fifteen years of Mushroom research

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in

Kerala Agricultural University
(1976 to 1990)

Editors
M. Aravindakshan
M. C. Nair
C. Gokulapalan

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English

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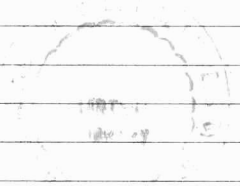

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Foreword

Kerala is bestowed with a diversity of climatic conditions and soil types making it a natural abode of different types of mushrooms. The study of the mushroom flora of Kerala is only in its exploratory stage. The cultivation of edible mushrooms has been gaining momentum in almost all the developed Asian countries and it will go a long way in increasing the range of protein rich food available to the common man. In a State such as Kerala, where intensive utilization of land for paddy cultivation is practiced, the cultivation of edible mushrooms will be of much help in the very efficient and economic conversion of agricultural waste into consumable high quality protein rich biomasss.

Concentrated efforts have been made at the College of Agriculture, Vellayani, under the Kerala Agricultural University, to conduct a systematic survey of the natural mushroom flora and research on the cultivation of the common edible species suitable for Kerala. The survey brought to light new records for the country. Techniques suitable for cultivation of tropical species such as paddy straw mushroom and the oyster mushroom have also been perfected.

The results of nearly fifteen years work on mushrooms conducted in the Kerala Agricultural University is summarised and presented in this publication which I trust will be perused with interest by all those who have an inclination for mushrooms.

Vellanikkara,
7-1-1991

E. G. Silas,
Vice-Chancellor
Kerala Agricultural University

MUSHROOM RESEARCH IN KERALA AGRICULTURAL UNIVERSITY - AN OVERVIEW

The Kerala Agricultural University came into existence in 1972. Research on mushrooms was initiated during 1976, with trials on paddy straw mushroom (Volvariella volvacea). Techniques suitable for its cultivation has been standardised and popularised in the state during the period. Organised research on this tropical mushroom was carried out by Dr. S. Bhavani Devi, for her doctoral programme under the chairmanship of Dr. M. C. Nair, at the College of Agriculture, Vellayani. In this pioneer attempt, about fifty species of native mushrooms of Kerala were also collected and described and out of this sixteen were new records for the country. A procedure for collection and identification of Agarics with a description chart was published by Dr. M. C. Nair and Dr. S. Bhavani Devi, in the monograph 'Beneficial Fungi and their Utilization'. This has been much acclaimed as a useful publication by the workers in this field. The technique developed for the cultivation of paddy straw mushroom holds good even today for raising this tropical mushroom.

During the eighties, when the oyster mushroom gained global importance, the group of workers at the College of Agriculture, Vellayani took up programmes of research on this mushroom. The study conducted by Dr. M. Suharban, for her doctoral programme was a monographic investigation of the genus Pleurotus. She could collect, identify and describe twenty out of the forty known species under this genus and based on the study a key for grouping the species has also been developed. The study helped to identify the species of Pleurotus suitable for cultivation under humid tropical conditions. The two programmes on paddy straw mushroom and oyster mushroom were partly financed by the Kerala State Committee on Science, Technology and Environment.

In the succeeding years other workers also evinced interest in research on various aspects of other groups of mushrooms. Trials on the cultivation of Coprinus sp., by D. Geetha and the work on Termitomyces species carried out by G. S. Sreelatha Nair, are two such important studies.

A project fully financed by the Dept. of Science & Technology, Govt. of India on the 'Mushroom Flora of Kerala' under the leadership of Dr. S. Bhavani Devi, made a detailed survey of the agaric wealth of Kerala.

Standardisation of techniques for the cultivation of the different types of mushroom was followed by organisation of training programmes for the Officers of the Departments of Agriculture, Forests etc., besides the one for the growers. As a result of these efforts, now mushroom cultivation is recognised as an employment generative avocation in different parts of the State.

The above mentioned programmes mainly centered around the College of Agriculture, Vellayani and the University has expanded its activities in the sphere to some centres like the Regional Agricultural Research Stations, Kumarakom, RARS Pilicode, RARS Pattambi, NARP (SR) Special Station, Kottarakkara, Rice Research Station, Kayamkulam and Cropping Systems Research Centre Karamana, Thiruvananthapuram. In all these centres work is mainly on the cultivation aspects of the oyster mushroom, besides organisation of regular training programmes for the growers.

The Indian Council of Agricultural Research, has recently sanctioned an ad hoc project on Tropical mushrooms in the College of Agriculture. The Kerala Agricultural University intends to develop this centre as the Regional Centre for Tropical Mushrooms.

The work carried out by the Kerala Agricultural University during the past fifteen years is summarised in the following pages. It is hoped that this publication will be useful to all those engaged in research and development activities in mushroom.

Editors

1.1.1991
Vellanikara

1. MUSHROOM GROWING - A HISTORICAL PERSPECTIVE

In ancient Greek and Roman literature there are references about mushrooms and their uses. As early as 300 B.C. Theophrastus recorded mushrooms as valuable food. During the centuries which followed, many well known Romans including Cierom, Juvenal and Pliny considered the mushrooms as an expensive delicacy. During the reign of Caesar there was a law on grading and selling of mushrooms. The crop growing in wild state continued to be used as food and it had provided tasty dishes throughout the middle ages without any knowledge of the true food value of this fungus.

It was in the seventeenth century during the reign of Louis XV (1638-1715) that mushrooms were gradually brought under cultivation as a crop, grown in caves near Paris. The earliest known account of mushroom cultivation was written by a French man, de Tournefort in 1707. Briefly, the system consisted of preparing horse manure and placing spores collected from wild mushrooms into it. Although on many occasions the crop did not appear, this method of cultivation was widely accepted till the period of second world war. Around 1800 the French were growing mushrooms underground in the quarries. In 1808 Rehandot planted mushroom in caves, twenty miles long and harvested an average of 3,000 lb of mushroom daily. But it took several years to spread the technique of cultivation to England, America and other countries. One of the reasons for this was that for a long time the French kept their methods of artificial cultivation of mushrooms as a closely guarded secret.

Early mushroom growers in England took great interest and around 1800 Brick spawn was being exported to many countries including America and even Australia. Today in England mushrooms rate high among the agricultural produce.

In 1893 the French brought a drastic change in the cultivation of mushrooms when sterilised or pure culture mushroom spawn was produced at the Pasteur Institute in Paris. Thus France could dominate the world in the cultivation of mushrooms.

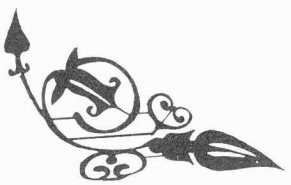
Mushroom growing in Eastern countries dates back to 1953, when the mushroom culture was initiated in northern Taiwan. With successful experimental results, commercial growing started immediately in 1955 especially in the central parts of the island. Other major mushroom growing countries are Korea, Japan and India.

