin
i. CV - riboflavin
j. CV - thiamine
k. Vitamin-free control.

95% cofidence limits.

Fig.3.18 : EFFECT OF VARIOUS NITROGEN SOURCES ON THE VEGETATIVE GROWTH OF S.RUGOSO-ANNULATA AFTER 4 WEEKS OF INCUBATION.



a. Peptone

b. Ammonium nitrate

c. Ammonium bicarbonate

d. Ammonium tartrate

e. Urea

f. Casein hydrolysate

g. Asparagine

h. Sodium nitrate

i. Potassium nitrite

j. Nitrogen-free control.

95% confidence limits

(c) CARBON : NITROGEN RATIO :

The optimum C : N ratio was 80 : 1 (Fig. 3.19) a high value when compared with 17 : 1 of A.bisporus but lower than that of P.sajor-caju (90 : 1) and comparable with 75 : 1 of V.volvacea (Chang-Ho and Ho, 1979).

(d) EFFECT OF FATTY ACIDS AND THEIR ESTERS :

The supplementation of the medium with fatty acids and their esters exerted more sugnificant effect on the growth of S.rugoso-annulata than P.sajor-caju (Fig. 3.20). Palmitic acid, myristic acid, linoleic acid and its ester and methyl oleate supported the greatest yield. Stearic acid, ethyl oleate, methyl palmitate and oleic acid caused moderate growth increase. The remaining esters showed only little growth increase compared with the control. Ethyl acetate proved to be inhibitory to growth.

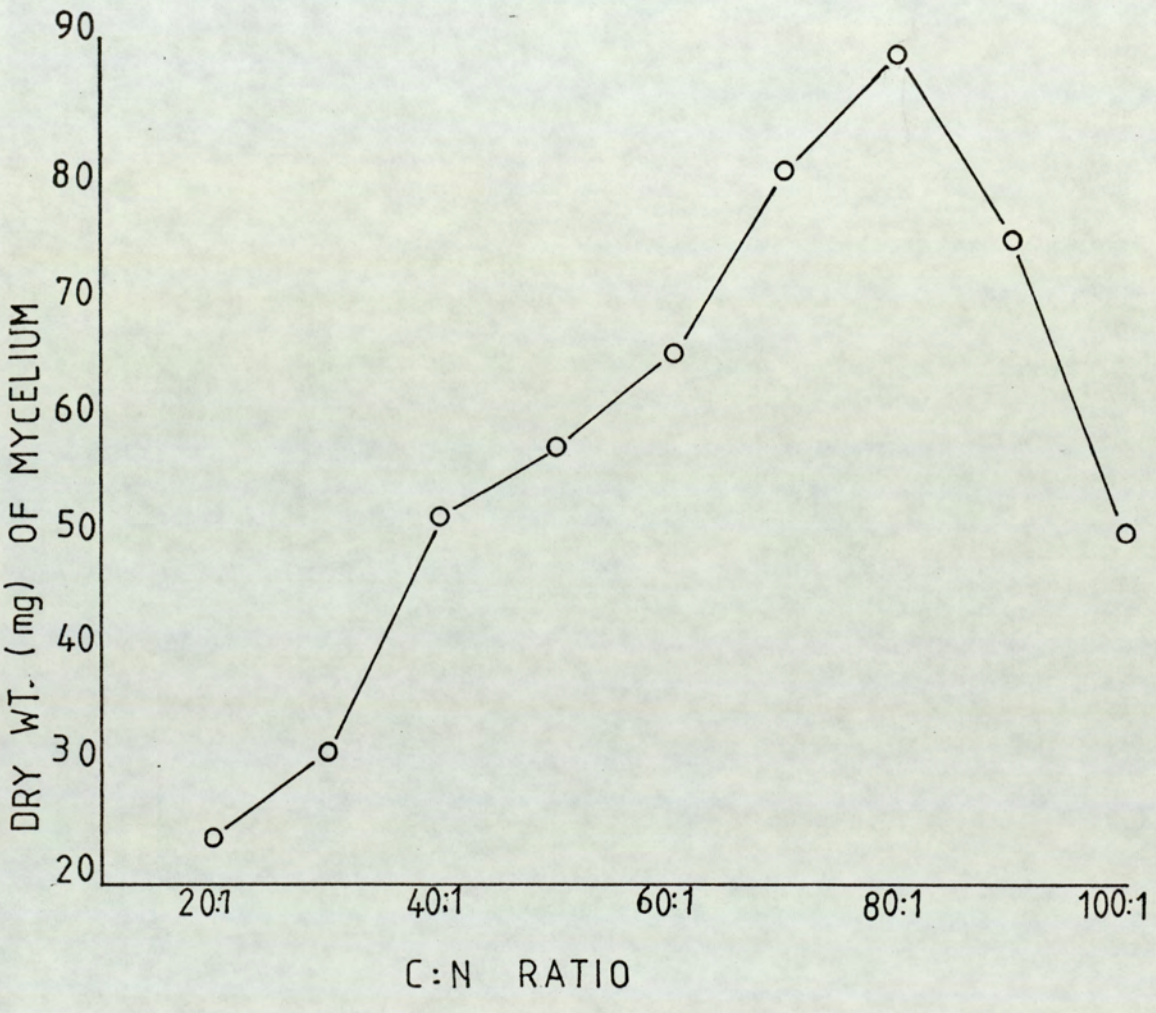
(e) INFLUENCE OF TRACE ELEMENTS AND ASH :

The smallest yield was obtained from the trace element-free control and the iron-free treatment. The flasks without copper and molybdenum produced the greatest growth, but the other treatment including the "complete" trace element solution did not influence growth to any extent. (Fig. 3.21)

(f) EFFECT OF OXYGEN AND CARBON DIOXIDE CONCENTRATION :

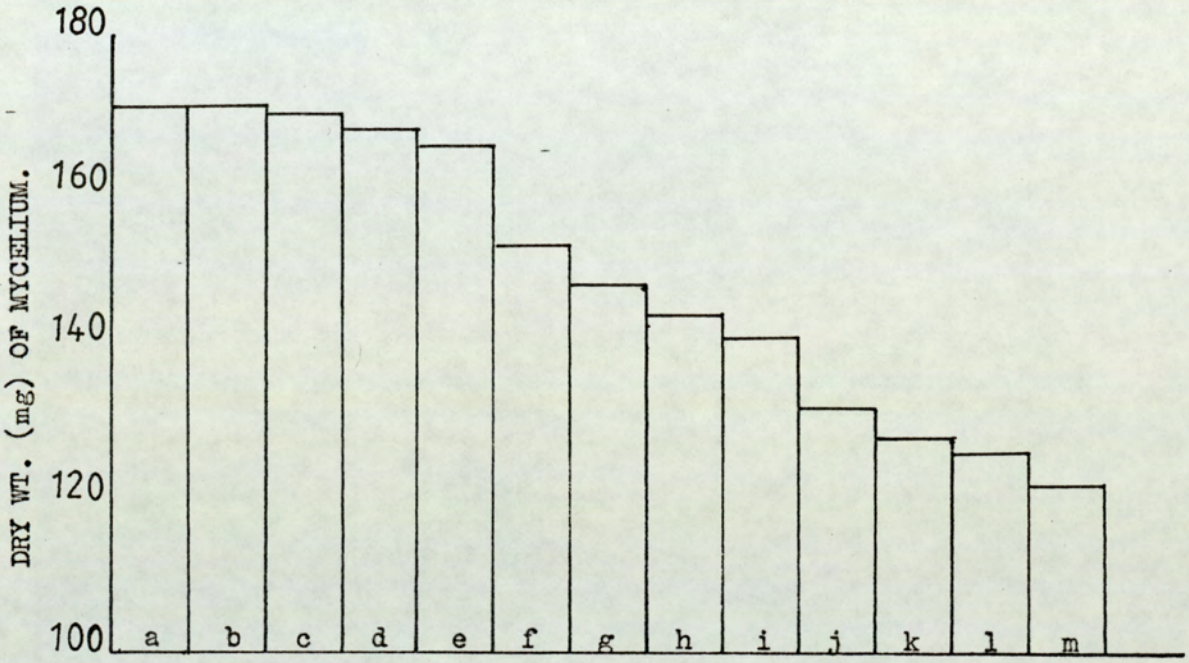
Between 0.4% and 4.7% oxygen there was a rapid increase in growth with increasing oxygen concentration (Fig. 3.22). Increase in growth became less apparent from 4.7% to 12.3% from which the increase rose to 20.4%.

Fig.3.19 : OPTIMUM CARBON : NITROGEN RATIO FOR THE VEGETATIVE GROWTH OF S.RUGOSO-ANNULATA AT 1g/LITRE (0.19gN/L) AFTER 4 WEEKS OF INCUBATION.



95% confidence limits.

Fig.3.20 : EFFECT OF VARIOUS FATTY ACIDS AND ESTERS ON THE VEGETATIVE GROWTH OF STROPHARIA RUGOSO-ANNULATA.



a. = Palmitic acid

b. = Myristic acid

c. = Linoleic acid

d. = Ethyl linoleate

e. = Methyl oleate

f. = Stearic acid

g. = Ethyl oleate

h. = Methyl palmitate

i. = Oleic acid

j. = Methyl stearate

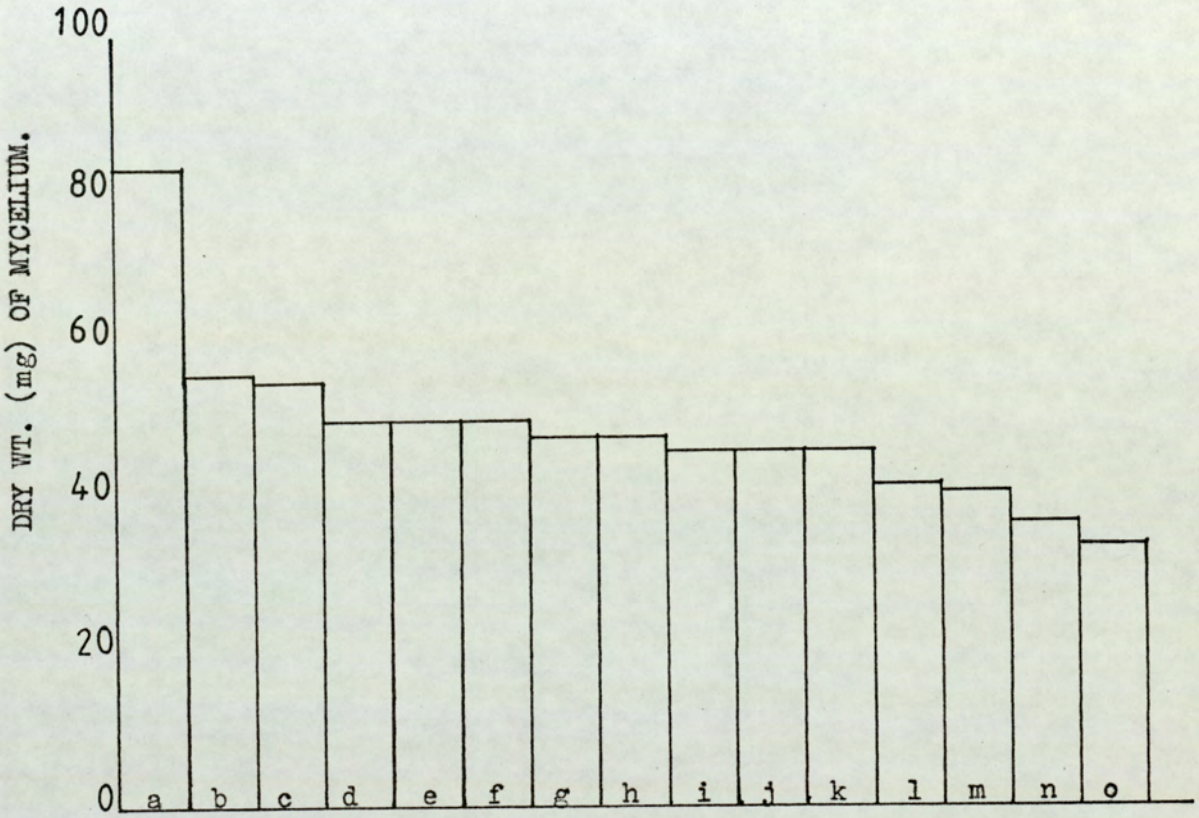
k. = Myristic acid methyl ester

l. = Lipid-free control

m. = Ethyl acetate.

95% confidence limits

Fig.3.21 : EFFECT OF TRACE ELEMENTS AND ASH ON THE VEGETATIVE GROWTH OF S.RUGOSO-ANNULATA IN A DEFINED LIQUID MEDIUM AFTER 40 DAYS OF INCUBATION.

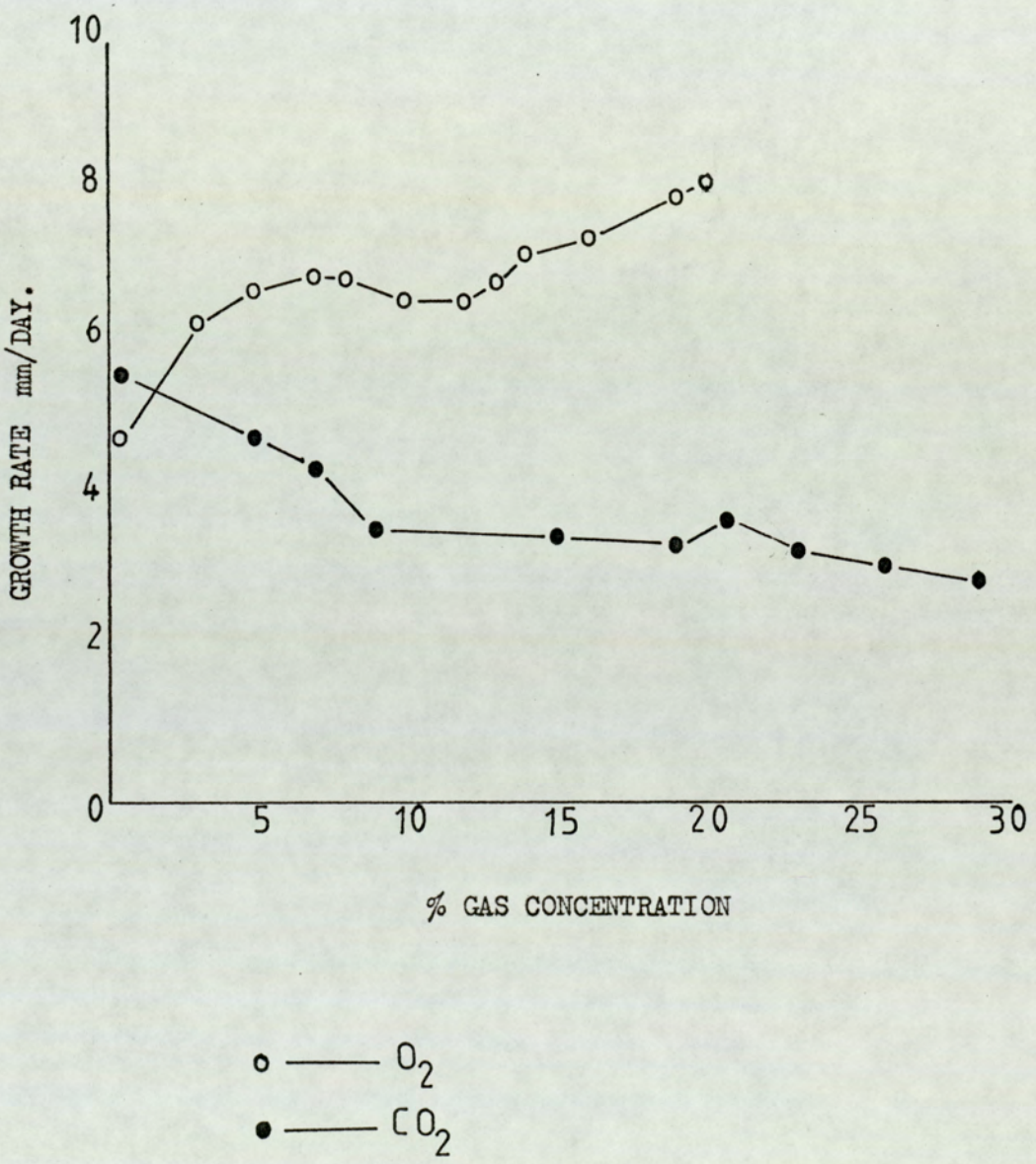


Supplements to the basal medium (BM)

a. Thiamine
b. 'Complete' mineral soln.(CM) - Mo
c. CM - Cu
d. Malt extract ash
e. CM
f. CM - Mn
g. CM - Ca
h. CM - Zn
i. Biotin
j. Rice straw ash
k. CM - Li
l. Wheat straw ash
m. Yeast extract ash
n. CM - Fe
o. BM only

| 95% confidence limits.

