MONOGRAPHIC STUDIES ON EDIBLE SPECIES OF PLEUROTUS AND STANDARDISATION OF THE TECHNIQUES FOR LARGE SCALE CULTIVATION

By

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THESIS

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for the degree
DOCTOR OF PHILOSOPHY
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Department of Plant Pathology
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DECLARATION

I hereby declare that this thesis entitled "Monographic studies on edible species of *Pleurotus* and standardisation of the techniques for large scale cultivation" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to one of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "Monographic studies on edible species of *Pleurotus* and standardisation of the techniques for large scale cultivation" is a record of research work done independently by Sat Cuharban under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

INTRODUCTION

Higher fungi - the mushrooms - have been used by man from time immemorial and their use for culinary purposes is closely related to the history of mankind. Use of mushrooms, as evidenced by literature, dates back to 3000 B.C., in India. Mycophagy was known to be existing also during the time of ancient Greek and Roman civilizations. Of the nearly half a lakh species of fungi technically described, 2000 species are known to be edible. However, hardly about 25 of them are widely accepted as items of food and among them about a dozen have been commercially exploited.

Cultivation of mushrooms, started first in Asiatic countries more than 2000 years ago with Lentinus edodes and Volvariella volvacea. Cultivation of Agaricus bisporus, the European mushroom was started in France in the Seventeenth Centuary. Today, edible fungi are cultivated world wide under various climatic conditions on diverse agricultural and forestry waste materials. It is now widely accepted that mushrooms are potential contributors of the world's food supply, since they have the ability to transform nutritionally valueless waste into highly acceptable nutritious food.

World production of mushrooms is currently on the increase and at present there is an awareness throughout the world to utilize fungal biomass for protein supplementation. It has been rightly pointed out by Zadrazil and Grabbe (1981) that introduction of mushroom cultivation techniques in developing countries depends on good communication of scientific knowledge. The development of a practice has to be based on local conditions; simple methods worked out in local research organizations in combination with a well organized extension programme seem to be more profitable and desirable than the introduction of highly sophisticated systems offered by industrialised countries.

Species of Pleurotus are rather a new introduction into the group of edible fungi as their cultivation started only during the beginning of this century (Falck, 1917; 1919). Cultivation of this much cherished group of tropical lignicolous fungi, commonly called the Oyster mushrooms, experienced a momentum when Bano & Srivastava (1962) from C.F.T.R.I., Mysore, developed the polybag method of cultivation utilizing paddy straw as substrate for P.flabellatus. It gained much popularity in India after Jandaik and Kapoor (1974) isolated P.sajor-caju from stumps of Euphorbia royleana from the foot hills of the Himalayas and developed techniques for its commercial cultivation.

Considering the above facts, a monographic study on the genus Pleurotus along with standardisation of techniques suitable for Kerala conditions was aimed at in the present study. Species of Pleurotus naturally occurring in Trivandrum area were collected and identified and a comparative study was done with all the available cultivable species of Pleurotus for selection of suitable strains for Kerala Conditions.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The genus Pleurotus was erected by Fries (1821), who characterised the genus giving emphasis on the gross morphology of the basidiocarp and grouped it among Hymenomycetes. The name derived from the Greek Word Pleuro means an ear or side. The Friesian era, with emphasis on the gross morphology of basidiocarp in the systematics of Hymenomycetes held good for a very long period, eventhough a shift to the emphasis on the internal microscopic structure had gained attention of Mycologists like Patouillard (1900). With the advancement of the study of the group Agaricales, much information on the characters of the species of Pleurotus has also been accumulated and this has lead to narrowing down the circumscription of the genus Pleurotus. The systematic position of the genus also was subjected to much change from Tricholomataceae to Polyporaceae and also in the creation of a narrow artificial 'family group' Pleurotaceae. The latest edition of 'Dictionary of the fungi' (Hawksworth et al., 1983) groups the genus Pleurotus under Polyporaceae.

The genus, though erected in 1821 by Fries was validly published by Kummer (1871) with P.ostreatus (Jacq.: Fr.) Kummer, as type species. However, Quélet (1886) also recharacterised the genus and both these are now considered as a homonym and synonym. Singer (1975) grouped the genus under Polyporaceae, tribe Lentineae, along with other four genera Phyllotopsis Gilbert & Donk: Singer, Panus Fr., Lentinus Fr. and Geopetalum Pat. These traditional genera of this group have been shown to be artificial in their limits. Much overlapping of species, especially under Pleurotus, Lentinus and Panus have been known to occur. The pioneer work of Corner (1932) which started with detailed hyphal analysis of the the basidiocarp anatomy of Polyporales in general, culminated in 1981 in the publication of the monographic paper on the genera, Lentinus, Panus and Pleurotus (Corner, 1981). He strongly advocated for retaining the lentinoid fungi in Polyporales separating them from Agaricales. He suggested their place as in between the hydnoid and polyporoid groups. Corner (1981) redefined these related genera almost entirely on the basis of hyphal analysis. More or less similar type of study on

the hyphal system was carried out by Stankovicova (1973) also. The importance of the perennial dikaryotic secondary mycelium in the life cycle of Basidiomycetes has long been recognised. The tertiary generative mycelium, which takes part in the basidia, basidiospores and also in the sterile massive structure of the carpophores of these Basidiomycetes was first recognised by Buller (1915) and this has been critically analysed and adopted in the case of Polyporales by Corner (1932). In the monographic treatment of the Lentinean group, Corner (1981) clearly designated the sporocarp of Lentinus as complicated with skeletobinding cells and that of Panus containing unbranched skeletal hyphae lacking the binding process while Pleurotus as comprising of monomitic hyphal system, of rarely dimitic with sclerified tapering terminal elements. Thus modern Mycologists define and delimit the genus Pleurotus (Fr.) Kummer, as follows.

'Pileus, flabellate to dimidiate, attached to substrate by a short, thick, lateral stipe, or often stipe absent, occasionally stipe eccentric or central,

even within a single collection upper surface pigmented or not. Gills decurrent, sometimes with a tooth, crowded to moderately spaced, edge entire. Stipe cylindric, solid, with or without an annular veil. Context white, fleshy, soft or firm, often water soaked, never gelatinised, generally soon decaying. Hyphal system monomitic, with hyaline non-inflated generative hyphae with thin or thick walls, septate with clamp connections. Spore print white cream or pale lilac, spores cylindrical, 4 to 15 µm long, hyaline thin walled, inamyloid. Basidia clavate to clavatecylindric. Cheilocystidia present, forming a sterile lamella edge. Pleurocystidia present or absent; but never metuloidal. Hymenophoral trama hyaline, subregular to irregular. Sub hymenial layer well developed. Pileal surface undifferentiated. Saprophytes on dead wood.

The development of the carpophores are reported to be metavelangiocarpous, or gymnocarpous to slightly stipitangiocarpous according to Reijnders (1952).

The genus Pleurotus has gained great economic importance in recent years; eventhough some species of

the same were in cultivation from the beginning of this century (Zadrazil, 1978). The sclerotium of P.tuberregium Fr.Singer serves as food and P.ostreatus (Jacq.: Fr.) Kummer; P.citrinopileatus Singer; P.flabellatus (Berk. & Br.) Sacc., P.sajor caju (Fr.) Singer, are known to be commercially cultivated (Singer, 1975). P.griseus Peck. was reported to yield the antibacterial antibiotic Pleurotin (Yoshioka et al., 1971). Some related forms of P.ostreatus like P.dryinus (Pers.: Fr.) Kummer; are noted for their occasional parasitism on trees, and most species are wood destroyers, particularly the destructive P.calyx in South America. P.olearius (Fr.), C. Gillet, is known to be poisonous (Mavetie, 1975) and the spores of P.florida Eger, is known to be allergic to human beings (Eger et al., 1979); which resulted in the discontinuation of cultivation of this much preferred species.

The genus Pleurotus, as delimited currently (Pegler, 1976; Singer, 1986) contains fifty species (Hawksworth et al., 1983). Out of this nearly twenty five species are known to occur in India-Nepal area

(Pegler, 1976; Bilgrami et al., 1979, 1981; Sathe et al., 1981; Sarbhoy et al., 1986). Several species were first collected by Sir J.D. Hooker, mainly from West Bengal, Sikkim, Nepal area during the last century and subsequently they were identified and described by Berkeley (1850; 1852; 1854 a b). These specimens were deposited in the Kew Herbarium, U.K. The first lot of species reported by Berkeley included the following nine species. P.anserinus Berk., on dead wood from Jallapahar, Darjeeling, P.dryinus (Pers.)Fr. on standing tree collected from Kashmir, P.eöus (Berk.)Sacc. and P.hapalosclerus Berk. on tree trunks, Darjeeling, P.ninguidus Berk. on dead timber, Sikkim, P.petaloides (Bull.)Fr on dead wood, Nepal, P.placentodes Berk. from Birchwood, Sikkim, P.salignus (Pers.) Fr. from Sikkim and P.verrucaris Berk on dead wood from Darjeeling. P.placentodes (Berk.) Sacc., was also collected by Hooker from Betula wood, Sikkim in 1848 (Pegler, 1976). This pioneer study by Hooker and Berkeley was followed by those of others and Cooke (1888) identified P.platypus Cooke & Massee collected from tree trunks from Nepal. Massee (1892-1912) described some species of Pleurotus for the first time from India, which included P.cretaceus Massee on wood in Madhya Pradesh, and P.membranaceus Massee from tree trunks from Pune.

Graham (1915) from erstwhile Madhya Pradesh recorded P.cretaceus and P.sapidus Kalchbr, as occurring in India. He also recorded P.fimbriatus Bott. and P.cornucopiae (Paulet: Pers) Rolland for India (Graham, 1915). Hennings (1901) recorded P.subpalmatus Fr., on the ground, on roots of trees at Mussoorie, Uttar Pradesh. Bose (1920) recorded P.flabellatus (Berk. & Br.) Sacc. on dead wood or on the ground in Hooghly district, West Bengal. However, Pegler (1976) pointed out that this mushroom was first recorded from India by Massee (1899). Again this mushroom was recorded from Calcutta on dead tree Caesalpinia pulcherrima (Banerjee, 1947), in Ladakh by Kaul (1979), in Dras-Zoji-La Pass, Kashmir by Watling and Gregory (1980), Dehradun, Uttar Pradesh, by Puri et al. (1981). The type species of the genus, P.ostreatus (Jacq.: Fr.) Kummer, which was first recorded as Agaricus ostreatus Jacq. was reported for the first time from India from Sonamarg in Kashmir by Murrill (1924). This was again recorded on Picea morinda in West Bengal by Bose and Bose (1940), in Baroda by Moses. (1948), in Lucknow, Uttar Pradesh by Ghosh et al. (1974). Vasudeva (1960) and Kaul and Kachroo (1974) reported

P.dryinus for Kashmir and Sinha and Padhi (1978) for Orissa. P.ebus (Berk.) Sacc. was reported from Mysore by Singh and Rajarathnam (1977) and also for West Bengal (Roy & Samajpathy, 1980). P.sajor-caju (Fr.) Singer, first recorded as Lentinus sajor-caju by Fries (1838) was reported for India at the beginning of this century by Llyod during 1904-1919, according to Butler and Bisby (1931). This was further recorded for Bengal by Banerjee (1947) and again by Bose (1920) from the Foot hills of Himalayas by Jandaik and Kapoor (1975); Jandaik (1976); for Kerala by Natarajan (1978) and Sathe and Daniel (1980); from Tamil Nadu (Sivaprakasam and Kandaswamy, 1980). The distribution of this mushroom in different parts of India was reported by various workers. From Bengal (Bose & Bose, 1940); Kadala and Bombay (Chopra & Chopra, 1955); Culcutta, West Bengal (Chandra, 1974) Tamil Nadu, Nilgiri district (Pegler, 1975), Pune (Nair & Kaul, 1980).

P.squarrosulus (Mont.) Singer was recorded by Bose and Bose (1940) from West Bengal, Kadala and Bombay (Chopra & Chopra, 1955); Nilgiris, Tamil Nadu (Pegler, 1976) P.eryngii (De.:Fr.) Quél, was recorded for Kashmir (Wassen, 1969; Agha, 1974; Jandaik, 1976; Watling & Gregory, 1980; Rawal & Singh, 1980).

Chakravarty and Purkayastha (1976) recorded P.pulmonarius (Fr.) Quél, from trunks of tree in West Bengal. Horak (1980) reported P.lutescens (Fr.) Bresadola on rooting branches and sticks in Quercus spp; P.platypus (Cooke & Massee)Sacc., was recorded from Jammu Tawi by Watling & Gregory (1980).

The monographic studies of Agaricales of South West India by Sathe and his co workers based at Pune, resulted in the discovery of three species of Pleurotus, from Maharashtra viz., P.columbinus Quél apaud Bres from living guava trees from Pune, P.ciliosmus (Berk. apud Hessey)Sacc., from wood in Puttankudi, P.flabellatus (Berk. & Br.) Sacc., from Pune on wood (Sathe & Deshpande, 1980). Another species of Pleurotus viz., P.ostreatus from Agumbe, Karnataka by Sathe and Kulkarni (1980).

Bhavani Devi (1982) recorded five species of Pleurotus from Kerala. They were P.cornucopiae, P.ostreatus, P.platypus, P.spathulatus Pent.; P.squarrosulus. Out of these five species P.spathulatus was new record for the country.

As commented by Singer (1975) a modern monographic study of the genus Pleurotus is lacking and in this context, the efforts of Pegler (1976) are to be highly commended. He gave revised descriptions of the Pleurotus species collected and deposited at Kew Herbarium from the Indian sub-continent (India, Nepal and Pakistan) during last century by Sir J.D. Hooker. He divided them into two groups according to the spore size, small spores of 6 to 9 µm long which were more characteristic of typical subtropical species, and those with spores over 10 µm in length which are more typically north temperate flora and confined to Himalayan localities. He included ten species in his monographic work and properly redescribed them. They were P.anserinus (Berk.) Sacc., P.eöus (Berk.) Sacc., P.flabellatus (Berk. & Br.) Sacc., P.fossulatus (Cooke)Sacc., P.aff.gemmellarii (Inzeg) Sacc., P.membranaceous Massee, P.ninguidus (Berk.) Sacc., P.ostreatus (Jacq.: Fr.) Kummer, P.placentodes (Berk.) Sacc., P.platypus (Cooke & Massee)Sacc. Pegler (1976) has excluded four species which were recorded under Pleurotus, like the P.apalosclerus (Berk.) Sacc., collected and deposited at Kew gardens (Hook, 1850). This specimen has been identified by Pegler (1976) as

Armillaria mellea (Vahl: Fr.) Karst. P.dryinus (Pers.: Fr.) Kummer, collected by Thompson (1848), from Kashmir, which has been recorded by Berkeley (1854 b) and specimen deposited in the Kew Herbarium. Pegler (1976) reported that the sporocarps were too immature and unrecognisable for proper identification of the species correctly. The third one, viz., P.nepalensis Corner apud Baltour-Browne collected from Nepal on critical examination by Pegler (1976), has been identified as a species of Phyllotopsis Singer. P.verrucarius (Berk.) Sacc. collected by Hooker from Darjeeling was found to be Lentinus badius (Berk.)Berk.

MATERIALS AND METHODS

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A preliminary survey on the occurrence of species of *Pleurotus* was conducted by collecting the same from different localities in Trivandrum district. Collection and identification of the specimens were carried out following the procedure outlined by Bhavani Devi (1982) and utilized the data sheet prepared for the same (Nair & Bhavani Devi 1985) (Appendix I). The techniques described by Watling (1973) and Sathe *et al.* (1980) were also followed in enumerating the characters of the collected mushrooms. Identification of the specimens was done by comparing the characters published in literature and in case of doubtful identity the same was confirmed by sending the specimens for identification by experts in the field. Identification was confirmed by Professor Natarajan of the Centre for Advanced Studies in Botany, University of Madras, who also critically commented on the first reports of *Pleurotus* spp for India (Natarajan, 1987). Specimens were collected from the field with all the different stages of development as far as possible and the general observations like locality, type of substrate,

date of collection etc., were recorded in the field itself and specimen transferred to the laboratory by packing in waxed paper sheets. The collections were serially numbered and details recorded.

Before making detailed microscopic observation, all the external characters as per the data sheet appended were enumerated. The spore prints were made where ever possible and for this purpose either the pileus was removed and placed over clean plane microscope slides, with gills facing the slides, or the entire mushroom was placed in a vertical jar in such a way that clean slides could be placed underneath the gills. The pileus was kept moist by placing wet cotton over it. Slides were removed after sufficient deposit accumulated over this and the slides were gently dried and stored in cabinets. The whole pileus was cut off from the stipe and the same was placed over black paper, gills downward and the spore print was collected in paper also. The collections were properly dried in a vegetable dryer of the make Sigg, Dorrex utilising a forced circulation of hot air at 50°C.

The microscopical characters were studied either with free hand section mounted in lactopheol or from the tissue macerations. For this purpose the tissue was first placed in 10 per cent potassium hydroxide solution for a while before being stained. The stain was allowed to react for about 10-15 minutes and the specimens mounted in 10% potassium hydroxide. The tissue was macerated by keeping a piece of the same on glass slides and gently tapping with the blunt end of a needle.

Macrochemical and metachromatin reaction of various parts of the basidiocarps were studied following methods of Watling (1971) and Singer (1975). The test was carried out on the surface context of pileus, stipe and stipe apex and base. Fresh tissue from pileus, apparently one centimeter square was dissected from the sporocarp with clean single edge razor blades and placed in the depression in a porcelain plate. A few drops of Melzer's reagent were applied and the reaction indicated by colour change was recorded. Colour of spores was determined at a magnification of 650 x in water mounts. Measurements of all structures were made from mounts in 10% KOH at magnification of either 650 or 1250 x.

Melzer's reaction of spore mass was detected following the method described by Watling (1971). Small portion of spore print was transferred to a clean slide and mounted in Melzer's solution and colour change was noted under microscope. The reaction was graded as amyloid if positive and inamyloid if negative.

Information on edibility was gathered from various literature utilized for identification of fungi verified with Tanaka's Cyclopaedia of Edible Plants (Tanaka, 1976).

Photographs of the habitat of mushrooms were taken on the collection spot itself where ever possible otherwise dried exsiccati were photographed in the laboratory. Single lens reflex camera (Pentax K 1000) with close up lens was used for the purpose. Microscopic characters were recorded using a camera lucida attachment on the microscope.

The following microscopic characters were studied where ever possible:

1. Hymenophoral habit.types.

The manner of growth of mushroom body (Hymenophore) was recorded. Type of attachment of pileus and stipe was also observed and recorded.

2. Hymenophoral trama.

The type of sterile tissue, making the main core of gills and the arrangement of the hyphae within the tissue has been studied in detail.

3. Spore morphology.

The type of spore, its shape, its wall, smooth or ornamented, its colour reaction etc., were recorded.

4. Cystidia.

The characters of the sterile elements present on surface of pileus, on gill and in stipe were studied in detail. The details of the cystidia present on the gill surface and edge, viz., pleurocystidia, and cheilocystidia were studied.

5. Hyphal system and clamp connection.

The type of hyphal system, whether monomitic or dimitic and presence of clamp connections were also studied in detail.

Based on the characters the collections were identified and the most commonly occurring species are presented under results.

RESULTS

RESULTS

Pleurotus citrinopileatus Singer

Sporophores on tree trunks, occurring in large numbers.

PILEUS: central, broad, whitish, 8 to 12 cm in diameter, at first spathulate, then reniform, often sessile with a narrow basal attachment or sometimes with a short stipe, often many on a single central stipe, smooth, cream coloured or light yellow, margin irregular.

GILLS: adnate, white, smooth, moderately crowded, number per cm 10, decurrent.

STIPE: absent or very short, often central, 3 to 10 x 2 to 6 mm, cylindric, solid, white tomentose.

SPORES: basidiospores white to cream coloured, smooth, cylindrical, 8 to 12.5 x 3.0 to 3.5 µm in size.

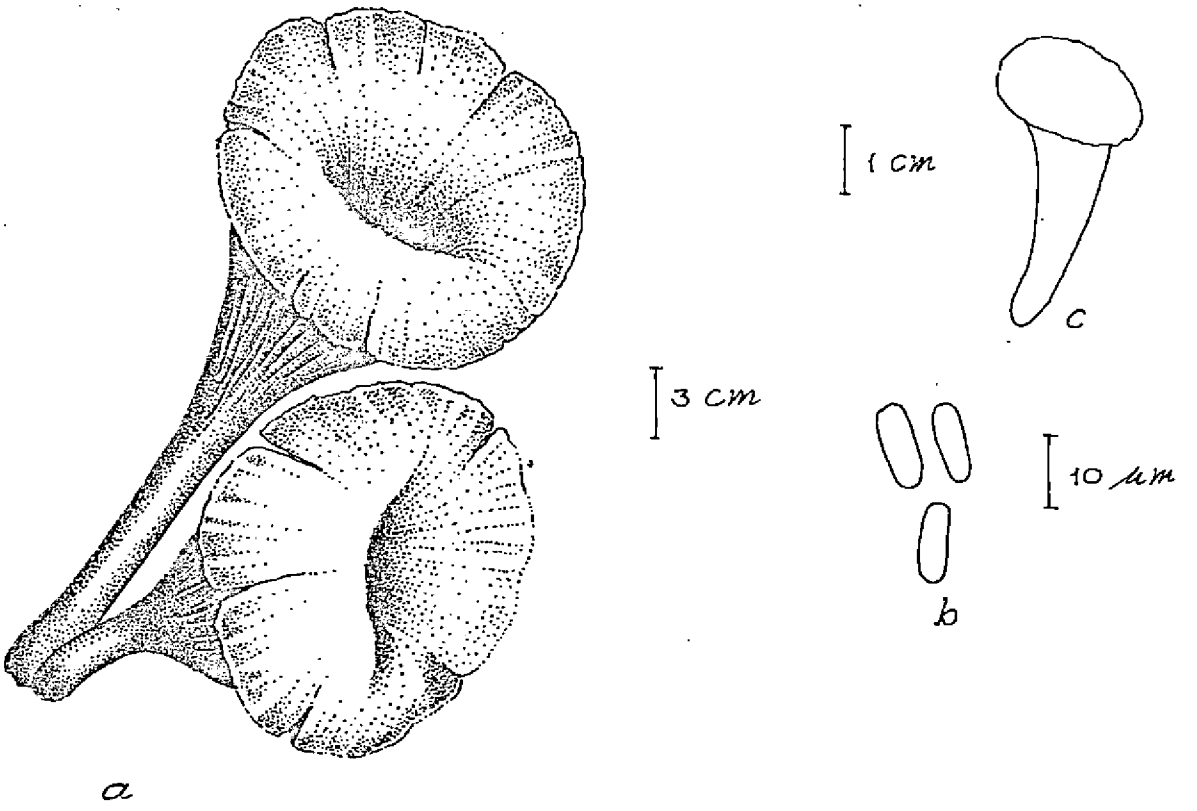


FIG. I. a. *Pleurotus citrinopileatus*

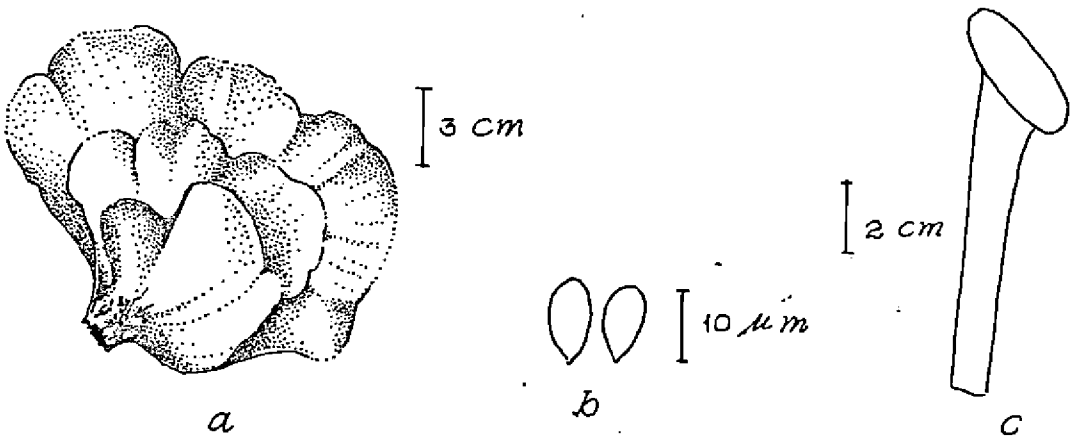


FIG. I. b. *Pleurotus cornucopiae*

PLATE. I. *Pleurotus citrinopileatus* - Spore print

PLATE. II. *Pleurotus citrinopileatus*

hyphal system monomitic, hyaline, uninflated, generative hyphae 2.5 to 10 µm in diameter, either thin walled or with a slightly thickened wall, septate with clamp connections.

SPORE PRINT: white (Plate No.I).

EDIBILITY: edible

collected from Rice Research Station Moncompu from Anacardium occidentale during June, 1986 (Plate No.II, Fig. I a).

Pleurotus cornucopiae (Paulet: Pers.) Rolland

Sporophores in groups on logs.

PILEUS: wide, convex to funnel shaped, depressed at maturity, 5 to 15 cm, smooth, white, pale ochreous - brown or yellowish, downy first soon turn glabrous, margin decurved or involute when young later expanded.

GILLS: whitish, crowded at pileus margin, with intermediates, less so near stipe on which they are decurrent, often form a net like pattern, 18 to 20 per cm at the margin.

STIPE: small, 3 to 8 x 0.7 to 1.5 cm, often ramified nearly central to fairly eccentric, whitish, almost completely covered with usually anastomosed extension of gills.

flesh thick soft and spongy.

PLATE. III. *Pleurotus cornucopiae*

PLATE. IV. *Pleurotus dryinus*

SPORES: elliptical, smooth, white or yellowish when young finally turning brownish 10 to 11 x 4 to 5 µm.

SPORE PRINT: light pink to pale lilac.

hyphal system monomitic hyaline, generative hyphae septate with clamp connections.

EDIBILITY: edible (Pacioni, 1981).

collected from Palode, Trivandrum from dried up Anjili logs (Artocarpus hirsuta), during September-October, 1983 (Plate No.III, Fig.I b).

Pleurotus dryinus (Pers.: Fr.) Kummer

Sporophores on tree trunk, solitary, eccentric or sub lateral.

PILEUS: broad, whitish, 5 to 10 cm in diameter, convex to flattened finally expanded, with central depression, whitish at first later buff yellowish when old, surface floccose when young and also covered with dirty brown down which breaks up into spot - like adpressed scales, becoming greyish brown sometimes with edge appendiculate because of the membranous and downy velar remains, margin inrolled.

GILLS: not so close, deeply decurrent, down even up to ring zone, whitish when young, ochrous grey when old and the edges darken with age. number per dm 36.

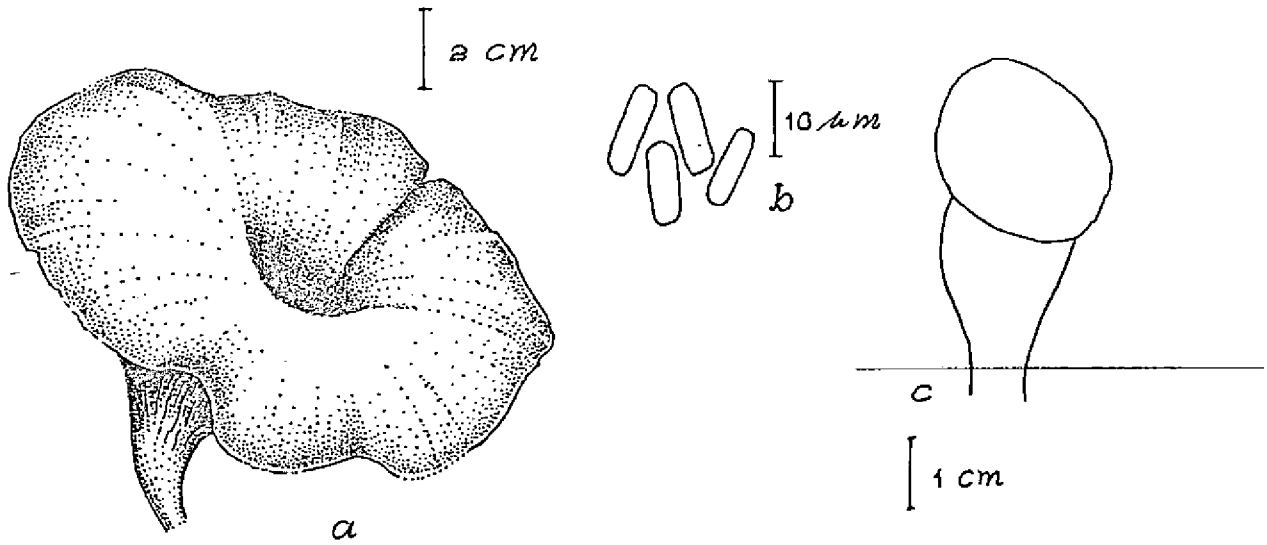


FIG. II. a. *Pleurotus dryinus*

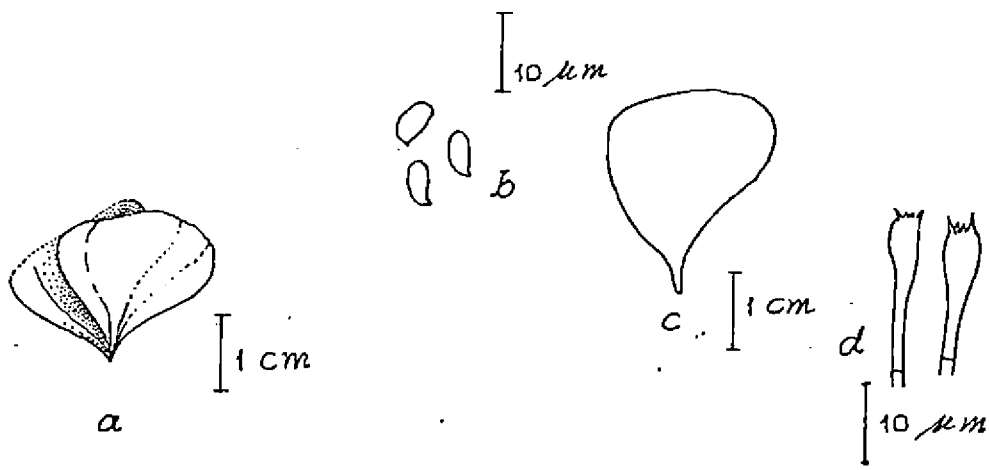


FIG. II. b. *Pleurotus eous*

STIPE: very short and stout, lateral or eccentric, 2.0 to 3.0 cm long 1 to 2.0 cm thick, white almost woolly, downy, squamulose with tapered base, some times with ring-like velar remains, flesh thick and dry.

VEIL: scarcely conspicuous on stem, but appendiculate round the margin when young, disappearing with expansion of pileus.

SPORES: besidiospores hyaline, cylindrical, smooth, 12 to 13 x 3 to 4 µm.

hyphal system dimitic, generative hyphae thin walled, septate with clamp connections, skeletal hyphae without clamp connections.

EDIBILITY: edible (Atkinson, 1961).

collected from College of Agriculture, Vellayani from dried up camphor tree (Cinnamomum camphora), during September 1984. (Plate No.IV, Fig.II a).

Pleurotus eöus (Berk.) Sacc.

Sporophores in groups on tree trunks, caespitose, imbricate.

PILEUS: usually spathulate at least when young, flabelliform when old, pink or flesh coloured, coriaceous, glabrous, up to 9 cm in diameter, margin incurved, hyphae radially parallel, slightly agglutinated.

GILLS: crowded, decurrent, whitish or creamish, narrow, thin, lamellulae of four different length.

STIPE: absent, even if present very small.

context 1 to 2 mm thick soft fleshy to brittle.

SPORES: besidiospores cylindrical, thin walled, 6 to 8 x 2.5 to 3.5 µm hyaline, inamyloid.

basidia narrowly clavate bearing four sterigmata, pleurocystidia absent.

PLATE. V. *Pleurotus eöus*

hymenophoral trama irregular, hyaline with interwoven hyphae, mostly thin walled.

EDIBILITY: edible and can be cultivated (Singh & Rajarathnam, 1977).

collected from Palode, Trivandrum district on logs of Anjili (Artocarpus hirsuta). June, 1986 (Plate No.V, Fig.II b).

Pleurotus flabellatus (Berk. & Br.) Sacc.

Sporophores in groups.

PILEUS: creamy white, smooth, hygroscopic, slender, mostly flabelliform, sometimes reniform with an attenuated base more rarely with an eccentric or central stipe, caespitose, surface convex or depressed white to ivory coloured at maturity, slightly tomentose towards the base other wise glabrous, margin at first incurved, then undulate.

GILLS: decurrent, narrow, moderately crowded, white, thin, up to 4 mm broad, edge concolourous, entire, number per cm 18.

STIPE: absent, if present very small, typically lateral but occasionally eccentric, 0.5 to 3 x 0.5 to 1 cm in size, irregular.

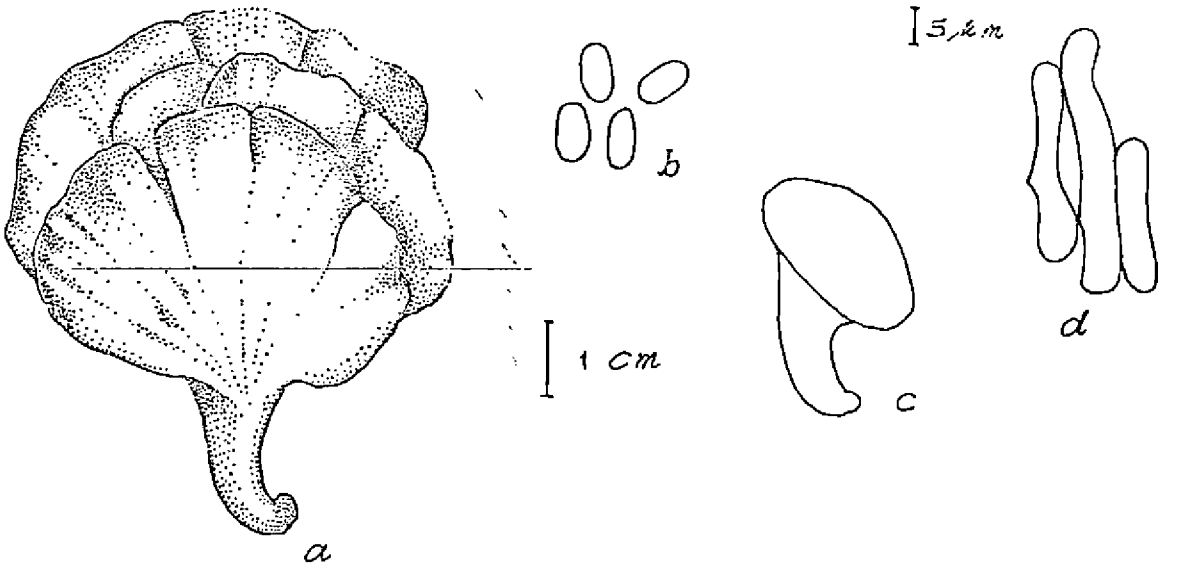


FIG. III.a. *Pleurotus flabellatus*

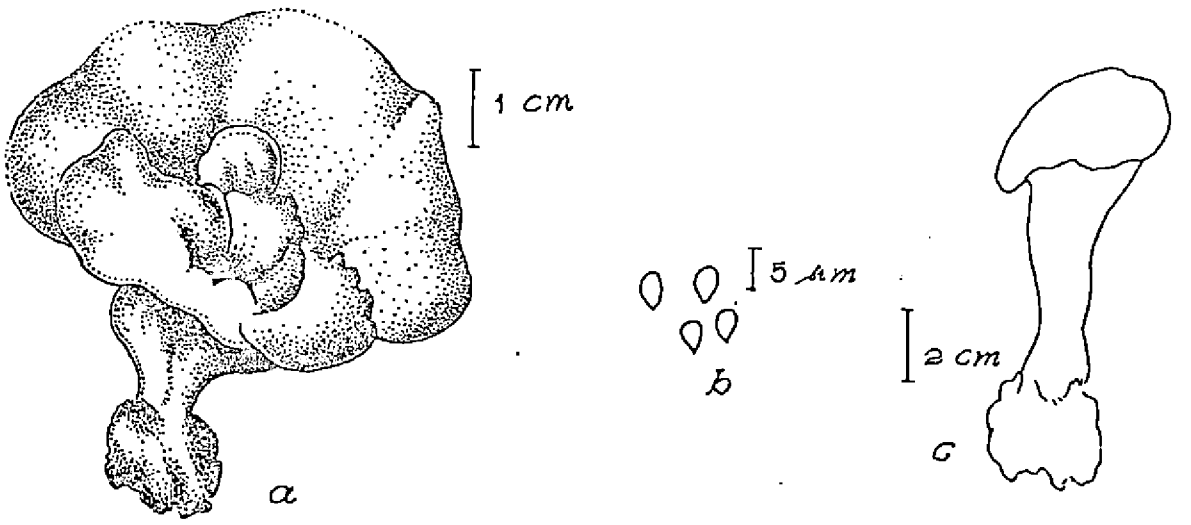


FIG. III.b. *Pleurotus lignatilis*

PLATE VI a. *Pleurotus flabellatus* on tree

VI. b. *Pleurotus flabellatus*

VI. c. Spore print

flesh, fleshysoft and slender.

