STRAIN IMPROVEMENT IN OYSTER MUSHROOM (*Pleurotus* spp.)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "**Strain improvement in oyster mushroom (*Pleurotus* spp.)** " 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 of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,

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CERTIFICATE

Certified that this thesis entitled "**STRAIN IMPROVEMENT IN OYSTER MUSHROOM (*Pleurotus* spp.)**", is a record of research work done independently by Miss. ANITHA. R 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

Mushrooms have attracted the attention of man from very ancient times. Their utilization as food as well as medicine were mentioned in ancient literature. Nutritional characteristics of mushrooms such as high protein, plenty of fibre, zero cholesterol and low calorie make them very attractive to those who are concerned about their diet.

Malnutrition in terms of 'protein deficiency' is becoming a major hazard in India and other developing countries. So the cultivation of edible mushrooms not only help in recycling agricultural wastes but also fill up protein gap prevalent among large population of the country.

Kerala state is blessed with a tropical climate and a variety of natural flora and agricultural resources as well as rich diversity of cultivated crops. The vast amount of agricultural wastes coupled with tropical climate can be successfully exploited for the commercial cultivation of various species of oyster mushroom. The great advantage of mushroom is its capacity to convert commercially valueless organic substances into high protein food. The State is also blessed with a rich natural wealth of tropical mushrooms suitable for domestication . Improving the qualities of available strains by scientific means can overcome the problems like erratic yield and incidence of pests and disease.

Under the above scenario the present programme was taken up with the major objective of evolving new strains of *Pleurotus* spp. of high yield and built in resistance / tolerance to major pests and weed moulds through hybridization and mutation techniques. This work envisages the evolution of an array of cultivable strains for the cultivator's preferential choice which will lead to steady and enhanced production of oyster mushroom in the State.

REVIEW OF LITERATURE

1. REVIEW OF LITERATURE

1.1 Collection and description of native flora of *Pleurotus* spp.

Species of *Pleurotus* were first collected by Sir. J. D. Hooker, mainly from West Bengal, Sikkim and Nepal areas during the last century and subsequently they were identified and described by Berkeley (1850 ; 1852 ; 1854). The first lot of Berkeley included nine species namely, *P. anserinus* Berk., on dead wood, *P. dryinus* (pers.). Fr. on standing tree collected from Kashmir, *P. eous* (Berk) Sacc., *P. hapalosclerus* Berk. both on tree trunks, *P.ninguidus* Berk. on dead wood, Sikkim; *P.petaloides* (Bull.) Fr. on dead wood, Nepal; *P. placentodes* (Berk.) Sacc. from Birch wood, Sikkim; *P. salignus* (Pers.) Fr. from Sikkim and *P. verrucaris* Berk. on dead wood from Darjeeling. This pioneer study by Hooker and Berkeley was followed by those of others.

P. sajor - caju (Fr.) Singer first recorded as *Lentinus sajor - caju* by Fries (1838) was reported from India at the beginning of this century by Lloyd during 1904 - 1919.

Graham (1915) from erstwhile Madhya Pradesh recorded *Pleurotus cretaceus* (Jacq) Fr. and *Pleurotus sapidus* Kalchb as occurring in India. He also recorded *P. fimbriatus* Bott. and *P. cornucopiae* (Paulet ex Pers.) Roll from India.

Bose (1920) recorded *P. flabellatus* Berk. & Br. Sacc. on dead wood or on the ground in Hoogly district, West Bengal and *P. sajor-caju* was also collected from West Bengal.

The type species of the genus, *P. ostreatus* which was first recorded as *Agaricus ostreatus Jacq.*, reported for the first time from India from Sonamarg in Kashmir by Murrill (1924).

Bose and Bose (1940) recorded *Pleurotus ostreatus* on *Picea morinda* in West Bengal and *Pleurotus squarrosulus* (Mont.) Singer was recorded from West Bengal.

Banerjee (1947) recorded *Pleurotus flabellatus* from Culcutta on dead tree *Caesalpinia pulcherrima* and *P. sajor - caju* was recorded from West Bengal. Moses (1948) recorded *Pleurotus ostreatus* from Baroda in Lucknow. *Pleurotus squarrosulus* (Mont.) Singer from Bombay (Chopra and Chopra, 1955).

In 1974 *Pleurotus squarrosulus* (Mont.) Singer was reported from Culcutta (Chandra), *Pleurotus ostreatus* from Uttar Pradesh (Ghosh *et al.*) and *P. dryinus* from Kashmir (Kaul and Kachroo).

Pegler (1976) reported that about 25 species of *Pleurotus* are known to occur in India. The herbarium materials of the collection of *Pleurotus* made by Hooker available at Kew were re-examined by Pegler (1976) who divided them into two groups according to the spore size. Those with spore size of 6 to 9 µm long were grouped in sub-tropical and tropical species and those with spore size over 10 µm length were grouped into temperate species. He included ten species in his monographic work and properly redescribed them. They were *P. anserinus* (Berk.) Sacc., *P.eous* (Berk.) Sacc., *P. flabellatus* (Berk. & Br.) Sacc., *P. fossulatus* (Cooke) Sacc., *P. aff gammellari* (Inzeg)

Sacc., *P. membranaceous* Massee., Sacc., *P. ninguidus* (Berk.) Sacc., *P. ostreatus* (Jacq : Fr.) Kummer, *P. placentode* (Berk.) Sacc., *P. platypus* (Cooke & Massee) Sacc.

The monographic studies of Agaricales of South West India by Sathe and Deshpande (1980) based at Pune, resulted in the discovery of three species of *Pleurotus* from Maharashtra *viz.*, *P. columbinus* Quel apud Bres from living guava tree from Pune, *P. euosmus* (Berk. apud Hessey) Sacc., from wood in Puttankudi, and *P. flabellatus* (Berk. & Br.) Sacc., from Pune on wood. Sathe and Daniel (1980) reported *P. sajor-caju* from Kerala and from Tamil Nadu by Sivaprakasam and Kandaswamy (1980).Another species of *Pleurotus, viz.*, *P. ostreatus* from Agumbe, Karnataka by Sathe and Kulkarni (1980).

Watling and Gregory (1980) recorded *P. flabellatus* (Berk. & Br.) Sacc. in Dras - Zoje - Lapass, Kashmir and from Dehradun, UttarPradesh by Puri *et al.*,(1981).

Bhavani Devi (1982) recorded and described five species of *Pleurotus* from Kerala namely; *Pleurotus* cornucopiae, *P. ostreatus*, *P. platypus*, *Pleurotus spathulatus* Pent., and *Pleurotus squarrosulus*, out of which *Pleurotus spathulatus* Pent. was a new record to the country.

Suharban (1987) conducted a detailed monographic study on *Pleurotus* spp. of Kerala and twenty species of *Pleurotus* were recorded and described in detail and a key for their identification has been devised. The species described were *P. citrinopileatus* Singer, *P. cornucopiae* Paulet : (Pers.)

Rolland, *P. dryinus* (Pers. ex. Fr.) Kummer, *P.oeus* (Berk.) Sacc., *P.flabellatus* (Berk.& Br.) Sacc., *P.luteoalbus* Beeli, *P.lignatilis* (Pers ex Fr.) Kummer, *P. mastricatus* Fr., *P. opuntiae* (Dur. & Lev.) Sacc., *P. ostreatus* (Jacq. : Fr.) Kummer, *P. petaloides* (Bull ex Fr.) Schulz., *P. platypus* (Cooke and Massee) Sacc., *P. pometi* (Fr.) Quel., *P. pubescens* peck., *P. pulmonarius* (Fr.) Quel., *P. salignus* (Pers ex Fr.) Kummer, *P. serotinus* Fr., *P. Squarrosulus* (Mont.) Singer, *P. subpalmatus* Fr., and *P. Ulmarinus* (Bull. ex. Fr.) Quel.

Geetha and Sivaprakasam (1993) reported *P. djamor* (Fr.) Boedijin a new edible species from Tamil Nadu, for the first time in India.

Balakrishnan (1994) conducted an intensive survey on *Pleurotus* spp. in selected localities of Thiruvananthapuram district and eight species of Pleurotus were recorded and among the eight species, *P. petaloides* (Bull. Fr.) Schulz and *P. sapidus* (Schulz. apud Kalchb) Sacc. syn. *P. cornucopiae* (Paulet ex pers.) Rolland were described in detail, domesticated and were reported to be amenable for large scale cultivation in Kerala.

1.2 Morphological characters of newly collected native isolates

1.2.1 *Pleurotus petaliodes* (Bull,. ex Fr.) Schulz

Suharban (1987) stated that the sporophores of this mushroom occur in groups and are imbricate. The pileus resembles the petals of flowers, which measured to 5 cm in diameter. The gills are linear, unequal and decurrent. Stipe absent or small. Basidia clavate with four sterigmata. Cheilocystidia

crowded and hyaline and measured 20 to 26 x 2 to 5 µm.. The species has been reported as edible.

1.2.2 *Pleurotus eous* (Berk.) Sacc.

Purkayastha and Chandra (1985) have given the morphological description of this species. Sporophores aggregated in tufts, sometimes growing on dead portion of living trees, usually sessile and imbricate. Pileus spathulate 3.5-9 cm in diameter pink flesh or cream coloured. Gills decurrent stipe small or absent, radially parallel. Basidia clavate with four sterigmata. Basidiospores hyaline, cylindrical, thin-walled. 6.0 - 8.0 x 2.5 - 3.5 µm spore print white.

1.3 Studies on Strain improvement programmes

1.3.1 Strain mixing

Moessner (1969) combined eight multispore cultures in various combination by growing pairs of strains in the same agar plates. The mycelium from the junction of two cultures was used for preparing and production of mushroom.

Kneebone *et al.* (1972) attempted to obtain new strains by mycelial anastomosis of several strains inoculated together into flask filled with grain and observed that most of the combinations yielded better.

Fusion of colonies of different strains induced by nicotinic acid in submerged cultures often produced superior yielding spawns (Stoller, 1974). Fritsche (1981) used two strains with different cap colour and fruit body morphology for strain mixing characters of the recombinant mushroom which showed intermediate cap colour.

1.3.2 Hybridization

1.3.2.1 Preparation of monospore cultures

Eger (1978) described the procedure of single spore isolation and preparation of monospore cultures. Single gemlings were selected from agar plates which were seeded earlier. The single gemlings were located and picked out under the microscope and transferred to separate plates.

1.3.2.2 Characters of monospores of different species of *Pleurotus*

Klingman (1943) showed that fertile single spore cultures varied in their growth rate, appearance of mycelia, sporophore morphology and productivity and Fritsche (1972) reported that single spore cultivars of *Agaricus bisporus* selected were yielded better than their parent strains.

In *Pleurotus sapidus* and *Pleurotus ostreatus* (Wang and Anderson, 1972) a correlation has been shown between the growth and yield of dikaryons and the fruiting behaviour of their component monokaryons.

Kneebone *et al.* (1974) selected a dozen of monospore cultures of which are higher yielding and selection of vigorous, high yielding, early fruiting monosporous isolates of *Volvariella volvaceae* have been tried by Chang *et al.* (1981).

In India the first attempt at raising of single spore isolates was made by Kumar and Munjal (1981). They selected 30 single spore isolates on the basis of rate of growth and the isolates gave 12.3 per cent to 21.98 per cent increased yield over the parent strain.

A correlation has been reported between monokaryotic fruiting and yield in *P. flabellatus* (Samsudin and Graham, 1984) and in *P. sajor-caju*

(Singh and Mehta, 1983) in 35 per cent of the monokaryotic isolates, small pinheads were formed and they have never developed into fruit bodies.

Bhandal and Mehta (1985) raised more than 200 single spore isolates and they were selected with regard to mycelial characteristics, spawn run, yield and sporophore characters.

Kumar and Munjal (1981) carried out single spore isolation by raising 51 single spore isolates from strain 11 (*Agaricus bisporus*) and five high yielding isolates could be obtained. Morphologically maximum variations in monospore isolates were recorded in stipe length and pileus : stipe weight ratio, while minimum in pileus diameter, stipe diameter and average weight of mushroom (Bhandal and Mehta, 1985). The variation was attributed to environmental and genotypic differences.

Bisko and Kholondy (1989) have used monospore selection method for obtaining high productive strains of *P. ostreatus*. The selection indexes at monokaryotic and dikaryotic stages included mycelial growth rate, primodia formation, utilization of different nutrient sources, temperature independence of mycelial growth and fruit body formation, productivity and biological value of fruit bodies. Radial growth of *P. ostreatus* dikaryon depend on the growth of monokaryon composing them (Bisko and Kosman, 1989).

Single spore isolates produced a strandy and silky type of mycelial growth with a growth rate greater than that of the parent (Suman, 1993). Monokaryotic fruiting was observed in *P. ostreatus* (Takehara *et al.*, 1993).

Bhandal and Mehta (1994) opined that it might be possible to isolate monokaryotic mycelia tolerant to specific problems like disease resistance colour etc. and intermating of such isolates was likely to give desirable ones.

Pal and Singh (1994) reported that monokaryons established from single basidiospores of *P. sajor-caju* on potato dextrose agar showed wide variation for several colony characteristic and growth out of 19 monokaryons, best were very slow growing ones as compared to the parent culture.

1.3.2.3 Pairing of compatible monospore cultures

Development of heterokaryons in *P. ostreatus* and related species has been reported by various workers (Croft and Smichen, 1965 ; Eugenio and Auderson, 1968). Mating of interstock monokaryons in *P. ostratus* has resulted in dikaryons with higher fruit body yield (Wang and Anderson, 1972). In *Pleurotus sajor-caju* dikaryotic hybrids were obtained by the somatic recombination of interstock monokaryons (Roxon and Jong, 1977).

According to Bresinsky *et al.* (1987) high amount of variability can be generated by intermating of different strains of *Pleurotus*. Due to the existence of multiple allelism in incompatibility factors, monokaryotic mycelia of *P. ostreatus* derived from different fruit bodies (representing different strains collected from different locations / host) intermated at a 100% rate compared to an inbreeding capacity of only 25%.

Martonffy (1987) released two varieties developed by crossing among different strains of *P. florida*. Ghosh and Chakravarty (1991) opined that in *Pleurotus sajor - caju* selective dikaryotization exert some exciting changes over the traditional cultures. Improvement in quality characters governs fast

colonising ability leading to crop earliness, reduced percentage of bud mortality, size, shape, colour of pileus and also protein content of the fruit bodies.

According to Singh (1994) interstrain mating groups or biological species occur in many heterothalic basidiomycetes population. Mating within a single biological species occurs readily while intergroup matings are wholly or partially incompatible. He pointed out that populations belong to the same species when they are able to interbreed and to produce viable offspring, provided that an absence of the infertility is caused only by genetic parameter operating in the entire sexual life cycle.

Pahil *et al.* (1994) conducted strain improvement studies of high temperature tolerant cultivated and wild species of *Agaricus.* Inter and intrastrain hybridization of these strains (W 19, W 20 and W 2-F) resulted in dikaryons producing higher yield, a more regular weekly flushing pattern and better quality fruit bodies.

1.3.3.1 Interspecific hybridization

Elliotd (1972) reported the methodology for mating single spore isolates. The resultant recombinant was then tested for desirable characters. The use of cross breeding in *Agaricus bisporus* has been confirmed by Raper and Raper , 1972.

Pleurotus sapidus and *Pleurotus florida* have been reported to intermate with *Pleurotus ostreatus* (Anderson *et al.*, 1973 ; Eger *et al.*, 1976). *P. abolonus* and *P. cystidiosus* were interbreedable through confirmations between monokaryotic mycelia of both taxa resulted in a 50% compatibility compared to an expected 75% or 100% compatibility in case of interstrain

crosses within a single species. In interspecific hybridization a collection may be categorized as different species because it may be showing different morphology on different host or under locations of morphological and physiological differences between two species.

