

**STUDIES ON THE GENETIC AND BIOCHEMICAL FACTORS
GOVERNING SPAWN RUN AND FRUCTIFICATION OF
A CULTIVATED AND A DOMESTICATED MUSHROOM**

**A THESIS
SUBMITTED TO THE
BIDHAN CHANDRA KRISHI VISWAVIDYALAYA
FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
PLANT PATHOLOGY**

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Dedicated to
My Beloved Mother

Bidhan Chandra Krishi Viswa Vidyalyaya

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This is to certify that the work recorded in the thesis entitled "STUDIES ON THE GENETIC AND BIOCHEMICAL FACTORS GOVERNING SPAWN RUN AND FRUCTIFICATION OF A CULTIVATED AND A DOMESTICATED MUSHROOM" submitted by Shri Nandalal Ghosh for the award of the Degree of Doctor of Philosophy in Plant Pathology of the Bidhan Chandra Krishi Viswavidyalaya, is the faithful and bonafide research work carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

D. K. Chakravarty
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Chapter-1

INTRODUCTION

INTRODUCTION

The genus Pleurotus is widely distributed one with lignicolous, saprophytic habit. Pleurotus ostreatus and a few other species in this genus have long been an article of diet with the people both in the West and in the East. The history of its artificial cultivation is relatively short (Block et al., 1958, 1959). The most popular oyster mushroom under cultivation in India is P.sajor-caju (Jandaik, 1974). This mushroom has greyish brown shade and can be grown under a temperature range of 20-26°C (Kaul, 1983) and hence its cultivation is restricted to summer months in hills and winter months in plains of India. The author during his survey of mushroom flora of Kalimpong subdivision of Darjeeling district of West Bengal found a species of Pleurotus growing on the decaying wood logs of Alnus nepalensis (Alder) and Juglans regia (walnut). This mushroom producing creamy white fruit bodies was identified as P.citrinopileatus and was domesticated by the author.

Cultivation of Pleurotus sajour-caju is gaining popularity in different corners of our country because of its easy method and low cost of cultivation, short duration of cropping and higher yield. But temperature sensitiveness and pigmentation of fruit bodies are some of the problems hindering its wide acceptance by the commercial growers. (In the present investigation, studies were undertaken on the genetic and biochemical factors

governing spawn run and fructification of the newly domesticated P.citrinopileatus and the established P.sajor-caju, to find out an improved variety of oyster mushroom free from pigmentation, tolerant to wider temperature and of higher nutritive value, which will be more acceptable to commercial growers.)

The investigations on P.sajor-caju included (i) understanding sexuality of the mushroom by isolating monosporous cultures and studying their fertility pattern from their mating behaviour, (ii) grouping of monosporous cultures in different compatibility groups, (iii) development of heterokaryons by recombination of suitable monosporous lines, and (iv) characterisation of heterokaryons.

The studies on P.citrinopileatus centred around (i) selection of proper ecological variant, suitable for cultivation, (ii) test of temperature tolerance, (iii) feeding trials of experimental animals, and (iv) determination of nutritive value of the mushroom.

Chapter – 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

I. Basidiospores and its germination

Basidiospores, the basic sexual units of basidiomycetous fungi originate externally on the specialised cells called basidia. They represent the segregating products of meiosis. As a generalised scheme, a basidiospore on germination produces haploid homokaryotic mycelium with an inherent deficiency in its nuclear set up. So, it bears a tendency to fuse with a compatible partner and produces a fertile dikaryon. Variation in desirable characters often occurs in a population. Breeding is the only controllable means by which desired genetic traits from different stocks can be combined. The efficiency of breeding of a mushroom species depends on its sexuality, fruiting competence, viability and germinability of spores, and relatively short and predominantly haploid life cycle that can be controlled in laboratory condition.

Pleurotus sajor-caju is a heterothallic species and tetra-sterigmatic-quadrifid in nature. Spores germinate easily in specially amended nutritional media. Spores preserved in dry condition in refrigerator retained their viability even after one year. Using vacuum-drying tube preservation method, a successful attempt by Rhodes (1950a,b), Soriano (1950, 1951) and Akai and Asada (1961), it was found possible to keep spores of various micro-organisms viable for a certain period of time. Hu and Song

(1967) successfully got the viability of basidiospores of Agaricus campestris, preserved by vacuum-drying method under various temperatures between -4°C to 25°C , barely after one year. According to Sarazin (1955) spores of cultivated mushrooms when well desiccated, remained viable for 3-5 years, but germination frequency decreased with age. Kneebone (1968) reported the amazing success in germinating spores of Agaricus bisporus after 36 years of storage at approximately 0°C . The success proved the evidence of potential longevity of mushroom spores.

Germination of basidiospores of cultivated mushroom was first reported by Costantin and Matruchot (1893). Spores found to germinate better when sown in sufficiently dense masses rather than in isolated condition. Sarazin (1955) reported maximum germination of over 80% after 48 hours of incubation at 28°C . Ferguson (1902) succeeded in stimulating the germination of spores by growing mycelium on the adjoining area of culture media. He was later supported by Kligman (1950). According to Raper (1976b) spore viability in Agaricus bitorquis the desirable feature for breeding of strains, varies from less than 5% to over 90%. Byun et al. (1981) obtained germination of basidiospores of Agaricus bisporus best on water agar.

Chang (1969), Chang and Chu (1969a) obtained the germination of basidiospores of Volvariella volvacea by using a specially formulated medium. They maintained an incubation temperature of 40°C for 48 hours to get maximum germination of spores. According to Santiago (1983) the frequency of basidiospore

germination of Volvariella volvacea in liquid culture was higher in complex media (malt extract) than in defined media (Czapek Dox). The temperature range ideal for germination was 35°C with pH optima of 7.0 and 6.5 respectively in complete and minimal media.

II. Isolation of monosporous culture

Isolation of single spore or monosporous germling to raise the monosporous cultures was considered as one of the important steps before entering further complexity of breeding work. Although breeding work scarcely struck to reports, scientists had advanced several methods for the isolation of monosporous cultures conveniently. Fritsche (1964) in his breeding work with Agaricus bisporus isolated monosporous cultures simply by dilution plating and isolation of tiny colonies assuming single spore origin. This method only facilitated to develop large number of monosporous lines but could not ensure its single spore origin. Sarazin (1955) recommended the use of micromanipulator to isolate single spore or germinated single spore from a mass inoculum of spores.

The use of cutter lens in isolation of single spore is a general practice in biological science. It has also been implicated largely in isolation of single spores in mushroom breeding. Chang and Yau (1971) isolated single spores of Volvariella volvacea after germination of spores on agar plates. Single germinated spores were cut from the medium under microscope by means of a 'spore cutter' as described by Raper (1963). Eger

(1978) in his breeding work with Pleurotus ostreatus recommended similar method of isolation by examining single germlings on the agar surface and cut them out under microscope.

III. Determination of sexuality and incompatibility groups

Pleurotus sajor-caju is heterothallic in nature with tetrapolar incompatibility. It means, basidiospore develops haploid homokaryotic mycelium bearing single allele of the two factors and is practically self sterile. Fertility can be reinstated by mating with a right partner carrying a compatible allele that is complementary for each other. The net outcome of such a compatible reaction leads to develop a fertile dikaryon. On progress the dikaryon reaches the stage of sporulation when two non-identical nuclei fuse in the basidium followed by the meiotic segregation of incompatibility alleles. The four basidiospores thus develop on the basidium receive one haploid post meiotic nucleus each and thus completes the cycle.

A comprehensive review of life cycle and sexual mechanism in fungi was presented by Esser (1966), Raper (1960, 1966a) and Whitehouse (1949). Koltin et al. (1972) and Raper (1953, 1966b) explored the detail of sexuality in Basidiomycetes. After a painstaking study with thousands of basidiomycetous fungi Raper (1966b) came to the conclusion that heterothallism predominates in Basidiomycetes. Further analysis of the same was represented as, 65% to be heterothallic under bifactorial control, 25% under heterothallic unifactorial control and the rest 10% to be covered by either primary or secondary homothallic forms.

The same token was found applicable in case of edible mushroom fungi with which laid our breeding interest. Because, it is heterothallic species which opened huge scope of outbreeding and possible means to make a new combination in gene pool. Unfortunately, the widely cultivated high prized mushroom Agaricus bisporus was reported to bear secondary homothallism (Jiri, 1965; Evans, 1959; Sarazin, 1938; Colson, 1935; and Sass, 1928). In contrast to Agaricus bisporus, Agaricus bitorquis represents unifactorial heterothallism as revealed by Raper (1976a,b) and Raper and Kaye (1978), through the method of genetic analysis.

Bensaude (1917, 1918) established heterothallic mode of sexuality in Coprinus fimetarius. Mounce (1922) evidenced to prove its bifactorial incompatibility which was confirmed later by other scientists also.

Heterothallic sexuality in Flammulina velutipes was suggested first by Kniep (1920) on the basis of observation on self sterile and cross fertile mycelium of monosporous isolates. Also, bifactorial incompatibility was documented by Su (1973); Takemaru (1961b, 1957a); Aschan (1954); Oddoux (1953); Brodie (1936); Heldmaier (1929); Zattler (1924); and Vandendries (1923).

Barnett (1937) designated Auricularia auricula as heterothallic species with unifactorial control, while another related species Auricularia polytricha was found to be heterothallic in nature with bifactorial compatibility (Su, 1973).

In shiitake mushroom, Lentinus edodes, bifactorial heterothallism was confirmed by Tanaka and Koga (1972).

Japanese mushroom Pholiota nameko was reported as heterothallic with unifactorial control except occasional formation of binucleate spores (Arita, 1973; Arita and Takemaru, 1962).

Anderson (1982) studied the sexual incompatibility system and nuclear cycle of Clitocybe tabescens. It was reported to bear bifactorial heterothallism as determined by mating tests among monosporous progenies of a single fruit body.

Pleurotus ostreatus, a very closely related mushroom fungus to Pleurotus sajor-caju, bears bifactorial heterothallism as evidenced by Vandendries (1933). His analysis was based on clamp formation after compatible matings of monosporous isolates. Bifactorial heterothallism in Pleurotus ostreatus was supported by Eger (1974), Anderson et al. (1973), Eugenio and Anderson (1968) and Terakawa (1960). Terakawa (1960, 1957) in addition to his support on sexuality, particularly focussed on the phenomenon of nuclear migration in relation to fully compatible and hemicompatible incompatibility factors. Kaufert (1936) studied heterothallic sexuality of Pleurotus corticatus with bifactorial control. More recently, Jong and Peng (1975) pointed out a common identity between Pleurotus cystidiosus and Pleurotus corticatus.

IV. Selection of parent lines

In a breeding programme when large number of monokaryons are to be considered, compatible matings in all possible

combinations and study of post recombination trials become cumbersome and time consuming. So, it seemed wise to select desirable parent monosporous lines through preliminary studies to make it easy and effective. Sathe and Nagarkar (1983) reported the best incubation temperature for the tropical oyster mushroom, Pleurotus sajor-caju, to be 25-27°C. This temperature sensitivity was reported as the major hinderance against its wide popularisation in tropical and subtropical climates. In breeding programmes development of temperature tolerant recombined variety was tried by selecting temperature tolerant parent stocks. Eger et al. (1976) successfully produced dikaryons with different temperature sensitivity in Pleurotus ostreatus. They selected monokaryons of temperature sensitive German and temperature insensitive American strains as parents.

Although majority of characters inherent in monokaryons remain obscure until dikaryotization, some of them bear good desirable characters which help easy selection of suitable parent lines. Eger (1974) implied the method of fruiting induction on the monokaryons of Florida isolate of Pleurotus ostreatus. In a period of ten days a small fraction of monokaryons formed primordia. This small fraction was obviously considered as the monokaryons with good fruiting competence. Homokaryotic or haploid fruiting was also reported in several species of Hymenomyces (Leonard and Raper, 1969; Takemaru, 1961; Buller, 1941; and Brodie, 1936). In Pholiota nameko however, most of the monokaryotic mycelia found to produce haploid fruit bodies on saw-dust rice bran medium (Arita, 1968a).

Pileus colour was considered as a striking characteristic along with other qualitative characters as slow and fast producing strains in Agaricus bisporus (Riber Rasmussen, 1959). It was suggested to consider both the slow and fast growing lines during selection of parents. Eger (1974) reported that slow growth may be linked with incompatibility factors as in Pleurotus ostreatus, or with characteristics that may be important in breeding work.

Strain differences in Agaricus bisporus was noted by Fritsche and Sengbusch (1961) on the points of yield, weight per mushroom and quality.

Eger et al (1976) developed a sporeless dikaryon, "F 42 x11" in Pleurotus ostreatus through di-mon mating. This 'sporeless' character was found as dominant factor in Florida isolate of the dikaryotic parent.

Of course, Pleurotus sajor-caju was reported as an efficient coloniser and fast growing mushroom fungus but its light to deep brown colour, large size of the fruitbodies and high bud mortality (Ghosh and Chakravarty, 1986) were considered as demerits. These points of demerit could be replaced only by trials of mutation and selective breeding.

V. Trials of recombination

The science of mushroom is mainly based on the studies and achievements on cultivated mushroom Agaricus bisporus. Often

attempts were made to raise a suitable variety showing better performance over the existing stocks. All those done merely through selection of best ecological variants of natural occurrence or from homothallic monosporous lines of a population. But the concept of breeding and genetic manipulation remained almost unravelled. However, breeding and genetic manipulation were proved as the only controllable means to combine desired genetic traits of different stocks and to develop a new recombined variety.

In 1962 at the 5th International Congress on Mushroom Science, Moessner reported his successful bid to develop a new strain of Agaricus bisporus by hyphal anastomosis between different strains. Fritsche (1964) reported the results of her attempts on crossing two strains of cultivated mushroom. 'Hu' a commercial strain with normal fruit bodies and blond cap was crossed with the strain '59' with white fruitbodies but deformed shape. Recombined variety developed successfully with normal shaped white fruitbodies. This proved the feasibility of combination breeding in cultivated mushrooms. Raper (1966a, b) was of opinion that in somatic recombination exchange of genes between two different nuclei occur occasionally and give rise to two new gene sets. Recombination of characters by hyphal anastomosis was demonstrated by Chang and Ling (1970). Chang and Yau (1971) also showed evidence to prove that paired atypical monosporous mycelia in V.volvacea could produce fruitbodies while unpaired mycelia could not.

In crop plants hybrids were found to give higher yield and luxurious growth compared to the parents. It was proved possible in Pleurotus ostreatus also. Dikaryons resulted from interstock matings performed better with higher fruitbody yield than intra-stock matings (Eugenio and Anderson, 1968). Dikaryotic mycelia grew considerably faster than their parental monokaryons as noted by Croft and Smichen (1965) in Flammulina velutipes, a tetrapolar heterothallic species. Smichen (1965) observed significant variation of growth rate of monosporous cultures of the species isolated from single fruitbody. He carefully studied the variation in growth rate of dikaryotic mycelium obtained by crossing compatible monokaryons, their ability to form fruitbodies, number of primordia and sporophores, and time necessary for primordia initiation. The prospect of exploitation of heterosis by synthesis of heterokaryon as advanced by Kneebone et al. (1972) and Raper et al. (1972) for Agaricus bisporus seemed possible as the way of improving the performance of hyphal growth. Fritsche (1978) described the variation in characteristics of K46, a recombined variety of Agaricus bitorquis developed by a cross between Horst K26 and K32. Comparative cropping trials of the recombined and recombinant lines showed slight difference in total yield/m².

Successful attempt by Eger et al. (1976), to hybridize temperature sensitive German and temperature insensitive American Pleurotus ostreatus led to develop recombined variety with changed temperature sensitivity. Through the crosses with monokaryons they got four types of dikaryons as : (i) whole fruiting process

temperature sensitive as German stock, (ii) insensitive as American stock, (iii) primordia formation insensitive but sporophore development sensitive to elevated temperatures, (iv) primordia initiation and initial sporophore development temperature sensitive but pileus expansion and basidiospore production insensitive. On this idea it was suggested that fruiting process in Pleurotus was not controlled by a single gene rather by several genes as in Coprinus macrorhizus (Takemaru and Kamada, 1971).

Royse and May (1982) made comparisons of genetic relatedness among a series of commercial and research maintained lines of Agaricus brunnescens to determine the best lines from which to generate homokaryons (breeding stock) for crossing. They stressed on comprehensive and practical approach to the development of improved cultivars of commercial mushroom by increasing genetic variability within and among the lines available. They also viewed on the possibility to produce selected intra-specific heterokaryons from homozygous single spore progeny with noble genotypes.

Anderson (1982) observed that compatible pairings of monosporous isolates of Clitocybe tabescens were distinguishable from incompatible pairings on the basis of macroscopic colony morphology and presence or absence of clamp connections and dikaryotic cells.

Liao (1984) successfully bred a new variety of shiitake mushroom, L 107, derived from the cross between Taiwan and

Japanese cultivated strains (K53 x D54) of Lentinus edodes. The recombined isolate produced a large economic yield and proved suitable for cultivation in low areas. The fruitbodies were large and normal.

VI. Selection of ecological variants

Selection as a method of choosing the best individuals from its population is a general practice in crop improvement. Upto date development in mushroom crop by raising improved cultivars has made possible mainly by selection of best ecological variants. Such variation in nature came through the way of spontaneous mutation, chance mating ensuring a new combination of characters and finally through the natural selection. For instance, all white mushrooms employed in commercial cultivation of Agaricus bisporus arose as the chance cluster in the bed of cream strain (Kligman, 1950). Fritsche (1976) found greater variability among the isolates of Agaricus bitorquis collected from different origins. Mushroom with desirable round cap and relatively long stipe was screened out of the majority with flat cap and short, thick stipe. Eger et al. (1976), based on the studies conducted, vehemently recommended the Florida and German isolates to belong in the same species of Pleurotus ostreatus. Florida isolate grows at the temperature of 4-24°C while German isolate do so only below 15°C. If so, the same species in different habitat adapted selectively to different temperature sensitivity.