Fig.3.22 : EFFECT OF OXYGEN AND CARBON DIOXIDE CONCENTRATION ON THE VEGETATIVE GROWTH OF S.RUGOSO-ANNULATA.



Carbon dioxide on the other hand suppressed growth. Growth inhibition was particularly marked as the concentration increased from 0.48 to 9.2%, but became more gradual until 29.3%. Within the concentrations used, increase in oxygen and carbon dioxide concentrations respectively increased and inhibited the growth of S. rugoso-annulata.

(g) GROWTH ON SOLID NATURAL SUBSTRATES :

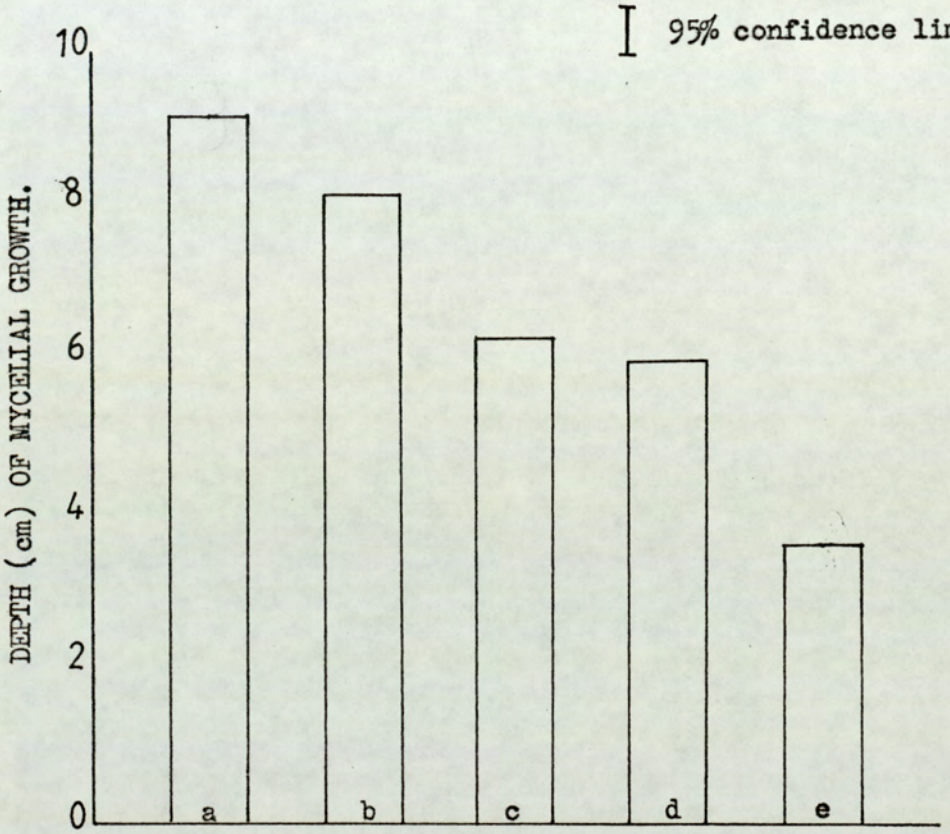
Like P. sajor-caju, S. rugoso-annulata showed distinct preference for cereal straw as a substrate (Fig. 3.23). Less growth occurred on Lawsons cypress and larch litter. Growth on sycamore, oak, and chestnut leaf litter was too irregular to be accurately measured and there was no growth at all on the remaining substrates.

3.3.2. REPRODUCTIVE STAGE

(a) EFFECT OF VARIOUS CASING MATERIALS :

In view of the preference for cereal straw, in particular wheat straw, as a growth substrate by this fungus, the following experiment was performed with wheat straw. For fruit-body formation of some fleshy fungi, a combination of factors are required to induce fruit-body formation. Some species, for example A. bisporus, require to be covered at the appropriate stage, with a layer of casing soil in order to induce fruit-bodies. Some others, for example P. sajor-caju, do not require casing but do require some illumination for induction of fruit-body formation. As for S. rugoso-annulata Szudyga (1978) stated that covering of the substrate with casing soil was absolutely necessary for fruit-body formation. Lelley (personal communication) said that casing soil was not necessary. The following experiment was therefore done in order to determine whether or not a casing layer was required for fructification; and if so, what casing formulations were most suitable.

Fig.3.23 : VEGETATIVE GROWTH OF S.RUGOSO-ANNULATA ON VARIOUS SOLID NATURAL SUBSTRATES IN GLASS TUBES.



a. Wheat straw

b. Rice straw

c. Lawson's cypress

d. Larch leaf litter (fresh)

e. Larch leaf litter (one year old)

NO GROWTH IN THE FOLLOWING

f. Pine needles

g. Mushroom compost

h. Old newspaper

i. Fir leaf litter

GROWTH IN THE FOLLOWING TOO IRREGULAR TO BE ACCURATELY MEASURED.

j. Sycamore litter

k. Oak litter

l. Chestnut litter

The method has already been described in Section 2.9. Six different casing formulations were used viz :

(i) Peat + humus soil mixture of pH 6.0 (not sterilized).

(ii) Peat + humus soil mixture of pH 6.0 (sterilized initially by autoclaving at 1kg / cm^2 for 1 hour for 3 consecutive days, but exposed on application; pH= 5.9 after the heat treatment).

(iii) Peat + humus of pH 5.0.

(iv) Humus only of pH 7.5.

(v) Peat only of pH 4.0.

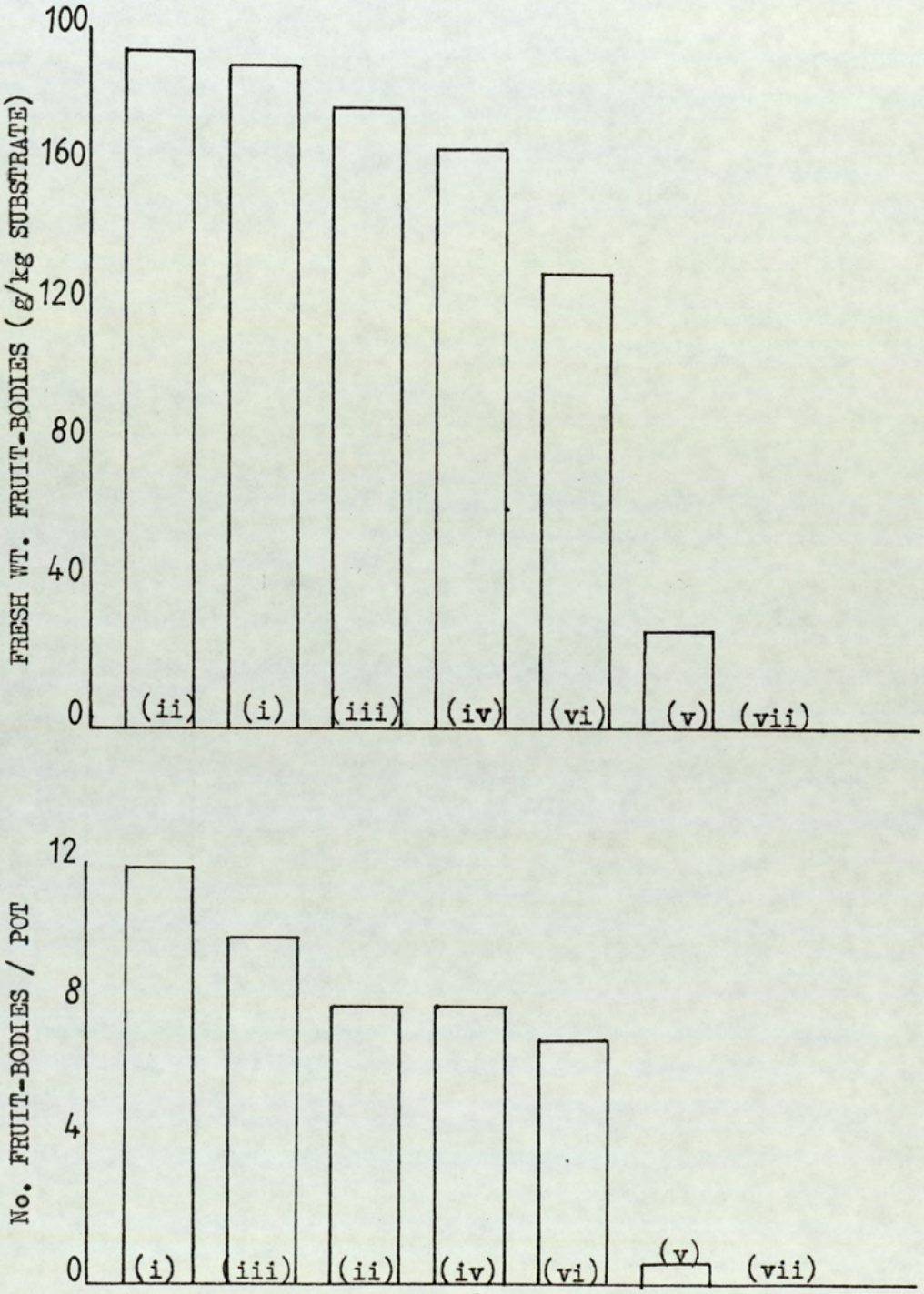
(vi) Peat + chalk (pH 7.5) as for A. bisporus.

All the above materials except (ii) above were applied without sterilization. At 7-day intervals bacteria were isolated from (i) & (ii) and estimation of bacterial populations done by the method described in Section 2.12. Determination of the dominant colony types was based on colour.

Electrical conductivity, as well as pH, were also measured as described in Section 2.11. on the same day as bacterial isolations.

Based on yield (g/kg), the pots covered with initially autoclaved soil (ii) gave the greatest yield, closely followed by (i), (iii), (iv) and (v), in that order. But in terms of number of fruit-bodies the highest number was obtained from (i) followed, in decreasing order, by (iii), (ii), (iv) and (v). Whichever of the above criteria was used, the poorest yield was obtained from (vi) ie the pots covered with peat only. Considering the time taken to produce primordia, the earliest was again (i), on which primordia appeared after 21 days from the time of casing. This was followed (in increasing order of time length) by (iii), (iv), (vi), (ii) and again lastly (v). These results are summarized in Fig. 3.24.

Fig.3.24 : YIELD OF S.RUGOSO-ANNULATA ON VARIOUS CASING MATERIALS AFTER 4 MONTHS OF INCUBATION.



(i) Peat/humus soil untreated (pH6)
(ii) Peat/humus soil heat-treated (pH5.9)
(iii) Peat/humus soil (pH 5.0)
(iv) Humus only
(v) Peat only (pH 4.0)
(vi) Peat/$CaCO_3$ (pH 7.5)
(vii) Control (no casing)

Fig.3.25 : pH AND ELECTRICAL CONDUCTIVITY CHANGES IN THE PEAT/HUMUS SOIL CASING LAYER OF S. RUGOSO-ANNULATA - DURING 70 DAYS OF INCUBATION

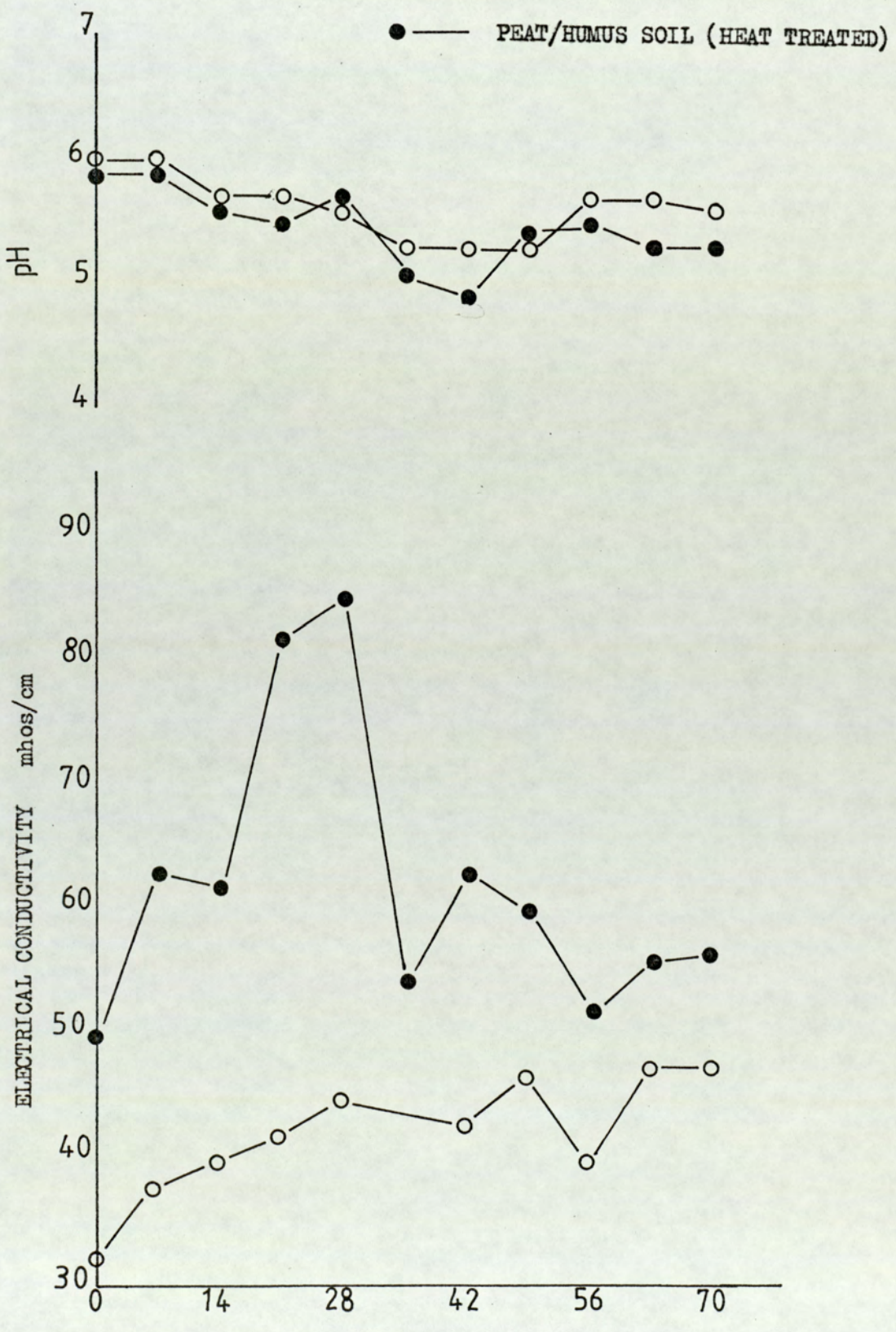


Fig.3.26 : BACTERIAL POPULATION IN THE PEAT/HUMUS CASING LAYERS OF S.RUGOSO-ANNULATA DURING 70 DAYS OF INCUBATION.