Three European *Pleurotus* species namely *P. ostreatus*, *P. columbinus* and *P. pulmonarius* were interbreedable with *P. sapidus* (Manning, 1977) and *P. columbinus* was interbreedable with *P. florida* (Hilber, 1978).

May and Royse (1988) collected sixty cultures of different *Pleurotus* species from world wide sources and studied interspecific allozyme variation. Takehara *et al.* (1993) opined that interspecific protoplast fusion can be performed between *P. ostreatus*, *P. pulmonarius* and *P. cornucopiae* var. *citrinopileatus*. The strains obtained have slower growth rates than their parents.

Bhandal and Mehta (1994) suggested interspecific hybridization for developing new variability in the absence of sufficient germplasm or desirable traits within a species.

1.3.4 Mutation Breeding

Bhandal and Mehta (1994) opined that in the absence of sufficient germplasm basidospores, non - sexual spores or hyphal segments can be exposed to physical or chemical mutagens to create variants. Mutations and strain variation in *Pleurotus* influenced by the substrate were known (Hilber, 1982).

1.3.4.1 Gamma Irradiation

In irradiation mutation, radiations like X-rays, UV and gamma rays are used to increase the frequency of mutation. Spores or hyphal fragments are

exposed to irradiation for variable period to determine the dose at which highest percentage of mutants among survivors are isolated (Pandey and Tewari, 1994)

Zhu *et al.* (1994) studied the mutagenesis of protoplasts from *Pleurotus sapidus*. Three isolates irradiated with UV - radiation produced no spores and others produced only a few spores.

Teow *et al.* (1995) conducted cross breeding after irradiating the monosporous isolates with gamma rays. The two selected hybrids were better yielders than their wild parents signifying that mutagenesis followed by cross breeding was an effective means of strain improvement of edible mushroom through the hybridization of genes of agronomic importance.

1.3.4.2. Chemical mutation

The use of certain chemicals as mutagens which can act either directly on pre-existing DNA or function as analogues to the DNA - bases has been reported in mushroom breeding (Fincham and Day, 1971)

Calam (1969) described the general methodology of chemical treatment for mutation. Suspension of spores or cells were prepared in buffer (pH 6-7) and to this the mutagen was added. The chemicals are usually used at a concentration of 0.5 M. The suspension cell should be diluted approximately and plated on agar medium to yield distinct individual colonies. Generally a kill of 99% happened. Several slopes from each culture should be made and carefully examined for morphological effects and good growth.

Breeding of the sporeless mutant is one of the objectives of genetic improvement in *Pleurotus* spp. A number of workers reported sporeless mutants in *Plerotus* spp. (Ohira,1979; Chang *et al.*,1985)

According to Visscher and Pompen (1985) the completly sporeless strain Somycel 3210 produced very small fruit bodies with long stem does not appear to be promising for Dutch growers.

1.3.5 Growth of *Pleurotus* species in different solid media

Potato dextrose agar (PDA) and Potato dextrose agar fortified with yeast extract supported maximum growth of *P. sajor-caju* and this medium was followed by Czapek (Dox) agar + casein hydrolysate and carrot potato agar with respect to the maximum mycelial growth of *Pleurotus* spp. (Jandaik and Kapoor, 1975).

Rangad and Jandaik (1977) observed that, yeastal PDA supported maximum mycelial growth followed by PDA in the case of *Pleurotus* Spp. namely *P. eryngii*, *P. cornucopiae*, *P. ostreatus* and *P. florida* and malt agar in the case of *P. eryngii*.

Zadrazil (1978) reported that, *Pleurotus* species grow well on several synthetic nutrient media. He suggested the media with the following composition as a good nutrient media for growth. Malt extract soybean flour 100 g; Peptone 1 g; KH_2PO_4. 0.5 g; $MgSO_4$. 7 H_2O 0.5 g; Feelz (1 % solution) 1ml. yeast extract 0.0 1g; agar agar 15 g; water 1 liter.

Subarhan (1987) reported that among the solid media tested, *Pleurotus* species showed maximum growth on oats agar medium which was followed by potato dextrose agar (PDA).

Ramos (1967) reported that addition of coconut milk in a liquid medium enhanced the mycelial growth in the case of *Volveriella volvaceae*.

Balakrishnan (1994) conducted an experiment to study the effect of coconut milk on the mycelial growth of *Pleurotus* species. The study revealed that oats agar blended with 40% coconut milk (modified oats agar) has supported the maximum growth of all the species tested followed by common oats agar medium. PDA was found effective for all the species except for *P. petaloides*.

Best mycelial growth was obtained when cultured in malt yeast (MY) medium (1 % malt extract, 0.4 % yeast extract at PH 6 and at > 80 % relative humidity (Eguchi *et al.*, 1994).

Fasidi and Olorunmaiye (1994) assessed the mycelial growth of *Pleurotus tuber-regium* in a basal medium supplemented with various compounds. Of the carbohydrates tested, glucose gave the best results followed by manitol, maltose and dextrin. All these significantly enhanced mycelial growth. Cellulose gave the poorest results. Of the N compounds tested, yeast extract produced maximum mycelial growth which was comparable to that obtained with glucose.

1.3.6. Reaction to insect pests and weed moulds of selected mutants, dikaryons, native isolates

The cultivated mushrooms are generally attacked by pests like sciarids, phoridss and mites (Fletcher *et al.* 1986; Nita Bahl, 1988; Sandhu and Brar. 1980). The nature of damage brought about by these pests has been described

by various workers. Kneebone (1968) reported that, mushroom flies and their larvae reduce the crop and lower the quality of mushrooms. Phorid larvae are known to cause damage by feeding mainly on the mycelium but sometimes they also tunnel into the mushrooms (Atkins, 1971).

Szudyga (1978) reported severe occurrence of dipteran flies on *P. sajor-caju*. Sandhu and Brar (1980) observed 32.7 per cent infestation of mushroom beds in Punjab by sciarid flies. Moorthy *et al.* (1991) observed the occurrence of *Megaselia* spp, a phorid fly in Tamil Nadu for the first time in India on oyster mushroom beds. The maggots feed on the mycelium causing extensive damage in spawn running stage and the decayed beds were found contaminated with moulds like *Trichoderma* spp.

Rajan *et al.* (1991) in Kerala reported the occurrence and heavy damage by a beetle *Staphylinus* spp. on oyster mushrooms for first time in India. The beetle feeds on both the pileus and stipe of oyster mushrooms resulting in great economic losses.

Occurrence of cecids on mushrooms has been reported for the first time from India. Unlike other musrhroom flies, the adult midges may never be noticed until swarming took place. Young larvae feed on growing mycelium. Under heavy infestation the larvae moved upwards, fell down the bags in heaps. This pest has a rapid rate of multiplication which is obviously a potential danger and to prevent serious outbreaks, Cecids must be kept out of the early spawn run at all costs (Johal and Kaushal, 1991).

Sharma and Jandaik (1980) reported that the green mould (*Trichoderma viride*) is a damaging competitor mould in *Pleurotus* beds. Bharadwaj and

Seth (1983) reported, the *Trichoderma viride* as a common contaminant of spawn and noticed both as a serious competitor as well as a pathogen during spawn run and cropping of mushrooms. A serious out break of the green mould (*Trichoderma* spp.) which causes heavy loss in mushroom industry was reported by Staunton (1987).

Das and Suharban (1991) reported that many fungi, mainly species of *Trichoderma, Aspergillus, Penicillium, Mucor, Rhizopus, Chaetomium* and *Coprinus* were found constantly attacking the beds of oyster mushrooms in Kerala. These organisms were found to inhibit the spawn run at various stages of growth, sometimes resulting in complete failure.

1.3.7. Organoleptic studies

A study conducted by Desai *et al.* (1991) revealed that consumer acceptability of *P.sajor-caju* was poor due to the tough texture of the stipe and unattractive colour of the pileus but its flavour was found to be good.

In a comparitive study Balakrishnan (1994) showed that *P. sapidus, P.membranaceous* and *P.petaloides* obtained maximum consumer acceptability with respect to colour, appearance and flavour. Overall acceptability of these species was significant when compared to the standard species *P.sajor- caju* and *P. flabellatus* which were found inferior in all the qualities.

MATERIALS AND METHODS

MATERIALS AND METHODS

2.1 Collection and description of native flora of *Pleurotus spp.*

Field survey was conducted in selected areas of Thiruvananthapuram and Kollam districts to collect native species of *Pleurotus.* Identification of the newly collected specimens was carried out following the procedure outlined by Sathe *et al.* (1980) and Nair and Bhavani Devi (1986). Comparison of the morphological characters was done following the published works in literature (Natarajan and Manjula, 1983 : Purkayastha and Chandra, 1985, Suharban, 1987 : Balakrishnan, 1994).

Specimens were collected from the field at different stages of development and the general observations like locality, type of substrate, date of collection etc. were recorded in the field itself and the specimens transferred to the laboratory by packing in waxed paper sheets. The collections were serially numbered and details recorded. The detailed characters were enumerated following the technique and proforma developed by Nair and Bhavani Devi (1986).

Spore prints were made by putting the pileus over clean and sterile microscope slide with gills facing towards the slide. Spore prints were also prepared on black / white sheets of paper which were incubated in humid chamber.

2.2 Morphological characters of newly collected native isolates

The micro characters were studied either with free hand sections mounted in lactophenol or from tissue maceration. For this, the tissue was

first kept in 10 per cent potassium hydroxide solution for five seconds before being stained. The stain was allowed to act for about 10-15 minutes and the specimens mounted in 10 per cent potassium hydroxide. The tissue was macerated by placing it on a glass slide and gently tapping with blunt end of a needle.

Macrochemical and metachromatin reactions of various parts of the basidiocarps were studied following the methods of Watling (1971) and Singer (1975). The test was carried out on the surface context of pileus, stipe, stipe apex and base. Fresh tissue (one cm square) was dissected out and placed in the depression in a porcelain plate. A few drops of Melzer's regent were applied and the reaction indicated by colour changes was recorded. Melzer's reaction of spore mass was detected following the method of Walting (1971). Small portion of spore print was transferred to clean slide and mounted in Melzer's solution and colour changes was noted under microscope. The reaction was graded as amyloid if positive and non-amyloid if negative.

All the microscopic characters were recorded using a camera lucida attachment on the microscope.

2.3 Studies on strain improvement programmes

2.3.1 Strain mixing

Commonly cultivated species of *pleurotus* namely *Pleurotus sajor - caju* (Psc - 1) *P. florida* (Pf - 1), *P. platypus* (Pts - 1) *P. eous* (Pe - 1), *P. citrino pileatus* (Pc - 1) and Ananthan (H - 1) were selected for the study. Cultures of these species were prepared in PDA slants and were purified by repeated subculturing.

Strain mixing was done by two methods

1. Mixed spawn prepared by inoculating cultures of two different species together in to one packet / bottle of spawn substrate.

Paddy grain was used as the spawn substrate. The paddy grain was pre-soaked for six hours and then boiled in fresh tap water. Boiling was stopped when the outer husk of the grain was just split. The grains were then drained and cooled by spreading over a gunny piece. Then the substrate was mixed with calcium carbonate at the rate of 50 g for 1 Kg grain substrate. The processed grain substrate was then filled in drip bottles and polypropylene packets provided with an one inch PVC ring at the neck and plugged with non-absorbant cotton. The bottles and packets filled with the substrate were sterilized at 1.02 Kg / cm^2 pressure for two hours in an autoclave.

Mixed spawn was prepared by inoculating cultures of two different species together in one packet of spawn substrate. Like wise mixed spawn the culture mixing was done in all given possible combinations of the selected species. Three replications were given for all the treatments and they along with control were incubated at laboratory conditions (28 ± 1^0C). Spawn with an age of twenty five days growth was used to layout beds using paddy straw as substrate and following the standard polybag technique of cultivation. Three replications were given for each treatment including control of Spawn prepared out of each parent culture and incubated at dark for spawn run. Beds were transferred to cropping room of the mushroom house after complete spawn run was attained and polythene wrap removed and watered twice or thrice daily as required. Observations namely number of days for full spawn

run, number of days taken from spawning to first harvest, sporocarp yield (from three flushes), incidence of pest and diseases and sporocarp characters etc., were recorded.

2. Mixed spawn prepared by mixing different fully grown spawn

After completing full spawn growth, spawn mixing was done by mixing spawn of different species in all the possible combinations in equal proportions. Beds were laid out on paddy straw using the mixed spawn at the rate of 3 beds / treatment along with the control beds. The fruiting criteria were recorded and compared.

2.3.2 Hybridization

2.3.2.1 Preparation of Monospore cultures

Medium matured medium sized and healthy sporocarp of each parent strain obtained from paddy straw beds was selected for spore extraction. The stipe was cut and removed with a clean blade. The pileus was then fixed on a piece of fresh banana pseudostem with pins as gill side faced outwards. This sporocarp was then kept over sterile petridishes so as to face the gill side of the pileus towards the sterile side of the dish without touching the inner surface of the dish. After 10 minutes the pileus was removed and dilute spore suspension was prepared by adding the spores in sterile distilled water. The dilution of the suspension was adjusted so as to obtain only 5-8 spores in a microscopic field under low power of a microscope. One loop of such dilute spore suspension was streaked on sterile plain Agar in Petriplates and incubated at (28 $\pm$ 1^{0}C) for 24 hours. Single spores were allowed to

germinate and the germlings were marked under microscope.Then they were picked by spatula like needle and transferred to sterile PDA slants. About 10-15 such single spore isolates were selected from each parent strain and assigned a number to each monokaryon (MK) culture.

2.3.2.2 Cultural characters of monospores of different species of *Pleurotus*

The selected monospore cultures were inoculated in sterile PDA slants and incubated at laboratory conditions (28 ± 1^{0}C). On the seventh day of inoculation observations like radial growth of mycelium, colony colour, type of mycelium were recorded and tabulated. (Plate 1)

2.3.2.3 Pairing of compatible monospore cultures

The monospore cultures of different stocks were initially tested for their compatibility pattern by growing them in petri plates containing PDA. Four single spore isolates were selected from each parent strain for mating studies. and they were grown on PDA in petri plates. Five mm disc of 10 day old cultures of these monokaryons of each breeding set were placed at a distance of two cm in petriplates containing Potato dextrose agar medium. In this manner mating and of the monokaryon cultures of plates one to four of each parent were performed separately as given under. The compatible pairs were marked and selected out by this preliminary trials.

Monokaryon germlings paired Monokaryon germlings paired

Pts -1	(1)	(2)	(3)	(4)	Pfs -1	(1)	(2)	(3)	(4)
Pc-1 (1)	-	-	-	-	H-1 (1)	-	-	+	-
(2)	-	+	+	-	(2)	-	-	-	-
(3)	+	-	-	-	(3)	-	-	-	-
(4)	-	-	-	-	(4)	-	-	-	+

The compatible pairs selected from the preliminary trials were paired by growing them in sterile PDA slants at 2 cm apart for each pair. The plates were then incubated at 25 ± 1°C in an AC room. Periodical observations were made on the mating behaviour of the mycelium on the plates. The successful matings of the mycelium has shown a thick stranded form at the junction of contact of the monoculture pairs (Plate 2). Such dishes were selected, small portion from the junction of contact of the mycelium were cut out and grown on PDA slants. The resulted cultures were subjected to microscopic observation to confirm the dikaryotization by the presence of clamp connections in the growing mycelium.

The dikaryons thus obtained were brought in to grain spawn following the method described elsewhere in this chapter. Twenty five days old fully grown spawn were then subjected to cultivation trials using paddy straw as the substrate following the standard method.

Observations on the fruiting behaviour of each dikaryon were made, data tabulated and analysed statistically.