Kaul and Kachroo (1975) made an evaluation test of yield among 18 strains of Agaricus bisporus. RRL 89 and RRL 106 were selected for their consistently highest yield. Gapinski and Glebionek (1978) carried out a comparative study among the strains of A1, A4 and A8 (from Switzerland), B2 (a local selection) and S53 (from France). Highest total yield along with best quality was observed in case of locally selected B2 strain. You et al. (1978) selected a new strain of Agaricus bisporus, No.703, based on its resistance to wet bubble and bacterial bloch disease. It gave an increased yield when cropping method was modified with increased bed depth. Chung et al. (1981) carried out an experiment for the selection of highest yield and good quality oyster mushroom strain for breeding. The strains were screened on the basis of their agro-ecological behaviour. Bisko (1982) reported the fundamentals of primary selection and developed highly productive strains of Agaricus bisporus for surface cultivation. Royse and May (1982) studied with 34 commercial and 162 research maintained lines of Agaricus brunneus to determine the best lines for crossing. They also studied with genetic relatedness among the lines and its application to selective breeding.

VII. Physical factors

Mushroom fungi thrive in a wide range of ecological conditions but its satisfactory performance needs to prevail ideal conditions at the successive stages of growth. Among others, temperature, light, humidity and aeration are of worth mentioning.

(a) Temperature :

The performance of mushroom fungi depends largely on the incubation temperature maintained in the micro-environment of mushroom house. For both of the mycelial growth and fructification mushroom fungi has a critical temperature requirement. Below that critical temperature growth becomes slower and above that level it has been proved to be injurious.

Different strains of Pleurotus ostreatus found to grow at the temperature range of 4-30°C. Eger et al. (1976) observed that American strain of the mushroom grew well at the temperature of 4-25°C whereas German one grew well only below 15°C. Quimio (1977) recommended 28-30°C temperature for the best growth of this mushroom on saw dust substrate ammended with rice bran, oat meal or mud press. Platt et al. (1982) obtained early and best yield of the mushroom grown on cotton straw substrate at 39°C compared to wheat straw substrate. Ginterova et al. (1982) grew Pleurotus ostreatus on different agricultural wastes at 18-23°C for 35 days. Fructification obtained naturally at 8-14°C or in phytotron with 12 hours light-dark cycle at 18°C during light period and 8°C during the dark period. Imbernon et al. (1983) made a comparative study on crop earliness, yield and average weight of Pleurotus ostreatus, and obtained earliest and best total yield at 12°C.

It has been found that, after the vegetative phase of colonisation, there is need to lower the bed temperature for an effective stimulation towards sexual phase. Ferri (1970)

reported the appearance of fruitbodies of Pleurotus ostreatus in Italy during autumn when temperature came down to 15°C. Schmaus (1972) observed temperature requirement of 22-24°C for vegetative growth while fruiting needed lowering of temperature to 4-14°C. Zadrazil and Schliemann (1975) recommended fructification of this mushroom upto 15°C when their colour remained unaffected. Zadrazil (1976, 1978) established that mycelial growth rate below 15°C was linear and an acceleration phase occurred at 15-20°C which gradually dropped off between 20-30°C. Cayrol (1978a) prescribed an average temperature of 15°C for Pleurotus ostreatus and 25°C for Pleurotus florida. Cayrol (1978b) used steam sterilized straw substrate for Pleurotus ostreatus, colonised at 25°C for three weeks and then transferred to aerated growing chamber with a temperature of 13°C. Fructification obtained after 7 days. Bisht and Harsh (1983) obtained good crop of Pleurotus ostreatus from used-tea-leaves substrate. Inoculated substrate was incubated at 25°C for colonisation, while fructification obtained at room temperature of 15-22°C.

Zadrazil and Schliemann (1975) observed the effect of temperature on Pleurotus florida. It had produced normal fruitbodies between 5-26°C. At higher temperature the variations observed were : less fleshy cap, consistency finer and slightly brittle, water content greater, spore formation faster and more marked and colour paler. Slow growing caps at low temperature contained more yellow and brown pigment. Oso (1977) reported the results of physiological studies on temperature requirements

and its impact on fruitbody development in Pleurotus tuber-regium. Delmas and Mamoun (1980) standardised optimum temperature for growth and fructification of Pleurotus cornucopiae as 23°C and 15-25°C respectively. Later in 1983 they reviewed and recommended the growing temperature between 21-23°C. Mehrotra et al. (1982) obtained economic yield of Pleurotus sajor-caju grown on waste paper at a temperature of 11-15°C. Cayrol (1982) grown Pleurotus pulmonarius on cereal straw substrate maintained at temperatures of 25°C and 20-26°C, for spawn run and fructification respectively. Sathe and Nagarkar (1983) obtained good yield of Pleurotus sajor-caju incubated at 25-27°C for 15 days. According to Imbernon et al. (1983) best early and total yield obtained in Pleurotus columbinus at 12°C and in P. pulmonarius at 20°C. Ghosh and Chakravarty (1986) reported the temperature tolerance of 15-35°C in Pleurotus citrinopileatus with optimum growth and yield between 25-30°C.

Stanek and Rysava (1971) and Vessey and Toth (1970) recommended a cold shock to the substratum to induce the first flush in Pleurotus ostreatus. Zadrazil and Schneiderreit (1972) reported maximum mycelial growth of the introduced species of Pleurotus at 30°C and fruiting formed at 19-25°C. Jandaik and Kapoor (1975b) observed best mycelial growth of Pleurotus sajor-caju at 25°C. Zadrazil (1976) studied the relation of mycelial growth of some species of Pleurotus with temperature. Optimum growth was recorded for Pleurotus eryngii as 25°C compared to 30°C for Pleurotus ostreatus and Pleurotus florida. In studies

on the effect of temperature on mycelial growth of some species of Pleurotus, Balazs and Szabo (1979) showed that optimum temperature for the best known Pleurotus proved to be 25°C. Growth rate decreased with decreasing temperature and stopped at 10°C. Maximum heat tolerance observed upto 40°C.

(b) Light :

Mushroom fungi governs two successive stages in its life cycle. Vegetative growth phase or colonisation is followed by sexual phase in the form of fructification. This transition from vegetative phase to sexual phase is induced by light. As darkness stimulate vegetative growth (Cayrol, 1982; Delmas and Mamoun, 1980; Chakravarty and Mallick, 1979), this condition can be imposed by placing the bed in dark growing chamber or by covering with black polythene sheet. When the spawn run is over it needs to expose in light to get good fruitbodies. Zadrazil and Schneiderei (1972) and Trandaf et al. (1974) studied the effect of light on growth of Pleurotus species. Kurtzman (1978) reported that initials formed in shake culture of Pleurotus sapidus developed into typical fruitbodies only when it was placed under low intensity fluorescent light. Eger (1970a) observed increase in the number of primordia with increased duration and intensity of light in Pleurotus florida. Eger et al. (1974) also observed similar response of light on primordia initiation and fruitbody development in P.ostreatus.

Delmas and Mamoun (1980) recommended probable best growth of Pleurotus cornucopiae in darkness and best fruiting in

continuous light (below 250 lux). Ginterova et al. (1982) reported fructification of Pleurotus ostreatus in natural condition at 8-14°C or in phytotron with alternate 12 hour light-dark cycles provided by 18° and 8°C temperature respectively. Cayrol (1982) conducted spawn run of Pleurotus pulmonarius for 15 days in darkness followed by its exposure in a growth room. Sporophores appeared 20 days late with lighting at 60 lux for 8 hours/24 hours. Schmaus (1972) reported fruiting induction in Pleurotus ostreatus by 5000 lux light from neon tubes. Gyuroko (1972) advanced 10,000 lux-hr. light necessity for basidiocarp development in the same species. Durand and Roger (1982) studied spectral sensitivity of the development of Coprinus congregatus. Spectral sensitivity for photo-induction of primordia formation, photoinhibition of primordia development and photomaturatation in primordia was quite similar, suggesting a common photoreceptor during fruitbody morphogenesis. Most effective wavelengths were in blue (445 nm) and wavelength >510 nm were ineffective. The action spectrum was similar to many blue light responses in which flavoprotein was postulated as photoreceptor.

(c) Relative humidity :

Beside other factors, high humidity is essential for optimum colonisation and yield of mushroom. Rise in temperature and circulation of fresh air during fruiting phase subsequently increase the rate of evaporation and thus impart cultural adversity to the fungi. Maintenance of essentially high humidity in cultivated mushroom is done by casing the substrate with garden

soil. Inoculated substrate wrapped with polythene (paper) during colonisation and regular irrigation at fruiting phase are the ways to maintain requisite humidity in different stages of oyster mushroom cultivation.

Relative humidity referred suitable for different species of Pleurotus is tabulated accordingly.

<u>Pleurotus</u> spp.	60-80% (Zadrazil, 1978)
<u>Pleurotus</u> spp.	80% (Hilber, 1977)
<u>P.abolonus</u>	90-95% (Han <u>et al.</u> , 1977)
<u>P.eryngii</u>	85-95% (Cailleux and Diop, 1976b)
<u>P.flabellatus</u>	70-80% (Bano <u>et al.</u> , 1979)
<u>P.florida</u>	85% (Block <u>et al.</u> , 1958)
	75-90% (Kalberer, 1976)
	80-95% (Zadrazil, 1973)
<u>P.sajor-caju</u>	85-90% (Jandaik and Kapoor, 1976a)

Ferri (1970) reported the adverse effect of draught on the production of Pleurotus ostreatus. Schmaus (1972) obtained reportedly good vegetative growth of Pleurotus ostreatus within 2-3 weeks under high humidity. Cayrol (1982; 1978b) prescribed transfer of inoculated substrate in growing room with RH of 90%. Cheng and Han (1977) advanced the view of significant effect of temperature and humidity on growth and formation of fruitbody in Pleurotus species. Jandaik and Kapoor (1976a) studied the effect of environmental factors on Pleurotus sajor-caju and found 75-80% humidity to be optimum. There was no fruiting below 65% humidity and abnormal development resulted in saturated

atmosphere. Delmas and Mamoun (1983) referred maximum RH for the cultivation of Pleurotus cornucopiae on barley and rice straw substrate.

(d) Aeration :

Mushroom fungi need circulation of fresh air particularly during fruiting phase. Because, increased degree of fresh air inside the mushroom bed is necessary for the induction of physiological changes from vegetative growth phase of spawn run to fructification. In spawn run phase it needs meagre aeration which means to preserve the favourable factors of increased humidity and CO₂ concentration. According to Ginterova (1973) Pleurotus required less aeration than many other organisms. He found little difference in growth of submerged cultures with only sixteen hours shaking a day with those of continuous ventilation. Kurtzman (1978) obtained fruitbody initials of Pleurotus sapidus at a late of 30 days in shake culture when exposed to ventilation. Cayrol (1978b) reported that Pleurotus ostreatus produced fruitbodies after 70 days when inoculated substrate was placed in aerated growth chamber.

Several authors demonstrated the effect of high carbon-dioxide concentrations on growth of mushroom fungi (Zadrazil, 1978; Kurtzman, 1979). Zadrazil (1975) reported that mycelia of at least several species of Pleurotus grew faster with increase in CO₂ concentration approximately upto 22%. Lack of proper aeration in mushroom bed, any way, favours the growth of anaerobic microorganisms and as an ultimatum the mushroom

fungi found to be eliminated. Insufficient ventilation (1-2 vol % CO₂) induced a bunched growth regeneration in Pleurotus ostreatus (Zadrazil, 1978).

VIII. Chemical analysis of mushroom

Mushroom as a choice food has been established undoubtedly because of its good food value. The food value is mainly shared by protein with additional factors as fat, carbohydrate, minerals and vitamins. Proximate composition of mushroom has been analysed following the standard methods as referred by Association of Official Analytical Chemists (Horowitz, 1980). Analytical reports on proximate composition showed significant variation from species to species, often in the same species as well. Beside intrinsic compositional variability it was partially attributed to the analytical methods followed.

(a) Protein :

It is considered as the major attribute to the composition of mushroom. Protein content in mushroom runs a wide range based on inherent and agro-climatic factors. Dzamic et al. (1985) analysed the chemical composition of some edible mushrooms and led the view of, chemical composition depends on species, variety, soil, subregion and other agro-ecological factors. Protein content was found to vary from 4-9% in the species of Auricularia to as high as 44% in Agaricus bisporus based on dry weight (FAO, 1972; Adriano and Cruz, 1933). Maggioni et al. (1968) reported 21% crude protein in the mushroom of Agaricus bisporus produced

from "Houser" spawn while those from "Somycel 87" spawn contained 27-35% protein. They also found that, mushroom grown on compost supplemented with ammonium sulphate or a mixture of urea and ammonium sulphate had no difference in crude protein content. Analytical reports on protein content of different species of Volvariella volvacea found to run between 21-34% (Lee and Chang, 1975; FAO, 1972; Bano et al., 1971; Adriano and Cruz, 1933). Chang (1964) found little difference in protein content of Volvariella volvacea grown on different composts. Kulesh et al. (1978) studied commercial cultivation on thirteen local and foreign (Russian, Japanese, Hungarian, French and East German) strains of Agaricus bisporus under similar conditions of growth. Protein content analysed for different strains ranged from 3.98 to 6.14% (fresh weight).

Bano et al. (1963) averaged the protein content of Indian varieties of Pleurotus species as around 21.6%. Adriano and Cruz (1933) got the protein percentage of 27.4% in Pleurotus ostreatus, while FAO report (1972) mentioned it to be 30.4%. Singh and Rajarathnam (1977) reported protein content of Pleurotus eous to be 33%. Bano et al. (1981) presented the analytical data of Pleurotus eous and Pleurotus florida with protein content around 25-27% (N x 6.25) which showed a conformity with the value reported earlier for Pleurotus flabellatus. Ogundana and Fagade (1982) analysed the nutritive value of some Nigerian edible mushrooms. According to them Termitomyces sp. contained upto 31% protein and in Pleurotus tuber-regium that was of 14.6%. Kress (1985) compared the nutritional value of

edible fungi like Agaricus bisporus, Lentinus edodes, and Pleurotus ostreatus with plant and animal foods. Edible fungi were high in protein content (17.5-30.4%) in relation to most vegetables.

Purakayastha and Nayak (1977) studied (on) the effect of wheat bran and maize meal supplements on yield and protein content of Calocybe indica. Fruitbodies contained 13% protein. Zadrazil (1980a) made a successful bid to increase nitrogen content in fruitbodies of Pleurotus sajor-caju growing on wheat straw substrate mixed with additives like NH_4NO_3 , soyabean meal and alfalfa meal in different proportions. Nitrogen percentage in the fruitbodies in case of only wheat straw was 4.1% but the value increased to 8.9% in case of supplementation with soyabean meal (70:30). It meant to increase the protein content from 17.9 to 38.9% (dry weight). Bahl (1982) reported better protein percentage of 43% in Volvariella volvacea grown on straw bed enriched with gram (Cicer arietinum) and arhar (Cajanus cajan) powder. Zakhary et al. (1984) analysed the mushroom of Volvariella volvacea grown on orange juice waste-bagasse medium and protein content reported to be 21.5% (Total N = 4.92%, non-protein N = 1.64%). Rajarathnam et al. (1986) observed that supplementation of substrate with organic nitrogen after spawn run or supplementation with spent straw increased the yield and fruitbody protein content of Pleurotus flabellatus. Supplementation with casein gave highest protein content in both the cases.

Paranjpe et al. (1979) made a qualitative and quantitative analysis of various tissues of Agaricus bisporus at different morphogenetic stages and found significantly higher soluble protein content in cap than in other tissues. The protein profiles of caps, stalks, pins or primordia and the mycelium were found to be different when studied by polyacrylamide-gel electrophoresis. Miljkovic and Dzamic (1984) tabulated protein contents with an average of 25.67% in Boletus edulis var. edulis, 25.05% in Boletus edulis var. aereus and 12.31% in Cantharellus cibarius var. pallesens. They also pointed out the highest protein content at the earliest growth stage of mushroom. Kosson and Bakowski (1984) analysed the protein and amino acid contents of sporophores at the three developmental stages according to the diameter of the cap : I < 25 mm; II 25-40 mm; III > 40 mm. Protein content in the strain somycel-53 of Agaricus bisporus increased significantly during growth cycle.

(b) Fat :

Crude fat content of mushroom attains a wide range of less than 1% to as high as 20% of the dry weight. On an average the value runs between 2-8%. The crude fat means to include all classes of lipids along with free fatty acids, mono-, di-, and triglycerides, sterols, sterol esters and phospholipids. According to FAO (1972) the lowest fraction of fat (0.2%) was reported in case of Tremella fusiformis. On the contrary, fat content in Volvariella esculenta recorded as 20.6% and proved

highest in the series of mushroom varieties (Adriano and Cruz, 1933). In cultivated mushroom Agaricus bisporus fat content was found to vary between 1.7-8.0% (FAO, 1972; Maggioni et al., 1968). Dzamic et al. (1985) studied the chemical composition of Boletus edulis and Cantharellus cibarius and found the lipid content to vary from 6.26-8.24% and concluded that chemical composition depended on variety and agroecological factors.

Straw mushroom Volvariella volvacea grown on paddy straw supplemented with gram and arhar dal powder was analysed by Bahl (1982) and she recorded a fat content of 6.38% (dry weight). But, Zakhary et al. (1984) got only 2.71% fat (ether extract) from the same species of mushroom grown on orange juice waste-bagasse medium.

Bano et al. (1963) made a generalised recommendation of fat content in Indian varieties of Pleurotus species to be around 7.2%. According to FAO report (1972) Pleurotus ostreatus contained 2.2% fat. Bano et al. (1981) cited an analytical data on Pleurotus eous and Pleurotus florida with fat content of 1.08% and 1.65% respectively. Kress (1985) carried out detailed analytical studies on the nutritional value of edible fungi like Agaricus bisporus, Lentinus edodes and Pleurotus ostreatus. He recommended its very low fat content compared to vegetables and animal food. Huang et al. (1985) made a thorough study on the total lipid content of Volvariella volvacea, Pleurotus sajor-caju, Tremella fusiformis, Lentinus edodes, Agaricus bisporus and Auricularia auricula. They got the result ranging from 0.6-3.1%.