SPORES: basidiospores cylindric, oblong-cylindric, hyaline, thin walled, smooth inamyloid 6 to 8.3 x 3 to 4 µm in size.

SPORE PRINT: cream (Plate No.VI a).

cheilocystidia present, crowded pleurocystidia absent.

hyphal system monomitic, hyaline, generative hyphae 3 to 8 µm in diameter, highly branched with slightly thickened wall, septate and with clamp connections, hymenophoral trama irregular.

EDIBILITY: edible. Bano (1971).

collected from standing Ciba cotton tree (Ceiba pentandra) from College of Agriculture, Vellayani, October, 1986. (Plate No.VI b & c, Fig.III a).

Pleurotus lignatilis (Pers.: Fr.) Kummer

Sporophores caespitose on logs, sometimes solitary also.

PILEUS: broad, flabellate, 3 to 10 cm in size, mostly eccentric rarely central, occasionally lateral, fleshy, thin, often umbilicate, margin at first involute then expanded.

GILLS: adnate, crowded very much upto 3 mm, broad, entire, number per cm 32.

STIPE: 5 to 8 cm long, hollow, always thin, unequal, curved, flexuous, whitish.

flesh, fleshy and soft.

SPORES: 4 to 5 µm x 2-3.5 µm in size, whitish, elliptical.

SPORE PRINT: cream.

EDIBILITY: edible.

collected from Palode from fallen logs of Artocarpus incisa, during September-October, 1985 (Plate No.VII, Fig.III b).

PLATE. VII. *Plenrotus lignatilis*

PLATE. VIII. *Pleurotus luteoalbus*

Pleurotus luteoalbus Beeli

Sporophores caespitose.

PILEUS: at first spathulate and then reniform, 5 to 8 x 4 to 5 cm in size, sessile, often with a very narrow attachment with a short stipe, surface brightly coloured in different shades of yellow, smooth, glabrous, dry with fine radial striae, margin thin and involute.

GILLS: moderately crowded, adnate, decurrent, white, pink when dried, thin, 3-6 mm wide, edge concolorous, number per cm 32.

STIPE: absent or very short, lateral 3 to 10 x 2 to 6 mm, cylindrical, solid, white and tomentose.

flesh tough and hard, cream when fresh, light brown when dried.

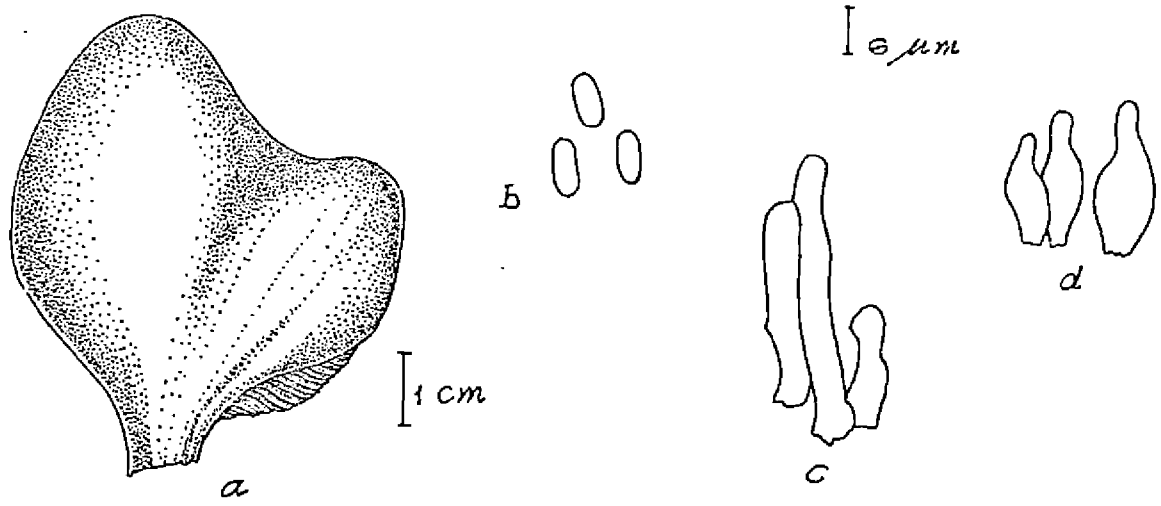


FIG. IV.a. *Pleurotus luteoalbus*

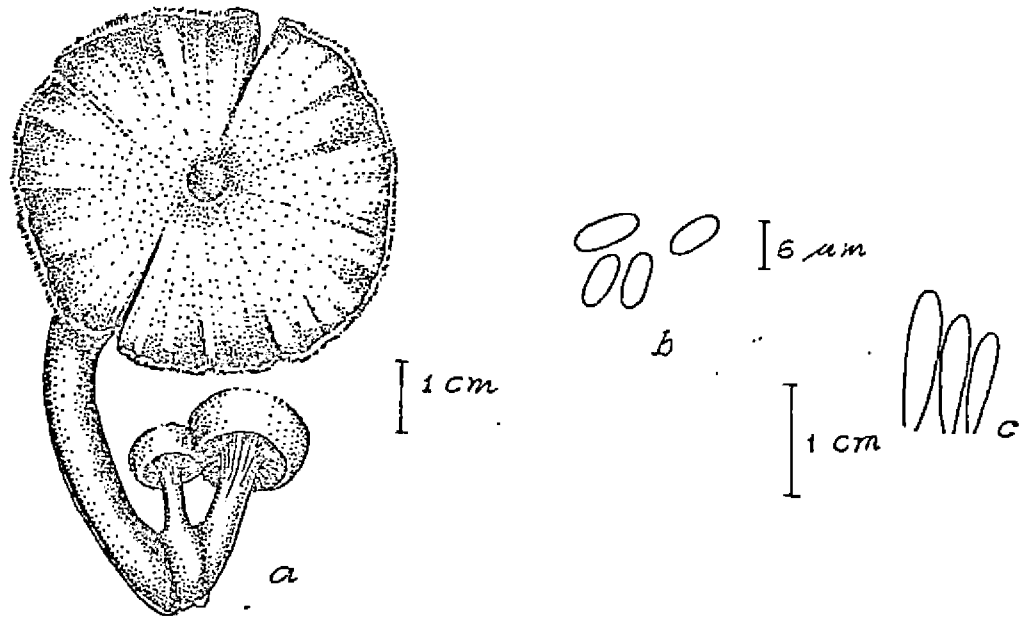


FIG. IV. b. *Pleurotus mastrucatus*

SPORES: basidiospores cylindric, hyaline, thin walled 5 to 7.5 x 2.8 to 3.5 um in size.

SPOREPRINT: cream

cheilocystidia abundant, irregularly fusoid, pleurocystidia numerous, extending beyond basidial layer, thin walled, with a rounded apex, hymenophoral trama regular to irregular,

monomitic hyphal system of hyaline uninflated generative hyphae 2.5 to 10 µm in diameter, mostly thin walled or at times with slightly thick walled hyphae which is septate and with clamp connections.

EDIBILITY: edible.

collected from base of mango tree, (Mangifera indica) from College of Agriculture, Vellayani, September, 1986 (Plate No.VIII, Fig.IV a).

Pleurotus mastrucatus Fr.

Sporophores solitary or in groups on old trunks.

PILEUS: upto 5 cm long and 2.5 - 3 cm broad, sessile, at first resupinate then expanded and horizontal, often lobed, upper stratum of pileus gelatinous, brown with squarrose or errect squamules.

GILLS: decurrent, broad, upto 3 mm wide, grayish white, number per cm 10-12.

STIPE : small 2 to 4 x 0.8 x 1.5 cm, nearly central whitish, cylindric, solid,

context fleshy, soft, consisting of firmly inter woven thin walled hyphae of 12 to 8 µm diameter.

SPORES: oblong, cylindrical, hyaline 6 to 9 µm x 4.5 µm in size Pleurocystidia present.

SPORE PRINT: white

EDIBILITY: edible and of good flavour.

collected from decayed coconut roots, inside a well, Punkulam, Vellayani, November, 1986 (Plate No.IX, Fig. IV b).

Pleurotus opuntiae (Dur. & Lév) Sacc.

Sporocarps in large numbers, imbricate.

PILEUS: subglobose to flabelliform or often spathulate, 4 to 10 cm in diameter, surface whitish, turn yellowish when old, paler along the margin, with a thin repent epicutis of parallel thin walled hyaline hyphae, glabrous, margin becoming lobed or splitting.

GILLS: crowded, decurrent, whitish to cream coloured, 3 mm broad, with lamellulae of three different lengths , number per cm 24.

STIPE: lateral, usually very short 1 to 2 cm x 4 to 8 mm in size, cylindric, solid and surface white pubescent, when young, soon turn glabrous.

context fleshy, white inamyloid, homogenous, with a monomitic hyphal system of hyaline mostly thin to slightly

PLATE. IX. *Pleurotus mastrucatus*

PLATE. XI. *Pleurotus opuntiae*

PLATE. X. *P. opuntiae* - spore print

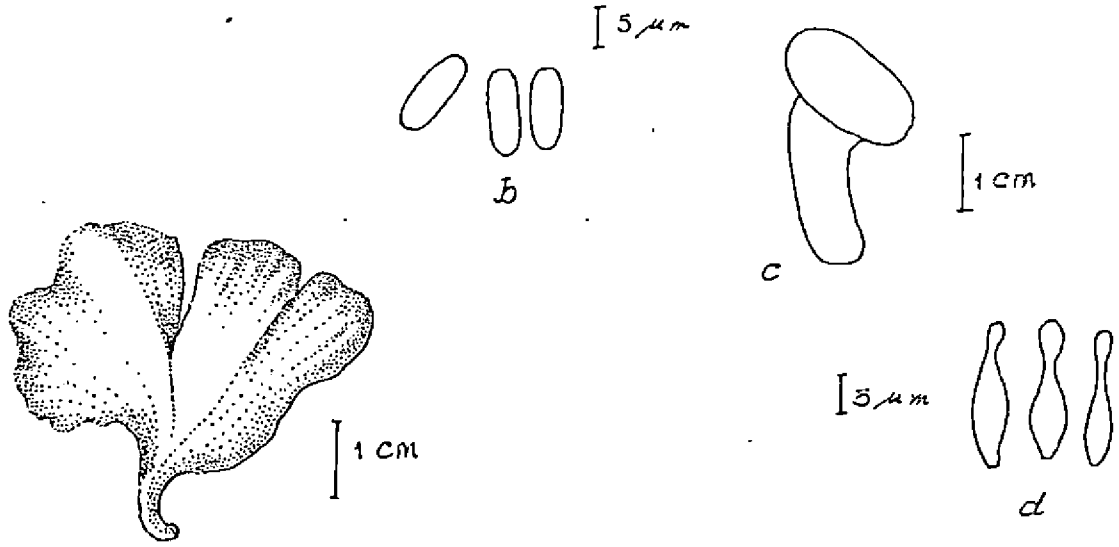


FIG. V. a. *Pleurotus opuntiae*

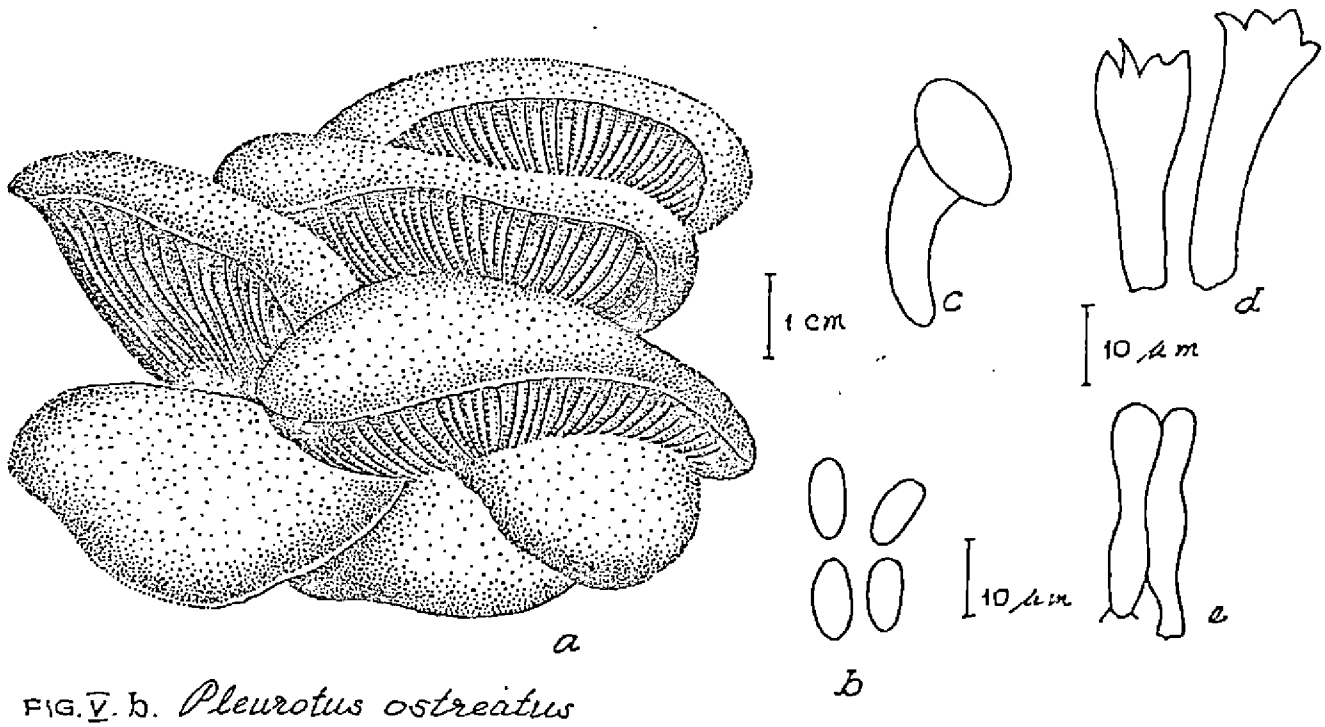


FIG. V. b. *Pleurotus ostreatus*

thick walled generative hyphae 2.5 to 12 µm diameter, with prominent clamp connections.

SPORES: basidiospores cylindric, hyaline, thin walled, contains refractive guttulate contents, 8.5 to 12.5 x 3.8 to 5 µm.

SPORE PRINT: white

pleurocystidia absent, chielocystidia present, lecythiform, bearing a small capitellum, hyaline, thin walled, hymenophoral trama completely irregular (Plate No.X).

EDIBILITY: edible

collected from oil palm waste (Elaeis guineensis) from Palode, June, 1985 (Plate No.XI, Fig.V a).

Pleurotus ostreatus (Jacq.: Fr.) Kummer

Sporophores on tree trunks in clusters, usually hygrophanous.

PILEUS: button shaped when young, soon expanding, spathulate to reniform, mostly typical shell-shaped, often some what dimidiate, fleshy, measuring 7 to 18 cm across, slightly depressed at attachment to stipe, smooth or slightly cracked, shiny and glabrous, some times cuticle torn into squamules, white, colour often variable, some times grey dark violaceous, brownish when young turn dingy when old and in many cases often white when old.

GILLS: not crowded, unequal, decurrent along stipe, long anastomosing at base, initially white, white or ivory white to yellow when dry, number per cm 18.

STIPE: absent or very short, eccentric or lateral, rarely central 1.0 to 3.0 cm long and 0.5 to 2.0 cm thick, passing gradually into one side of pileus, firm, whitish, hairy at base.

flesh white, soft, spongy, with pleasant smell 0.5 to 1.5 cm thick near stipe.

BASIDIA: clavate, 30 to 38 x 6 to 8 µm, tetrasterigmatic,

SPORES: basidiospores white, pale cream coloured, or shaded with pale lilac, inamyloid, oblong or sub cylindircal 8.6 to 12.5 x 3 to 4 µm in size.

SPORE PRINT: white to lilac.

hyphal system monomitic, generative hyphae thin walled, septate with clamp connections, some times sclerified generative hyphae also was found.

EDIBILITY: edible

collected from stump of Mango tree (Mangifera indica) from Moncompu, June, 1984. (Plate No.XII, Fig.V b).

Pleurotus petaloides (Bull.: Fr.) Schulz.

Sporophores in groups, imbricate.

PILEUS: wedge shaped to spathulate, irregularly petaloid, resembling petals of flowers, 2.5 to 10 cm long 1.0 to 5.0 cm in diameter, dingy brown, becoming pale, dimidiate, finely plumose towards the base, margin involute when young but expanded later.

GILLS: very crowded, decurrent, very narrow, soft, linear, unequal, sometimes bifurcating edge, entire, white first, finally ash coloured, number per cm 32.

STIPE: absent or small like an extension of pileus 3 to 8 x 3 to 4 mm in size, solid, firm, compressed.

context thin, white, duplex with a thin upper layer which is gelatinised consisting hyphae of 2 to 4 µm diameter,

PLATE. XII. *Pleurotus ostreatus*

PLATE. XIII. *Pleurotus petaloides*

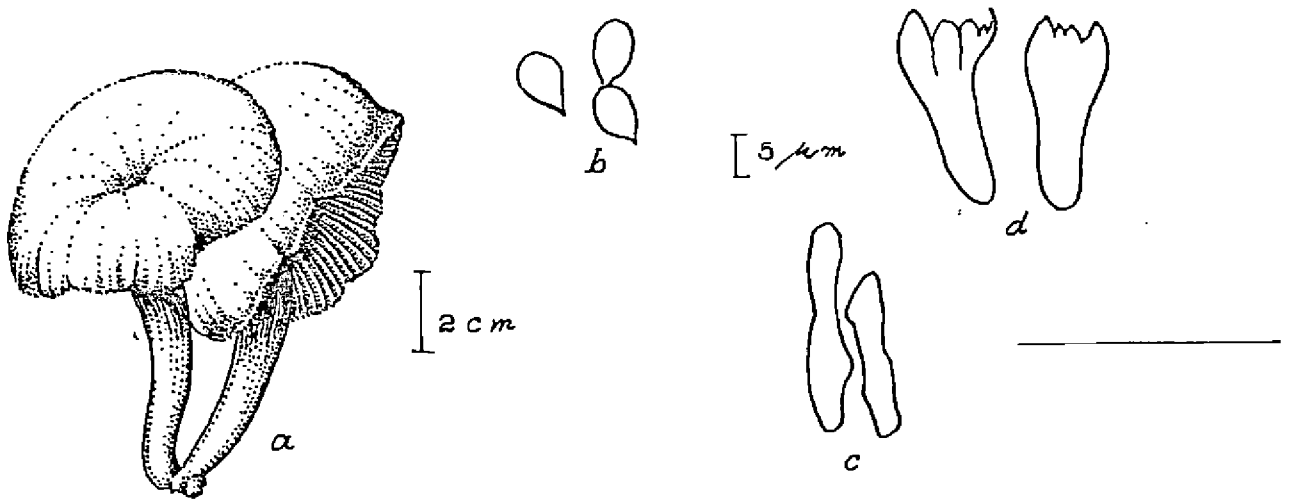


FIG. VI. a. *Pleurotus petaloides*

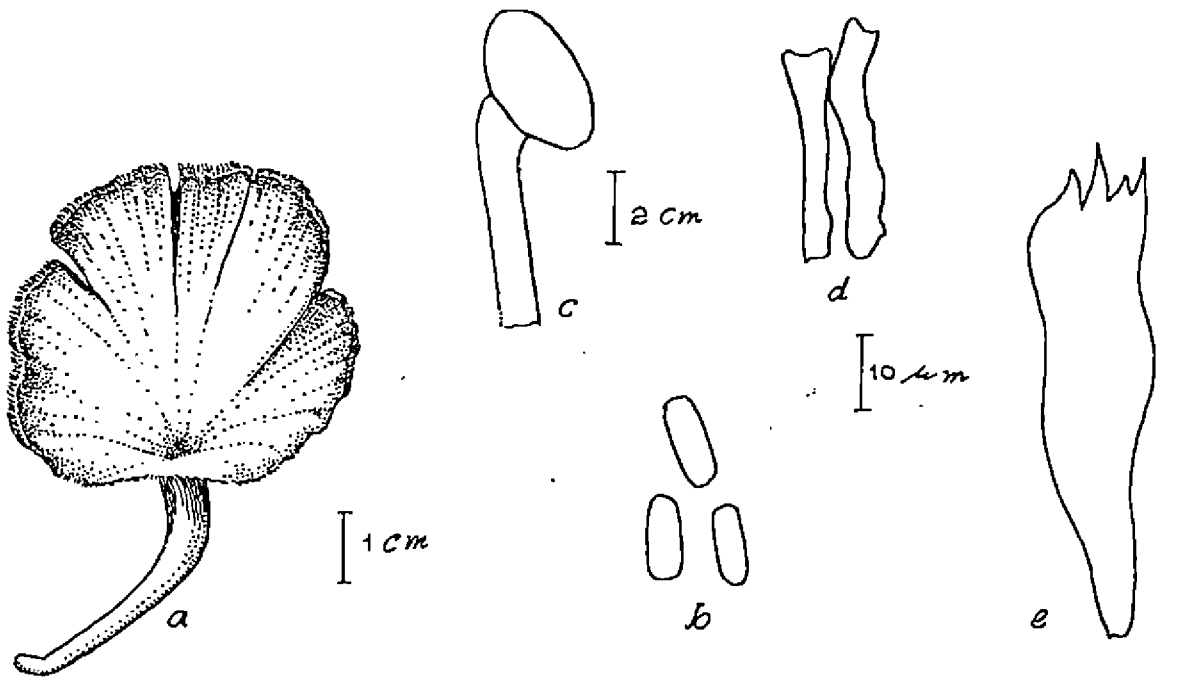


FIG. VI. b. *Pleurotus platypus*

loosely interwoven in a hyaline matrix and a lower layer of more tightly woven and not gelatinised hyphae.

SPORES: basidiospores ovoid, ellipsoid to broadly ellipsoid, hyaline, inamyloid, thin walled, with large refractive guttules, 7 to 9 x 4.5 to 5.7 µm.

BASIDIA: clavate, 22 to 27 x 5 to 7 µm, bearing four sterigmata, cheilocystidia crowded 20 to 26 x 2 to 5 µm, sinuate, hyaline, thin walled.

hymenophoral trama sub-regular, hyaline, non-gelatinised.

EDIBILITY: edible.

collected from stump of coconut tree (Cocos nucifera) from Thiruvallam, Trivandrum, November, 1986 (Plate No.XIII, Fig.VI a).

Pleurotus platypus (Cooke & Massee) Sacc.

Sporophores caespitose on logs.

PILEUS: convex, then depressed at maturity, sometimes subinfundibuliform, fleshy, surface tawny brown, smooth and glabrous, 2.5 to 8 cm in diameter, margin incurved and deeply lobed.

GILLS: moderately crowded deeply decurrent, white, 2 to 3 mm broad, with numerous lamellulae, number per cm 28.

STIPE: eccentric or lateral ascending with a bulbous ~~bulbous~~clavate base, 1.5 to 5 x 0.4 to 1 cm, paler than pileus, rugose often with hairy base and concolourous with pileus.

context upto 3 mm thick, fleshy, consisting of firmly interwoven thin walled hyphae 2 to 8 µm diameter with prominent clamp connections, flesh white, soft, spongy 0.5 to 1.5 cm thick near the stipe.

SPORES: basidiospores hyaline, oblong to cylindrical, thin walled, 8.5 to 13.5 x 2.5 to 3.5 µm.

BASIDIA: clavate to cylindrical, hyaline, thin walled, four spored 6.5 to 12.5 x 2.0 to 2.5 µm.

SPORE PRINT: white.

hymenophoral trama irregular to sub-regular, consisting of thin walled hyphae up to 2.5 mm thick, pleurocystidia absent subhymenial layer 5 to 6 µm wide, interwoven.

EDIBILITY: edible.

collected from Thiruvallam from rotten wooden piece (unidentified), November, 1986 (~~Plate No.CMFRI~~, Fig.VI b).

Pleurotus pometi (Fr.) Quél.

PILEUS: white fleshy, soft, sub-flaccid, irregular, 2 to 4 cm diameter, involute, convex, smooth,

GILLS: decurrent, crowded, white, smooth number per cm 24.

STIPE: eccentric, solid 5 to 8 cm long tough and ascending.

SPORES: basidiospores hyaline, globose, 2.0 x 3.0 µm thin walled.

SPORE PRINT: white.

context thick, fleshy consisting of firmly interwoven thin walled hyphae 2 to 6 µm diameter.

EDIBILITY: edible.

collected from old coconut waste (Cocos nucifera) from Thiruvallam, Trivandrum, November, 1986 (Fig.VII a).

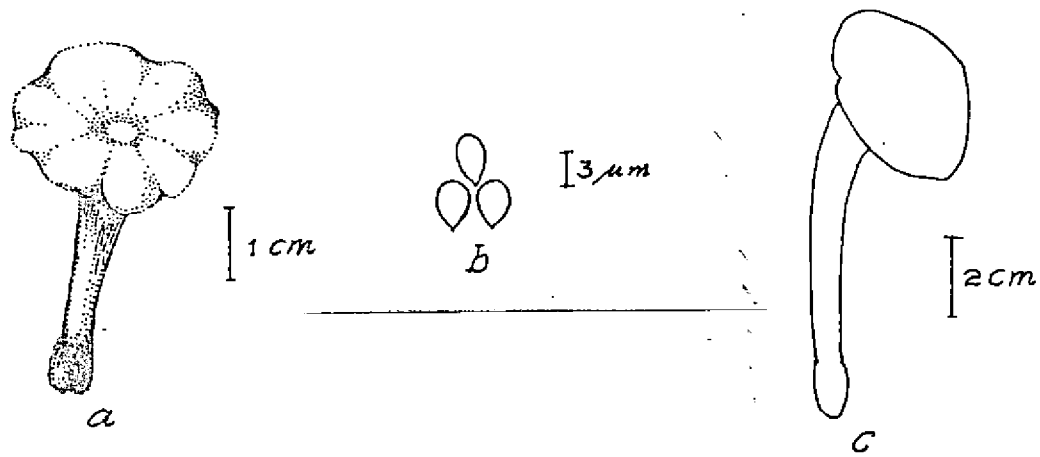


FIG. VII. a. *Pleurotus pometi*

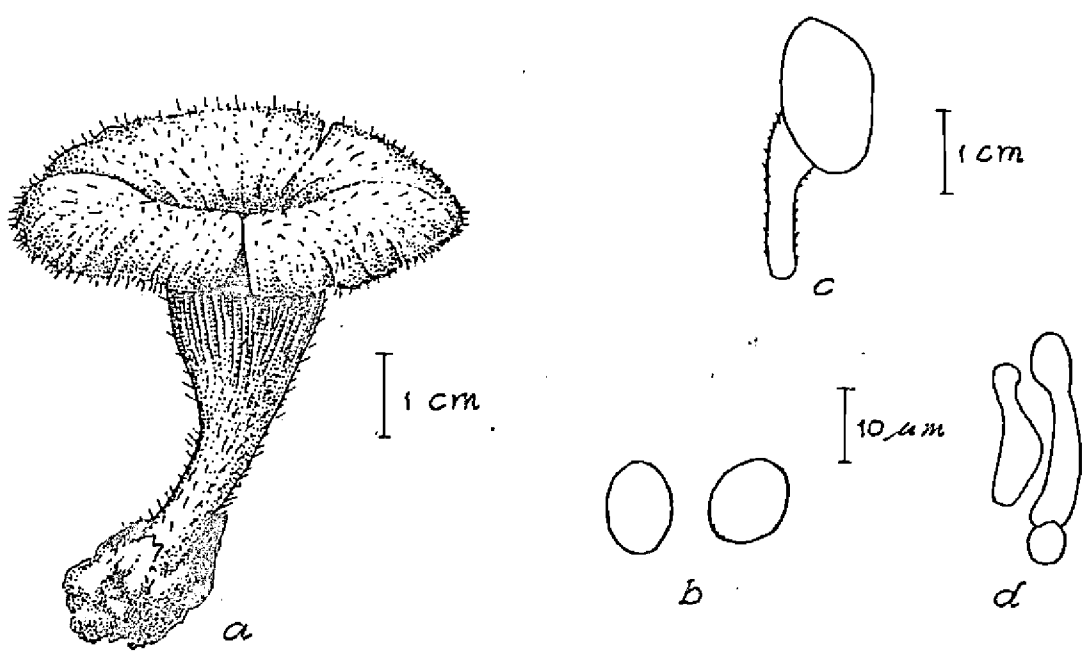


FIG. VII. b. *Pleurotus pubescens*

Pleurotus pubescens Peck

PILEUS: fleshy, convex, 4 to 8 cm broad sub-orbicular, pubescent, slightly yellowish.

GILLS: broad, sub distant, rounded behind, sinuate, pallid tinged reddish, number per cm 30.

STIPE: short, firm, curved, eccentric, more or less of the colour of pileus 2 to 3 cm long.

context fleshy, soft and pink coloured.

SPORES: globose and tuberculate, 8 to 10 µm x 2 to 3.5 µm in size.

SPORE PRINT: white

EDIBILITY: edible

collected from oil palm (Elaeis guineensis) bunch waste, Palode, Trivandrum, October, 1986 (Plate No.XIV, Fig.VII b).

Pleurotus pulmonarius (Fr.) Quél.

Sporophores solitary or caespitose.

PILEUS: white to cream coloured, convex, reniform or infundibuliform, plane or reflexoconchate at margin, 5 to 9 cm in diameter, fleshy, glabrous.

GILLS: crowded, whitish or cream coloured, turning lemon yellow, decurrent, smooth edge entire, not branched or anastomosing at the base, number per cm 14.

STIPE: very short, solid, exactly lateral, villose, sometimes attenuated towards the base, veil not formed. flesh, white, fleshy.

SPORES: basidiospores cylindrical, 8 to 12 x 2 to 3 µm in size.

SPORE PRINT: white

EDIBILITY: edible.

[illegible] hymenophoral trama irregular, hyphae rarely monomitic.

collected from Palode, Trivandrum from oil palm waste (Elaeis guineensis) October 1986. (Fig. VIII a).

PLATE. XIV. *Pleurotus pubescens*

PLATE. XV. *Pleurotus saligneus*

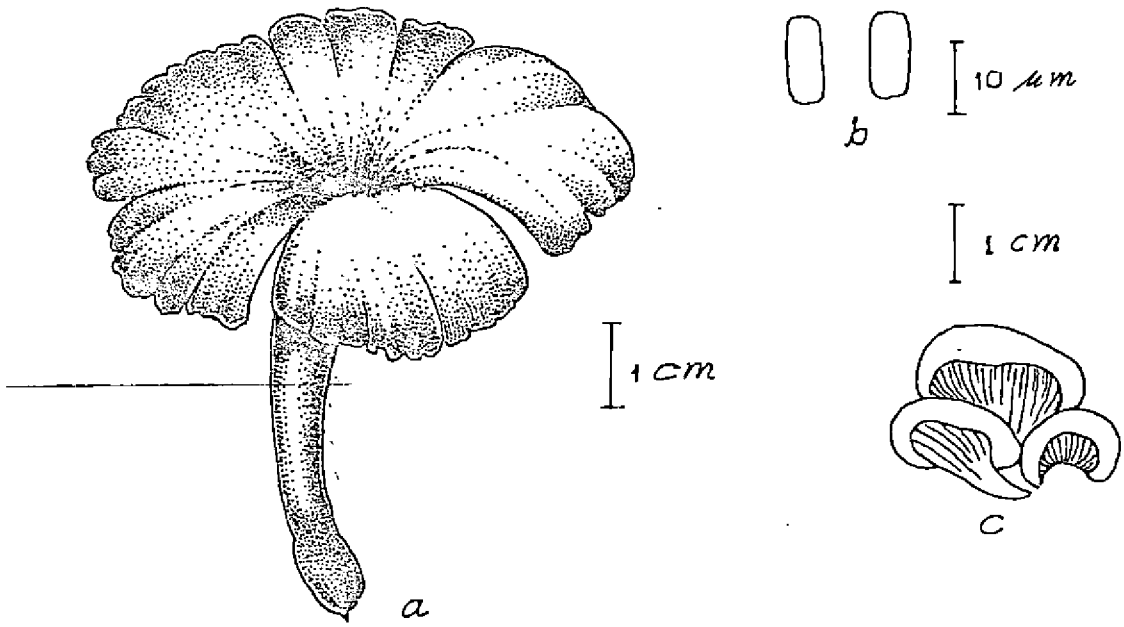


FIG. VIII. a. *Pleurotus pulmonarius*

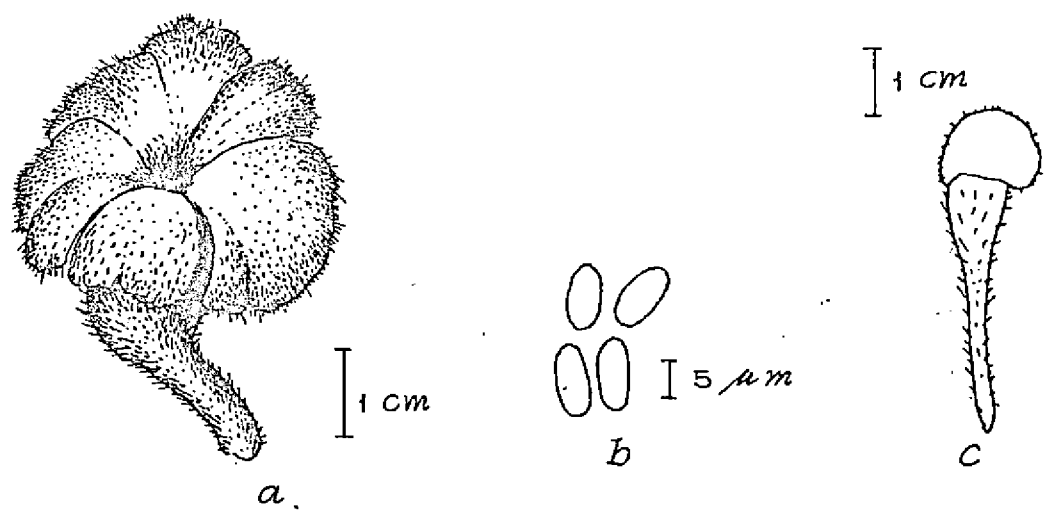


FIG. VIII. b. *Pleurotus salignus*

Pleurotus salignus (Pers.: Fr.) Kummer

Sporophores solitary, very rarely caespitose.