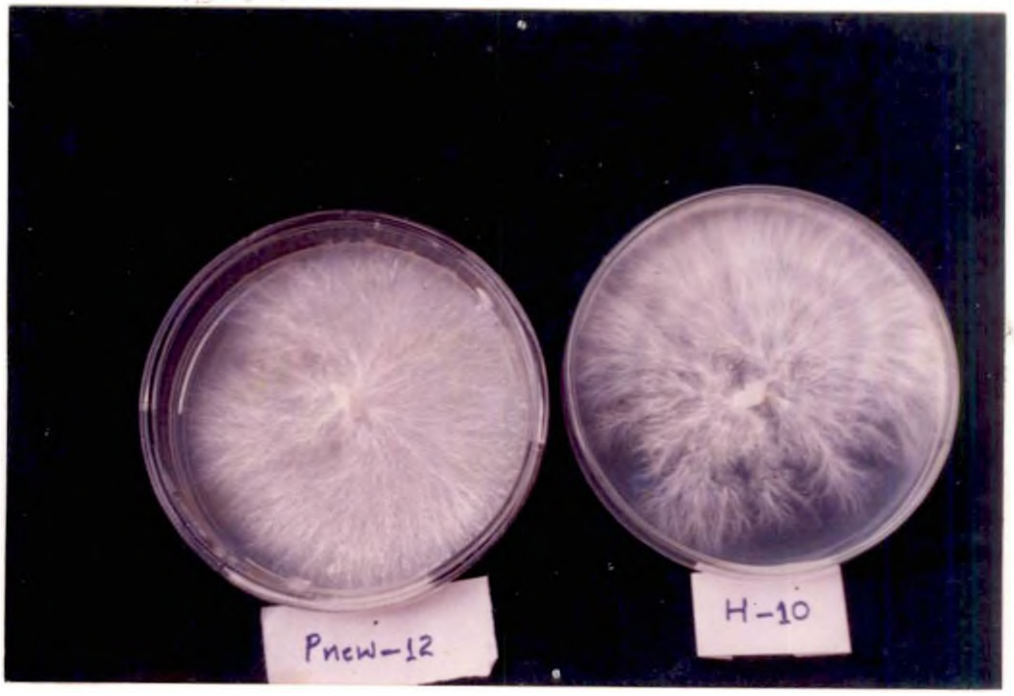
2.3.3 Mutation Breeding

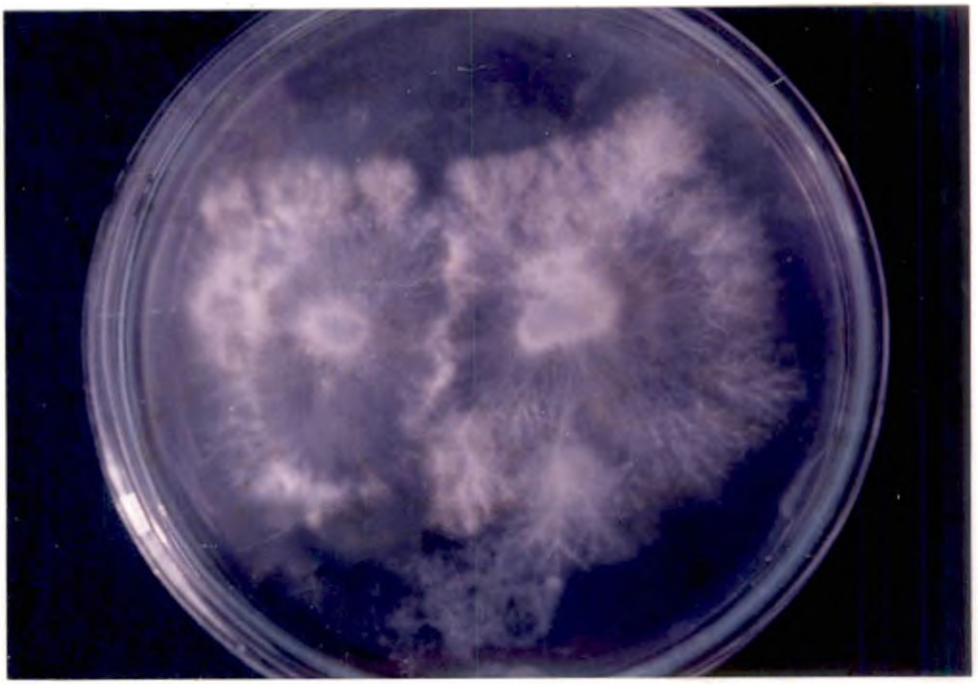
2.3.3.1 Gamma irradiation

Irradiation was done by subjecting selected cultures of *Pleurotus* spp., to gamma irradiation at the Radio Tracer Laboratory of Kerala Agricultural University at Vellanikkara campus. Pure cultures of the selected species and monokaryons were inoculated on Potato dextrose agar medium and incubated at laboratory conditions (28 ± 1°C). On the seventh day of inoculation the

Plate 1 Monospore cultures

Plate 2 Successful mating of the mycelium


PnCW-12
H-10



cultures were subjected to different levels of gamma irradiation viz., 0.5 Kr, 1.0 Kr, 1.5 Kr, 2.0 Kr and 2.5 Kr. Three replications were kept for each treatment. The irradiated cultures were transferred to PDA slants and incubated at laboratory conditions along with control.

Spawn was prepared using the irradiated cultures in paddy grain substrate. Beds were laid out using paddy straw as substrate. Two replications were kept for each treatment along with control. After complete spawn run the beds were transferred to cropping room of the mushroom house and polythene cover removed. Watering was done twice / thrice daily as required.

Observations on the number of days from spawning to first harvest, average sporocarp yield (from three flushes), sporocarp characters and incidence of pest and diseases were recorded and tabulated.

2.3.3.2 Chemical mutation

Chemical mutagens namely, ethyl methane sulphonate (EMS) and methyl methane sulphonate (MMS) were utilized for chemical mutation. Three concentrations viz. 500 ppm, 1000 ppm and 2000 ppm of the two chemicals were prepared by using distilled sterile water.

2.3.3.2.1 Treatment of cultures with chemical mutagens

The selected cultures were inoculated on PDA slants and incubated at room temperature (28 ± 1^{0}C). On the seventh day of mycelial growth, five ml each of the desired concentrations of the above two chemicals were poured in to the culture tubes of different species separately and kept undisturbed for 30

minutes. In another set, the treated cultures were kept for 60 minutes. Each treatment was replicated thrice. At the end of the required time of the treatment, the chemicals from the slants were decanted and repeatedly washed with distilled water. The treated cultures were subcultured in PDA slants and incubated at laboratory conditions.

2.3.3.2.2 Treatment of spores with chemical mutagens

Medium matured, medium sized healthy sporocarps of the test fungus were harvested. Sporocarp of each species was placed separately on sterile slide placed inside sterilized petridish with gill side downward to extract spores. After 10 minutes the sporocarp was removed and five ml each of the desired concentrations of the above two mutagens were poured over the spore print prepared on the slide in petridishes containing spores of different species separately and kept for 30 minutes. In another set of the same experiment the spores were treated for 60 minutes in the similar manner. Each treatment was replicated thrice and done for all the selected species of *Pleurotus*.

Few drops of spore suspension prepared as above were poured into sterile petridishes and then sterile PDA was poured over it aseptically and tilted well in order to spread the spores uniformly on the medium. The dishes were incubated at laboratory conditions (28 $\pm$ 1^{0}C). On the fifth day of incubation mycelial growth from petridishes were transferred to fresh PDA slants. Two such transfers were again made in an interval of seven days to confirm the purity of the cultures.

The resulted cultures of the above experiments were brought into spawn using paddy grains as substrate. Beds were laid out using the above spawn in paddy straw substrate in uniform size. Observations were made on number of days of spawn run, number of days from spawning to first harvest, sporocarp yield, sporocarp characters and nature of reactions to pests and competitor weed moulds.

2.3.4 Comparative cultural characters of various *Pleurotus* spp. and their mutants

To assess the comparative mycelial growth of the selected species of *Pleurotus*, four standard solid media viz., potato dextrose agar (PDA), tapioca dextrose agar (TDA), oats agar (OA) and yeast extract agar (YEA) medium were used. The media were prepared following the standard method. 60 ml of each media were taken in 250 ml conical flask. Then 40 ml coconut milk (filtered extract of 12 month old coconut endosperm) were added to each of the flask and swirled well for proper mixing of the contents. The modified media along with control media were sterilized at 1.02 kg cm^{-2} pressure for 20 minutes.

The melted media were poured into sterile petridishes and inoculated by 5 mm culture discs of the selected species cut out from an actively growing culture. Likewise all the selected species of *Pleurotus* were inoculated in all the modified media along with control and incubated at laboratory conditions

(28 ± 1°C). Mycelial growth was measured on fifth and seventh day of inoculation as radial growth. The data were tabulated and analysed.

2.3.5 Reaction of selected mutants, dikaryons and native isolates to insect pests and weed moulds

A separate replicated trial was conducted during the month of November - December, 1997 to evaluate the type of reactions of the newly evolved dikaryons, mutants and native isolates to the commonly occuring pests and competitor moulds.

Uniform size beds were laid out in paddy straw substrate using grain spawn of the desired dikaryons mutants and native isolates replicataed thrice. The beds were incubated in a dark room under identical conditions. Observations were made at three days interval on the beds for noting the incidence of various pest and competitor moulds. Observations were recorded in a graded manner according to the severity. The different grades used to note the severity were

(i) - (no incidence) (ii) + (mild incidence)

(iii) ++ (severe incidence) (iv) +++ (very severe incidence)

After the spawn run is over the beds were transferred to the cropping room polythene cover removed and watered daily as required

2.3.6 Organoleptic studies

Organoleptic test was conducted to evaluate the comparative quality of selected dikaryons, mutants and native isolates. Both the raw and cooked

mushrooms were subjected to comparison following the Kruskal - Wallis statistical analysis (Siegel and Castellan, 1988). In the raw mushrooms, appearance, colour, flavour and taste were assessed with a panel of five judges.

The selected dikaryons, mutants and native isolates were separately cooked with equal quantity of meat masala. The cooked samples were subjected to sensory evaluation with a panel of 5 judges with respect to appearance, colour, texture and taste of each item of mushroom.

RESULTS

3. RESULTS

3.1 Collection and description of native flora of *Pleurotus* spp.

Attempts were made to collect native flora of *Pleurotus* spp. by doing intensive survey for the same during monsoon periods. Among the various isolates made, two efficient ones were described and identified as listed under Table 1.

Evaluation on growth and fruiting behaviour of these two isolates were done following the standard methods. It was found that these isolates were amenable for large scale cultivation under Kerala conditions. Studies on quality parameters and consumer acceptability have revealed that these isolates were comparatively better than other species of *Pleurotus* obtained from various mushroom research centers of the country. The cultures procured from various sources and those which were included in the present study have been listed in Table 2.

Table 1. Native species of *Pleurotus*

Isolate No	Name of mushroom species collected and identified	Substrate	Location and period of collection
Pet - 2	*P.petaloides* (Bull. ex Fr) Shculz	Dead stumps of *Cocos nucifera*	Kollam, December, 1996
Pe - 1	*P.eous* (Berk.) Sacc.	Dead stumps of *Albizia lebeck*	Thiruvananthapuram June - 1996

Table 2. Cultures included in the study

Sl. No	Isolate No	Name of species	Sources
1	Psc - 1	*Pleurotus sajor-caju* (Fr.) Singer	N.C.M.R.T., Solan
2	Psc - 2	*P. sajor-caju* (Mutant - M_2)	T.N.A.U., Coimbatore
3	Pf - 1	*P. florida* Eger	College of Agriculture, Killikulam, Tamilnadu
4	Pf - 3	*P. florida* Eger	I.I.H.R. Bangalore
5	Pts - 1	*P. platypus* (Cook & Massee) Sacc.	Tamil Nadu
6	Pc - 1	*P. citrinopileatus Singer*	Tamil Nadu
7	Pfb - 1	*P. flabellatus* (Berk & Br.) Sacc.	C.F.T.R.I., Mysore
8	Pfh - 1	*P. florida* Eger	Holland
9	H - 1	Ananthan - an interstock hybrid developed at college of Agriculture, Vellayani	
10	Pet - 2	*P. petaloides* (Bull. ex Fr.) Schulz	Native isolate
11	Pe - 1	*P. eous* (Berk.) Sacc.	Native isolate

3.2 Morphological characters of newly collected native isolates

Morphological characters of the newly collected native species of *Pleurotus* were studied in detail as explained under materials and methods. The description of these isolates which were subsequently included in the detailed studies are summarised below;

(Plates 3 & 4 and Fig. 1 & 2).

3.2.1 (i) *Pleurotus petaloides* (Bull. ex Fr.) Schulz

Habitat - Dead stumps of *Cocos nucifera*

Sporophores - Usually in bunches or solitary

Pileus - Spathulate, petaloid or imbricate. 3.5 - 10 cm. in size and more or less dimidiate, white when fresh, turn yellowish at over maturity. Margin of the pileus often involute at first later expanded.

Gills - Decurrent, soft, linear and equal.

Stipe - 1.5 - 2.5 cm X 0.75 - 1.0 cm size. Context thin and white.

Basidia - Clavate with four sterigmata

Spores - Ovoid to ellipsoid, hyaline, non-amyloid thin walled and measured 6-9 x 5-6 µm spore mass creamy white.

Edibility - Edible

3.2.2 (ii) *P. eous* (Berk.) Sacc.

Habitat - Dead stumps of *Albizia lebeck*

Sporophores - Small stiped or sessile

Pileus - Usually sessile, solitary or gregarious imbricate 3.5 - 9.0 cm in size initially spathulate, flabelliform when old pink, flesh cream coloured coriaceous, margin incurved.

Stipe - Absent, even if present very small1. 2 - 1.4 X 0.5 - 0.7 size, context light pink / cream

Gills - Whitish or creamish, radially parallel

Basidia - Clavate with four sterigmata

Spores - Cylindrical, spore print creamy white, spores measured 6 + 0.8 x 2.5 - 3.5 µm in size, hyaline