(c) Carbohydrate :

It is a major occupant in the dry matter of mushroom which includes a series of related compounds in association. Detailed analysis on Agaricus bisporus showed the overall picture of the associated members of carbohydrate. They were pentoses (xylose, ribose), methyl pentoses (rhamnose and fucose), hexoses (glucose, galactose and mannose), disaccharides (sucrose), amino sugars (glucosamine and N-acetyl glucosamine), sugar alcohols (mannitol and inositol), sugar acids (galacturonic acid and glucuronic acid) as well as unidentified uronides and methyl sugars (Holtz, 1971; Hughes et al., 1958; McConnell and Esselen, 1947). Different species of mushroom reported to cover a wide variation in total carbohydrate content of 31.7% in Volvariella esculenta to as high as 95% in Tremella fusiformis (FAO, 1972; Adriano and Cruz, 1933).

A higher degree of carbohydrate content was reported in Pleurotus species ranging from 46.6% to 81.8% (FAO, 1972; Adriano and Cruz, 1933; Mendel, 1898) as compared to around 60% in cultivated mushroom Agaricus bisporus (Maggioni et al., 1968; Watt and Merrill, 1963; Pilat, 1954; McConnell and Esselen, 1947; Anderson and Fellers, 1942). Bano et al. (1963) recommended an average value of 60.5% carbohydrate in Indian varieties of oyster mushrooms. Further analysis of carbohydrate by Bano (1967) reported 4.2% soluble carbohydrates, 1.66% pentosans and 32.26% hexosans on dry weight basis. Bano et al. (1981) made a comparative estimation on the composition of Pleurotus eous and

Pleurotus florida; got 59.18% and 58.04% carbohydrate respectively. Ogundana and Fagade (1982) studied on the nutritive value of Termitomyces sp. and Pleurotus tuber-regium, and obtained the value of 32% and 18.6% carbohydrate.

Through detailed analysis of carbohydrates of paddy straw mushroom Zakhary et al. (1984) presented the data of 6.07% reducing sugars, 4.23% non-reducing sugars, 19.2% starch, 5.29% glycogen and 9.86% mannitol. Dzamic et al. (1985) reported the chemical composition of some edible mushrooms and found the sugar contents to vary from 34.1-51.6%. Kress (1985) made a comparative study on the nutritive value of edible fungi with that of plant and animal foods. He concluded with low carbohydrate contents in edible fungi compared to most vegetables.

(d) Minerals :

Mushroom on complete ignition left behind a grey mass of ash averaged 5-10% of the dry weight (Sawaya et al., 1985; Bahl, 1982; Bano et al., 1981, 1963; FAO, 1972; Maggioni et al., 1968). It was found to include all the minerals and heavy metals available in the soil or substrate. According to the report as tabulated by Bano et al. (1981b) it is evident that potassium and phosphorus are the major constituents of ash of Pleurotus species. The same was also true for Agaricus and other edible mushrooms (Bourlier, 1978; Korbonskaya and Amanova, 1975). Excepting Lentinus edodes, calcium content was insignificant in all other mushrooms, but appreciable amount of iron was found to ^{be} present in Pleurotus species as well as in

Agaricus campestris. Compared with most vegetables and animal foods edible mushroom contain very poor amount of calcium (Kress, 1985). Santoprete et al. (1984) were of opinion that age of fruitbody affects the amount and distribution of elements in the mushroom. Bano et al. (1981b) further pointed out higher degree of copper content in all the mushrooms. Cadmium and lead contents were also remarkable. Of the heavy metals zinc content was highest in all species of oyster mushroom although its availability in substrate was very low. This showed a tendency of Pleurotus spp. to accumulate zinc in the sporophores. Accumulation of zinc in sporophores was observed in other mushrooms also (Grabbe and Domsch, 1976). Still the concentration of heavy metals like lead, cadmium, copper and zinc in Pleurotus species were found well within the prescribed limits under Food Product Order and Prevention of Food Adulteration Act (P.F.A. Act (1954), 1979; Sixth Report of FAO and WHO, 1972; F.P.O., 1955).

IX. Nutritional studies :

Mushroom as a food has been considered since the early food gathering stage. Still contradictory opinions were expressed regarding the nutritive value of edible mushroom. Some authors considered them to be devoid of any nutritive value. Information evaluating nutritive value of edible mushroom was struct to report in 1898 by Mendel followed by the contribution of Konig (1903) and so on.

Nutritional value of mushrooms to human diet has been derived from the empirical determination of its composition especially of the protein content. Quackenbush et al. (1935) stated that "Although most of the interest has been directed to the protein fraction, little can as yet be said with certainty regarding the actual proteins present and their digestive and nutritive properties". Feeding trials with rats confirmed the nutritive value of mushroom and also focussed on its inability to serve as a sole source of protein (Antal et al., 1969; Fitzpatrick et al., 1946; Quackenbush et al., 1935). Anderson and Fellers (1942) carried out feeding trials with rats considering mushroom (Agaricus bisporus) as the sole source of protein. They observed 30% increase in body weight compared to the casein fed group after 6 weeks. But when 20% of the casein was replaced with mushroom protein, body weight increased by 84%. Lintzel (1943; 1941) equated the nutritive value of mushroom to that of animal muscle and vegetable proteins. He prescribed 100-200 g of mushroom (dry) to maintain the nutritive balance in human being.

In absence of properly designed biological method of feeding studies with animals, nutritional studies sustains difficulty to evaluate mushroom composition in terms of nutrition. As an alternative, several methods have been advanced at least to make a predictive analysis. Following quasibiological method Mendel (1898), Morner (1886), and Saltet (1885), separately tried for the legitimate information on the digestibility of crude mushroom protein using pancreatic and gastric extracts.

Purely biochemical method for predicting nutritional value based on their content of essential amino acids are also available (FAO, 1973; 1970). Oser (1959, 1951) proposed Essential Amino Acid Index to rate dietary protein in terms of a highly nutritive reference protein.

Bell et al. (1958) observed improvement in body weight of experimental mice fed with a diet containing 50% mushroom mycelia of Tricholoma nudum. Guha and Banerjee (1973) reported that body weight of mice fed on mycelial powder of Agaricus bisporus for 8 weeks was lower than those supplied with equivalent amount of (% N₂ basis) casein. Chandra and Purakayastha (1976a) compared the gain in body weight of mice fed with mycelial powder of several mushrooms. They observed significantly increased body weight of mice in case of Calocybe indica and Termitomyces eurhizus compared to other mushrooms. Digestibility coefficient of Volvariella volvacea was reported as 70% and 84% respectively by Munjal and Seth (1977) and Thayumanavan and Manickam (1980). Vijayakhader and Pandya (1981) reported overall digestibility of 87% in Volvariella volvacea. Samajpati (1979) obtained increased body weight of experimental mice when fed with the diet supplemented with mycelia of P.sajor-caju and P.ostreatus. No morphological or histopathological abnormality was noticed. Bano et al. (1981) calculated EAA-Index and Nutritional Index along with prediction of Biological value of Pleurotus eous and Pleurotus florida. In vitro digestibility of the two species was 89% and 79% compared to 87% and 63% in Pleurotus flabellatus and Pleurotus sajor-caju. Sawaya et al.

(1985) studied the nutritive value of truffles of Saudi-Arabia and reported in-vitro digestibility of 82.8-86.7%.

X. Amino acids :

It is apparent that mushrooms in general contain all the essential amino acids as well as most commonly occurring non-essential amino acids and amides. Of the total amino acid content an average of 25-40% essential amino acid was reported with a range of 10-50% (Miljkovic and Dzamic, 1984; Kalberer and Kunsch, 1974; LeRoux and Danglot, 1972; Maggioni and Renosto, 1970; Bano et al., 1963). About 25-35% of the total amino acids comes as free amino acids while the remainder exists in the form of protein. Sato et al. (1985) analysed 113 kinds of mushroom and obtained 20 free types of protein amino acids along with 11 non protein amino acids. Though considerable difference was found among species of mushroom, the average free amino acid was $213 \mu \text{mol/g}$ dry matter. They found that alanine, glutamic acid and glutamine were very high and were dominant amino acids in the free amino acid pool. Contents of non-protein amino acids were commonly low. Kress (1985) reported that edible fungi were high in essential amino acid content compared to most vegetables and animal foods.

B'Klova (1978) reported similar amino acid composition in different strains of cultivated mushroom. According to him phenylalanine and tyrosine contents were relatively low, whereas lysine and tryptophan contents were high. Sobberg and Remedios

(1979) were of opinion that some fungi were especially rich in certain amino acids. For instance, Agaricus bisporus with 7.9% lysine-N/total N and Lactarius torminosus with 12.23% arginine-N/total N. Quantitative determination of common edible mushroom as conducted by Oka et al. (1981) accounted that 60% of the total nitrogen and about 70% of the ethanol extractable nitrogen was attributed by the free amino acids and their derivatives. They demarkated alanine, glutamic acid, proline and arginine as predominant protein amino acids. Zakhary et al. (1984) analysed 14 amino acids from paddy straw mushroom of which 7 were essential amino acids. Methionine, arginine, histidine and serine were absent. Tryptophan concentration was higher than in any other mushroom or legume seeds. Bano et al. (1963) obtained 17 amino acids (essential and non-essential) in Pleurotus flabellatus while 18 amino acids were reported from the related species Pleurotus sajor-caju by Jandaik and Kapoor (1976b). In the later species amino acid composition was compared with Pleurotus ostreatus and Agaricus bisporus. Total amino acid content was found highest in Pleurotus sajor-caju and lowest in Agaricus bisporus. Bano and Rajarathnam (1982) tabulated a comparative data on the essential amino acid composition of some species of Pleurotus (Bano et al., 1981; Jandaik and Kapoor, 1976b; Kalberer and Kunsch, 1974; Bano et al., 1963) along with the commonly cultivated mushrooms. Pleurotus contain slightly less tryptophan than Agaricus bisporus and Volvariella diplasia. Some species of Pleurotus (P. eous, P. florida and P. flabellatus) contain higher amount of isoleucine, valine, lysine (except P. flabellatus) and threonine as against A. bisporus (FAO, 1972). Again,

phenylalanine and tyrosine are slightly lower but cystine and methionine are very low in concentration. Oka et al. (1984) studied in detail the free amino acid profile of Pleurotus ostreatus. Alanine, glutamic acid, valine, glutamine, glycine and leucine were predominant protein amino acids and occurred in free form. They also determined that the sum of N of 3-abundant amino acids and ornithine (non-protein) accounted about 40% of the total amino acid nitrogen.

Cultural practices, age of the fruitbody, substrate used and availability of nutrition often found to prove its impact on mushroom composition. Hughes et al. (1958) observed decreasing value of tyrosine in Agaricus bisporus with each successive break while other amino acids remained unchanged. Maggioni et al. (1968) found that mushrooms cultivated on compost supplemented with urea plus ammonium sulphate exhibited low amino acid content with limited proline and arginine production. Increased production of methionine, aspartic acid, valine and alanine obtained when the compost was supplemented with ammonium sulphate alone. Kosson and Bakowski (1984) reported that type of compost influenced the amino acid composition of the mushroom according to strain. There was a tendency to increase lysine content in the sporophores during successive stages of growth.

Like other biochemical components of mushrooms the amino acid composition was reported to be in the constant state of flux. Under short-term storage, free amino acid content was increased by 5% (LeRoux and Danglot, 1972). According to

Minamide et al. (1985) total free amino acid content became almost double during storage at 20°C for 5 days and the increase was notable in material without stipe end. Remarkable increase was observed in case of alanine, leucine, valine, isoleucine and γ -amino butyric acid.

Chapter—3

MATERIALS AND METHODS

MATERIALS AND METHODS

I. Source of authentic cultures of mushroom fungi :

(a) Pleurotus sajor-caju :

Isolate of the fungi was received from Type Culture Collection, Indian Agricultural Research Institute, New Delhi, in 1982.

(b) Pleurotus citrinopileatus :

(i) Collection :

Intensive survey was conducted in the different localities of the Kalimpong sub-division of Darjeeling district of West Bengal frequently during 1984 and 1985 to witness the seasonal occurrence of the mushroom species. Mushroom samples were collected from different localities in different times for isolation of tissue to develop the mycelial culture for future research work.

(ii) Isolation of tissue :

Freshly collected mushrooms of different stages of maturity were brought to the laboratory and thoroughly washed with sterile distilled water to remove superficial dirt. Under aseptic condition pieces of interior tissue, particularly from the dividing zone (i.e. junction of stipe with pileus) was cut with the inoculation needle, dipped in the disinfectant as

sodium hypochlorite (NaOCl) or 0.1% mercuric chloride (HgCl_2) solution. The disinfected bits of tissue were washed with sterile water, when necessary, transferred to slants containing PDA or MEA medium and kept for incubation at 25°C for a week. Fresh growth of mycelium emerged from the inoculated bits of tissue. Repeated tip culture from the fresh growth followed by microscopic examination led to the isolation of pure culture of the mushroom fungi.

II. Collection and preservation of spores :

Collection of basidiospores and their preservation were considered to be the basic tools for conducting breeding work. In this regard fruitbodies at the right stage of maturity were collected and cleansed of superficial dirt followed by the cut off of stipe at the midway. The fruitbodies were then placed with gills downward, on the glossy surface of a thick sterilized art paper. The operation was conducted in a sterile chamber. After 24 hours, huge spores were found deposited on the paper. Fruitbodies were then removed, papers with deposited spores were folded and allowed to air dry in sterile condition. Papers with deposited spores were rolled conveniently, inserted into small vials, plugged with sterile cotton, labelled and finally put into spacious glass tubes containing dry silica gel. The large tubes were plugged with rubber cork and sealed with a thick layer of paraffin. Whole lot was kept preserved within the available facilities of refrigerator. Preservation of spores had opened huge scope to work with this, as a stock material round the year.

III. Germination of spores :

The basidiospores underwent germination of as high as 98-100% when freshly collected. It was found to retain its germinability of about 90% even after one year of preservation in refrigerator. The spores could be germinated in liquid as well as semisolid media (Fig.1). Germination of spores was found possible only when the germinating medium was supplemented with 5-10% or more amount of fruitbody decoction.

(a) Preparation of fruitbody decoction :

Ten grams of freshly collected fruitbodies were squashed well in a mortar and pestle with addition of neutral sand to facilitate the crushing operation. The squashed matter was then mixed with 100 ml distilled water and boiled for few minutes, filtered immediately through ordinary filter paper. The filtrate was taken in a 150 ml conical flask, mixed with 100 ppm chloramphenicol group of antibiotic and sterilized in autoclave at 15 p.s.i. for 15 minutes. This sterile decoction was kept for future use.

IV. Isolation of single spore/single germinated spore :

Spores were subjected to the pretreatment with fruitbody decoction for 2 hours. These pretreated spores as such or germinated spores, after 12-24 hours, were isolated in one celled stage (Fig. 2). Isolation was conducted by self-styled 'capillary isolation pipette'. Isolation by dilution plating, one colony one monosporous line, or isolation by cutter lens



Fig.1. Germinating basidiospores of Pleurotus sajor-caju (magn. 646x)



Fig.2. Isolated single spore germling of Pleurotus sajor-caju (magn. 3764x)

mechanism may often raise confusion. But as the isolation method by 'capillary isolation pipette' opened huge scope of correction by microscopic examination, and left no chance of ambiguity the same was followed in experimental step forwards.

(a) Preparation of capillary isolation pipettes :

Minimum thickness ordinary glass tubes (4-5 mm dia) were cut into small pieces (5"-6"). Now, middle part of the tube was placed on a flame. After a while that part became softened and right at this stage the two ends of the tube were pulled mildly in opposite direction. As a matter of fact, the softened portion started elongation and ultimately formed a fine capillary tube which was broken at suitable position to get two 'capillary isolation pipettes'.

(b) Method of isolation :

Before starting the operation a series of isolation pipettes were washed with distilled water, dried and followed by placing a non-absorbent cotton plug at the even hollow end. The isolation pipettes were placed in groups in plugged culture tubes and were sterilized in autoclave as usual. In a well sterilized inoculation chamber an isolation pipette was taken aseptically and the plugged end was inserted in one end of matched rubber tube. Spore/germinated spore suspension was diluted to the level so that fine droplets by isolation pipette got maximum of one or two spore(s)/germinated spore(s). Very small amount of suitably diluted spore/germinated spore suspension

was sucked by placing mouth on the plugged distal end of the rubber tube. Now, very small droplets (smaller than the low power microscopic field) were placed in series on a sterilized slide. The droplets were examined sequentially and only those with single spore/germinated spore were marked. A fresh isolation pipette adjusted as earlier and the fine tip of the capillary end was touched on the droplet. The liquid medium along with the object entered into the capillary tube by capillary action which was then placed on other part of the slide for correction. After checking 3-4 times and on confirmation the droplet was taken into the pipette followed by a little more sterile water and placed aseptically in poured petriplate in a marked area. Fresh isolation pipettes were used and changed as per convenience. Care has been taken particularly during the transfer of a finally checked spore droplet.

V. Raising of monosporous lines :

Petri-plates with isolated spores in the marked areas, were incubated at suitable temperatures for 4-5 days till the tiny colonies were recognised. Immediately after the appearance of the colonies, it were transferred into slants with PDA/MEA medium.

Some spores germinated earlier, some after 48 hours of incubation; some grew faster while others slowly. So spores germinated at different times and of different mode of growth were considered to maintain random collection of variants from the population.

VI. Maintenance of culture :

Following the above principles sixty monosporous lines were raised with available desired behavioural patterns. Monosporous lines thus collected were purified by repeated tip culture followed by microscopic examination and were labelled properly with numerical identity. Continuous growing phase of the lines was maintained by routine subculturing of the isolates at an interval of one month in PDA/MEA medium. Incubation temperature was maintained around 25°C.

VII. Study of comparative growth pattern :

The monosporous lines in culture showed different mode of growth behaviour. Nature of mycelial growth on the interface of supplied medium reflects the inherent character of its growth and colonisation efficiency in the substrate. So as a basic point of selecting parent lines it is of benign importance to go through the detailed study on the cultural behaviour of the isolated monosporous lines.