In India H.W. Newtown was the first to grow mushrooms in 1886. But the species cultivated by him is not known. Knowledge about mushrooms, their habit, occurrence, medicinal value, edibility etc.,were described in ancient Indian works of medicine. However, methods of their artificial cultivation were not mentioned in those works. In 1908 Sir David Prain made a thorough study on the possibilities of mushroom industry in India and he reported that there was a ready market for edible mushrooms in Burma and India, Later the possibilities of mushroom industry in India were suggested by Bose (1921). However, serious efforts in this direction were not made till Baker (1934) first

M.C. Nair

gave a detailed account of the cultivation of *Volvariella* in Burma. Experimental cultivation of paddy straw mushroom was first undertaken in India by the Department of Agriculture of the erstwhile Madras Presidency during 1939-1945. Thomas *et al.* (1943) first introduced *V. diplasia* in Tamil Nadu and did pioneering work in its cultivation. They obtained the spawn from Burma. Kannaiyan and Prasad (1976) reviewed all the work carried out in India on the edible species of *Volvariella* especially on the standardisation of techniques for their large scale cultivation. Attempts on the cultivation of *Agaricus bisporus* were made in 1961 by the Department of Agriculture, Himachal Pradesh in collaboration with Indian Council of Agricultural Research. As a result of these efforts, mushroom industry was established in Himachal Pradesh, Delhi and Jammu & Kashmir in the North and Tamil Nadu and Karnataka in the South. Today in India the three groups of mushrooms, namely *Agaricus bisporus* (White button), *Volvariella* species (Paddy straw mushroom) and *Pleurotus* species (Oyster mushroom) are being cultivated.

In Kerala earlier accounts are not available on any attempt on the cultivation of mushroom. During 1976 preliminary trials on the artificial cultivation of paddy straw mushroom *Volvariella volvacea* were started in the Department of Plant Pathology, College of Agriculture, Vellayani, Kerala and the detailed studies conducted thereafter helped to standardise the techniques for the cultivation of the mushrooms in Kerala, which have been recommended to the farmers for adoption on a wide scale.



2. MUSHROOM FLORA OF KERALA

Kerala is bestowed with a diversity of climatic conditions and soil types and hence we have an abundant wealth of mushrooms. The study of the Agaric flora of the State is at the exploratory stage and any systematic study made in this direction will be basic to the understanding of this group of fungi for the State.

Collections made during the monsoon seasons indicated the occurrence and distribution of almost all type species in the State.

The survey conducted in the monsoon seasons for the consecutive years revealed that the growth, occurrence and distribution of different species mainly depended on the rainfall which maintains the adequate moisture content in the soil.

It was also observed that based on the periodicity of occurrence and their abundance, the flora can be grouped as pre monsoon flora, monsoon flora and post monsoon flora. Large number of different species were found to grow luxuriantly during the pre monsoon periods (10-15) days after the first showers), their number decreased during the actual rainfall period, while post monsoon periods (10-15 days after the actual rainfall) also supported the growth of a number of species including the highly esculent species like *Termitomyces robustus*, *Pleurotus ostreatus* etc. Lignicolous species like Schizophyllum commune was found to occur even 20-25 days after the rainy

season. Very little collections were made 30 days after the monsoon period.

The frequency of occurrence of the same species continuously from different localities of the State irrespective of the soil type indicated their cosmopolitan nature of occurrence. The different species belonging to in the following genera can be listed as the common flora of the State, *Lepiota, Macrolepiota, Coprinus, Marasmius, Pleurotus, Termitomyces, Agaricus, Psathyrella, Cortinarius, Schizophyllum* and *Tricholoma.*

Different species of *Lepiota* ranging from very small size to large (0.5 cm - 15 cm) occur in large number throughout the State. Though *Macrolepiota procera* and *Macrolepiota rachodes* occur abundantly they are not considered as edible and hence are not popular among the people of the State. Lepiotas are locally known as "Pranthankoon" - the mad mushrooms. It is believed that these mushrooms cause some hallucinogenic effect and allergies and hence people discard them as inedible. This may be due to the lack of scientific basis in identifying *Lepiota* from *Chlorophyllum* which closely resembles Lepiota and differs mainly in spore colour. Detailed studies have to be taken to understand the biology and toxic nature of these common "parasol tombs" of Kerala.

Members belonging to the following genera viz., Coprinus, Marasmius, *Psathyrella, Schizophyllum* and *Cortinarius* occur in large numbers throughout the State. Species of *Coprinus* and *Psathyrella* can be grouped under the pre monsoon flora while different species of *Marasmius* and *Cortinarius* occur during the monsoon and post monsoon periods. Though *Marasmius oreades* occurs abundantly in pastures and fields, it is not collected and consumed by local people.

Different species of *Agaricus* occur in large numbers during the pre monsoon showers. Though most of them are edible they are not consumed by the people of the State. Species like *Agaricus campestris* and *A arvensis* with its excellent edible characters, consistency and size reveal their potentialities for commercial cultivation. More work has to be done on this group to select and isolate local species suited for the climatic conditions of the State.

Among the different species of *Pleurotus* collected, some appears to be very promising and suitable for large scale cultivation, viz., *Pleurotus opuntiae* collected from oil palm bunch waste, *P cornucopiae* obtained from the fencing stumps of *Jatropha gladulifera, P. ostreatus* from coconut husk and old logs. Though all the species of *Pleurotus* are of common occurrence *Pleurotus squarrosulus* is found to be the most common species occurring in large numbers throughout the State during both monsoon periods. Locally it is known as 'Marakoonu' since they are seen growing an old stumps, logs and even on branches of the living trees. *P. citrinopileatus* was found occurring the Cashew (*Anacardium occidentale*) tree trunks in large numbers while *P. dryinus* was found solitarily on the stump of a dried up Camphor tree (*Cinnamomum camphora*). *P. eous* was found in groups on the stumps of *Artocarpus hirsuta* after the pre monsoon showers. *P. flabellatus* was found to occur in massive fleshy groups on an old, dying Ceiba cotton tree (*Ceiba pentandra*) *P. lignatilis* was collected from fallen logs of *Artocarpus incisa* where it was seen occurring in groups.

P. luteoalbus was found occurring in small groups at the base of a old mango tree (*Mangifera indica*). Another species, *P. mastrucatus* was found growing in association with the roots of a coconut tree (*Cocos*

nucifera) on the insides of a well during the post monsoon period. *P. petaloides* was found occurring on a decaying coconut tree stump while *P. platypus* was found occurring on an unidentified rotting piece of timber wood.

P. pometi was found occurring naturally on old coconut husk waste. One of the hairy species of *Pleurotus, P. pubescens* was found occurring abundantly on oil palm bunch waste. Other species of *Pleurotus*, including *P. salignus, P. serotinus* and *P. pulmonarius* were also found to occur abundantly on oil palm bunch waste.

It was observed that unlike all *Pleurotus* sp. *P. opuntiae* putforth fruiting bodies 9-10 days after inoculation of the substrate with grain spawn. Preliminary steps to cultivate this species on paddy straw was successful.