Edibility - Edible

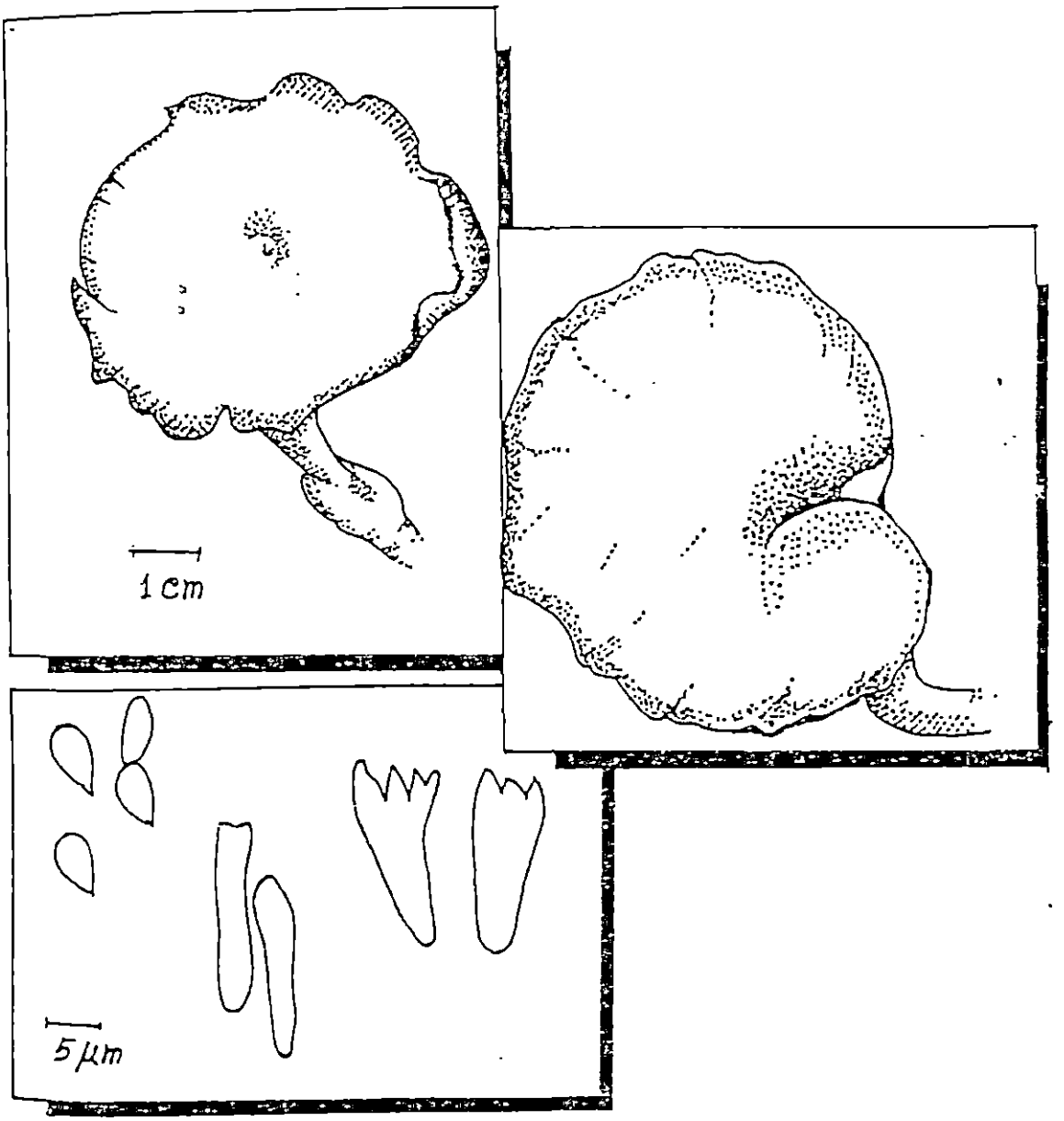


Fig. 1. *Pleurotus petaloides*

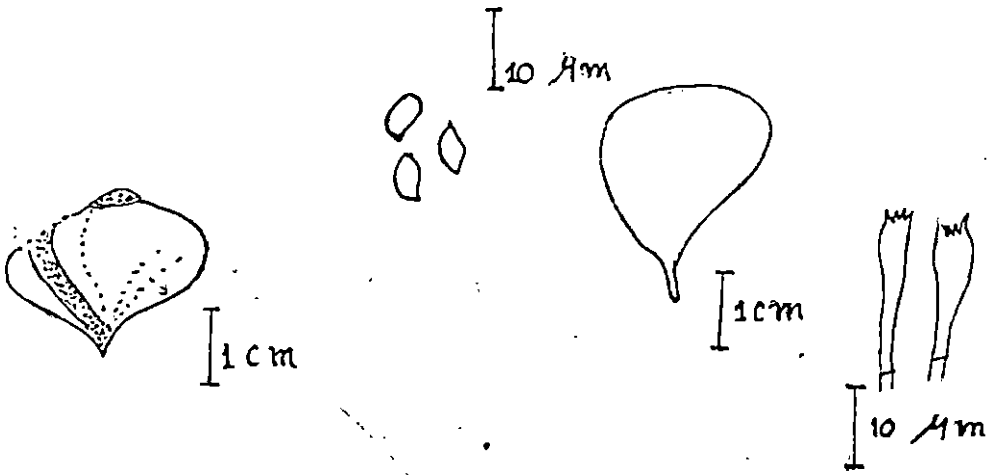
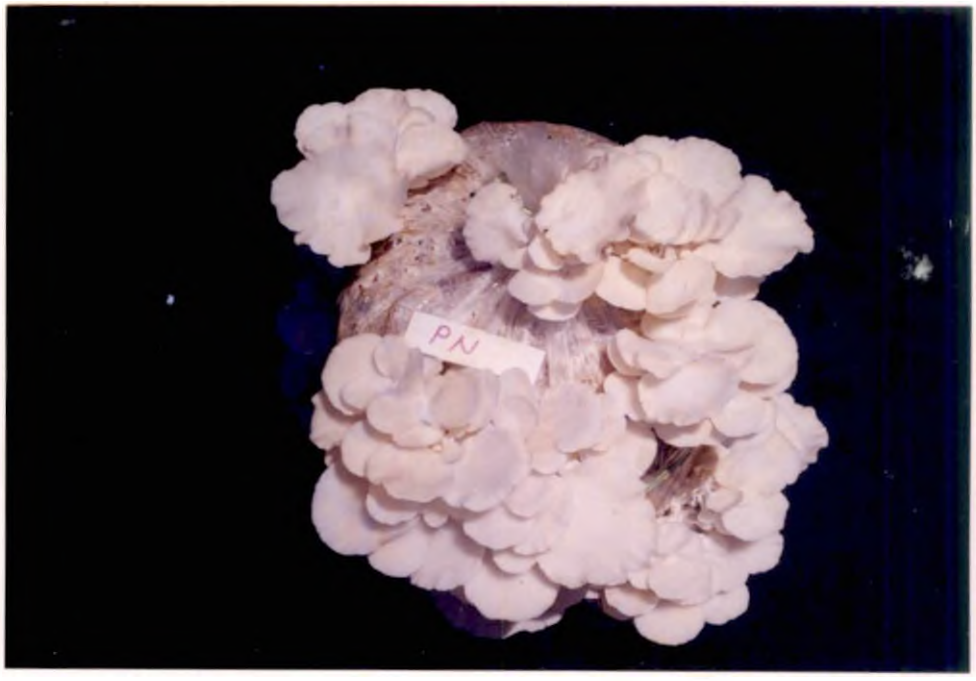


Fig. 2. *Pleurotus eous*

Plate 3 Native isolate - *P. petaloides*

Plate 4 Native isolate - *P. eous*


P. pulmonarioides
Local


PN

3.3 Strain improvement programmes

3.3.1 Strain Mixing

Mixed spawn was prepared and cultivation trials conducted following the methods described under materials and methods.

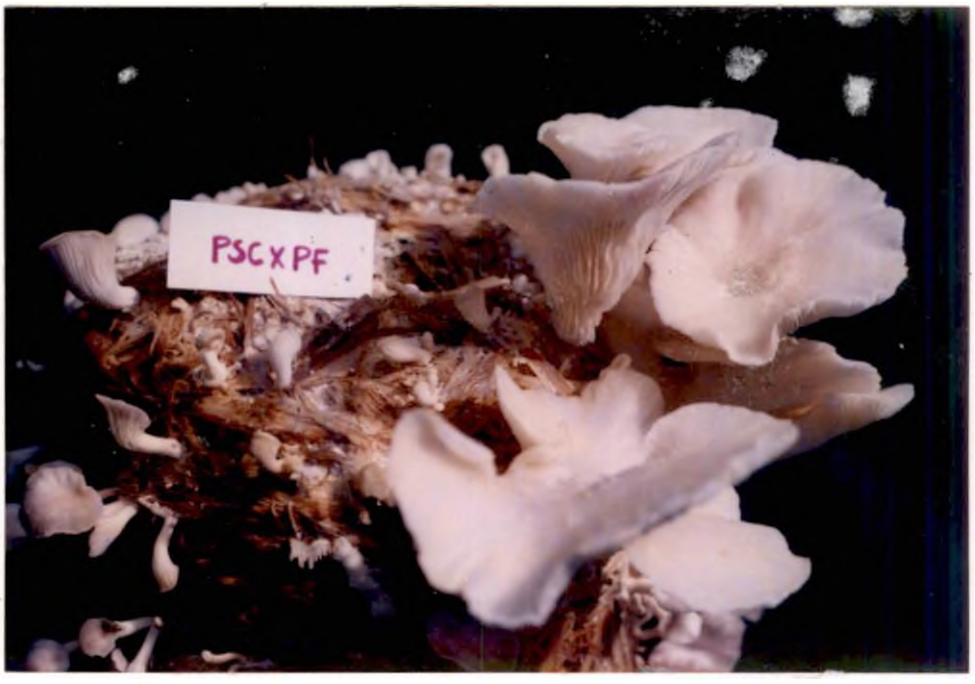
The recombined cultures (mixed spawn prepared as described under 2.3.1.1) have a spawn run period of 15 - 20 days. No recombined cultures showed any marked difference in the case of spawn run compared to their parents. The recombined culture *P. sajor-caju* (Psc - 1) X *P. florida* (Pf-1) recorded the maximum yield (Pate 5) and it was on par with one of the parents *P. sajor-caju* (Psc -1) and superior than the other parent *Pleurotus florida* (Pf -1) *Pleurotus sajor-caju* (Psc - 1) X *P. Platypus* (Pts -1) was found to be superior than platypus (pts -1) and inferior than *Pleurotus sajor-caju* (Psc -1). All the other recombined cultures showed poor performance compared to their parents (Table. 3).

Sporocarp colour of *Pleurotus sajor-caju* (Psc - 1) X *P. florida* (Pf -1) was cream which is intermediate between the parents (gray and white respectively). It was superior to that of *P. sajor-caju* (Psc -1)which has poor market acceptability due to gray colour (Plate 6).

Strain mixing by spawn of two different species as described under 2.3.1.2 was found to have no significance in strain improvement of *Pleurotus* spp. All the recombined cultures showed poor yield as compared to their parents (Table 4).

Plate 5 ***P. sajor-caju*** **(Psc - 1) x** ***P. florida*** **(Pf -1) mixed spawn - bed**

Plate 6 ***P. sajor-caju*** **(Psc - 1) x** ***P. florida*** **(Pf -1) mixed spawn - sporocarp**


PSC x PF


PSC x PF
PSC

Table 3. Comparative performance of recombined cultures of mixed spawn

Sl. No	Name of species and isolate number	No. of days for spawn growth	Average sporocarp yield / kg substrate (g)	Average pileus diameter (cm)	Average stipe length (cm)	Colour
1	*Pleurotus sajor - caju* (Psc - 1) X *P. florida* (Pf-1)	17.33	595.00	7.1 - 8.9	1.5-1.8	Cream
2	*P. sajor - caju* (Psc - 1) X *P. platypus* (Pts - 1)	16.00	580.00	5.9 - 7.5	1.5-1.8	Light grey
3	*P. sajor - caju* (Psc - 1) X *P. citrinopileatus* (Pc -1)	20.00	490.00	6.1 - 6.9	1.5-2.0	Light grey
4	*P. sajor - caju* (Psc - 1) X Ananthan (H-1)	15.00	464.67	5.1 - 6.8	1.5-1.7	Cream
5	*P. sajor - caju* (Psc - 1) X *P. eous* (Pe - 1)	15.33	456.67	6.5 - 6.8	0.5-0.9	Light grey
6	*P. sajor - caju* (Psc - 1)	17.67	585.0	4.5 - 5.2	1.2-1.6	Light grey
7	*P. florida* (Pf-1)	14.33	475.33	6.7 - 8.9	1.3-1.5	White
8	*P. platypus* (Pts - 1)	15.33	446.67	5.5 - 7.8	0.6-1.4	White
9	*P. citrinopileatus* (Pc -1)	21.33	370.33	4.4 - 5.0	0.4-0.5	White
10	Ananthan (H-1)	11.33	533.33	8.0 - 9.5	3.0-3.5	Creamy white
11	*P. eous* (Pe - 1)	15.00	481.67	9.5 - 10.7	0.5-0.8	Light pink
	CD (0.05)	1.28	84.87			

Table 4. Comparative performance of recombined cultures of mixed spawn

Sl. No	Name of species and isolate number	No. of days for spawn growth	Average sporocarp yield / kg substrate (g)
1	*Pleurotus sajor - caju* (Psc - 1) X *P. florida* (Pf-1)	17.33	375.00
2	*P. sajor - caju* (Psc - 1) X *P. platypus* (Pts - 1)	16.33	361.00
3	*P. sajor - caju* (Psc - 1) X *P. citrinopileatus* (Pc -1)	19.33	335.00
4	*P. sajor - caju* (Psc - 1) X Ananthan (H-1)	15.00	355.67
5	*P. sajor - caju* (Psc - 1) X *P. eous* (Pe - 1)	15.66	366.67
6	*P. sajor - caju* (Psc - 1)	17.67	585.0
7	*P. florida* (Pf-1)	14.33	475.33
8	*P. platypus* (Pts - 1)	15.33	446.67
9	*P. citrinopileatus* (Pc -1)	21.33	370.33
10	Ananthan (H-1)	11.33	533.33
11	*P. eous* (Pe - 1)	15.00	481.67
	CD (0.05)	1.78	128.99

3.3.2 Hybridization

3.3.2.1 Preparation of monospore culutres

Viable spores extracted from healthy medium sized and medium matured sporocarps were subjected to germination in sterile PDA medium and healthy germlings observed were picked out and cultured. Such monospore cultures were used for the hybridization following the techniques explained under the materials and methods.

3.3.2.2 Cultural characters of monospores of different species of *Pleurotus*

The monospore cultures were randomly selected and inoculated in PDA medium and incubated. The colony characters and the radial growth of the mycelium were observed on the seventh day of inoculation. The data was statistically analysed and presented in Table. 5.

It was observed that the monospore cultures of *P. eous* (Pe. 1) - MK 11 and *P. citrinopileatus* (Pc - 1) MK - 3 recorded the maximum mycelial growth followed by *P. eous* (Pe -1) MK - 12 and Ananthan (H - 1) MK - 10. The cultures, *P. platypus* (Pts - 1) MK - 1, *P. eous* (Pe - 1) MK - 5, P. platypus (Pts - 1) MK 6 and *P. platypus* (Pts - 4) showed very poor mycelial growth.

3.3.2.3 Pairing of compatible monospore cultures

Random tests were conducted initially to locate compatible monospore isolates. The compatible ones so located were finally subjected to pairing following the techniques as described under materials and methods. The dikaryons thus obtained were subjected to detailed observations. Period of

Table 5 **Cultural characters of monospores of different species of *Pleurotus***

Sl. No	Name and No. of isolate	Monospore cultures	Radial growth of mycelium (cm) in PDA	Mycelium type and colour of colony
1	*P. eous* (Pe - 1)	MK - 1	2.30	Strandy, cream
2	*P. eous* (Pe - 1)	MK - 5	1.80	Strandy, cream
3	*P. eous* (Pe - 1)	MK - 6	2.75	Strandy, cream
4	*P. eous* (Pe - 1)	MK - 9	3.00	Strandy, cream
5	*P. eous* (Pe - 1)	MK - 10	4.00	Strandy, cream
6	*P. eous* (Pe - 1)	MK - 11	4.35	Strandy, cream
7	*P. eous* (Pe - 1)	MK - 12	4.25	Strandy, cream
8	*P. florida* (Pf -1)	MK - 1	4.15	Fluffy, white
9	*P. florida* (Pf -1)	MK - 3	4.10	Fluffy, white
10	*P. florida* (Pf -1)	MK - 6	4.20	Fluffy, white
11	*P. florida* (Pf -1)	MK - 7	4.20	Fluffy, white
12	*P. citrinopileatus* (Pc -1)	MK - 2	3.80	Fluffy, white
13	*P. citrinopileatus* (Pc -1)	MK -3	4.35	Fluffy, white
14	*P. platypus* (Pts -1)	MK - 1	1.90	Fluffy, white
15	*P. platypus* (Pts -1)	MK - 2	2.35	Fluffy, white
16	*P. platypus* (Pts -1)	MK - 3	2.35	Fluffy, white
17	*P. platypus* (Pts -1)	MK - 4	1.75	Fluffy, white
18	*P. platypus* (Pts -1)	MK - 6	1.80	Fluffy, white
19	Ananthan (H -1)	MK - 3	3.90	Strandy, white
20	Ananthan (H -1)	MK - 9	4.05	Strandy, white
21	Ananthan (H -1)	MK - 10	4.25	Strandy, white

CD (0.05) - 0.499 MK - monospore cultures (the number indicate the random number assigned to monospore cultures)

vegetative phase and yield characters were recorded (Table 6).