(a) Comparative study on radial growth :

Poured petri-plates with PDA medium were inoculated with uniform mycelial disc (4 mm dia) cut by a cork-borer from the fresh growth of previously grown mycelial cultures. Mycelial discs were placed at the centre of the poured plates. The inoculated plates were kept for incubation at the fixed temperature of 25°C for a period of 10 days. After the schedule of

incubation the area covered by the mycelial growth was measured from four different angles as :

- i) maximum diameter
- ii) at right angle to maximum diameter
- iii) minimum diameter
- iv) at right angle to minimum diameter.

Finally, from the average diameter, the area covered by the mycelial growth was calculated and compared against the wild culture of Pleurotus sajor-caju. Uniform nutritional as well as physical factors were maintained throughout the experimental procedure.

(b) Comparative study on growth in liquid culture :

The amount of mycelial mass which was of importance in assessing the efficiency of substrate colonisation by the fungi could not be satisfied by radial growth measurement. The right way to focus this area was to study the comparative growth in liquid culture and to collect and determine the dry mycelial mass.

150 ml conical flasks with 25 ml PD-broth medium were inoculated with uniform mycelial disc (4 mm dia) cut from the freshly grown mycelial cultures. Inoculated flasks were incubated at 25°C for a period of 10 days. After the incubation phase the mycelial mats were filtered separately through dry, preweighed filter paper. The filter papers with mycelial mat

were dried to constant weight in hot air oven (105°C), cooled in a desiccator and weighed. Weight of dried mycelial mat was calculated by subtracting the value of filter paper weight from total weight of filter paper with mycelial mat and compared against the wild culture of Pleurotus sajor-caju as check.

VIII. Study of temperature tolerance :

Petri-plates poured with PDA medium were inoculated with mycelial disc (4 mm dia) cut from the fresh growth of mycelial culture of all the monosporous lines separately. Inoculated plates were incubated at 25°C for 5 days to allow initiation of fresh mycelial growth. Next, they were subjected to the pre-fixed temperatures of increasing order from 25-40°C. The calculated area covered by the mycelia in a period of 5 days after the initiation phase opened an idea about the temperature sensitivity of the cultures concerned.

IX. Determination of cardinal temperature :

With the increasing temperature beyond an optimal range growth of mycelium gradually depleted and at certain temperature growth of mycelium became totally checked. This extremity of temperature tolerance or cardinal temperatures of the selected lines were estimated by measuring the point of horizontal line of growth in semisolid PDA medium. The assessment was supported by constant or decreasing weight of dry mycelial mat in liquid PD-broth medium.

X. Induction of fruitbody primordia :

Petriplates with ground straw (through 10 mesh sieve) amended PDA medium were inoculated with the mycelial culture of monosporous lines and incubated. When the mycelial growth just covered the surface, petriplates were exposed to light. To maintain the humidity, plates were placed inside the moist chamber with sufficient facility of air exchange. Cultures were observed for a period of 4 weeks to allow initiation of fruitbody primordia. Temperature optima considered was 25°C.

XI. Classification of cultures into incompatibility groups :

The series of monosporous lines showed diversified behavioural patterns. Just before stepping toward the crossing reactions to be conducted, the monokaryons were classified into incompatibility groups. Initially 12 monosporous lines were exposed to a series of mating reactions in all possible combinations. For each test reaction two choice lines were placed side by side in a poured petriplate. After 5-10 days of incubation at 25°C the meeting zone was observed to note the change in mycelial behaviour, particularly the vigour of the mycelium. If compatible mating took place it led to develop a dikaryon with vigorous, fluffy mycelium. This type of macroscopic change in compatible mating, especially the change in growth pattern has been confirmed by Raper (1966a, b) in Schizophyllum commune and other tetrapolar hymenomycetes. Each mating reaction was confirmed by microscopic examination of hyphal anastomosis at the meeting zone followed by marking

clamp connections in the new growth of secondary mycelium.

The mating tests where compatibility had been confirmed were marked with the sign '+'. A detailed scheme thus produced had developed a clear picture of classifying the 12 initially considered monosporous lines into four distinct groups. Next, one representative line of each group had been considered as 'tester strain'. Using these tester strains in mating tests the whole lot of collection of the monosporous lines were grouped accordingly.

XII. Selection of parent monosporous lines :

The success in breeding work to develop a recombinant variety depends largely on the right selection of parent lines. Nine monosporous lines were selected on the basis of their growth pattern, temperature tolerance and primordia initiation.

XIII. Isolation of recombined culture and its maintenance :

Selected parents of different incompatibility groups were exposed to the trials of recombination in all possible combinations against the members of its compatible group. The trial was conducted by placing the choice partners in twos in poured plates. The recombined secondary mycelium from the meeting zone was isolated and maintained separately in growing phase as in the method mentioned in section VI.

XIV. Methods of cultivation :

A. Preparation of spawn of mushroom fungi :

a) Preparation of wheat grain spawn in bottles :

One kg healthy wheat grains were washed thoroughly in tap water and boiled with 1.5 litre of water for about 30 minutes till they became soft. Before splitting, the grains were taken out and spread on the wire netting. Excess water was drained out and the grains were mixed with 13.33 g calcium carbonate and 3.33 g calcium sulphate (gypsum). Two hundred grams each of such boiled grains were taken in 500 ml milk bottles and plugged with non-absorbent cotton. Bottles were then sterilized by autoclaving at 15 p.s.i. for 1 hour each for two consecutive days. After cooling the bottles to room temperature they were inoculated under aseptic condition with 1 cm disc of pure mycelial culture of mushroom fungi raised on PDA/MEA medium. The inoculated bottles were incubated at 25°C. After about 10-15 days all the grains became covered with mycelial growth and the spawn was thus ready for use.

b) Preparation of wheat grain spawn in polypropylene packets :

Polypropylene packets (25 cm x 10 cm) of 100 gauge thickness was considered as an alternative to milk bottles for the preparation of wheat grain spawn. Polypropylene packets were filled each with 200 g of boiled wheat grains after mixing with requisite amount of calcium carbonate and calcium sulphate as earlier. Each of the grain filled packet was inserted into another packet of same size. A corrugated iron sheet ring or

a thick iron wire ring (3.5 cm wide x 3.0 cm dia) was placed in the inner space of the neck (about 2.5 cm below the upper edge) and the margin of both the packets as a whole was inserted through the ring to form a consistent neck with open mouth. The open mouth was plugged with non-absorbent cotton, sterilized at 15 p.s.i. for 1 hour each on two consecutive days. The method of inoculation and after care were same as the method described earlier.

B. Cultivation proper :

(a). Substrate :

Well dried paddy straw of golden yellow colour (not otherwise primarily colonised with mold fungi) was considered as the basic substrate for the cultivation of mushroom. This selection was based on its suitability for utilization by the mushroom fungi, low price and easy availability throughout the year.

(b) Pretreatment :

Paddy straw chopped into smaller bits (1 cm length) was soaked in clean tap water for a period of 24 hours. After proper soaking excess water was drained out till hand squeezing found no more water to come out of it. This pretreated substrate was then inoculated accordingly.

(c) Sterilization of substrate :

When study of comparative spawn run period, recombination trials and expression of characters for selection etc. were

concerned it was needed to avoid contamination to eliminate the chance of error in phenotypic expression of characters. Thus presoaked straw (300 g) was packed in polypropylene packet (25 cm x 15 cm), plugged and sterilized at 15 p.s.i. for 30 minutes each in two consecutive days. On the next day the substrate was inoculated with the wheat grain spawn conveniently.

(d) Inoculation of substrate :

(i) Inoculation in polypropylene packet :

Polypropylene packet with sterilized substrate was inoculated aseptically with wheat grain spawn at the rate of 3% (wet wt/w). Inoculation was done with the help of a surgery spoon made of stainless steel. After inoculation the spawn was mixed with the substrate and homogenised by making the packet upside down and by hand squeezing from outside.

(ii) Inoculation in nylon net :

2.5 kg soaked straw was mixed thoroughly with 75 g of wheat grain spawn. The whole mass of substrate with spawn was packed in a nylon net (45 cm x 30 cm) with mild pressure so as to provide sufficient compactness within the substrate.

(iii) Inoculation in polythene bag :

Eight kg of wet, pretreated substrate was mixed homogeneously with 240 g of wheat grain spawn. Now, the whole mixture was packed into large polythene bag (75 cm x 60 cm) with pressure

to provide requisite compactness. The open mouth was tied with a rubber band and kept for incubation.

C. After care :

Inoculated mushroom fungi in small polypropylene packet or large polythene bag need optimum air exchange to induce better spawn run, because creation of anaerobic condition, any way, means to stimulate the anaerobic organisms, particularly in unsterilized substrate, which ultimately cease the growth of mushroom fungi.

Small polypropylene packets were provided with cotton plug at the neck which had served the purpose to allow necessary air exchange. But in case of large polythene bags, increased demand of aeration due to increased width of the substrate mass was satisfied by making several small holes all around the surface.

Containers as polypropylene packets, polythene bags or nylon nets were wrapped with black polythene for a period of 10-20 days as needed in different cultural types. Black polythene served the purpose of protecting the fungi from the reach of light, mulched the moisture around saturation point and at the same time increased CO₂ concentration in that microenvironment. All these factors favoured better vegetative growth. After the period of spawn run when white mycelial growth covered the substrate the containers were unveiled out of black polythene. Polypropylene or polythene bags were torn out and the colonised blocks were placed on the wooden rack inside the moist chamber.

Polythene nets were hung on a hook inside the chamber. Regular irrigation was maintained once or twice a day to keep the substrate moist.

XV. Nutritional studies

Following the standard method of medical feeding test on white mice, an experiment was conducted to determine the quality of protein and degree of digestibility. The test animals were male white mice of Swiss-strain and the test material was oven dried fruitbodies of newly introduced mushroom species, Pleurotus citrinopileatus. To compare its standard as edible mushroom the well recommended variety of oyster mushroom Pleurotus sajor-caju was considered as check. Again, all the groups of mice supplied with 'mushroom diet' were challenged against 'normal diet' groups that were supplied with a complete food combination.

Normal diet as recommended bears the following additives :

Wheat flour	-	1080	g
Gram flour	-	720	g
Maize flour	-	1080	g
Dried whole milk	-	360	g
Wheat bran	-	360	g
Common salt	-	18	g
Multimin	-	36	g
Vitablend	-	0.2	g

The experiment was intended to supply mushroom as a major protein source in case of 'mushroom diet' irrespective of the

protein fraction in wheat flour. So to avoid complexity the charter was modified as follows :

Wheat flour	-	2880	g
Milk powder	-	360	g
Wheat bran	-	360	g
Common salt	-	18	g
Multimin	-	36	g
Vitablend	-	0.2	g

In order to formulate the mushroom diet, the estimated amount of protein of milk powder was replaced by mushroom protein by adding requisite amount of mushroom powder (ground through 60 mesh sieve). Also, the fat content of milk powder was replaced by requisite amount of 'Amul butter'.

The feeding trial was conducted for a period of 40 days, splitted in 4 phases of 10 days each. During the first phase all the mice were supplied with normal diet to develop normal food habit in laboratory condition. In the second phase the mice were separated into respective diet groups of 10 mice, divided in two cages of 5 each. In this phase 'mushroom diet' groups were supplied with mixed diet (Normal diet : Mushroom diet :: 1:1). In the third and fourth phases they were allowed full mushroom diet. The amount of food supplied was at the rate of 4 g, 5 g, 6 g and 7 g (dry wt.) per mouse per day respectively in the four phases.

The increase in body weight reflects the overall picture of health of a developing organism. So the body weight of mice of respective groups were accounted before and after each diet phase.

XVI. Digestibility and assimilation :

The digestibility and rate of assimilation means to direct the quality of a protein food. Certain fraction of protein is always shared by non-essential amino acids which do not come into use in assimilation. This non-essential fraction of protein varies in different sources and thus proves the variation in protein quality of the same. Based on this idea test organisms at the third and fourth phases (full mushroom diet phase) were maintained in a specially designed cage, usually in use in medical feeding test. The cages were with arrangements to collect the excreta and food left on each day. Excreta and food left for each phase were collected separately, dried in hot-air oven till constant weight, ground into powdery masses and passed on the analytical processes.

XVII. Chemical analysis :

A. Collection and drying of sample :

Collection of mushroom sample at the particular stage of harvest and its proper drying was prerequisite to go further into analytical processes. For drying, definite amount of sample was taken and kept for drying in hot-air oven at 105°C for 72 hours or more if necessary, till it reached its constant

weight. After each drying, samples were cooled in a desiccator and weighed, and moisture content of the samples were determined.

B. Determination of total nitrogen using Micro-Kjeldahl method :

Reagents :

- i) Sulphuric acid (analytical reagent grade)
- ii) Sodium hydroxide solution (50%)
- iii) Boric acid solution (4%)
- iv) Mixed indicator (Bromocresol green - methyl red)
(0.2% methyl red in alcohol : 0.1% bromocresol green in alcohol :: 1:3)
- v) Digestion mixture (K_2SO_4 : $CuSO_4$:: 10:1)

Procedure :

500 mg of ground dried sample was taken in a Kjeldahl digestion flask (100 ml) for each replication. To it, added 4-5 g of digestion mixture followed by addition of 10 ml of concentrated sulphuric acid (H_2SO_4) in such a way as to wash down any solid adhering in the neck. The flask was shaken gently to mix the sample well with the acid. Open mouth of the flask was closed with a loosely fitting reagent bottle stopper and the flask was then placed in a fume hood in inclined position at an angle of 30-45°.

The flask with its content was heated gently till frothing ceased and then heated strongly until it formed a clear solution with bluish green in colour. Heating continued for

another 30 minutes with occasional shakings. Digested material was left for cooling. When cold, 40 ml of distilled water was added slowly, swirled and then allowed to stand till the content became cool. The digest was transferred in 100 ml volumetric flask and made upto the mark with distilled water. Ten ml of this solution was transferred to the vacuum mantle of micro-distillation unit. To it, added 50 ml of sodium hydroxide solution (50%) and steam distilled the ammonia for 5-10 minutes. About 100 ml of distillate was collected in a 150 ml conical flask containing 15 ml boric acid solution (4%) with indicator. Resulting ammonium borate in collection flask was titrated against 0.05 (N) sulphuric acid to the pink end point. Amount of acid needed was recorded.

For the determination of a blank, the above method was followed exactly with only omission of the mushroom sample.

$$\text{Calculation : } \% \text{ N} = \frac{(V_2 - V_1) \times N \times 0.014 \times 100}{\text{Sample weight (g)}}$$

where, V_2 = Volume in ml of standard acid used in titration.

V_1 = Volume in ml of standard acid used in blank titration.

N = Normality of the standard acid used.

C. Determination of crude protein :

Crude protein content was determined from the total nitrogen content as estimated by the Micro-Kjeldahl method (A.O.A.C. 47.021; Horwiltz, 1980) using the conversion factor ($N \times 6.25$).

However, recent studies showed that the digestibility of mushroom protein is around 70%. This reduced coefficient can be explained by the fact that mushroom contain a significant amount of non-protein nitrogen in their chitinous cell wall. Thus, a closer approximation of digestible protein content of mushroom can be obtained using the conversion factor equal to 70% of $(N \times 6.25)$ or $(N \times 4.38)$ (Crisan and Sands, 1978).

D. Determination of crude fat :

Crude fat content of mushroom has been estimated by prolonged ether extraction of dried sample in a Soxhlet apparatus. The extraction was done following the standard method as referred by A.O.A.C. 7.056, (Horwitz, 1980).

Reagents :

- i) Analytical reagent grade, anhydrous ether.
- ii) Fused calcium chloride.
- iii) Sodium metal.

Procedure :

(a) Dehydration of ether :

To remove every trace of water from ether it was poured in a loosely stoppered round bottom flask with sufficient amount of fused calcium chloride. Left till hydrogen evolution ceased followed by transfer of ether in another loosely stoppered dry bottle. Small pieces of carefully cleaned metallic sodium was dropped into it. When hydrogen evolution stopped, the dehydrated ether was stored in a brown bottle and kept protected from light.

(b) Extraction :

Two grams of ground well dried mushroom was taken in the fold of a filter paper placed in a funnel. Washed primarily with five 20 ml portions of distilled water to remove water soluble components. The sample was then dried to constant weight at 105°C. Dried sample was scraped out of the filter paper and placed in the Soxhlet apparatus. About 100 ml of dried ether was poured to run the extraction. The apparatus was placed on a water bath with temperature regulated around 70-80°C. The condenser was connected with a scoop pump meant for supplying ice water and temperature of the condenser was maintained always below 20°C to increase the condensation rate.

Extraction was continued for 5-6 hours. All the fatty and fat soluble materials came out of the sample and remained soluble in ether. This ether extract was taken in a round bottom flask and the ether was removed using a rotary evaporator. The flask was then placed in oven at 100°C and dried to constant weight, cooled in a desiccator and weighed.

Calculation :

$$\% \text{ crude fat} : \frac{\text{Amount of ether extract (g)} \times 100 (\%)}{\text{Sample weight (g)}}$$

E. Determination of carbohydrate :

Several colorimetric methods (Hodge and Hofreiter, 1962) are available for the determination of carbohydrate. Among them, the phenol sulphuric acid method (Dubois et al., 1956) was

followed in determining the carbohydrate content of mushroom sample. Because, phenol sulphuric acid method is simple, accurate, sensitive and widely applicable. Virtually, all classes of sugars can be determined. Moreover, the reagents used were inexpensive, readily available and stable. During the process a stable colour is produced and the results are reproducible.

Reagents :

- i) Phenol 5% (w/v) in distilled water.
- ii) Sulphuric acid (AR)
- iii) Standard solution of Glucose (0.1%)
- iv) Dehydrated ether.