The highly esculent and large mushroom, viz. *Tricholoma georgi* also known as *Calocybe gambosa* occur during the North East pre monsoon showers. All collections were made from the basins of coconut palm indicating the mycorrhizal nature of its growth. Though it is not a popular edible mushroom, some people in a particular region of the State collect and consume this mushroom.

All species of *Termitomyces* recorded in the present study occur abundantly in the forest soil and in the plains of the State during the post monsoon periods of the N.E.monsoon. It is found to be the most common edible species collected and consumed by the people of the State. Locally it is known by different names in different regions like Uppukoon, Arikoon, Mazathandan, Perumkala, Nilampulappan, etc. *Termitomyces heimii* occurs in hundreds in the forest area during the season and hence tribal people collect and sell it in the local market.

Boletus edulis, the mycorrhizal mushroom is observed to grow under the jack fruit trees during the pre monsoon summer shower (April-May) from1984 onwards. Locally it is known as "Pannikoon". Though it has got an undesirable smell, people consume it adding onions while preparing the recipes.

Occurrence of gastromycetous fungi like *Calvatia gigantia*, in large numbers on lawns during the pre monsoon season indicate their mycorrhizal association with grass. Because of its resemblance with egg locally it is known as 'Muttakoon'. Though it is not a popular edible fungus, people in some part of the State collect and consume it.

There are nearly 2000 species of mushrooms reported to be edible, among which about a dozen or more are safely consumed in different parts of India. However the utilisation of the natural mushrooms necessitates detailed descriptions of the agaric flora of each locality which helps the local mycophagist to identify the edible ones. Though the people of Kerala too collect and consume some of the edible mushrooms during the monsoon seasons, this work forms the pioneer effort to collect, describe, identify and catalogue the edible mushroom flora of the State.

3 OYSTER MUSHROOM

Six species of *Pleurotus*, viz., *P. citrinopileatus, P. flabellatus, P. florida, P. opuntiae, P. ostreatus* and *P. sajor-caju* were tested for the comparative efficacy for cultivation under Kerala conditions. Beds were

M. Suharban, C. Gokulapalan & M.C. Nair

MAJOR ACHIEVEMENTS

Identification and cataloguing of edible mushroom flora of Kerala.

New records of fungi for India, especially nine species of *Pleurotus*, the Oyster mushroom

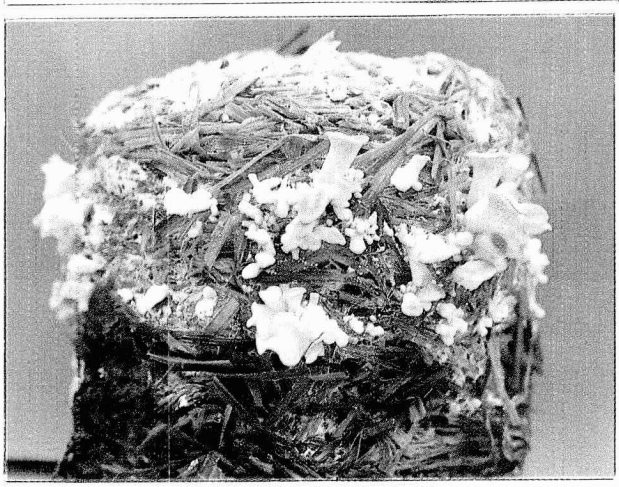
Identification of species of *Pleurotus (P. sajor-caju, P. citrinopileatus, P. florida & P. opuntiae, Volvariella (V.volvacea)* and *Coprinus (C.lagopus)* suited for cultivation in Kerala.

Standardisation of techniques for the cultivation of the Oyster Mushroom, Paddy straw mushroom and Ink caps as suited to Kerala conditions.

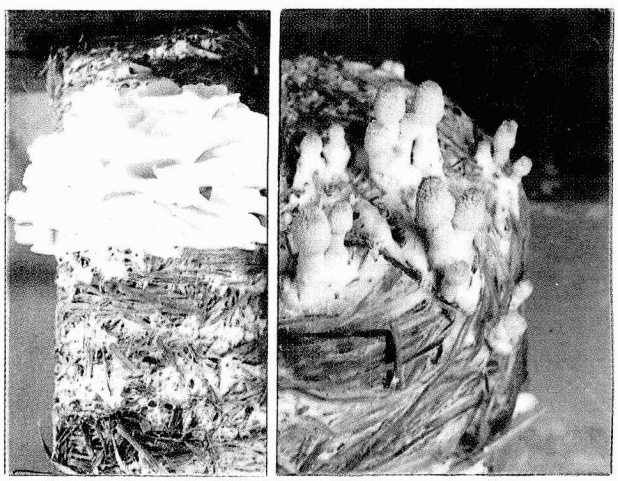
Intensive training programmes on identification and cultivation of edible mushrooms with emphasis on *Pleurotus* and *Volvariella* for Agricultural Officers, Forest Rangers and Growers.