Maximum sporocarp yield was recorded by the dikaryon (No : 6) *P. florida* (Pf -1) MK -7 x Ananthan (H - 1) Mk - 10 (531.0g/kg substrate) (Plate 7) which was significantly superior to its parent *P. florida* (pf -1) MK 7 and on par with the parent Ananthan (H - 1) - MK -10. Sporocarp yield of the dikaryons (N0 : I and No : II) *P. sajor-caju* (Mutant M2) PSC - 2) - MK - 2 x *Pleurotus platypus* (Pts - 1) MK - 1 and *P. platypus* (Pts - 2) MK - 2 x *Pleurotus citrinopileatus* (PC - 1) MK - 2 (Plate 8) were significantly superior yielders than their respective parents and sporocarp yield of both these were on par. All the other dikaryons showed poor performance.

Stipe length and pileus size of the dikaryon *P. sajor-caju* (Psc -1) (Mutant M2) MK - 2 x *Pleurotus platypus* (Pts - 1) MK -1 was found to be more when compared to its parents. No other dikaryon showed any improvement in stipe length.

3.3.3 Mutation breeding

3.3.3.1 Gamma irradiation

Gamma irradiation of selected species and monokaryons of *Pleurotus* spp. was conducted as explained under materials and methods. The irradiated cultures were evaluated for their growth and fruiting behaviour. The data were analysed and presented in the table 7.

Gamma irradiation has influenced certain *Pleurotus* species and their monokaryons in their sporocarp yield. *Pleurotus florida* (Pf - 1). MK - 1 (monokaryon) was found to be the best yielder compared to the other species.

Table 6 **Growth and yield of dikaryons**

Dikaryon No.	Name of crosses	No. of days from spawning to first harvest (days)	Average sporocarp yield / Kg substrate (g)	Average pileus diametre (cm)	Stipe length (cm)	Colour
1	*P. platypus* - Pts -1(MK -1) x *P. eous* - Pe -1 (MK -1)	20.33	370.00	5-5.5	0.7-1.5	White
2	*P. platypus* - Pts -1(MK -2) x *P. eous* - Pe -1 (MK -1)	20.33	350.00	5.7-6.5	4.2-4.5	White
3	*P. platypus* - Pts -1(MK -2) x *P. citrinopleatus* - Pc -1 (MK -2)	19.33	468.33	7.5-7.9	1.9-2.5	Creamy white
4	*P. platypus* - Pts -1(MK -3) x *P. citrinopleatus* - Pc -1 (MK -2)	18.66	405.00	6.5-6.7	1.5-2.0	White
5	*P. florida* - Pf -1 (MK -7) x Ananthan H -1 (MK -9)	19.33	350.00	4.5-5.1	1.5-1.7	White
6	*P. florida* - Pf -1 (MK -7) x Ananthan H -1 (MK -10)	15.33	531.33	5.5-8.9	1.2-1.5	Cream
7	*P. platypus* - Pts -1(MK -2) x *P.florida* - Pf -1 (MK -7)	15.00	370.00	5-5.5	0.7-1.0	White
8	*P. platypus* - Pts -1(MK - 3) x *P.eous* - Pe -1 (MK -1)	18.33	320.00	5.7-7.9	1.2-1.6	Cream

9	*P. florida (Pf-1)* (MK -1) x *P. citrinopileatus* Pc -1 (MK -2)	19.66	292.67	5.5-6.8	1.5-1.7	White
10	*P. sajor - caju* (Mutant μ2) Psc -2 (MK -1) x *P. florida* Pf -1 (MK - 7)	19.33	336.67	6.1-7.2	1.8-2.1	White
11	*P. sajor - caju* (mutant M_2) Psc -2 (MK -2) x *P.platypus* Pts -1 (MK -1)	17.67	463.33	8-10.6	7.5-8.75	Creamy white
12	*P. platypus* - Pts -1(MK - 1)	19.00	340.00	4.5-6.2	1.5-2.5	White
13	*P. platypus* - Pts -1(MK -2)	18.00	318.93	4.7-6.3	2.0-2.5	White
14	*P. platypus* - Pts -1(MK - 3)	16.67	401.67	4.5-5.8	1.8-2.5	White
15	*P. eous* Pe -1 (MK -1)	18.00	310.00	5.8-6.9	0.8-1.3	Light pink
16	*P. citrinopileatus* - Pc -1 (MK - 2)	21.00	333.33	4.5-5.8	1.8-2.7	White
17	*P. florida* Pf -1 (MK -7)	14.67	413.33	6.5-8.1	2.6-2.9	White
18	Ananthan H -1 (MK-9)	14.00	495.33	5.0-8.5	0.7-1.2	white
19	Ananthan H -1 (MK -10)	13.33	495.00	4.5-8.3	0.6-0.9	Cream
20	*P. sajor - caju* (mutant M_2) Psc -2 (MK -2)	18.00	300.00	5.5-7.25	6.5-7.3	Greyish white
	CD (0.05)	3.04	61.62			

The number in the paranthesis denotes the random numbers assigned to monospore cultures

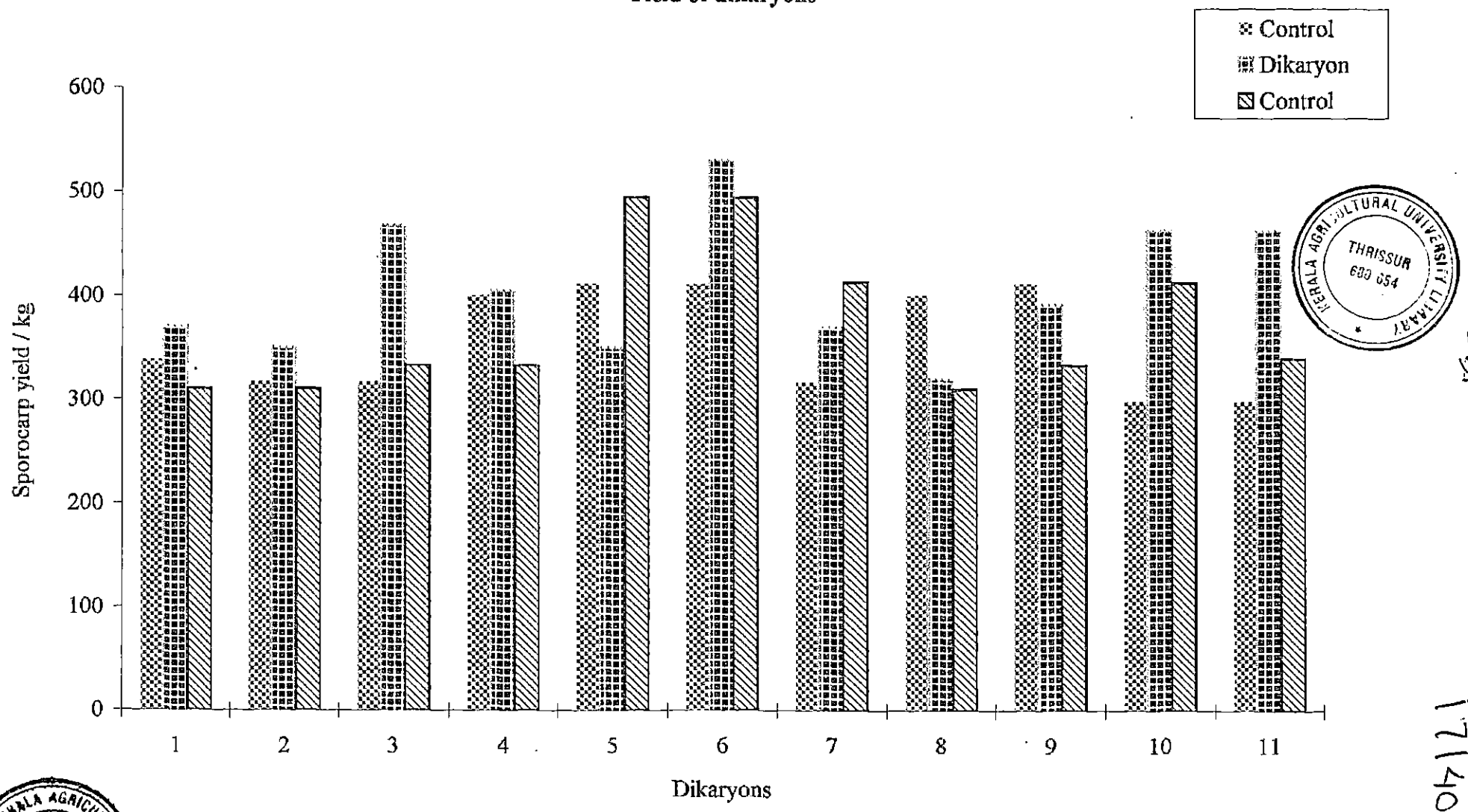


Plate 7 *P. florida* (Pf-1) MK - 7 x Ananthan (H -1) MK -10

Plate 8 *P. platipus* (Pts-2) MK-2 x *P. citrinopileatus* (Pc-1) MK-2


CROSS. PF. 7x H-10

Table 7. Influence of Gamma irradiation on sporocarp yield

Sl. No	Name of species	Isolate No.	Sporocarp yield / Kg substrate (g) at different level of γ - irradiation					Mean yield of un-irriadiated cultures (control)
			0.5kr	1.0 kr	1.5 kr	2.0 kr	2.5 kr	
1	*P. florida*	Pf -1	339.00	330.00	476.67	563.00	472.33	466.67
2	*P. florida*	Pf -2	314.00	361.67	381.67	416.67	481.00	421.67
3	*P. platypus*	Pts -1	318.33	343.33	373.33	390.00	411.67	392.00
4	*P. citrinopileatus*	Pc -1	258.33	406.67	275.00	266.67	322.00	366.67
5	Ananthan	H -1	365.67	299.00	569.00	233.33	390.00	523.33
6	*P. sajor - caju*	Psc-1	268.33	287.33	309.33	248.33	387.33	371.67
7	*P. sajor - caju* Mutant M -2	Psc-2	211.67	203.00	315.00	392.00	350.00	391.67
8	*P. florida* (MK -1)	Pf -1	345.67	390.00	583.00	543.33	589.67	495.00
9	*P. florida* (MK -4)	Pf -1	390.00	313.33	316.67	341.67	283.33	305.00
10	Ananthan (MK - 6)	H -1	266.67	293.33	421.67	471.67	441.67	482.67
11	*P. eous* (MK - 2)	Pe -1	238.33	355.00	303.33	229.00	311.67	401.67
12	*P. eous* (MK - 10)	Pe -1	271.67	307.33	271.67	343.33	338.33	340.00
13	*P. eous* (MK - 12)	Pe -1	225.00	212.33	616.67	190.00	318.33	320.00
	Mean		285.50	315.56	377.02	356.07	399.79	406.00

CD (0.05) species - 30.97 Levels - 21.04 S X L - 75.87

The number in the paranthesis denotes the random numbers assigned to monospore cultures.

Yield of *Pleurotus florida* (Pf_3) was lesser than *Pleurotus florida* (Pf - 1) MK - 1 but significantly superior than Ananthan (H - 1) MK-6. *P. eous* (Pe - 1) MK - 12 have showed the poorest performance with respect to yield. *P. citrinopileatus* (Pc - 1), Ananthan (H -1) MK-6, *P. eous* (Pe - 1) - MK-2 & 12 etc were found to have no effect on yield due to irradiation.

In *P. florida* (Pf - 1) gamma irradiation at 2.0 Kr level showed higher yield than all other levels of irradiation. As the level of gamma irradiation increased from 0.0 level to 2.5 Kr level sporocarp yield also increased in the case of *P. florida* (Pf - 2) and *P. platypus* Pts -1). Ananthan (H-1) gave maximum yield at 1.5 Kr level Yield of *P. florida* (Pf -1) MK -1 have shown a positive effect to gamma irradiation even though there was no significant difference among various levels of irradiation.

3.3.3.1.1 Sporocarp characters of the selected mutants

Detailed observations were recorded on the sporocarp characters of the irradiated mutants in addition to their quantitative yield. A preliminary screening was made based on the desirable characters of the sporocarp. The results are summarised in Table 8.

Sporocarp of *Pleurotus florida* (Pf-1) at 2.0 Kr and *P. florida* (Pf-1) MK -1 at 2.5 Kr level of irradiation have shown some peculiar characters. The sporocarps were found to be branched from a single basal stipe. The stipe was long and thick. Pileus unopened having distant free gills with lesser spore load. Similarly, Ananthan at 1.5 Kr level has produced soft and smaller sporocarps than its original unirradiated culture. Irradiation resulted in smaller and unattractive sporocarps in the case of *P. eous* (Plates 9 & 10).

Plate 9 *P. florida* (Pf-1) MK-1 2.5 Kr

Plate 10 Ananthan (H-1) 1.5 Kr


FL. MK 1 2.5 KV


H. 1.5 KA

Table 8. Sporocarp character of the selected mutants

Sl. No	Name of the species and No. of isolate	Level of r - irradiation (kr)	Average pileus diameter (cm)	Average stipe length (cm)	Colour	Special features
1	*P. florida* (Pf -1)	2.0 kr	7 - 10 cm	4.5 - 7.0	Creamy white	Sporocarp in bunches - stipe thick and long - pileus unopened bud like long structure
2	*Pleurotus florida* (Pf -1) MK-1	1.5 kr	7.2 - 7.8	2.7 - 3.1	Creamy white	
3	*P. florida* (Pf -1) MK - 1	2.5 kr	6.1 - 6.9	3.9 - 7.0	Creamy white	Sporocarp in bunches - stipe thick and long - pileus unopened bud like long structure
4	Ananthan (H- 1)	1.5 kr	4.5 - 6.5	1.5 - 2.5	Cream	More soft and smaller
5	*P. florida* (Pf -1)	Control	7:5 - 9.8	2.5 - 4.0	Creamy white	
6	*P. florida* (Pf -1) MK - 1	Control	6.5 - 7.2	2.1 - 2.5	Creamy white	
7	Ananthan (H - 1)	Control	5.5 - 8.2	2 - 3.1	Cream	

3.3.3.2 Chemical Mutation

3.3.3.2.1 Treatment of cultures with chemical mutagens

Selected cultures of *Pleurotus* species were treated with chemical mutagens as described under materials and methods. The resulted mutants were subjected to cultivation trials and the data were statistically analysed and presented in the table 9.

No significant effect was noticed in any of the mutants with respect to the vegetative growth compared to the parent cultures.

Maximum sporocarp yield was recorded by the mutant of *P. petaloides* (pet - 1) treated at 2000ppm with Ethyl Methane Sulphonate (EMS) (IH) followed by *P. florida* (Holland Pfh -1) at 1000 ppm (½H). In *P. florida* (Holland Pfh) treatment of EMS at 2000 ppm (1/2 H) has significantly increased the sporocarp yield as compared to its parent culture. Chemical treatment in *P. petaloides* (Pet -1) was found to be highly significant in increasing the soprocarp yield. On the other hand, the data revealed that chemical mutation have not shown any marked difference in sporocarp yield of *P. sajor - caju* (Psc-1), *P. platypus* (pts -1) and Ananthan (H -1). Sporocarp yield of *P. eous* (Pe -1) 1000 ppm EMS (1/2 H) was on par with its control treatment.