Procedure :

250 mg of ground dried mushroom was taken in a Soxhlet apparatus and subjected to ether extraction for two hours to make the sample totally fat free. This extracted mushroom was transferred to an evaporating dish and dried to constant weight. The sample was taken in a 250 ml conical flask fitted with a reflux condensor and mixed with 100 ml of distilled water and 10 ml of concentrated hydrochloric acid. The flask was placed on a water bath and refluxed for 3 hours, cooled, filtered and then transferred to a 250 ml volumetric flask and made upto the mark with distilled water. Ten ml of this solution was pipetted to a 100 ml volumetric flask and further diluted to the mark to get the sample solution. A dilution gradient of standard solution (0.1%, 0.05%, 0.04%, 0.03%, 0.02%, 0.01%) was done.

One ml each of the sample solution and standard solutions were pipetted separately in test tubes. To each tube added 1 ml of 5% phenol followed by the addition of 5 ml of concentrated sulphuric acid using fast delivery pipette. The stream of acid was directed against the liquid surface to obtain good mixing. Tubes were allowed to stand for 10 minutes, shaken and then placed at a temperature of 25-30°C for 20 minutes. A distinct colour was developed which remained stable for several hours. Reading (OT/OD) was taken in a colorimeter at 490 nm.

Calculations :

A standard curve was plotted from the readings of the standards and the amount of carbohydrate present in the sample was calculated and expressed in terms of percentage weight.

$$\begin{aligned}\% \text{ carbohydrate} &= \frac{\text{Concentration (mg/ml)} \times 250 \text{ ml}}{\text{Sample weight (g)} \times 1000 \text{ (mg/g)}} \times \frac{100 \text{ (ml)}}{10 \text{ (ml)}} \times 100 (\%) \\ &= \frac{\text{Concentration} \times 250}{\text{Sample weight (g)}} (\%) \end{aligned}$$

F. Estimation of mineral composition :

The ground mushroom samples were digested in a tri-acid mixture of H_2SO_4 : HClO_3 : HNO_3 :: 2:1:9) and various elements therein were analysed using atomic absorption spectrophotometer (Philips, model-SP-9), following the standard method as advanced by Jackson, M.L. (1967).

G. Analysis of amino acids :

Amino acid content of mushroom samples were analysed using Technicon Sequential Multipurpose Amino Acid Analyser following the method of Moore and Stein (1963) which was later modified by Gopinathan et al. (1986).

Chapter—4

RESULTS AND DISCUSSION

RESULTS - I

A. Studies on the effect of preservation of basidiospores of *Pleurotus sajor-caju* at different temperatures on germination

Isolation of monosporous lines is a tedious and time consuming procedure. To work with a large number of monosporous lines needs steady supply of viable basidiospores. Availability of fresh, viable spores may not be possible for a long period of time and then feasible alternative is to preserve the spores conveniently. Basidiospores of *Pleurotus sajor-caju* was found to retain its viability for a long period of time even in the available space of refrigerator. To determine a better possible way of preservation of basidiospores it was kept in different conditions of room temperature (25°-30°C) and refrigerator (0°C). Effect of preservation on the viability was tested at every three months interval. Percentage germination of spores preserved at different conditions observed upto one year is represented in Table 1, Fig. 3.

The result shows that germination of spores started earlier than 12 hours and it increased upto a maximum limit within 24 hours. In case of spores preserved at room temperature it became totally non-viable only after three months. This quick loss of viability was indicated by the sharp depletion in the percentage of germination. Spores when freshly collected or preserved at 0°C upto one month showed 100% germination. Increased period of

preservation at 0°C led only to very slow loss of viability of spores which was indicated by high rate of germination of 88.7% even after one year.

Table 1. Studies on the effect of preservation of basidiospores of Pleurotus sajor-caju at different temperatures on germination.

Period of preservation (month)	% of germination of spores preserved at -			
	25-30°C		0°C	
	Incubation period		Incubation period	
	12 hrs	24 hrs	12 hrs	24 hrs
Fresh	92.0	100.0	92.0	100.0
1	56.0	71.5	84.4	100.0
3	12.2	21.5	79.6	96.2
6	Nil	Nil	81.8	94.5
9	Nil	Nil	82.9	95.5
12	Nil	Nil	68.2	88.7

B. Study of comparative growth pattern of monosporous lines by measuring circular growth on semisolid medium :

Monosporous cultures raised by single basidiospore isolation showed a wide diversification in its growth behaviour under cultural condition. To develop a clear picture of differential growth pattern on semi-solid media, cultures were inoculated in poured petri-plates and exposed to the favourable conditions of growth for a period of ten days. Inoculum density and physical as well as nutritional factors were maintained constant. After

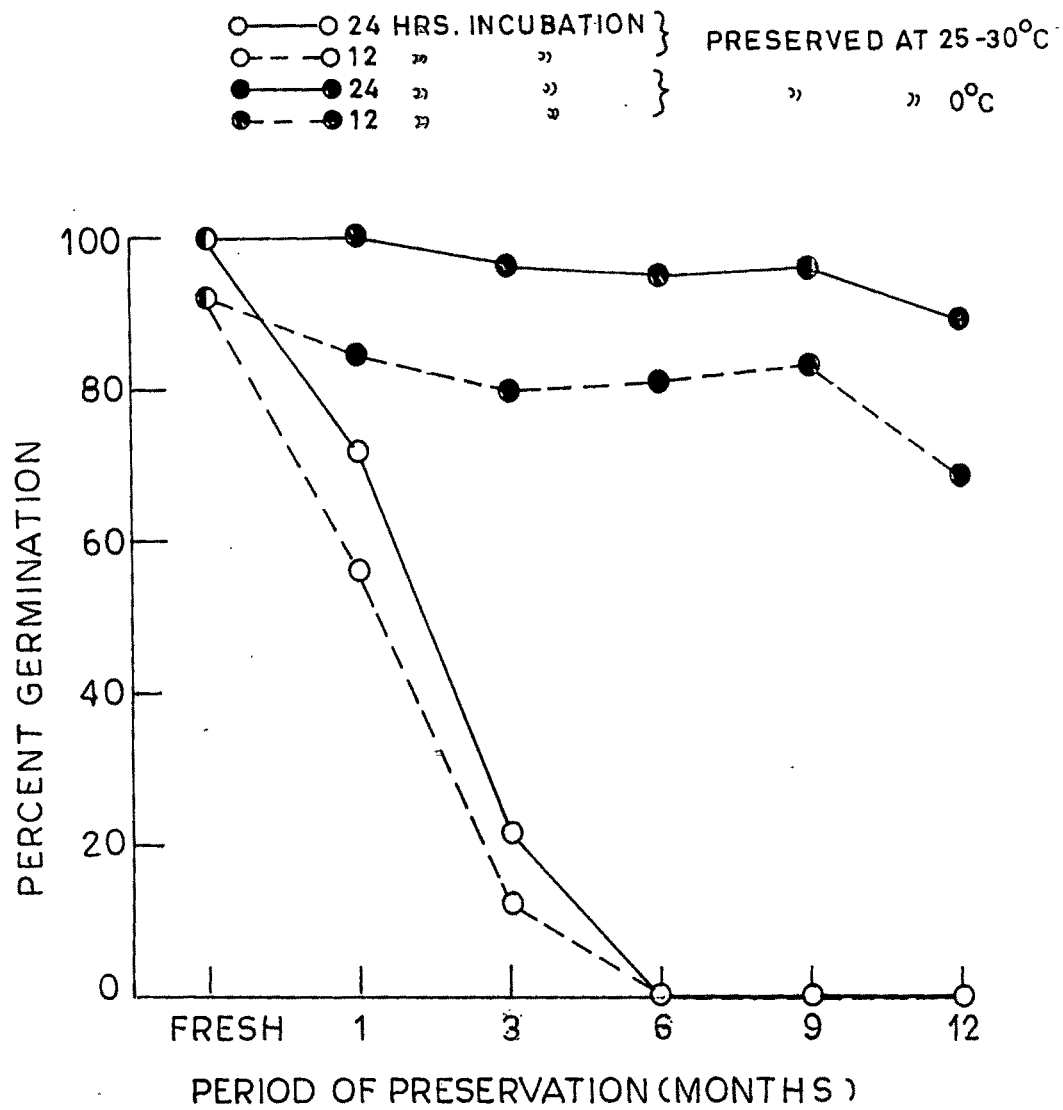


Fig.3 : Studies on the effect of preservation of basidiospores of P. sajor-caju at different temperatures on germination.

the period of incubation area of growth covered by the mycelia were determined. Calculated area of growth in different monosporous lines are represented in Table 2.

The result shows a wide range of growth difference starting from a minimum of 1.63 sq.cm. to as high as 98.87 sq.cm. compared to 96.07 sq.cm. growth area in wild culture. In general the cultures harboured slow to medium slow growing behaviour with rare occurrence of fast growing cultures. The area of growth of individual culture was compared against the wild culture of Pleurotus sajor-caju and percentage areas to that of the wild culture were calculated. From the calculated data of percentage value it is apparent that most of the cultures could not attain even 50% growth to that of the wild culture. Only the monosporous cultures as PS₃, PS₂₀, PS₂₆, PS₂₇, PS₃₇, PS₄₇, PS₅₇, PS₅₉, PS₆₀ and PS₆₄ exceeded 50% growth level with striking performance of growth observed in PS₂₇ and PS₆₄. These two cultures established 102.91 and 94.47% of growth respectively.

Table 2. Study of comparative growth pattern of monosporous lines by measuring circular growth on semi-solid medium.

Culture No.	Diameter of growth (cm) after 10 days	Area covered (cm ²)	% area compared to wild culture
PS ₃	8.52	57.01	59.34
PS ₅	6.23	30.48	31.73

Table 2 contd.

Culture No.	Diameter of growth (cm) after 10 days	Area covered (cm ²)	% area compared to wild culture
PS ₉	7.76	47.29	49.22
PS ₁₀	4.09	13.14	13.68
PS ₁₁	6.56	33.79	35.17
PS ₁₂	3.30	8.55	8.90
PS ₁₆	7.23	41.05	42.73
PS ₁₈	7.04	38.92	40.51
PS ₁₉	7.79	47.66	49.61
PS ₂₀	8.09	51.40	53.50
PS ₂₁	6.89	37.28	38.80
PS ₂₂	7.80	47.78	49.73
PS ₂₃	4.27	14.32	14.90
PS ₂₄	3.57	10.01	10.42
PS ₂₅	6.62	34.41	35.82
PS ₂₆	8.70	59.45	61.88
PS ₂₇	11.22	98.87	102.91
PS ₂₈	2.88	6.51	6.78
PS ₂₉	7.77	47.42	49.36
PS ₃₀	4.50	15.90	16.55
PS ₃₁	7.24	41.16	42.84
PS ₃₂	7.50	44.18	45.99
PS ₃₃	7.20	40.71	42.37
PS ₃₄	5.61	24.72	25.73

Table 2 contd.

Culture No.	Diameter of growth (cm) after 10 days	Area covered (cm ²)	% area compared to wild culture
PS ₃₅	5.23	21.48	22.36
PS ₃₆	6.60	34.21	35.61
PS ₃₇	9.39	69.25	72.08
PS ₃₈	1.44	1.63	1.70
PS ₃₉	5.38	22.73	23.66
PS ₄₀	2.88	6.51	6.78
PS ₄₁	5.14	20.75	21.60
PS ₄₂	6.52	33.39	34.75
PS ₄₃	6.19	30.09	31.32
PS ₄₄	6.33	31.47	32.76
PS ₄₅	6.48	32.98	34.33
PS ₄₆	7.58	45.13	46.98
PS ₄₇	8.25	53.46	55.65
PS ₄₈	5.51	23.84	24.81
PS ₄₉	3.24	8.24	8.58
PS ₅₀	5.78	26.24	27.31
PS ₅₁	6.48	32.98	34.33
PS ₅₂	6.26	30.78	32.04
PS ₅₃	4.43	15.41	16.04
PS ₅₄	6.96	38.04	39.60
PS ₅₅	3.50	9.62	10.01
PS ₅₆	5.00	19.63	20.43
PS ₅₇	8.00	50.26	52.32

Table 2 contd.

Culture No.	Diameter of growth (cm) after 10 days	Area covered (cm ²)	% area compared to wild culture
PS ₅₈	5.53	24.02	25.00
PS ₅₉	9.14	65.61	68.29
PS ₆₀	8.22	53.07	55.24
PS ₆₁	7.65	45.96	47.84
PS ₆₂	7.48	43.94	45.74
PS ₆₃	4.44	15.48	16.11
PS ₆₄	10.75	90.76	94.47
PS ₆₅	6.61	34.31	35.71
PS ₆₆	6.26	30.78	32.04
PS ₆₇	5.58	24.45	25.45
PS ₆₈	7.51	44.30	46.11
PS ₆₉	6.00	28.27	29.43
PS ₇₀	5.88	27.15	28.26
PS (Wild)	11.06	96.07	-

C. Classification of monosporous lines on the basis of growth pattern :

To facilitate the method of selection of suitable monosporous lines for recombination it seemed useful to classify the whole lot of monosporous lines into small groups based on similarity of growth patterns. In the present investigation a total

of 60 monosporous lines were splitted into four major groups on the basis of percentage growth compared to the wild culture. The groups presented in Table 3 are (i) Slow growing lines (1-25%); (ii) Medium slow growing lines (26-50%); (iii) Medium fast growing lines (51-75%) and (iv) Fast growing lines (76-100% or more). An overview on the table shows that majority of the cultures were slow to medium slow in nature. Medium fast growing lines were less available with chance occurrence of fast growing lines.

Table 3. Classification of monosporous lines on the basis of growth pattern.

Slow growing lines (1-25%)	Medium slow growing lines (26-50%)	Medium fast growing lines (51-75%)	Fast growing lines(76-100% or more)
PS ₁₀	PS ₅	PS ₃	PS ₂₇
PS ₁₂	PS ₉	PS ₂₀	PS ₆₄
PS ₂₃	PS ₁₁	PS ₂₆	
PS ₂₄	PS ₁₆	PS ₃₇	
PS ₂₈	PS ₁₈	PS ₄₇	
PS ₃₀	PS ₁₉	PS ₅₇	
PS ₃₄	PS ₂₁	PS ₅₉	
PS ₃₅	PS ₂₂	PS ₆₀	
PS ₃₈	PS ₂₅		
PS ₃₉	PS ₂₉		
PS ₄₀	PS ₃₁		

Table 3 contd.

Slow growing lines (1-25%)	Medium slow growing lines (26-50%)	Medium fast growing lines (51-75%)	Fast growing lines (76 - 100% or more)
PS ₄₁	PS ₃₂		
PS ₄₈	PS ₃₃		
PS ₄₉	PS ₃₆		
PS ₅₃	PS ₄₂		
PS ₅₅	PS ₄₃		
PS ₅₆	PS ₄₄		
PS ₅₈	PS ₄₅		
PS ₆₃	PS ₄₆		
PS ₆₇	PS ₅₀		
	PS ₅₁		
	PS ₅₂		
	PS ₅₄		
	PS ₆₁		
	PS ₆₂		
	PS ₆₅		
	PS ₆₆		
	PS ₆₈		
	PS ₆₉		
	PS ₇₀		

D. Study on comparative growth pattern of monosporous lines
by measuring dry mycelial mass grown on liquid medium :

Mycelial mass of mushroom fungi in culture often reflects
the behaviour of mode of colonisation and length of spawn run

period. The monosporous lines though generally slow growing in nature, sometimes open scope for the selection of fast growers. In the present experiment Potato-Dextrose (PD) broth media was inoculated with monosporous cultures and allowed to grow for a period of ten days. For all the cultures similar inoculum density and physical as well as nutritional factors were maintained. Dry mecelial mass obtained after ten days of growth were weighed. Net weight of mycelial growth of different lines as well as their growth in comparison to that of the wild culture, expressed in percentage value, are presented in Table 4.

The result shows that net weight of dry mycelial growth of different lines varied from 91.70 mg to as high as 228.45 mg; while in wild culture it was 236.83 mg. When compared to wild culture, the growth was found to run between 38.71% to 96.46% with a general tendency to lie within the range of 50-80%.

Table 4. Study on comparative growth pattern of monosporous lines by measuring dry mycelial mass grown on liquid medium.

Culture No.	Net wt. of dry mycelial mass (mg)	% dry mycelial mass compared to wild culture
PS ₃	175.13	73.95
PS ₅	188.05	79.40
PS ₉	185.10	78.16
PS ₁₀	175.87	74.26
PS ₁₁	100.95	42.62

Table 4 contd.

Culture No.	Net wt. of dry mycelial mass (mg)	% dry mycelial mass compared to wild culture
PS ₁₂	135.25	57.11
PS ₁₆	192.87	81.44
PS ₁₈	201.62	85.13
PS ₁₉	142.87	60.32
PS ₂₀	153.62	64.86
PS ₂₁	218.82	92.39
PS ₂₂	175.02	73.90
PS ₂₃	132.12	55.79
PS ₂₄	91.70	38.72
PS ₂₅	208.25	87.93
PS ₂₆	196.75	83.08
PS ₂₇	228.45	96.46
PS ₂₈	143.87	60.75
PS ₂₉	192.20	81.15
PS ₃₀	129.87	54.84
PS ₃₁	148.75	62.81
PS ₃₂	186.82	78.88
PS ₃₃	111.02	46.88
PS ₃₄	119.70	50.54
PS ₃₅	197.38	83.34
PS ₃₆	180.85	76.36
PS ₃₇	173.91	73.43

Table 4 contd.

Culture No.	Net wt. of dry mycelial mass (mg)	% dry mycelial mass compared to wild culture
PS ₃₈	94.50	39.90
PS ₃₉	142.32	60.09
PS ₄₀	120.15	50.73
PS ₄₁	141.87	59.90
PS ₄₂	147.95	62.47
PS ₄₃	120.91	51.05
PS ₄₄	175.38	74.05
PS ₄₅	185.87	78.48
PS ₄₆	192.62	81.33
PS ₄₇	157.51	66.51
PS ₄₈	173.87	73.41
PS ₄₉	104.72	44.22
PS ₅₀	119.12	50.30
PS ₅₁	144.82	61.15
PS ₅₂	149.82	63.26
PS ₅₃	165.37	69.83
PS ₅₄	145.66	61.50
PS ₅₅	121.63	51.36
PS ₅₆	116.05	49.00
PS ₅₇	171.87	72.57
PS ₅₈	164.51	69.46
PS ₅₉	158.12	66.76

Table 4 contd.