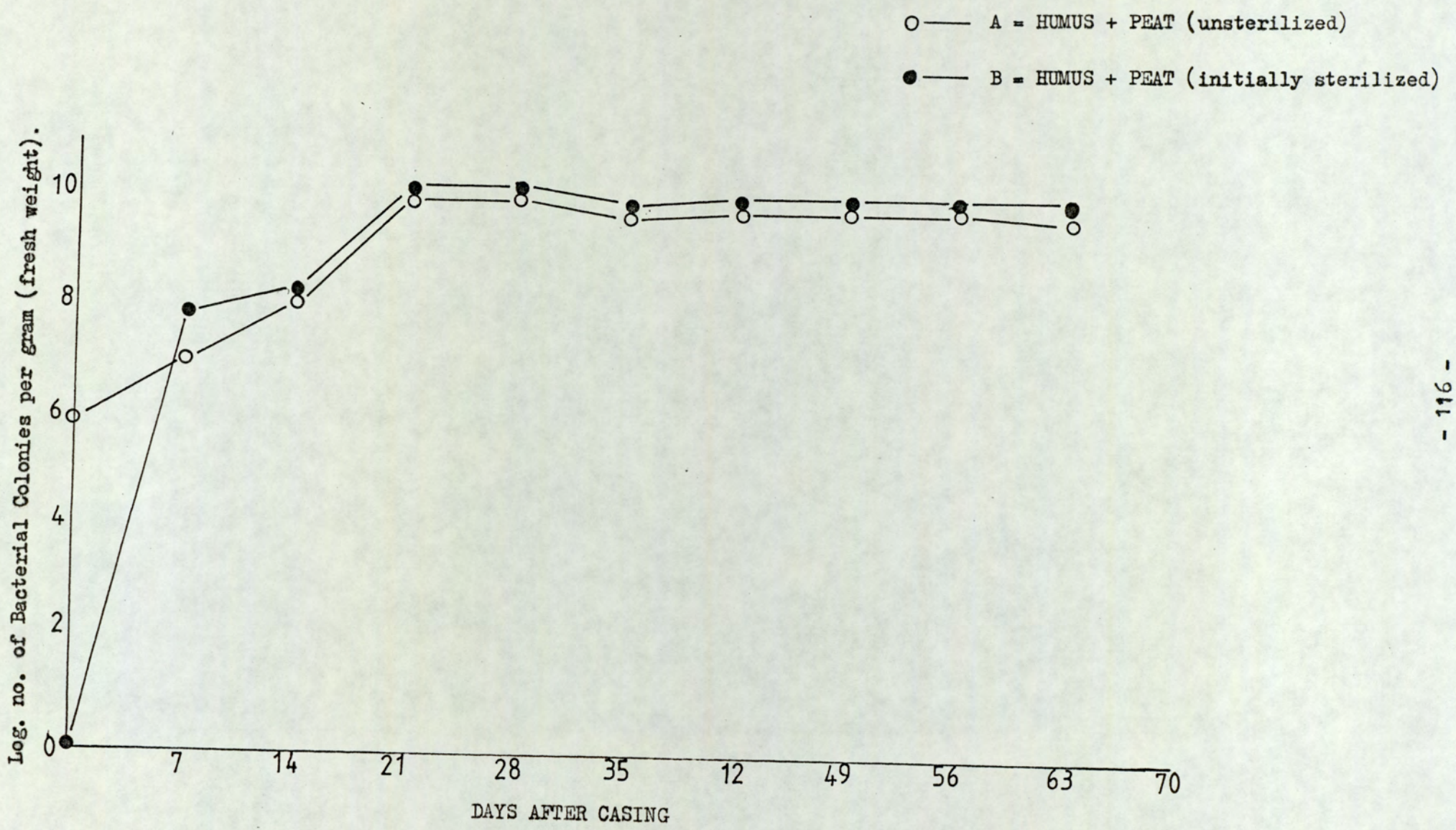
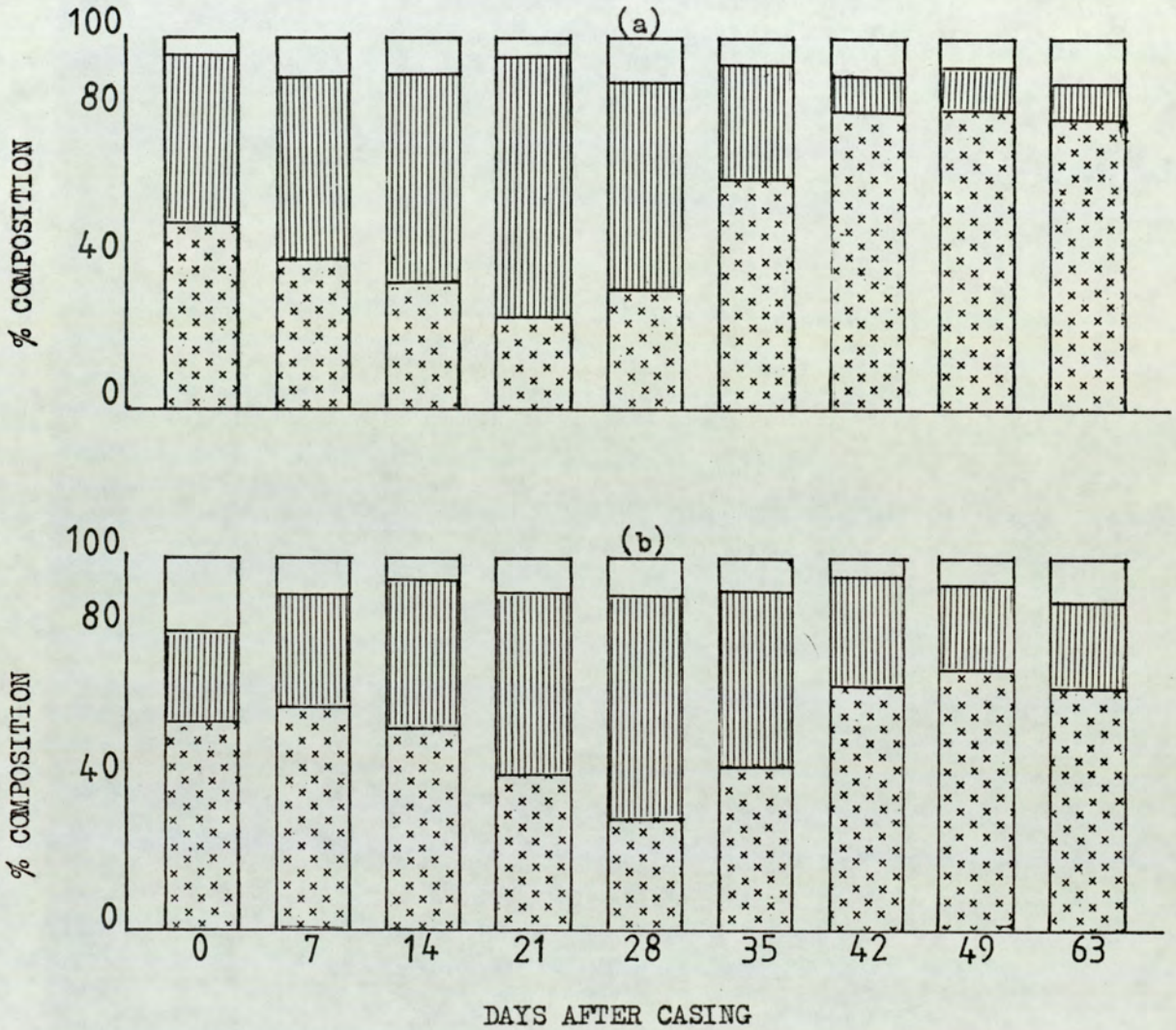


Fig.3.27 : % COMPOSITION OF THE BACTERIAL FLORA OF THE PEAT/ HUMUS SOIL CASING LAYER OF S.RUGOSO-ANNULATA DURING 63 DAYS OF INCUBATION.



(a) = Casing soil (heat-treated)

(b) = Casing soil (untreated)

TYPE A (CREAM)

TYPE B (YELLOW)

TYPE C (OTHERS)

Within the experimental period of 4 months, the control ie. the one without any casing, did not produce any primordia. However, the pots were not discarded but rather continually watered. One out of the five uncovered pots, nevertheless, produced one fruit-body after a rather prolonged incubation period of 7 months.

As expected, no bacteria were isolated from treatment (ii) on day 0, just after sterilization, but it was quickly infected and the bacterial population increased to 90×10^6 colonies per gram fresh weight on day 7. This was higher than the population in (1), 121×10^5 per gram fresh weight. By the 21st day the difference between the populations in the two treatments narrowed down considerably and remained approximately steady, throughout the experiment. Their populations were high during fruit-body development (Fig. 3.26).

There were two dominant bacterial types :- type A (cream) and type B (yellow). All the others, including an actinomycete species, were grouped together as type C. The individual bacterial populations, especially types A and B fluctuated more or less in a regular manner. The greatest number of type B (and incidentally the smallest number of type A) occurred at the time of the first flush (Fig. 3.27).

There was much fluctuation in the electrical conductivity in both treatments (more so in (ii)) but the highest values were obtained at the time of fruit-body formation (Fig. 3.25).

There was gradual fall in pH in both treatments, again more so in (ii). The lowest values in both cases occurred about the 42nd day (Fig. 3.25).

(b) EFFECT OF MICROFLORA AND AERATION ON FRUCTIFICATION :

It is now generally agreed that A. bisporus primordium formation is triggered off by the activities of the micro-flora in the casing layer, as reviewed in Section 1.10. Some other fleshy fungi like P. sajor-caju produce fruit-bodies in axenic cultures. The role of micro-flora in fruit-body formation of S. rugoso-annulata has not been studied. Hence the following experiment was designed to investigate the influence of microbial activity in the fructifiaction of this fungus.

A secondary aim of this experiment was to study the aeration requirements for fructification. The effect of oxygen and carbon-dioxide concentration on its vegetative growth was described in Section 3.31.

The procedure has already been described in Section 2.10. The quantity of the casing material used was 110.0g per flask (fresh weight) and the pH after sterilization was 5.4.

There was uniform growth of mycelium in all the flasks up to the time of casing. In the later part of incubation, however, there was profuse mycelial growth in the sterile casing soil which, after 20 weeks of incubation, did not produce any fruit-body or primordia. On the other hand, there was scanty and strandy mycelial growth in the non-sterile casing soil. Primordia appeared in all the aerated flasks with non-sterile casing soil after 12 weeks. Some of the primordia developed into mature fruit-bodies (see Table 3.5 and Plate 3.5) In the non-aerated flasks with non-sterile casing soil, primordia appeared on the 14th week but these failed to develop further.

(c) STUDIES WITH DIVIDED PETRI-PLATES :

It has been shown above that primordia and fruit-bodies only formed in non-sterile cultures. But the role of the micro-flora of

TABLE 3.5

EFFECT OF MICRO-FLORA AND AERATION ON FRUIT BODY FORMATION OF STROPHARIA RUGOSO-ANNULATA.

	NON-STERILE			STERILE			
	No.of primordia	No.of Fruit-bodies	Wt.of F-bodies /flask	No.of primo-rdia	No. of F-bodies	Wt. of F-bodies/ flask	
flask i	42	0		0	0	-	AERATED
" ii	36	0		0	0	-	
" iii	32	2		0	0	-	
" iv	34	2		0	0	-	
Mean	36	1	30.65g	0	0	-	
Time before primordium appearance	12 weeks			-			
Remarks :	Scanty and strandy mycelial growth in the casing layer in all 4 flasks			Profuse mycelial growth in the casing layer in all 4 flasks.			
flask i	5	0	-	0	0	-	NON - AERATED
" ii	8	0	-	0	0	-	
" iii	3	0	-	0	0	-	
" iv	9	0	-	0	0	-	
Mean	6.25	0	-	0	0	-	
Time before primordium appearance	14 weeks			-			
Remarks :	Scanty and strandy mycelial growth in the casing layer in all 4 flasks.			Profuse mycelial growth in the casing layer in all 4 flasks.			

Plate 3.5 Necessity of micro-flora in fruit-body formation S.rugoso-annulata.

Note developing fruit-bodies in the non-sterile cultures and absence of fruit-bodies in the sterilr cultures.

the casing layer is not yet clear. This experiment was therefore performed in an effort to encourage S.rugoso-annulata to produce primordia in sterile agar plates and so provide some evidence to explain the requirements for primordium formation.

When the vegetative medium in one half of the plate had been completely colonized (Section 2.2.(b)) by the mycelium, the reproductive medium was poured into the second half. Various reproductive media were prepared from the following ingredients :-

$NaNO_3$	0.6g
KCl	0.02g
$MgSO_4.7H_2O$	0.02g
KH_2PO_4	0.04g
$FeSO_4.7H_2O$	0.08mg
$CaCO_3$	1.0g
Agar	20.0g
Deionized water	1l.

Modified from Cresswell, 1980.

The above salts were used in sequential single additions. Another set of reproductive media consisted of the amino acids shown below :-

Arginine, aspartic acid, cysteine, phenylalamine, tryptophane and isoleucine. These were added singly into water agar at the concentration of 100 μg per litre. All treatments were adjusted to pH 5, 6, and 7. The control was water agar.

After three months primordia were observed in some plates. The results are summarized in the following table :-

Table 3.5 : Primordium formation in divided Petri-plates

Treatment	pH	Mean No. of Primordia
$NaNO_3$	5	2
Salt additions up to $FeSO_4.7H_2O$	5	2
Salt additions up to KH_2PO_4	6	2
Salt additions up to $CaCO_3$	5	3
H_2O agar only	5	1
H_2O agar + tryptophane	5	2

There was no primordia formation in other treatments.

3.3.3. CONCLUSIONS.

EFFECT OF VITAMINS AND YEAST EXTRACT ON GROWTH :

S. rugoso-annulata, like P. sajor-caju, showed a definite requirement for thiamine. Absence of thiamine suppressed growth to nearly the same extent as the vitamin-free control. Conversely the other single vitamin depletions supported growth to approximately equal extent and comparable with the complete vitamin treatment. It may therefore be inferred that no other vitamin tried was necessary for the growth of S. rugoso-annulata.

NITROGEN SOURCES :

Organic and ammonium sources of nitrogen were preferred to other sources. This was also found to be so with P. sajor-caju. Sodium nitrate and potassium nitrite proved to be toxic, in agreement with other evidence of poor utilization of nitrates and nitrites by basidiomycetes. Ammonium nitrate seemed to be the exception, but like other basidiomycetes, the ammonium ion was probably preferentially utilized. Ammonium nitrate should therefore be regarded as an ammonium source of nitrogen and not a nitrate source. It has been shown earlier (Section 3.31) that ammonia suppresses

the utilization of the nitrate ion. Since removal of ammonia from a solution also causes a reduction in pH, therefore only the fungi capable of growing at low pH might be able to utilize the nitrate ion left after the ammonium ion has been removed. Zadražil (1980) increased the decomposition of straw by some basidiomycetes including S. rugoso-annulata by supplementing the substrate with ammonium nitrate.

There was virtually no growth in the nitrogen-free control- suggesting that it is incapable of fixing atmospheric nitrogen.

CARBON : NITROGEN RATIO :-

The optimum C : N for S. rugoso-annulata was determined to be 80 : 1. Although this is quite high compared with those of such fleshy fungi as A. bisporus, L. nuda, L. saeva and C. gambosum as cited earlier, it is quite comparable with the optimum C : N ratios of P. sajor-caju and V. volvacea. Chang-Ho and Ho (1979) determined the best C : N ratio of V. volvacea to be 75 : 1 but stated that C : N ratios ranging from 32 : 1 to 150 : 1 were almost as effective. Therefore like P. sajor-caju and V. vovacea this fungus would be expected to utilize cereal straw very well with little or no supplementation with exogenous nitrogen.

FATTY ACIDS AND ESTERS :

The various fatty acids and the respective esters increased growth of S. rugoso-annulata. Wardle & Schisler (1969) Obtained a significant stimulation of growth of A. bisporus when esters of oleic and linoleic acids were added to their medium. They also attributed the growth increase caused by the addition of oils, to oleic and linoleic acids contained therein. The results also agree with Wright's results (Wright 1976) on the stimulation of growth of L. nuda and L. saeva by oleic and linoleic acids.

TRACE ELEMENTS :

There was a requirement for iron, but copper and molybdenum did not seem to be required for growth. Elimination of each of the remaining metals impaired growth to some extent, not as much as the suppression of growth by the absence of iron. The necessity of iron has been observed by many investigators (Section 1.7.).

The yields were generally low except in the treatment in which thiamine was substituted for the mineral solution. The low yield might therefore have been due to the absence of thiamine or the drastic fall in pH in the treatments.