PILEUS: shell shaped 5 to 8 cm broad, sooty ash colour or ochraceous, fleshy, compact, spongy somewhat dimidiate, pulvinate when young, here and there strigose, incurved,

GILLS: horizontal, decurrent, branched, in middle-crowded, dingy, often eroded at the edge, not glandular, slightly bluish, number per cm 26.

STIPE: always short, 2 to 3 cm long, firm, more or less tomentose, glabrous.

SPORES: oblong or cylindrical, 8 to 10 x 3 to 4 µm in size.

SPORE PRINT: creamy white.

EDIBILITY: edible.

collected from harvested oil palm bunch waste (Elaeis guineensis) from Palode, Trivandrum, during June 1986 (Plate No.XV, Fig.VIII b).

Pleurotus serotinus Fr.

Sporophores solitary or caespitose and imbricate, variously coloured dingy yellow.

PILEUS: fleshy, 3 to 8 cm broad, compact, convex or nearly plane, viscid when young and moist, dimidiate or kidney shaped or sub orbicular, colour variable from yellowish green brownish green or olive green.

GILLS: crowded, decurrent, up to 3.5 mm wide, whitish or yellowish, often branched, edge entire, number per cm 16.

STIPE: very short or almost wanting, lateral, thick, yellowish beneath and minutely tomentose or squamulose with blackish points.

flesh thick white and gelatinous under cuticle.

SPORES: basidiospores minute, elliptical, 5 µm long x 2 to 3 µm broad, amyloid.

SPORE PRINT: cream

collected from Oil palm Elaeis guineensis inflorescence, Palode, Trivandrum, November, 1986 (Plate No.XVI, Fig.IX a).

PLATE. XVI. *Pleurotus serotinus*

PLATE. XVII. *Pleurotus squarrosulus*

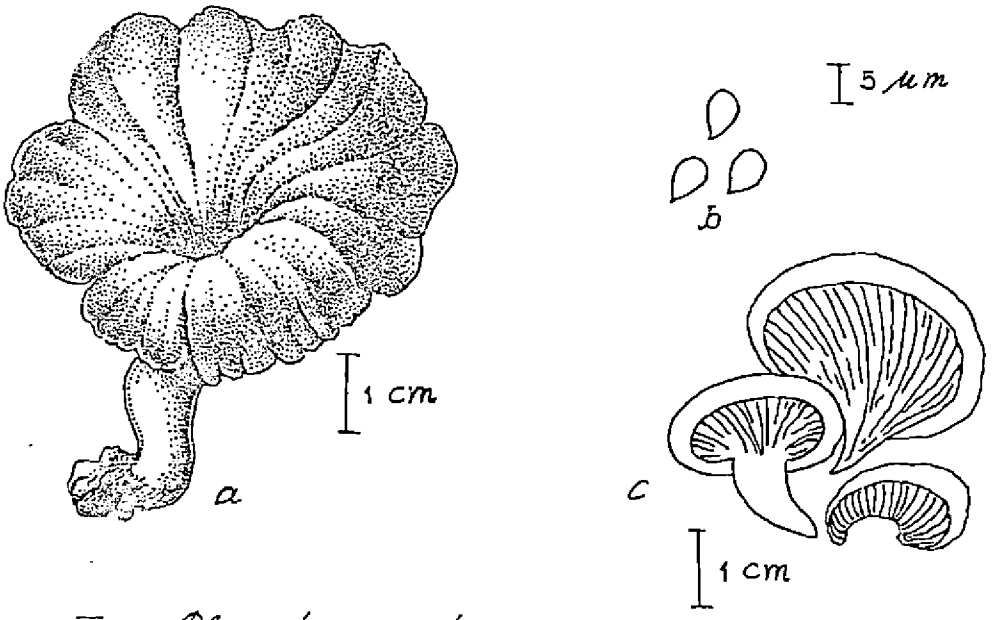


FIG. IX. a. *Pleurotus serotinus*

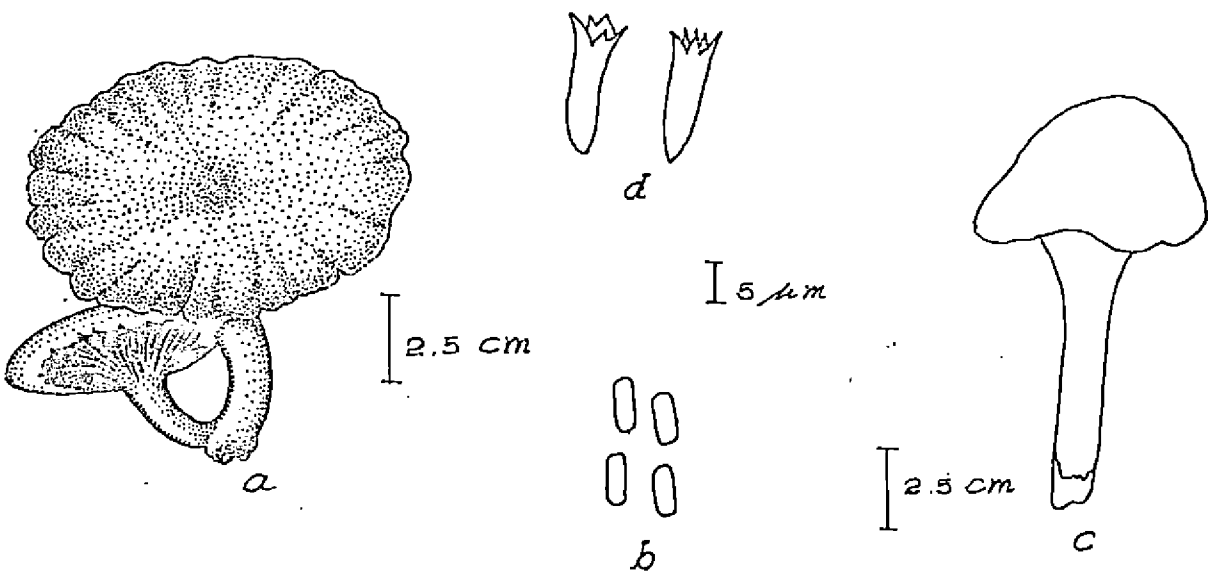


FIG. IX. b. *Pleurotus squarrosulus*

Pleurotus squarrosulus (Mont.) Singer

Sporophores generally in clusters on logs.

PILEUS: circular or shell shaped, centrally stipitate, 2 to 10 cm wide, white to cream coloured coriaceous becoming stiff on drying and flexible when fresh, squamose to squarrose with small concentrically arranged innate scales which may be concolorous or slightly darker, straight when fresh, becoming involute on drying, margin thin, regular or lobed.

GILLS: crowded, deeply decurrent, white to pale buff, concolorous with the pileus with gills of four length, arcuate, thin, 2 to 3 mm wide, slightly interveined towards the base, edge finely denticulate, number per cm 38.

STIPE: typically central, rarely eccentric, cylindrical, 1.2 to 7 x 2 to 5 cm in size attenuated towards the base, whitish at first, brown at maturity surface covered with irregular flocculose, squamules,

usually without annulus and volva, however, in young ones, velar remnants can be observed.

context upto 2 mm thick, fleshy, coriaceous, white with a dimitic hyphal system of generative and binding hyphae, generative hyphae thin walled 2 to 4 µm in diameter, hyaline, occasionally branching with prominent clamp connections at the septa, binding hyphae 2 to 10 µm in diameter well developed and the dominant hyphal type with a subhyaline wall upto 2.5 µm thick and numerous side branches of limited growth which taper distally to 1.0 µm in diameter.

SPORES: basidiospores cylindric, hyaline, thin walled, 5 to 7.5 x 1.7 to 2.5 µm in size.

SPORE PRINT: pale cream

BASIDIA: clavate, cylindrical bearing four sterigmata, 15 to 20 x 3.5 to 4.2 µm in size pleurocystidia and cheilocystidia absent, cystidiole abundant, 15 to 27 x 4 to 8 µm clavate to fusiform.

EDIBILITY: edible when fresh and young.

collected from Rubber (Hevea braziliensis) College of Agriculture, Vellayani June, 1986. (Plate No.XVII, Fig.IX b).

Pleurotus subpalmatus Fr.

Sporophores often tufted with imbricate caps on tree trunks.

PILEUS: convex first then flattened 5 to 14 cm, pinkish in colour, relative to stipe eccentric, margin remain involute for a long period, covered with a thick wrinkled gelatinous cutiole, very much astringent.

GILLS: crowded, broad, soft adnate, sinuate connected by veins, dingy, number per cm 40,

STIPE: whitish, when young, turn orange brown, short fibrillose, striate pruinose, solid, eccentric, lateral, but the pileus is always marginate behind.

SPORES: basidiospores subspherical to nearly globose, spinulose 5 to 7 µm x 2 to 3.5 µm in size, pinkish or salmon coloured.

SPORE PRINT: cream

EDIBILITY: not edible - bitter taste.

collected from dried mango tree (Mongifera indica) from College of Agriculture, Vellayani during September, 1986 (Plate No.XVIII, Fig.X a).

PLATE. XVIII. *Pleurotus subpalmatus*

PLATE. XIX. *Pleurotus ulmarius*

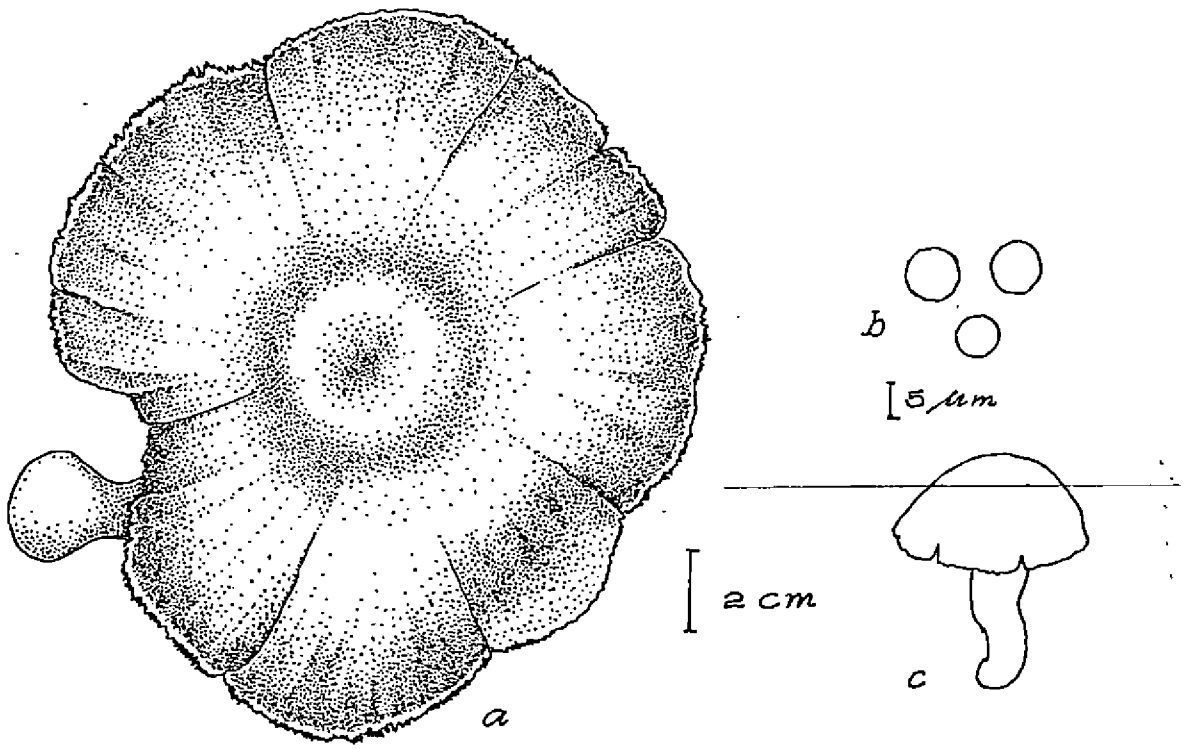


FIG. X. a. *Pleurotus subpalmatus*

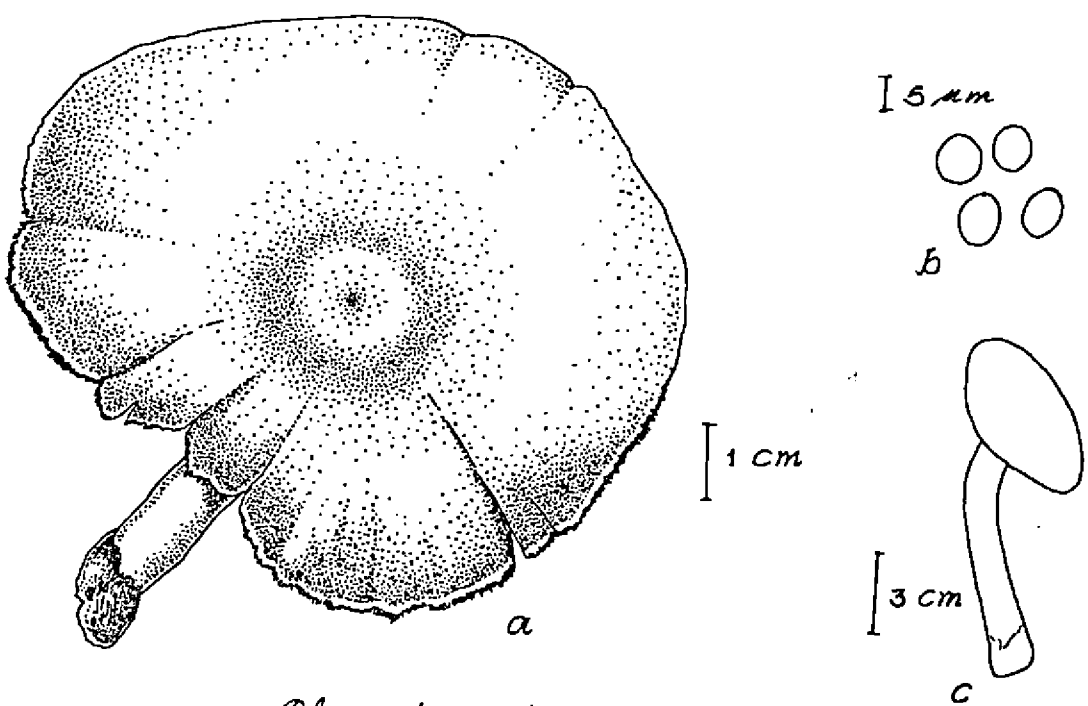


FIG. X. b. *Pleurotus ulmarius*

Pleurotus ulmarius (Bull.: Fr.) Quél.

Sporophores solitary or caespitose on tree trunks.

PILEUS: first convex, then plane, 7.5 to 12.5 cm in size, often marbled with round spots, fleshy, compact, horizontal, moderately regular although more or less eccentric, smooth, white first then pale ochre.

GILLS: sinuate, horizontal, rounded behind, slightly adnexed, broad, some what crowded, whitish to pale ochre, number per cm 14-16.

STIPE: eccentric or almost laterally attached to the pileus, curved, ascending, tomentose at the base, rarely villous through out, whitish, varying in length often very long 5 to 15 cm, often tomentose.

SPORES: basidiospores are nearly globose, 5 µm long and 5 to 6.5 µm broad, white.

SPORE PRINT: cream white

EDIBILITY: when young and small it is tender and of acceptable flavour.

collected from dried up Mango tree (Mangifera indica), College of Agriculture, Vellayani, July, 1986 (Plate No.XIX, Fig.X b).

DISCUSSION

DISCUSSION

A preliminary survey conducted in and around the College of Agriculture, Vellayani and in some parts of Trivandrum district and adjoining areas revealed the occurrence of a number of *Pleurotus* species. They were found to occur mostly during the north east monsoon period, preferring dead and rarely living stumps of various trees as substrates. Twenty species, which were more common and properly identified are described in this study. All these collections were preserved and deposited in the Agaricales collections of the Department of Plant Pathology, College of Agriculture, Vellayani.

The details of occurrence of the twenty species of *Pleurotus* described here can be summarised as follows:

Sl No	Name	Substrate	Period of collection
1	*P.citrinopileatus* Singer	Stumps of *Anacardium occidentale*	June, 1986

2	P.cornucopiae (Paulet: Pers.) Rolland	Dried up anjili log (Artocarpus hirsuta)	October, 1983
3	P.dryinus (Pers.: Fr.) Kummer	dried up Camphor tree (Cinnamomum camphora)	September, 1984
4	P.ëöus (Berk.) Sacc.	logs of Anjèli (Artocarpus hirsuta)	June, 1986
5	P.flabellatus (Berk. & Br.) Sacc.	Standing Ciba cotton (Ceiba pentandra)	October, 1986
6	P.luteoalbus Beeli	Base of Mango tree (Mangifera indica)	September, 1986
7	P.lignatilis (Pers.: Fr.) Kummer	Fallen logs of Artocarpus incisa	October, 1985
8	P.mastrucatus Fr.	decayed coconut roots inside a well (illegible)	November, 1986
9	P.opuntiae (Dur. & Lév) Sacc.	Oil palm waste (Elaeis guineensis)	June, 1985
10	P.ostreatus (Jacq.: Fr.) Kummer	logs of Mangifera indica	June, 1984
11	P.petaloides (Bull.: Fr.) Schulz.	Stump of Cocos nucifera	November, 1986
12	P.platypus (Cooke & Massee) Sacc.	Rotten wood pieces	November, 1986
13	P.pometi (Fr.) Quél	Coconut Stump waste	November, 1986
14	P.pubescens Peck.	Oil palm waste	October, 1986
15	P.pulmonarius (Fr.) Quél.	Oil palm waste	October, 1986

16	P.saligmus (Pers.: Fr.) Kummer	Oil palm waste	June, 1986
17	P.serotinus Fr.	Oil palm waste	November, 1986
18	P.squarrosulus (Mont.) Singer	from standing rubber tree (Hevea brailiensis)	June, 1986
19	P.subpalmatus Fr.	dried up mango tree	September, 1986
20	P.ulmarius (Bull.: Fr.) Quel.	dried up mango tree	July, 1986

This is the first elaborate monographic study on the collection and identification of species of Pleurotus from India after the sustained effort of Sir J.D. Hooker, in the middle of 19th Century from Darjeeling - Sikkim - Nepal area. Bhavani Devi (1982) in a preliminary survey, as part of the floristic study of Agaricales of Kerala recorded five species of Pleurotus, viz., P.cornucopiae, P.ostreatus, P.platypus, P.squarrosulus and P.spathulatus Pers., as occurring in Kerala and out of this P.spathulatus was ranked as a new record for the country. In the present study except P.spathulatus all the other four species were recorded. The present study confirms the occurrence of these four species in the State and also added the other sixteen species of Pleurotus as new records to the Agaric wealth of Kerala. Out of the twenty species recorded in

the present study, the following eight species are new records for India (Natarajan, 1987).

1	P.luteoalbus	2	P.lignatilis
3	P.mastrucatus	4	P.petaloides
5	P.pometi	6	P.pubescens
7	P.serotinus	8	P.ulmarius

Eventhough mention has been made about the occurrence of P.citrinopileatus (Sivaprakasam, 1986; Purushothama, 1987) P.opuntiae (Nair & Bhavani Devi, 1986), and P.subpalmatus (Butler & Bisby, 1931) in the country their occurrence has not been validly published sofar.

The first detailed floristic study of Pleurotus flora of India as mentioned above was by Sir J.D. Hooker in the West Bengal - Sikkim - Nepal area during the last century and all these specimens deposited in the Royal Botanic Gardens, Kew, England were subsequently examined by Pegler and ten species were validly published from this collections (Pegler, 1976). These include P.ninguidus (Berk.) Saco., P.membranaceus Massee,

P.flabellatus, P.eöus, P.anserinus (Berk.) Sacc., P.placentodes (Berk.) Sacc., P.platypus, P.gemmellarii (Inzeng) Sacc., P.ostreatus and P.fossulatus (Cooke) Sacc.

Characterisation of species in Pleurotus has been attempted by many workers from time to time (Eger, et al., 1979; Pegler, 1976). However, according to Singer (1975) a modern monographic study of the genus is absolutely essential. Some of the presently defined species like P.ostreatus are also often pointed out to be very poorly defined, as it is described as a complex species (Eger et al., 1979). Shape and type of pileus, size and shape of spores etc., are often employed in delimiting the species complex in Pleurotus groups. Most of the species of Pleurotus have white spores except very few like P.cornucopiae with pink to lilac spores.

Among the species recorded, P.citrinonileatus, P.cornucopiae, P.dryinus, P.eöus, P.flabellatus, P.ostreatus and P.platypus are considered to be good esculent species (Tanaka, 1976).

P.citrinonileatus is characterised by a broad spathulate pileus with a very short stipe to almost sessile.

P.cornucopiae has an umbilicate to infundibuliform pileus strongly decurrent and repeatedly anastomosing gills that give the long and more or less centrally attached stipe a rigid appearance to its base which is considered as a diagnostic character of the species. While P.ostreatus has a strong and short decurrent gills; with few or no anastomoses, eccentric and frequently laterally attached stipe, which are relatively short. P.cornucopiae has been first recorded from India from erst while Madhya Pradesh as P.sapidus (Graham, 1915) later, on, it has been recorded from Baroda by Moses (1948) and from Calcutta by Bose and Bose (1940).

P.cöus was first recorded for India from the collections of Hooker and identified by Berkeley (1847). It has been first collected from the hot valleys of Sikkim. The species can be easily identified by the pinkish tints of the pileus and the small narrow spores. This is a highly prized esculent species and Singh and Rajarathnam (1977) from Mysore succeeded in its cultivation. This mushroom is known to be distributed in Sikkim (Hooker, 1848 - vide Pegler, 1976), Mysore (Singh & Rajarathnam, 1977) and in West Bengal (Roy & Samajpati, 1980).

P.flabellatus, first recorded from Calcutta on dead wood, by Bose (1920) and again on dead wood of Caesalpinia pulcherima by Banerjee (1947). It is domesticated for large scale cultivation at C.F.T.R.I., Mysore by Bano and Srivastava (1962). It is known to be distributed in other parts of India also as in Mysore (Bano, 1971), Katra forests - Jammu (Kaul & Janardhanan - vide Watling & Gregory, 1980), West Bengal (Thakur, 1980) and in Maharashtra (Sathe & Deshpande, 1980). Petch (1924) described it in detail from Sri Lanka. In the present study it was recorded from Ciba Cotton (Ceiba pentandra) during the North East monsoon period.

P.luteoalbus recorded in the present study is a new record for India. It is a common mushroom in many of the African countries Pegler (1977). This species first described by Beeli (1928) as Claudopus, because of the discolouration on drying of the lamellae, but on subsequent microscopic examination of the fungus, it revealed its true Pleurotus nature. This is a spectacular species occuring in large clusters on dead wood, fallen trunks etc. It is easily recognisable

by its brilliant yellow coloured pileus. It some what resembles P. citrinopileatus but differs from it in its larger spores and strongly developed stipe.

P.lignatilis recorded from fallen logs of Artocarpus incisa is a new record for the country and is known to be an edible species of immense potential. This is a common species in U.S.A. (McIlvaine & Macadam, 1973) and in many parts of Africa (Pegler, 1977). It can be easily distinguished by its dingy white pileus.

P.mastrucatus recorded from a well on decaying coconut roots is a new record for the country. Originally described by Fries (1821), it is known to occur in many parts of America and Europe and is known to be edible with a good flavour (McIlvaine & Macadam, 1973). The mushroom is readily recognisable by its brown squarrosely scaly pileus. However, opinion differs regarding its identity and according to Singer (1975) it is Hohenbuehelia mastructatus (Fr.) Sing.

P.opuntiae, originally described as Agaricus opuntiae was revalidated by Saccardo (c.f. Pegler, 1977) is a common species in Africa (Pegler, 1977) and so far not validly published for India.

P.ostreatus, the type species of the genus is a common mushroom in India, recorded in the current survey also, as occurring on stumps of mango tree. This mushroom was first identified for India from the collections of Hooker by Berkeley (1854). It is known to occur in Sikkim (Berkeley, 1854), West Bengal (Bose & Bose, 1940). Baroda (Moses, 1948) Kashmir (Murrill, 1924), Jammu (Kaul & Kachroo, 1974), Lucknow (Ghosh et al., 1974) and in Karnataka (Sathe & Kulkarni, 1981). A good edible species,it can be easily distinguished by the caespitose nature of the spathulate pileus, which may in most cases be hygrophanus also.

P.petaloides recorded from coconut stump during the North East Monsoon period is a new record for India. However, it has been collected by Hooker from Nepal and Berkeley (1856) identified the same as P.petaloides.

The dingy brown pileus which resambles the petals of flowers gained the species the name. Schulzer (1866) from Germany named the same fungus as Hohenbuchelia petaloides and opinion differs on this.

P.platypus collected from rotten wood pieces is a good edible species and recorded from Nepal by Cooke (1888). It was recorded from Jammu-Tawi also by Watling and Gregory (1980).

P.pometi is a new record for India. This mushroom originally described by Fries (1821) can be distinguished by the white fleshy and soft subflaccid pileus. However, according to Kummer (1871) this is a synonym of P.dryinus.

P.pubescens recorded from oil palm waste during north east monsoon period, is a new record for India, and the species is characterised by the pubescent pileus.

P.pulmonarius recorded from Oil palm waste during the north east monsoon period is already a known fungus for India, which has been first recorded from West Bengal

by Chakraverty and Purkayastha (1976). This is an edible mushroom and can be easily distinguished by the white to cream coloured glabrous pileus.

P.saliensus recorded from Sikkim by Berkeley (1856) has been recorded in the present survey from the oil palm waste during the South west monsoon period. This can be identified by the shell shaped pileus which is ash coloured or ochraceous.

P.serotinus, again a mushroom associated with oil palm waste, generally observed during the north east monsoon period, is a new record for India. This is characterised by a reniform pileus.

P.squarrosulus previously described as Lentinus subnudus and subsequently as L.squarrosulus appear as caespitose in logs. Recorded from rubber in the present study, this is not an edible species, since it is leathery. First time for India this fungus had been recorded by Bose and Bose (1940) from West Bengal, Chopra and Chopra (1955) from Bombay and again from Calcutta by Chandra (1974).

Pegler (1975) pointed out that Lentinus squarrosulus described from Nilgiris by Montague (1842) was P.squarrosulus and it is to be considered as first record of this fungus for the country. In current literature the mushrooms P.squarrosulus is considered as a synonym of Lentinus squarrosulus (Singer, 1961)

P.subpalmatus first described by Fries (1821) characterised by a dingy white pileus, isolated from mango stumps, has been recorded for India by Hennings (1901) from Utter Pradesh. This mushroom is of no economic importance.

P.ulmarius with whitish or cream coloured pileus, which was collected from dried up Mango tree logs is the first record for the country. This is also not a good edible species. However, the correct identity of this fungus is on debate and according to Singer (1975) it is Hypsizygus tessulatus (Bull.: Fr.) Sing. and in European species often as a Lyophyllum.

Out of twenty species of Pleurotus described above, thirteen of them were collected during the

north east monsoon period. Bhavani Devi (1982) also recorded that species of Pleurotus occur mostly during the north east monsoon period. It is also interesting to note here that during the survey five of the species were collected from Oil palm waste. It is already known that oil palm harbours a very rich mycoflora (Turner, 1971) and already species of Pleurotus viz., P.opuntiae, P.pubescens, P.pulmonarius, P.saliŋnus and P.serotinus were recorded from Oil palm (Turner, 1971).

A commendable attempt has been made by Pegler (1976) to redescribe the species of Pleurotus, collected from the Indian sub continent, by Sir J.D. Hooker utilising the herbarium at Kew. By validating only ten species from this collection he developed a simple key for designating the species. According to Pegler (1976) the Pleurotus species of the Indian sub continent can be divided conveniently into two groups according to their spore size, those with small spores 6-9 µm long which are more characteristic of the tropical, sub tropical species and those with spores over 10 µm in length which are more typical of the North temperate flora, confined to

Himalayan localities. Following Pegler (1976) an attempt is made here to fit a simple Key for the diagnosis of the twenty species of Pleurotus described in the present survey. The majority of the flora (fourteen) are with spores less than 10 µm in size falling under the group tropical and sub-tropical flora and six of them with spores longer than 10 µm, which may be considered as the temperate flora. Kerala is blessed with a diversity of climate and is rich in its diverse flora. All these have contributed to an ideal condition for a very rich natural macromycete flora, making the State a paradise for mushroom hunters.

KEY TO THE PLEUROTUS FLORA OF KERALA

1. Spores less than 10 µm in Size
   2. Pileus whitish or cream coloured
      5. Gills decurrent, crowded
         6. Stipe very small, eccentric, spores oval
            1. P.pometi
         6'. Stipe very long, central, spores cylindrical
            2. P.squarrosulus
         7. Stipe very small, spores cylindrical
            3. P.flabellatus
         7'. Stipe small, spores globose
            4. P.ulmarius

2'. Pileus ochraceous or pink coloured

8. Pileus typically shell shaped, gills decurrent, crowded, stipe short and tomentose, spores oblong to cylindrical.

5. P.seligmus

8'. Pileus typically flabelliform, gills decurrent, crowded, stipe absent

6. P.eöus

3. Pileus dingy white

9. Gills adnate, crowded, stipe present, spores elliptical

7. P.lignatilis

9'. Gills adnate, crowded, stipe absent, or very small, spores globose.

8. P.subpalmatus

3'. Pileus dingy brown

10. Gills decurrent and crowded, stipe absent or small, basidiospores ovoid to elliptical.

9. P.petaloides

10'. Gills decurrent moderately crowded, basidiospores oblong to cylindrical.

10. P.platypus

4. Pileus typically spathulate, gills adnate, moderately crowded, stipe short or absent spore cylindrical.

11. P.luteoalbus

4'. Pileus not spathulate, but convex or nearly plane.

11. Pileus smooth, gills decurrent crowded, stipe very small, spores minute and elliptical.

12. P.serotinus

11'. Pileus pubescent gills sinuate, moderately crowded, stipe absent, spores cylindrical

13. P.pubescens

12. Pileus lobed, upperstratum of pileus gelatinous, brown with squamules.

14. P.mastrucatus

1'. Spores more than 10 µm in size.

13. Hyphal system monomitic.

14. Pileus spathulate

15. Pileus fairly large (8-15 cm) gills adnate and crowded, spores subcylindrical.

15. P.citrinopileatus

16. Pileus medium size (5-12 cm) gills decurrent, crowded, stipe very short

15'. Spores sub-cylindrical and spore print white to lilac.

16. P.ostreatus

16'. Spores cylindrical and ~~spore~~ spore print pure white.

17. P.opuntiae

14. Pileus infundibuliform, gills decurrent and crowded, stipe very short.

17. Spores elliptical

18. P.cornucopiae

17'. Spores cylindrical

19. P.pulmonarius

13'. Hyphal system dimitic

Pileus convex to flattened gills decurrent, not crowded, stipe very short, spores cylindrical

20. P.dryinus

PART II

CULTIVATION OF *PLEUROTUS* SPECIES

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The group of wood destroying saprophytic fungi commonly known as Oyster mushrooms, though sometimes appearing as parasite, is wide spread both in the temperate and tropical zones. Cultivation of *Pleurotus* Spp. started first by the begining of this century on tree stumps, logs etc. (Falck, 1917). The technique has been further refined using sawdust as substrate (Block *et al.*, 1958) and later, on straw bits which was first employed in India (Bano & Srivastava, 1962). The first domesticated species under *Pleurotus* was *P.ostreatus*, but in India in the recent past *P.sajor-caju* (Fr.) Singer, has gained importance as a commercial species. A brief review of the works carried out in the attempt to domesticate different species of *Pleurotus* is given below.

A. *Pleurotus ostreatus* (Jacq.: Fr.) Kummer

In the modern era, the large scale popularisation of the species depends mainly on the works of Block *et al.* (1958) who originated the technique of cultivation using saw dust as substrate at Florida in U.S.A. *P.ostreatus* is more or less as typical temperate zone fungus which comes

to fruit under natural conditions at 15°C. Strains which are less affected by temperature (fructification at 15-20°C) are also known to occur (Stanĕk & Ryšava, 1971). The species vary in its colour from dark blue, white, cream to brown, yellow, pink etc. The pileus is attached to the stem at the side, and it is typically shell shaped and later depressed. According to Moser (1967) there are two subspecies - Var - salignus (Pers.: Fr.) Konr. and Maubl and Var. pulmonarius (Fr.) the lamellae whitish or grey and decurrent, stipe short, eccentric or lateral. The spores 8-12 µm x 3-4 µm in size, hyaline and spore deposit white to lilac grey. The cultivation of the species on tree stumps and logs started by Falck (1917; 1919). His crude technique has been further refined by others like Passecker (1959); Luthard (1969); Vessey and Toth (1970). Zadrazil and Grabbe (1981) reviewed the cultivation of Pleurotus spp., on logs using spawn based on saw dust or grains. The inoculum is not pressed into holes as in shiitake, but spread over the surface of a cut and then covered by thin slices of wood. Afterwards the stumps were piled up and protected against drying. They were also put in layers in pits covered with branches, plastic foil and soil during the growth of mycelium. Later on,

they were separately placed half dry into the soil for fructification (Luthard, 1969; Lelley et al., 1976; Steineck, 1973; Chang & Hayes, 1978). The foundation for industrial production of substratum and fruit bodies was developed by Junkova (1971), Stanek and Ryseva (1971); Heltay et al. (1971); Zadrazil and Schneidereit (1972); Zadrazil (1973 a, b); Kalberer and Vogel, (1974). In India also cultivation of this mushroom on logs of wood had been attempted. Branches of Euphorbia royleana (Kaul & Janardhanan, 1970); non resinous wood, like Poplar, Acasia, Willow etc., were found to be very much suitable for the same in Kashmir by Dhar (1976), and species of Quercus and Poplar were also reported to support the growth, by Krishna (1978), Pant and Bhatt (1985) in a recent report compared some of the logs of some common trees and reported that chestnut tree (Aesculus indica) also supported good growth of P.ostreatus, ~~P.florida~~.

P. florida

This species is more or less similar to P.ostreatus but the pileus is some what smaller and finer in structure

(Block et al., 1959; Eger, 1965 a, b). The colour varies from that of P.ostreatus and it changes with change in temperature. At low temperature, the pileus is light brown, and as temperature raises, it turns to paler to pallid yellow or white (Zadrazil & Schliemann, 1975). Usually this mushroom recorded a higher yield than P.ostreatus in temperate zones (Kalberer & Vogel, 1974). The question of species rank to this mushroom is still in debate and according to Block et al. (1959), this is the same as P.ostreatus. In a detailed comparative study Eger (1965, a) stated that this mushroom differed in several properties from the European P.ostreatus and according to the description of Singer (1949) P.florida resembled P.floridanus Singer. Conditions for the cultivation of this species were described by Block et al. (1959) and were extended by Zadrazil and Schneidereit (1972), Zadrazil (1973 a, c). P.florida was successfully cultivated in the plains of India by Khanna and Garcha (1981) using chopped paddy straw as substrate. In Punjab the crop was raised in fruit baskets during mid December to March. They recorded a cumulative yield of 32 per cent of fresh mushrooms in 104 days.