Culture No.	Net wt. of dry mycelial mass (mg)	% dry mycelial mass compared to wild culture
PS ₆₀	201.77	85.20
PS ₆₁	159.58	67.38
PS ₆₂	122.70	51.81
PS ₆₃	124.83	52.71
PS ₆₄	222.50	93.95
PS ₆₅	176.20	74.40
PS ₆₆	117.87	49.77
PS ₆₇	163.60	69.08
PS ₆₈	118.37	49.98
PS ₆₉	158.65	66.99
PS ₇₀	200.25	84.55
PS (Wild)	236.83	-

E. Test of temperature sensitivity among the monosporous lines :

To study the variation in temperature sensitivity among the monosporous lines, young mycelial cultures were developed and exposed to the temperatures between 25° and 38°C. At the initiation phase poured petriplates with Potato-Dextrose-Agar (PDA) medium were inoculated with monosporous cultures and incubated at 25°C for five days. Following the initiation phase, area of growth of each culture was demarkated along its circumference

from the lower surface of petriplates and transferred to the treatment phase. In treatment phase, replicates of each culture were separated in five groups and were placed for incubation at 25°, 30°, 34°, 36° and 38°C for five days. Diameter of fungus occupied radial growth of individual culture at different temperatures were measured. Net increased area of growth at the treatment phase was calculated by subtracting the value of initial growth from total growth area. Calculated area of growth in response to increasing temperatures are presented in Table 5.

From the available data it is apparent that monosporous lines as PS₄₀, PS₅₅ and PS₅₇, unlike rest of the lines, were very much temperature sensitive and found to cease out their growth at 30°C. These cultures were found active at 25°C temperature and thus maximum tolerance lay between 25° and 30°C. At 34°C growth of another 12 monosporous lines were found to be arrested. The cultures were PS₁₀, PS₂₀, PS₂₈, PS₂₉, PS₃₀, PS₃₈, PS₄₂, PS₄₃, PS₄₈, PS₅₈, PS₆₃ and PS₆₇ whose maximum level of tolerance might be considered to lie between 30°-34°C. The remaining 45 lines though survived even at 34°C, growth activity became slowed down with varying degrees compared to their optimum growth at 25°C. No one of the cultures was found to continue its growth at 38°C and thus maximum temperature tolerance for the last 45 lines remained within 34°-38°C.

Table 5. Test of temperature sensitivity among the monosporous lines.

Cultures	T E M P E R A T U R E (net area of growth (cm ²) after 5 days)				
	25°C	30°C	34°C	36°C	38°C
PS ₃	45.36	44.42	18.55	4.50	Nil
PS ₅	21.32	18.86	8.24	2.46	Nil
PS ₉	34.73	32.97	26.42	0.51	Nil
PS ₁₀	10.18	7.55	Nil	Nil	Nil
PS ₁₁	22.88	23.76	3.46	Nil	Nil
PS ₁₂	7.06	5.30	3.80	0.82	Nil
PS ₁₆	29.22	27.34	5.72	3.86	Nil
PS ₁₈	35.43	32.98	10.52	Nil	Nil
PS ₁₉	33.39	30.19	9.51	2.35	Nil
PS ₂₀	36.42	28.08	Nil	Nil	Nil
PS ₂₁	26.42	23.33	9.08	0.86	Nil
PS ₂₂	33.90	33.39	28.08	4.91	Nil
PS ₂₃	11.64	5.22	0.19	Nil	Nil
PS ₂₄	7.79	6.51	5.23	Nil	Nil
PS ₂₅	24.63	24.10	3.80	Nil	Nil
PS ₂₆	40.48	38.48	11.94	6.16	Nil
PS ₂₇	64.18	63.90	30.19	9.49	Nil
PS ₂₈	4.87	1.77	Nil	Nil	Nil
PS ₂₉	34.10	6.97	Nil	Nil	Nil
PS ₃₀	10.69	4.52	Nil	Nil	Nil
PS ₃₁	28.27	33.18	1.76	Nil	Nil

Table 5 contd.

Cultures	T E M P E R A T U R E (Net area of growth (cm ²) after 5 days)				
	25°C	30°C	34°C	36°C	38°C
PS ₃₂	32.07	30.19	21.24	6.15	Nil
PS ₃₃	27.15	9.62	5.39	2.54	Nil
PS ₃₄	20.42	11.94	2.19	Nil	Nil
PS ₃₅	16.69	14.90	2.35	Nil	Nil
PS ₃₆	24.48	24.63	9.62	1.55	Nil
PS ₃₇	46.57	47.17	16.04	4.83	Nil
PS ₃₈	1.43	0.19	Nil	Nil	Nil
PS ₃₉	16.47	15.90	8.29	0.68	Nil
PS ₄₀	5.64	Nil	Nil	Nil	Nil
PS ₄₁	16.04	4.60	2.21	Nil	Nil
PS ₄₂	23.93	5.64	Nil	Nil	Nil
PS ₄₃	21.89	3.46	Nil	Nil	Nil
PS ₄₄	19.56	22.90	16.47	2.83	Nil
PS ₄₅	23.84	13.85	3.94	0.91	Nil
PS ₄₆	31.07	30.97	18.85	4.39	Nil
PS ₄₇	35.47	34.42	26.42	6.41	Nil
PS ₄₈	17.05	6.16	Nil	Nil	Nil
PS ₄₉	6.88	6.15	4.90	2.46	Nil
PS ₅₀	18.93	13.20	4.22	Nil	Nil
PS ₅₁	19.48	13.85	0.78	Nil	Nil
PS ₅₂	20.43	16.04	15.14	1.83	Nil
PS ₅₃	11.46	6.15	0.44	Nil	Nil

Table 5 contd.

Cultures	T E M P E R A T U R E (Net area of growth (cm ²) after 5 days)				
	25°C	30°C	34°C	36°C	38°C
PS ₅₄	25.97	27.33	0.78	Nil	Nil
PS ₅₅	8.04	Nil	Nil	Nil	Nil
PS ₅₆	14.18	13.20	5.11	1.55	Nil
PS ₅₇	35.89	Nil	Nil	Nil	Nil
PS ₅₈	19.63	5.93	Nil	Nil	Nil
PS ₅₉	52.08	45.12	9.62	3.67	Nil
PS ₆₀	39.59	36.85	13.85	4.80	Nil
PS ₆₁	32.67	31.66	3.98	0.86	Nil
PS ₆₂	29.41	28.09	2.13	Nil	Nil
PS ₆₃	10.89	5.22	Nil	Nil	Nil
PS ₆₄	66.62	66.76	25.16	4.39	Nil
PS ₆₅	26.24	25.33	11.34	3.90	Nil
PS ₆₆	22.99	23.58	13.85	0.52	Nil
PS ₆₇	17.50	16.33	Nil	Nil	Nil
PS ₆₈	35.04	34.00	21.48	2.21	Nil
PS ₆₉	21.64	18.09	2.40	Nil	Nil
PS ₇₀	21.24	22.90	10.29	4.99	Nil
PS _{Wild}	69.30	65.75	42.97	3.97	Nil

F. Induction of primordia of bud in the haploid monosporous cultures of *Pleurotus sajor-caju*.

Formation of fruitbody primordia occur very rarely in monosporous cultures of *Pleurotus sajor-caju*. The cultures that can

produce such primordia may be demarkated as good fruiterers. All the 60 monosporous lines under consideration were tested for their ability to produce fruitbody primordia. In this experiment straw ammended PDA medium was inoculated with monosporous cultures and all the conditions favourable for mushroom cultivation were maintained in aseptic condition to avoid unwanted chance crossing and confusion in result. After a period of 17 to 23 days of incubation, primordia was found to appear only in 7 members out of the 60 lines tested. Number of primordia, their nature of distribution and ultimate development was observed carefully. The observations were recorded in Table 6.

Table 6. Induction of primordia of bud in the haploid monosporous cultures of Pleurotus sajor-caju.

Monosporous cultures	Period of incubation (days)	No. of primordia	Distribution	Development
PS ₃	17	12	Thinly scattered	Very small albino fruitbody
PS ₁₈	22	26	Scattered	-
PS ₃₆	22	18	Clumped	-
PS ₄₆	23	64	Uniformly distributed	Only few formed very small fruit bodies
PS ₄₈	19	31	Scattered	-
PS ₆₄	19	72	Uniformly distributed	Few formed lanky fruit bodies
PS ₆₆	20	9	In small patches	-

The result shows that number of primordia in cases of PS₄₆ and PS₆₄ was higher than in other lines. Though primordia formed after around 20 days of time no one could lead to develop normal fruitbodies, rather rarely formed lanky or unhealthy, albino fruitbodies. Mostly the primordia looked scorchy brown which ultimately became rotten and lost.

G. Determination of sexuality by random crossing among the monosporous lines

For the determination of sexuality in Pleurotus sajor-caju, the monosporous lines developed were tested for their mating behaviour among themselves. Primarily, 12 monosporous lines were considered for inoculation in pairs side by side on PDA medium of poured petriplates in all possible combinations. Within a maximum of 7 days emerging young mycelial hyphae from both the inocula met at the space between them. The zone of contact was observed carefully. The testing monosporous lines were of slow growing in nature but produced morphologically distinguishable, fluffy mycelial growth, the so called incompatibility bridge, at the zone of contact which indicated compatible matings (Fig. 4). The compatible matings were confirmed by microscopic examination of hyphal fusion and occurrence of clamp connection in the dikaryotic mycelia (Fig. 5). In the present investigation 12 monosporous lines as PS₃, PS₅, PS₉, PS₁₀, PS₁₁, PS₁₂, PS₁₆, PS₁₈, PS₁₉, PS₂₀, PS₂₁ and PS₂₂ were tested for the mating reactions. Mating response of each line against the rest 11 lines is represented in Table 7. Compatible matings leading to the formation of

(a)



(b)

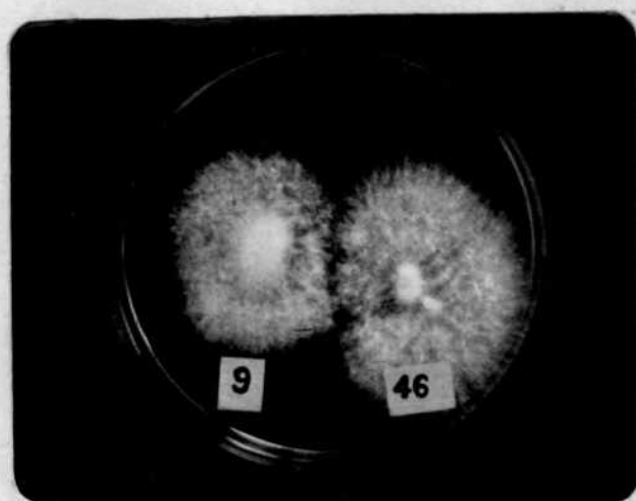


Fig.4. Test of recombination among the monosporous lines of P.sajor-caju

- (a) Compatible mating ($PS_3 \times PS_{18}$) showing fluffy cluster of dikaryotic mycelia at the zone of contact
- (b) Incompatible mating ($PS_9 \times PS_{46}$) with hyaline zone in between

dikaryotic mycelia are indicated by '+' sign when '-' sign stands for incompatible mating. Two sets of mating response are indicated by a diagonal line that are mirror image for each other.

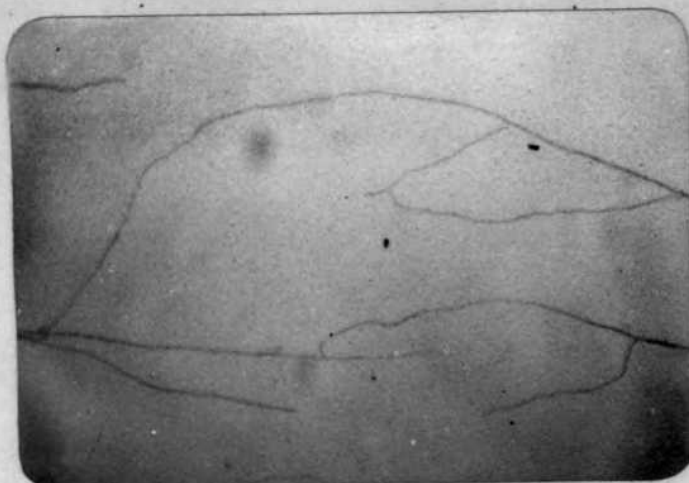
Table 7. Determination of sexuality by random crossing among the monosporous lines.

	3	5	9	10	11	12	16	18	19	20	21	22
3	-	+	-	-	-	-	-	+	-	-	-	+
5	+	-	-	+	-	-	+	-	-	+	-	-
9	-	-	-	-	-	+	-	-	+	-	-	-
10	-	+	-	-	-	-	-	+	-	-	-	+
11	-	-	-	-	-	+	-	-	+	-	-	-
12	-	-	+	-	+	-	-	-	-	-	+	-
16	-	+	-	-	-	-	-	+	-	-	-	+
18	+	-	-	+	-	-	+	-	-	+	-	-
19	-	-	+	-	+	-	-	-	-	-	+	-
20	-	+	-	-	-	-	-	+	-	-	-	+
21	-	-	-	-	-	+	-	-	+	-	-	-
22	+	-	-	+	-	-	+	-	-	+	-	-

H. Classification of monosporous lines into incompatibility classes.

Based on the mating response as represented in Table 7, monosporous lines with similar mating behaviour were sorted out and were rearranged in such a manner as in Table 8 to classify sexually identical cultures in a particular group. Twelve monosporous lines under consideration were thus classified into

(a)



(b)



(c)



Fig.5. Hyphal anastomosis in compatible matings of monokaryons

- | | | |
|-------|-------------------------------------|---------------|
| (a) | PS ₃ x PS ₁₈ | (magn. 381 X) |
| (b) | PS ₂₇ x PS ₄₆ | (magn. 86 X) |
| (c) | PS ₂₇ x PS ₄₆ | (" 1113 X) |

four distinct groups in Table 9. Group I includes the lines PS₃, PS₁₀, PS₁₆ and PS₂₀; Group IV compatible with Group I includes PS₅, PS₁₈ and PS₂₂. Similarly, PS₉, PS₁₁ and PS₂₁ belong to Group II and its compatible Group III includes PS₁₂ and PS₁₉. Occurrence of such four distinct classes of monosporous lines proved tetrapolar incompatibility in Pleurotus sajor-caju.

Table 8. Rearrangement of monosporous lines with similar sexual response.

	3	10	16	20	9	11	21	12	19	5	18	22
3	-	-	-	-	-	-	-	-	-	+	+	+
10	-	-	-	-	-	-	-	-	-	+	+	+
16	-	-	-	-	-	-	-	-	-	+	+	+
20	-	-	-	-	-	-	-	-	-	+	+	+
9	-	-	-	-	-	-	-	+	+	-	-	-
11	-	-	-	-	-	-	-	+	+	-	-	-
21	-	-	-	-	-	-	-	+	+	-	-	-
12	-	-	-	-	+	+	+	-	-	-	-	-
19	-	-	-	-	+	+	+	-	-	-	-	-
5	+	+	+	+	-	-	-	-	-	-	-	-
18	+	+	+	+	-	-	-	-	-	-	-	-
22	+	+	+	+	-	-	-	-	-	-	-	-

Table 9
Classes tester

I	II	III	IV
3	9	12	5
10	11	19	18
16	21		22
20			

Table 10
Classes tester with incompatibility factors

I(A_1B_1)	II(A_1B_2)	III(A_2B_1)	IV(A_2B_2)
3	9	12	5
10	11	19	18
16	21		22
20			

To determine the incompatibility factors governed by the above mentioned classes, further detailed studies were conducted. Arbitrarily Group I was considered to bear A_1B_1 factor which automatically assigned its compatible partners in Group IV to bear the factors different at both the loci as A_2B_2 . To know the incompatibility factors governed by Class II and Class III, the method as advanced by Eger (1974) was followed. It is based

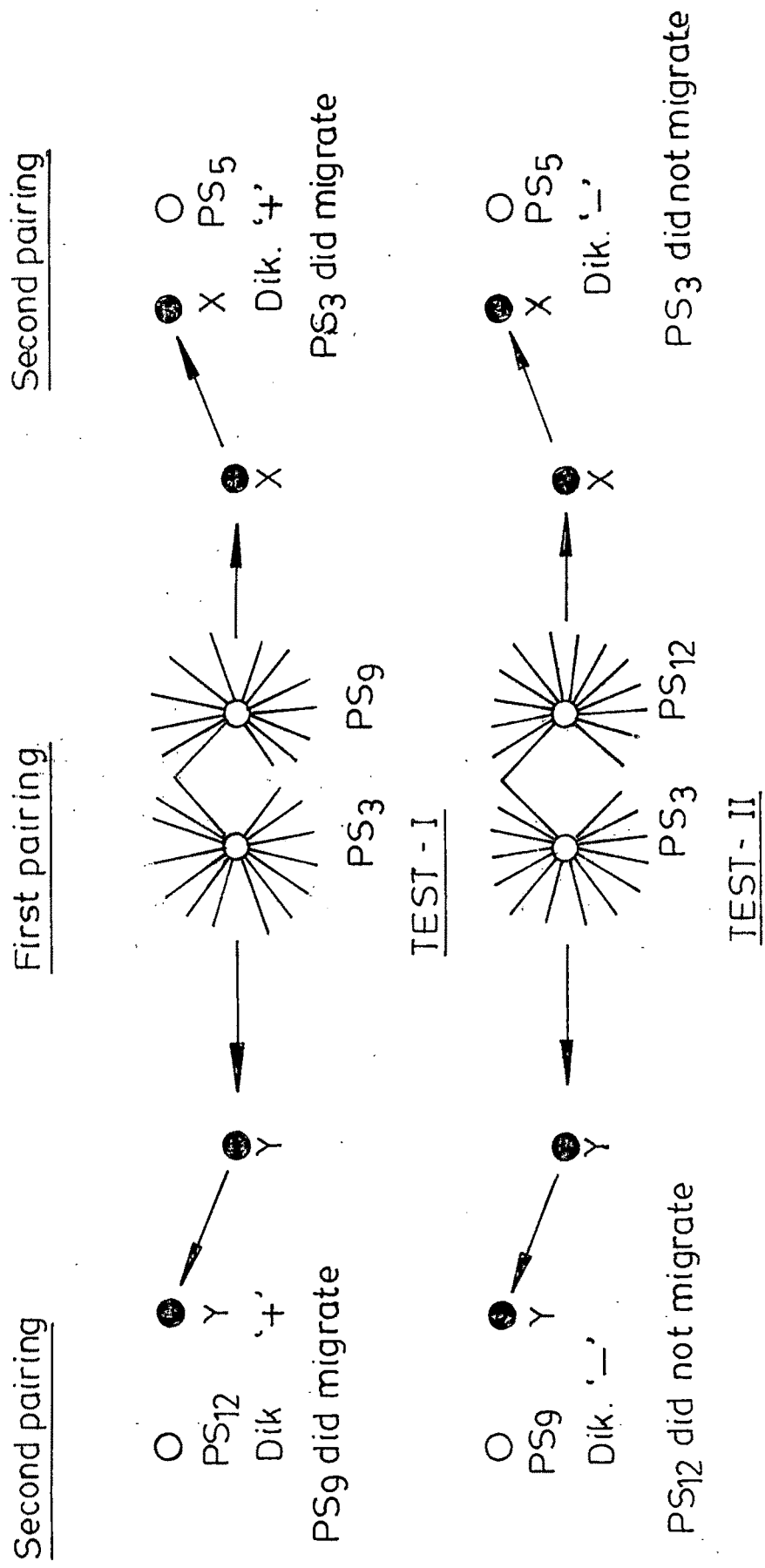


Fig.6 : Determination of common A and B factors in single incompatibility class.

on the principle, where A factors are equal, nuclear migration from one mate to other will occur (Raper, 1966b).