INFLUENCE OF OXYGEN AND CARBON DIOXIDE :

The growth of S. rugoso-annulata was suppressed by low oxygen concentration. Overall, the rate of growth increased with increase in oxygen concentration between 0.48% and 20.4%. On the contrary, growth was suppressed by increasing concentrations of carbon dioxide from 0.48% to 29.3%. However, there was high tolerance of carbon dioxide up to 29.3%. It was not possible to determine the effect of these gases at 0% concentration. It could not therefore be known whether this fungus would be able to grow in complete absence of oxygen or what effect carbon dioxide concentrations less than 0.48% would have on its growth. In natural situations neither oxygen nor carbon dioxide is likely to be a limiting factor for growth.

High carbon dioxide concentrations suppressed primordium formation. While it took 12 weeks for primordia to appear in aerated flasks (Table 3.4), primordia developed in non-aerated flasks after 14 weeks. Moreover, there were numerically less primordia (6.25 in average) in the non-aerated flasks than the aerated ones (average = 36). No

primordia developed into mature fruit-bodies in non-aerated flasks, but fruit-bodies developed in aerated flasks.

GROWTH ON SOLID NATURAL SUBSTRATES :

The conclusions reached on the growth of P. sajor-caju on natural substrates (Section 3.2.1) also apply for S. rugoso-annulata. However, S. rugoso-annulata appeared to be more fastidious in its colonization of substrates than the former. Out of twelve different substrates, measurable growth could be obtained from only five, the greatest growth occurring on wheat straw and rice straw. Pleurotus sajor-caju grew very well and produced good yield of fruit-bodies on composted wheat straw, but Stropharia rugoso-annulata failed to grow on it. This was probably because the nitrogen content of the compost was unsuitable. Since its optimum C : N ratio was determined to be 80 : 1, lower than that of P. sajor-caju (90 : 1), S. rugoso-annulata would be expected to grow better on compost with C : N ratio of 17 : 1, than P. sajor-caju. It seemed likely that it was more sensitive to nitrogen concentration and had a narrower substrate range than P. sajor-caju.

As suggested in Section 3.3.2, pH might have been a limiting factor in its failure to grow on compost. Its optimum pH was determined to be 5.0 while the compost had a pH of 7.5.

FRUIT-BODY FORMATION ON VARIOUS CASING MATERIALS :

The mixture of humus and peat of pH between 5 and 6 proved to be the best casing medium. Difference in yield on the same casing formulation but different pH between 5 and 6 was not significant. Yields on humus soil only (pH 7.5) and the conventional peat + chalk mix (pH 7.5) were only moderate. By far the poorest yield was obtained from peat only (pH 4.0). This result is consistent with the report of Szudyga (1978).

Variations in pH seemed to be a major operating factor in this experiment.

Heat treatment did not appear to affect the bacterial population or composition of the casing layer. Although this treatment depressed the number of fruit-bodies produced, individual fruit-bodies were heavier and total yield was not affected.

FRUIT-BODY FORMATION IN FLASKS :

Primordia and mature fruit-bodies were produced on non-sterile casing soil but failed to form on sterile casing soil. This proved the necessity of micro-organisms in the formation of fruit-bodies. Particularly interesting was the fact that there was profuse mycelial growth in the sterile casing soil and only scanty growth in the non-sterile one, suggesting a relationship between the suppression of growth and micro-organisms. This observation was also made by Eger (1963) and Hayes *et al* (1969) for *A. bisporus*.

Conclusions about aeration have been drawn earlier.

STUDIES WITH DIVIDED PETRI-PLATES :

Some primordia were formed after protracted incubation in the divided Petri-plate technique. However, no conclusions are possible since their occurrence was sporadic. The results may suggest a relationship with the presence of some salts in the reproductive medium. Since the few primordia that did form did so only at low pH, it may also suggest a possible inter-relationship with this factor.

The Petri-plate test using two media representing both

a vegetative and a reproductive medium, may prove useful in future studies aimed at stimulating or inhibiting numbers of primordia.

DISCUSSION AND GENERAL CONCLUSIONS.

DISCUSSION AND GENERAL CONCLUSIONS.

The preliminary part of this work consists of basic physiological experiments to determine the temperature and pH optima of thirteen known edible basidiomycetes, and to assess the ability of these species to utilize some of the commonest sources of carbon : cellulose, lignin and starch for growth. Initial trials were also made to determine the capability of these fungi to fructify in agar plates and in uncomposted straw. The results of the preliminary experiments formed the background for more detailed study on Pleurotus sajor-caju and Stropharia rugoso-annulata. Further physiological requirements for the vegetative growth of these two species were assessed in Sections 3.2.1. and 3.3.1. as a pre-requisite for the investigations on the conditions most favourable for fruit-body formation in Sections 3.2.2. and 3.3.2.

UTILIZATION OF CELLULOSE, LIGNIN AND STARCH.

Both fungi were efficient in utilizing cellulose, lignin and starch. Pleurotus sajor-caju (Jandaik and Kapoor,1974; and Roxon and Jong,1977) and Stropharia rugoso-annulata (Overholts, 1930, and Pearson 1946) are both saprophytes on decaying plant materials. In view of the fact that these are predominantly cellulosic and lignin environments, their ability to degrade cellulose and lignin might be considered to be of importance for their existence in such substrates. The results (Fig.3.7&3.8) indicate faster utilization of cellulose and starch by P.sajor-caju than S.rugoso-annulata. P.sajor-caju has been grown on a variety of substrates by various workers (Section 1.3.5) but the best results were obtained from banana pseudostems and this was attributed to the high starch content of this material.

These species are therefore equiped with the necessary

enzymes to enable them to utilize a wide range of plant materials in natural environment including straw. P. sajor-caju proved to be more versatile in the utilization of different plant materials. S. rugoso-annulata preferred unsupplemented straw for growth to any other substrates tried. It was actually isolated from decomposing farm waste straw (Pearson, 1946).

NITROGEN SOURCES :

P. sajor-caju was observed to respond more readily to supplementation with nitrogen sources than S. rugoso-annulata. Although P. sajor-caju grew well and produced fruit-bodies on unsupplemented straw, substrate decomposition was accelerated, the yield of fruit-bodies as well as nitrogen content of the fruit-bodies increased when the straw was enriched with extraneous nitrogen sources (Jandaik and Kapoor, 1974; Zadrazil, 1980). On the contrary, supplementation of straw with exogenous nitrogen resulted in small increase in lignin decomposition, but further increases in the nitrogen concentration depressed lignin decomposition by S. rugoso-annulata (Zadrazil, 1980). Nitrogen addition also either did not affect the growth and yield of S. rugoso-annulata or actually depressed yield (Szudyga, 1978; Zadrazil, 1979 & 1980).

S. rugoso-annulata therefore prefers a substrate with low nitrogen content and straw meets this low nitrogen-level requirement : rice straw has 0.8% N (Chang-Ho and Ho, 1979); 0.63% - 0.97% N (Chang, 1972); wheat straw contains 0.72% N (Ramasamy and Verachtert, 1979). The carbon : nitrogen ratio is 100 : 1. This balance of carbon and nitrogen places no restriction on the metabolism and supports high rates of growth and reproduction of S. rugoso-annulata with an optimum C : N ratio of 80 : 1. Although P. sajor-caju (optimum C : N ratio 90. : 1) also grows well on straw, for its maximum growth and reproduction the addition of an

exogeneous nitrogen source is necessary to bring the C : N ratio closer. Although high yields of P.sajor-caju achieved on banana pseudostems was attributed to high starch content of the medium, it could also have been due to the higher nitrogen content in the banana tissues (1.3% N, Chang-Ho and Ho,1979) than in straw. Composted straw (mushroom compost) supported good growth of this species, though to a lesser extent than uncomposted straw, but failed to support the growth of S.rugoso-annulata, due perhaps to the relatively high nitrogen content of compost and consequently low C : N ratio. Another reason was that the pH of the compost was also above what has been determined as optimal.

EFFECT OF VITAMINS :

Both species showed definite requirement for thiamine but not for any other vitamin tried. Also for both species yeast extract supported more growth than a 'complete' vitamin mix, probably because the former contained more amino-acids that could be directly assimilated by both fungi, or it contained more vitamins than in the 'complete' vitamin mix or the same vitamins but in more suitable concentrations. It might also have contained certain hormones and other unidentified growth substances that favoured growth and reproduction.

According to Cochrane (1958) vitamins are "organic molecules required in small amounts and not, so far as we know , used as sources of either energy or structural materials of protoplasm". Most vitamins have a catalytic function in the cell as co-enzymes or constituents of co-enzymes. Absence of thiamine completely prevented the formation of primordia in cultures of P.sajor-caju (Plate 3.4). Most fungi which require thiamine or biotin need more of the vitamin for reproduction than for vegetative growth. The primary use of thiamine is for the regulation of carbohydrate metabolism (Lilly and Barnett,1951).

It may accelerate sugar utilization and therefore hasten the onset of reproduction.

Thiamine occurs naturally in wood, litter and other plant materials including straw and therefore is unlikely to be limiting to growth in cultivation houses.

OXYGEN AND CARBON DIOXIDE REQUIREMENTS :

Both P.sajor-caju and S.rugoso-annulata showed tolerance to high concentrations of carbon dioxide during the vegetative phase of growth. Growth of P.sajor-caju even took place at 29.3%, though at a lower rate. This high tolerance is advantageous in most practical situations, including growing houses, since high carbon dioxide concentrations would not be a serious obstacle to growth. On the contrary it would act as a shield against contaminating micro-organisms which either cannot grow or die off at such concentrations (Zadrazil, 1974).

S.rugoso-annulata was more sensitive to changes in concentrations of oxygen. Its growth was suppressed at low concentrations to a greater extent and increased with increasing concentrations of oxygen more than that of P.sajor-caju which was not affected within the concentration range tested, even at semi-anaerobic levels. It could not be determined whether or not the growth of these fungi would cease under anaerobic situations. However, it is unlikely that oxygen could be a limiting factor for growth in natural situations.

In the reproductive stage, however, oxygen and carbon dioxide have more critical effects, the latter being detrimental at high concentrations. Therefore the need to reduce the concentration of carbon dioxide and increase the level of oxygen by adequate ventilation is therefore absolute. Poor ventilation caused clumping of under-developed fruit-

Plate 3.6 Fruit-body regeneration of P.sajor-caju in a poorly aerated and poorly illuminated condition.

bodies as well as fruit-body regeneration (Plate 3.6) in P. sajor-caju. In S. rugoso-annulata lack of ventilation caused a reduction in the number of primordia and also prevented further development of the formed primordia into fruit-bodies. The inhibitory effects of high carbon dioxide concentrations was first recorded by Lambert in 1933. He noted that concentrations more than 1.0% was injurious to fruit-body formation in A. bisporus and advocated the need for adequate ventilation in growing houses. Since then numerous workers have confirmed the deleterious effects of high carbon dioxide concentrations and emphasized ventilation requirements. A little carbon-dioxide is, however, stimulative and 0.01 - 0.1% range has been suggested as being optimal for fructification in A. bisporus (Long and Jacob, 1969).

Many investigations have shown that ventilation removes not only carbon dioxide but also certain harmful gaseous metabolites produced by the mycelium. Stoller (1952a) attributed stem elongation and pileus retardation of A. bisporus to the presence of the volatile metabolites emitted from the compost and casing layer. The substances, he postulated, prevented the development of the thickenings at hyphal tips into sporophores and reasoned that the function of the casing layer was to provide an alkaline, oxygen-rich medium to destroy these volatile inhibitors.

Hayes et al, 1969, demonstrated stimulative effect of the volatiles on bacteria colonizing the casing layer and suggested that bacteria could utilize these substances as carbon sources; and Hayes and Nair (1974) confirmed the above effect on Pseudomonas species. Furthermore, the latter workers demonstrated a link between the occurrence of casing layer Pseudomonad bacteria and the structure of the casing soil. A more open casing allowed a greater degree of aeration in the micro-environment

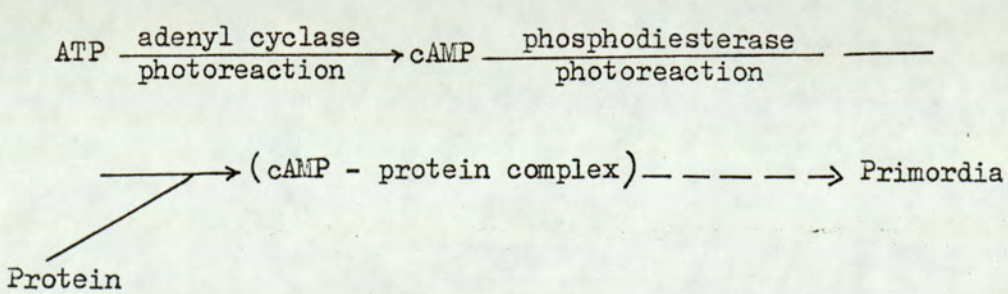
of the casing layer which influenced the numbers and composition of Pseudomonas species which are required for fruit-body formation to occur.

LIGHT AND FRUIT-BODY FORMATION IN P.SAJOR-CAJU :

Pleurotus sajor-caju has been shown to require light for the induction of primordia and also for the development of primordia into fruit-bodies. When an actively growing colony was exposed to light, retarded growth resulted and this preceded the formation of P.sajor-caju fruit-bodies. The stimulatory effect seems likely to be due to an inhibition of hyphal elongation (Lilly and Barnett,1951) stopping the flow of nutrients towards hyphal tips, and consequently giving rise to a localised concentration of nutrients. This in turn gives rise to greater tissue formation leading to the formation of primordia

Light also increases the temperature of an object and some authors including Cochrane (1958) have speculated that the initiation effect might be due to temperature increase. Many species require a lowering of temperature for production of fruit-bodies eg. A.bisporus and S.rugoso-annulata. In the experiments described, temperature was kept constant at 25°C. This hypothesis does not therefore apply at least to Pleurotus sajor-caju.

The effect of light on the formation of fruit-bodies has recently been related to the metabolism of adenosine 3 : 5 - cyclic monophosphate (cyclic AMP or cAMP) by Uno, et al (1974). The main principles of their postulate can be summarized as follows :-



They showed that light stimulated the accumulation of cAMP in a fruit-body-forming monokaryons and dikaryons of Coprinus macrorhizus which normally requires light for fructification. No fruit-bodies occurred in the absence of cAMP and the light-induced enzymes. This nucleotide then combined with a protein to give cAMP-protein complex, an intermediate complex in primordium morphogenesis. However, the mechanism involved in the final induction of primordia in the presence of this complex remains to be elucidated and its relevance to P. sajor-caju established.

FRUIT-BODY FORMATION IN STROPHARIA RUGOSO-ANNULATA.

In sharp contrast to P. sajor-caju, fruit-body formation was not affected by light in Stropharia rugoso-annulata. However, the application of casing layer was essential for the formation of fruit-bodies, although a single fruit-body did form in one un-cased culture after a prolonged incubation of 7 months. This is comparable to A. bisporus, the common edible mushroom which is widely cultivated.

In A. bisporus culture, the casing layer performs many functions (Hayes et al,1969; Hayes,1978; Yeo and Hayes,1979; Cresswell and Hayes,1979). It serves as a mechanical support for the developing fruit-bodies and functions in maintaining the correct water relations for the culture as a whole. But recent researches by the above workers have drawn attention to the significance of biological (bacteria) and some chemical factors which are associated with fruit-body formation of A. bisporus in the casing layer. These factors appear to be independent of the environmental factors, temperature and carbon dioxide concentration which are also critical for fruit-bodies to form (Tschierpe and Sinden,1964; Long and Jacobs,1969).

This study confirmed that a casing layer is essential for the formation of fruit-bodies in S. rugoso-annulata and thus this provides a basis for comparisons between A. bisporus and S. rugoso-annulata and the associated chemical and biological properties of a casing layer.