CULTIVATION OF EDIBLE MUSHROOMS

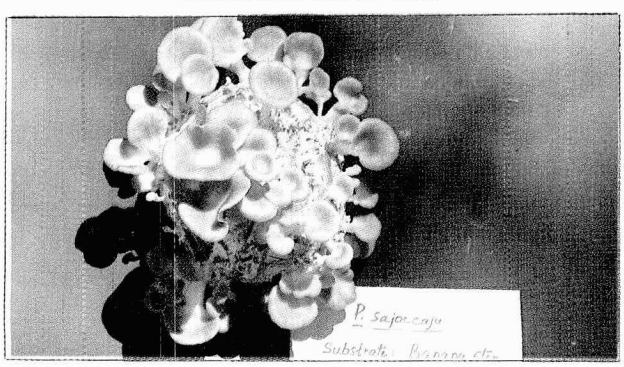
VOLVARIELLA VOLVACEA

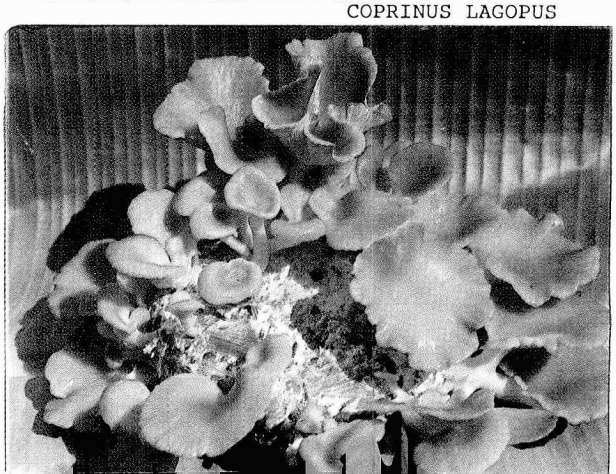


PLEUROTUS SAJOR-CAJU

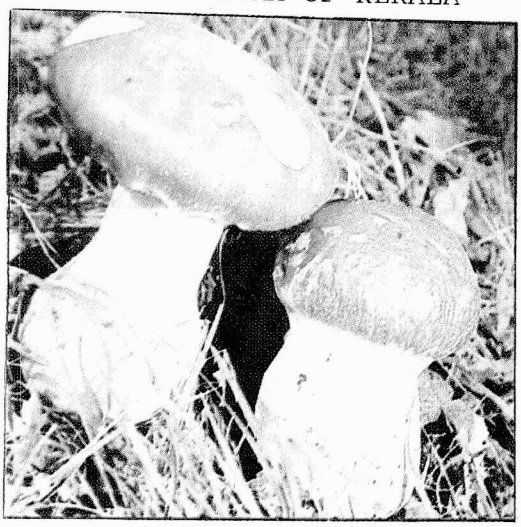


COPRINUS LAGOPUS



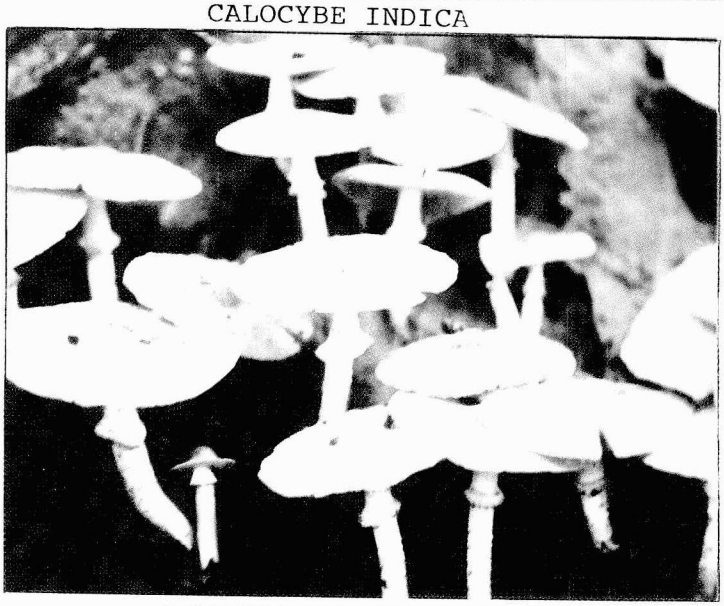


MUSHROOM FLORA OF KERALA



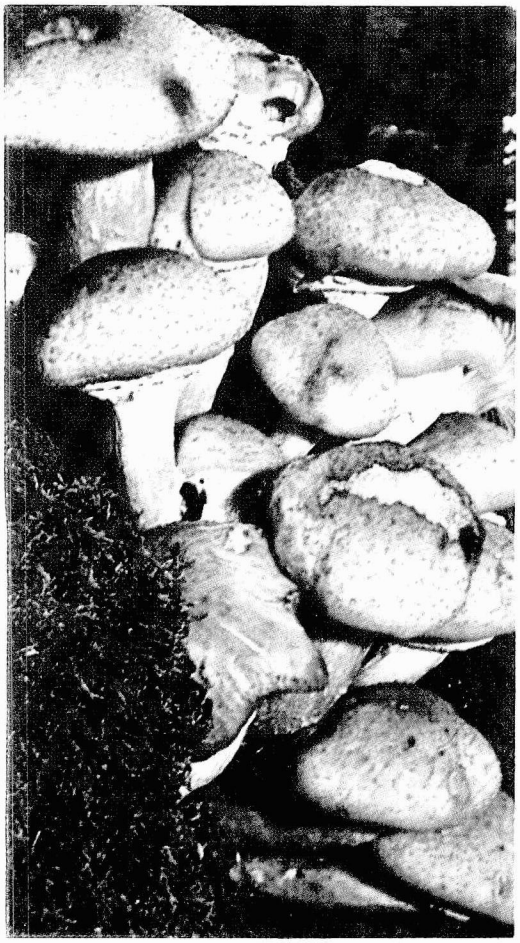
AMANITA CAESEREA

CALOCYBE INDICA

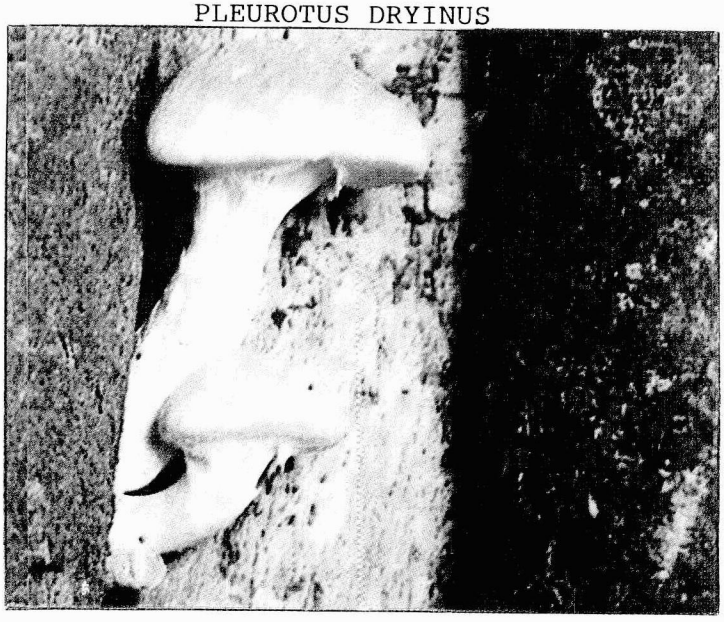


TERMITOMYCES HEIMII

ARMILLARIA MELLEA



PLEUROTUS DRYINUS



PROJECTS IN MUSHROOMS IMPLEMENTED IN KERALA AGRICULTURAL UNIVERSITY

1. Survey of the edible mushroom flora of Kerala and exploring the possibility of their large scale multiplication (1978-81) - funded by Kerala State Committee on Science, Technology & Environment. (Project Leader : Dr. M.C. Nair)

2. Studies on the edible mushrooms of Kerala with special reference to paddy straw mushroom *Volvariella* sp. (1982) - Ph.D Programme of Dr. S. Bhavani Devi - Chairman : Dr. M.C. Nair

3. Investigations on the edible species of *Coprinus* and standardisation of techniques for its large scale cultivation (1982) - P.G. Project of Smt. D.Geetha, Chairman : Dr. M.C. Nair

4. Monographic studies on edible speries of *Pleurotus* and standardisation of the techniques for large scale cultivation. (1987) - Ph.D. programme of Dr. M. Suharban - Chairman : Dr. M.C. Nair

5. Mushroom flora of Kerala - (1984-87) funded by Dept. of Science & Technology, Govt. of India - Project Leader : Dr. S Bhavani Devi

6. Trials on large scale cultivation of *Pleurotus* spp. under the agroclimatic conditions of Kerala. (1987-90), funded by Kerala State Committee on Science, Technology & Environment, - Project Leader : Dr. M.C. Nair

7. Biology of *Termitomyces* species and standardisation of its cultivation techniques. (1985-88) P.G. Programme of Smt. G.S. Sreelatha Nair - Chairman : Dr. S. Bhavani Devi

8. Standardisation of techniques for the cultivation of Tropical species of mushrooms (1989) - ongoing Ph.D. Programme of Sri. B. Balakrishnan, Chairman : Dr. M.C. Nair