As according to the Fig.6, PS₉ of Class II was mated in the first pairing with PS₃ bearing A₁B₁ factors. In the second pairing X and Y inocula from PS₉ and PS₃ side were mated with PS₅ and PS₁₂ respectively. In both the cases dikaryotisation took place which proved the occurrence of common A factor in PS₃ and PS₉. As PS₃ bears A₁B₁ factor PS₉ must be with the factors of A₁B₂. Conversely, in the next observation PS₃ was mated with PS₁₂. X and Y inocula from PS₁₂ and PS₃ side were subjected for pairing with PS₅ and PS₉ respectively but in no case dikaryotisation occurred. This proved non-migration of nuclear factor from one mate to the other which otherwise meant for the occurrence of common B factor in PS₃ and PS₁₂. So, PS₁₂ includes the incompatibility factors of A₂B₁. With the demarkation of incompatibility in PS₉ and PS₁₂ all the four classes of monosporous lines were properly mentioned with their incompatibility factors as represented in Table 10.

PS₃, PS₉, PS₁₂ and PS₅ were considered as the tester strains in the respective incompatibility classes and by their mating reactions with rest of the unclassified lines all of them were separated with ease into their respective classes. The incompatibility classes with their belonging monosporous lines were presented in Table 11. Five monosporous lines, PS₃₁, PS₃₂, PS₃₉, PS₆₁ and PS₆₅ were found not to response in mating test against any of the tester strain and thus faced difficulty

Table 11. Monosporous lines classified in respective incompatibility groups

I(A ₁ B ₁)	II(A ₁ B ₂)	III(A ₂ B ₁)	IV(A ₂ B ₂)	V(Disputed)
PS ₃	PS ₉	PS ₁₂	PS ₅	PS ₃₁
PS ₁₀	PS ₁₁	PS ₁₉	PS ₁₈	PS ₃₂
PS ₁₆	PS ₂₁	PS ₂₄	PS ₂₂	PS ₃₉
PS ₂₀	PS ₂₃	PS ₃₀	PS ₂₅	PS ₆₁
PS ₂₆	PS ₂₈	PS ₃₄	PS ₂₇	PS ₆₅
PS ₂₉	PS ₃₆	PS ₃₅	PS ₄₅	
PS ₃₃	PS ₃₈	PS ₄₁	PS ₄₈	
PS ₃₇	PS ₄₄	PS ₄₂	PS ₅₂	
PS ₄₀	PS ₄₉	PS ₅₁	PS ₅₈	
PS ₄₃	PS ₅₆	PS ₅₄	PS ₇₀	
PS ₄₆	PS ₆₀	PS ₅₇		
PS ₄₇		PS ₅₉		
PS ₅₀		PS ₆₃		
PS ₅₃		PS ₆₆		
PS ₅₅		PS ₆₈		
PS ₆₂				
PS ₆₄				
PS ₆₇				
PS ₆₉				

to include them in any of the classes. These were probably mutants or accidentally dikaryotised forms and so were placed separately in disputed class V.

I. Criteria for selection of parent monosporous lines :

Many of the desirable inherent characters in monosporous lines remain obscure and awaits expression only after dikaryotisation. But recombination trial among the compatible lines in all possible combinations seems tedious and becomes almost impossible when working with a long list of monosporous lines. So, selection of suitable lines with careful consideration of available characters of all groups may bring about a good chance of effective recombination saving much time and labour. In the experiment under consideration 9 monosporous lines were selected as parent lines based on characters studied earlier as (i) growth pattern, (ii) optimum temperature of growth, (iii) maximum temperature tolerance and (iv) primordia formation. The selected lines with their basis of selection are presented in Table 12.

Table 12. Criteria for selection of parent monosporous lines.

Selected parent lines	Growth pattern	Optimum temperature for growth (°C)	Maximum temperature tolerance (°C)	Primordia formation	Incompatibility class
PS ₃	Medium fast	25-30	36	+	A ₁ B ₁
PS ₉	Medium slow	25-34	36	-	A ₁ B ₂

Table 12 contd.

Selected parent lines	Growth pattern	Optimum temperature for growth (°C)	Maximum temperature tolerance (°C)	Primordia formation	Incompatibility class
PS ₁₈	Medium slow	25-30	34	+	A ₂ B ₂
PS ₂₃	Slow	25	34	-	A ₁ B ₂
PS ₂₇	Fast	25-30	36	-	A ₂ B ₂
PS ₃₅	Slow	25-30	34	-	A ₂ B ₁
PS ₄₆	Medium slow	25-30	36	+	A ₁ B ₁
PS ₅₉	Medium fast	25-30	36	-	A ₂ B ₁
PS ₆₄	Fast	25-30	36	+	A ₁ B ₁
PS _W	Fast	25-30	36	-	

As according to the presented characters PS₃, PS₄₆ and PS₆₄ belonged to the A₁B₁ incompatibility class with medium fast, medium slow and fast growing behaviour respectively. They bore similar optimum temperatures for growth as well as extremity of tolerance. PS₃ and PS₆₄ were primordia formers. Regarding A₂B₂ factors PS₁₈ was medium slow growing in contrast to the fast growing nature of PS₂₇. The later one harboured remarkably long range of temperature tolerance from 25° to 36°C. Here PS₁₈ was primordia former. PS₉ and PS₂₃ with medium slow & slow growing habit were belonged to A₁B₂ group. PS₂₃ was found too temperature sensitive compared to PS₉ and others. PS₃₅ and PS₅₉

of A_2B_1 group were slow and medium fast growing lines with maximum temperature tolerance of 34°C and 36°C respectively.

J. Study of changes in growth pattern in recombined cultures compared to parent lines :

(a) Study of comparative circular growth :

With the exception of very rare cases monosporous lines in general are slow growing in nature. But the recombined cultures were found to reinstate the vigour of mycelial growth at least upto a standard level. Studies on radial growth gives an idea about the rate of colonisation and crop earliness of mushroom fungi. In this experiment recombined cultures were allowed to grow on PDA medium for 10 days. After this period of growth, fungal occupied area was calculated by measuring the diameter of the radial growth. Finally, percentage area covered by the fungi was determined against the growth of wild culture. The growth behaviour of recombined cultures along with the recombining parents are furnished in Table 13, Fig. 7 and 8.

Result shows that when both the parents were slow growing in nature, recombined cultures produced were with maximum fastness in the mode of growth compared to both the parents, but when any of the parents was fast growing then growth increased only upto certain limit. Again, growth of recombined cultures when compared with the wild culture $PS_3 \times 18$, $PS_9 \times 59$, and $PS_{27} \times 46$ showed increased growth behaviour over the standard.

A = PS₃ ; B = PS₉ ; C = PS₁₈ ; D = PS₂₃ ; E = PS₂₇ ; F = PS₃₅ ; G = PS₄₆ ; H = PS₅₉ ; I = PS₆₄ ; W = WILD CULTURE
 1 = PS₃ X 18 2 = PS₃ X 27 3 = PS₉ X 35 4 = PS₉ X 59 5 = PS₁₈ X 46
 6 = PS₁₈ X 64 7 = PS₂₃ X 35 8 = PS₂₃ X 59 9 = PS₂₇ X 46 10 = PS₂₇ X 64

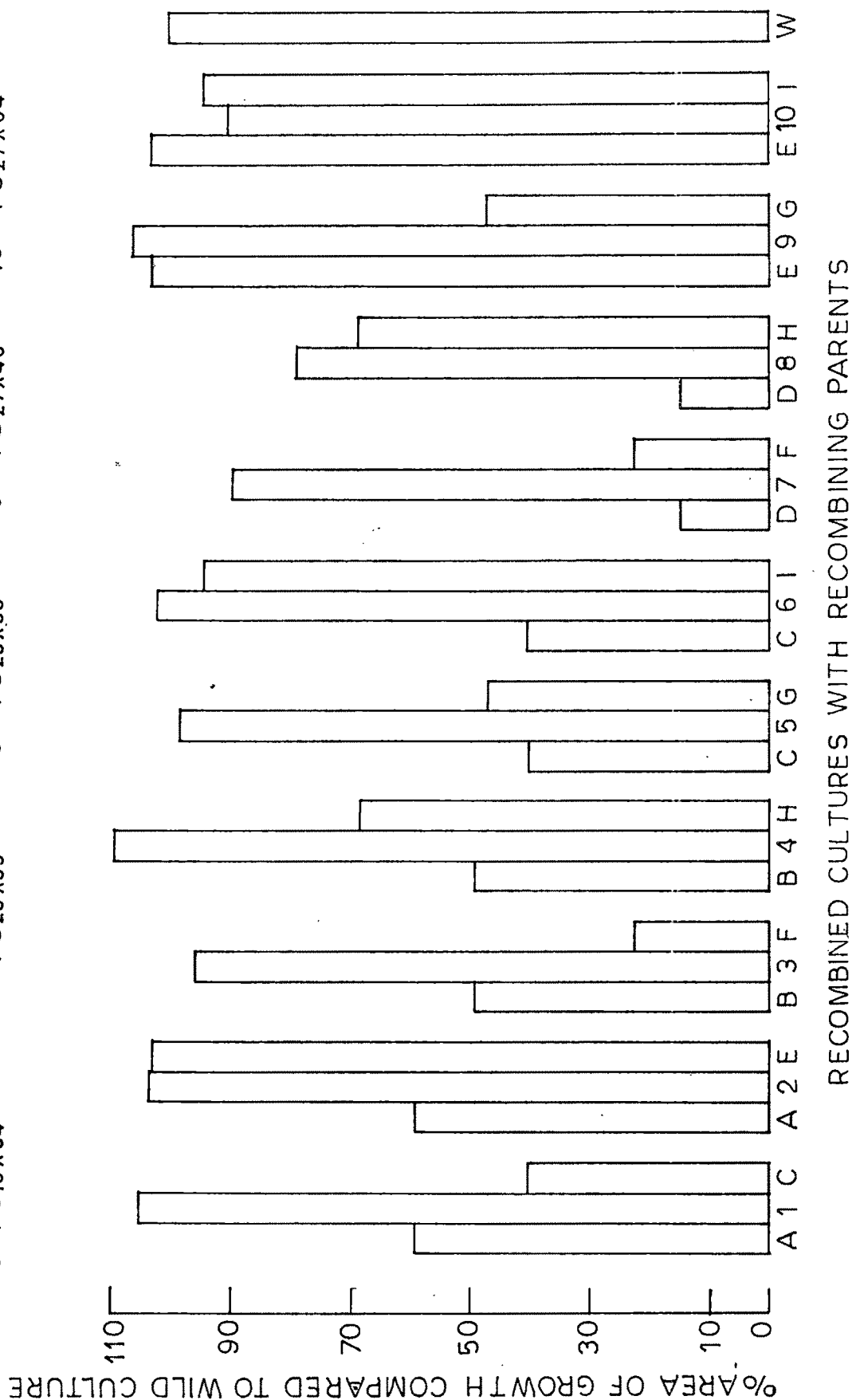
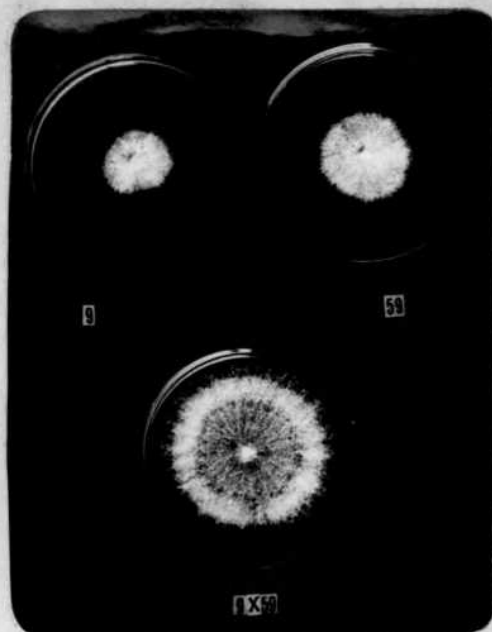


Fig.7 : Study of changes in growth pattern in recombined cultures compared to parent lines by measuring circular growth.

(a)



(b)

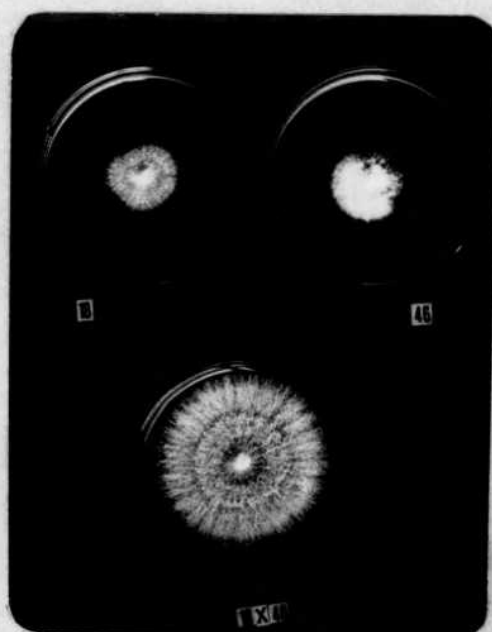


Fig.8. Growth behaviour of recombined cultures side by side with the recombining parents

(a) PS₉ x 59

(b) PS₁₈ x 46

PS₃ x 27 and PS₁₈ x 64 were with mode of growth parallel to the wild culture. PS₉ x 35, PS₁₈ x 46, PS₂₃ x 35 and PS₂₇ x 64 showed marginal reduction with much slower growth observed in case of PS₂₃ x 59.

Table 13. Study of changes in growth pattern in recombined cultures compared to parent lines by measuring circular growth.

Recombined cultures	Diameter of growth (cm)	Area of growth (πr^2) (cm ²)	% area of growth compared to wild culture		
			Parent-I	Parent-II	Recombined culture
PS ₃ x 18	11.80	109.36	59.34	40.51	105.65
PS ₃ x 27	11.69	107.33	59.34	102.91	103.69
PS ₉ x 35	11.25	99.40	49.22	22.36	96.02
PS ₉ x 59	12.00	113.10	49.22	68.29	109.26
PS ₁₈ x 46	11.40	102.07	40.51	46.98	98.61
PS ₁₈ x 64	11.60	105.68	40.51	94.47	102.10
PS ₂₃ x 35	10.86	92.63	14.90	22.36	89.49
PS ₂₃ x 59	10.20	81.71	14.90	68.29	78.94
PS ₂₇ x 46	11.83	109.91	102.91	46.98	106.18
PS ₂₇ x 64	10.90	93.31	102.91	94.47	90.14
PS _(Wild)	11.48	103.51	-	-	-

(b) Study of growth by measuring dry mycelial mass grown in liquid culture :

To find out the change in nature of growth leading to the efficiency in production of mycelial mass in liquid medium, recombined cultures were inoculated in the Potato-Dextrose (PD) broth medium. Mycelial mass produced after 10-days was harvested and dried to constant weight. Percentage value of the dry mass based on the standard of wild culture of Pleurotus sajor-caju was calculated. To get an overview of the change in percentage value of mycelial mass of recombined cultures, percentage growth of recombining parents were represented side by side in Table 14, Fig. 9.

Regarding the production of mycelial mass, the recombined cultures showed a tendency to emulate the efficiency as established by the wild culture. A further detail showed marginal dropping in percentage weight of mycelial mass in $PS_9 \times 35$, $PS_{18} \times 64$ and $PS_{23} \times 59$ with a minimum of 87.44% in $PS_{23} \times 35$. While in other cultures it showed equal or increased performance with a maximum production of mycelial mass of 234 mg by $PS_3 \times 18$ as against 224.52 mg in wild culture. As per percentage value of mycelial mass in recombined cultures compared to the recombining parents, efficiency found to overlap in all the cases.

Table 14. Study of changes in growth pattern in recombined cultures compared to parent lines by measuring dry mycelial mass grown in liquid culture.