In A. bisporus optimum fruit-body formation occurs at pH 7.0 or above, and peat the most widely adopted casing medium is adjusted to this optimum by the addition of calcium carbonate. In this study, peat when used alone or when used with carbonate was found to

be a relatively inferior casing material (Fig. 3.24). However, the addition of calcium carbonate raised the pH level from 4.0 to 7.5 and this resulted in a significant improvement to fruit-body formation. A humus soil with a pH of 7.5 was superior to a peat and calcium carbonate mix (pH 7.5) and fruit-body yield was further improved by admixing peat and humus soil. Best yields were given with this mixture at pH 6.0 while at pH 5.0 yields were depressed. These results suggest that unlike a casing soil for A.bisporus culture, a soil in the acid range is more favourable, with an optimum at pH 6.0.

The recent work of Yeo and Hayes (1979) has associated the onset of fruit-body formation in A.bisporus with accumulation of soluble salts immediately following application of the casing layer. A similar occurrence was shown in this study with S.rugoso-annulata. Although salt levels were lower in a peat/humus soil mixture, the pattern of accumulation (Fig. 3.25) followed a similar trend to that of A.bisporus culture. Yeo and Hayes (1979) showed that potassium and sodium were the major ions accumulating, with smaller amounts of water soluble iron.

In this study the ions contributing to increasing conductivity were not measured. It was noted however, that autoclaving a peat/humus mixture before application caused a significantly higher conductivity, suggesting a release of salts by this extreme heat treatment. Higher salt levels in the casing soil have been associated with lower numbers of fruit-bodies forming (Hayes,1981, in press) in A.bisporus. Similarly with S.rugoso-annulata (Fig. 3.24) the number of fruit-bodies were significantly reduced by heat treatment and consequently higher salt levels in the casing layer. As with A.bisporus the total weight of fruit-bodies was not affected. Therefore the individual

weight of fruit-bodies were greater in heat treated soil.

The pattern of bacterial populations in the culture casing layer subsequent to its application in A.bisporus has been studied by Cresswell and Hayes (1979). Typically, populations increase to a maximum at the time of fruit-body initiation and primordium formation. Subsequently numbers remain more or less constant throughout cropping. An almost identical pattern was shown for S.rugoso-annulata (Fig. 3.26) and as in the studies of Cresswell and Hayes, after heat treatment, numbers increased rapidly after application.

Bacteria are required to be present in the casing layer for fruit-body formation in A.bisporus (Eger,1961; Hayes et al,1969). This fact was also shown to be true for S.rugoso-annulata (Plate 3.5).

Thus it can be seen that some factors associated with fruit-body formation in S.rugoso-annulata are also common to those in A.bisporus culture. The major difference appears to be the optimum pH requirement. A more detailed study and understanding of such factors may be of importance in maximizing productivity of S.rugoso-annulata. Studies with A.bisporus for example have shown that the biological component of a casing soil may be highly specific (Hayes et al,1969; Cresswell and Hayes,1979). Some bacterial isolates stimulate numbers of primordia while others do not. Within isolates which stimulate primordia, a proportion seemingly are able to cause development of primordia into fruit-bodies, suggesting a complex interrelationship between the bacterial flora in the normal casing layer. There is also evidence which shows that of all the ions which may be detected in the casing layer, it is only the element, iron in the water soluble form which is influenced by the casing soil micro-flora (Hayes,1981 in press). Low levels of water soluble iron are maintained by the

bacterial populations, while in axenic cultures which do not form fruit-bodies, levels increase progressively with time. This suggests that the formation of A. bisporus fruit-bodies may be inhibited by high levels of water soluble iron and that bacteria function in maintaining optimal levels.

Parallel work at the University of Aston Mushroom Science Laboratories by Hussain and Shandilya has shown that bacteria may also contribute to the chemical environment of the casing layer, by maintaining critical levels of auxin, giberellin and cytokinins, which are known to influence fruit-body initiation in Petri-plate culture.

There remains therefore an interesting and productive area for research with S. rugoso-annulata, and comparative studies with A. bisporus may provide new and useful information on the mechanisms of fruit-body formation in Agarics requiring a casing layer for the completion of their life cycles. The fact that primordia can form in low numbers in simple Petri-plate culture as found in this study (Table 3.5) as with A. bisporus, offers the opportunity of precise and definitive investigation and should accelerate progress and understanding of the phenomenon.

PROSPECTS FOR COMMERCIAL CULTIVATION.

Evidence from this work suggests that both Pleurotus sajor-caju and Stropharia rugoso-annulata have good potential for commercial cultivation.

Both basidiomycetes are primary saprophytes able to initiate the decomposition of plant materials. It has been shown that both utilize unfermented cereal straw for growth and fruit-body production. Therefore the elaborate process of composting is not a necessity. S.rugoso-annulata does not even tolerate the traditional mushroom compost. P.sajor-caju, however, requires supplementation of the straw substrate with an extraneous nitrogen source. Its nitrogen requirement is not high and a little addition of various farm wastes is enough. In contrast to S.rugoso-annulata, it grows on a wide range of industrial and farm wastes and compost is also used in its cultivation.

The two species are tolerant of high carbon dioxide concentrations, a characteristic which is useful in keeping off other micro-organisms. Despite the relatively slow growth, S.rugoso-annulata was observed to be resistant to disease organisms and pests, an advantage over A.bisporus which requires intensive husbandry. P.sajor-caju grows rapidly and fully colonizes the substrate, minimizing competition and infection. Fruit-bodies appear 10 - 14 days of incubation.

Pleurotus sajor-caju does not require casing for fruit-body formation. S.rugoso-annulata in contrast, does require casing but a humus soil may function adequately as a casing layer.

All the above features make the cultivation of these basidiomycetes relatively simple and less expensive than the cultivation of A.bisporus.

Under laboratory conditions the yield of S.rugoso-annulata was 254.5 g/Kg (= 20.36 Kg/m^2 where 1m^2 contains 80 Kg of fresh straw substrate). The yield of P.sajor-caju was 122 g/Kg (or 9.76 Kg/m^2). Compared with the normal yield of A.bisporus, about 15 Kg/m^2, the yield of S.rugoso-annulata is very good while that of P.sajor-caju is fairly low.

There are varying data on the nutritional value of various flesh fungi (for comprehensive review, see Chang and Hayse,1978). The different results are mostly due to the method of cultivation (or source of isolation in the case of wild types) of the test fungi and method of analysis.

The nutritional value of these two fungi has not been adequately studied but the works of Szudyga (1978), Samajpati (1979) and Botham (1981) suggest that they compare favourably with A.bisporus and vegetables. Both also possess acceptable palatability and culinery qualities.

In addition to the edible fruit-bodies it has been shown that the used substrate may be beneficial. Kirk and More (1972) cited by Zadrazil (1979) stated that the increase in digestibility of straw for purposes of animal feed depended on the lignin content of straw. Because of the high ligninolytic ability of these two fungi the lignin content of the fermented straw is considerably less after fructification; the simple carbohydrate and protein content increases. This mixture of fermented substrate, fungal mycelium (and fruit-bodies if not harvested) can be used for animals (Zadrazil,1979). Citing Torev, et al (1969) he stated that this substance had similar feed value as feed yeast, fish meal and other conventional feed components. Like the used compost of A.bisporus it can also be used in crop farms to

increase soil fertility.

For commercial viability much work, especially breeding development is needed to further improve the quality of the fruit-bodies and cultivation methods. As fruit-body formation and other life processes are governed by genetic factors, the need for improved strains for commercial use cannot be over-stressed. More rapidly growing strains of Stropharia rugoso-annulata are desired, and sporeless strains of Pleurotus sajor-caju will be more preferable. The fruit-body of P. sajor-caju is gymnocarpous and like P. ostreatus Eger (1978), produces spores as soon as the gills appear. The release of spores from the early stages of development till harvest can cause the spread of viruses and also may cause an allergy among the workers (Eger, 1974 and Jandaik, 1976). Fortunately many varieties of S. rugoso-annulata exist and unlike A. bisporus it is tetrasporous. P. sajor-caju is also tetrasporous, as well as heterothallic (Roxon and Jong, 1977). These are advantages in breeding work.

Pleurotus sajor-caju has the added advantage of growing and fructifying at high temperatures (it produces fruit-bodies even at 30° C, with optimum at 25° C), which makes its cultivation particularly suited for warm tropical and sub-tropical regions. S. rugoso-annulata requires cooling for fructification and, although the simplicity of its cultivation may offset the cost of cooling, it is highly recommended for exploitation in cooler regions.

APPENDICES.

Appendix 1.1.(a) Effect of temperature on vegetative growth.

(i) A.arvensis, incubation period : 2 weeks.

Temp.°C	Dry Wt. of mycelium (mg)					
	R1	R2	R3	R4	R5	MEAN
5	10.9	12.1	9.9	10.8	13.9	11.52
10	15.2	12.5	12.2	14.1	12.9	13.38
15	24.5	23.1	21.5	21.8	24.0	22.98
20	30.9	31.2	28.6	34.3	29.2	30.84
25	34.5	35.2	34.9	30.0	31.9	33.3
30	18.2	17.0	14.5	16.2	16.0	16.38
35	13.0	12.4	12.8	13.5	12.9	12.92

ANOVAR Summary

Total sum of squares : 2480.98

Between treatment SS : 2409.96

Within treatment SS : 71.02

Between treatment MS : 401.66

Within treatment MS : 2.54

The F ratio : 158.13

95% confidence limits $\bar{x} \pm 1.46$

From Table : Fp = 0.01(6,28) = 3.5

Highly significant.

Where :

SS = sum of squares

MS = mean square

F ratio = variance ratio.

R = replicate

(ii) A. bisporus, incubation period : 5 weeks.

Temp. °C	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
5	12.2	14.3	15.7	14.9	10.3	13.48
10	20.9	23.2	25.8	23.1	25.3	23.66
15	28.8	30.4	30.6	26.3	24.8	28.18
20	55.8	59.0	63.7	54.4	55.2	57.62
25	62.9	70.5	59.6	68.5	63.4	64.98
30	13.0	17.1	15.9	13.2	18.4	15.60

ANOVAR Summary

Total sum of squares : 12308.65

Between treatment SS : 11181.67

Within treatment SS : 1126.98

Between treatment MS : 2236.33

Within treatment MS : 46.96

F ratio : 158.13

95% Confidence Limits $\bar{x} \pm 5.9$

From table Fp = 0.01 (5,24) = 3.9.

(iii) A. bitorquis, incubation period : 2 weeks

Temp.°C	Dry Wt. (mg) of mycelium					
	R1	R2	R3	R4	R5	MEAN
5	4.5	4.7	4.4	3.5	128.6	4.28
10	19.0	16.1	17.1	17.1	165.2	17.33
15	22.6	22.1	22.6	24.9	142.3	23.05
20	26.8	27.2	26.0	29.6	162.5	27.40
25	31.0	28.1	29.2	29.9	171.4	29.55
30	38.6	30.4	34.7	38.9	149.9	35.65
35	22.9	26.4	18.9	25.4	129.7	23.40

ANOVAR Summary

Total sum of squares : 2523.91

Between treatment SS : 2421.04

Within treatment SS : 102.87

Between Treatment MS : 403.51

Within treatment MS : 4.9

F ratio : 82.35

95% Confidence Limits $\bar{x} \pm 2.3$

From table Fp = 0.01 (6,28) = 3.5

Highly significant.

(iv) A.aggregatum, incubation period : 2 weeks.

Temp.°C	Dry wt. (mg) of mycelium.				
	R1	R2	R3	R4	MEAN
10	5.1	4.6	3.8	4.2	4.43
15	15.5	16.0	17.7	14.3	15.88
20	36.3	33.2	33.0	29.5	33.0
25	42.0	46.2	44.2	44.2	44.15
30	38.0	33.2	37.1	35.2	35.88
35	6.5	6.0	7.0	6.9	26.40
40	4.6	5.1	5.7	5.5	5.23

ANOVAR Summary

Total sum of squares : 6685.48

Between treatment SS : 6631.61

Within treatment SS : 53.87

Between treatment MS : 1105.27

Within treatment MS : 2.57

F ratio 430.07

95% Confidence Limits $\bar{x} \pm 1.67$

From table Fp = 0.01(6,21) = 3.8

Highly significant.

(v) C.comatus, incubation period : 15 days.

Temp.°C	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
5	6.9	8.4	7.2	8.7	7.6	7.76
10	12.7	13.4	17.0	12.1	12.2	14.08
15	18.9	18.1	21.2	17.7	20.3	19.24
20	22.2	25.4	22.8	23.8	26.5	24.14
25	36.8	37.9	40.0	37.4	38.2	38.06
30	35.8	32.8	33.4	30.7	29.6	32.46
35	8.0	8.9	7.3	9.3	9.0	8.50

ANOVAR Summary

Total sum of squares : 4133.42

Between treatment SS : 4061.42

Within treatment SS : 72.0

Between treatment MS : 676.9

Within treatment MS : 2.57

F ratio 263.39

95% Confidence Limits $\bar{x} \pm 1.45$

From table Fp = 0.01(6,28) = 3.5.

Result is highly significant.

(vi) L. nuda, incubation period : 16 days.

Temp.°C	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
5	5.9	6.8	7.2	5.8	6.9	6.52
10	13.5	13.9	15.3	14.6	13.9	14.24
15	19.6	20.4	23.8	19.9	20.9	20.92
20	36.8	35.3	32.2	31.9	30.8	33.40
25	34.9	36.6	37.8	34.0	36.4	35.94
30	10.6	9.8	8.5	10.9	8.6	9.68
35	5.2	4.9	3.6	3.7	3.9	4.26

ANOVAR Summary

Total sum of squares : 4913.19

Between treatment SS : 4856.57

Within treatment SS : 56.62

Between treatment MS : 809.43

Within treatment MS : 2.02

F ratio 400.71

95% Confidence Limits $\bar{x} \pm 1.3$

From table Fp = 0.01(6,28) = 3.5

Highly significant.

(vii) L. saeva, incubation period : 2 weeks.

Temp.°C	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
5	11.1	14.0	11.6	10.7	18.5	13.18
10	15.1	13.4	13.4	12.3	14.3	13.70
15	23.0	22.8	20.6	27.1	29.8	24.66
20	32.2	32.2	31.1	25.5	29.0	30.0
25	31.6	36.6	34.6	37.2	42.7	36.54
30	14.0	16.7	14.4	16.7	15.8	15.52
35	13.8	14.1	14.5	15.9	11.0	13.86

ANOVAR Summary

Total sum of squares : 2875.96

Between treatment SS : 2656.55

Within treatment SS : 219.41

Between treatment MS : 442.76

Within treatment MS : 7.84

F ratio 56.47

95% Confidence Limits $\bar{x} \pm 2.38$

From table Fp = 0.01(6,28) = 3.5

Highly significant.

(viii) M.conica, incubation period 16 days

Temp.°C	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
5	6.01	7.31	7.61	9.21	8.51	7.73
10	14.30	14.50	13.01	14.30	15.10	14.24
15	18.20	22.90	24.40	26.10	20.80	22.48
20	34.30	33.90	31.40	32.10	34.01	33.14
25	36.01	38.80	32.60	39.10	35.90	36.48
30	5.11	4.91	4.81	4.11	3.91	4.57

ANOVAR Summary

Total sum of squares : 4442.17

Between treatment SS : 4360.01

Within treatment SS : 82.16

Between treatment MS : 872.0

Within treatment MS : 3.42

F ratio 254.97

95% Confidence Limits $\bar{x} \pm 1.67$

From table, Fp = 0.01(5,24) = 3.9

Highly significant.

(ix) M.vulgaris, incubation period : 3 days.

Temp.°C Dry wt. (mg) of mycelium.

	R1	R2	R3	R4	R5	MEAN
5	5.51	7.21	8.31	7.01	7.21	7.05
10	12.10	17.30	13.01	15.50	13.50	14.28
15	18.50	20.50	20.10	21.01	19.50	19.92
20	39.20	37.90	36.90	30.70	30.30	35.0
25	45.80	52.60	48.01	53.80	43.10	48.66
30	21.30	19.60	21.20	19.70	20.80	20.52
35	5.61	5.91	6.21	7.11	9.31	6.83

ANOVAR Summary

Total sum of squares : 7184.35

Between treatment SS : 6995.69

Within treatment SS : 188.66

Between treatment MS : 1165.95

Within treatment MS : 6.74

F ratio 172.99

95% Confidence Limits $\bar{x} \pm 2.35$

From table, Fp = 0.01(6,28) = 3.5

Highly significant.