P.eryngii (Dc.: Fr.) Quél.

(= C fuscus (Batt.) Bres.)

P.eryngii belongs to the typical subtropic flora and it is wide spread in the steppe regions of U.S.S.R. (Vasilkov, 1955). It is one of the parasitic species of Pleurotus, as it attack the roots of Eryngium campestre and Laserpitium latefolium as well as Ferula sp. (Kreisel, 1961). The pileus is reddish brown, grey brown to dirty yellow, slightly squamulose, 4 to 8 cm wide. The fruit body is very big and weigh about 300 to 400 g. The lamellae are white or grayish and decurrent. The stipe is whitish, 3 to 10 cm long, the spores are hyaline 8 to 11 µm x 4 - 5 µm in size. The technique of cultivation of the species has not reached to industrial dimensions (Zadražil, 1978).

P.cornucopiae

This is another species under cultivation. Under natural conditions, this mushroom can be seen growing on wood, Quercus, Fagus, Ulnus etc. The species is described else where in this work and its cultivation is only in an experimental stage (Kreisel, 1961).

P.eöus

This species collected wild has been domesticated and put under cultivation utilizing chopped paddy straw as substrate in India (Singh & Rajarathnam, 1977). It prefers a temperature of 21-35°C and a humidity range of 65 to 100 per cent. Proper ventilation is essential for fruit body production. Initially the colour of fruit body is pink which gradually fades with age. This fungus has been successfully grown in nylon nets, earthern pots and wooden trays containing chopped paddy straw with or without gram dhal powder (Purkayastha & Jana, 1983).

P.fossulatus

Puri et al. (1981) attempted to cultivate this species on pre-soaked and chopped paddy straw and wheat straw supplemented with Bengal gram powder. The optimum temperature for fruiting was found to be 20 ± 1°C and they also reported that light was essential for fruiting. Addition of nitrogenous materials like pulses to the substrate, hastened fruit body formation and paddy straw was found to be superior to wheat straw as substrate.

P.flabellatus

This mushroom was first commercially cultivated in India by Bano and Srivastava (1962). They have developed for the first time, paddy straw bits as suitable substrate for cultivation of Pleurotus species. Enhancement of yield was noticed by adding various pulses and cereal grains in powdered form (Bano, 1971).

P.sajor-caju (Fr.) Singer

This mushroom which was originally named as Lentinus sajor-caju by Fries (1838) is reported from various parts of India. From South Andaman Island (Cooke, 1981); Bengal (Bose, 1920; Banerjee, 1947); North and Central India (Bagchee, 1954); Foot hills of Himalayas (Jandaik & Kapoor, 1975; Jandaik, 1976), Tamil Nadu (Bhaskaran et al., 1978; Sivaprakasam & Kandaswamy, 1980) Kerala (Sathe & Daniel, 1980). This mushroom, though originally described as a species under Lentinus, Singer (1951) changed the name as P.sajor-caju and now the taxonomic limits of Lentinus and Pleurotus are well marked and P.sajor-caju is characterised by the infundibuliform to cyathiform pileus

with trimitic hyphal system and the presence of an annulus on the stipe. Later on, the characters of P.sajor-caju and Lentinus sajor-caju were studied by Jandaik and Kapoor (1975) and by Natarajan (1978). According to Pegler (1976) the characters of P.sajor-caju agreed well with that of P.platypus, but not with L.sajor-caju. This fungus is heterothallic and tetrapolar (Roxon & Jong, 1977), the somatic cells are multinucleate whereas stipe and pileus cells are binucleate (Jandaik & Kapoor, 1979). This species has become so popular in India after the experimental cultivation of the same by Jandaik (1974); Rangaswamy et al. (1975) and Chakravarthy and Sarkar (1978). The cultivation of this mushroom has been so popular in many parts of India currently and the same was originally isolated from stump of Euphorbia royleana and the optimum temperature for fruit body formation is 25°C and can fructificate upto 30°C also. Cultivation of this mushroom on paddy straw bits followed by the compact poly bag method developed by Bhaskaran et al. (1978) is very popular in the country. Effect of various organic substrates on the production of sporocarps of P.sajor-caju was studied by many workers (Kandaswami & Rangaswami, 1978; Chakravarthy & Sarkar, 1982 a, b; Jana & Purkayastha, 1983).

P.sajor-caju also has been successfully cultivated on logs as reported by Chakravarthy and Sarkar (1982 a). The logs were collected, dried in sun for 15 days and soaked in water for 7 days. These logs were inoculated after making holes (2 cm diameter and 2 cm in depth) at a distance of 7.5 cm on the surface of logs. Each hole is filled with wheat grain spawn covered with polythene bags and incubated at 22-30°C and irrigated at an interval of 5 days. Polythene bags were removed when the primordia began to develop. The inoculated logs were sprinkled with water whenever necessary. Trials of Chakravarthy and Sarkar (1982a) showed that out of nine types of logs used in their study, logs of Mangifera indica and Artocarpus lakoocha Supported maximum growth of the mushroom, while it was medium on logs of Hymeodictylon and it was very low in logs of Eugenea jambolana, Zizyphus auritiana and in Casuarina equisitifolia. It could not infect logs of Erythrina variegata, Psidium guajava and Trewia nudiflora.

The spawn run period also varied according to the type of log from 70 to 93 days. Fruitification appeared from end of January to beginning of February and appeared

in two flushes. The total yield from each type of log was directly correlated with extend of infection of logs. The highest yield per log was recorded in Mangifera indica (179 g) followed by Artocarpus lakoocha and the lowest yield was per log of Casuarina equisitifolia (27 g). The detailed studies carried out at Tamil Nadu Agricultural University, Coimbatore for the large scale cultivation of P.sajor-caju starting with the works of Rangaswami et al. (1975) helped to perfect a suitable technique for the same.

Nutritional Studies

The comparative ability of Pleurotus spp. in their mycelial growth varies. P.ostreatus, P.florida, P.cornucopiae and P.salmoneo stramineus are characterised by rapidity of growth and high saprophytic colonization ability of the mycelium. These attributes facilitate a speedy penetration of the substratum by the fungus which simplifies the cultivation as a whole.

The detailed nutritional studies carried out by many workers clearly indicate that the Pleurotus species grow well on several synthetic nutrients (Zadražil, 1978).

Various individual nutrient components on mycelial growth of P.ostreatus has been investigated by Koch (1958), Staněk and Ryšava (1971), Volz (1972), Kurtzman (1974) and of P.florida (Eger, 1965 a,b,; 1970 a,b; Poppe, 1973). Many workers observed starch as best carbon source for many edible fungi, including Pleurotus spp. Madelin (1956) and Srivastava and Dano (1970) observed starch and glucose as good carbon sources for P.flabellatus and xylose as poor carbon source. Hashimoto and Takahashi (1974) also reported Pentose such as xylose and arabinose as poor carbon source for P.ostreatus. They also reported preferential utilization of ammoniacal nitrogen. Kikan and Rao (1983) conducted physiological studies of the four edible mushrooms to the effect of different carbon and nitrogen sources. They found that different strains will have varying response on different carbon and nitrogen sources and they found starch as best carbon source for two strains of P.ostreatus and among nitrogen sources organic forms were found to be more suitable. By consolidating the results of various nutritional trials, Zadražil (1978) suggested the following media as a good nutrient media for growth of Pleurotus spp. Malt extract Soyabean flour 10 g, peptone 1 g; KH_2PO_4 0.5 g; $MgSO_4$ 7 H_2O 0.5 g; Feelz (1% solution) 1ml; yeast extract 0.1 g; agar agar 15 g; water 1 litre.

Under natural conditions Pleurotus species grow mostly on dead and rarely on living parts of plants which are generally poor in nutrients and vitamins. It has been well documented that for both mycelial growth and fruit-body development on lignin cellulose (C:N relationship 1:50 - 100-500), materials such as Maize cobs, straw of all cereals, paper, wood shavings, sawdust, vegetable wastes, as well as food industry wastes are sufficient (Kedyk & Smotlachova, 1959; Bano & Srivastava, 1962; Block, 1965; Eager, 1965 a; Schanel, et al., 1966).

The effect of environmental factors, on the growth of Pleurotus has also been studied. According to Zadrazil and Schneidereit (1972) ~~and~~ spread of mycelial growth of Pleurotus is related to temperature. They also reported differences between species in their relation to temperature. ~~They also reported differences, between Pleurotus species, in their relation to temperature.~~

Effect of Co_2 on the growth of different species of Pleurotus has also been worked out. Schanel (1970) reported that for Pleurotus ostreatus and Zadrazil (1975 a) for P.ostreatus, P.florida & P.eryngii, mycelial growth was found to be stimulated by Co_2 concentration in the air.

Pleurotus spp. are known to grow well and rapidly under submerged culture. This method has been pointed out by many as suitable for industrial production of mycelium (Starka, 1955; Jennison, 1955; Worgan, 1968). A low concentration of sulphate liquor can be used as sole source of substrate for mycelium and fruit body formation (Zadrazil, 1974 b). This method has been pointed out to be useful for economic disposal fof papermill waste, however, according to Worgan (1968) cultivation of Basidiomycetes in fermentors is mostly used in physiological studies in order to determine single parameters for biomass production, or to produce special enzymes.

SPAWN PRODUCTION

When Agaricus bisporus and Volvariella volvacea were first cultivated, freshly prepared substrate was inoculated with small amount of old spent compost (Singer, 1961). In recent times special sterilized substrates inoculated with pure cultures derived from spores have been used. Wedge shaped, round wedge shaped or rod shaped pieces of wood (1 cm diameter) or 2 cm in length are often used for inoculating logs (Singer, 1961). They are soaked in water, supplemented with nutrients, sterilized,

inoculated and incubated at 24 to 28°C. These are then inserted into the holes drilled into the wood logs. Alternatively pastes of sawdust and rice bran, cotton seed and minerals containing thriving colonies of mycelia which are pressed into wooden slices which are nailed to the face of logs and stumps (Luthard, 1969). A break through in the use of spawn was made by Sinden (1932; 1934) who first introduced grain spawn in the cultivation of mushrooms. Different kinds of grain, wheat, rye, millet etc., are cooked, dried and mixed with 1:3 per cent weight of calcium sulphate and calcium carbonate. The addition of Gypsum and calcium carbonate prevents grains from clogging (Stoller, 1962). Spawn bottles were filled with the substrate and sterilized for 2 to 3 hours at 121 to 135°C. The substrate after sterilization should contain 40 to 50 per cent moisture and pH around 7.5. After 7 to 10 days of incubation the containers are shaken and spawn of Pleurotus spp. is reported to be ready by 10 days. Lemke (1972) stated that fully grown spawn in polythene bags with few holes can be stored upto an year under 2°C.

In contrast to organic carriers, perlite has been used as an inorganic material for spawn preparation (Lemke, 1972).

More or less the general pattern of spawn preparation is followed in the case of Pleurotus spp. also. As pointed out by Huhnke et al. (1973) spawn of Pleurotus spp. can be easily prepared using anaerobically fermented wheat grains. Liquid spawn (mycelium in submerged culture) had also been used for production of sporophores in P.ostreetus as reported by Kostadinov et al. (1971).

Addition of nitrogenous compounds to grain spawn base was found to be of no influence on sporophore yield in Pleurotus sajor-caju (Krishnamohan, 1975). Rangad and Jandaik (1977) studied the effect of different spawn substrates and storage conditions in yield of Pleurotus spp. They found that freshly prepared spawn produced maximum yield in a number of species of Pleurotus they have tested. The yield of sporocarp was more or less the same from spawn kept for two months either at room temperature or in refrigerated condition.

Thapa et al. (1978) used polypropylene bags for spawn production of Pleurotus which was found to be cheap and effective.

Cultivation of *Pleurotus* species

The cultivation of *Pleurotus* species as a group of edible mushrooms has gained popularity in recent years. Their ability to excrete hydrolysing and oxidising enzymes (Toyama & Ogawa, 1974; Rajarathnam *et al.*, 1979) has enabled them to flourish over a wide range of natural lignocellulosic waste materials like sawdust (Block *et al.*, 1958) paddy straw (Bano & Srivastava, 1974), News paper wastes (Hashimoto *et al.*, 1974), wheat straw (Zadrazil, 1974) and hulled maize cobs (Sivaprakasam & Kandaswamy, 1981). The work of Block *et al.* (1959) opened up new avenues in the area of mushroom cultivation. They found that *P.ostreatus* yielded sporocarps (1-2 lb/lb of sawdust) in two weeks in sterile sawdust fortified with oat meal. Staněk and Ryšava (1971) successfully produced *Pleurotus* mycelium and fruit body on a substratum fermented with *Streptomyces thermovulgaris*. Bano and Srivastava (1962) utilized paddy straw bits for the cultivation of *P.flabellatus* and this is considered as a break through in the cultivation of *Pleurotus* spp. and helped much in industrialising their production. Zadrazil and Schneidereit (1972) determined that both aerobic and anaerobic fermentation of a straw substratum were suitable for *Pleurotus* cultivation.

Synthetic white mushroom compost (Humfeld, 1972) with a relatively high nitrogen content proved to be suitable for both P.ostreatus and P.florida. The relationship of type of fermentation to yield was studied by Kalberer and Vogel (1974) and Kalberer (1974). Fermentation of substratum was reported to be not necessary for the cultivation of this mushroom. A number of agricultural and industrial wastes were tested for their ability to support Pleurotus sporocarp production.

Commercial cultivation of Pleurotus species in India gained momentum when Jandaik (1976) introduced the cultivation of P.sajor-caju and he found that this fungus can grow on various substrates. With less nitrogen content various substrates were tried by Garcha et al. (1980) and they found that cereal straw invariably supported maximum yield of P.florida and P.sajor-caju and 48 to 60 per cent biological efficiency was recorded for them. They also tried various agricultural wastes from maize, bajra, mentha, groundnut stalks, berseem and bagassed and vegetable wastes of chilli, bhindi, and potato. Besides the cereal straw, they found that bagassee and mentha also were suitable with a biological efficiency of 18.46 per cent.

Sivaprakasam (1980) investigated the use of several materials for Pleurotus sajor-caju like waste paper, bagasse of sugarcane, hulled cobs of maize, rice straw, coir waste of coconut pericarp, wood shavings etc. Results clearly indicated that waste paper, sugarcane bagasse, hulled maize cobs and rice straw were suitable for sporocarp formation. Sivaprakasam and Kandaswamy (1981) also reported several substrates, like waste paper, begasse of sugarcane, hulled cobs of maize, straw of rice, dried flowers of Delonix regia, coir waste of coconut pericarp, wood shavings and ragi waste were tried as bedding materials for Pleurotus sajor-caju, Roberto et al. (1982) used Citronella bagasse and coffee pulp as substrate for producing oyster mushroom. However, processing the substrate was found to be more costly. Cotton straw was used as a substrate for cultivation of Pleurotus Sp. by Platt et al. (1982) and they got an yield of 600 to 700 g/kg of dry straw. In Kenya also Nout and Keya (1983) found that cotton hull waste was superior as a substrate than banana leaves, sawdust etc., for Pleurotus sajor-caju.

The safest method of substratum preparation was reported to be the "Till method" developed for Agaricus bisporus (Sengbusch et al., 1971). Spawn running under sterile

conditions in plastic bags, which were to be opened after mycelial permeation for development of fruit bodies (Toth, 1970). A simplification of Till method is to subject the substratum to heat (100 or 80°C); (Junkova, 1971); (Zadražil & Schneidert, 1972), then to cool and spawn.

The sterilization process is replaced by pasteurisation or fermentation process at temperature 40°C to 90°C (Zadražil & Grabbe, 1981). The risk of contamination can be further lessened by hot water treatment of 65°C for 10-15 minutes. This procedure followed by Bano et al. (1979) and Kurtzman (1979) for cultivation of Pleurotus spp.

For the fruit body production horizontal (beds) or vertical (Walls) surfaces are recommended. Appearance of fruit body was reported to be dependant on the kind of surface used. Fungi develop central stem on beds, while on walls stems are eccentric or lateral. Walls or beds can be built from well permeated straw blocks. For mycelial growth period, the spawned substratum is packed in plastic foil and pressed into rectangular blocks. The width of walls are depending on the climatic conditions, but it should not be more than 30 cm (Zadražil, 1973 a, c). In order to simplify the method, containers were developed (Zadražil, 1974 a).

For incubation the sacks, boxes or containers are placed in a dark room of uniform and constant temperature. During this period the temperature of culture is reported not to exceed 33°C, at the centre of the container. Though, regulation of humidity was reported to be not necessary it must be between 60 to 80 per cent.

Nutritional value

Crisan and Sands (1978) enumerated the data available on the proximate constituents of various mushrooms including Pleurotus spp. A comparative account of the nutritive value of different species of mushroom with that of egg has been discussed by Kannayan and Ramaswamy (1980).

Jandaik and Kapoor (1975) reported the nutritive value of the mushroom Pleurotus sajor caju. Starton (1984) reported the nutritional value of mushrooms. Compared with most of the vegetables, mushrooms supply a slightly higher percentage of protein. Like all non animal foods, however, the protein in mushrooms lacks some of the essential amino acids. Mushroom have almost no fat (Starton, 1984).

Mushroom contain a small amount of complex carbohydrates and no sugar. They have lower carbohydate content than most vegetables with just 3.8 g/100 g serving. Mushrooms are an excellent source of vitamins especially the B complex vitamin, riboflavins, niacin, thiamin and pantothenic acid. Riboflavin is needed for healthy skin, eyes and body tissues; niacin is also vital for healthy skin (Starton, 1984).

The sporophores of Pleurotus was found to contain 24.18 per cent of crude protein on dry weight basis (Rangaswamy et al., 1975). Nine amino acids were detected in the mature sporophores of the fungus (Jandaik, 1976 b). Subsequently 18 amino acids were detected in mature sporophores were also found to contain 33.57 per cent of crude protein, 2.26 per cent of fat, 0.285 per cent of reducing sugars, 0.121 per cent of starch, 0.06 per cent phosphorus, 2.58 per cent of potassium, 0.04 per cent of calcium, 0.16 per cent magnesium, 0.20 per cent of sodium, 0.02 per cent of copper, 0.002 per cent of Zinc, 0.004 per cent of Manganese and 0.01 per cent of iron on dry weight basis (Jandaik, 1977).

MATERIALS AND METHODS

MATERIALS AND METHODS

Six species of *Pleurotus* were tested for their comparative efficacy for cultivation under Kerala conditions. They were:

1. *P.citrinopileatus*
2. *P.flabellatus*
3. *P.florida*
4. *P.opuntiae*
5. *P.ostreatus*
6. *P.sajor caju*

Out of this, *P.citrinopileatus* and *P.sajor-caju* were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore; *P.florida*, *P.flabellatus* from the Department of Plant Pathology, College of Agriculture, Pune and *P.opuntiae* was isolated from oil palm waste, collected from C.P.C.R.I., Regional Station, Palode, Trivandrum district.

A. CULTURAL CHARACTERS OF SPECIES OF *PLEUROTUS*.

1. Growth of *Pleurotus* species on various solid media under laboratory conditions.

In order to find out the best medium for radial growth of *Pleurotus* species, three different species of *Pleurotus*, viz., *P.flabellatus*, *P.sajor-caju* and *P.ostreatus* were used.

Culture discs of 5 mm in diameter cut out from seven day old culture of the respective test fungus were used for inoculation. Various solid media, viz., Oats agar, Potato dextrose agar, Malt extract, Richards medium and Czapek's medium were used. The composition of the media used are given in Appendix II. The media were prepared and sterilized by autoclaving at 1.02 kg/cm^2 for 15-20 minutes. The media before solidification were poured into sterile petri dishes of 9 cm diameter and allowed to solidify. After inoculation the dishes were incubated at room temperature (28 ± 2°C). One set of dishes was placed under oridinary light over laboratory tables and the other set of dishes was wrapped with black paper and incubated in complete darkness. Three replications were kept for each treatment and the colony diameter was measured at every 24 hour interval for 6 days.

2. Growth of different species of Pleurotus in different media in shake culture.

Six different species of Pleurotus, viz., P.sajor-caju P. florida, P.flabellatus, P.opuntiae, P.ostreatus and P.citrinopileatus were used in this study. Three different media, Potato dextrose, Oats and Malt extract broths were used.

The composition used was the same as used in the previous experiment, except for the omission of two per cent agar-agar in each media. The liquid media were prepared and 50 ml of each medium was dispensed in 250 ml conical flask and autoclaved at 1.02 kg/cm^2 for 15 minutes. The media were then inoculated by 5 mm culture disc of the fungi, cut out from an actively growing culture and kept in a EMENVEE Rotary shaker at 200 x 260 rpm (Stroke/minute) for a period of about one month. After one month the pellets formed were filtered through a Whatman No.1 filter paper and dried in an oven at 70°C. The dry weights was taken until a constant weight was obtained. Three replications were maintained for each treatment.

3. Influence of different nitrogen sources on the growth of Pleurotus spp.

Six different forms of Nitrogen viz., Ammonium nitrate, Peptone, Ammonium carbonate, Sodium nitrate,

Ammonium chloride and Potassium nitrate were substituted in Malt extract broth so as to give the same per cent of nitrogen in each case. Fifty ml of the respective medium was taken in each 250 ml Erlenmeyer flask, sterilized and were inoculated as described earlier, with P.sajor caju, P.ostreatus and P.flabellatus and incubated at room temperature (28 ± 2°C) for 10 days. The mycelial mat was filtered through a whatman No.1 filter paper and dry weights were taken after drying at 70°C, till a constant weight was obtained. Three replications were maintained in each case.

4. Influence of different carbon sources on the growth of Pleurotus spp.

As in the above case, the three different species of Pleurotus were grown in media with various carbon sources, viz., Lactose, Xylose, Arabinose, Galactose, Mannose, Fructose, Inositol and Glucose were substituted in media without agar agar, to give the same per cent of carbon in each case. Fifty ml of each medium was taken in 250 ml conical flask and autoclaved at 1.02 kg/cm^2.

The medium was then inoculated by 5 mm culture discs of the fungi cut out from an actively growing culture and incubated at room temperature (28 ± 2°C). After 10 days, the mycelial mat was filtered and dry weight was taken after drying at 70°C till a constant weight was obtained. Three replications were maintained in each case.

5. Effect of different hydrogen ion concentration in the media on growth of Pleurotus spp.

Potato dextrose broth was prepared and initial pH was adjusted to 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5 and 7 by adding 0.1 N hydrochloric acid or 0.1 N sodium hydroxide, as the case may be. Fifty ml of each medium was taken in 250 ml Erlenmeyer flasks and autoclaved at 1.02 kg/cm^2. The medium was inoculated by a five mm culture disc of seven day old culture of Pleurotus sajor-caju, P.ostreatus and P.flabellatus, and incubated at room temperature (29 ± 2°C) for 10 days. The mycelial mat was filtered, dried at 70°C and dry weight was taken till constant weights were obtained. Three replications were kept in each case.

6. Effect of different temperature on growing various species of Pleurotus.

In order to assess the best temperature for the maximum growth of three Pleurotus species, viz., P.sajor-caju P.flabellatus and P.ostreatus five mm culture disc of actively growing seven day old culture of the fungi was inoculated in potato dextrose broth and incubated at different temperature, viz., 15°C, 20°C, 25°C, 30°C and 35°C. After ten days of incubation, the mycelial mat was filtered, dried at 70°C and dry weights were taken till two consecutive weights were equal. Three replications were kept for each treatment.

B. PREPARATION OF SPAWN.

1. Growth of various species of Pleurotus in spawn bottles on wheat grains as substrate.

Colourless empty milk bottles were used as containers. Wheat grains were boiled for 3 to 5 minutes with equal volume of water. The excess water present was drained off and they were dispensed at the rate of 300 g/bottle. Five to percent of calcium carbonate was

mixed thoroughly with the grain in each bottle. The filled bottles, were sterilized at 1.02 kg/cm^2 for 1 to 2 h per day consecutively for two days and allowed to cool down. Mycelial bits from seven day old actively growing pure culture of Pleurotus species viz., P.sajor-caju, P.florida, P.flabellatus, P.ostreatus, P.opuntiae and P.citrinopileatus were inoculated aseptically and incubated at room temperature (29 ± 2°C). Three replications were maintained in each case and mycelial growth of the fungi were measured and recorded at 7 day intervals for a period of twenty one days.

The spawn thus prepared was used to raise beds subsequently.

2. Growth of P.sajor-caju on grains of cereals and pulses as spawn substrates in different containers.

Four different containers, viz., Boost bottle, Horlicks bottle, 750 ml liquor bottle and 500 ml Milk bottle were used for producing spawn.

Cereals like Sorghum, Ragi, Bajra, Maize, and Wheat pulses like green gram, Red gram, Black gram, Horse gram

and Bengal gram were just boiled with equal quantity of water, excess water drained off and mixed with 5 per cent calcium carbonate thoroughly. All bottles mentioned above were filled with the above cereals and pulses, leaving 7 to 8 cm space at the top. The bottles were sterilized at 1.02 kg/cm^2 for 1 to 2 hours per day consecutively for two days and allowed to cool down. Mycelial bits from seven day old actively growing pure culture of the fungus P.sajor caju was inoculated aseptically and incubated at room temperature (29± 2°C) for a period of two weeks. Three replications were maintained in each case and the mycelial growth of the fungus was recorded after 7 and 14 days.

The spawn thus prepared were used for spawning beds.

3. Growth of Pleurotus sajor-caju in different oil cakes and agricultural wastes as substrates in different containers.

The different types of containers used in the previous experiment were used here also. Various oil cakes,

like Coconut oil cake, Gingelly oil cake, Ground nut oil cake, Rice bran, Saw dust and Paddy Chaff were used as substrates. The oil cakes and other substrates were powdered, moistened and filled in the containers leaving the top 7 to 8 cm space. They were then sterilized at 1.02 kg/cm^2 for 2 hours per day consecutively for 2 days and allowed to cool down. Mycelial bits from seven day old actively growing pure culture of the fungus, P.sajor-caju was inoculated aseptically and incubated at room temperature (29 ± 2°C). Three replications were maintained in each case and the mycelial growth of the fungus was recorded at an interval of 7 days, for a continuous period of 21 days.

4. Effect of temperature on the mycelial growth of P.sajor-caju in spawn bottles.

Spawn bottles were prepared as above using 750 ml liquor bottles and in fungus. The substrate was different pulses, viz., red gram, horse gram, bengal gram, green gram, wheat grain, paddy straw and dried salvinia. Inoculated bottles were incubated at various temperatures, viz., 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. Three replications were maintained in each case. After 14 days of incubation visual observation of the mycelial growth of the fungus was recorded and graded as follows:

+++++ Very good mycelial growth

++++ good mycelial growth

+++ moderate mycelial growth

++ poor mycelial growth

+ very poor

- no growth

The spawn prepared in each of the substrate was used to spawn paddy straw beds.

C. CULTIVATION OF PLEUROTUS spp.

1. Suitability of different species of Pleurotus for cultivation in Kerala.

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 raised following the poly bag method as described by Bhaskaran et al. (1978). However, a modified technique wherein instead of the paddy straw bits, straw made into small twists were also used for laying beds. Paddy straw was

cut into bits of 5 to 10 cm in length, or they may be made into small twists. These were thensoaked in water overnight, taken out, excess water removed and put into boiled water and continued to boil for about 15-20 minutes. They were then air dried and used for layout of beds. Polythene tubes, of 400-500 guage and 30 cm in diameter were cut into bits of 60 cm in length and they were used to layout the beds. Few small holes were made on them for permitting air circulation and the bottom tied. Treated straw were placed on the cover for about a height of about 15 cm. This layer was spawned by wheat grain spawn of the test mushroom and again a second layer was placed and spawned. In this way the whole cover was filled layer by layer and cover made as compact as possible and tightly tied to a compact mass. The bits or twists were used separately to layout the beds. Same quantity of straw was used for each bag. 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.

2. Influence of growth regulators in enhancing sporocarp formation in Pleurotus spp.

Beds were prepared following the standard poly bag method, as described above utilising all the above six species. Growth regulators; Indol acetic acid, Indole Butyric acid, Naphthalene acetic acid, and 2, 4D were sprayed just after removal of cover at 50 and 100 ppm concentration and sporocarp production assessed.

D. STANDARDISATION OF CULTIVATION TECHNIQUES FOR CULTIVATION OF PLEUROTUS SAJOR-CAJU.

In the preliminary trials it has been found that P.sajor-caju is more suited to Kerala conditions and hence detailed trials with this species has been carried out inorder to standardise a suitable technique for the same. Unless otherwise mentioned, beds were raised following the poly bag technique as detailed under C (1).

1. Comparative efficacy of different types of spawn on yield of P.sajor-caju.

The spawn prepared as detailed under B(2) - above

on various grains of cereals and pulses were used in the trial to raise beds of P.sajor-caju following the poly bag method as described under C(1) - above. The grain spawns used were those on sorghum, Ragi, Bajra, Maize, Paddy and Wheat while the pulses were Green gram, Red gram, Bengal gram, Horse gram and Black gram. The total yield under each treatment were recorded and compared.

2. Effect of various organic amendments on the yield response of P.sajor-caju.

The following six organic amendments at the rate of fifty g per bed with one kg of straw was also added while laying out beds, with wheat grain spawn. The amendments used were wheat flour, green gram powder, Bengal gram powder, Horse gram powder, Red gram powder and cowdung slurry in comperison with unamended control. The total yield of sporocarp from each treatment was recorded and compared.

3. Cultivation on logs.

Cultivation of P.sajor-caju was tried on logs of different trees, as suggested by Singer (1961), Kaul and

Jeanardhanan (1970) and Luthard (1969). Logs of the following fifteen trees and banana pseudostem were used for the trial. The logs used were:

1. Albizzia lebbec
2. Anacardium occidentale
3. Artocarpus heterophyllus
4. Artocarpus hirsuta
5. Caesalpinia pulcherima
6. Ceiba pentandra
7. Cocos nucifera
8. Enterolobium saman
9. Erythrina indica
10. Eucalyptus citriodora
11. Eugenia jambos
12. E.jambolana
13. Lichi chinensis
14. Mangifera indica
15. Persea americana
16. Banana pseudostem - Musa paradisiaca

The logs of these trees which were 5 - 10 cm in diameter were cut into pieces of about a meter in length. Holes, about 2 cm in diameter and depth at an interval of about 7 cm were made on the surface of logs for inoculation. These logs were sun dried, for a week and were kept immersed in water for 24 hours. After this in holes were filled with wheat grain spawn of P.sajor-caju and the logs kept vertically and covered with Polythene cover and incubated at room temperature (28 ± 2°C). These logs were irrigated at an interval of 5 days and kept for observation for a period of two years.

4. Influence of different types of bed for production of sporocarps of P.sajor-caju.

For different methods, viz., polythene wrapper bed (Zadrazil, 1974 a) cylindrical bed, tray method and tier method were tried in layout of beds for the cultivation of P.sajor-caju.

Paddy straw chopped into bits of 5-10 cm were pre-soaked for about 8-12 hours, drained off excess water and boiled for 15-30 minutes and excess water drained, cooled and used. Polythene sheet of 500 guage thickness with holes 10-15 cm apart were used in the first type. One kg of paddy straw was spread in the sheet with alternate spawing. Paddy straw was compactly pressed and covered in the sheet and tied to form a compact mass. In cylindrical bed method straw of 5-10 cm in length as described above in C(1) was filled upto a height of 15 cm in polythene tube of 500 guage thickness, 30 x 60 cm in size and with few small holes. This layer was spawned all around by wheat grain spawn of P.sajor caju. A second layer was placed over this and spawned. In this way the whole cover was filled layer by layer and tightly tied to a compact mass.

In the third method, wooden tray of size 60 x 40 x 15 cm was used. One kg paddy straw of the type described above was mixed initially with a bottle of wheat grain spawn of P.sajor-caju in another container and the same was spread in wooden tray tightly pressing the same to form a compact mass. The tray was then covered with polythene sheet.

In the tier method, a three tier system was followed to lay the mushroom bed. Each tier was made up of bamboo sticks of 1.5 m x 2.5 cm. In each tier, the bamboo sticks were nailed one by one leaving a gap of about 2 cm. The width of each tier thus made was 0.5 m. Beds were laid in each tier with treated paddy straw, of the above type leaving a space of about 5 cm on either side. First, a base layer was made with paddy straw. Spawning was done on the periphery all around. The process was repeated upto a height of about 8-10 cm. It was then covered with polythene sheet tightly pressing the bed. All the above types of beds were kept in darkness for one to two weeks for spawn running. Gunny screens were hanged around the mushroom shed and always kept moist to maintain a high percentage of relative humidity. Spawn run was completed within two weeks.

All covers were removed then and the beds were kept for a period of about one month for getting ~~in~~ complete yield.

5. Influence of size of polythene bag on yield of Pleurotus sajor-caju.

To assess the comparative efficacy of polythene bags of various sizes, six different sizes, viz., 15 x 25 cm, 25 x 35 cm, 35 x 45 cm, 45 x 55 cm, 55 x 60 cm and 55 x 65 cm of 500 guage thickness were used. Small holes of 0.5 cm diameter were made in the polythene bags 10-15 cm apart. Chopped and pre-treated straw as described earlier was used as substrate. The quantity of straw varied from 0.25 kg to 1.5 kg according to size of bed spawned and finally compactly tied at the top. They were incubated in darkness for spawn run. The beds were placed on a floor with wet sand to provide a relative humidity of 80-90%. The sand was frequently moistened to maintain proper humidity. The polythene bags were removed after completion of spawn run. The beds were moistened well and kept for yield. Three replications were maintained in each case.

6. Comparative efficacy of different containers for lay out of beds by P.sajor-caju.

Four different types of containers, viz., bamboo basket, earthen pot, wooden tray, and polythene bag of sizes 30 cm x 60 cm, 30 cm x 30 cm, 60 x 40 x 15 cm and 60 cm x 30 cm respectively were tested for their comparative efficiency in increasing the yield of P.sajor-caju.

Bits of paddy straw about 5-10 cm in size were soaked over night in water, drained off the excess water and immersed in hot boiling water for 15 - 20 minutes. Excess water was drained off and the straw was used for laying beds. Straw bits were spread into a thickness of 2 cm layers in all the four containers. Spawning was done on the periphery of the layers all around. Repeated the process leaving the top layer un-spawned. All the beds were tightly pressed in the containers and covered with polythene sheet except that in the polythene bag. The polythene bag was tied with jute twine.

Beds in all the four containers were incubated in darkness for spawn run. After completion of spawn run,

the covering was removed. The beds were moistened twice daily and kept for yielding.

7. Comparative efficiency of different types of straw beds in sporocarp formation by Pleurotus sajor-caju.

Four different types of beds were compared for their efficacy to support sporocarp formation. The beds were laid out using the same quantity of straw and each treatment replicated three times.

Rectangular beds with straw bundles.

Four kg straw was tied into bundles of 250 g each. The diameter of each bundle was about 8 cm and length of about 75 cm. The bundles were soaked well over night in clean water. After draining excess water, four bundles of the straw were placed in a plat-form side by side. Another four bundles were then placed cross wise over them and these four bundles formed one base layer. Small bits of spawn were placed all along the edges. Similarly the second layer was laid and spawned as described above. The bed was pressed from the top to compact it and covered with transparent polythene sheet to develop optimum temperature and humidity for growth and development of the fungus.