Sporocarp characters of the mutants

Observations were also made on the morphological characters of the mutants. The chemical mutation have shown some marked variations in sporocarp characters. The sporocarp of mutants of *P. sajor-caju* (psc -1) was found to be larger in size and multilobed. The mutant of *P. sajor - caju*

Table 9. Influence of chemical mutagens on growth and yield of *Pleurotus* spp. (Treatment of Cultures)

Mutant No	Name and No. of isolate	Name and doze of chemicals	Time in hour (H)	No. of days from spawning to first Harvest (days)	Average sporo carps yield / Kg substrate (g)	Average pileus diameter (cm)	Average stipe length (cm)	Colour	Special features
1	*P. sajor - caju* (PSC -1)	EMS 500 ppm	1/2 H	19.67	425.00	7.6 - 7.9	1.5 - 2.0	Light grey	
2	*P. sajor - caju* (PSC -1)	EMS 1000 ppm	1/2 H	18.67	403.33	9 - 10.2	2 - 2.5	Light grey	Large sporocarp with lobed margin
3	*P. sajor - caju* (PSC -1)	EMS 2000 ppm	1/2 H	19.67	270.00	5.8 - 7.9	2 - 2.3	Light grey	
4	*P. sajor - caju* (PSC -1)	EMS 2000 ppm	1 H	19.33	441.00	5.3 - 5.5	2.2 - 3.2	Light grey	Very smallsporo carp with lobed margin
5	*P. platypus* (Pts -1)	EMS 1000 ppm	1/2 H	19.33	388.33	3.9 - 4.5	3.1 - 3.5	White	
6	*P. platypus* (Pts -1)	EMS 2000 ppm	1/2 H	19.00	265.00	3.2 - 5.2	2.5 - 3.2	White	
7	Ananthan (H - 1)	EMS 500 ppm	1/2 H	21.00	306.67	5.5 - 6.1	2.4 - 2.6	White	
8	Ananthan (H - 1)	EMS 2000 ppm	1/2 H	14.33	538.33	5.5 - 8.7	1.7 - 1.9	White	
9	*P. florida* (Holland) (Pfh -1)	EMS 1000 ppm	1/2 H	17.00	310.00	7.9 - 8.9	3 - 4.2	Creamy White	
10	*P. florida* (Holland) (Pfh -1)	EMS 1000 ppm	1/2 H	15.67	605.00	7 - 10.2	3.2 - 5.0	Creamy White	With very few spores
11	*P. florida* (Holland) (Pfh -1)	EMS 2000 ppm	1/2 H	18.00	600.00	7.2 - 8.5	3.5 - 4.6	Greyish white	
12	*P. florida* (Holland) (Pfh -1)	EMS 2000 ppm	1 H	18.33	483.33	2.3 - 3.2	3.2 - 4.0	Creamy White	Very small sporocarp with long stipe

Mutant No.	Name and No. of isolate	Name and dose of chemical	Time in hour (H)	No. od days from spawning to 1st harvest	Average sporocarp yield per kg substrate (g)	Average diametre (cm)	Average stipe length (cm)	Colour	Special feature
13	*P. eous* (Pe - 1)	EMS 500 ppm	1 H	15.33	311.67	4.2 - 5.1	0.5 - 0.8	Light pink	
14	*P. eous* (Pe - 1)	EMS 1000 ppm	1/2 H	16.67	493.33	7.8 - 7.9	0.7 - 0.9	First pink then cream	
15	*P. petaloides* (Pet - 2)	EMS 500 ppm	1/2 H	16.33	575.00	7.5 - 10.2	2.9 - 3.1	White	
16	*P. petaloides* (Pet - 2)	EMS 2000 ppm	1 H	15.67	610.00	5.9 - 10.2	2.5 - 5.6	Creamy white	
17	*P. sajor - caju* (Psc -1)	Control		18.00	585.67	2.9 - 3.5	2 - 2.2	Light grey	
18	*P. platypus* (Pts -1)	Control		15.00	446.67	4.1 - 5.9	2.1 - 2.9	White	
19	Ananthan (H -1)	Control		12.67	533.33	8 - 9.5	2.0 - 3.5	White	
20	*P. florida* (Holland) Ft-1	Control		20.33	511.67	4.5 - 10.0	2 - 6.1	Greyish white	
21	*P. eou* (Pe -1)	Control		17.33	488.33	5.5 - 8.6	1 - 1.5	Light pink	
22	*P. petaloides* (Pet -2)	Control		16.00	538.00	7.5 - 8.6	2 - 4.5	White	
CD	(0.05)			2.92	57.16				

(psc-1) 2000ppm EMS (1H) produced very small sporocarps with lobed margin. From a single stipe 2-3 sporocarp could be obtained. sporocarp of the mutant, *Pleurotus florida* (Holland Pfh - 1) 1000 ppm EMS (1/2 H) was found to be with very few spores and *P.florida* (Holland Pfh -1) 2000 ppm EMS (IH) produced very small sporocarp with long stipe. The mutants of *P. petaloides* (pet 1) produced very large sporocarps than their original culture. Instead of pink colour in *P. eous* (pe -1) the treatment 1000ppm EMS (IH) produced sporocarp having cream colour from the every beginning (Plates 11, 12 & 13).

3.3.4.2.2 Treatment of spores with chemical mutagens

Viable spores of each of the culture were extracted and treated with chemical mutagens as explained under materials and methods. Nature of vegetative growth and fruiting behaviour of the resultant mutants were evaluated.

The mutant of *P.eous* (Pe - 1) treated with 1000ppm EMS (1/2 H) was considered as a good coloniser with a minimum spawn run phase which varied from 17 - 18 days. The mutant of *P. flabellatus* (Pfb-1) 1000ppm EMS (1/2 H) showed a minimum period of 15 days for first harvest from spawning followed by mutants of *P. eous* (Pe 1) 1000 ppm MMS (Methyl Methane Sulphonate) (1/2 H) and *P. flabellatus* (Pfb - 1) 1000 ppm EMS (1 H) as in Table 10.

In general, it was found that the mutants were inferior compared to their parent cultures with respect to the quality of sporocarp. However, the

Table 10. Influence of chemical mutagens on the growth of *pleurotus* spp.

(Treatment of spores)

Mutant No.	Name and No. of isolate	Name and dose of chemicals	Time in hours (H)	No. of days from spawning to 1st harvest (days)	Average sporocarp yield per Kg substrate (g)	Average pileus diametre (cm)	Average stipe length (cm)	Colour	Special features
1	*P. flabellatus* (Ptb -1)	EMS 1000 ppm	1/2 H	15.67	298.33	5.1 - 5.9	2.5 - 3.5	Greyish white	
2	*P. flabellatus* (Ptb -1)	EMS 1000 ppm	1 H	17.67	396.67	4.9 - 5.5	3.2 - 3.5	Greyish white	
3	*P. flabellatus* (Ptb -1)	MMS 1000 ppm	1/2 H	19.67	373.33	3.5 - 5.1	2 - 2.2	Greyish white	
4	*P. eous* (Pe -1)	EMS 1000 ppm	1 H	18.67	323.33	5.5 - 9.7	0.4 - 0.6	Light pink	
5	*P. eous* (Pe -1)	EMS 1000 ppm	1/2 H	17.00	410.00	6.0 - 8.1	0.6 - 0.9	Cream from the very beginning	Sporocarp in bunches and with lobed margin
6	*P. eous* (Pe -1)	MMS1000 ppm	1/2 H	20.00	458.33	7.5 - 9.5	0.5 - 0.7	Cream from the very beginning	Sporocarp with lobed margin
7	*P. flabellatus* (Ptb -1)	Control		19.67	527.67	3.5 - 4.75	0.75 - 1	Greyish white	
8	*P. eous* (Pe -1)			18.33	488.33	5.2 - 6.1	0.3 - 0.8	Light pink	
	CD (0.05)			2.206	81.81				

Plate 11 *P. petaloides* (Native isolate) 2000 ppm (1 H) EMS

Plate 12 *P. eous* (Native isolate) 500 ppm (1H) EMS

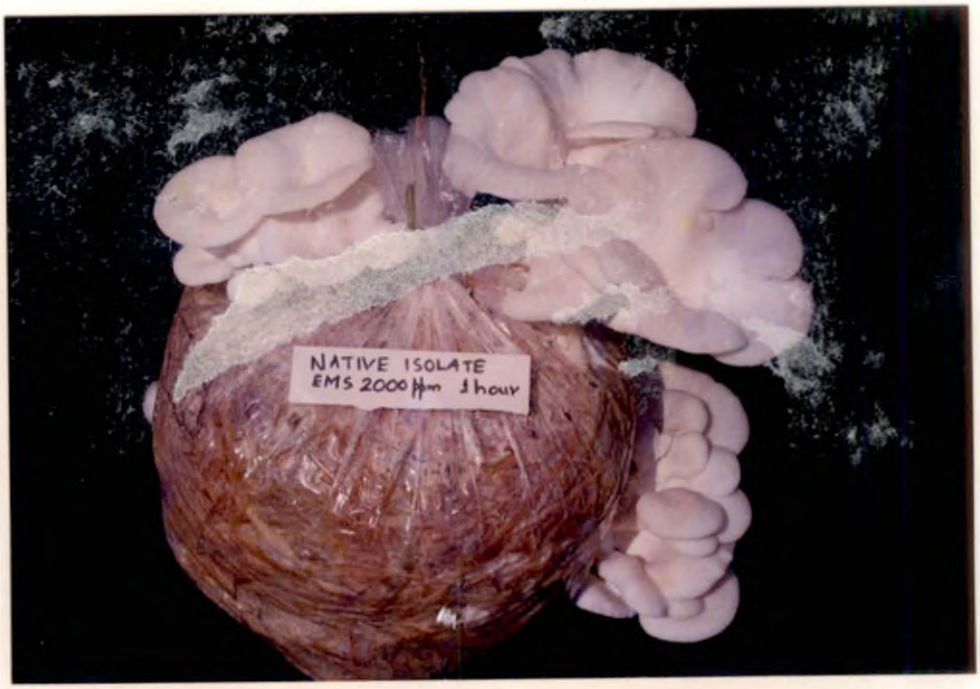

NATIVE ISOLATE
EMS 2000 Mm 3 hour

Plate 13 *P. eous*(Native isolate) 1000 ppm (1/2H) EMS


P. cous [T] C1 D2 T1

sporocarp of the mutant *P. eous* (Pe - 1) treated at 1000 ppm MES (1/ 2 H) showed cream colour as against the pink colour in the parent with lobed pileus margin.

3.3.5 Comparative cultural characters of various *pleurotus* spp. and mutants

Mycelial growth of selected cultures were evaluated in various standard media and their modified media by blending with 40 per cent coconut milk.

Among the various standard media tested, 0A medium has promoted maximum mycelial growth in all the cultures except for the mutant of Ananthan (H - 1) 1.5 Kr and *P. platypus* 0Awas significantly superior to all other media tested. This was followed by YEA Among the species evaluated, the mutants of *P. florida* (Pf -1) MK - 1 (2.5 Kr) showed maximum mycelial growth in oats agar and this was on par with the other mutant of the same species also (mutant - 2 kr.) PDA and Tapioca Dextrose Agar (TDA) were on par with respect to *P. florida* (Mutant 2 kr.) whereas for the mutant (2.5 kr) PDA and YEA were on par. TDA was found to be inferior for all the isolates tested.

Various media blended with coconut milk (modified media) was found to improve the quality of media with respect to the mycelial growth of all the species subjected to the evaluation. Modified YEA was found to influence more on mycelial growth for most of the cultures and this was followed by the modified OA medium. It was also noticed that the addition of 40 per cent coconut milk has improved the quality of other standard media namely PDA and TDA (Table 11).

Table 11. Comparative cultural characters of various *Pleurotus* spp. and their mutants

Sl. No	Isolate No.	Name of species / mutant	Radial growth of mycelium in cm							
			Media without coconut milk				Media with coconut milk			
			PDA	TDA	OA	YEA	PDA	TDA	OA	YEA
1	Pf -1	*P. florida* (Mutant, 2kr)	3.47	3.53	4.23	4.07	3.93	4.27	4.37	4.30
2	Pf -1	*P. florida* MK -1 (Mutant, 2.5 kr)	4.27	3.43	4.37	4.23	4.07	4.30	4.47	4.27
3	H -1	Ananthan	1.37	2.73	4.00	3.70	3.80	4.10	4.00	4.33
4	H -1	Ananthan (Mutant, 1.5 kr)	1.90	3.43	3.07	4.20	4.03	3.97	4.13	4.20
5	Pts -1	*P. florida* (Holland)	2.67	2.77	3.60	3.13	4.27	3.63	4.03	4.40
6	Pts -1	*P. platypus*	2.17	3.30	3.17	3.03	3.83	3.70	3.97	4.03
		Mean	2.64	3.20	3.74	3.73	3.98	3.99	4.16	4.26

CD (0.05)

Species - 0.22

Media - 0.06

M x S - 0.43

Various media

PDA - Potato dextrose agar

TDA - Tapioca dextrose agar

OA - Oats agar

YEA - Yeast etract agar

3.3.6 Reaction of selected mutants, dikaryons and native isolates to insect pests and common weed moulds (Healing)

Periodical observations were made in mushroom beds of dikaryons and mutants along with the native isolates for their resistant / tolerant reactions against insect pests and common weed moulds.

Important pests encountered were staphylinid beetle, phorid flies and cecid flies. The weed moulds commonly observed were the green mould (*Trichoderma* spp.) ink caps (*Coprinus* spp.) and *Aspergillus* spp. *Trichoderma* spp. were the frequently occurring competitor moulds both in the spawn bottles and mushroom beds during the spawn run phase. Regarding the competitor moulds the number of beds infested were observed and classified on the basis of the degree of reactions as explained under materials and methods.

In the case of insect pests the population of infestation during the spawn run phase and cropping phase were observed and classified according to the severity grades.

The mutants *P. florida* (Pf -1) 2. Kr and *P. florida* (Pf -1) MK -1, 2.5 Kr were tolerant to infection against all the insect pests and weed moulds tested except phorid files which cause mild infection in both the mutants during spawn run phase. The native isolates *P. eous* (Pe -1) and *P. petaloides* (Pet -2) and their mutants Viz *P.eous* (Pet -2) treated at 2000 ppm EMS (IH) showed better tolerance to all the insect pests and weed moulds.

Similarly Ananthan (H-1) Mutant 1.5 Kr was found to be tolerant against all the insect pests and weed moulds tolerant.

Table 12. Reaction to insect pests and weed moulds of selected mutants, dikaryons and native isolates

Sl. No	Name and No. of selected isolates	Nature and severity of incidence of insect pests			Nature and severity of incidence of weed moulds during spawn run phase		
		Phorids	Staphylinid beetle	Others	*Trichoderma* spp.	*Asperigillus* spp	*coprinus* spp.
1	*Pleurotus florida* (Pf -1) Mutant, 2Kr	+	-	-	-	-	-
2	*P. florida* (Pf -1) MK -1 Mutant, 2.5 Kr	+	-	-	-	-	-
3	Ananthan (H-1) Mutant, 1.5 Kr	-	-	-	-	-	-
4	*P. eous* (Pe -1) EMS 1000 ppm 1/2 H	-	-	-	-	-	-
5	*P. Petaloides* (Pet-2) EMS 2000ppm 1H	-	-	-	-	-	-
6	*P. florida* (Pfh-1) EMS 1000ppm 1H	+	-	+	-	-	-
7	*P. florida* (Pfh -1) EMS 2000ppm 1 H	+	-	+	++	-	-
8	*P. sajor-caju* (PSC -1) x *P. florida* (Pf-1)	+	+	-	+	-	-
9	*P. platypus* (Pts-1) MK-3 x *P. citrinopileatus* (PC-1) MK -2	+	+	-	-	-	-
10	*P. florida* (Pf-1) MK-7 x Ananthan (H-1) MK-10	+	-	-	+++	-	-
11	*Pleurotus petaloides* (Pet -2)	-	-	-	-	-	-
12	*P. eous* (Pe -1)	-	-	-	-	-	-

- No infection + mild infection ++ Severe infection +++ very severe infection

Sl No	Name and Number of selected isolates	Mature and severity of incidence of insect pests			Nature and severity of incidence of weed moulds during spawn run phase		
		Phorids	Staphylinid bettle	Others	*Trichoderma* spp	*Aspergillus* spp	*Coprinus* spp
13	*P. florida* (pf -1)	+	-	-	+++	-	-
14	*P. florida* (pf -1) MK -1	+	+	-	++	-	-
15	Ananthan (H -1)	-	-	-	-	-	-
16	*P. sajor - caju* (PSC -1)	+	-	-	+++	-	-
17	*P. florida* (Pfh -1)	+++	-	-	-	-	-
18	*P. platypus* (pts- 1) MK -2	+	+	-	+	-	-
19	*P. citrinopileatus* (Pc -1) MK -3	+	+	-	+	-	-
20	*P. florida* (pt -1) MK -7	+	-	-	++	-	-
21	Ananthan (H -1) MK - 10	-	-	-	-	-	-

- No incidence

+ Mild incidence

++ Severe incidence

+++ very severe incidence

The dikaryon (N0 :4) *P. platypus* (Pts -1) MK - 3 x *P. citrinopileatus* (Pc -1) MK -2 showed its tolerance to *Trichoderma* spp. while the dikaryon (No : 6) *P. florida* (Pf -1) MK -7 x Ananthan (H -1) MK -10 was highly susceptible to the infection of *Trichoderma* spp which showed very severe infection during spawn run period. Mild infection of phorid flies (during spawn period) and cecid files (during cropping period) were observed in the beds of mutants of *P. florida* (Pfh -1) *VIZ.*, *P. florida* (Pfh -1) EMS 1000 ppm (1H) and *P. florida* (Pfh -1) EMS 2000 ppm (1H). *Aspergillus* spp and *Coprinus* spp were not a problem in the cultivation of the tested mutants, dikaryons and native isolates (Table 12).