(x) Phallus impudicus, incubation period : 5 weeks.

Temp.°C	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
5	15.2	16.7	15.9	18.8	15.6	16.44
10	18.5	18.1	16.0	18.8	18.8	18.04
15	39.7	37.9	46.4	50.3	43.0	43.46
20	65.6	77.4	73.1	77.4	64.3	71.56
25	79.5	80.9	81.1	87.5	75.1	80.82
30	15.7	14.7	15.4	12.0	13.7	14.30
35	13.9	12.9	11.7	10.6	10.0	11.82

ANOVAR Summary

Total sum of squares : 25806.16

Between treatment SS : 25434.49

Within treatment SS : 371.66

Between treatment MS : 4239.08

Within treatment MS : 13.27

F ratio (calculated) 172.99

95% Confidence Limits $\bar{x} \pm 3.1$

From table, Fp = 0.01(6,28) = 3.5

Highly significant.

(xi) P.sajor-caju, incubation period : 10 days.

Temp.°C	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
5	17.60	19.10	18.20	17.40	16.90	17.84
10	21.20	22.70	23.20	21.01	22.10	22.04
15	26.80	26.80	28.30	24.50	22.20	25.72
20	33.40	37.40	29.10	32.01	29.90	32.36
25	52.40	51.20	47.10	51.20	52.40	50.86
30	56.40	59.70	62.20	60.40	55.90	58.92
35	20.01	18.01	18.40	20.10	19.80	19.26

ANOVAR Summary

Total sum of squares : 8027.24

Between treatment SS : 7902.69

Within treatment SS : 124.55

Between treatment MS : 1317.12

Within treatment MS : 4.45

F ratio 295.98

95% Confidence Limits $\bar{x} \pm 1.81$

From table, Fp = 0.01(6,28) = 3.5.

Highly significant.

(xi) P.cervinus, incubation period : 5 weeks.

Temp.°C	Dry wt. (mg) of mycelium					
	R1	R2	R3	R4	R5	MEAN
5	30.6	31.9	30.9	28.9	29.8	30.40
10	58.3	64.3	62.2	57.5	66.3	61.72
15	92.4	97.5	87.3	90.5	95.9	92.72
20	106.0	152.3	115.7	128.8	122.1	124.98
25	124.8	128.1	146.4	135.9	117.3	130.50
30	24.9	25.8	27.1	21.5	21.7	24.20
35	20.2	17.2	17.9	17.3	15.7	17.66

ANOVAR Summary

Total sum of squares : 70079.47

Between treatment SS : 68203.80

Within treatment SS : 1875.66

Between treatment MS : 11367.3

Within treatment MS : 66.99

F ratio (calculated) 169.7

95% Confidence Limits $\bar{x} \pm 7.0$

From table, Fp = 0.01(6,28) = 3.5

Highly significant.

(xiii) S.rugoso-annulata, incubation period : 7 days

Temp.°C	Dry wt. (mg) of mycelium					
	R1	R2	R3	R4	R5	MEAN
5	6.1	8.9	7.8	5.8	8.9	7.50
10	9.3	10.4	9.8	10.2	10.6	10.06
15	13.2	13.1	14.8	13.8	12.8	13.54
20	23.0	22.2	24.6	23.3	24.7	23.56
25	26.0	26.9	28.6	27.9	27.3	27.34
30	26.1	27.2	27.4	26.1	26.4	26.64
35	10.4	12.9	11.6	13.8	13.6	12.46

ANOVAR Summary

Total sum of squares : 2102.5

Between treatment SS : 2072.05

Within treatment SS : 30.45

Between treatment MS : 345.34

Within treatment MS : 0.79

F ratio (calculated) 438.2

95% Confidence Limits $\bar{x} \pm 0.8$

From table Fp = 0.01(6,28) = 3.5

Highly significant.

Appendix 1.1(b) : Effect of pH on vegetative growth of :

(1) A.arvensis, incubation period : 4weeks.

pH	Dry wt. (mg) of mycelium					
	R1	R2	R3	R4	R5	MEAN
2.8	8.01	8.41	8.81	8.51	6.81	8.44
5.2	15.50	20.01	19.10	17.60	12.10	18.05
5.8	22.30	23.30	28.40	21.60	13.01	23.90
6.1	21.50	25.60	23.30	24.10	14.80	23.63
6.7	7.11	6.91	8.91	7.81	18.90	7.69

Anovar Summary

Total sum of squares : 1053.86

Between treatment SS : 1002.22

Within treatment SS : 51.64

Between treatment MS : 250.56

Within treatment MS : 3.44

F ratio 72.84

95% Confidence Limits $\bar{x} \pm$ 1.87

From table, Fp = 0.01(4,20) = 4.4

Result. Highly significant

(ii) A. bisporus, incubation period : 3 weeks

pH	Dry wt. (mg) of mycelium					
	R1	R2	R3	R4	R5	MEAN
3.1	5.41	6.21	6.41	7.51	6.81	6.47
5.6	12.30	13.01	10.90	11.90	12.10	12.04
6.0	14.10	12.90	12.10	13.50	13.01	13.12
6.5	15.90	12.80	15.10	13.60	14.80	14.44
6.8	16.90	15.90	19.10	19.60	18.90	18.08
7.7	6.61	7.01	6.81	6.01	7.11	6.71
6.9	5.31	7.31	4.61	6.91	6.21	6.07

ANOVAR Summary

Total sum of squares : 682.82

Between treatment SS : 653.88

Within treatment SS : 28.94

Between treatment MS : 108.98

Within treatment MS : 1.03

F ratio 105.81

95% Confidence Limits $\bar{x} \pm 0.87$

From table, Fp = 0.01(6,28) = 3.5

Result. Highly significant.

(iii) A. bitorquis, incubation period : 9 days.

pH	Dry wt. (mg) of mycelium				
	R1	R2	R3	R4	MEAN
2.5	4.2	4.5	4.7	4.9	4.57
3.3	4.1	5.5	4.8	4.7	4.78
5.2	22.9	24.9	23.8	24.0	23.90
5.9	27.8	31.1	26.4	28.2	28.38
6.1	38.9	35.8	45.3	39.1	39.78
6.3	33.1	38.4	37.7	36.9	36.53
7.1	27.2	29.4	27.2	28.1	27.98
8.1	5.9	6.7	5.5	6.3	6.10
8.3	5.4	4.7	4.9	4.8	4.95

ANOVAR Summary

Total sum of squares : 6889.57

Between treatment SS : 6805.94

Within treatment SS : 83.63

Between treatment MS : 850.74

Within treatment MS : 3.1

F ratio 274.43

95% Confidence Limits $\bar{x} \pm 1.8$

From table, $F_p = 0.01(8,27) = 3.3$

Result. Highly significant.

(iv) A.aggregatum

pH	Dry wt. (mg) of mycelium				
	R1	R2	R3	R4	MEAN
3.1	13.1	12.1	13.6	15.1	13.48
5.1	27.2	28.7	23.5	28.3	26.93
5.3	30.0	27.0	29.1	30.1	29.05
6.1	22.3	22.5	24.5	24.5	23.45
6.5	28.5	29.8	31.1	31.1	29.88
7.0	29.3	27.9	33.7	33.9	31.20
7.6	25.3	24.0	22.9	21.1	23.33

ANOVAR Summary

Total sum of squares : 951.52

Between treatment SS : 878.36

Within treatment SS : 73.16

Between treatment MS : 146.39

Within treatment MS : 3.48

F ratio 42.07

95% Conidence Limits $\bar{x} \pm 1.94$

From table, Fp = 0.01(6,21) = 3.8

Result. Highly significant.

(v) C.comatus, incubation period : 2 weeks.

pH	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
3.0	5.7	5.7	5.5	5.1	128.6	5.50
5.3	15.1	18.5	22.3	24.0	165.2	19.98
5.7	22.5	24.5	26.9	26.2	142.3	25.03
5.9	26.4	24.1	26.1	22.4	162.5	24.75
6.3	23.1	23.5	24.3	2.9	171.4	18.45
6.8	11.2	8.8	9.1	12.0	149.9	10.28
8.0	5.7	5.8	6.6	7.4	129.7	6.38

ANOVAR Summary

Total sum of squares : 2062.62

Between treatment SS : 1660.36

Within treatment SS : 402.26

Between treatment MS : 276.73

Within treatment MS : 19.16

F ratio 14.44

95% Confidence Limits $\bar{x} \pm 4.6$

From table, Fp = 0.01(6,28) = 3.5

Result. Highly significant.

(vi) L.nuda

pH	Dry wt. (mg) of mycelium					
	R1	R2	R3	R4	R5	MEAN
2.3	20.9	23.8	22.1	21.4	128.6	22.05
5.0	33.3	28.6	30.5	34.4	165.2	31.70
5.5	30.2	40.0	38.8	36.2	142.3	36.30
5.8	32.6	25.7	28.3	28.5	162.5	28.78
6.4	41.6	42.9	43.2	41.2	171.4	42.23
6.9	28.8	27.4	28.5	29.6	149.9	28.58
7.5	17.9	17.3	17.5	20.7	129.7	18.35
8.3	20.7	17.0	20.3	20.0	166.7	19.50

ANOVAR Summary

Total sum of squares : 2069.23

Between treatment SS : 1940.5

Within treatment SS : 128.73

Between treatment MS : 277.21

Within treatment MS : 5.36

F ratio 51.72

95% Confidence Limits $\bar{x} \pm 2.41$

From table, Fp = 0.01(7,32) = 3.2

Result. Highly significant.

(vii) L. saeva, incubation period : 18 days.

pH	Dry wt. (mg) of mycelium				
	R1	R2	R3	R4	MEAN
2.9	5.2	4.5	5.7	5.7	5.28
5.2	25.1	22.2	31.4	31.0	27.43
6.0	32.1	34.6	32.5	31.9	32.77
6.7	23.6	27.7	26.4	29.3	26.75
7.0	28.6	24.1	33.2	31.3	29.30
7.3	28.6	31.0	29.1	26.8	28.88
8.2	17.5	15.0	17.9	18.2	17.15
8.5	14.8	15.4	13.6	15.0	14.70

ANOVAR Summary

Total sum of squares : 2629.45

Between treatment SS : 2481.21

Within treatment SS : 148.24

Between treatment MS : 354.46

Within treatment MS : 6.18

F ratio : 57.36

95% Confidence Limits $\bar{x} \pm 2.59$

From table, Fp = 0.01(7,24) = 3.4

Result. Highly significant.

(ix) M.conica, incubation period : 3 weeks.

pH	Dry wt. (mg) of mycelium.				
	R1	R2	R3	R4	MEAN
3.0	15.9	12.9	16.4	12.0	14.30
3.5	16.8	17.3	15.1	16.8	16.50
4.0	46.7	36.4	36.7	42.3	40.53
5.0	47.5	49.7	44.7	45.4	46.83
5.5	47.3	39.5	53.9	50.2	50.23
5.9	50.3	57.0	55.2	51.3	58.45
6.8	32.8	31.0	31.1	33.0	31.98
7.2	20.8	20.0	20.8	16.8	19.60
7.8	11.9	13.3	13.1	12.7	12.75

ANOVAR Summary

Total sum of squares : 8822.24

Between treatment SS : 8648.76

Within treatment SS : 173.48

Between treatment MS : 1081.09

Within treatment MS : 6.43

F ratio 168.13

95% Confidence Limits $\bar{x} \pm 2.6$

From table, Fp = 0.01(8,27) = 3.3

Result. Highly significant.

(viii) M.vulgaris, incubation period : 1 week.

pH	Dry wt. (mg) of mycelium				
	R1	R2	R3	R4	MEAN
2.8	7.3	7.6	8.0	9.6	8.13
3.0	10.1	9.6	8.3	10.0	9.50
5.0	82.3	82.2	88.7	95.4	87.15
6.0	89.9	87.8	91.0	84.7	88.35
6.4	86.0	89.1	93.9	88.2	89.30
7.4	63.1	63.9	64.7	66.4	64.53
7.7	21.7	20.0	20.4	21.8	20.98
8.0	15.9	15.7	15.4	17.7	16.18
8.6	12.4	12.5	15.0	16.1	14.0
8.9	6.4	6.5	7.4	5.5	6.45

ANOVAR Summary

Total sum of squares : 63971.65

Between treatment SS : 63744.71

Within treatment SS : 226.94

Between treatment MS : 6374.47

Within treatment MS : 6.88

F ratio 926.52

95% Confidence Limits : $\bar{x} \pm 2.68$

From table, Fp = 0.01(9,30) = 3.2

Result. Highly significant.

(x) Phallus impudicus, incubation period : 14 days.

pH	Dry wt. (mg) of mycelium				
	R1	R2	R3	R4	MEAN
3.0	9.0	8.6	9.6	8.8	9.0
5.3	28.2	30.1	29.9	26.4	28.65
6.0	28.3	29.8	26.1	29.7	28.48
6.7	8.8	9.4	9.0	8.2	8.85
7.0	4.3	4.5	4.4	4.5	4.43
7.3	3.5	3.7	3.8	3.8	3.70

ANOVAR Summary

Total sum of squares : 2712.48

Between treatment SS : 2693.22

Within treatment SS : 19.26

Between treatment MS : 538.64

Within treatment MS : 1.07

F ratio 503.4

95% Confidence Limits $\bar{x} \pm 1.09$

From table, Fp = 0.01(5,18) = 4.3

Result. Highly significant.

(xi) P.sajor-caju, incubation period : 9 days.

pH	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
2.3	9.1	9.2	8.8	8.4	128.6	8.88
3.0	9.8	8.8	8.7	9.3	165.2	9.15
4.1	33.1	32.2	26.6	29.9	142.3	30.45
5.5	39.8	37.5	39.7	39.2	162.5	39.05
6.0	23.8	18.9	19.9	19.6	171.4	20.55
6.4	16.8	12.6	12.8	13.1	149.9	13.83
7.4	12.2	14.5	13.0	12.7	129.7	13.10
7.7	12.2	12.0	10.9	11.8	166.7	11.73

ANOVAR Summary

Total sum of squares : 3444.58

Between treatment SS : 3384.33

Within treatment SS : 60.25

Between treatment MS : 483.48

Within treatment MS : 2.51

F ratio 192.62

95% Confidence Limits $\bar{x} \pm 1.65$

From table, Fp = 0.01(7,32) = 3.2

Result. Highly significant.

(xii) P.cervinus, incubation period : 5 weeks.

pH	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
3.0	8.7	7.7	10.9	10.0	9.2	9.3
5.3	142.5	143.9	154.1	143.9	139.4	144.9
6.0	186.9	177.7	185.4	177.1	183.8	182.9
6.3	164.1	157.7	147.7	162.6	153.9	157.2
6.9	95.7	97.9	88.8	80.4	81.5	88.86
7.3	90.4	61.0	60.3	85.2	76.0	74.58
8.0	25.1	38.8	28.9	34.7	30.2	31.54

ANOVAR Summary

Total sum of squares : 129974.59

Between treatment SS : 128466.15

Within treatment SS : 1508.43

Between treatment MS : 21411.03

Within treatment MS : 53.87

F ratio 398.1

95% Confidence Limits $\bar{x} \pm 6.3$

From table, Fp = 0.01(6,28) = 3.5

Result. Highly significant.

(xiii) S. rugoso-anmulata

pH	Dry wt. (mg) of mycelium.				
	R1	R2	R3	R4	MEAN
2.8	11.9	12.5	10.0	11.3	11.43
4.0	14.6	16.3	14.2	16.1	15.30
5.1	21.0	28.5	24.3	21.3	23.78
5.7	16.3	16.2	18.8	17.8	17.28
5.9	15.7	16.4	16.2	15.2	15.88
6.5	14.2	13.3	14.6	13.8	13.98
7.0	17.0	15.1	18.0	17.2	16.83
7.7	12.6	10.6	10.6	10.2	11.0
8.2	9.1	11.4	8.5	11.5	10.13

ANOVAR Summary

Total sum of squares : 624.64

Between treatment SS : 559.67

Within treatment SS : 64.97

Between treatment MS : 69.96

Within treatment MS : 2.41

F ratio 29.03

95% Confidence Limits $\bar{x} \pm 1.6$

From table, Fp = 0.01(8,27) = 3.3

Result. Highly significant.