Rectangular beds with straw twists.

Four kg of straw was made into twists of about 5 cm in diameter. These twists were immensed in water over night. The pre-soaked twists after draining excess water was placed on a raised wooden plat form in a zig-zag manner. Second twist was placed over the first row in the opposite direction which form the first layer of the bed. Spawn bits were placed along the periphery of the bed. The same procedure was followed for placing the remaining twists. The entire bed was compacted by pressing from the top and watered with a rose can to maintain proper moisture level. Finally the bed was completely covered with a transparent polythene sheet.

Rectangular beds with loose straw.

Rectangular beds were made with loose paddy straw of the same quantity as in the previous experiment. Four kg of paddy straw was kept immensed in clean water over night. Wooden planks were placed over a platform in such a way as to make a rectangular box with base and top open. Presoaked loose straw was spread at the base in 2 cm thickness and spawn bits were placed on the periphery all around.

Again another layer of loose straw of 2 cm thickness was made and spawned as before. Such layers were made in a similar way so as to form a rectangular shape. The wooden planks from the sides were removed after the bed was made compact by pressing from the top. The whole bed was covered with transparent polythene sheets and maintained as described earlier.

Hollow beds.

Hollow beds were laid following the method of Krishnamohan (1975). Four kg of presoaked straw in the form of twists were wound around a pot of 30 cm diameter, kept in the centre of the wooden platform. After pressing the bed, the pot was removed leaving a hollow centre. The bed was spawned on both inner and outer margins and the whole bed was covered with a polythene sheet.

8. Comparative efficacy of straw of different varieties of rice in supporting sporocarp formation.

In order to test the comparative efficacy of different varieties of paddy straw in the yield response of Pleurotus sajor-caju, paddy straw of Thriveni, Jaya,

Jyothi, T-9 and Kochu vithu were used. One kg paddy straw of each variety was used. The method adopted was as described above. Three replications were maintained in each case.

9. Effect of frequency of watering the mushroom beds on sporocarp production of Pleurotus sajor-caju.

In order to test the effect of watering on mushroom beds of Pleurotus sajor-caju, watering was done at an interval of 12 hours, 24 hours, 48 hours and no watering in the mushroom beds after opening the polythene cover. The relationship between watering and yield of mushroom was found to follow a quadratic relationship of the form $y = b_0 + b_1 t + b_2 t^2$ where y is the yield of mushroom in gms and 't' the interval of watering.

10. Seasonal variations in yield of Pleurotus sajor-caju.

Mushroom beds were raised during different periods of the year with Pleurotus sajor-caju from January to December 1986. The climatological parameters like temperature, relative humidity, rainfall etc., were noted and the effect of these parameters on yield was correlated.

E. NUTRITIVE VALUE OF DIFFERENT SPECIES OF *PLEUROTUS*

The percentage of protein, mineral constituents (Calcium, Magnesium, Iron and total Ash) vitamin A and C were analysed in six Species of *Pleurotus* viz., *Pleurotus squerrosulus*, *P.dryinus*, *P.cornucopiae*, *P.sajor-caju*, *P.petaloides* and *P.subpalmatus* in comparison with *Volvariella volvacea* was analysed. In the case of *Volvariella volvacea* fully developed buttons just before expansion was used as sample and in case of *Pleurotus* species sporocarps of more or less uniform size and age of development were used as sample.

1. Analysis of protein.

The nitrogen percentage in the sample was estimated by microkjeldahl digestion and distillation method as outlined by Jackson (1968). The percentage crude protein in the sample was determined by multiplying the nitrogen per cent with a constant 6.25.

2. Determination of ash content.

The determination of total ash was carried out as described by Raghuramulu *et al.* (1983). About 5 to 10 gram

of the sample was weighed accurately into a porcelin crucible. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 3.5 hours at about 600°C. It was then cooled in a dessicator and weighed. To ensure completion of ashing, the crucible was again heated in the muffle furnace for 30 minutes, cooled and weighed. This was repeated till two consecutive weights were the same and the ash was almost white or greyish white in colour.

3. Preparation of Ash solution.

Ash solution was prepared as described by Raghuramulu et al. (1983).

The ash was moistened with a small amount of deionised water (0.5 - 1 ml) and 5 ml of Hcl was added to it. The mixture was evaporated to dryness on a boiling water bath. Another 5 ml of Hcl was added again and the solution evaporated to dryness as before. 4 ml of Hcl and a few ml of water were then added and the solution warmed over boiling water bath and filtered into a 100 ml volumetric flask. After cooling, the volume was made up to 100 ml and suitable aliquots were used for the estimation of Fe, Ca and Mg.

4. Calcium.

Ten ml of the ash solution was diluted to 100 ml with distilled water. A few drops of methyl red indicator was added. The solution turned pink. The mixture was neutralised with concentrated ammonia till the pink colour changed to yellow. The solution was heated to boil and 10 ml of 4% ammonium oxalate was added. Both Mg and Ca precipitate. The mixture was then allowed to boil for a few minutes and few drops of glacial acetic acid was added till the colour of the solution was distinctly pink. The precipitate was allowed to settle down for 1 hour. The completion of the precipitation was tested by adding a few drops of ammonium oxalate through the sides of the beaker. The solution was filtered and the precipitate was washed with ammoniacal water (3% ammonia) till free of oxalate. Washing was continued till the final washing gave no precipitate with calcium chloride. The precipitate was washed down into the same beaker by adding sufficient amount of a warm solution of 2 N. H_2SO_4. The solution was then heated to about 70°C and titrated against 0.01 N $Kmno_4$.

5. Magnesium.

The method adopted for the estimation of magnesium was as described by Raghuramulu et al. (1983). Magnesium is converted to Magnesium pyrophosphate which is estimated gravimetrically. To the Ca free filtrate (obtained from the filtrate after precipitation of Ca as oxalate) was added 30 ml of conc. HNO_3 and the solution evaporated completely on a boiling water bath. 5 ml of conc. Hcl and 100 ml of water were then added and the solution stirred well with a glass rod. It was followed by the addition of 10 ml ammonium pyro phosphate solution and 5 ml of 10% sodium citrate solution and the mixture stirred. After adding 2 or 3 drops of methylred indicator the solution was neutralized with the addition of 1:4 dilute ammonia. Strong ammonia was then added, stirred vigorously and the mixture, left to stand over night filtered through Whatman No.40 or 44 filter paper and washed free from chloride using 1:10 dilute ammonia. The precipitate was dried and ashed in a weighed crucible and kept in a muffle furnace at 900°C for 2 hrs. then cooled in a desiccator and weighed to get Mg as its pyrophosphate.

6. Iron.

Iron was estimated by Wong's method.

To an aliquot (6.5 ml or less) of the ash solution enough water was added to make up to a volume of 6.5 ml followed by 1 ml of 30% H_2SO_4, 1 ml 7% potassium persulphate solution and 1.5 ml of 40% potassium thyocyanate solution. The red colour that developed measured within 20 minutes at 540 nm.

Iron was determined colorimetrically making use of the fact that ferric iron give a blood red colour with potassium thiocyanate.

7. Determination of Vitamin C.

Vitamin C (l-ascorbic acid) reduces the blue coloured redox indicator dye 2.6 dichlorophenol indophenol to a colourless solution. The excess unreduced dye in acid medium gives a rose pink colour during titration. Hence the attainment of rose pink colour during titration between the oxalic acid extract of the sample and the dye solution indicates the end point. The dye was standardized with a known standard of ascorbic acid solution.

Estimation of Carotene

The carotene present was extracted with petroleum ether and the intensity of the colour of the extract was compared with that of standard using a colorimeter.

Ten g of the sample was pulverized with 95 per cent ethanol. The suspension was refluxed for about ½ an hour in a boiling water bath. Filtered and filterate diluted with 2 ml of 85% ethanol. Extracted the solution repeatedly with petroleum ether using 20 ml each time and extraction done 3 or 4 times and carotene extracted in the petroleum ether. Pooled the ether extracts and concentrated under reduced pressure to a final volume of 2 - 4 ml.

Separation using alumina column

Two ml of the concentrated carotene extract was loaded on to a column of alumina (10 x 1 cm) containing 5% anhydrous sodium sulphate and eluted with petroleum ether containing 3% acetone. The volume of the elute is made up to 100 ml with ether.

Took different volumes of the standard carotene solution (2-8 ml) corresponding to 40 - 100 r (10 mg of the standard carotene was weighed and made upto 10 ml with ether. 2 ml of the stock standard solution was taken and made upto 50 ml with petroleum ether). The volume of all the solution was made up to 8 ml with petroleum ether. A blank was also prepared similarly by taking 8 ml of petroleum ether. The extract was considered to be unknown 8 ml of the made up extract was taken. The colour developed was read at 540 nm in a calorimeter.

F. PRESERVATION

1. Refrigeration.

One hundred gram of fresh mushrooms of *P. sajor-caju* were collected and stored in a refrigerator at 10-15°C. The samples were kept either in open plastic trays lined with paper or in poly bags of 500 and 150 guage. The shelf life of the mushrooms was observed after 24, 48 and 72 hrs. of incubation. Both visual observation and organoleptic tests were conducted to assess the quality of the mushroom. Three replications were maintained in each case.

2. Dehydration.

Two hundred and fifty g of mature mushrooms were collected, dried under sun for three consecutive days to reduce the moisture content to 5-6 per cent. Simultaneously other samples were also dehydrated keeping it in a Sigg Dorrex dehydrator continuously for 24 hours at a temperature of 55-60°C. The dehydrated samples were transferred to polythene bags of 150 guage thickness and sealed. Another set of dried samples were kept in air tight containers. Dried mushrooms kept open served as control. Visual observations were conducted at different periods after 1, 2, 3 and 6 months respectively, regarding the quantity of the sporocarps.

RESULTS

RESULTS

A. CULTURAL CHARACTERS OF SPECIES OF *PLEUROTUS*

1a. Growth of *Pleurotus* spp. on various solid media under laboratory conditions.

Comparative radial growth of three common species of *Pleurotus* for a period of 6 days incubated under ordinary laboratory conditions (28 ± 2°C) on three of the common laboratory media are given in table 1. There was no growth in CZapek's and Richard's media. The results show that there is not much variation in growth between the three different species. The best medium was found to be Oats agar followed by Potato dextrose agar.

Analysis of data showed no interaction for the three species and time. The trend of growth was observed to be the same for all the species. However, a significant interaction was observed for different media. With Potato dextrose agar there was 95 per cent increase in radial growth after 144 hours, which was 35 per cent for Malt

extract agar and 104 per cent for Oats agar. In PDA and Malt extract agar no significant difference in radial growth was seen after 24 hours of incubation. It was very low (0.6 cm) in Malt extract agar, while it was 1.44 and 1.39 for PDA and Oats agar respectively. No significant difference in radial growth was noticed after 120 hours and 144 hours in all the species in all the three media tested.

Table 1. Growth of Pleurotus spp. in different solid media under laboratory conditions on the sixth day of incubation (Growth in cm transformed values).

Sl. No.	Species	Potato dextrose agar M1	Malt extract agar media M2	Oats agar media M3	Mean
S1	Pleurotus ostreatus	2.030	1.85	2.22	2.03
S2	P.sajor-caju	2.039	1.88	2.20	2.04
S3	P.flabellatus	2.118	1.93	2.29	2.11
	Mean	2.06	1.89	2.24	

C.D. for comparison of 'SxM' means = 0.1439

C.D. for comparison of S or M means = 0.1173

Table 2. Growth of Pleurotus spp. in different solid media under darkness on the sixth day of incubation (Growth in cm transformed values).

Sl No	Species	Potato dextrose agar M1	Malt extract agar media M2	Oats agar media M3	Mean
S1	Pleurotus osreatus	2.06	2.10	2.21	2.12
S2	P.sajor-caju	1.89	1.92	2.02	1.95
S3	P.flabellatus	2.28	2.27	2.35	2.30
	Mean	2.08	2.10	2.20	

C.D. for comparison of S or M = 0.0067

C.D. for comparison of SxM means= 0.0115

1b. Growth of different Pleurotus spp. under darkness.

A comparative account of the radial growth of three different species of Pleurotus in three common solid media incubated under darkness under laboratory conditions (28 ± 2°C) is given in table 2. Significant difference in radial growth was observed between the three different species.

Maximum growth was recorded for P.flabellatus followed by P.sajor-caju and P.ostreatus. Oats agar was found to support maximum growth than PDA or Malt extract agar. A critical analysis of the data revealed that radial growth was found to increase with time for all species grown in different media. Up to three days the radial growth was more for P.flabellatus followed by P.sajor-caju and P.ostreatus while after the 4th day, the growth was found to be more or less similar for all the three species tested.

Significant interaction was found for media under different periods of time. In malt extract media the growth of all the three species was low from the very beginning. In general less growth was observed in Malt extract agar media at different periods of observation.

2. Growth of different species of Pleurotus in different media in shake culture.

Six different species of Pleurotus, viz., P.sajor-caju P.florida, P.flabellatus, P.ostreatus, P.ommtiae and P.citrinopileatus were employed in this study. Three different media were also used. The inoculated flasks were

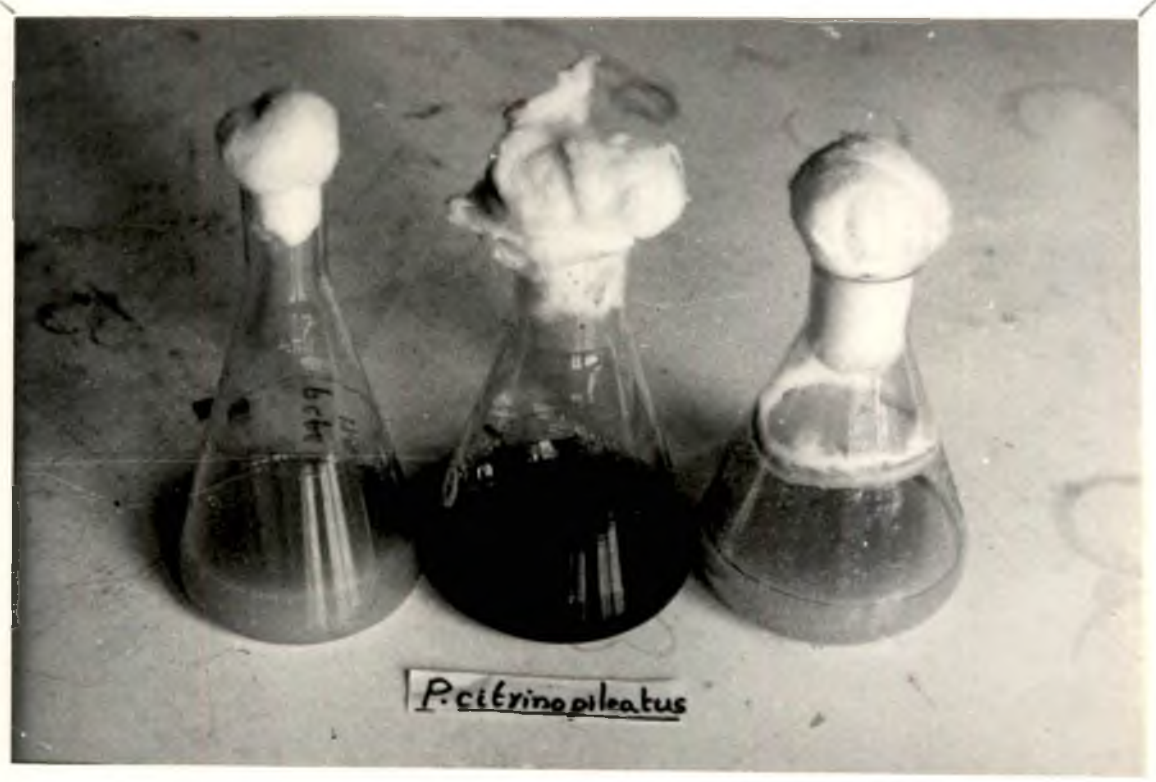
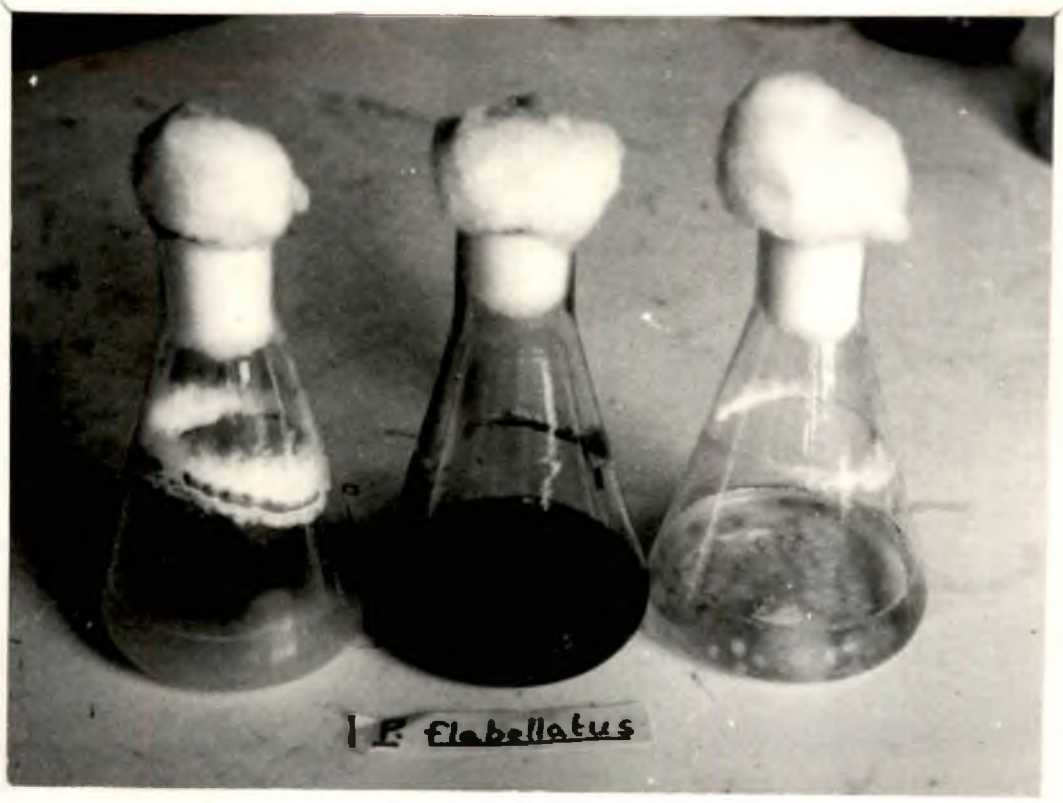


Plate XX. Growth of different species of Pleurotus in shake culture.



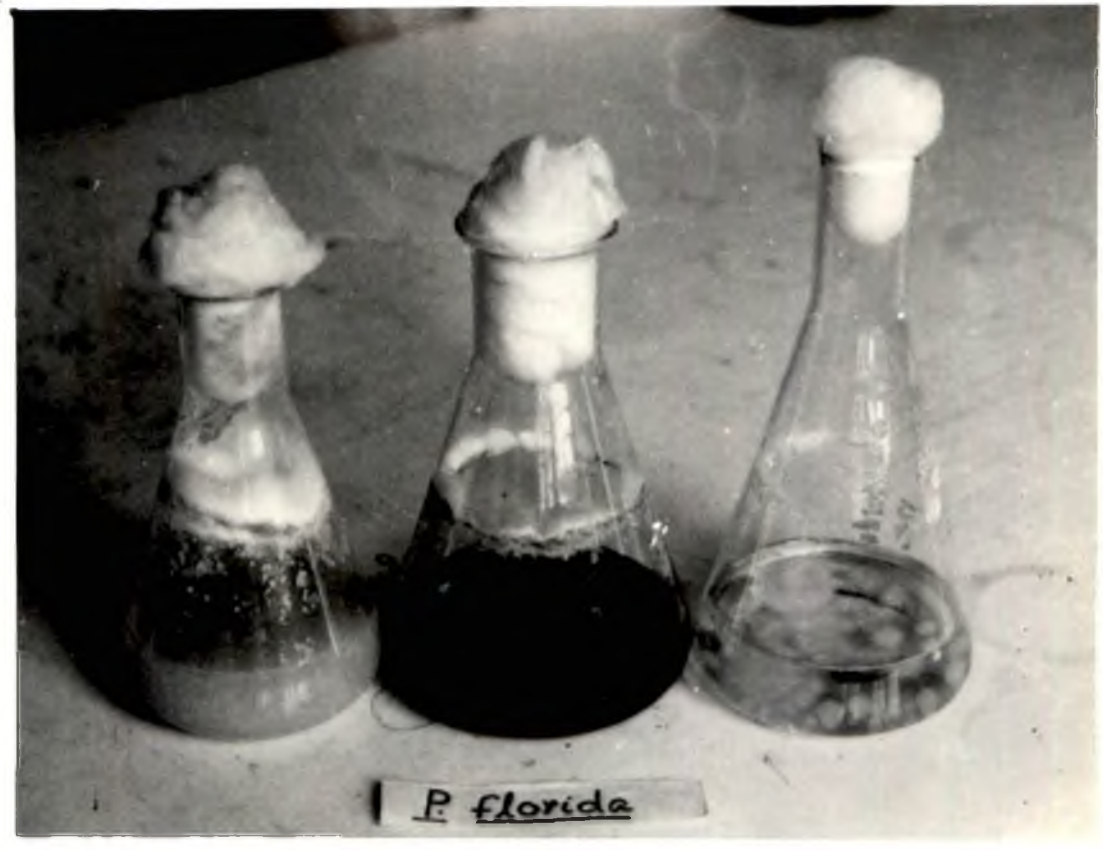
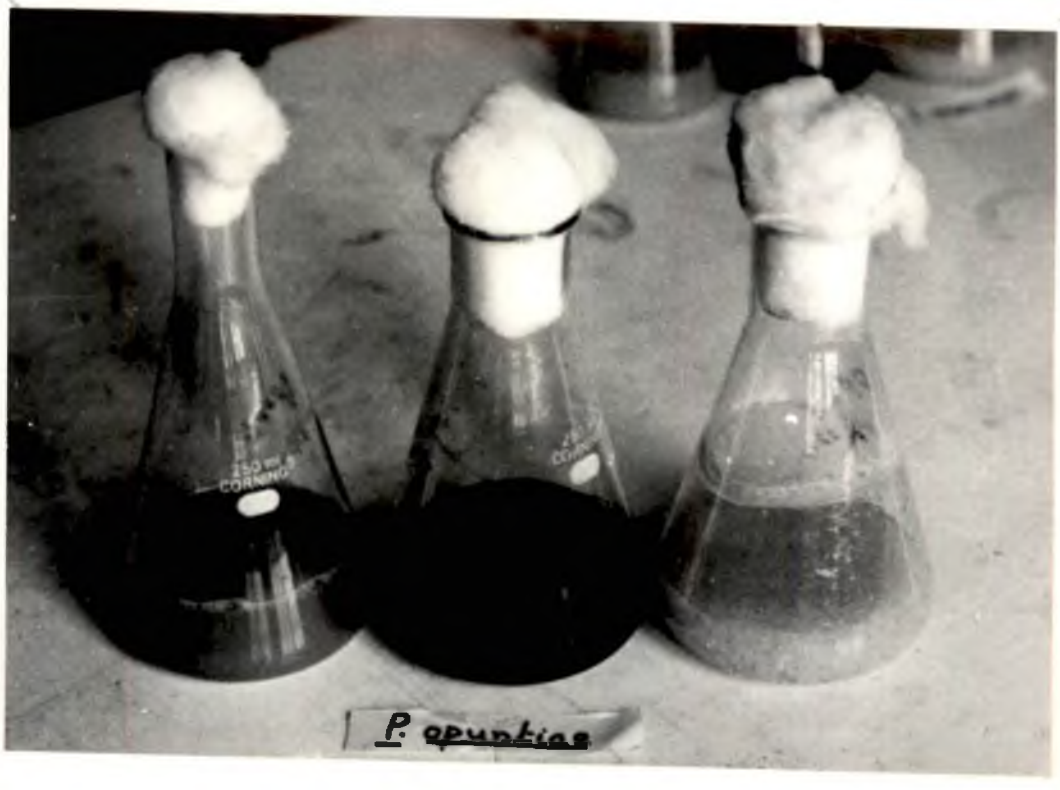


Plate XX. Growth of different species of Pleurotus in shake culture (Contd.)



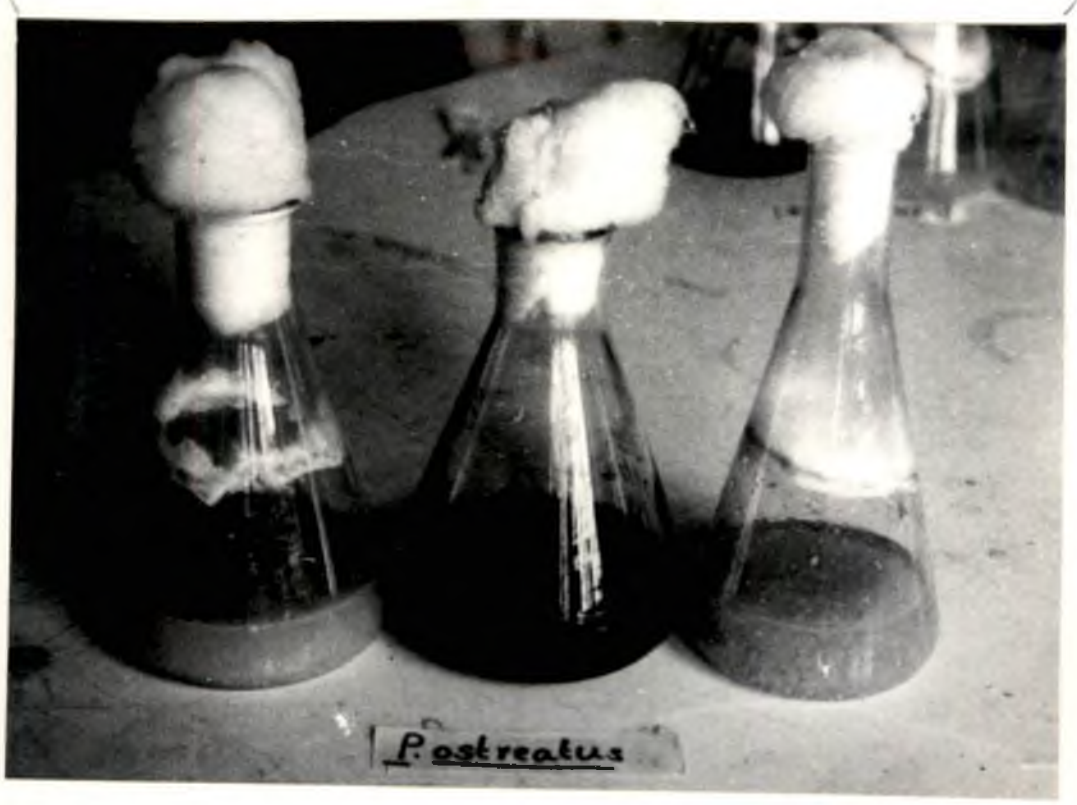


Plate XX. Growth of different species of Pleurotus in shake culture (Contd.)

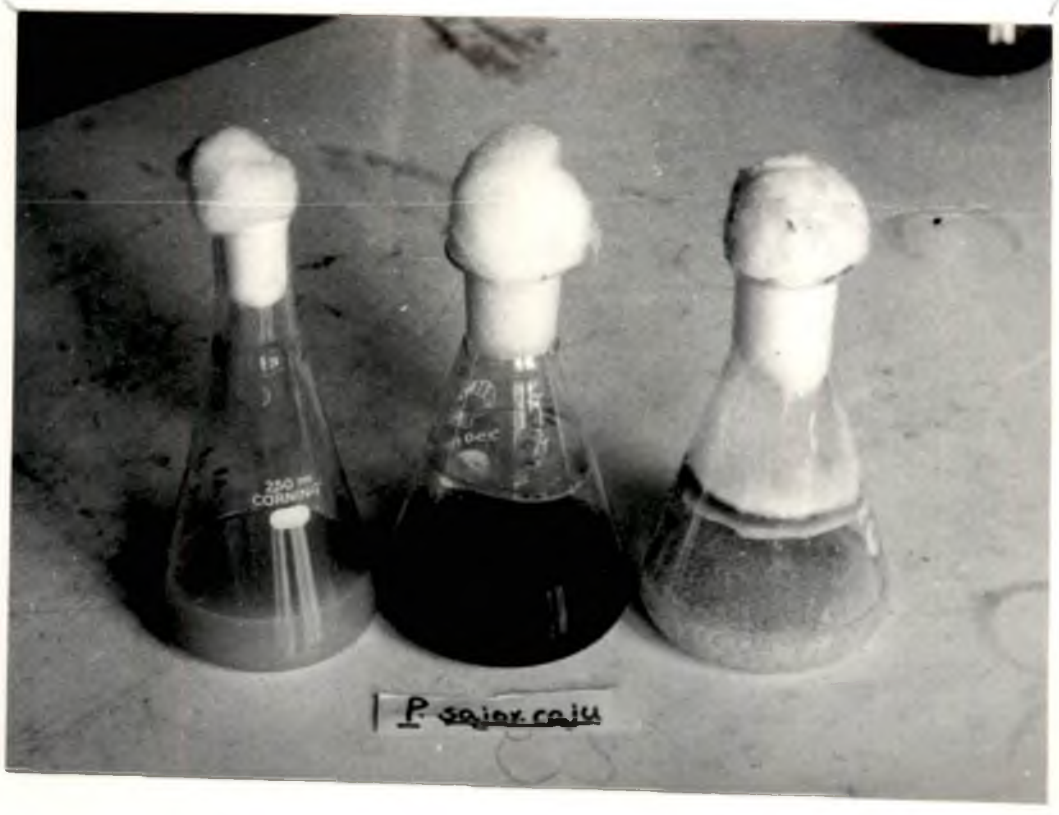
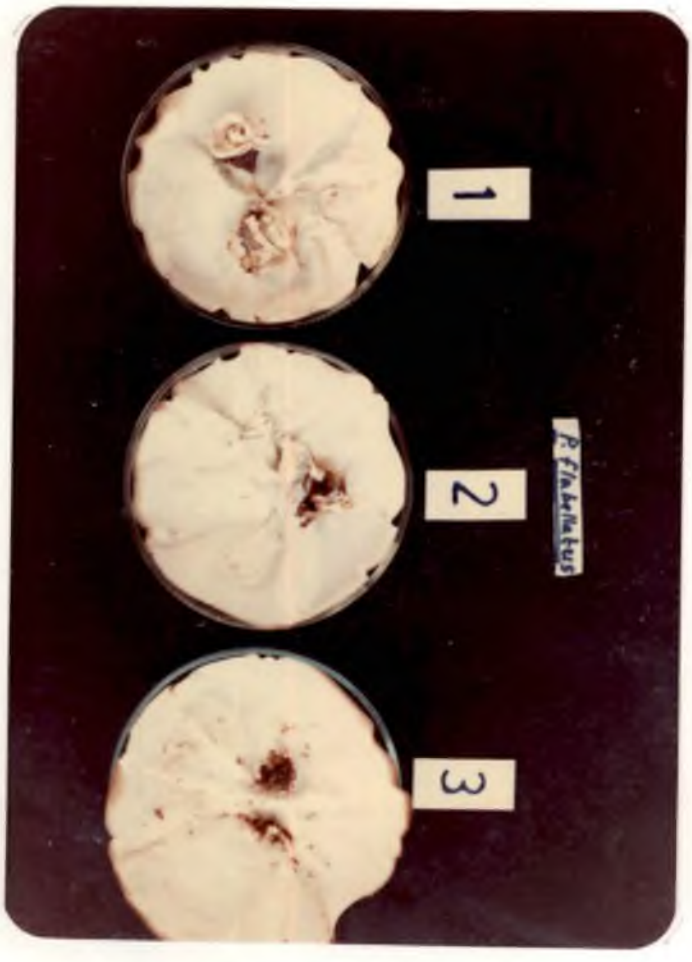


PLATE. XXI Mycelial dry wt. of different spp. of Pleurotus in shake culture

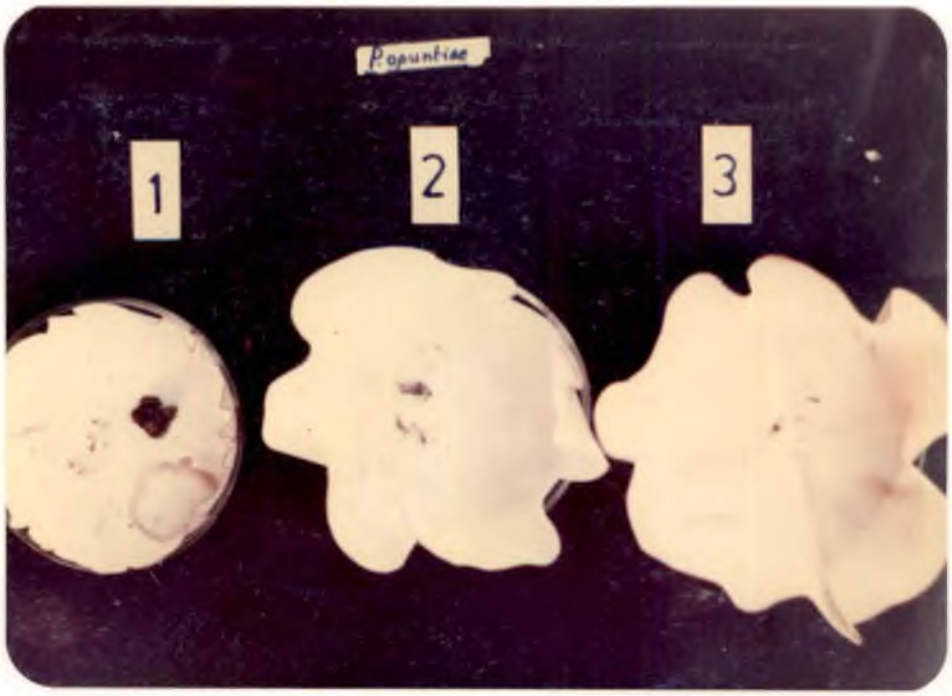
a. P. citrinopileatus



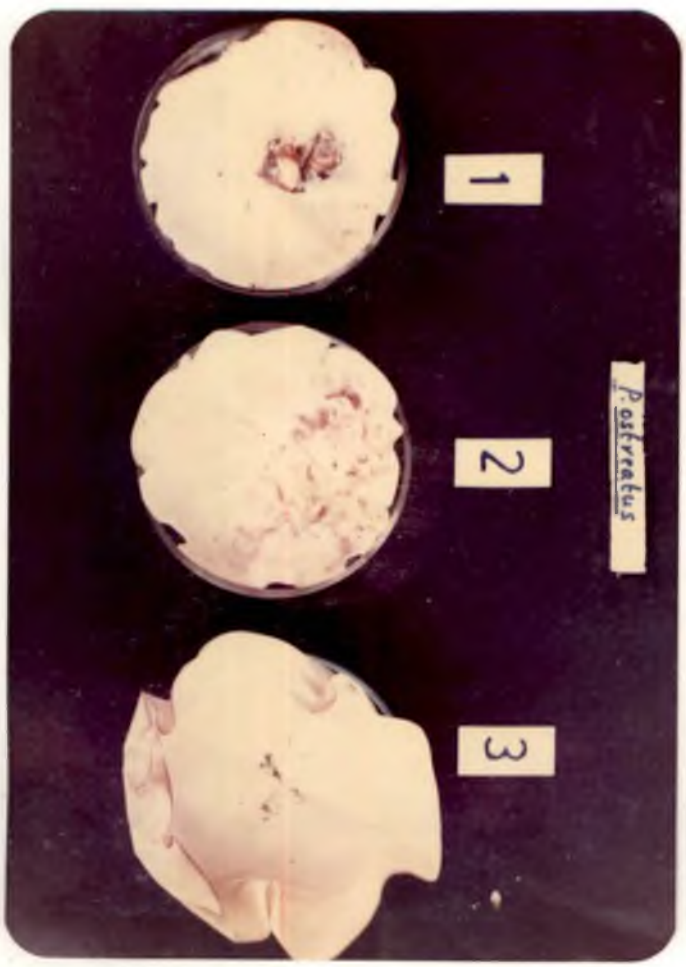
b. P. flabellatus

c. P. florida

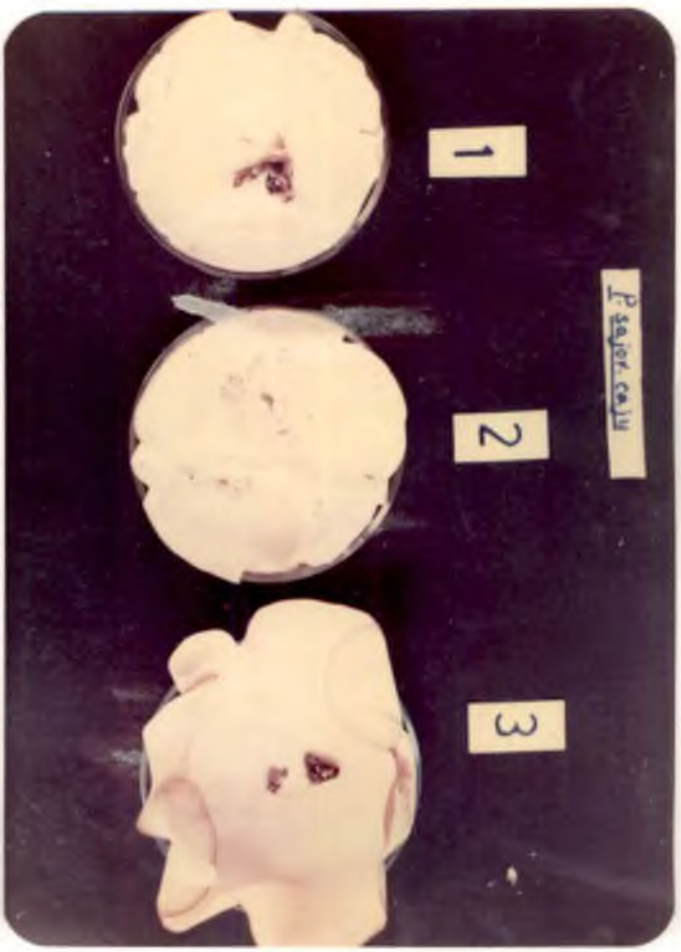
(CONT)



d. P. opuntiae



e. P. ostreatus



f. P. sajor-caju

incubated in a shaker. After a period of one month the pellets formed were filtered, dried and weighed. Maximum dry weight was recorded for P.flabellatus. The dry weight of P.florida and P.sajor-caju was not significantly different, but more than that of P.citrinopileatus, P.ostreatus and P.opuntiae. The dry weight of P.citrinopileatus was more than that of P.ostreatus and P.opuntiae. The potato dextrose broth supported maximum dry weight for all the species tested while it was less in Malt extract medium. For P.sajor-caju potato dextrose broth was the best medium. The dry weight of P.flabellatus was almost the same when grown in Oats broth and potato dextrose broth. Oats broth was the better media for P.ostreatus. Growth was found to be very poor in Malt extract media for P.opuntiae and P.citrinopileatus (Table 3, Plate 20 & 21).