9. Survey of mushroom flora of Kerala and standardisation of techniques for cultivation of tropical species (1989) Ongoing - ICAR *ad hoc* project - Project Leader : Dr. M.C. Nair

raised following a modified poly bag method. Paddy straw was cut into bits of 5 to 10 cm in length, or they were made into small twists. These were then soaked in water overnight, taken out, removed excess water and put into boiled water and continued to boil for about 15-20 minutes. They were then air dried and used for lay - out of beds. Polythene tubes, of 400-500 guage and 30 cm diameter were cut into bits of 60 cm in length and they were used to lay - out the beds. Few small holes were made on them for permitting air circulation and the bottom was tied. Treated straw was placed on the cover for about a height of about 15 cm. This layers was spawned by wheat grain spawn of the test mushroom and again a second layer was placed and spawned. In this way the whole tube was filled layer by layer and made as compact as possible and tightly tied to form a compact mass. The bits or twists were used separately to layout the beds. Same quantity of straw was used for each tube. The filled up bags were kept under darkness for spawn run for 7 to 10 days and once the spawn run was complete, the polythene cover was removed and the beds kept under a high humid atmosphere for sporocarp formation.

P. ostreatus did not grow well in this trial, probably because of the unfavourable climatic condition during the period of trial. The other five species were tested for their significant effects under bits and twists of paddy straw. The yield was significantly different among the five species tested. The highest yield was recorded for *P. sajor-caju* (775 g) followed by *P. opuntiae* (644 g) and *P. citrinopileatus* (523.75 g). The lowest yield was for *P. florida* (265 g), *P. flabellatus* (347 g) was better than *P. florida* (Table 1). Use of paddy straw as twists was found to be better than use of bits.

To find out the influence of different organic amendments on the yield of *Pleurotus sajor-caju* experiments were laid out with different organic amendments (Table 2). The amendments tried were Wheat flour, Green gram powder, Bengal gram powder, Horse gram powder, Red gram powder and Cowdung slurry. It was found that beds amended with Bengal gram powder recorded maximum yield. Green gram also was found to be equally effective as Bengal gram. Red gram powder was observed to be superior to wheat flour, Horse gram, Cowdung slurry and control. Wheat flour was better than Horse gram powder Cowdung slurry and control. Cowdung slurry was found least effective in yield response.

Cultivation of *Pleurotus sajor-caju* was tried in different logs of common trees. Fifteen trees, namely Mango (*Mangifera indica*), Coconut (*Cocos nucifera*), Cashew (*Anacardium occidentale*), Anjili (*Artocarpus hirsuta*), Albizzia (*Albizzia lebbec*) Caesalpinia pulcherima, Jamba (*Eugenia jambolana*), Avacado (*Persea americana*) Rose apple (*Eugenea jambosa*) Litchi (*Litchi chinensis*) Jack (*Artocarpus heterophyllus*) Erythrina (*Erythrina indica*) Ciba cotton (*Ceiba pentandra*) Raintree (*Enterolobium saman*), Eucalyptus (*Eucalyptus citriodora*) and Banana pseudostem (*Musa* spp.) were used for cultivation of *P. sajor-caju*. It was found that of the various plants tested, logs of Mango tree yielded the maximum of 389 g of fresh sporocarp followed by Cashew tree logs (269.7 g), followed by Banana pseudostem 236.3 g, Coconut log 169.7 g, and it was 106 g in Anjili log. Growth was found to be poor in logs of other trees. It is interesting to not that most of the trees when used as fresh, even spawn run was not there. But the same logs used after one year for inoculation resulted in sporocarp production.

Table 1. Comparative yield of sporo-carp of different species of *Pleurotus* raised in poly bags on paddy straw bits or twists (Average of 3 replications).

Type of substrate	*P. sajor-caju*	*P. florida*	*P. opuntiae*	*P. citrino pileatus*	*P. flabellatus*	Mean
			(yeld as g/kg of substrate)			
Paddy straw with bits	575.0	171.25	587.50	443.75	331.25	421.75
Paddy straw with small twists	975.0	353.75	700.00	603.75	362.50	600.00
Mean	775.0	265.0	643.0	523.75	346.87	

C.D. for comparison of bit and twists	=	27.14
C.D. for comparison of species	=	42.91
C.D. for comparison of straw types x species	=	60.69

Table 2. Effect of addition of various organic amendments on the yield of sporophores of *Pleurotus sajor-caju.*

Sl. No.	Amendments	Average yield in g/kg of substrate
1	Wheat flour	469.66
2	Green gram powder	632.16
3	Bengal gram powder	642.00
4	Horse gram powder	452.16
5	Red gram powder	604.51
6	Cowdung slurry	153.20
7	Control	277.10

C.D. = 22.1342

The proximate composition of major nutrients in six common Pleurotus species was analysed in comparison with *Volvariella volvacea* and are given in table 3. From the data it can be seen that percentage of protein was maximum in *P. squarrosulus* and it was minimum in *P. cornucopiae.* When compared to *Volvariella volvacea* all the constituents, viz., protein, calcium, Magnesium, Iron, Ash, Vit A and Vit C were more in all the six species of *Pleurotus* tested.

Visual observation of fresh sporocarps of *Pleurotus sajor-caju* kept under refrigeration revealed that by refrigeration, the mushroom started deteriorating with the accumulation of moisture in plastic bags and liquid started oozing from the mushroom which made them unfit for consumption and those kept in open polythene bags of 500 guage thickness remained fresh for five days. But when 150 gauge polythene bags were used it remained fresh for three days only. But those

Table 3. Proximate analysis of various species of *Pleurotus* compared to *Volvariella volvacea*

	Volvariella volvacea	*P. squarrosulus*	*P.dryinus*	*P.cornucopiae*	*P.sajor caju*	*P.petaloides*	*P.subpalmatus*
Protein %	10.72	15.46	3.50	2.85	12.85	6.60	8.71
Calcium mg/100g	6.40	8.18	7.43	7.28	8.41	6.02	10.04
Magnesium mg/100g	1.04	1.82	1.28	2.49	1.11	1.58	0.96
Iron mg/100g	1.32	1.82	1.92	2.34	2.85	3.11	2.90
Ash g/100g	2.67	3.14	1.98	2.94	3.12	2.16	3.04
Vit A mg/100g	2.01	502.80	427.3	321.8	262.50	167.80	397 .50
Vit C mg/100g	4.86	163.60	142.71	11.43	6.80	8.70	121.30

mushrooms covered with plain paper started discolouration after 24 hours. Organoleptic tests showed that no taste difference was experienced after cooking the samples kept for 120 hours of refrigeration. Those samples kept in closed bags showed that after 24 hours of preservation by refrigeration the mushroom started deteriorating with the accumulation of moisture.

Properly dehydrated mushroom by sun drying or dried in a drier, preserved effectively by keeping them in polythene bags. Visual observation of the dehydrated mushrooms kept in polythene bags and in air tight containers revealed that the samples were free of any mould growth. the samples kept open was found to be infected by common species of *Aspergillus, Penicillium* etc.