Appendix 2.1 (ai) : Effect of various vitamins and yeast extract on the vegetative growth of P.sajor-caju after 2 weeks.

Vitamins	Dry wt. (mg) of mycelium					
	R1	R2	R3	R4	R5	MEAN
CV	100.8	108.6	111.6	99.9	120.4	108.24
CV-T	49.7	75.5	66.1	45.8	58.9	296.00
CV-B	99.2	87.8	103.5	91.9	104.1	97.30
CV-I	118.7	105.9	126.7	108.9	97.8	111.60
CV-Pyr	93.4	112.0	95.9	114.4	104.4	104.02
CV-PA	102.1	107.4	98.9	92.8	111.9	102.62
CV-R	85.5	104.8	97.4	107.6	109.1	100.88
CV-NA	117.2	115.8	101.3	100.1	109.6	108.80
CV-FA	98.7	91.8	97.4	99.7	84.6	94.44
YE	225.4	258.5	257.3	218.1	232.7	238.40
-CV	36.2	21.7	22.7	33.4	39.0	30.60

ANOVAR Summary

Total sum of squares : 133423.04

Between treatment SS : 128456.75

Within treatment SS : 4966.29

Between treatment MS : 12845.68

Within treatment MS : 112.87

F ratio 113.81

95% Confidence Limits $\bar{x} \pm 9.58$

From table, Fp = 0.01(10,44) = 2.7

Result. Highly significant.

Key : CV = "Complete" vitamin solution
- = Elimination of the appropriate vitamin
T = thiamine; B = biotin; I = inositol
Pyr = pyridoxine; PA = pantothenic acid; R = riboflavin
NA = nicotinic acid; FA = folic acid
YE = yeast extract; -CV = vitamin-free control.

(aii) : Effect of various vitamins and yeast extract on the vegetative growth of P.Sajor-caju after 3 weeks.

Vitamins	Dry. wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
CV	220.5	263.0	218.6	230.9	222.7	231.14
CV-T	137.5	130.6	125.4	101.2	104.3	119.80
CV-B	177.4	201.5	176.8	182.9	194.1	186.54
CV-I	188.9	216.6	213.5	201.7	197.2	203.58
CV-Pyr	200.1	199.2	190.9	213.6	187.0	198.12
CV-PA	179.2	179.7	206.4	210.9	188.8	195.60
CV-R	178.7	190.0	182.3	188.4	197.5	187.38
CV-NA	172.6	206.6	204.1	210.1	192.3	197.74
CV-FA	178.9	209.4	221.2	205.3	180.0	198.96
YE	388.7	351.1	360.8	319.6	348.2	353.68
-CV	56.5	78.5	77.1	67.3	59.4	67.76

ANOVAR Summary

Total sum of squares : 252814.85

Between treatment SS : 242825.14

Within treatment SS : 9989.71

Between treatment MS : 24282.51

Within treatment MS : 227.04

F ratio 106.95

95% Confidence Limits $\bar{x} \pm$ 13.59

From table, Fp = 0.01(10,44) = 2.7

Result. Highly significant.

Appendix 2.1.(b) : Effect of various nitrogen sources on the vegetative growth of P. sajor-caju after 2 weeks.

Nitrogen Sources	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
CaS. hyd	207.2	225.3	232.1	299.9	230.8	219.06
Asparagine	91.0	81.8	88.1	85.6	90.4	87.37
NH_4HCO_3	88.3	98.2	89.5	88.5	82.4	89.38
Amm.tat	85.1	129.8	97.9	116.7	107.1	107.32
NH_4NO_3	94.0	85.4	94.7	81.1	78.8	86.80
$NaNO_3$	22.1	26.1	26.5	31.3	20.3	25.26
KNO_2	20.1	18.2	15.5	21.2	11.4	17.28
Urea	193.6	226.8	173.2	151.5	217.7	192.56
Peptone	129.7	130.2	124.9	127.9	118.3	126.20
N-free Contr.	17.9	16.3	14.8	14.5	14.2	15.54

ANOVAR Summary

Total sum of squares : 223438.63

Between treatment SS : 216903.75

Within treatment SS : 6534.88

Between treatment MS : 24100.42

Within treatment MS : 163.37

F ratio 147.52

95% Confidence Limits $\bar{x} \pm 11.52$

From table, Fp = 0.01(9,40) = 3.0

Result. Highly significant.

Appendix 2.1.(c) : Effect of Carbon : Nitrogen ratio on the Vegetative growth of P.sajor-caju (using glucose and asparagine as carbon and nitrogen sources respectively) after 2 weeks.

C : N ratio	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
10 : 1	38.7	37.1	49.5	47.9	33.5	41.34
20 : 1	51.1	52.9	56.9	66.8	55.9	56.72
30 : 1	57.6	54.8	62.9	68.2	62.6	61.22
40 : 1	64.5	58.3	63.9	59.8	67.4	62.78
50 : 1	69.6	72.5	70.7	67.0	61.9	68.34
60 : 1	67.5	82.9	71.4	85.0	79.9	77.34
70 : 1	106.3	104.4	96.5	99.4	86.2	98.56
80 : 1	132.1	92.3	138.9	110.2	108.1	116.32
90 : 1	150.0	125.5	137.8	114.6	120.9	129.76
100 : 1	90.6	125.0	128.8	123.0	110.1	115.50

ANOVAR Summary

Total sum of squares : 45023.67

Between treatment SS : 40754.98

Within treatment SS : 4268.69

Between treatment MS : 4528.33

Within treatment MS : 106.72

F ratio 42.43

95% Confidence Limits $\bar{x} \pm 9.31$

From table, Fp = 0.01(9,40) = 2.2

Result. Highly significant.

Appendix 2.1.(d) : Effect of various fatty acids and esters on the vegetative growth of P.sajor-caju after 2 weeks.

Fatty acids/ esters	Dry wt. (mg) of mycelium					
	R1	R2	R3	R4	R5	MEAN
Oleic acid	259.8	287.3	301.5	229.7	239.9	263.64
Methyl oleate	236.2	217.3	249.6	276.3	234.6	242.80
Ethyl oleate	256.8	203.9	239.1	223.9	229.4	230.62
Linoleic acid	209.8	223.2	288.5	238.6	213.7	234.76
Ethyl linoleate	212.3	229.0	211.6	173.8	219.4	208.22
Stearic acid	201.7	203.0	261.0	235.8	226.2	225.54
Methyl stearate	252.4	306.9	229.0	253.6	232.5	254.88
Palmitic acid	265.6	203.9	191.5	220.3	209.8	218.22
Methyl palmitate	233.0	287.7	182.5	249.8	245.2	239.64
Myristic acid	211.8	226.3	236.9	228.1	222.8	225.18
Myristic acid methy ester	195.6	237.7	219.0	238.7	220.6	222.32
Ethyl acetate	232.8	246.0	295.2	286.2	227.1	257.46
Lipid-free contr.	255.7	231.8	241.9	220.3	196.2	229.18

ANOVAR Summary

Total sum of squares : 51599.58

Between treatment SS : 15740.50

Within treatment SS : 689.60

Between treatment MS : 1311.76

Within treatment MS : 689.60

F ratio 1.9

95% Confidence Limits $\bar{x} \pm$ 23.45mg

From table, Fp = 0.01(12,52) = 2.5

From table, Fp = 0.05(12,52) = 1.9

Result. Not significant.

Appendix 2.1.(e) : Effect of trace elements on the vegetative growth of P.sajor-caju in a defined liquid medium after 3 weeks.

Trace element	Dry wt. (mg) of mycelium					
	R1	R2	R3	R4	R5	MEAN
CM	47.5	56.2	67.6	59.2	60.7	58.24
CM - Ca	77.9	75.1	78.8	79.4	71.5	76.54
CM - Mo	80.5	74.6	99.2	77.8	72.5	80.92
CM + Cu	86.8	90.5	78.4	79.9	80.1	83.14
CM + Mn	70.5	73.1	69.0	72.4	65.8	70.16
CM - Zn	78.2	75.1	78.0	76.8	70.9	75.80
CM + Fe	80.5	72.4	92.7	82.4	85.1	82.62
- CM	52.6	61.3	60.1	54.3	52.6	56.18
BM + T	176.3	184.3	158.0	169.1	158.8	169.30
BM + B	121.3	112.3	119.4	108.9	99.7	112.32
CM + Li	81.1	79.7	78.6	76.0	69.8	77.04
BM + Y	164.7	174.9	129.1	111.6	135.7	143.20
BM - W	68.7	76.4	78.2	64.6	70.5	71.68
BM + R	75.8	88.7	84.9	74.2	79.1	80.54
BM + M	98.5	80.7	82.5	91.6	85.3	87.72

ANOVAR Summary

Total sum of squares : 71720.94

Between treatment SS : 66438.7

Within treatment SS : 5282.24

Between treatment MS : 4745.62

Within treatment MS : 88.04

F ratio 53.9

95% Confidence Limits $\bar{x} \pm$ 8.39

From table, Fp = 0.01(4,60) = 3.7

Result. Highly significant.

KEY :

CM = "complete" mineral solution

- = minus

BM = basal medium

T = thiamine

B = biotine

Y.EXT = Yeast extract

M.EXT = Malt extract

W.S.= Wheat straw

R.S.= Rice straw.

Appendix 2.1.(f) : Effect of oxygen concentration on the vegetative growth of P.sajor-caju after 10 days.

% Conc.	Mycelial extension (cm)					
	R1	R2	R3	R4	MEAN	Growth rate mm/day
20.4	9.5	8.9	8.5	9.0	8.98	12.8
19.1	9.6	9.1	9.0	9.1	9.20	13.1
18.7	9.5	9.3	9.2	9.2	9.30	13.3
15.9	10.0	9.5	9.2	8.9	9.40	13.4
14.1	9.1	9.9	9.6	9.8	9.60	13.7
13.2	9.8	9.7	9.8	9.8	9.78	13.9
12.3	9.8	9.7	9.6	9.9	9.75	13.9
10.2	10.1	10.6	10.4	9.4	10.13	14.5
8.34	9.9	10.0	9.5	9.4	9.70	13.9
6.6	9.9	10.4	9.4	9.5	9.80	14.0
4.74	9.9	10.3	9.8	9.2	9.80	14.0
3.0	10.4	10.4	9.3	9.4	9.88	14.1
0.48	10.0	10.0	9.0	9.3	9.58	13.7

ANOVAR Summary

Total sum of squares : 10.79

Between treatment SS : 4.69

Within treatment SS : 6.1

Between treatment MS : 0.39

Within treatment MS : 0.16

F ratio 2.44

95% Confidence Limits $\bar{x} \pm 0.4$

From table, Fp = 0.01(11,36) = 20.

Result. Significant.

Appendix 2.1.(g) : Effect of carbon dioxide concentration on the vegetative growth of P.sajor-caju after 10 days.

% Conc.	Mycelial extension (cm)					Growth rate mm/day
	R1	R2	R3	R4	MEAN	
0.48	11.4	12.0	11.7	11.5	11.15	14.6
2.6	11.2	11.2	11.5	11.7	11.40	14.3
4.6	10.9	11.2	11.4	11.3	11.20	14.0
7.2	10.5	10.5	11.3	11.4	10.93	13.6
9.9	10.4	11.0	10.5	10.5	10.60	13.1
12.5	11.0	11.5	10.9	10.7	11.03	13.8
15.1	10.4	11.0	10.4	10.2	10.50	13.1
17.8	10.4	10.4	10.0	10.1	10.23	12.8
20.6	10.0	10.4	10.0	10.0	10.10	12.6
23.1	10.2	10.3	10.2	10.3	10.25	12.8
25.7	9.8	9.8	10.0	10.0	9.90	12.4
29.3	9.4	9.4	9.5	9.7	9.50	11.9

ANOVAR Summary

Total sum of squares : 20.92

Between treatment SS : 18.49

Within treatment SS : 2.43

Between treatment MS : 1.68

Within treatment MS : 0.07

F ratio 24.0

95 % Confidence Limits $\bar{x} \pm$ 0.26cm

From table, Fp = 0.01(11,36) = 2.7

Result. Highly significant.

Appendix 2.1.(h) : Vegetative growth of P.sajor-caju on various solid natural substrates after 12 days.

Substrate	Depth of growth (cm)					
	R1	R2	R3	R4	R5	MEAN
Oak L	4.1	4.7	4.3	4.9	5.3	4.66
Sycamore L	7.8	9.2	8.0	7.2	7.0	7.84
Chestnut L	4.3	4.2	4.6	4.7	4.0	4.36
Fir L	5.4	5.6	4.8	5.8	5.8	5.48
	0.5	0.9	1.5	0.7	0.6	0.84
W.S.	13.0	12.3	13.5	11.6	14.3	12.94
R.S.	13.5	14.3	14.5	14.5	14.8	14.32
L.C.L.	8.9	7.5	8.0	8.2	8.8	8.28
L.L.(fresh)	4.8	6.2	5.8	5.4	5.0	5.44
L.L.(old)	2.01	1.51	2.81	2.91	2.2	2.28
Comp.(st)	10.0	8.5	10.5	11.1	9.6	9.94
Comp.(unst.)	7.0	6.5	6.1	5.7	6.3	6.32
Old Newsp.	6.4	5.4	5.0	4.8	5.0	5.32

ANOVAR Summary

Total sum of squares : 920.67

Between treatment SS : 899.34

Within treatment SS : 21.33

Between treatment MS : 74.95

Within treatment MS : 0.41

F ratio 182.8

95% Confidence Limits $\bar{x} \pm 0.57$

From table, Fp = 0.01(12,52) = 2.5

Result. Highly significant.

Appendix 2.2.(a) : Influence of light on the growth and fruit-body formation of P. sajor-caju.

Effect of light on vegetative growth on agar medium after 7 days.

Condition of illumination	Colony diameter (cm)					
	R1	R2	R3	R4	R5	MEAN
Cont. light	6.3	6.3	6.5	6.2	6.2	6.3
Alt.light & dark	4.8	4.7	5.0	5.2	4.8	4.9
Cont. dark	2.9	2.6	2.8	2.7	3.0	2.8

ANOVAR Summary

Total sum of squares : 31.35

Between treatment SS : 31.03

Within treatment SS : 0.32

Between treatment MS : 15.52

Within treatment MS : 0.03

F ratio 517.33

95% Confidence Limits $\bar{x} \pm 0.17$

From table, Fp = 0.01'2,8) = 8.7

Result. Highly significant.

Appendix 2.2.(b) : To detect the time of optimum sensitivity of the mycelium of P.sajor-caju to light for primordium formation.

Growth condition		No. of initials	Mean	No. fruit-bodies	Mean
1.Exposed to light from start	i	1200		4	
	ii	1232		5	
	iii	1216	1175	5	4
	iv	1120	± 50	3	
	v	1112		3	
2.Exposed to light after 3 days in continuous dark	i	1192		5	
	ii	1216		3	
	iii	1184	1192	4	4
	iv	1280	± 62	4	
	v	1088		4	
3.Exposed to light after 5 days in continuous dark	i	1600		7	
	ii	1568		7	
	iii	1696	1597	8	8
	iv	1472	±74	9	
	v	1648		9	
4.Exposed to light after 7 days in continuous dark	i	2392		10	
	ii	2440		6	
	iii	2240	2384	9	8
	iv	2520	± 93	7	
	v	2328		8	

Growth condition		No. of initials	Mean	No. fruit-bodies	Mean
5. Exposed to light after 9 days in continuous dark	i	1960		6	
	ii	1992		6	6.20
	iii	1984	2018 ±48	9	6
	iv	2072		5	
	v	2080		5	
6. Exposed to light after 11 days in continuous dark	i	1520		4	
	ii	1688		5	6
	iii	1576	1624 ± 82	7	
	iv	1752		6	
	v	1584		8	
7. Exposed to light after 13 days in continuous dark	i	728		5	
	ii	872		3	
	iii	864		4	4.2
	iv	730	798 ±61	5	4
	v	792		4	
8. Exposed to light after 15 days in continuous dark	i	384		3	
	ii	320		2	
	iii	488	336 ±121	1	
	iv	116		2	
	v	408		2	
9. Exposed to light after 17 days in continuous dark	i	0		0	
	ii	0		0	
	iii	0	0	0	0
	iv	0		0	
	v	0		0	

Confidence Limit at 95% $\bar{x} \pm 1.96 \sqrt{\frac{\sigma}{N-1}}$

Appendix 3.1.(a) : Effect of various vitamins and yeast extract on the vegetative growth of S. rugoso-annulata.