Table 3. Growth of different species of Pleurotus in different media in shake culture (in g).

Media (M)	Species (S)	Pleurotus sajor-caju (S1)	P.florida (S2)	P.flabellatus (S3)	P.ostreatus (S4)	P.opuntiae (S5)	P.citrinopileatus (S6)	Mean
M1	Oats	1.98	1.70	2.98	1.95	1.49	1.89	1.998
M2	Potato dextrose	2.71	2.47	2.99	1.30	1.50	1.90	2.158
M3	Malt extract	1.86	2.47	2.50	0.74	0.78	0.76	1.521
	Mean	2.18	2.22	2.83	1.33	1.28	1.52	

C.D. for comparison of 'S' Means = 0.067
C.D. for comparison of 'M' Means = 0.048
C.D. for comparison of SxM Means = 0.117

Table 4. Influence of different nitrogen sources on the growth of *Pleurotus* spp. (weight in mg).

Sl No	Species	Ammonium nitrate (N1)	Peptone (N2)	Ammonium carbonate (N3)	Sodium nitrate (N4)	Ammonium chloride (N5)	Potassium nitrate (N6)	Control (N0)
1	*Pleurotus ostreatus* (S1)	0.2547	1.1166	0.8267	0.3107	0.226	0.4053	0.1250
2	*P.sajor-caju* (S2)	1.059	1.0807	0.4133	0.3423	0.2917	0.5293	0.1200
3	*P.flabellatus* (S3)	0.4067	1.145	0.15033	0.2023	0.255	0.527	0.2107
	Mean	1.1124	1.3903	0.2851	0.2575	0.4872	0.1519	

C.D. for comparison of 'N' means = 0.003257

C.D. for comparison of 'S' means = 0.002119

C.D. for comparison of NxS means = 0.005713

3. Influence of different nitrogen sources on the growth of *Pleurotus* spp.

Comparative growth of three different species of *Pleurotus* under different nitrogen sources are given in table 4.

The growth of the different species varied significantly in different nitrogen sources. *P.ostreatus* recorded the highest weight (11.5 mg) and *P.sajor-caju* the least (8.7 mg). Growth of various species in peptone showed a marked increase in weight compared to all other Nitrogen sources. Compared to control, significant higher growth was recorded for all the three species when grown in different Nitrogen sources. *P.ostreatus* recorded highest weight when grown in Ammonium nitrate, Sodium nitrate, Ammonium chloride and potassium nitrate, *P.sajor-caju* recorded highest weight when grown in Ammonium carbonate.

4. Influence of different carbon sources on the growth of *Pleurotus* spp.

All the three common species of *Pleurotus* utilised in the physiological studies were tested for their ability

to utilize different carbon sources. Growth was found to be more for P.major-caju than the other two species. However, growth was not found to be significantly different between P.major-caju and P.flabellatus and also between P.flabellatus and P.ostreatus. In all cases growth was significantly higher in all the carbon sources tested. Growth of the species was more when lactose was used as the carbon source but more or less similar results was obtained for Mannose also. Lactose was found to be superior to all other sources except Mannose which was found to be superior to Arabinose, Fructose, Xylose and glucose in growth response.

Significant interaction was found between the species grown in different carbon sources. Growth of P.major-caju was significantly low compared to P.ostreatus and P.flabellatus when sterile water was used. In lactose P.flabellatus performed well than P.ostreatus and P.major-caju for P.ostreatus xylose was found to be the best carbon source and for P.major-caju Arabinose was found to be the best carbon source. Inositol was also found to be better carbon source for P.major-caju. For P.ostreatus and P.major-caju Galactose and mannose were found to be better carbon sources (Table 5).

Table 5. Influence of different carbon sources on the growth of *Pleurotus* spp.

(weight in g)

Sl. No.	Species	Lactose (C1)	Xylose (C2)	Arabinose (C3)	Galactose (C4)	Mannose (C5)	Fructose (C6)	Inositol (C7)	Glucose (C8)	Control (C0)
1	*Pleurotus ostreatus* (S1)	0.5447	0.3250	0.4077	0.3653	0.3473	0.5770	0.4200	0.4093	0.2800
2	*P.major-caju* (S2)	0.4530	0.5047	0.4060	0.4757	0.5207	0.1430	0.4017	0.3750	0.2800
3	*P.flabellatus* (S3)	0.4683	0.3917	0.4773	0.4850	0.5257	0.5367	0.5427	0.1133	0.2083
	Mean	0.4887	0.4071	0.4303	0.4420	0.4629	0.4189	0.4548	0.2992	0.2561

C.D. for comparison of 'S' means = 0.016373

C.D. for comparison of 'C' means = 0.028373

C.D. for comparison of SxC means = 0.049144

Table 6. Effect of different hydrogen ion concentration on the growth of *Pleurotus* spp. (Weight in mg).

Sl. No.	Levels of pH	*P.ostreatus* (S1)	*P.sajor-caju* (S2)	*P.flabellatus* (S3)	Mean
1	4.5 (L_1)	33.20	28.43	28.60	30.08
2	5.0 (L_2)	43.73	41.90	35.90	43.84
3	5.5 (L_3)	80.40	80.13	86.70	82.41
4	6.0 (L_4)	68.17	60.67	74.10	67.64
5	6.5 (L_5)	50.90	33.20	57.30	37.13
6	7.0 (L_6)	37.77	30.10	38.47	35.44
	Mean	52.36	45.74	55.18	

C.D. for comparison of 'S' means = 0.04406

C.D. for comparison of 'L' means = 0.06232

C.D. for comparison of SxL means = 0.107947

Table 7. Effect of different temperatures on the dry weight of Pleurotus spp. after 2 weeks (weight recorded in milligrams)

Sl. No.	Temperature	P.ostreatus (S1)	P.sajor-caju (S2)	P.flabellatus (S3)	Mean
1	15°C (T_2)	33.43	30.50	30.10	31.34
2	20°C (T_2)	56.67 3	55.57 3	50.43 3	54.22 3
3	25°C (T_3)	72.87 1	78.90 1	82.57 2	78.11 1
4	30°C (T_4)	59.60 2	60.43 2	95.40 1	71.78 2
5	35°C (T_5)	23.13	19.07	28.07	23.42
6	Mean	49.14	48.89	57.31	

C.D. for comparison of 'S' means = 0.075794

C.D. for comparison of 'T' means = 0.097850

C.D. for comparison of SxT means = 0.169481

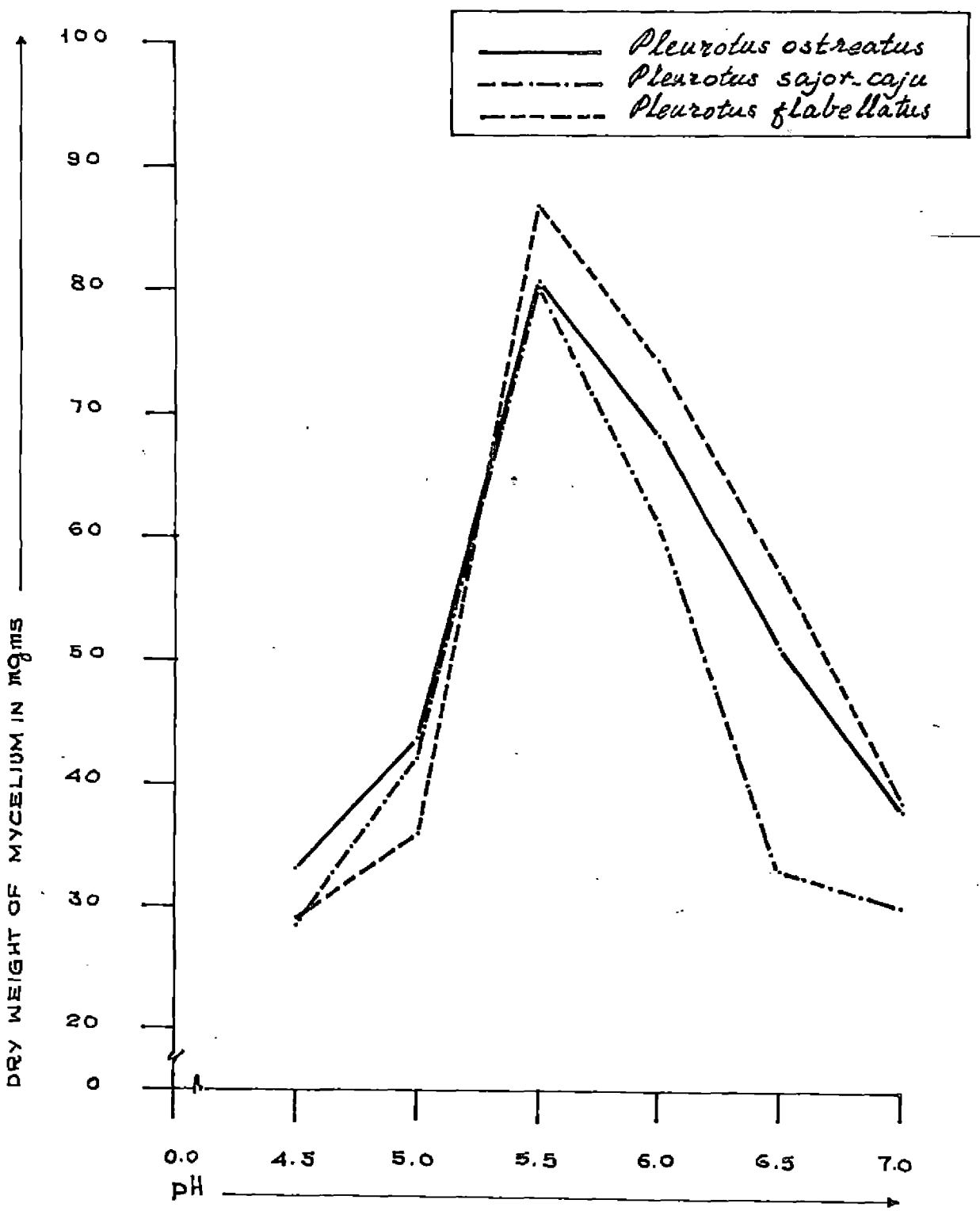
P.sajor-caju and P.flabellatus responded similarly in fructose and the growth of P.ostreatus was significantly low in fructose. When grown in xylose P.flabellatus and P.ostreatus gave better result than P.sajor-caju.

5. Effect of different hydrogen -ion concentration in the media on growth of Pleurotus spp.

The three common species of Pleurotus viz., P.sajor-caju, P.flabellatus and P.ostreatus were grown in various pH range starting from 3 to 7. No growth was observed in pH 3 to 4 and the growth rate in respect of other treatments are given in table 6.

Significant difference in dry weight was observed for the various species grown at different pH levels. When the average effect of pH levels are concerned maximum dry weight was observed at a pH of 5.5 (82.41 mg) and the minimum was recorded at a pH of 4.5 (30.08 mg). P.flabellatus when grown at a pH of 5.5 recorded the maximum weight of 86.7 mg and the minimum growth for P.sajor-caju grown at a pH of 4.5. At pH 4.5 all species recorded the lowest weight. Then a steady decrease in weight was observed from pH 6 to 7. Thus the

FIG. XI. EFFECT OF pH ON THE GROWTH OF *Pleurotus spp.*



optimum pH for all the three species tested was found to be 5.5 (Table 6; Fig.XI).

6. Effect of different temperature on the growth of various species of Pleurotus.

Three commonly cultivated species of Pleurotus viz., P.sajor-caju, P.flabellatus and P.ostreatus were inoculated in Potato dextrose broth and incubated under different temperatures ranging from 15°C to 35°C. The comparative growth was assayed by considering the final dry weight.

Among the three species grown, it was observed that there was a steady increase in dry weight with increase in temperature from 15°C to 25°C and then the growth declined in the case of P.ostreatus and P.sajor-caju. But in the case of P.flabellatus, maximum weight was recorded at 30°C and minimum at 35°C. Beyond 25°C, a rise in temperature recorded only decreased weight in the case of P.ostreatus and P.sajor-caju while in the case of P.flabellatus 30°C recorded maximum weight. The results showed that P.ostreatus and P.sajor-caju preferred an optimum temperature of 25°C and that in the case P.flabellatus it was 30°C (Table 7; Fig.XII).

FIG. XII. EFFECT OF DIFFERENT TEMPERATURES ON *Pleurotus spp.*

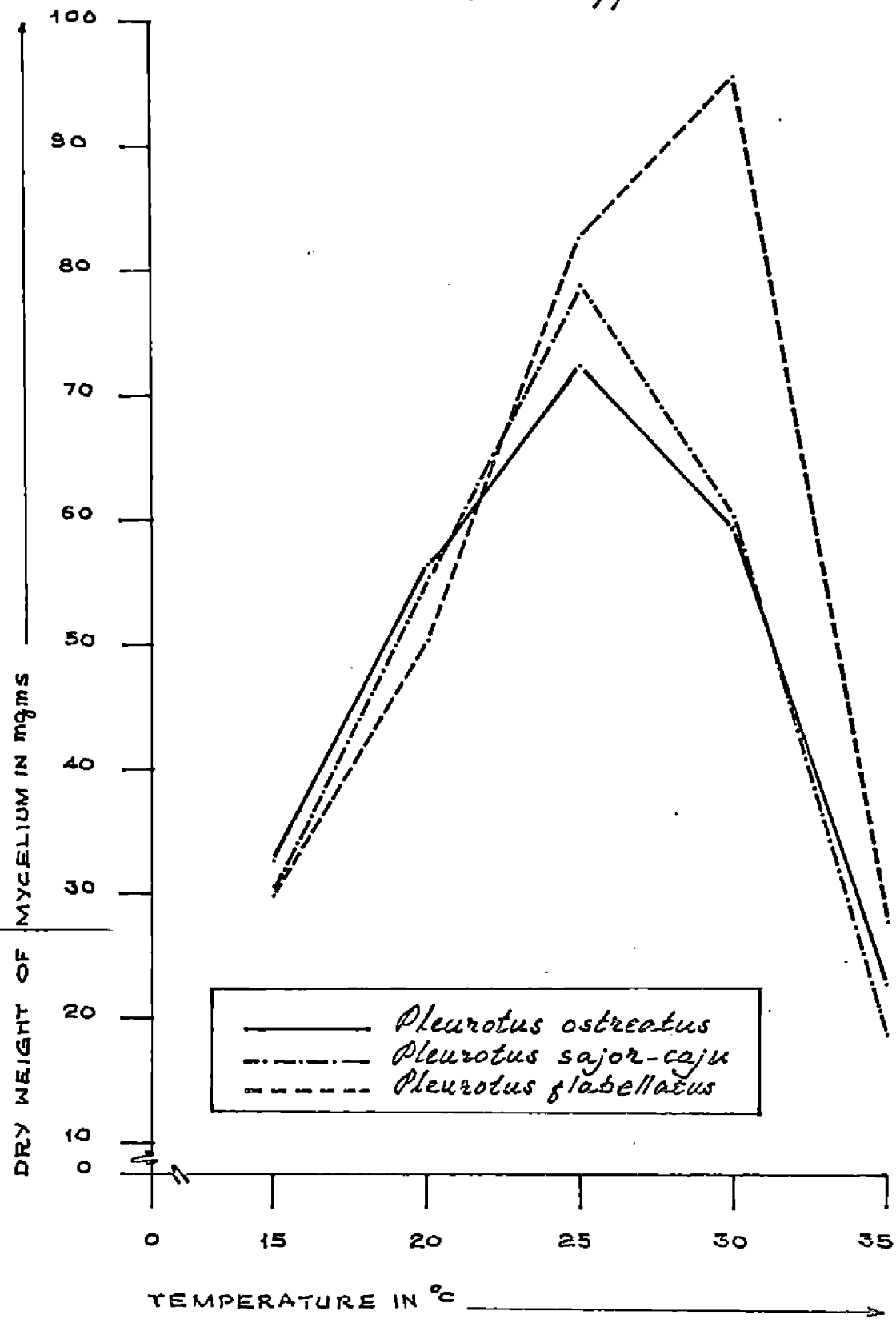


Table 8. Growth of various species of *Pleurotus* on wheat grains in spawn bottles at different intervals (Growth in cm after transformation).

Sl No	Days after inoculation	*P.sajor-caju* (S1)	*P.ostreatus* (S2)	*P.florida* (S3)	*P.opuntiae* (S4)	*P.citrino pileatus* (S5)	*P.flabellatus* (S6)	Mean
1	7 days(D_1)	2.83	2.96	1.81	2.89	2.74	2.80	2.67
2	14 days(D_2)	3.37	3.50	2.32	3.81	3.74	3.37	3.35
3	21 days(D_3)	3.87	3.87	2.32	3.87	3.87	3.77	3.60
	Mean	3.36	3.44	2.15	3.52	3.45	3.31	

C.D. for comparison of 'S' means = 0.055

C.D. for comparison of 'D' means = 0.039

C.D. for comparison of SxD means = 0.095

Table 9a. Growth of *Pleurotus sajor-caju* on grains of cereals and pulses as spawn substrates in different containers (Growth in cm).

Sl No	Containers	Sorghum (S1)	Ragi (S2)	Bajra (S3)	Maize (S4)	Wheat (S5)	Green gram (S6)	Red gram (S7)	Black gram (S8)	Horse gram (S9)	Bengal gram (S10)	Mean
1	Boost bottle (C1)	9.95	9.75	9.58	9.75	9.51	10.0	10.0	9.22	9.45	9.05	9.63
2	Horlicks bottle (C2)	9.25	9.50	9.65	9.90	9.43	9.50	8.90	7.55	8.25	7.85	8.97
3	Wine bottle (C3)	13.0	12.75	13.0	14.50	13.33	13.35	13.03	12.0	12.80	12.45	13.03
4	Milk bottle (C4)	7.03	6.90	6.93	7.15	6.28	6.70	6.40	6.02	6.17	6.40	6.60
	Mean	39.81	9.73	9.79	10.33	9.64	9.88	9.58	8.70	9.17	8.93	

C.D. for comparison of 'S' means = 0.5304
C.D. for comparison of 'C' means = 0.1335

B. PREPARATION OF SPAWN

1. Growth of various species of Pleurotus in spawn bottles on wheat grains as substrates.

Comparative growth of six different species of Pleurotus on wheat grain in spawn bottles during different intervals are given in (table 8).

Significant differences in growth was observed with respect to duration of incubation (Plate 22). Maximum growth was observed in Pleurotus opuntiae. Growth of P.opuntiae and P.ostreatus were on par with each other. Similarly growth of P.sajor-caju and P.flabellatus also was on par. But the growth of P.citrinopileatus and P.ostreatus were found to be superior to P.sajor-caju, P.flabellatus and P.florida. The growth of P.florida is significantly low compared to all other species. Maximum growth was observed in 21 days of incubation. It was observed that the growth of the culture in wheat grains increased with increase in the number of days except, P.florida where no change in growth was observed after the second week.

Plate XXII. Growth of various species of Pleurotus on Wheat grains.

Significant interaction was observed between species and time.

2. Growth of P.sajor-caju on grains of cereals and pulses as spawn substrates in different containers.

Grains of some of the common cereals and pulses were tested for their comparative efficacy to support growth of Pleurotus sajor-caju. The data presented in table 9a and 9b, Fig.XIII revealed that Maize was the best substrate than all others in supporting maximum growth of P.sajor-caju. This was followed by Sorghum, Bajra and Green gram. Black gram and Bengal gram were found to be the least effective substrate compared to all the other substrates except Horse gram.

Among the containers cylindrical wine bottle (750 ml) was found to be the best container and milk-bottle the least effective. Almost complete growth was achieved by the second week of incubation (table 9c). After the first week when the growth was measured, maximum was observed in wine bottle and minimum in milk-bottle but there was not much significant difference

FIG. XIII. GROWTH OF PLEUROTUS SAJOR-CAJU ON GRAINS OF CEREALS AND PULSES AS SPAWN SUBSTRATES IN DIFFERENT CONTAINERS

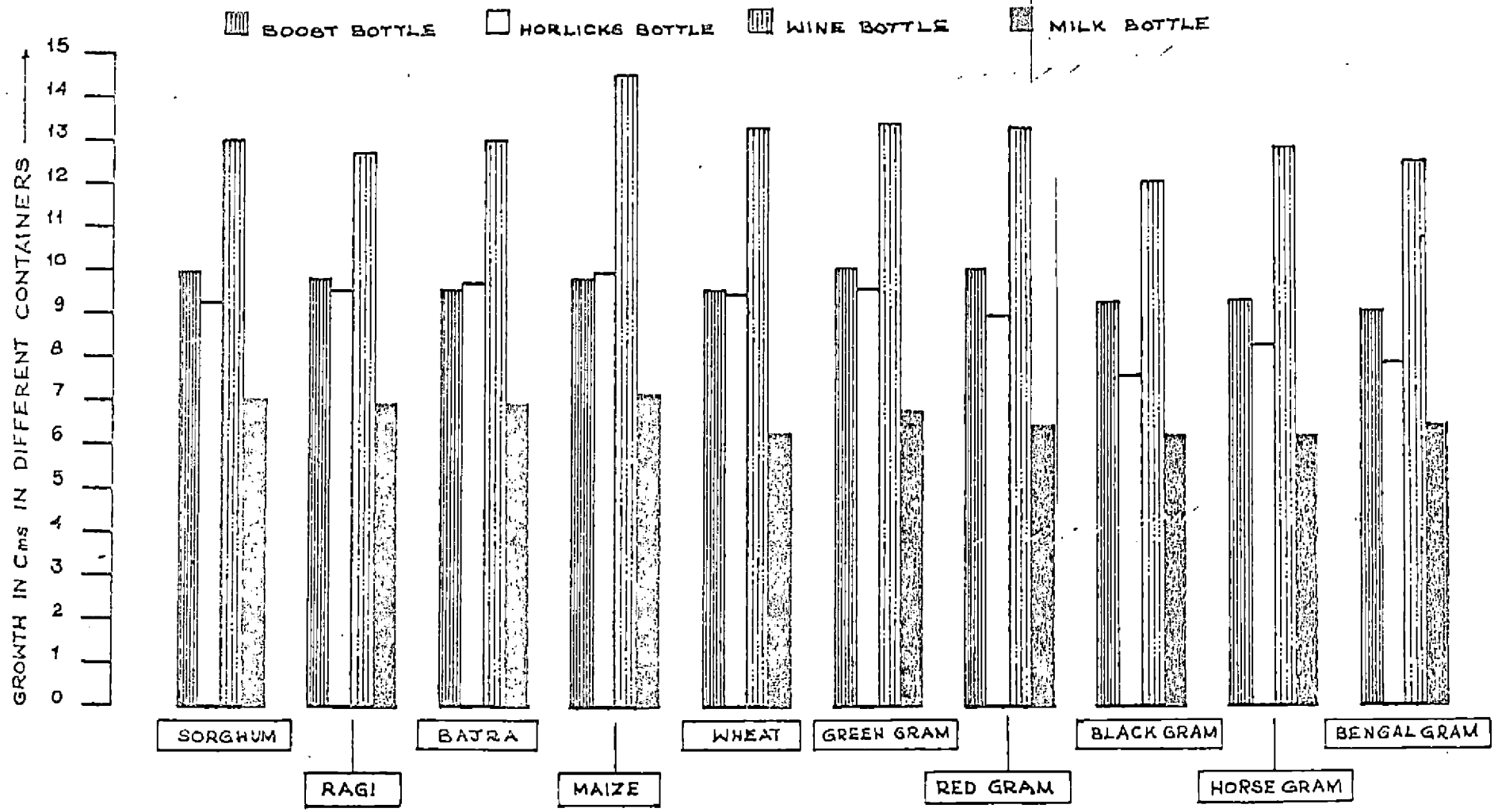


Table 9b. Growth of Pleurotus sajor-caju in grains of cereals and pulses (Growth in cm).

Sl No	Substrate	1st week	2nd week	Mean
1	Sorghum	6.25	13.36	9.81
2	Ragi	5.83	13.63	9.73
3	Bajra	6.31	13.28	9.79
4	Maize	6.65	14.00	10.33
5	Wheat	5.89	13.37	9.64
6	Green gram	6.27	13.51	9.86
7	Red gram	5.84	13.32	9.58
8	Black gram	4.93	12.47	8.70
9	Horse gram	5.21	13.13	9.17
10	Bengal gram	4.80	13.07	8.93
	Mean	5.80	13.31	

Table 9c. Growth of Pleurotus sajor-caju in different containers (Growth in cm).

Sl No		1st week	2nd week	Mean
1	Boost bottle	6.20	13.05	9.63
2	Horlicks bottle	6.10	11.85	8.97
3	Wine bottle	7.24	18.81	18.03
4	Milk bottle	3.66	9.54	6.60
	Mean	5.80	13.31	

C.D. for periods = 0.237
C.D. for PxC = 0.474

in growth in boost and Horlicks bottles but after second week boost bottle was found to be better than Horlicks bottle. In wine and milk bottle 160 per cent increase was observed after second week i.e., the growth was about more than two and a half times after second week in these bottles while in Boost and Horlicks bottle it was about twice.

3. Growth of Pleurotus sajor-caju in different oil cakes, and agricultural wastes as substrates in different containers.

Growth of Pleurotus sajor-caju on different substrates like oil cakes, rice bran and saw dust revealed that they differ in their ability to support the growth of Pleurotus sajor-caju. The data showed that the growth was high in wine bottle irrespective of the substrate except chaff, while growth was found to be better in Horlicks bottle filled with chaff. Growth was found to be poor in any of the substrates in Milk bottles.

Table 10a. Growth of Pleurotus sajor-caju in different Oil cakes and agricultural wastes as substrates in different containers during the first week (Growth in cm).

Sl No	Substrates	Boost bottle (C1)	Horlicks bottle (C2)	Wine bottle (C3)	Milk bottle (C4)	Mean
1	Coconut Oil cake (S1)	0.7071	1.1256	1.6930	0.6324	1.03945
2	Gingelly Oil cake (S2)	1.6632	1.5593	1.7793	1.3411	1.58575
3	Groundnut Oil cake (S3)	0.72536	0.91246	1.8348	0.5706	1.0108
4	Rice bran(S4)	0.8559	0.8159	1.3661	0.7047	0.93565
5	Saw dust (S5)	1.5915	1.5054	1.7979	0.5477	1.3606
6	Chaff (S6)	0.9821	1.6017	1.6831	0.9306	1.2994
	Mean	1.0875	1.2534	1.6924	0.7879	

C.D. for comparison of 'C' means = .0008604

C.D. for comparison of 'S' means = 0.0015818

C.D. for comparison of CxS means = 0.0126573

Table 10b. Growth of Pleurotus sajor-caju in different Oil cakes and agricultural wastes as substrates in different containers during the second week (Growth in cm).

Sl No	Substrate	Boost bottle (C1)	Horlicks bottle (C2)	Wine bottle (C3)	Milk bottle (C4)	Mean
1	Coconut Oil cake (S1)	2.7386	2.5754	2.8047	2.2063	2.5812
2	Gingelly Oil cake (S2)	2.7988	3.6469	2.8524	2.4012	2.9162
3	Groundnut Oil cake (S3)	2.1213	1.8965	2.7507	2.1984	2.2417
4	Rice bran(S4)	2.1050	1.9748	2.8751	2.1213	2.2690
5	Saw dust (S5)	1.5915	1.5054	1.7980	0.5477	1.3606
6	Chaff (S6)	3.2196	3.1198	3.7103	2.7863	3.2091
	Mean	2.4291	2.2977	2.7985	2.0434	

C.D. for comparison of 'C' means = 0.0010468

C.D. for comparison of 'S' means = 0.0019235

C.D. for comparison of CxS means = 0.0461731

Table 10c. Growth of Pleurotus sajor-caju in different Oil cakes and agricultural wastes as substrates in different containers during the third week (Growth in cm).

Sl No	Substrate	Boost bottle (C1)	Horlicks bottle (C2)	Wine bottle (C3)	Milk bottle (C4)	Mean
1	Coconut Oil cake (S1)	2.7386	2.5947	2.9381	2.2060	2.5860
2	Gingelly Oil cake (S2)	2.7988	2.7141	2.8577	2.4138	2.6961
3	Groundnut Oil cake (S3)	2.1678	1.8965	2.7598	2.2044	2.2571
4	Rice bran(S4)	2.1050	2.2710	2.8768	2.1446	2.3495
5	Saw dust (S5)	1.5915	1.5055	1.7980	0.5477	1.3607
6	Chaff (S6)	3.2197	3.1198	3.5770	2.7868	3.1758
	Mean	2.4369	2.3503	2.7790	2.0506	

C.D. for comparison of 'C' means = 0.0789495

C.D. for comparison of 'S' means = 0.0080576

C.D. for comparison of CxS means = 0.0055815

Table 11. Effect of temperature on the mycelial growth of *Pleurotus* in spawn bottle transformed means (x + 1).

Sl No	Substrate	Temperature of incubation in Celsius						
		10	15	20	25	30	35	Mean
1	Red gram	1.732	1.732	2.236	2.45	2.236	2.00	2.064
2	Horse gram	1.732	1.732	2.236	2.45	2.236	2.00	2.064
3	Bengal gram	1.821	2.00	2.236	2.45	2.236	2.00	2.124
4	Wheat grains	1.821	2.00	2.236	2.45	2.236	2.00	2.124
5	Green gram	1.732	1.732	2.236	2.45	2.236	2.00	2.064
6	Paddy straw	1.138	1.414	2.000	2.236	2.236	2.00	1.637
7	Salvinia	1.00	1.00	1.414	1.732	2.236	2.00	1.5611
		1.568	1.654	2.085	2.317	2.326	2.00	

C.D. for comparison between temperature = 0.0296
C.D. for comparison between substrate = 0.0320
C.D. for comparison between temperature x substrate = 0.0783

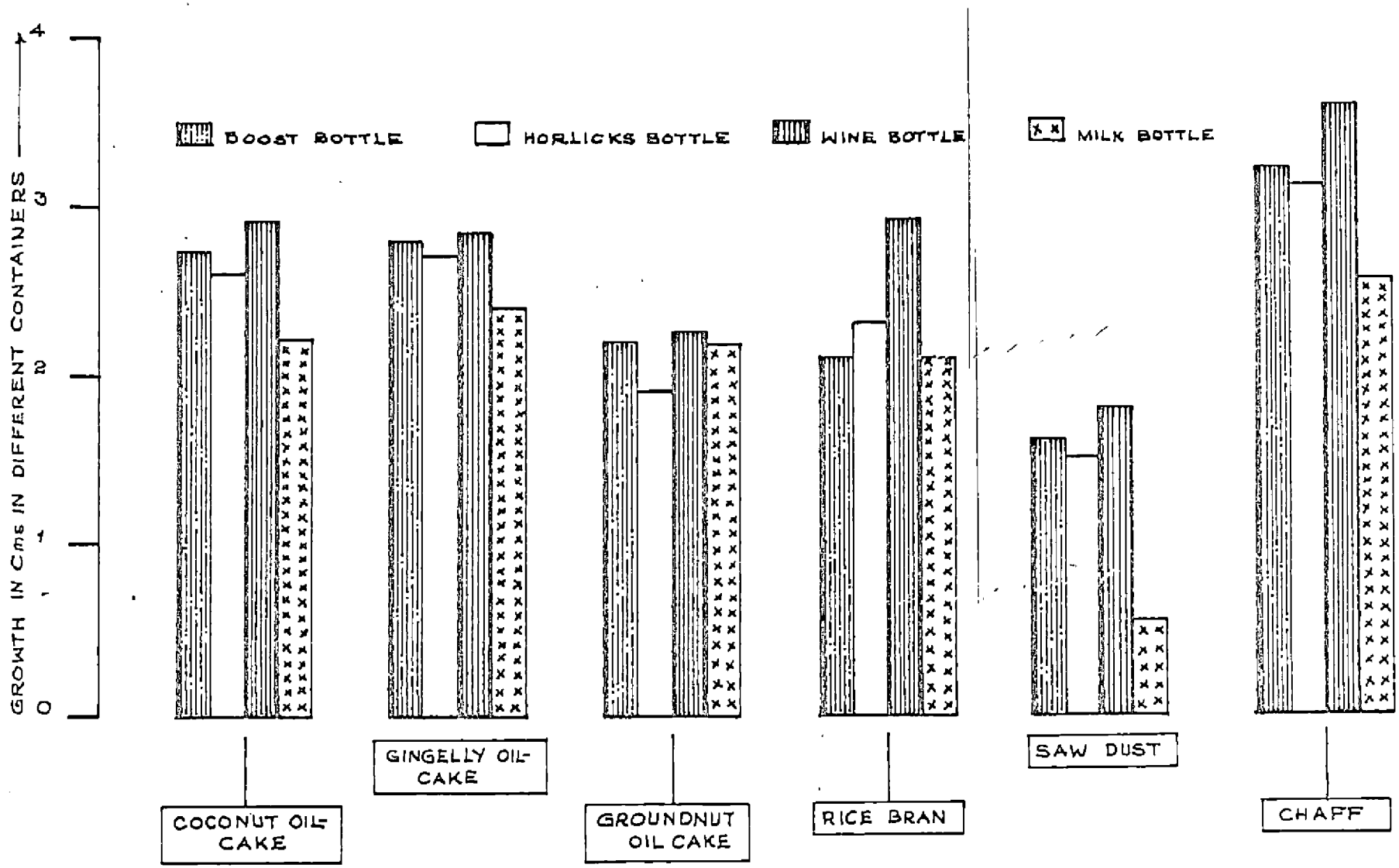
When gingelly oil cake was used, Horlicks bottle was found to perform less effective than growth in boost bottle and wine bottle, while no significant difference was observed for the growth in Boost bottle and Horlicks bottle filled with Rice bran. For all other oil cakes Horlicks bottle was found to be better than Boost bottle, though the average effect of Boost bottle was found to be better than Horlicks bottle.