4. PADDY STRAW MUSHROOM

Mushrooms, a heterogenous group of fleshy fungi, numbering thousands of species have been known to man ever since he became aware of his environment. They have been esteemed as a delicacy and hence formed one of the choiciest table dishes. Since mushrooms are liked for their innate flavour, irresistible taste and high food value, they are grown on a commercial basis in many parts of the world. Mushrooms are rich in proteins, vitamins and minerals, while poor in fat and carbohydrates. Their protein is considered to be of high quality with a better digestibility than other protein foods. In addition to the appetising flavour and nutritional value, the medicinal properties like antitumour, antibiotic and hypolipidemic activity attributed to them also have played a significant role in popularising mushroom cultivation. The utilisation of mushrooms as food or food additive is by the collection of naturally occurring edible species or by cultivating promising species amenable to cultivation under artificial conditions utilising the locally available substrates.

Due to the variations in the general climatic conditions between the northern and southern regions of India, the tropical mushroom, *Volvariella volvacea*, the choice of Asians is commonly cultivated in the south and the temperate mushroom, *Agricus bisporus* in the north. *Volvariella volvacea* which is better adapted to a warm humid condition is suitable for cultivation in Kerala also. It is

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known to possess edible qualities superior to those of *Agaricus* (Chang, 1974). Further it can be easily dried to marketed in a powder form for being used as food additive or for soup making. However, standardisation of its cultivation technique on scientific lines is the major limiting factor for its popularisation on a home scale basis in the state.

The following method has been perfected for the cultivation of paddy straw mushroom in Kerala. Ten kg of straw was made into twists of 10 to 15 cm in diameter and 10 m in length. These twists were made into small bundles and kept immersed in water for 15 hours. The pre-soaked twists after draining excess water were placed on a raised wooden platform (1m x 0.75 x 1m) in a zig-zag manner. The second twist was placed over the first row in the opposite direction which form the first layer of the bed. Two bottles of spawns were divided into small bits of 3-5 cm in size and placed 10 cm apart along the periphery of the bed. A total quantity of 150 g of coarsely powdered red gram was sprinkled uniformly over the spawn in each bed. The same procedure was followed for placing the remaining twists and they were spawned and sprinkled with redgram powder successively making altogether five layers. The entire bed was compacted by pressing from the top, and watered with a rose can to maintain proper moisture level (74.27 per cent). Finally the bed was completely covered with a transparent polythene sheet.

An albino mutant which occurred naturally in the experimental mushroom beds which resembled the parent type in all its characters except in possessing a white sporocarp was isolated into pure culture (type B). Further studies carried out revealed that this albino mutant breeds true, and in yield compared well with the parent grey type (type A). Both the parent and albino mutant were found to be more or less similar in their morphological characters and developmental morphology. The parent and albino type established well on oat meal agar medium. The nutritional status of the parent and albino are presented in Table 4.

The paddy straw mushroom beds were found to be severaly infected with *Rhizoctonia solani* resulting in a drastic reduction in yield. Symptoms of infection could be observed in beds five days after laying the beds and the hypha of *R. solani* could be seen as a whitish coating over the mycelium of *V. volvacea*. On examination of the whitish growth it was found to be the basidial state of *R. solani* (*Thanatephorus cucumeris*). Ten days after the appearance of the white growth, abundant dark brown sclerotia were observed on the growth. The organism was isolated on PDA and purified. The hyphae were initially creamy white and turned light brown at maturity. Mycelium was branched, septate 5.36 - 10.7 um wide. The sclerotia developed on the straw of the mushroom beds were only microsclerotia which were oval to round in shape measuring 675 - 700 um x 450 - 600mm. The white crust of fungal growth observed on the surface of straw represented the basidial state of the organism.

Weed fungi like *Coprinus lagopus* and *Psathyrella* sp. were also found to occur on mushroom beds. These weed fungi established in the beds quickly, over grew *Volvariella* and mature in five or six days after laying the beds. *Coprinus lagopus* has long, slender stipes with small delicate pileus with narrow deep fuscus to black lamellae which become liquefied by auto-deliquescence at maturity producing an ink like substance. *Psathyrella* sp. is a very delicate weed fungus found in the mushroom beds. They have very thin fills, delicate pileus and thread like slender stipes.

Table 4 Percentage composition of dry matter, protein, crude fibre and fat content of parent and albino mutant of *Volvariella volvacea* at different stage of development (G per 100 g of fresh weight)

Stages of development	Dry matter		Protein		Crude fibre		Fat	
	Parent	Albino	Parent	Albino	Parent	Albino	Parent	Albino
Pin head stage	11.20	11.20	1.21	1.20	0.34	0.34	0.73	0.73
Buttons	11.70	11.80	1.80	1.80	0.52	0.54	0.94	0.91
Egg	11.80	11.85	3.18	3.20	0.82	0.82	0.95	0.94
Elongation	11.00	11.10	3.18	3.20	0.90	0.91	0.96	0.96
Mature	11.00	11.00	2.42	2.42	0.91	0.90	0.96	0.96

Infestation of mushroom beds by nematode was evidenced by the presence of poorly developed buttons which turn brownish in colour, become soggy and eventually decay. Samples of infected buttons kept in a sterile petridish showed symptoms of decay by the oozing of brown liquid. Examination of this liquid revealed the presence of innumerable nematodes. The nematode was identified as *Acrobeloides* sp. Slightly infested spawn on incubation found to be decomposed and mixed with dark red liquid. In severe cases of infestation of the spawn the mycelium failed to develop. Infested buttons when used for isolating the fungus failed to develop the characteristic fluffly mycelial growth on oats agar media. Even the scanty hyphal growth appeared on media was soon invaded by the nematodes and finally a reddish mycelial partially fluid mass was observed on the cultures.

Large number of spring tails were collected from mushroom beds from the on basal part of the button and also the gill plate of the mature pileus. They were identified as *Lepidocyrtus* sp. and *Xenylla* sp. They were also found to attack the mushroom buttons as well as mature sporocarps by chewing holes on them. They were also found to damage the spawn bits before it is well established on the substratum.

During harvesting time it was observed that a large number of soft, pallid transparent insects were found to colonise under the pileus between the gills and inside the volva of mature opened mushrooms. They were collected and identified as *Glyptholaspis americana* Brel. and *Parasitus consanguineus* Ouds & Voigts. These insects were found to damage the fruiting bodies by chewing and making holes in them and likewise they destroyed the mycelium after spawning.

Chonocephalus heymonsi Stobbe and *Chonocephalus depressus* De Meijere were the two phorids found to occur in large numbers on mushroom beds. Rats are another potential threat to mushroom cultivation. During the present study it was found that they cause damage by frequently digging up the grain spawns from beds which in turn reduced the yield. They also cause destruction to the buttons by eating and making injuries to healthy ones, thereby making them unfit for use.