3.3.7 Organoleptic studies

Organoleptic studies of both raw and cooked mushroom of the selected dikaryons and mutants were done in comparison to the native isolates following the Kruskal - Wallis statistical methods using the scoring system as explained under materials and methods.

1. Comparative quality of raw mushrooms

In the case of raw mushrooms, *P. eous* (Pe - 1) obtained maximum rank mean with respect to appearance, colour and flavour and taste. Appearance, flavour and taste of *P. petaloides* (Pet - 2) 2000 ppm EMS (1) was significantly on par with *P.* eous (Pe-1). Colour of *P. petaloides* (Pet - 2) was equally ranked as *P. eous* (Pe - 1). With regard to the taste, *P. eous* (Pe -1) and *P. eous* 1000 ppm EMS (1/2 H) was statistically on par with *P. petaloides*

(Pet - 1) *P. florida* (Pf - 1), *P. florida* (Pf - 1) MK - 1 (2.5 kr.) *P. florida* (Pfh - 1) 1000 ppm EMS (1 H) and *P. florida* (Pfh - 1) 2000 ppm EMS (1 H).

All the quality parameters except flavour was acceptable for *P. florida* (Pf - 1). Except taste all the other qualities of the cross *P. sajor-caju* (Psc - 1) x *P. florida* (Pf - 1) was on par with *P. eous* (Pe - 1). Overall acceptability of the tested species were statistically superior when compared to the standard species *P. sajor-caju* (Psc - 1) and *P. florida* (Pfh - 1) which were found inferior in all the qualities Table 13).

2Comparative quality of cooked mushrooms

Pleurotus eous (Pe - 1) scored maximum rank mean with respect to appearance, colour, flavour and taste. *P. eous* (pe-1) EMS 1000 ppm 1 H also have taste equal to *P. eous* (Pe-1). Appearance and colour of *P. petaloides* (pet - 2) *P. petaloides* (pet-2) EMS 2000 ppm 1H, *P. florida* (Pf - 1), *Pleurotus florida* (Pf -1) mutant, 2.5 kr, *P. sajor-caju* (P-1) x *P. florida* (Pf - 1) and *P. florida* (Pf-1) MK 7 x Ananthan (H-1) MK - 10 were statistically on par with *P. eous* (Pe-1).

Maximum rank mean for texture was scored by *P. petaloides* (Pet-2) and *P. sajor-caju* (Psc-1) EMS 2000 ppm IH. Overall acceptability of cooked mushroom was poor for *P. sajor-caju* and *P. florida* (pfh-1)when compared to other species (Table 14).

Table 13. Comparative organoleptic characters of selected dykaryons, mutants and native isolates (comparative quality of raw mushroom)

Isolate number	Name of species	Rank means for overall acceptability N = 5			
		Appearance	Colour	Flavour	Taste
1	*Pleurotus sajor - caju* (PSC -1)	4.5	10.4	22.4	13.2
2	*Pleurotus florida* (Pf -1)	42.5	47.2	26.5	33.3
3	*P. florida* (Holland) Pfh -1)	20.9	18.6	18.3	17.8
4	*P. sajor - caju* (PSC -1) x *P. florida* (Pf -1)	30.5	34.6	32.4	27.0
5	*Pleurotus florida* (Pf -1) mutant, 2 kr	42.5	40.9	38.3	27.0
6	*P. florida* (Pf -1) (MK-1) mutant, 2.5 kr	48.5	47.2	50.1	39.6
7	*P. sajor - caju* (PSC -1) EMS 2000ppm 1H	20.9	47.2	10.1	22.4
8	*P. petaloids* (Pet -1) EMS 2000ppm 1 H	54.5	18.6	56.0	52.2
9	*P. florida* (Holland) Pfh -1) EMS 2000ppm 1000 ppm 1 H	23.3	34.6	18.3	33.3
10	*P. florida* (Holland) Pfh -1) EMS 2000ppm 2000 1 H	25.2	34.6	18.3	33.3
11	*P. eous* (Pe -1) EMS 1000ppm 1/2 H	48.5	40.9	56.0	58.5
12	*P. florida* (Pf-1) MK -7 x Ananthan (H-1) MK -10	32.9	24.9	44.2	28.7
13	*P. petaloids* (Pet -2)	42.5	53.5	50.1	52.2
14	*P.eous* (Pe -1)	54.5	53.5	56.0	58.5
	CD (0.05)	25.227	25.227	25.227	25.227

Table 14. Comparative quality of cooked mushroom

Isolate number	Name of species	Rank means for overall acceptability N = 5				
		Appearance	Colour	Flavour	Texture	Taste
1	*Pleurotus sajor - caju* (Psc -1)	7.4	7.7	42.6	15.8	24.0
2	*Pleurotus florida* (Pf -1)	44.1	34.1	25.1	36.8	10.7
3	*P. florida* (Holland) Pfh -1)	26.3	15.7	29.2	19.9	24.7
4	*P. sajor - caju* (Psc -1) x *P. florida* (Pf -1)	22.2	34.1	35.9	19.9	24.0
5	*P. florida* (Pf -1) mutant, 2 kr	58.5	47.7	42.6	49.6	24.0
6	*P. florida* (Pf -1) MK-1 mutant, 2 kr	35.2	52.6	35.9	32.7	29.7
7	*P. sajor - caju* (Psc -1) EMS 2000ppm 1H	15.9	11.9	49.3	30.4	10.7
8	*P. petaloides* (Pet -1) EMS 2000ppm 1 H	53.7	52.6	29.2	43.2	56.5
9	*P. florida* (Holland) Pfh -1) EMS 1000ppm 1000 1 H	26.3	30.3	35.9	19.9	33.7
10	*P. florida* (Holland) Pfh -1) EMS 2000ppm 2000 1 H	15.9	19.5	35.9	30.4	45.1
11	*P. eous* (Pe -1) EMS 1000ppm 1/2 H	53.7	47.7	25.1	49.6	56.5
12	*P. florida* (Pf-1) MK -7 x Ananthan (H-10)	30.4	57.5	25.1	43.2	39.4
13	*P. petaloides* (Pet -2)	48.9	47.7	49.3	49.6	56.5
14	*P.eous* (Pe -1)	58.5	57.5	35.9	56.0	56.5
	CD (0.05)	25.227	25.22	25.227	25.227	25.227

DISCUSSION

DISCUSSION

In the present study attempts were made to domesticate native wild flora of *Pleurotus* spp., by conducting intensive survey during the monsoon periods. The native species were identified and subjected to preliminary study to explore their suitability to bring them under cultivation. Two species were found amenable for large scale cultivation under Kerala conditions, viz., *Pleurotus eous* and *P. petaloides*. These species were described and subjected to detailed studies along with the other cultures procured from various mushroom research centres of the country. The species included in the study are as summarised below.

Sl.No	Isolate No	Name of species	Source
1	Psc-1	*Pleurotus sajor-caju* (Fr.) Singer	N.C.M.R.T., Solan
2	Psc-2	*P. sajor-caju* (Mutant-M_2)	T.N.A.U., Coimbatore
3	Pf-1	*P. florida* Eger	College of agriculture, Killikulam, Tamilnadu
4	Pf-3	*P. florida* Eger	I.I.H.R., Bangalore
5	Pts-1	*P. platypus* (Cook & Massee) Sacc.	TamilNadu
6	Pc-1	*P. citrinopileatus* Singer	TamilNadu
7	Pf-1	*P. flabellatus* (Berk & Br.) Sacc.	C.F.T.R.I., Mysore
8	Pfh-1	*P. florida* Eger	Holland
9	H-1		Anathan - An interstock hybrid developed at College of Agriculture , Vellayani
10	Pet-2	*P. petaloides* (Bull. ex Fr.) Schulz	Native isolate (from dead wood)
11	PC-1	*P. eous* (Berk.) Sacc.	Native isolate (from dead wood)

Earlier workers have reported the occurrence of different *Pleurotus* spp., in Kerala which are collected in the present study. Suharban (1987) in a monographic study on *Pleurotus* spp., of Kerala, recorded and described 20 species which included *Pleurotus eous* and *P. petaloides*. Balakrishnan (1994) recorded the occurrence of *Pleurotus petaloides*. The occurrence of the two collected native isolates in Kerala has been confirmed by the present observations. Quality parameters and consumer acceptability of *Pleurotus petaloides* were comparatively better than other species of *Pleurotus* obtained from various mushroom research centres of the country (Balakrishnan, 1994).

Morphological characters of selected native isolates

Pleurotus eous (Berk.) Sacc collected from the dead stumps of *Albezia lebeck* during south - west monsoon, in the present study has been collected earlier by Hooker and identified by Berkely (1850) as *P. eous*. Sporophores aggregated in tufts, sometimes on dead portions of living trees and were usually sessile or imbricate. Pileus initially spathulate, flabelliform when old. Stipe absent; even if present very small. Gills decurrent, radially parallel. Pink, flesh cream coloured. The detailed observations made in the present study are found to be in full agreement with the description of the species made by Purkayastha and Chandra (1985) and Suharban (1987).

P. petaloides (Bull. ex Fr) Schulz collected from the dead stumps of *Cocos nucifera* during the North - East monsoon period, in the present study has been collected earlier by Berkeley (1850) and described as *P. petaloides*. The pileus which resembles the petals of flower gained the species the name

'petaloides'. The detailed morphological characters of the speices recorded in the present study fully agreed with the descriptions given by Suharban (1987).

Strain improvement programmes

The objective of strain improvement programme is to improve the selected strains of *Pleurotus spp.* by standard hybridization and mutation techniques so as to develop high yielding strains and also resistant / tolerant ones against the most damaging pests and competitor weed moulds. In the present study an attempt was made to achieve the above objectives by following techniques like strain mixing, hybridization and mutation.

Strain mixing

Mixing of the commercially available strains for formation of 'hybrid' cultures appears to be easiest and most tempting. Mixed spawn was prepared in the case of *Agaricus bisporus* by inoculating cultures of two different species together in to one packet / bottle of the spawn substrate (Kneebone *et al.*, 1972).

A similar attempt was made for *Pleurotus* spp. in the present study. The recombined culture of *Pleurotus sajor-caju* (Psc -1) x *P. florida* (Pf -1) recorded the maximum yield and it is on par with one of the parents, *P. sajor-caju* (Psc - 1) and superior to the other parent *P. florida* (Pf - 1). Sporocarp yield of *P. sajor-caju* (Psc - 1) x *P. platypus* (Pts -1)was found to be superior to one of the parents *P. platypus* (Pts -1). All the other recombined cultures showed poor performance compared to their parents. Kneebone *et al.* (1972) obtained similar results. They reported that most of the combinations yielded less than one or both of the experimental parental strains.

Fritsche (1972) had used two strains with different cap colour and fruit body mophology for strain mixing. She was able to obtain 'recombinant mushrooms' having intermediate characters. The present observation has also shown that the sporocarp colour of the recombined culture *P. sajor-caju* (Psc -1) x *P. florida* (Pf -1) was cream which is intermediate between the parents (grey and white respectively). It is superior to that of the *P. sajor caju* which has lesser acceptibility in market due to its grey colour.

No combination has out yielded the parental strain. This may be due to inter strain competition.

Hybridization

The major aim of hybridization programme is to combine characters from different strains and to create more variability in the existing germplam. Development of heterokaryons in *P. ostreatus* and related species has been reported by various workers (Croft and Smichen, 1965; Eugenio and Anderson, 1968). Mating of interstock monokaryons in *P. ostreatus* has resulted in dikaryons with higher fruit body yield (Wang and Anderson, 1972). In *P. sajor-caju* dikaryotic hybrids were obtained by the somatic recombination of interstock monokaryons (Roxon and Jong, 1977).

Ghosh and Chakravarthy (1991) opined that in *P. sajor-caju* selective dikaryotization exert some exciting changes over the traditional cultures. Pahil *et al.* (1994) conducted strain improvement studies of high temperature tolerant cultivated and wild species of *Agaricus* through inter and intra strain hybridization.

The interstrain and interspecific hybridization involve isolation of single spores and intermating of such monokaryotic isolates from different strain / species. Due to existence of multiple allelism in incompatibility factors, monokaryotic mycelia of *P. ostreatus* derived from different fruit bodies (representing different strains collected from different locations / host) intermated at 100 per cent rate compared to an inbreeding capacity of only 25 per cent (Bresinsky *et al.*, 1987).

Cultural characters of monospore cultures of different species of *Pleurotus*

Ten to fifteen monospore cultures from each parent strain were prepared by following the method suggested by Eger (1978). Cultural characters of the monospore cultures were studied. The characters studied were, mycelial growth, colour and type of mycelium. Klingman (1943) reported that fertile single spore cultures varied in their growth rate and appearance of mycelia. Monospore culture of *P . eous* (Pe-1) MK-11 and *P. citrinopileatus* (Pc-1) MK-2 recorded the maximum mycelial growth followed by *P. eous* (Pe-1) MK-12 and Ananthan (H-1) MK-10. *P. eous* (Pe-1) MK-11 has a strandy growth of mycelium. In India the first attempt of raising single spore isolates was made by Kumar and Munjal (1981). They selected 30 single spore isolates on the basis of rate of growth. In the present study also the important characcter considered in the selection of monospore cultures were growth rate.

Pairing of compatible monospore cultures

The compatible monokaryons were subjected to pairing by following the technique as described under materials and methods. Among the dikaryons, two were selected for detailed studies. Among this one dikaryon obtained (*P.platypus* (Pts-1) MK 3 X *P. citrinopileatus* (Pc-1) MK 2) showed maximum desirable characters, viz., attractive white colour, higher yield, good consumer acceptability and tolerant to *Trichoderma viride.* Balakrishnan (1994) reported that one dikaryon could be obtained by interstock hybridisation of monokaryons of *Pleurotus* spp. (cross between weed mould tolerant mutant of *P. petaloides* and its wild parent strain) which showed high adaptability to the agroclimatic conditions of Kerala and this got all the desirable characters, namely, attractive white sporophores, higher yield, good consumer acceptability, tolerent to *Trichoderma viride* and steady yielder althrough the year. The observations made in the present study have a similar correlation.

Mutation breeding

Bhandal and Mehta (1994) has suggested that in the absence of sufficient germplasm basidiospores, non-sexual spores or hyphal segments can be exposed to physical or chemical mutagens to create variants.

Irradiation

Irradiation was done to evolve efficient mutants. Spores and hyphal fragments were exposed to gamma irradiation for variable periods. Pandey and Tewari conducted similar study during 1994.