In general, wine bottle is observed to be the best container and gingelly oil cake the best substrate (Table 10a, b, c & Fig. XIV).

4. Effect of temperature on the mycelial growth of Pleurotus sajor-caju in spawn bottles.

In order to find out the effect of temperatures on Pleurotus sajor-caju in spawn bottles containing various substrates like Red gram, Horse gram, Bengal gram, Wheat grain, Green gram, Paddy straw and Salvinia, an experiment was carried out by keeping spawn bottles at various temperature viz., 10°C, 15°C, 20°C, 25°C, 30°C and 35°C the results are presented in table 11.

FIG. XIV. GROWTH OF PLEUROTUS SAJOR-CAJU IN DIFFERENT OIL CAKES AND AGRICULTURAL WASTES AS SUBSTRATES



The growth of the fungus was on an average same in Red gram, Horse gram, and Green gram. In Bengal gram and Wheat grains maximum growth was observed and these two substrates were found to be the best for the growth of Pleurotus sajor-caju. The lowest growth (1.7 cm) was observed in Salvinia. Growth was found better in paddy chaff ~~straw~~ than Salvinia.

The fungus was found to grow well at a temperature of 25°C and then growth was stunted. The fungus responded differentially ~~with~~ in each substrate at a given temperature. The growth was similar in Red gram, Horse gram and Green gram at all temperature. In Horse gram and Bengal gram there was significant growth even at 15°C. It did not grow at the lowest temperature of 10°C in paddy straw and Salvinia. Its growth started only at 20°C in Salvinia and continues to grow till 30°C. Maximum growth was recorded at 25°C in Red gram, Horse gram, Bengal gram, Wheat grain and Green gram.

Plate XXIII. Suitability of different species of Pleurotus for cultivation in Kerala.

Plate XXIII. Suitability of different species of Pleurotus for cultivation in Kerala(Contd.)

PLATE . XXIII . e *P. florida*

PLATE . XXIII . f. *P. ostreatus*

C. CULTIVATION OF *PLEUROTUS* Spp

1. Suitability of different species of *Pleurotus* for cultivation in Kerala.

Six common species of *Pleurotus* viz., *P.sajor-caju* *P.ostreatus*, *P.florida*, *P.opuntiae*, *P.citrinopileatus* and *P.flabellatus* were used in this study. Polythene bags with bits as well as with small twists of straw was spawned as detailed under materials and methods. The yield recorded under each species is given in table 12.

P.ostreatus did not grow well in this trial, probably because of the unfavourable climatic condition of 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* (Plate 23). Use of paddy straw as twists was found to be better than use of bits.

Species grown in twists gave an average yield of 600 g compared to 422 g in bits. Significant interaction was observed between the type of paddy straw (twists or bits) and species. For all the species yield was found to be better in twist except P.flabellatus. In P.flabellatus no significant difference in yield was observed in bits and twists. When grown in bits the yield was 331 g while in twist it was 363 g, which were on par. For P.florida though the yield was comparatively less, it was more than double in twists (359 g) as compared to bits (171 g) (Table 22 Fig.XV).

Table 12. 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.flo-rida	P.opun-tiae	P.citrino pileatus	P.flabe-llatus	Mean
			(yield 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 bits and twists = 27.14
C.D. for comparison of species = 42.91
C.D. for comparison of straw type x species = 60.69

FIG. XV. EFFECT OF USING PADDY STRAW AS BITS AND SMALL TWISTS IN SUPPORTING SPOROCAP FORMATION IN *Pleurotus spp.*

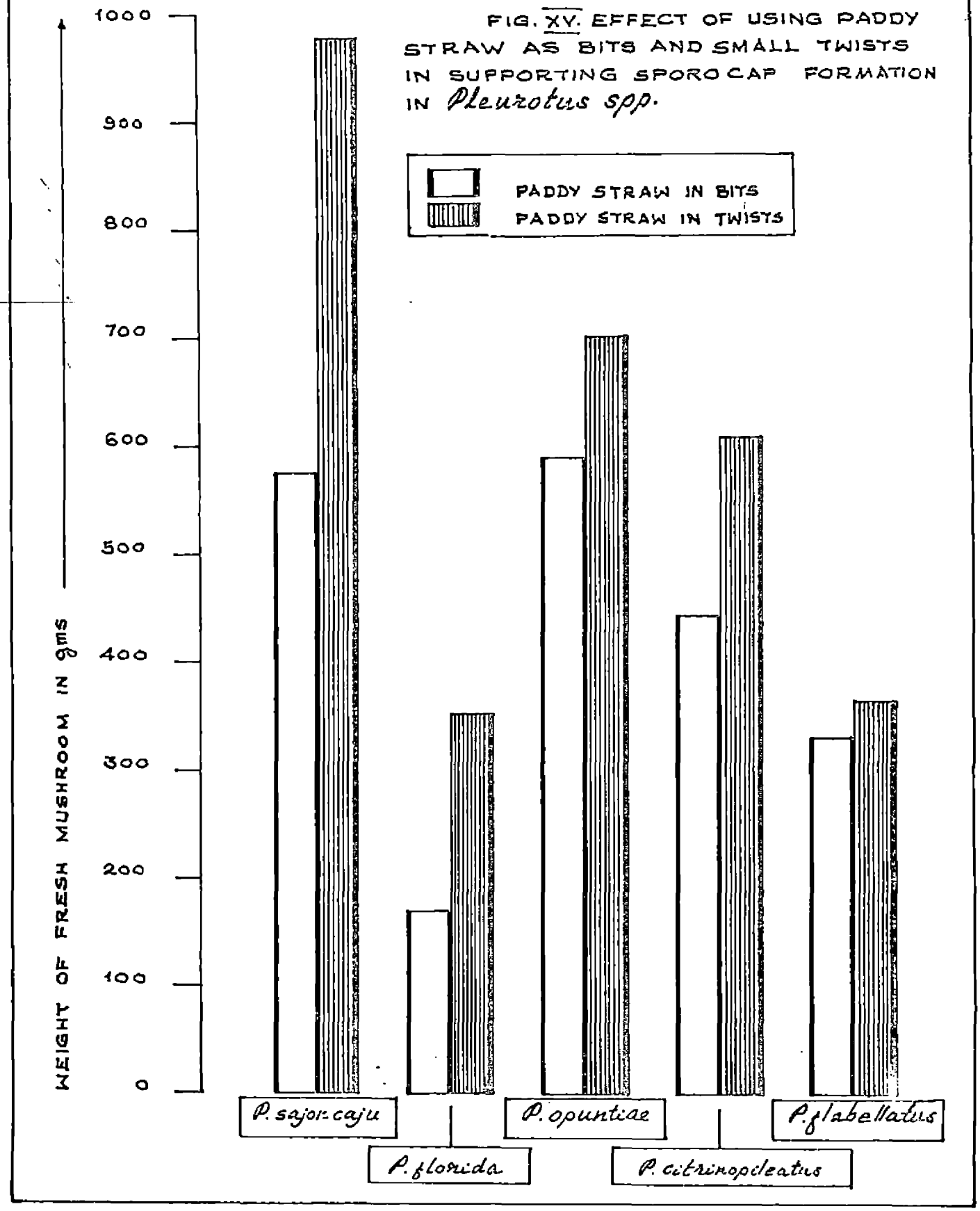


PLATE . XXIV . Influence of growth regulators on yield of P. Sajor-caju

2. Influence of growth regulators in enhancing sporocarp formation in Pleurotus spp.

Six species of Pleurotus viz., P.sajor-caju, P.florida, P.ostreatus, P.flabellatus, P.opuntiae and P.citrinopileatus were raised following polythene bag method. After the spawn run when bags were opened, the growth regulators were sprayed as detailed under Materials and Methods. (to be included).

It was observed that of the various growth regulators used, Indole butyric acid 50 ppm was found to be most effective one in increasing the yield (1275 g), followed by 2, 4-D 100 ppm (1045 g), IAA 100 ppm (730 g), IBA 100 ppm (670 g), IAA 50 ppm (515 g), and 2, 4-D 50 ppm (310 g) (Table 13); (Plate 24).

Sporocarp development was found arrested in the case of all other species tried.

Table 13. Influence of growth regulators on yield of P.sajor-caju

Sl No	Growth regulators and concentrations tested	Yield in g/kg of substrate
1	N.A.A 50 ppm	0
2	N.A.A. 100 ppm	0
3	IAA 50 ppm	515
4	IAA 100 ppm	730
5	IBA 50 ppm	1275
6	IBA 100 ppm	670
7	2,4-D 50 ppm	310
8	2,4-D 100 ppm	1045
9.	Control	775

D. STANDARDISATION OF TECHNIQUES FOR CULTIVATION OF PLEUROTUS SAJOR-CAJU

1. Comparative efficacy of different types of spawn on yield of P.sajor-caju.

The spawn produced on each of the 6 different cereals viz., Sorghum, Bajra, Ragi, Maize, Paddy and Wheat and five Pulses, viz., Green gram, Red gram, Bengal gram, Horse gram and Black gram was used to spawn the standard beds of paddy straw. 21 days old spawn was used to layout the conventional straw beds. The average yield obtained for various cereal grains spawns are presented in table 16 and those with pulses in table 17. Among the cereals it was found that Maize spawn was superior to all the rest followed by Bajra, Ragi, Sorghum, Wheat and Paddy. Maximum yield of mushrooms was recorded from beds spawned with maize spawn (585.74 g) followed by Bajra (565 g) Ragi (553 g) Sorghum (548.54 g) wheat (336.98 g) and paddy (335.24 g). The yield of sporocarps obtained from beds laid out with paddy spawn was very low (Table 14).

Table 14. Average yield of sporocarp of *P.sajor-caju* from beds laid with spawn on grains of different cereals.

Sl No	Type of spawn	Mean yield in g/kg of substrate
1	Sorghum	548.54
2	Ragi	553.00
3	Bajra	565.00
4	Maize	585.74
5	Paddy	335.24
6	Wheat	336.98

C.D. = 3.6548

Spawn raised in various pulses viz., Green gram, Bengal gram, Red gram, Horse gram and Black gram also varied in their effect on sporocarp production. It was found that spawn prepared with Horse gram gave maximum yield compared to others. This was followed by Green gram, Bengal gram, Red gram, and Black gram in the order of their efficiency. Black gram spawn was found to be the least effective one in yield response (Table 15).

Table 15. Average yield of sporocarp of P.sajor-caju from beds laid out with different pulse spawn.

Sl No	Type of spawn	Mean yield (g/kg of substrate)
1	Green gram	531.10
2	Red gram	375.33
3	Bengal gram	473.08
4	Horse gram	536.41
5	Black gram	321.76

C.D. = 1.2603

2. Effect of various organic amendments on the yield response of Pleurotus sajor-caju.

To find out the influence of different organic amendments on the yield of Pleurotus sajor-caju experiments were laid out with different organic amendments and the data obtained are given in table 16. 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 flour was observed to be superior to wheat flour, Hoarse gram, Cowdung slurry and control. Wheat flour was better than Horse gram flour, Cowdung slurry and control. Cowdung slurry was found least effective in yield response.

Table 16. 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

3. Cultivation on logs.

Cultivation of Pleurotus sajor-caju was tried in different logs of common trees. Fifteen trees,

namely Mango (Mangifera indica), Coconut (Cocos nucifer), Cashew (Anacardium occidentale), Anjili (Artocarpus hirsuta), Albizzia (Albizzia lebbec) Caesalpinia (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. The logs were uniformly spawned as described under materials and methods. 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 (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 (Table 17). It is interesting to note 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 17. Average yield of *P.sajor-caju* Sporocarps from logs of different trees.

Sl No	Name of plant	Yield in g/log
1	Mango (*Mangifera indica*)	389.0
2	Coconut (*Cocos nucifera*)	169.7
3	Cashew (*Anacardium occidentale*)	269.7
4	Anjili (*Artocarpus hirsuta*)	106.0
5	Albizzia (*Albizzia lebbec*)	37.7
6	Caesalpinia (*Caesalpinia pulcherima*)	14.0
7	Jamba (*Eugenia jambolana*)	25.3
8	Avacado (*Persea americana*)	29.0
9	Rose apple (*Eugenia jambosa*)	0.0
10	Litchi (*Litchi chinensis*)	30.3
11	Jack (*Artocarpus heterophyllus*)	17.3
12	Erythrina (*Erythrina indica*)	0
13	Ciba cotton (*Ceiba pentandra*)	11.7
14	Raintree (*Enterolobium saman*)	0
15	Eucalyptus (*Eucalyptus citriodora*)	0
16	Banana pseudostem (*Musa paradisiaca*)	236.3

4. Influence of different types of bed for production of sporocarps of P.sajor-caju.

Various methods of cultivation of P.sajor-caju with paddy straw as substrate were tested. They were as detailed under materials and methods, polythene wrapper bed method, cylindrical bed method, tray method and tier method. The first two cultivation methods were found to be better than the other two methods and no significant difference in yield was observed with these two methods (Table 18).

Table 18. Influence of different types of bed for production of sporocarps of Pleurotus sajor-caju.

Sl No	Type of bed	Average yield g/kg of substrate
1	Polythene wrapper bed	461.7
2	Cylindrical bed	451.7
3	Tray method	320.0
4	Tier method	268.3

C.D. = 14.89

5. Influence of size of polythene bags on yield of P.sajor-caju.

Six different sizes of polythene bag were tested for their comparative effect on production of sporocarps of Pleurotus sajor-caju. The quantity of paddy straw used varied from 0.25 to 1.5 kg in the different sizes of polythene bags viz., 15 x 25 cm, 25 x 35 cm, 35 x 45 cm, 45 x 55 cm, 55 x 60 cm and 55 x 65 cm. It was found that polythene bag of size 35 x 45 (cm) recorded highest yield and was equally effective with polythene bag of size 25 x 35 cm and 45 x 55 cm. Polythene bag of size 55 x 60 cm was significantly superior to sizes 55 x 65 and 15 x25 in their yield performance Table 19.

Table 19. Influence of size of polythene bag on yield of P.sajor-caju.

Sl No	Size of polythene bag in cm	Average yield in g/kg of substrate
1	15 x 25	194.43
2	25 x 35	396.67
3	35 x 45	442.22
4	45 x 55	398.33
5	55 x 60	336.00
6	55 x 65	268.89

C.D. = 58.75

Plate XXV. Poly bag method

6. Comparative efficacy of different containers for lay out of beds by Pleurotus sajor-caju.

Four different containers was used to lay out beds. They were Bamboo basket Earthen pot, wooden tray (60x40x15 cm) and polythene bag of 60 cm x 30 cm.

Polythene bag filled with 1 kg straw was found to be the best container for this mushroom production, which gave an average yield of 426 g of mushroom (Plate 25). In the other three methods growth was found to be poor. The least effective one was wooden tray. Earthen pots and bamboo baskets were found to be better than wooden tray for production of mushroom (Table 20).

Table 20. Comparative efficacy of different containers for lay out of beds by Pleurotus sajor-caju.

Sl No	Type of containers	Average yield in g/kg of substrate
1	Bamboo basket	130.33
2	Earthen pot	115.00
3	Wooden tray	93.00
4	Polythene bag	426.33

C.D. = 6.15

7. Comparative efficacy of different types of straw beds on sporocarp production by P.sajor-caju.

Four different methods of beds viz., rectangular beds with bundles of paddy straw, solid rectangular beds with twists of straw, Rectangular beds with twists of straw were tried to see whether they can support sporocarp formation. None of the methods was found to be effective. Out of this, rectengular beds with twists of straw gave negligible yield (Table 21).

Table 21. Comparative efficiency of different types of straw beds in sporocarp formation by P.sajor-caju.

Sl No	Type of bed	Average yield in g/kg of substrate
1	Rectangular beds with bundles of paddy straw	17.67
2	Rectangular beds with twist of paddy straw	28.67
3	Rectangular beds using loose straw	0.0
4	Hollow round beds with twist of straw	0.0

C.D. = 6.1474

8. Comparative efficacy of straw of different varieties of rice in supporting sporocarp formation.

The comparative efficacy of the straw from five different common varieties of rice in supporting sporocarp formation was tested and the data presented in table 22.

Straw from the variety Kochuvithu recorded maximum yield. The minimum yield was recorded from Jyothi which was on par with that of Jaya and Thriveni.

Table 22.

Sl No.	Variety of rice	Fresh weight of sporocarps g/kg of substrate
1	Thriveni	298.63
2	Jyothi	297.25
3	Jaya	311.00
4	T_9	368.00
5	Kochuvithu	376.25

C.D. = 8.99

9. Effect of frequency of watering the mushroom beds on sporocarp production in *Pleurotus sajor-caju*.

Maximum yield was observed when the watering was done at an interval of 24 h. Watering at 48 hours of interval was found to be better than 12 hours. In the control (no watering) only 97 g of mushroom was recorded which was very poor compared to others.

The relationship between the interval of watering and the yield of mushroom was found to follow a quadratic nature of response as given below:

$\hat{y} = 100.21 + 66.256 - 1.23\ t^2$ when t stand for the interval of watering. It was found that watering at an interval of 27 hrs will give optimum yield (Table 23).

Table 23. Effect of frequency of watering the mushroom beds on sporocarp production in *Pleurotus sajor-caju*.

Sl No	Frequency of watering	Mean yield (g/kg of substrate)
1	Watering at 12 hrs interval	731.25
2	Watering at 24 hrs interval	968.75
3	Watering at 48 hrs interval	896.75
4	No watering	97.00

C.D. = 55.24

10. Seasonal variation in yield of P.major-caju.

To see the effect of seasonal variations on yield of Pleurotus, beds were laid during all months of the year. The climatological parameters as described in materials and methods were also recorded. The data presented in table 24; ~~Fig. XVI~~. The correlation between yield and the climatological factors x1, x2, x3, x4 and x5 were worked out and are given below:

r(y, x1) = -.2125
r(y, x2) = -.3411
r(y, x3) = -.378
r(y, x4) = -.3027
r(y, x5) = -.3103

Maximum yield was recorded during January, February and March and the lowest yield recorded during May. But there was not much reduction in yield between different periods of the year.

Table 24. Yield of sporocarps of *P.sajor-caju* recorded during different periods of the year.

Month		Average yield in g/kg of substrate	Room temperature		R.H.	
			Maximum	Minimum	F.N.	A.N.
January	1986	1000 g	31.9	20.3	71.55	59
February	1986	1000 g	32.5	21.4	72.0	68
March	1986	1000 g	33.8	24.2	81.0	72
April	1986	850 g	33.9	23.4	87	60
May	1986	800 g	34.5	22.8	85	63
June	1986	1000 g	33.1	22.3	89	73
July	1986	950 g	32.1	22.7	86	70
August	1986	900 g	30.2	22.5	80	71
September	1986	900 g	31.3	21.6	85	65
October	1986	900 g	31.6	24.2	80	69
November	1986	950 g	30.5	21.0	89.3	65
December	1986	950 g	31.5	21.7	78.7	58.3

No significant correlation was observed between yield and climatological parameters at 5% level of significance. How ever, the temperature, relative humidity at forenoon and rainfall were found to have a negative effect on yield. Relative humidity at afternoon was found to have a positive relationship. The yield and climatological parameters were related by the multiple linear regression equation as given below:

$y = 1280\ \ 1050 - 4\ 4.8537\ x\ 1 - 29.5688\ x\ 2 - 1.9389\ x3 + 10.0400\ x4 - 8.9621\ x5$ when x1, x2, x3, x4 and x5 stands for the climatological parameters in the given order.

x1 = Maximum temperature

x2 = Minimum temperature

x3 = R.H. (F.N)

x4 = R.H. (A.N)

x5 = Rain fall

x6 = Yield

The above parameters were found to explain the variation in yield which was explained by the above Mean linear regression equation (MLR equation).

E. NUTRITIVE VALUE OF DIFFERENT SPECIES OF *PLEUROTUS*

The proximate composition of major nutrients in six common *Pleurotus* species were analysed in comparison with *Volvariella volvacea* and are given in table 25. 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 proximate constituent viz., protein, calcium, Magnesium, Iron, Ash, Vit A and Vit C are more in all the six species of *Pleurotus* tested.

F. PRESERVATION

1. Refrigeration.

Visual observation of fresh sporocarps of *Pleurotus sajor-caju* kept under refrigeration revealed that samples by refrigeration, the mushroom started deteriorating with the accumulation of moisture in plastic bags and a liquid started oozing from the mushroom which made them unfit for consumption. Those kept in open polythene bags of 500 guage

thickness remained fresh for five days. But when 150 guage polythene bags were used it remained fresh for three days only. But those mushroom covered in 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.

2. Dehydration.

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.

Table 25. 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 %	10.725	15.4688	3.5063	2.8575	12.850	6.600	8.7118
Caloium mg/100 g	6.400	8.18	7.43	7.28	8.41	6.02	10.04
Magnesium mg/100g	1.041	1.82	1.281	2.49	1.110	1.58	0.962
Iron mg/100 g	1.32	1.82	1.925	2.34	2.851	3.11	2.904
Ash g/100g	2.67	3.14	1.988	2.94	3.12	2.16	3.04
Vit A mg/100 g	2.017	502.80	427.3	321.8	262.50	167.80	397.50
Vit C mg/100 g	4.86	163.60	142.71	11.43	6.80	8.70	121.30

DISCUSSION

DISCUSSION

The *in vitro* physiological studies with few species of *Pleurotus* revealed that they are adopted to a wide variety of environmental conditions. Effect of light and darkness on the mycelial growth of three species of *Pleurotus* showed best growth in Oats agar media under darkness. Under this condition even pin heads appeared in the culture media. Growth was found to be less when exposed to light irrespective of the media. This shows that light is not essential during early stages of mycelial growth. These results are in confirmity with the findings of Qadir and Khatoon (1983) who found that light had an inhibitory effect on the mycelial growth of *Pleurotus* spp. Puri *et al.* (1981) observed that light is an essential factor in the development of fruit bodies in *Pleurotus fossulatus*. Buller (1915) clearly demonstrated the marked differences in fruit body development in *Coprinus sterquilinus* under exposure to light and those under darkness. He had also reported that light inhibits the development of the tapering pseudorhizal stipe in *Coprinus sterquilinus*.

Growth of six species of Pleurotus viz., P.opuntiae, P.ostreatus, P.florida, P.flabellatus, P.sajor-caju and P.citrinopileatus in various liquid media in shake culture resulted in the formation of hyphal pellets which revealed that interuption or continuous rotation will result in active growth of the culture of Pleurotus. Kurtzman (1978) observed the formation of fruit bodies of P.sapidus in flasks kept stationary after shaking for 30 days. Solomon (1981) remarked that the fermenters used for mushroom culture have employed slow speed agitation, a feature which in itself tends to favour pellet formation. Agitation besides simply suspending particles also transfers oxygen from the supplied air stream into the culture medium.

Studies on the effect of different sources of carbon on the growth of Pleurotus species revealed that growth was significantly higher in all the carbon sources tested. In general Lactose and Mannose were found to support maximum growth of Pleurotus species. Kikon and Rao (1980) reported that starch supported maximum growth of P.ostreatus and P.florida. Madelin (1956) and Srivastava and Bano (1970) also observed that starch and glucose were good carbon sources for the growth of edible fungi viz.,

Coprinus lagopus and P.flabellatus. In the present studies, arabinose, fructose, xylose and glucose were found to be poor carbon sources for all the species of Pleurotus tested and this is in conformity with the findings of Hashimoto and Takahashi (1974); Kikon and Rao (1980) and Qudir and Khatoon (1983). The differential response of the strains of Pleurotus spp. to various carbon sources was attributed to the structural variation among the compounds (Steinburg, 1942) or to the change of metabolic pathways to a particular sugar (Elheridge, 1955). In this context it is interesting to note the final pH of the growth substrate after the experiment with glucose, the pH of the culture filtrate decreased and became too much acidic, while with mannose and lactose the pH had not changed much even though growth was maximum in these media. It might be due to utilization of slowly hydrolysable compounds accompanied by accumulation of few organic acids compound to glucose as suggested by Cochrane (1955).

P.sajor-caju, P.ostreatus and P.flabellatus when grown in different Nitrogen sources, growth of the species recorded highest under peptone. P.ostreatus recorded highest dry weight when grown with ammonium nitrate,

sodium nitrate, ammonium chloride and potassium nitrate as nitrogen sources. On the other hand, P.sajor-caju recorded maximum growth when grown in ammonium nitrate and peptone. P.flabellatus recorded highest weight when ammonium carbonate was given as nitrogen sources. All the species of Pleurotus tested had shown preferential utilization of ammoniacal nitrogen for vegetative growth (Table 4) as the case with many other fungi (Madelin, 1956; Srivastava & Bano, 1970). Kikon and Rao (1980) suggested that the immediate assimilation of ammoniacal nitrogen for the synthesis of amino acids and protein might be one of the reasons for the better growth of the Pleurotus group of fungi. The poor growth in nitrate nitrogen was attributed to the toxic effect exerted by Pyruvic acid accumulated in the mycelium as suggested by Nord and Mull (1945). The growth of the three species of Pleurotus in the medium devoid of nitrogen indicated their ability to use/fix atmospheric nitrogen. The findings of Zadrazil (1974), Rangad and Jandaik (1977) and Rangaswamy et al. (1975) on the Nitrogen fixing ability of different species of Pleurotus have strengthened this observation. Effect of different carbon and nitrogen sources on the mycelial

growth of P.sajor-caju, in liquid media indicated that there is a non-significant difference in the mycelial yield (Qadir & Khatoon, 1983).

In vitro studies carried out to assess the optimum pH range for the growth of three species of Pleurotus revealed that these fungi can grow profusely at a wide pH from 4.5 to 7.0, optimum range for the growth being 4.5 to 6.5. Chandra and Purkayasta (1977) also reported similar results with edible mushrooms P.eryngii, P.cornucopiae, P.ostreatus and P.florida grown in various liquid media failed to grow at pH 4 or below and maximum growth in all the species was recorded when pH was adjusted to 5-6 (Rangad & Jandaik, 1977b). P.flabellatus (Srivastava & Bano, 1970) P.sajor-caju (Jandaik & Kapoor, 1975) and P.ostreatus (Hashimoto & Takahashi, 1974) had reported more or less similar results. All the species failed to grow in alkaline medium (Rangad & Jandaik, 1977b).

The comparative growth of Pleurotus sajor-caju, P.ostreatus and P.flabellatus when incubated under various temperatures from 15°C to 35°C revealed that

the growth was mainly influenced by the temperature at which the cultures were incubated. The data on the average dry weight of mycelium at different temperatures revealed that the optimum temperature for maximum growth of the fungus was between 15 to 30°C though the organism succeeded in growing from 15°C to 35°C. Similar results were reported in the case of Volvariella volvacea by Rangaswamy (1956) and Muthukrishnan (1971). According to Chang and Chu (1969) the optimum temperature for the growth of the mycelium was 30 - 35°C, the best being 32°C. They also observed reduction in growth at 20°C and at 40°C. According to Singh (1983) Pleurotus sajor-caju could be grown successfully at a temperature range of 19.1°C to 30.5°C and he observed considerable reduction in yield at a temperature below 19.1°C and above 30.5°C. Ranged and Jandaik (1977b) reported that P.cornucopiae, P.eryngii, P.ostreatus and P.florida failed to grow at 10°C or below. They had reported that maximum growth of P.eryngii, P.cornucopiae and P.ostreatus was recorded at 25°C where as P.florida produced highest growth at 30°C. Similar results were reported for P.ostreatus and P.flabellatus by Block et al. (1959); Srivastava & Banu, 1970). All the Pleurotus spp., studied failed to grow at 35°C or above, except P.florida which could grow moderately at this temperature (Rangad & Jandaik, 1977b).

Comparative growth of six different species of Pleurotus viz., P.opuntiae, P.ostreatus, P.florida, P.flabellatus, P.sajor-caju and P.citrinopileatus in wheat grains as spawn substrate revealed that growth of P.opuntiae was faster than the rest of the five species and minimum growth was recorded for P.florida. The growth rate of P.opuntiae and P.ostreatus were observed to be on par with each other so also the growth of Pleurotus sajor-caju and P.flabellatus was almost the same. Maximum growth was recorded by 21 days of incubation. Sinden (1932) first introduced the grain spawn with the addition of calcium salts for the cultivation of Agaricus bisporus. Purkayastha et al. (1980) found that wheat grain spawn was the best for the production of fruit bodies of Volvariella volvacea. Suharban et al. (1978) also reported the efficacy of wheat grain for the maximum production of sporocarps of V.volvacea. Bhavani Devi (1982) observed maize grain spawn as the best for maximum yield of Volvariella, followed by wheat grain, so also she had found that among the various substrates tried, grains in general supported good mycelial growth and high quality spawn in V.volvacea.

Comparative efficacy of various containers and substrates used for spawn production revealed that maize was the best substrate among all others except Sorghum, Bajra and Green gram. This is in agreement with the findings of Bhavani Devi (1982) who reported maize as the best substrate for maximum yield of Volvariella volvacea. Nout and Keya (1985) also reported that maize kernels supported the growth of P.sajor-caju better than wheat. They also enlightened the added advantage of maize since it do not lump and agglomerate as quickly as wheat does. The maize spawn performed very well as an inoculum. They also observed that the mycelium of P.sajor-caju had a thin and sickly appearance on Sorghum. Black gram and Bengal gram were found to be the least effective substrate compared to all other pulses in supporting mycelial growth of Pleurotus except Horse gram. Among the containers tried Wine bottle was found to be the best container and Milk bottle least effective one in supporting mycelial growth of P.sajor-caju. This can be attributed to the difference in space in the bottles. Thapa et al. (1979) reported the efficacy of Poly propylene bags to substitute glass bottles for spawn production.

Different oil cakes, Rice bran, Saw dust and Chaff were tested for their comparative efficacy in supporting mycelial growth of Pleurotus sajor-caju in containers like Boost bottle, Wine bottle, Horlicks bottle and Milk bottle. It was observed that growth of P.sajor-caju increased with duration in incubation. When the effect of containers were concerned maximum growth was observed in wine bottle. Growth was found to be better in Horlicks bottle and Boost bottle than Milk bottle. As far as substrates were concerned maximum growth was observed in chaff and lowest in ground nut oilcake. The growth of Pleurotus sajor-caju was more when grown in Wine bottle with all substrates except gingelly oil cake.

The data revealed that oil cakes will not support the growth of mushroom and may not be a suitable substrate for spawn production. Nout and Keya (1983) also found saw dust inferior to cotton mill waste, banana leaves, filter mud and coir dust in growing P.sajor-caju.

Comparative growth of P.sajor-caju on various substrates at different temperatures ranging from 10°C to 35°C revealed that growth of P.sajor-caju was on an average same

in Red gram, Horse gram, and Green gram. Maximum growth was observed in Bengal gram and Wheat grain and these two substrates were found to be the best for the growth of the fungi. Growth was very scanty in Salvinia. This is in confirmity with the findings of Bhavani Devi (1982).

P.sajor-caju responded differentially within each substrate at given temperature its growth was similar in substrates like Red gram, Horse gram and Green gram at all the temperature levels. In Horse gram and Bengal gram there was significant growth even at 15°C. Maximum growth was recorded at 25°C in substrates like Red gram, Horse gram, Bengal gram, Wheat grain and Green gram. Krishna-Mohan (1975) reported the optimum temperature for the growth of paddy straw mushroom in spawn bottles to be between 30 to 35°C. Bhavani Devi (1982) also has reported the same results.

In the present investigation, Maize spawn was found to be superior to Sorghum, Ragi, Bajra, Paddy and Wheat in increasing the yield. This is in confirmity with the findings of Bhavani Devi (1982) in the case of Volvariella volvacea. Purkayastha et al. (1980) found that

wheat grain spawn was the best for the production of fruit bodies of V.volvacea. Nout and Keya (1983) reported that Maize kernels supported the growth of P.sajor-caju better than Wheat. They also enlightened that maize spawn performed very well as an inoculum. They had also reported that Sorghom did not perform well in supporting mycelial growth of Pleurotus spp.

Various pulses when used as spawn substrate, it was found that spawn prepared in Horse gram gave maximum yield followed by Green gram, Bengal gram, Red gram and Black gram in the order of their efficiency.

Studies on the suitability of organic amendments for sporocarp production indicated that maximum yield (642 g) was obtained when Bengal gram was used followed by Green gram, Red gram, Wheat flour and Horse gram. The yield was significantly poor with cowdung slurry (153.2 g). Zakia Bano and Rajaratnam (1978) reported that maximum yield of P.sajor-caju was obtained from supplementing the straw substrate with horse gram powder and yeast mud. They had observed that increase in yield was generally associated with reduction in protein content of the

mushroom and the highest protein contents recorded were from relatively low yielding substrates. Increase in yield and protein content of the fruit bodies, have been reported by Schisler and Sinden (1962 a, b, 1966) in the context of Agericus cultivation and supplementation of the compost with nitrogen sources.

Trials on the cultivation of Pleurotus sajor-caju was tried on logs of common trees. Fifteen trees were tried and it was observed that of the various logs tested logs of Mango tree yielded the maximum quantity of sporocarp followed by cashew tree. Chakraverthy and Sarkar (1982) also reported best results on logs of Mangifera indica and Artocarpus lakoocha. Dhar (1976) found that in Kashmir valley, non resinous woods like poplar acacia and willow have been found suitable for growing P.ostreatus, and Paul et al. (1983) from Himachal Pradesh found that Horse chestnut tree can also be used for the cultivation of P.ostreatus.

When spawn raised in various cereals were used for raising beds, it was found that Maize spawn was superior to all others in maximising the yield, followed by Bajra,

Ragi, Sorghum, Wheat & Paddy. Nout and Keya (1983) reported the efficacy of Maize in supporting maximum growth of P.sajor-caju. Similar results were reported by Bhavani Devi (1982) in the case of Volvariella volvacea.