Vitamins	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
CV	55.0	55.2	52.8	60.5	68.1	58.3
CV - T	27.2	28.5	26.4	34.9	27.8	29.0
CV - B	45.6	41.7	39.8	49.2	42.8	43.8
CV - I	48.5	51.4	47.3	43.0	48.1	47.7
CV - Pyr	45.5	49.1	49.2	38.2	38.9	44.2
CV - PA	52.3	49.0	34.8	35.1	53.4	44.9
CV - R	47.6	32.3	49.6	46.3	40.2	43.2
CV - NA	47.0	41.3	48.5	51.2	42.6	46.1
CV - FA	43.7	44.1	53.6	39.8	41.2	44.5
YE	69.4	66.0	72.3	93.4	81.5	76.5
- CV	23.9	23.8	25.7	22.3	24.6	24.1

ANOVAR Summary

Total sum of squares : 110.40

Between treatment SS : 73.98

Within treatment SS : 16.42

Between treatment MS : 9.40

Within treatment MS : 0.37

F ratio 25.20

95% Confidence Limits $\bar{x} \pm 0.55$

From table, Fp = 0.01(10,44) = 2.4

Result. Highly significant.

where $\bar{x}$ = mean of sample.

Appendix 3.1.(b) : Effect of various nitrogen sources on the vegetative growth of S.rugos-annulata after 4 weeks.

Nitrogen sources	Dry wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
Cas. hyd.	55.7	48.5	49.2	49.5	46.5	49.88
Asparagine	43.6	34.8	31.4	38.4	32.2	36.08
NH_4HCO_3	69.9	63.9	65.5	61.8	65.3	64.28
NH_4NO_3	77.4	81.2	65.9	72.6	71.7	73.76
$NaNO_3$	9.0	8.0	9.3	10.0	10.3	9.30
KNO_2	9.1	9.0	9.1	9.2	8.1	8.90
Urea	64.7	66.9	61.0	60.2	61.5	62.86
Peptone	75.6	79.6	78.8	76.3	78.5	77.76
Amm.Tatrate	64.9	63.9	65.5	61.8	65.3	64.28
N-free contr.	7.8	7.0	6.4	6.7	7.3	7.04

ANOVAR Summary

Total sum of squares : 37040.58

Between treatment SS : 36591.45

Within treatment SS : 449.13

Between treatment MS : 4065.72

Within treatment MS : 101.65

F ratio 40.00

95 % Confidence Limits $\bar{x} \pm 9.09$

From table, Fp = 0.01(9,40) = 3.00

Result. Highly significant.

Appendix 3.1.(c) : Effect of carbon : nitrogen ratio on the vegetative growth of S.rugoso-annulata (using glucose and asparagine respectively as carbon and nitrogen sources) after 4 weeks.

C : N ratio	Dry Wt. (mg) of mycelium					
	R1	R2	R3	R4	R5	MEAN
20 : 1	33.4	17.6	20.2	23.1	23.6	23.58
30 : 1	24.5	23.8	41.8	33.2	31.1	30.88
40 : 1	45.2	49.1	57.6	51.8	56.9	52.12
50 : 1	53.7	50.1	59.2	65.9	61.7	58.12
60 : 1	70.3	58.8	62.7	64.7	73.3	65.96
70 : 1	68.5	86.5	73.8	90.5	89.2	81.70
80 : 1	85.5	93.0	84.6	103.3	93.3	91.94
90 : 1	83.4	68.1	72.4	74.8	82.3	76.20
100 : 1	51.4	44.9	59.7	49.8	44.9	50.08

ANOVAR Summary

Total sum of squares : 22294.85

Between treatment SS : 20587.45

Within treatment SS : 1707.4

Between treatment MS : 2573.43

Within treatment MS : 47.43

F ratio 54.26

95% Confidence Limits $\bar{x} \pm 6.25$

From table, Fp = 0.01(8,34) = 30

Result. Highly significant.

Appendix 3.1.(d) : Effect of fatty acids and esters on the vegetative growth of S.rugoso-annulata after 5 weeks.

Fatty acid & ester	Dry Wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
i	142.0	129.0	155.3	152.5	128.6	141.48
e	170.8	170.6	151.8	170.7	165.2	165.82
g	167.0	138.4	130.8	160.4	142.3	147.78
c	170.4	158.6	176.0	183.1	162.5	170.12
d	170.2	169.6	155.3	175.2	171.4	168.34
f	150.0	146.4	146.8	169.9	149.9	152.60
j	118.0	122.3	155.4	132.6	129.7	131.60
a	182.9	165.9	171.6	169.2	166.7	171.26
h	143.0	135.8	166.3	134.1	140.7	143.98
b	183.9	163.9	172.1	177.1	158.6	171.12
k	128.6	125.2	131.4	125.8	127.9	127.78
m	140.7	106.8	100.2	134.8	129.2	122.34
l	136.9	138.4	98.3	126.0	128.9	125.70

ANOVAR Summary

Total sum of squares : 28257.3

Between treatment SS : 20950.2

Within treatment SS : 7307.1

Between treatment MS : 1745.85

Within treatment MS : 140.52

F ratio 12.42

95% Confidence Limits $\bar{x} \pm$ 10.71 From table, Fp = 0.01(12,52) = 2.5

Pp = 0.05(12,52) = 1.9

Result. Significant.

Appendix 3.1.(e) : Effect of trace elements on the vegetative growth of S.rugoso-annulata in a defined liquid after 40 days.

Trace element	Dry Wt. (mg) of mycelium.					
	R1	R2	R3	R4	R5	MEAN
CM	56.1	48.0	45.6	47.8	47.2	48.94
CM - Ca	32.9	51.9	47.8	48.8	59.9	48.26
CM - Mo	53.0	51.2	67.6	59.1	50.5	56.28
CM - Cu	66.5	46.4	60.7	52.1	47.5	54.64
CM - Mn	48.6	50.3	41.4	58.8	43.7	48.56
CM - Zn	53.6	41.0	48.8	53.5	42.5	47.88
CM - Fe	31.7	34.1	38.4	39.6	43.4	37.44
CM - Li	42.7	43.8	41.0	52.1	50.8	46.08
- CM	36.5	35.5	31.9	37.2	30.8	34.38
-CM + T	99.1	121.2	88.9	82.5	74.2	93.18
-CM + B	52.4	49.3	40.0	47.8	42.8	46.46
BM + Y.EXT.ASH	39.5	36.1	44.2	42.7	42.4	40.78
BM + M.EXT.ASH	50.7	42.8	43.8	52.9	53.6	48.76
BM + W.S. ASH	34.3	56.4	37.2	32.3	51.8	42.40
BM + R.S. ASH	49.5	44.0	45.2	56.0	35.9	46.12

ANOVAR Summary

Total sum of squares : 16352.98

Between treatment SS : 12593.7

Within treatment SS : 3759.28

Between treatment MS : 899.55

Within treatment MS : 62.65

F ratio 14.36

95% Confidence Limits $\bar{x} \pm 7.08$

From table, Fp = 0.01(14,60) = 2.5

KEY

CM = "complete" mineral solution

\- = minus

BM = basal medium

T = thiamine

B = biotin

Y.EXT = Yeast extract

M.EXT = Malt extract

W.S. = Wheat straw

R.S. = Rice straw

Appendix 3.1.(f) : Effect of oxygen concentration on the vegetative growth of S.rugoso-annulata after 12 days.

% Conc.	Mycelial extension (mm)					Growth rate(mm/day)
	R1	R2	R3	R4	MEAN	
20.4	9.7	9.8	9.9	10.1	9.88	8.2
19.8	9.6	9.2	9.6	9.9	9.58	8.0
18.72	9.8	9.0	10.1	9.4	9.58	8.0
15.9	8.9	8.7	9.1	8.8	8.88	7.4
14.1	8.1	8.2	9.0	9.0	8.58	7.2
13.2	8.2	8.3	8.0	7.9	8.10	6.8
12.3	7.8	8.2	7.6	7.9	7.88	6.6
10.2	7.6	7.7	8.2	8.1	7.90	6.6
8.34	8.8	7.6	8.5	8.2	8.27	6.9
6.6	8.5	8.2	8.6	8.2	8.38	6.9
4.74	8.0	8.1	7.7	8.1	7.98	6.7
3.0	7.6	7.7	7.5	7.6	7.60	6.3
0.48	5.0	5.9	6.1	6.2	5.80	4.8

ANOVAR Summary

Total sum of squares : 57.74

Between treatment SS : 53.39

Within treatment SS : 4.35

Between treatment MS : 4.45

Within treatment MS : 0.11

F ratio 40.45

95% Confidence Limits $\bar{x} \pm 0.33$

From table, Fp = 0.01(12,39) = 2.7

Result. Highly significant.

Appendix 3.1.(g) : Effect of carbon dioxide concentration on the vegetative growth of S.rugoso-annulata after 12 days.

% Conc.	Mycelial extension (mm)					Growth rate (mm/day)
	R1	R2	R3	R4	MEAN	
0.48	68.0	67.0	70.0	65.0	67.50	5.6
4.6	57.0	60.0	56.0	56.0	57.25	4.8
7.2	57.0	53.0	51.0	51.0	53.0	4.4
9.2	43.0	40.0	45.0	44.0	43.0	3.6
15.1	45.0	40.0	39.0	45.0	42.25	3.5
17.8	45.0	40.0	40.0	40.0	41.25	3.4
20.6	44.0	40.0	45.0	47.0	44.0	3.7
23.1	43.0	33.0	40.0	44.0	40.0	3.3
25.7	40.0	36.0	38.0	35.0	37.25	3.1
29.3	34.0	34.0	38.0	35.0	35.25	2.9

ANOVAR Summary

Total sum of squares : 3898.78

Between treatment SS : 3661.78

Within treatment SS : 237.0

Between treatment MS : 406.86

Within treatment MS : 7.9

F ratio 51.5

95% Confidence Limits $\bar{x} \pm 2.87$

From table, Fp = 0.01(9,30) = 3.2

Result. Highly significant.

Appendix 3.1.(h) : Vegetative growth of S.rugoso-annulata on various natural substrates after 12 days.

Substrate	Depth of mycelial growth					
	R1	R2	R3	R4	R5	MEAN
R.S	7.8	8.5	8.4	8.2	8.0	8.18
W.S.	9.9	8.8	9.0	9.5	8.9	9.22
L.C.L.	7.0	6.7	6.0	5.8	5.9	6.28
L.L.(fresh)	6.0	6.2	5.8	6.1	5.9	6.0
L.L.(old)	3.8	3.7	3.6	3.5	3.6	3.64

ANOVAR Summary

Total sum of squares : 95.32

Between treatment SS : 92.82

Within Treatment SS : 2.5

Between treatment MS : 23.21

Within treatment MS : 0.13

F ratio 178.58

95% Confidence Limits $\bar{x} \pm 0.34$

From table, $F_p = 0.01(4,20) = 4.4$

Result. Highly significant

KEY :

R.S. = Rice straw

W.S. = Wheat straw

L.C.L. = Lawsons Cypress leaf litter

L.L.(fresh) = Larch leaf litter (fresh)

L.L.(old) = Larch leaf litter (one year old).

Appendix 3.2.(a) : COMPARISON OF THE YIELD OF S. RUGOSO-ANNULATA ON VARIOUS CASING MATERIALS.

Casing Material		Fresh Wt.(g) of mush/Kg of substrate	Mean	No. of fruit-bodies	Mean	Days from Casing to pinhead formation
1. Peat + humus	i	187.90		13		
pH 6.0	ii	193.44	188.79	8	12	21
	iii	169.00	±14.7	12	±29	
	iv	204.80		15		
2. Peat + humus	i	158.34		5		
pH 6.0	ii	241.26	193.30	12	8	27
(sterilized initially)	iii	150.56	±44.7	4	±4.0	
	iv	223.02		11		
3. Peat + humus	i	139.40		12		
pH 5.0	ii	196.62	179.37	4	10.25	22 ± 5.0
	iii	170.98	±30.6	9	±5.0	
	iv	210.46		16		
4. Humus pH 7.0	i	229.96		15		
	ii	140.24	165.39	7	8.0	23 ± 4.7
	iii	131.64	±43.7	6		
	iv	159.72		4		
5. Peat pH 4.3	i	141.48		1		
	ii	-	28.3	-	0.25	31
	iii	-	±	-	±	
	iv	-		-		

6. Peat + $CaCO_3$	i	199.58		9		
pH 7.5	ii	213.08	128.8	16	7.25	24
	iii	18.90	±93.2	1	±6.6	
	iv	83.64		3		
7. No Casing	i	-		-		
(Control)	ii	-	0	-	0	
	iii	-		-		-
	iv	-		-		

ANOVAR Summary of the yield in weight.

Total sum of squares : 129268.41

Between treatment SS : 72218.32

Within treatment SS : 57049.58

Between treatment MS : 14443.76

Within treatment MS : 3169.42

F ratio 4.56

From table, Fp = 0.05(5,18) = 2.8

Result : Significant.

Appendix 3.2.(b) : CHANGES IN pH IN PEAT/HUMUS SOIL CASING LAYER OF S. RUGOSO-ANNULATA DURING A 63-DAY INCUBATION PERIOD.

Days after casing	Peat/humus soil not treated	Peat/humus soil heat-treated
0	6.0	5.8
7	6.0	5.8
14	5.7	5.6
21	5.7	5.5
28	5.6	5.7
35	5.3	5.1
42	5.3	4.9
49	5.3	5.4
56	5.7	5.5
63	5.7	5.3

Appendix 3.2.(c) : CHANGES IN THE ELECTRICAL CONDUCTIVITY OF PEAT/HUMUS SOIL CASING LAYER OF S. RUGOSO-ANNULATA DURING A 63-DAY INCUBATION PERIOD

Days after casing	Electrical conductivity	
	Peat/humus soil not treated	Peat/humus soil heat-treated
0	32	50
7	38	63
14	40	62
21	42	82
28	45	85
35	43	54
42	47	63
49	40	60
56	42	52
63	48	56

Appendix 3.2.(d) : BACTERIAL POPULATION AND COMPOSITION IN THE PEAT/HUMUS SOIL CASING LAYER OF S.RUGOSO-ANNULATA DURING A 63-DAY INCUBATION PERIOD.

Days after casing	Untreated casing			Heat-treated casing		
	No. of colonies	% Composition		No. of colonies	% Composition	
0	$102X10^4$	A	55	0	-	
		B	25			
		C	20			
7	$121X10^5$	A	60	$90X10^6$	A	50
		B	30		B	45
		C	10		C	5
14	$170X10^6$	A	54	$190X10^6$	A	40
		B	40		B	49
		C	6		C	11
21	$149X10^8$	A	42	$171X10^8$	A	32
		B	49		B	58
		C	9		C	10
28	$162X10^8$	A	30	$189X10^8$	A	25
		B	60		B	70
		C	10		C	5
35	$76X10^8$	A	44	$100X10^8$	A	32
		B	47		B	56
		C	9		C	12
42	$112X10^8$	A	60	$126X10^8$	A	62
		B	35		B	31
		C	5		C	7
49	$109X10^8$	A	64	$123X10^8$	A	80
		B	28		B	10
		C	8		C	10

56	$90X10^8$	A	70	$120X10^8$	A	81
		B	23		B	11
		C	7		C	8
63	$84X10^8$	A	65	$91X10^8$	A	78
		B	23		B	9
		C	12		C	12

Where :

Type A = cream

Type B = yellow

Type C = others.

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