Experiments were conducted to evaluate the efficacy of different substrates for spawn production. Twelve different substrates

Table 5 Comparative efficacy of spawn produced on different substrates for the production of sporocarps of *Volvariella volvacea* (Yield expressed in kg/bed of 10 kg spawn)

	Substrates used for spawn preparation											
Sl. No. of beds	Cotton seed	Maize grain	Cholam grain	Paddy straw	Half filled paddy grain	Coco-nut pith	Rice bran	Paddy husk	Dried Salvia-nia	Spent Lemon grass	Wheat grain	Saw dust
1.	2.00	2.25	1.00	2.45	2.50	0.20	0.95	1.00	2.00	2.45	2.50	0.50
2.	2.10	2.50	0.95	2.41	2.00	0.40	1.00	1.20	1.50	2.50	2.50	0.20
3.	2.50	1.90	0.80	2.25	2.49	0.25	0.96	0.25	1.20	2.50	2.40	0.25
4.	1.95	2.40	1.11	2.25	2.40	0.20	0.50	0.95	1.45	2.40	2.40	0.20
Mean	2.05	2.50	0.96	2.40	2.34	0.26	0.85	0.83	1.53	2.46	2.45	0.29

C.D. at 5 per cent level = 0.5841

were used and yield date was recorded (Table 5). When maize was used as substrate there was a steady increase in the development of mycelium from fifth day onwards while good growth was observed only from 15th day onwards in the case of spawn raised on half filled paddy grain, cotton seed, wheat, paddy straw and spent lemongrass. Spawn prepared on cholam grain showed only moderate growth throughout the experimental period, whereas, very poor growth was noticed in the case of rice bran, coconut pith, paddy husk and salvinia.

The main draw back for the commercial cultivation of *Volvarialla volvacea* is the poor keeping quality of the buttons. It deteriorates quickly under ordinary methods of storage. However, the present study showed that this mushroom can be stored for longer periods by following some of the common preservation techniques, provided, they are harvested at the proper stage. The mushrooms in egg stage contained less polyphenol oxidases and hence could be stored for a longer period. The degree of browning is related to the percentage content of the enzyme polyphenol oxidase. Mushrooms harvested at the button stage can be stored safely for very long periods under 5 percent braine. The trials on the storage of the mushrooms harvested at different stages of growth under refrigerated conditions revealed that the mushroom harvested in the late button stage and early egg stage can be stored without any deterioration for 48 hours in open trays. In polythene bags it deteriorated quickly. Dehydration, the most common and economical method of preservation of vegetables is found to be applicable in the case of paddy straw mushroom also. Both sun dried and those dried in the dehydrator by hot air were found to be free from any contamination when stored in polythene bags of 100 gauge thickness. These dried mushrooms can also conveniently be stored in powder form. The results indicated that preservation in brine and dehydration followed by powdering can be adopted for processing the product under commercial cultivation, while keeping the fresh buttons at low temperature in a refrigerator can be recommended for household purpose for 48 hours.

5. BIOLOGY OF *TERMITOMYCES* SPECIES

A state wide survey was conducted during the South West and North East monsoon periods in 1984-85 and nine species of *Termitomyces* were collected, identified and recorded from thirty two localities. Among the nine species (Table 1) of *Termitomyces* collected and identified *T. heimii, T. clypeatus* and *T. microcarpus* var. *santalensis* were the first records for Kerala.

Detailed description of the morphological and microscopical characters of the nine species were recorded in the data sheet along with the ethnomycological and gastronomic data collected from the local people. Information collected from the local people, however, revealed that all the *Termitomyces* species were actually being consumed in the region under survey and that each species are known locally as Uppukoon, Arikoon, Parambinkoon, Nilampulappan.

During the survey, observations on the frequency and intensity of occurrence of the nine species showed that *T. microcarpus T. microcarpus* var. *santalensis* and *T. robustus* were the most commonly and abundantly occurring mushrooms distributed throughout the state irrespective of soil type. Observations on their habit of occurrence also revealed that *T. robustus* and *T. striatus* were always seen solitary above the hypogeal termite combs, while *T. microcarpus* and *T. microcarpus* var. *santalensis* occurred in widely scattered groups of more than hundred sporocarps above the scattered termite combs. *T. heimii* occurred in groups of more than hundred sporocarps above the partly epigeal termitaria *T. clypeatus T. globulus T. radicatus* and *T. perforans* also appeared in well scattered groups of 25-50 and 10-25 sporocarps above the subterranean combs.

The results relating to the periodicity of occurrences of different species of *Termitomyces* indicated a post monsoon (July, October) maxima for the six species belonging to subgenus Eutermitomyces and a monsoon (June, September) maxima for the two species, viz., *T. microcarpus* and *T. microcarpus* var. *santalensis* of the subgenus *Pratermitomyces.*

Studies condcuted to observe the different stages of growth and development of *T. robustus* from mycelial stage till maturity revealed that different stages of development can be divided into eight viz., spherule, colvebud, primordial stage, epigeal elongation and mature. The first four stages of development were hypogeal and took 192 h to attain the 4th stage of development viz., pseudorrhiza stage. Pseudorrhiza formed the major part of the sporocarp. The next four stages of development viz., epigeal button, epigeal egg, epigeal elongation and mature stage were epigeal and took 96 h to attain the maturity stage. Critical observations of the different stages of growth and development revealed that pseudorrhiza and the perforatorium played an important role in the hypogeal development of the sporocarp. It was also observed that the length of the pseudorrhiza mainly depended on the depth and location of the termitaria in the soil. *In vitro* studies on the developmental morphology of *T. robustus* revealed the abundant growth of the mycelia of *Xylaria* in all the experiments.

The data relating to the temperature and humidity of the comb of *T. robustus* and surrounding soil showed insignificant

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Table. 1. Periodicity of occurrence and distribution of Termitomyces sp

Sl. No.	Species	Intensity	Period
1.	T. robustus	+	June, July
2.	T. heimii	++++	Sept. Oct.
3.	T. radiatus	++	July
4.	T. clypeatus	+++	July
5.	T. globulus	+++	July
6.	T. striatus	++	Oct.
7.	T. perforans	++	June, July
8.	T. microcarpus	++	June, July
9.	T. microcarpus var. santalensis	++++	Sept. Oct.

++++	50 - 100	and more sporocarps
+++	25-50	do
++	10-25	scattered
+	1-10	solitary

variations. Maximum and minimum temperature recorded inside the comb and surrounding soils were 31.2, 28.1, 29.5 and 27.0°c respectively. The humidity recorded was 100 and 90 per cent

A comparative study of the chemical composition of the combs of *T. robustus* and *T. heimii* showed little differences in the total moisture content, cellulose, carbon, nitrogen and pH.

Species belonging to *Odontotermes* was found to be the most common termite associated with different species of *Termitomyces* in Kerala. (Table 2). *O. obesus* was always found to be associated with *T. microcarpus.*

The beetle *Amblyopus cinctipinnis* was found to be a common pest of *Termitomyces.* The beetle was found to infest and feed on emerging and mature sporocarps and turn them unfit for consumption. The most commonly isolated fungi from termitaria were species of *Aspergillus* and *Xylaria.*

It was found that more number of sporocarps emerged from irrigated plots than from non irrigated ones. Among the eight media tested, Rebecca's selective medium was found to be the only medium suited for the mycelial growth of *T. robustus. In vitro* studies conducted showed that cellulose was the best carbon source for the growth of the fungus. The maximum protein content of 28.99 g/ 100 g dry matter was observed for *T. heimii* (Table 3) while the minimum protien content was observed for *T. robustus* (19.84 g/100 g).