Spawn prepared on various pulses, when used for raising mushroom beds it was found that Horse gram spawn was found to give maximum yield per kg of paddy straw followed by spawn prepared with green gram, Bengal gram, Red gram and Black gram. Zakia Bano and Rajaratnam (1982) reported the efficacy of Horse gram powder in recording maximum yield of P.sajor-caju when used as an amendment.

Studies on the influence of different types of straw beds for the maximum production of sporocarp of P.sajor-caju revealed that none of the four methods tried were effective.

Studies on the influence of different containers in supporting sporocarp production revealed that polythene bag was the best container which gave an average yield of 426 g of mushroom. Zakia Bano and Nagarajan (1977) reported that from the point of obtaining higher yield in a shorter duration

this technique of cultivating P.flabellatus in paddy straw packed in polythylene bags with vents is quite advantageous. They had also remarked that the cost of polyethylene bags works out very low as compared to the cost of earthern pots or wooden trays. The labour involved in this method of cultivation is less as compared to the conventional methods. They had concluded that the technique of cultivating P.flabellatus in Polyethylene bags with vents indices high yield in a short time and hence offers a very profitable commercial feasibility. Bhaskaran et al. (1978) also reported with P.sajor-caju that in compact poly bag method the mushroom yield per kg of paddy straw was nearly twice than that under the bamboo basket method. They had attributed the main reason for such increase of mushrooms in bag method to the more surface area of exposed sides than in bamboo baskets method for emergence of mushrooms.

Various methods of lay out of beds showed that polythene wrapper bed method was superior to others. Polythene wrapper bed method recorded the highest yield of sporophores followed by cylindrical bed method. Bhaskaran et al. (1978) found that yield of mushroom was twice in cylindrical bed method than that of bamboo

basket method. Sivaprakasam and Kandaswamy (1980) also found that polythene wrapper bed method to be superior to other methods and supports the results of this study. They also reported the reason for increase of yield in polythene wrapper bed and cylindrical bed methods to more surface area of exposed sides and compactness of the bed which helps in retaining moisture.

Different sizes of polythene bag when tested for their comparative effect on production of sporocarp revealed that increasing paddy straw from 1.0 to 1.5 kg did not increase the yield significantly. ZakiaBano and Nagarajan (1987) reported that the size of bag was found to influence the yield of P.flabellatus. They got maximum yield in polythene bag of size 35 x 53 cm. They also reported that cultivation of mushroom in earthern pots or wooden trays gave 8 to 10 crops in an year where as cultivation in polythene bags resulted in 20 to 24 crops per year. Efficacy of straw of local varieties and improved varieties were compared for their ability to support sporocarp formation. The results clearly indicated that straw of local varieties of rice were better than high yielding varieties of rice in supporting higher sporocarp production. Studies of

Krishna Mohan (1975) also showed that tall, hand threshed and rigid straw gave increased yield over dwarf cattle threshed and flexible straw. More or less same results were obtained by Rath (1976) also who compared the yield and other characters of sporocarp formation in mushroom beds laid out with straw of high yielding and local varieties where he obtained higher yield with local varieties. He concluded that the high yielding varieties though not suitable for mushroom cultivation, is often not economical for mushroom culture since the yield they produced was lower than that obtained from local varieties. It is proper to note that the straw of the high yielding varieties tends to decompose earlier than that of local varieties when kept under high moisture conditions for longer period.

In the present study comparative efficacy of straw made into bits and small twists were tested for making beds with six species of Pleurotus viz., P.sajor-caju, P.ostreatus, P.florida, P.opuntiae, P.citrinopileatus and P.flabellatus. The data revealed that P.ostreatus did not grow at all in any of the types of beds. The climatological factors prevailed may not be suitable for the fungus to grow at the time of experimental cultivation.

The other five species grew well giving promising yields. P.sajor-caju recorded highest yield of 775 (g) establishing its suitability for cultivation. P.opuntiae recorded an yield of 644 g which adds one more mushroom to suit under Kerala conditions. Use of Paddy straw as twists were found to be better than use of bits. Species grown in twists gave an average yield of 600 g compared to 422 g in bits. The probable reason can be in twists the mycelial run will be more. The beds with twists have more surface area. The compactness of the bed also kept it free from drying and retained more moisture. The mycelial growth was also better. As a result mushroom freely appeared on all the sides, thus increasing their number.

Cultivation of P.sajor-caju in different periods of the year revealed that the mushroom can be successfully cultivated in Kerala all through the year on paddy straw. Maximum yield was recorded during January, February and March and the lowest yield recorded during May. The high temperature prevailed in April-May may be the reason for the low yield. The temperature and relative humidity of the forenoon, together with rainfall were found to have a negative effect on yield. It is to be pointed out

that 80-90 per cent relative humidity around the mushroom beds is to be maintained by frequent sprinkling with water. Singh (1981) found that highest yield was obtained at a temperature range of 20.5°C - 30°C and 70.32 - 80.57 per cent relative humidity during November and December. However, in the months of March and April the yields were poor mainly because of higher temperatures and lower relative humidity.

The maintenance of moisture content of mushroom beds is an important factor for maximum sporocarp production. The initial moisture content in the bed achieved by soaking straw for appropriate periods is maintained by frequent watering. Watering of the bed was done at different intervals and the data clearly revealed that watering the straw bed every 24 hours supported more sporocarp formation than watering every 12 or 48 hours. The yield from control beds (without watering) indicated that frequent watering is a must for sporocarp production. Garcha (1976) reported that beds were watered twice a day, morning and evening during summer under Punjab conditions for maximum sporocarp production.

The over all effect of growth regulators on the yield of Pleurotus spp. in general revealed that IBA 50 ppm was superior in yield response over others. As against this 2,4-D 50 ppm and NAA 50 and 100 ppm were found to exert even slighty adverse effect on yield. In short, the plant growth regulators were found to have little effect on yield response even though IBA 50 ppm had very good effect on yield. Bohus (1959) also found enhancement of mycelial growth by IAA in only one of the several strains of common cultivated mushroom tested by him. Similarly, Urayama (1956) and Nagimoto and Konishi, (1960) also failed to detect growth promoting action of the exogenously supplied IAA on the Agaricus stipe.

Rawal and Singh (1980) found that use of growth regulators at 1 ppm concentration like NAA, 2,4-D, MH, IAA, Indole 3-yl propionic acid, IPA and GibberPllic acid did not support growth of Pleurotus species like P.eryngii, P.florida, P.ostreatus, P.sajor-caju and P.sapidus.

A comparative analysis of the proximate constituents with six species or Pleurotus compared with that in

Volvariella volvacea is given in table 27. The data revealed that maximum per cent of protein in P.squarrosulus and minimum in P.cornucopiae. Among the six species of Pleurotus, P.subpalmatus contained maximum of 10.04 mg of calcium and P.petaloides the least (6.02), Maximum quantity of magnesium was found in P.cornucopiae (2.49 mg) and P.subpalmatus the least (0.962 mg). P.petaloides contained maximum iron i.e., 3.11 mg, and 1.82 mg in P.squarrosulus the least. The quantity of Vit.A and Vit.C was maximum in P.squarrosulus while it was found to be least in P.petaloides and P.sajor-caju respectively.

The protein content of P.sajor-caju was higher than that recorded in Auricularia polytricha, Lentinus sp., Pleurotus sp. and P.opuntiae Adriano and Cruz (1933), Pleurotus sp. (Bano et al., 1963) and V.volvacea (Anon., 1972). The protein content of P.sajor-caju is much higher than in common vegetables which ranges from 7.6% in potato to 18.4% in cabbage but lower than hen's egg and goat meat which contained 50.6% and 83% protein respectively (Arkoyed, 1966; Bano 1976). Thus the nutritional value of sporophores of P.sajor-caju can be considered as, intermediate between vegetable and egg and meat proteins.

The high ash content of P.sajor-caju indicated higher mineral incorporation in the sporophores (Janardhanan & Husain, 1975). The content of minerals like phosphorus and calcium essential for human nutrition was higher than in many fruits and vegetables (Arkoyed,1966).

The sporophores of P.sajor-caju contained minerals like Calcium, Magnesium & Iron. Sivaprakasam (1978) had reported the presence of minerals like Phosphorus, Potassium, Sodium, Calcium, Magnesium, Iron, Copper, Zinc and Manganese. He had also reported that the sporophores of P.sajor-caju contained appreciable amount of ascorbic acid which supports the present study.

Refrigeration and dehydration were tried and found promising for preservation of Pleurotus sajor-caju. Adsule et al. (1981) reported several solution for enhancing the shelf life of mushroom. They had found that water containing 0.5% citric acid and 0.2% potassium metabisulphite solution preserves Oyster mushroom for three months without under going spoilage. Jandaik and Sharma (1983) reported that storage of different Pleurotus species in perforated polythene bags is effective in

reducing moisture loss as compared to unpacked conditions in which loss of moisture was about 32 - 35 per cent at 15°C - 18°C and 6 per cent at 6°C - 8°C after 72 hours. They had found no loss of moisture in packed bags but the fruiting bodies were slimy due to accumulation of moisture. When fruit bodies were stored at different temperatures in perforated bags, the loss of moisture was less, appearance as well as change in colour were unnoticeable. Similar results were also reported by Nichols and Hammond (1975) and Cho et al. (1985).

Jandaik and Sharma (1983) found that sun dried fruit bodies have 3 to 4 per cent moisture in comparison to 2 per cent moisture in fruit bodies dried mechanically (Hot air) at 40 - 45°C. They had also reported that the change in colour was also slight in sun drying as drying in hot air oven (55 - 60°C), which resulted in dark brown colour of the fruit bodies. The fruit bodies dried in the sun or mechanically at 45°C were acceptable organoleptically where as drying of fruit bodies of Pleurotus at 55 - 60°C resulted in burnt taste and were found unacceptable organoleptically. The time required for rehydration with luke warm water (45 - 50°C) was 12 - 15 minutes while at ambient temperature (25 - 30°C) 1 to 2 hours. The dried products can easily be stored for 120 days in sealed polythene bags.

SUMMARY

SUMMARY

A preliminary survey conducted in and around the College of Agriculture and parts of Trivandrum district revealed the occurrence of a number of species of Pleurotus. Each of the collection was identified by comparing the enumerated characters with those described in literature and confirmed by Professor K. Natarajan, Centre for Advanced Studies in Botany, University of Madras. Twenty of the Pleurotus spp., properly identified were further subjected to detailed comparative study. Based on these an attempt was also made to formulate a convenient 'Key' for the identification of the common Pleurotus spp. of Kerala. The majority of the species of Pleurotus (fourteen) are with spores less than 10µm in size falling under the group tropical and sub tropical flora and six of them with spores larger than 10 µm which are considered as temperate flora.

Out of the 20 species described, the following eight species are new records for India, in addition to P.citrinopileatus and P.opuntiae, which are also not validly published for India.

1. P.luteoalbus
2. P.lignatilis
3. P.mastrucatus
4. P.petaloides
5. P.pometi
6. P.pubescens
7. P.serotinus
8. P.ulmarius

Among the species recorded P.citrinopileatus, P.cornucopiae, P.dryinus, P.eöus, P.flabellatus, P.ostreatus and P.platypus are considered to be good esculent species.

Of the few edible mushrooms cultivated in India, the Pleurotus spp. was found to be better adopted for the warm humid conditions of Kerala. Hence a study was undertaken to standardize the cultivation practices under Kerala conditions.

In vitro physiological studies with few species of Pleurotus revealed that they are adopted to a wide variety of environmental conditions.

Growth of six species of Pleurotus viz., P.opuntiae, P.ostreatus, P.flabellatus, P.sajor-caju and P.citrinopileatus in various liquid media in shake culture showed that as a result of continuous rotation hyphal pellets will be formed.

Studies on the effect of different sources of carbon on the growth of Pleurotus species revealed that growth was significantly higher in all the carbon sources tested. All the species of Pleurotus tested had shown preferential utilization of ammoniacal nitrogen for vegetative growth. The optimum temperature for maximum growth of the fungus was between 15 to 30°C and they can grow profusely at a wide pH range of 4.5 to 6.5.

Comparative study with six different species of Pleurotus viz., P.opuntiae, P.ostreatus, P.florida, P.flabellatus, P.major-caju and P.citrinopileatus revealed that wheat grains as best substrates for spawn.

Comparative efficacy of various containers and substrates used for spawn production revealed that maize was the best substrate among all others except sorghum, Bajra and Greengram. Among the containers tried, Wine bottle was found to be the best container and Milk bottle the least effective one in supporting mycelial growth of P.major-caju.

Different oilcakes, rice bran, sawdust and chaff were tested for their comparative efficacy in supporting

mycelial growth of Pleurotus sajor-caju in containers like Boost bottle, Wine bottle, Horlicks bottle and Milk bottle. The data revealed that oil cakes will not support the growth of mushroom and may not be a suitable substrate for spawn production.

Comparative growth of Pleurotus sajor-caju on various substrates at different temperature ranging from 10°C to 35°C revealed that growth of P.sajor-caju was on an average same in Red gram, Horse gram and Green gram. Maximum growth was observed in Bengal gram and wheat grain and these two substrates were found to be best for the growth of the fungi.

In the present investigation, Maize spawn was found to be superior to Sorghum, Ragi, Bajra, Paddy and Wheat in increasing the yield.

Various pulses when used as spawn substrate, it was found that spawn prepared in Horse gram gave maximum yield followed by Green gram, Bengal gram, Red gram and Black gram in the order of their efficiency.

Studies on the suitability of organic amendments for sporocarp production indicated that maximum yield was obtained when Bengal gram was used. The yield was significantly poor with cowdung slurry.

Trials on the cultivation of Pleurotus sajor-caju was tried in logs of common trees. Of the various logs tested, logs of Mango tree yielded the maximum quantity of sporocarp.

When spawn raised in various cereals were used for raising beds, it was found that Maize spawn was superior to all others in maximising the yield.

Spawn prepared on various pulses, when used for raising mushroom beds it was found that horse gram spawn was found to give maximum yield per kg of paddy straw.

Studies on the influence of different containers in supporting sporocarp production revealed that polythene-bag was the best container.

Various methods of layout of beds showed that polythene wrapper bed method was superior to others.

Different sizes of polythene bag when tested for their comparative effect on production of sporocarp revealed that increasing the quantity of paddy straw from 1.0 to 1.5 kg did not increase the yield significantly. Efficacy of straw of local varieties and improved varieties were compared for their ability to support sporocarp formation. The result indicated that straw of local varieties of rice were better than high yielding varieties of rice in supporting higher sporocarp production.

Comparative efficacy of straw made into bits and small twists were tested for making beds with six species of Pleurotus, viz., P.sajor-caju, P.ostreatus, P.florida, P.opuntiae, P.citrinopileatus and P.flabellatus. P.sajor-caju recorded highest yield of 775 g per kg of substrate establishing its superiority for cultivation in Kerala.

Cultivation of P.sajor-caju in different periods of the year revealed that the mushroom can be successfully cultivated in Kerala all through the year on paddy straw. Watering of the bed was done at different intervals and the data clearly revealed that watering the straw bed every 24 hours supported most sporocarp formation than watering every 12 or 48 hrs.

The overall effect of growth regulators on the yield of Pleurotus spp. in general revealed that IBA 50 ppm was superior in yield response over other growth regulators.

A comparative analysis of the proximate constituents with six species of Pleurotus compared with that in Volvariella volvacea revealed that maximum per cent of protein in P.squarrosulus and minimum in P.cornucopiae. P.subpalmatus contained maximum of 10.04 mg of calcium and P.petaloides (2.49 mg) and P.subpalmatus the least (0.962 mg). P.petaloides contained maximum iron 3.11 mg and 1.82 mg in P.squarrosulus the least. Vit.A and Vit.C was maximum in P.squarrosulus while it was found to be least in P.petaloides and P.sajor-caju respectively. The sporophores of P.sajor-caju contained minerals like calcium, Magnesium and Iron.

Refrigeration and dehydration were tried and found promising for preservation of Pleurotus sajor-caju for short periods.

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* Original not seen

APPENDICES

APPENDIX I

DATA-SHEET

Sl.No, ... Date of collection: .....198...

Collected by: Locality (Village/Taluk/Dist.)

Final Identification:

(Confirmed by .....)

Taxonomy:

Order:

Family:

GENERAL

Common name: Local name:

Soil type: Vegetation:

Substrate:

Season: Temp. R.H. Rainfall:

Any other information on climate:

Others:

Habitat : Terrestrial/lignicolous/Epixylose/Coprophilous/ Humicolous.

Habit : Solitary/Scattered/Caespitose/Gregarious.

PILEUS

Shape: When young: Conical/Spherical/Campanulate/Convex.
At maturity: Infundibuliform/Umbonate/Broadly umbonate/Campanulate/Umbilicate/Aplanate/Conical/Petaloid/Flabelliform/Mucronate/Depressed/Dimidiate/Resupinate.

Size: When young: .....
At maturity: .....

Colour: .....

Texture: Soft/Brittle/Fleshy/Coriaceous/Hygrophanous/Fragile/Cartilaginous/Membrneous.

Surface : Smooth/Scaly/Rugose/Rugulose/Visdd/Striate/ Dry/Squamulose/Velutinous/Pubescent/ Strigose/Sulcate/Tomentose/Alveolate/ Farinose/Floccose/Punctate/Rivose/Rivulose.

Margin : Serrate/Serrulate/Smooth/Undulate/Reflexed/ Involute/Fimbriate/Incised/Lobed/Revolute.

Context : Colour: Before cutting:
After cutting:

Colour changes with;

1 Melzer's reagent : Amyloid/Pseudoamyloid/Inamyloid.
2 Green Vitriol : .....
3 Phenol : .....
4 Sulphovanilin : .....

GILLS

Arrangement : Remote/Free/Decurrent/Adnate/Adnexed/Sinuate.

Shape : Rounded anteriorly or posteriorly/Lanceolate/ Ventricose/Reticulate.

Texture : Soft/Brittle/Coriaceous/Waxy/Thick/Papery/ Opaque.

Margin : Smooth/Wavy/Serrate/Fimbriate/Dentate.

Size : Number per cm.

Gill trama : Regular/Irregular/Bilateral/Inverse.

Cystidia

1 Pilocystida Size:
2 Pleurocystidia 1
3 Cheilocystidia 2
4 Caulocystidia 3
4

Shape:

a. Ventricose/ b. Clavate/
c. Filiform/ d. Napiform/
e. Lageniform/ f. Rostrate/
g. Encrusted/ h.Ramified/
i. Lanceolate/ j. Pyriform/
k. Granulate/ l. Pointed/
m. Beaked/ n. Capitate/
o. Lacythiform p. Cylindrical

1 2 3 4

VEIL

Type	: Present/Absent	Universal/Partial
Colour	:	.....
Texture	:	Membraneous/Fleshy/ Smooth/Coriaceous
Position	:	

ANNULUS Present/Absent

Size	:	.....
Texture	:	Fleshy/Coriaceous/Papery/Thin.
Colour	:	.....
Attachment	:	Superior broad/Medial pendulous/ Inferior/ Narrow fragments/Appendiculate/Fibrillose/ Movable.

STIPE

Present (Stipitate)/Absent(sessile)

Size	:	Length : ..... Diameter : .....
Shape	:	Clavate/Obclavate/Cylindrical/Solid/Hollow/ Slender/Short.
Attachment to Pileus	:	Lateral/Eccentric/Central/Resupinate.
Surface	:	Glabrous/Scaly/Pubescent/Velutinous/ Squamose/Tomentose/Fibrillose.
Colour	:	Before cutting: ..... After cutting: .....
Reaction with Melzer's reagent	:	Amyloid/Pseudoamyloid/Inamyloid.
Basal Part	:	Globular/Annular stripes/Fusoid/Bulbous/ Sheathing Bulbous/Marginately depressed bulb/ Pseudorhizoid/Rhizines/Rhizemorphoid.

VOLVA

Present/Absent	: Persistent/Evanescent
Shape	: Free/Lobed/Irregular/Cup like.
Colour	: .....
Texture	: Soft/Fleshy/Tough/Papery.
Odour:	: Before cutting: ..... After cutting: .....
Taste	: Acrid/Mealy/Acidulous/Blunt.

SPORE PRINT

Colour	: .....
Other details	: .....

BASIDIA

Size	: .....
Shape	: .....
Sterigmata	: No.1, 2/ /4 /

SPORES

Colour	: .....
Reaction with:	
Cotton blue	: Cyanophilic/Acyanophilic.
Melzer's reagent	: Amyloid/Pseudoamyloid/Inamyloid.
Shape	: Ovate/Elliptical/Globose/Sub globose/ Apiculate/Cylindrical/Fusiform/ Angular/Echinulate/Verrucose/ Reticulate/Tuberculate/Ovoid/ Obtusely fusiform/Allantoid/Guttulate/ Pipshaped/Pyriform/Pedicillate/ Muriform/Filiform.

Other characters of sporea: (fig.)

ANY OTHER DETAILS:

APPENDIX II

Composition of the Media and reagents used for the study

1. Potato dextrose agar medium

Pealed and Sliced Potato	= 250 g
Dextrose	= 20 g
Agar agar	= 15 g
Distilled water	= 1000 ml
pH	= 6.0-6.5

2. Oat meal agar medium

Oats	= 40 g
Agar agar	= 15 g
Distilled water	= 1000 ml

3. Malt extract medium

Malt extract	= 25 g
Agar agar	= 15 g
Distilled water	= 1000 ml

4. Richards Medium

Potassium nitrate	= 10 g
Potassium dihydrogen phosphate	= 5 g
Magnesium sulphate	= 2.5 g
Fernic chloride	= 0.02 g
Sucrose	= 50.0 g
Agar	= 15.0 g
Distilled water	= 1000 ml
pH	= 6.6 to 7.2

5. Czapek's agar

Sucrose	= 30 g
Sodium nitrate	= 2 g
Dipotassium phosphate	= 1 g
Magnesium sulphate	= 0.5 g
Potassium chloride	= 0.5 g
Ferrous sulphate	= 0.01 g
Agar	= 15 g
Distilled water	= 1000 ml

6. Melzer's reagents (Melzer, 1934)

Potassium iodide	= 1.5 g
Iodine	= 0.5 g
Water	= 20.0 ml
Chloral hydrate	= 22 g

7. Potassium hydroxide = 3 per cent

8. Hydrochloric acid = 11 M

APPENDIX III

Growth of Pleurotus spp in different Solid media under laboratory conditions on the sixth day of incubation. (Analysis of variance table).

Source	S.S.	df	Mean Sum of squares	F Calculated
Total	62.33	161		
Treatment		53		
Species(S)	0.2063	2	0.1032	2.21
Media(M)	3.3000	2	1.6500	35.41 **
S x M	0.0167	4	0.0042	<1
T(period)	49.9560	5	9.9912	214.40 **
S x T	0.0626	10	0.0063	<1
M x T	3.4812	10	0.3481	7.47 **
S x M x T	3.2749	20	0.1637	3.51 **
Error	5.0323	108	0.0466	

** Significant at 0.01 level

C.D. for S or M = 0.0830

C.D. for T = 0.1173

APPENDIX IV

Growth of Pleurotus spp in different solid media under darkness

Source	S.S.	df.	Mean sum of square	F Calculated
Total	60.3229	161		
Treatment				
Species (S)	0.4409	2	0.2205	735.00
Media (M)	3.3851	2	1.6926	5642.00
S x M	0.0248	4	0.0062	20.6667**
T (period)	52.8437	5	10.5687	35.229**
S x T	0.0952	10	0.0095	31.6667**
M x T	3.3156	10	0.3316	1105.3333**
S x M x T	0.1825	20	0.0091	30.3333**
Error	0.0351	108	0.0003	

C.D. for S or M = 0.0067
C.D. for T = 0.0094
C.D. for S x M = 0.0115
C.D. for M x T = 0.063
C.D. for S x T = 0.0163

** Significant at 0.01 level.

APPENDIX V

Growth of different species of Pleurotus in different media in Shake culture (in g)

Source	S.S.	df	Mean sum of squares	calculated F
Species (S)	17.02	5	3.410	685.11
Media (M)	3.96	2	1.982	398.13**
S x M	4.85	10	0.490	97.58**
Error	0.18	36	0.005	

** Significant at 0.01 level.

C.D. for S = 0.067
C.D. for M = 0 048
C.D for S x M= 0.117

APPENDIX VI

Influence of different nitrogen sources on the growth of *Pleurotus* spp

Source	Sum of squares	df	Mean sum of squares	F calculated
Total	7.3207	62		
Treatment				
Nitrogen sources(N)	5.4364	6	0.9061	75508.3337**
Species(S)	0.1923	2	0.0962	8016.6667**
N x S	1.6915	12	0.1410	11750.00**
Error	0.0005	42	0.000012	

** Significant at 0.01 level
C.D. N = 0.003257
C.D. for S = 0.002119
C.D. for NxS = 0.005713

APPENDIX VII

Influence of different carbon sources on the growth of *Pleurotus* spp

Sources	Sum of squares	df	Mean sum of squares	F Calculated
Total	1.1472	80		
Treatment	1.1463	26	0.101576	5975.1**
Error	0.0009	54	0.000017	

** Significant at 0.01 level

C.D. for S = 0.016373
C.D. for C = 0.028373
C.D. for C x S= =.049144

APPENDIX VIII

Effect of different hydrogen-ion concentration on the growth of *Pleurotus* spp

Sources	Sum of squares	df	Mean sum of squares	F Calculated
Total	19572.84	53		
Treat	19572.69	17	9276.67	
Species	18085.60	2	9042.80**	
pH levels	845.28	5	169.06**	
Species x pH	641.81	10	64.81**	
Error	0.15	36	0.0042	

** Significant at 0.01 level.

C.D. for Species = 0.04406
C.D. pH levels = 0.06232
C.D. Sp x pH levels=0.107947

APPENDIX IX

Effect of different temperatures on the dry weight of *Pleurotus* spp after 2 weeks

Sources	Sum of squares	df	Mean sum of squares	F Calculated
Total	23758.23	44		
Treatment	23757.92	14	5840.6375**	
Error	0.31	30	0.010330	

** Significant at 0.01 level

C.D. for S = 0.075794
C.D. for T = 0.097850
C.D. for SxT = 0.169481

APPENDIX X

Growth of various species of *Pleurotus* in Wheat grains. (Square root transformation)

Sources	Sum of squares	df	Mean sum of squares	F Calculated
Treatments				
Species (S)	16.46	5	3.290	740.00**
Days (D)	11.04	2	5.520	240.67**
S x D	0.92	10	0.090	20.56**
Error	0.24	54	0.004	

** Significant at 0.01 level

C.D. for comparison of S Means = 0.055
C.D. for comparison of D Means = 0.039
C.D. for comparison of SxD Means= 0.095

APPENDIX XI

Growth of Pleurotus sajor-caju on grains of cereals and pulses as spawn substrates in different containers

(Growth in cm)

Sources	S.S.	df.	M.S.	F Calculated
Total	5095.644			
Grain (V)	51.059	9	5.6732	7.073*
Container (C)	1265.690	3	421.898	526.056**
V x C	22.268	27	0.825	1.028
Period	3386.259	1	3386.259	4222.268**
V x P	8.293	9	0.922	1.149
C x P	340.104	3	113.369	141.351**
V x C x P	21.676	27	0.802	434.835**
Error	0.295	160	0.0018	

* Significant at 0.05 level
** Significant at 0.01 level

C.D. for container = 0.1335
C.D. for grains = 0.5304
C.D. for periods = 0.237
C.D. for P x C = 0.474

APPENDIX XII

Growth of *Pleurotus sajor-caju* in different oil cakes and agricultural wastes as substrates in different containers-Pooled Anova

Sources	Mean sum of square	df	F Calculated
Period (W)	27.9661 x 3	2	41.9492
Container (C)	3.1726 x 3	3	3.1726
W X C	1.8811 x 3	6	0.9406
Substrate (S)	10.7916 x 3	5	2.1583
W x S	5.1910 x 3	10	1.5573
C x S	15.6942 x 3	15	3.1388
W x C x S	32.7936 x 3	30	3.279363
Error (pooled)		144	0.0007276

C.D. (C)	= 0.010373
C.D. (S)	= 0.012712
C x S	= 0.03114
W	= 0.01557
W x C	= 0.01798
W x S	= 0.02202

APPENDIX XIII

Effect of temperature on the mycelial growth of Pleurotus sajor-caju in spawn bottle. $\sqrt{x+1}$ transformation.

Sources	M.S.	df.	F.calculated
Total	4.6245	125	
Temperature (T)	1.9460	5	846.09**
Substrates (S)	2.4358	6	1059.04**
T x S	0.2404	30	104.52**
Error	0.0023	84	

** Significant at 0.01 level
C.D. for comparison between temperatures = 0.0296
C.D. for comparison between substrates = 0.0320
C.D. for " Temperature x substrate=0.0783

APPENDIX XIV

Comparative yield of sporocarp of different species of Pleurotus raised in poly bags on paddy straw bits or twists.

Source	Sum of squares	df	Mean sum of squares	F Calculated
Total	3789495	39		
Treatment	1268250	9	207583.30	117.5111
A (Species)	1399472	4	349868.00	198.0573**
B (Straw type)	317731	1	317731.00	179.8447**
A x B	151047	4	37761.75	21.37659
Error	52995	30	1766.50	

** Significant at 0.01 level
C.D. for comparison of bits and twists = 27.14
C.D. for comparison of species = 42.91
C.D. for comparison of straw type x species = 60.69

APPENDIXXV

Average yield of sporocarp of P.sajor-caju from beds laid with spawn on grains of different cereals

Source	Sum of squares	df	Mean sum of squares	F Calculated
Total	208592.6	17		
Treatment	208541.8	5	41708.36	9860.13
Error	50.8	12	4.23	

C.D. = 3.6548

APPENDIX XVI

Ranks T_4 T_3 T_2 T_1 T_6 T_5

Average yield of sporocarp of P.sajor-caju from beds laid out with different pulse spawn

Source	Sum of squares	df	Mean sum of spares	F Calculated
Total	111599.4	14		
Treatment	111594.6	4	27898.65	58122.187
Error	4.8	10	0.48	

C.D. = 1.2603

Ranks T_4 T_1 T_3 T_2 T_5

APPENDIX XVII

Effect of various organic ammendments on the yield response of *Pleurotus sajor-caju*

Source	Sum of squares	df	Mean sum of squares	F Calculated
Total	1842496.5	20	336.	
Treatments	1840185.5	6	3066975.8	18579.84
Error	2311.0	14	165.07	

C.D. = 22.1346

Ranking T_3 T_2 T_5 T_1 T_4 T_7 T_6

APPENDIX XVIII

Cultivation on logs of trees

Source	Sum of squares	df	Mean sum of squares	F Calculated
Total	628268.82	47		
Treatment	623185.48	15	41545.698	261.5**
Error	5083.34	32	158.85	

C.D. = 20.9935

Ranking T_1 T_3 T_{16} T_2 T_{14} T_5 T_{10} T_8 T_7 T_{11} T_6 T_{13} T_9 T_{12} T_{15}

APPENDIX XIX

Influence of different types of bed for production of sporocarps of P.sajor-caju

Source	Sum of squares	df	Mean sum of squares	F Calculated
Total	83873	11		
Treatment	83373	3	27791	444.6**
Error	500	8	625	

C.D. = 14.89

** Significant at 0.01 level

APPENDIX XX

Influence of size of polythene bags on yield of Pleurotus sajor-caju

Source	Sum of squares	df	Mean sum of squares	F Calculated
Total	140554.7118	17		
Treatment	127467.5266	5	25493.5053	23.3757*
Error	13087.1852	12	1090.5988	

C.D. = 58.75

** Significant at 0.05 level

APPENDIX XXI

Comparative efficacy of different containers for lay out of beds by *Pleurotus sajor-caju*

Source	Sum of squares	df	Mean sum of squares	F Calculated
Total	223347.67	11		
Treatment	223326.33	3	74442.11	27901.8**
Error	21.34	8	2.668	

C.D. = 3.0754

Ranking T_4 T_1 T_2 T_3

APPENDIX XXII

Comperative efficacy of different types of straw beds on sporocarp production by *Pleurotus sajor-caju*

Source	Sum of squares	df	Mean sum of squares	F Calculated
Total	1877	21		
Treatment	1791	3	597.00	56.004
Error	86	8	10.66	

C.D. = 6.1474

APPENDIX XXIII

Comparative efficacy of straw of different varieties of rice in supporting sporocarp formation

Source	Sum of squares	df	Mean sum of squares	F Calculated
Total	24537.20	19		
Treatment	24003.00	4	6000.7500	
Error	534.20	15	35.6133	168.57**

C.D. = 8.9924

Ranking $\underline{V_5\ V_4}\ V_3\ V_1,\ V_2$

APPENDIX XXIV

Effect of frequency of watering the mushroom beds on sporocarp production in *Pleurotus sajor-caju*

Source	Sum of squares	df	Mean sum of squares	F Calculated
Total	1906033.5	15		
Treatment	1889909.2	3	629969.73	468.84
Error	16124.3	12	1343.6916	

C.D. = 55.2355

MONOGRAPHIC STUDIES ON EDIBLE SPECIES OF PLEUROTUS AND STANDARDISATION OF THE TECHNIQUES FOR LARGE SCALE CULTIVATION

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement

for the degree

DOCTOR OF PHILOSOPHY

Faculty of Agriculture

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ABSTRACT.

Mushrooms have long been recognized as a food because of their nutritive value and flavour and hence are grown on commercial scale on many parts of the world. In recent years cultivation of Pleurotus species are becoming popular in many of the tropical areas. No effort has been made in the past to collect, identify, describe and catalogue the Pleurotus mushroom flora of Kerala and standardise the techniques for their large scale cultivation. The present study was therefore initiated to achieve the above objectives.

Collection, identification and description of the Pleurotus flora naturally occurred in and around the College of Agriculture and parts of Trivandrum district were carried out with the help of a data sheet. Twenty species of Pleurotus properly identified were further subjected to detailed study. A key was constituted to enable easy identification of all the twenty described species.

Out of the twenty species described, eight species are new records for India.

Among the common cultivable species of Pleurotus, P.sajor-caju was found to be the most suitable mushrooms for the warm humid climatic condition of Kerala.

The optimum temperature for maximum growth of this mushroom was found to be between 15 to 30°C.

Comparative efficacy of various containers and substrates used for spawn production revealed that Maize and empty wine bottle were the best substrate and container respectively for spawn production.

Trials on cultivation of P.sajor-caju on logs of common trees revealed Mango tree log to be the best in getting higher yield.

Polythene bag was found to be the best container in supporting maximum sporocarp formation. Straw of local varieties of rice were better than high yielding varieties of rice in supporting sporocarp, formation.

Comparative efficacy of straw made into bits and small twists with six species of Pleurotus revealed higher yield of 775 g per kg of substrate with spawn of Pleurotus-sajor-caju establishing its superiority for cultivation in Kerala.

Cultivation of P.sajor-caju in different seasons of the year revealed that this mushroom can be successfully cultivated in Kerala all through the year on paddy straw.

The over all effect of growth regulators on the yield of *Pleurotus* spp. in general revealed that I.B.A. at 50 ppm was superior in enhancing yield over other growth regulators.

A comparative analysis of the proximate constituents of six species of *Pleurotus*, compared with that of *Volvariella volvacea* revealed that maximum per cent of protein was in *P.squarrosulus* and minimum in *P.cornucopiae*. The sporophores of *P.sajor-caju* contained minerals like calcium, magnesium and iron.

Refrigeration and dehydration were found promising for preservation of *Pleurotus sajor-caju*.