Pluerotus florida (Pf-1) MK- 1 subjected to irradiation at 2.5 Kr was found to be the best yielder compared to other species. Gamma irradiation showed a positive effect on sporocarp yield of *P. florida* (Pf-1) MK-1 even though there was no significant difference among various levels of irradiation. As the level of gamma irradiation increases from 0.0 level to 2.5 Kr level the sporocarp yield was also increased in the case of *P. florida* (Pf-3) and *P. Platypus* (Pts-1). Ananthan(H-1) gave maximum sporocarp yield at 1.5 Kr level of irradiation. Teow *et al.* (1995) conducted cross breeding after irradiating the monospore isolates with gamma rays. The two selected hybrids were better yielder than their wild parents. The observations made in the present study also agree with the above findings.

Sporocarps of *P. florida* (Pf-1) at 2.0 Kr and P. florida(Pf-1) MK -1 at 2.5 Kr level of irradiation have shown some peculiar characters. The sporocarps were found to be branched from a single basal stipe. The stipe was long and thick. Pileus unopened having distant free gills with lesser spore load. Similarly, Ananthan at 1.5 Kr level has produced more soft and smaller sporocarps than its original unirradiated culture.

Chemical mutation

Fincham and Day (1971) reported the use of certain chemicals as mutagens which can act directly on pre-existing DNA or function as analogues to the DNA -bases in mushroom breeding. In the present study two chemicals, viz., Ethyl methane sulphonate and Methyl methane sulphonate as suggested by Fincham and Day (1971) which act directly on pre-existing DNA were used.

Spores and cultures of the selected *Pleurotus* spp., were treated with

three different concentrations of the mutagens viz., 500 ppm, 1000 ppm and 2000ppm for half hour and one hour. The treated spore or cultures were transferred to fresh agar medium after the required time and incubated. Each mutated culture was carefully examined for the morphological changes and growth (Calam, 1969)

Maximum sporocarp yield was recorded by the mutant *P. petaloides* (Pet-2) treated at 2000 ppm with EMS (1 H) followed by *P. florida* (Pfh-1) at 1000 ppm with EMS (½H). Treatment of *P. florida* (Pfh-1) at 2000 ppm EMS (1/2 H) has significantly increased the sporocarp yield as compared to its parent culture.

The mutants showed some marked variations in sporocarp characters. The sporocarp of *P. saju-caju* (Psc-1) was found to be larger in size and multilobed. Another mutant *P. sajor-caju* (Psc-1) 2000 ppm (1H) produced very small sporocarp with lobed margin. From a single stipe 2-3 sporocarp could be obtained. The sporocarp characters of the S.H mutant culture of *P. sajor-caju* (Psc-1) reported by Balakrishnan (1994) also agree with the above findings.

The mutant *P. florida* (Pfh-1) treated at 1000 ppm with EMS (1/2 H) was found to be with lesser spores. The same isolate treated at 2000 ppm of EMS produced very small sporocarp with long stipe. Breeding for sporeless mutant is one of the objectives of genetic improvement in *Pleurotus* spp. A number of workers reported sporeless mutants in *Pluerotus* spp. (Chang *et al.*, 1985; Ohira, 1979). According to Visscher and Pompen (1985) the completely sporeless strain Somycel 3210 produced very small fruit bodies with long stem

does not appear to be promising for Dutch growers. The findings of the present study have similar correlation with the findings of the above workers.

Cultural characters of various *Pleurotus* spp., and mutants

Among the various standard media tested OA medium has promoted maximum mycelial growth in all the cultures except for the mutant of Ananthan (H-1) 1.5 kr and *P. platypus*. OA was significantly superior to all the other media tested. This was followed by YEA medium Suharban (1987) reported OA medium as the best medium for *Pleurotus* spp., followed by PDA as observed in the present study. Rangad and Jandaik (1977) suggested that PDA fortified with yeast extract agar for better growth of most of the species of *Pleurotus*.

An attempt was made to study the effect of coconut milk on the mycelial growth of *Pleurotus* spp. Modified yeast extract agar blended with 40 per cent coconut milk was found to influence more on mycelial growth of most of the cultures and this was followed by the modified OA medium. It was also noticed that the addition of 40 per cent coconut milk has improved the quality of other standard media namely PDA and TDA. It was also observed that the coconut milk blended media has influenced mycelial growth in majority of the species of *Pleurotus* tested. Coconut milk is reported to be the richest natural source of growth substances especially kinetin. It also contains many vitamins in addition to the essential amino acids. This rich nutrient status of the coconut milk might have enhanced the mycelial growth as observed in the present study. It has been reported that addition of coconut endosperm in a liquid medium enhanced the mycelial growth in the case of

Volvariella volvaceae (Ramos, 1967) which is in full agreement with the present study.

Reaction of dikaryons and mutants against insect pests and common weed moulds

Incidence of insect pests and competitor weed moulds in *Pleurotus* spp., were reported earlier by several workers. *Megaselia* sp., a phorid fly is reported for the first time in India on Oyster mushroom beds by Moorthy *et al.* (1991). The maggots feed on the mycelium *of P. citrinopileatus* Singer and *P. sajor- caju* (Fr.) Singer and cause extensive damage in spawn running stage. Johal and Kaushal (1991) reported the occurrence of cecids on mushroom beds of *Pleurotus* spp. for the first time in India. It has been reported that the phorids are one of the most important pests of mushroom in India (Shandilya *et al.*, 1975). Szudyga (1978) reported severe occurrence of these dipteran flies on *P.sajor-caju*. Rajan *et al.* (1991) reportd the unusual damages of oyster mushrooms by a beetle, *Staphylinus* spp., in Kerala for first time in India. In the present study only mild infestation of phorid flies and cecid flies were observed in the mushroom beds of mutants viz., *P.florida* (Pfh-1) treated with 1000 ppm of EMS (1H) and *P.florida* (Pfh-1) treated with 2000 ppm EMS (1H) as against very severe infection in their control treatment. Mild infestation of *staphylinus* sp. was observed in the dikaryons *P.sajor-caju* (Psc-1) , *P. florida* (Pf-1) and *P.platypus* (Pts-1)MK 3 X *P. citrinopileatus* (Pc-1) MK-2 while in their parents severe to very severe infestation was observed.

The mutant of the hybrid Ananthan 1.5 Kr showed resistance against all the insect pests and competitor moulds considered in the study. The tolerance of this hybrid against *Trichoderma* spp., was reported earlier by Balakrishnan (1994). This tolerance may be due to the ~~mutation~~ segregation of the gene responsible for tolerance against *Trichoderma* spp., from the hybrid to its mutant.

The native species of *Pleurotus* which were collected and domesticated during the course of the study and the mutants developed have shown tolerance against all the insect pests and competitor moulds. This shows their inherent desirable quality to support large scale cultivation.

Sharma and Jandaik (1980) reported that the green mould (*T. viride*) is a damaging competitor in *Pleurotus* beds. Bharadwaj and Seth (1983) and Staunton (1987) have also reported *T. viride* as the common contaminant mould of both spawn as well as *Pleurotus* beds. Das and Suharban (1991) reported that many fungi, mainly species of *Trichoderma, Aspergillus, Penicillium, Mucor, Rhizopus, Chaetomium, and Coprinus* were found constantly associated with the beds of oyster mushrooms in Kerala. These organisms were found to inhibit the spawn run at various stages of growth, sometimes resulting in complete crop failure. The spawn run was completely distorted by their competition for food materials and by production of toxic materials. The dikaryon *P.sajor-caju* (Psc-1) X *P. florida* (Pf-1) showed mild infection of *Trichoderma* spp., as against very severe infection observed in its parents. The dikaryon *P. platypus* (Pts-1) MK-3 X *P. citrinopileatus* (Pc-1) MK-2 showed tolerance against *Trichoderma* sp while its parents were highly susceptible to the fungus.. This may be due to the expression of tolerance

which was recessive in the parents.

Organoleptic studies

A study conducted by Desai *et al.* (1991) revealed that pink *Pleurotus* has significantly high acceptance for its attractive flesh colour while *P. sajor-caju* has poor acceptability due to its brown grey colour. Texture of pink *Pleurotus* was firm, crisp and melting while *P. sajor-caju* did not have melting texture. Taste and flavour of both the mushrooms were evaluated as good. Overall acceptance of pink *Pleurotus* was high as compared to *P. sajor-caju.*

In a comparative study Balakrishnan (1994) showed that *Pleurotus sapidus, P. membranaceous* and *P. petaloides* were obtained maximum rank means with respect to colour, appearance and flavour. Overall acceptability of these species were significant when compared to the standard species *P. sajor-caju* and *P. flabellatus* which were found inferior in all the qualities. With regard to the cooked mushroom,colour and acceptance of different species differed significantly, whereas flavour, texture and taste of all the species were on par. In the present study it was proved that overall consumer acceptability of all the tested species of *Pleurotus* was significant as compared to the stndard species *P. sajor-caju.*

SUMMARY

SUMMARY

An intensive attempt was made to domesticate wild native tropical mushrooms by local collection from selected areas of Thiruvananthapuram and Kollam districts. The native species were identified and subjected to preliminary study to explore their suitability to bring them under cultivation. Two species were found amenable for large scale cultivation under Kerala conditions viz., *P. eous* and *P. petaloides*. Those species were described and subjected to detailed studies, along with the other cultures procured from various mushroom research centres of the country.

Studies on strain improvement programmes

1. Strain mixing

An attempt was made to evolve hybrids by strain mixing. The recombined strain *P. sajor-caju* (Psc -1) x *P. florida* (Pf -1) recorded the maximum sporocarp yield and it was superior to one of the parents. The recombined strain showed intermediate sporocarp caharacters between the parents. *P. sajor - caju* (Psc - 1) x *P. platypus* yielded better than *P. platypus*. None of the recombined cultures showed any improvement in spawn growth compared to their parents.

Hybridization

Cultural characters of monospores showed that the cultures *P. eous* (Pe -1) MK - 11 and *P. citrinopileatus* (Pc -1) MK -2 recorded the maximum

mycelial growth followed by *P. eous* (Pe -1) MK -12 and Ananthan (H -1) MK -10.

Pairing of compatible monospore cultures

Attempts were made to evolve dikaryons (Hybrids) by pairing compatible monospore cultures. The dikaryon *P. platypus* (Pts -1) MK -3 x *P. citrinopileatus* (Pc -1) MK -2 showed maximum desirable characters viz. attractive white colour, higher yield good consumer acceptability. The hybrid *P. florida* (Pf -1) MK -7 x Ananthan (H - 1) MK - 10 showed poor performance when compared to their parents.

Mutation Breeding

Irradiation

An attempt was made to develop mutants of *Pleurotus* spp., by gamma irradiation. The mutant *P. florida* (Pf -1) MK-1 2.5 Kr was a best yielder compared to other species. Gamma irradiation has a positive effect on *P. florida* (Pf -1) MK-1. Sporocarp yield of *P. floirida* (Pf -3) and *P. platypus* (Pts -1) increased as the level of irradiation was increasesd. Ananthan (H -1) gave maximum sporocarp yield at 1.5 Kr level of irradiation with attractive colour.

Sporocarps of *P. florida* (Pf -1) at 2.0 Kr and *P. florida* (Pf -1) MK -1 at 2.5 Kr level of irradiation have shown some peculiar characters. The sporocarps were found to be branched from a single basal stipe. The stipe was long and thick. Pileus was unopened having distant free gills with lesser spore

load. Similarly, Ananthan at 1.5 Kr level produced sporocarps which were more soft and smaller than its original unirradiated culture.

Chemical mutation

Chemical mutation was done by treating the cultures and spores of different species of *Pleurotus*. Maximum sporocarp yield was recorded by the mutant *P. petaloides* (Pet -1) treated at 2000 ppm with EMS (IH) followed by *P. florida* (Pfh -1) at 1000 ppm with EMS (½H). Treatment of *P. florida* (Pfh -1) at 2000 ppm EMS (1/2 H) has significantly increased the sporocarp yield as compared to its parent culture.

The mutants showed some marked variation in sporocarp characters. The sporocarp of mutants of *P. sajor-caju* (Psc -1) was found to be larger in size and multilobed. Another mutant *P. sajor caju* (Psc -1) 2000 ppm EMS (1 H) produced very small sporocarp with lobed margin. From a single stipe 2-3 sporocarp could be obtained. The mutant of *Pleurotus florida* (Pfh -1) treated at 1000 ppm with EMS (1/2 H) was found to have lesser spores. The same isolate treated at 2000 ppm of EMS produced very small sporocarp with long stipe.

Cultural characters of various *Pleurotus* spp., and mutants

Among the various media tested Oats agar medium (OA) has promoted maximum mycelial growth in all the cultures except for the mutant of Ananthan (H-1) 1.5 Kr and *Pleurotus platypus*. OA was significantly superior to all other media. This was followed by yeast extract. Modified yeast extract agar

(YEA blended with 40 per cent coconut milk) was found to influence more on mycelial growth for most of the cultures and this was followed by the modified OA medium. It was also observed that the coconut milk blended media has influenced speedy mycelial growth in majority of the species of *Pleurotus* tested. Another observation is that addition of 40 per cent coconut milk has improved the quality of other standard media also namely, Potato dextrose agar (PDA) and Tapioca dextrose agar (TDA).

Reaction of dikaryons and mutants against insect pests and common weeds moulds

In the case of insect pests, mild infection of phorid flies and cecid flies were observed in mushroom beds of mutants viz. *Pleurotus florida* (Pfh -1) EMS 1000 ppm (0.1 H) and *Pleurotus florida* (Pfh -1) 2000 ppm (0.1 H) as against very severe infection in their control treatments. The mutant Ananthan (H-1) 1.5 Kr showed tolerance against all the insect pests and competitor moulds. The native species of *Pleurotus* which are collected during the course of study and their mutants showed minimum damages by insect pests. The dikaryon *Pleurotus sajor - caju* (Psc-1) X *Pleurotus florida* (Pf -1) showed only mild incidence to *Trichoderma* spp compared to the parent ones.

Organoleptic studies

In the case of raw mushrooms, *P. eous* (Pe-1) and *P. petaloides* (Pet-2) treated at 2000 ppm EMS (I H) showed maximum acceptability with respect to

appearance, colour and flavour. With regard to the taste, *P. eous* (Pe-1) and *P. eous* treated at 1000 ppm (1/2 H) ranked first. In the case of cooked mushrooms *P. eous* (Pe-1) got maximum rank with respect to appearance, colour, flavour and taste. Overall acceptability of all the tested species were statistically superior when compared to the standard species.

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STRAIN IMPROVEMENT IN OYSTER MUSHROOM (*Pleurotus* spp.)

By

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ABSTRACT OF THESIS

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ABSTRACT

Attempts were made to collect native flora of *Pleurotus* spp., by doing intensive surveys for the same during monsoon periods. Among the various isolates made two efficient ones were identified and described namely, *Pleurotus eous* and *P. petaloides*. Evaluation of growth and fruiting behaviour of these isolates done following the standard method and it is proved that these isolates were amenable for large scale cultivation under Kerala conditions. The two selected isolates along with the other standard cultures procurred from various mushroom research centres of the country were subjected to detailed observations. Strain improvement programmes have been done following strain mixing hybridization and mutation techniques. One recombined strain was developed through strain mixing which has got good yield and intermediate sporocarp characters between the parents. Physical mutation was done by irradiating them with gamma radiation. Among the mutants three effective mutants were selected for their better sporocarp yield, steady yielding character and resistance against insect pests and competitor weed moulds. In the chemical mutation, one mutant having small sporocarp with long stipe and other mutant having sporocarp with very few spores have been developed. In hybridization, one hybrid was developed by pairing compatible monospore cultures of two different species of Pleurotus which have got maximum desirable characters among the developed hybrids. Cultural characters of the selected isolates were studied and the effect of coconut milk in enhancing mycelial growth were proved. Periodical observation of the incidence of insect pests and competitor weed moulds in the mutants, hybrids and native isolates have been

consumer acceptability have proved that the native isolates were comparatively better to the other species of *Pleurotus* obtained from various mushroom research centres of the country. Organoleptic studies of selected hybrids and mutants have been proved that they have better acceptability in the market as compared to the standard species, *Pleurotus sajor-caju.*