Studies on the preservation of the sporocarps revealed that dehydrated sporocarps and powdered samples of the same can be preserved in closed polythene covers

Table. 2 Termites associated with *Termitomyces sp*

Sl. No.	*Termitomyces sp*	*Termite Species*
1.	*T. robustus*	*Oontotermes brunneus*
2.	*T. heimii*	*O. malabaricus*
3.	*T. clypeatus*	*O. redemani*
4.	*T. radicatus*	*O. obesus*
5.	*T. microcarpus*	*O. obesus*
6.	*T. striatus*	*O. sp*

and air tight containers for 10 months. Preservation in various concentrations of brine indicated that microbial contamination is very low at five, six, and seven per cent concentrations kept for four weeks. Observations also showed that blanched specimens kept in sterilised glass jars remained fresh up to five months in room temperature. Sporocarps could be pickled and stored for six months without microbial spoilage.

Table. 8 Nutritive value of different *Termitomyces sp* (9/100 g DM)

	Protein	Carbon	Fat	Crude fibre	Ash
T. robustus	19.84	52.23	6.4	5.0	7.0
T. heimii	28.99	33.10	2.5	8.8	10.5
T. clypeatus	23.84	53.92	3.5	8.0	8.5
T. radicatus	22.30	49.1	4.2	3.1	12.0
T. microcarpus	20.4	46.1	3.8	3.2	10.81
T. microcarpus var. santalensi	50.0	4.8	3.2	0.741	9.27
T. globulus	22.1	49.2	3.9	3.1	11.9

6. THE INK CAPS

The mushrooms of the genus *Coprinus* are commonly known as ink caps and are characterised by black spore deposits and the conversion of cap and gills into black inky fluid. They vary in size and grow on dung, recently manured ground, humus and wood. Most of the larger ones are edible in immature stages. They must be picked before they mature and used almost at once. These mushrooms have excellent flavour and fine texture, but for culinary purpose, they are not as popular as other mushrooms because of the dirty colour of the spores and smaller size of fruit bodies.

Species of *Coprinus* are suited for the cultivation under the tropical conditions utilising various farm and industrial waste products. They are found to occur on cowdung manure, paddy straw and guinea grass stumps and among these *Coprinus lagopus* (Fr.) Fr. is found to be the best growing one on paddy straw beds. Very limited studies have been carried out so far on the morphology, nutrition and suitability of the large scale cultivation of any species of *Coprinus*. An attempt has been made to present the different aspects of cultivation of the ink caps.

A survey was conducted in and around the campus of College of Agriculture, Vellayani, during May-June 1982 and the commonly occurring species of *Coprinus*, viz., *C. lagopus* (Fr.) Fr. *C. disseminatus* (Pers. ex Fr.) S.F. Gray and *C. ephemerus* (Bull. ex. Fr.) Fr. were collected.

Studies were conducted to observe the different stages of development mushroom from spawning till maturity. The developmental stages of mushroom is divided into five stages viz., pinhead stage, tiny button stage, button stage, elongation stage and mature stage. Mushrooms of pinhead stage of size 4 mm began to appear on the bed, after 72 hours (3 days) of spawning. The pinhead stage remained as such for two more days. After 120 hours (5 days) of spawning, the pinheads attained a size of 5-6 mm. The vertical section showed that the pileus and stipe length of 2-2.5 cm and pileus diameter of 1.5-3 cm. The vertical section revealed differentiation of stipe and pileus. After 150 hours of spawning, the buttons attained a stipe length of 4-5 cm and pileus diameter of 2-5-4 cm. The colour of the buttons changed from white to ashy grey during afternoon hours. After 158 hours of spawning, the buttons reached the elongation stage, with a stipe length of 10-15 cm. During the mature stage (160 hours after spawning) the pileus was expanded and splitted radially. Autodigestion of the pileus started after 162-163 hours, from the periphery of the gills towards the centre and the gills liquified into a black inky fluid. The expansion of the pileus, following autodigestion was observed during late nights. After the completion of the autodigestion, the stipe collapsed and the whole fruit body fell to the ground.

In order to study the influence of different types of straw bed for the maximum production of sporocarps of *C. lagopus*, rectangular beds were laid using 4 kg of paddy straw as twists and chopped straw. A significant increase in yield (704 g) was obtained from rectangular beds laid out with paddy straw twists. The yield was very poor (378 g) from beds laid out with chopped straw.

The maximum yield was obtained (525 g) from conventional paddy straw beds. Beds prepared with a mixture of chopped straw and paper at a ratio of 3:1 yielded only

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508.33 g (Table 1). From a single banana pseudostem 120 g was obtained, while no yield was obtained from beds laid out with Salvinia.

Visual observations of fresh sporocarps of *C. lagopus* kept under refrigeration revealed that the samples which were kept in open polythene bags remained fresh after 48 hours of storage and they started shrinking after 72 hours of storage. Visual observations of the samples kept in closed polythene bags showed that, after 24 hours of preservation, the buttons started deteriorating with the accumulation of moisture in plastic bags and they liquified producing a bad smell.

Properly dehydrated mushrooms could be preserved effectively by keeping them in polythene bags. Visual observation of the dehydrated mushrooms kept in polythene bags and in air tight containers revealed that the samples were free from the attack of microorganisms, while the samples kept open were found to be infected by common species of *Aspergillus, Penicillium* and Bacteria.

Fresh mushrooms at button stages were harvested, cleaned and preserved in different concentrations of brine (1 to 7 per cent). The duration of storage was 6 weeks. Visual observation of the preserved mushroom at different concentrations of brine revealed that the mushrooms retained more or less the same original colour of the materials preserved. The microbial assay of preserved mushrooms conducted at weekly intervel showed that it was free from any bacterial growth in those preserved on 5, 6 and 7 per cent brine up to 4 weeks. The population of bacteria and fungi are more in 1 to 4 per cent brine. The results indicated a gradual reduction in bacterial population as the concentration of brine increased. Actinomycetes were absent in all the treatments throughout the experimental period.

The nutritive value of the sporophore of *C. lagopus* in button stage was assessed. The data indicated that moisture formed the major constituent of mushroom (91.25 per cent), followed by carbohydrate (5.095 per cent) and protein (1.529 per cent). The crude fibre content was o.625 per cent and fat content was 0.576 per cent.

Table 1
Effect of different substrates on the yield of *C. lagopus*

Sl. No.	Substrates	Fresh Wt. of mushroom (g) (average of of 4 replications)
1.	Paddy straw twists	525.00 (substrate - 3 kg)
2.	Chopped straw and paper	508.33 (")
3.	Salvinia	——
4.	Banana pseudostem	120.00 (one complete pseudostem)

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