Recombined cultures	Wt. of dry mycelial mass (10 days) (mg.)	% dry mycelial mass compared to wild culture		
		Parent-I	Parent-II	Recombined culture
PS ₃ x 18	234.00	73.95	85.13	104.22
PS ₃ x 27	225.80	73.95	96.46	100.57
PS ₉ x 35	204.25	78.16	83.34	90.97
PS ₉ x 59	230.67	78.16	66.76	102.74
PS ₁₈ x 46	224.25	85.13	81.33	99.88
PS ₁₈ x 64	217.62	85.13	93.95	96.93
PS ₂₃ x 35	196.33	55.79	83.34	87.44
PS ₂₃ x 59	209.03	55.79	66.76	93.10
PS ₂₇ x 46	228.70	96.46	81.33	101.86
PS ₂₇ x 64	225.00	96.46	93.95	100.21
PS (Wild)	224.52	-	-	-

K. Studies on the changes in circular growth pattern in the recombined cultures of di-mon mating :

To determine the growth pattern in recombined cultures of di-mon mating it were inoculated in poured petriplates of PDA medium. After 10 days of growth at 25°C, diameter of the fungal occupied area was measured and the area covered was

A=PS₃; B=PS₉; C=PS₁₈; D=PS₂₃; E=PS₂₇; F=PS₃₅; G=PS₄₆; H=PS₅₉; I=PS₆₄; W=WILD CULTURE
 1=PS_{3X18} 2=PS_{3X27} 3=PS_{9X35} 4=PS_{9X59} 5=PS_{18X46}
 6=PS_{18X35} 7=PS_{23X35} 8=PS_{23X59} 9=PS_{27X46} 10=PS_{27X64}

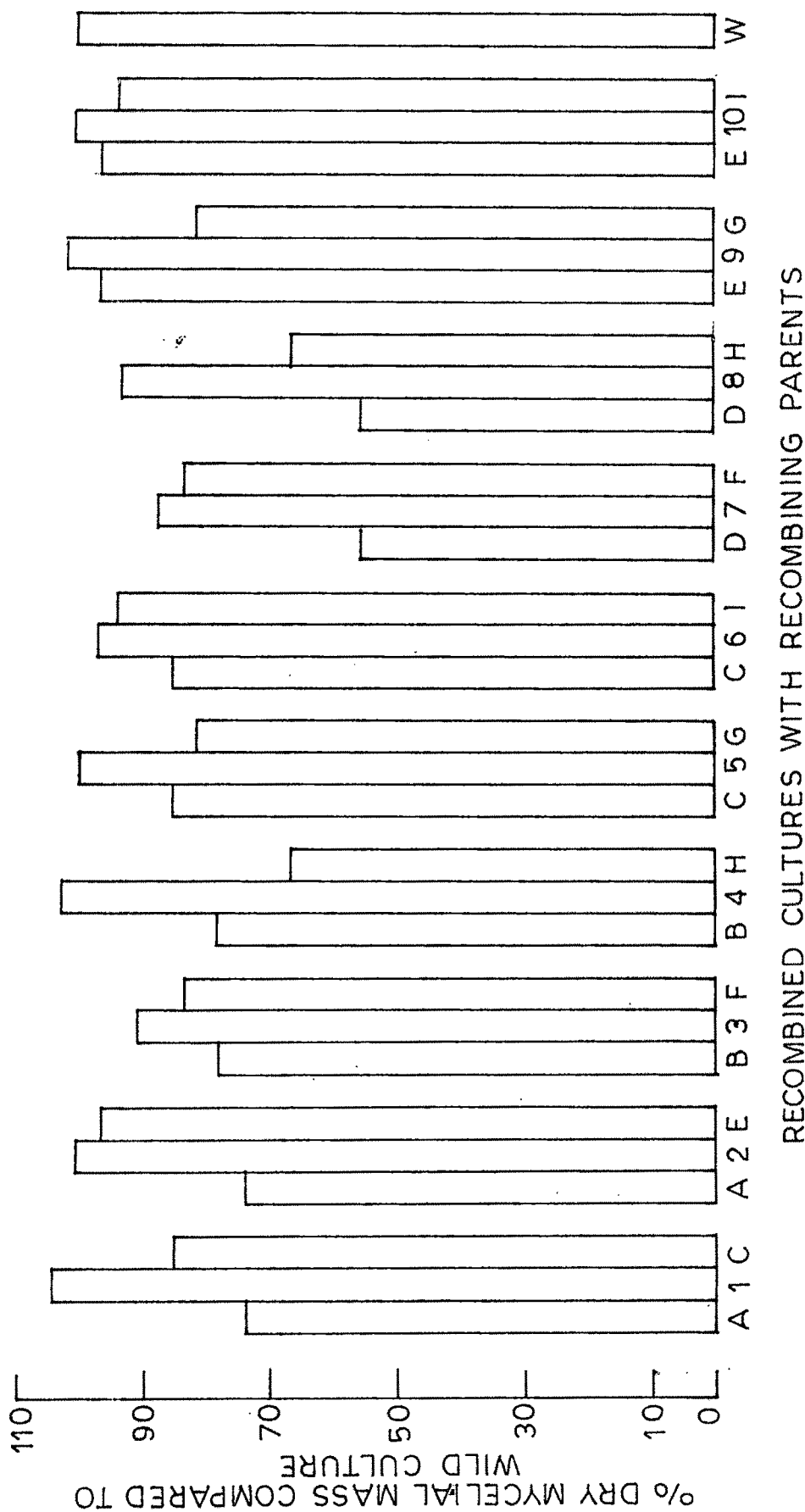


Fig.9: Study of changes in growth pattern in recombined cultures compared to parent lines by measuring dry mycelial mass grown in liquid culture.

calculated. The area of growth of recombined cultures were compared to that of wild culture. Also, percentage value of growth to that of the wild culture was determined. Area of growth of recombined cultures along with its percentage value are presented side by side with the recombining parents as in Table 15, Fig. 10.

PS₅₉ x W with 108.99 sq.cm growth area overlapped the area covered by the wild culture. Growth in PS₂₇ x W parallels wild culture with a marginal reduction observed in case of PS₉ x W. When percentage area of growth was considered, one of the recombining parents in all the combinations was its wild culture and in no case percentage value exceeded the standard to a very large extent. PS₅₉ x W with 105.29% growth proved more efficient coloniser than wild culture. PS₂₇ x W and PS₉ x W with 101.22% and 97.40% growth were placed slightly above and below the standard respectively.

Table 15. Study on the changes in circular growth pattern in the recombined cultures of di-mon mating.

Recombined cultures	Diameter of growth (cm)	Area of growth (πr^2) (cm ²)	% area of growth compared to wild culture		
			Parent-I	Parent-II	Recombined culture
PS ₉ x W	11.33	100.82	49.22	100.0	97.40
PS ₂₇ x W	11.55	104.77	102.91	100.0	101.22
PS ₅₉ x W	11.78	108.99	68.29	100.0	105.29
PS (Wild)	11.48	103.51	-	-	-

A = PS₉ ; B = PS₂₇ ; C = PS₅₉ ; W = WILD CULTURE

1 = PS₉ x W ; 2 = PS₂₇ x W ; 3 = PS₅₉ x W

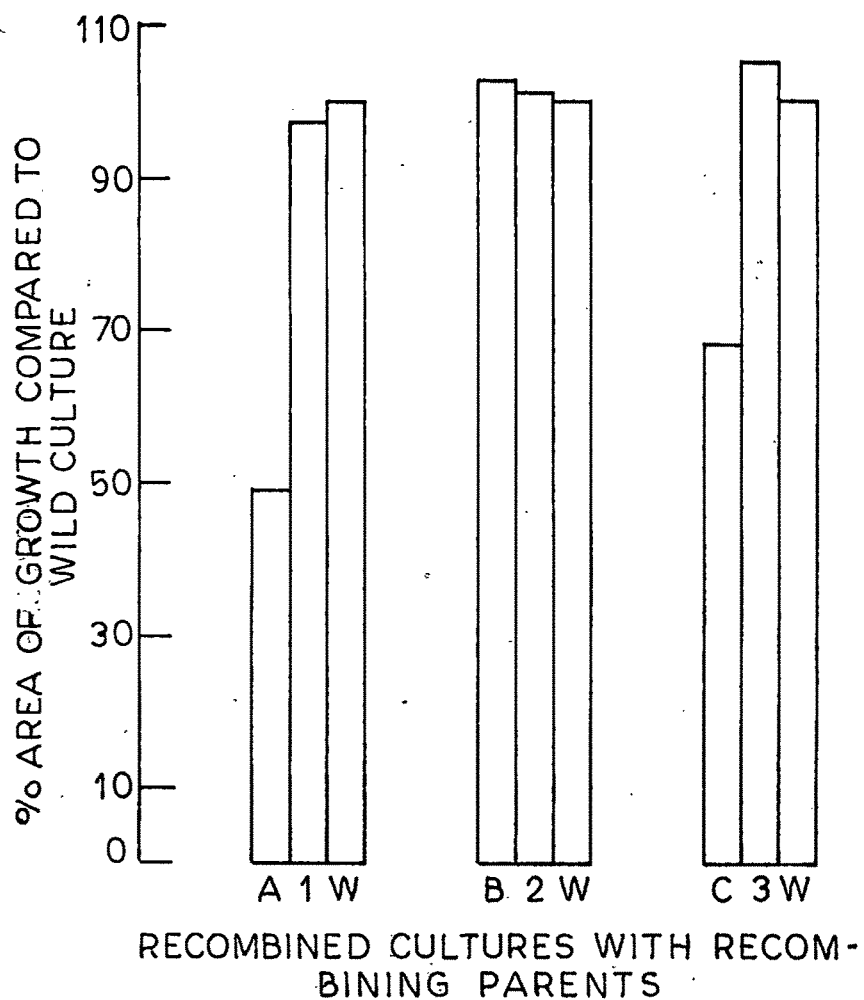


Fig 10: Study on the changes in circular growth pattern in the recombined cultures of di-mon mating.

L. Trials of cultivation of recombined cultures considering spawn run, bud mortality and yield of mushroom :

Recombined cultures raised from the crossing trials among selected parent monosporous lines in all possible combinations were subjected to the cultivation practices to see the changes in characters, if any, over the wild culture. In the following experiment wheat grain spawn of recombined cultures were inoculated in sterilized straw substrate and incubated at 25°C. Spawn run period and further details of fruitbody formation, bud mortality and yield along with fruitbody quality were thoroughly studied. Yield data has been statistically analysed following "Randomised Block Design". Experimental results are tabulated in Table 16.

The result shows an average of about 16 days spawn run period was required for most of the recombined cultures with a minimum of 14 days in case of $PS_3 \times 27$ while 21 days was considered maximum in case of $PS_{23} \times 59$. All the recombined cultures developed much higher number of buds compared to the wild culture, but with a varying degree of bud mortality. In general all were with a high bud mortality. $PS_9 \times 59$, $PS_{18} \times 46$ and $PS_{18} \times 64$ showed more than 70% bud mortality while $PS_3 \times 18$, $PS_9 \times 35$, $PS_{23} \times 35$, $PS_{23} \times 59$ and $PS_{27} \times 64$ lay between 60-70%. $PS_{27} \times 46$ with 58.46% bud mortality behaved similarly to the wild culture. On the contrary, the only culture $PS_3 \times 27$ showed too less bud mortality of 46.03% in relation to 57.89% in wild culture of Pleurotus sajor-caju.

No recombined culture exhibited its excellence of remarkably higher yield, rather, most of the cultures hardly managed to cross even the yield level of wild culture. The difference among the yield of cultures, $PS_{27 \times 46}$ (56.33 g), $PS_3 \times 27$ (55.5 g), PS_W (54.33 g), $PS_{18 \times 46}$ (53.67 g) and $PS_9 \times 59$ (50.0 g) respectively were statistically not significant. The rest of the cultures showed much less yield in comparison with that of the yield values of wild culture.

Table 16. Trials of cultivation of recombined cultures considering spawn run, bud mortality and yield of mushroom.

Recombined cultures	Spawn run period (days)	No. of buds	No. of fruit-bodies	Bud mortality (%)	Yield (g)
$PS_3 \times 18$	18	75	25	66.67	46.00
$PS_3 \times 27$	14	63	34	46.03	55.50
$PS_9 \times 35$	17	45	16	64.44	39.50
$PS_9 \times 59$	15	72	19	73.61	50.00
$PS_{18 \times 46}$	16	51	15	70.59	53.67
$PS_{18 \times 64}$	17	59	17	70.19	43.67
$PS_{23 \times 35}$	19	56	22	60.71	34.50
$PS_{23 \times 59}$	21	53	20	62.26	22.33
$PS_{27 \times 46}$	15	65	27	58.46	56.33
$PS_{27 \times 64}$	18	65	20	69.23	47.33
$PS_{(Wild)}$	16	38	16	57.89	54.33
CD(0.05)					4.65

M. Assessment of fruitbody quality of recombined cultures :

Under similar preconditions of growth distinct changes observed in the fruitbody quality of recombined cultures. Based on the cultivation trials conducted earlier, an overall assessment of qualitative characters was done and represented in Table 17, Fig. 11.

Although, weight of average fruitbody depends on the amount of substrate used, in the present experiment only $PS_{18 \times 46}$ produced larger fruitbodies like that of the wild culture. Average weight decreased to around 2.5 g in cases of $PS_{9 \times 59}$, $PS_{18 \times 64}$, $PS_{9 \times 35}$ and $PS_{27 \times 64}$. Further reduction observed in $PS_{27 \times 46}$, $PS_{3 \times 18}$, $PS_{3 \times 27}$ and $PS_{23 \times 35}$ respectively with least minimum in $PS_{23 \times 59}$. Reduction in average fruitbody weight was found to be due to the increase in number of fruitbodies. Stipe length varied from 1-3.5 cm in most of the cases even in wild culture. But stipe length in $PS_{3 \times 27}$, $PS_{3 \times 18}$ and $PS_{23 \times 59}$ gradually reduced to the minimum of 0.5-1.5 cm in the later one. Remarkable change in pileus colour was noted in many of the recombined cultures. $PS_{3 \times 27}$, $PS_{18 \times 46}$ and $PS_{27 \times 46}$ were with white to creamy white fruitbodies in contrast to whitish brown or greyish-brown fruitbodies of wild culture as well as in $PS_{3 \times 18}$, $PS_{9 \times 35}$, $PS_{9 \times 59}$, $PS_{23 \times 35}$, $PS_{23 \times 59}$ and $PS_{27 \times 64}$. $PS_{18 \times 64}$ produced fruitbodies with deep brown in colour. $PS_{3 \times 27}$, $PS_{9 \times 35}$, $PS_{9 \times 59}$, $PS_{18 \times 46}$ and $PS_{27 \times 46}$ had fruitbodies with entire margin while in other cultures it was slightly wavy to wavy in nature.

PS₁₈ x 64 produced fruitbodies with striated lines of ornamentation on the pileus. Regarding fruitbody thickness PS₃ x 18, PS₃ x 27, PS₂₃ x 35 and PS₂₇ x 46 were with lesser thickness than normal, while PS₁₈ x 64 looked comparatively thick. Flavour in all the cultures but PS₃ x 18, PS₂₃ x 35 and PS₂₃ x 59 was normal.

Table 17. Assessment of fruitbody quality of recombined cultures.

Recombined cultures	Average fruit-body wt (g)	Stipe length (cm)	Pileus colour	Pilear margin	Thickness (T)	Flavour (F)
PS ₃ x 18	1.84	0.5-1.8	Normal	Slightly wavy	T-	F-
PS ₃ x 27	1.63	0.5-2.6	White	Entire	T-	F
PS ₉ x 35	2.49	1.5-2.5	Normal	Entire	T	F
PS ₉ x 59	2.63	1.0-2.7	Normal	Entire	T	F
PS ₁₈ x 46	3.58	1.25-2.5	Creamy white	Entire	T	F
PS ₁₈ x 64	2.57	1.0-3.5	Brown	Striated & wavy	T+	F
PS ₂₃ x 35	1.57	1.0-2.1	Less brown	Slightly wavy	T-	F-
PS ₂₃ x 59	1.12	0.5-1.5	Less brown	Slightly wavy	T	F-
PS ₂₇ x 46	2.09	1.0-2.9	Creamy white	Entire	T-	F
PS ₂₇ x 64	2.37	1.5-3.0	Less brown	Wavy	T	F
PS _W	3.39	1.25-3.0	Normal (Whitish-brown)	Entire	T	F

(a)



(b)

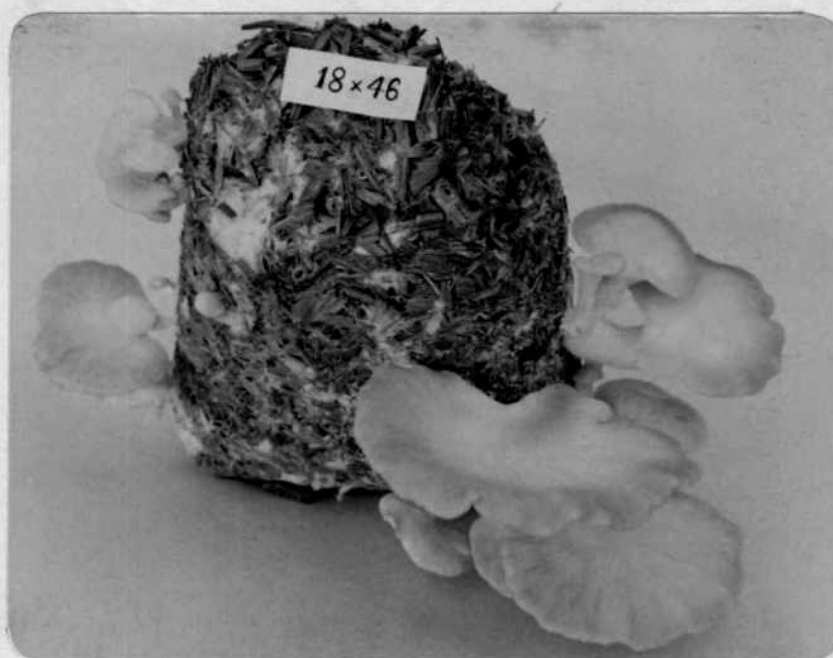
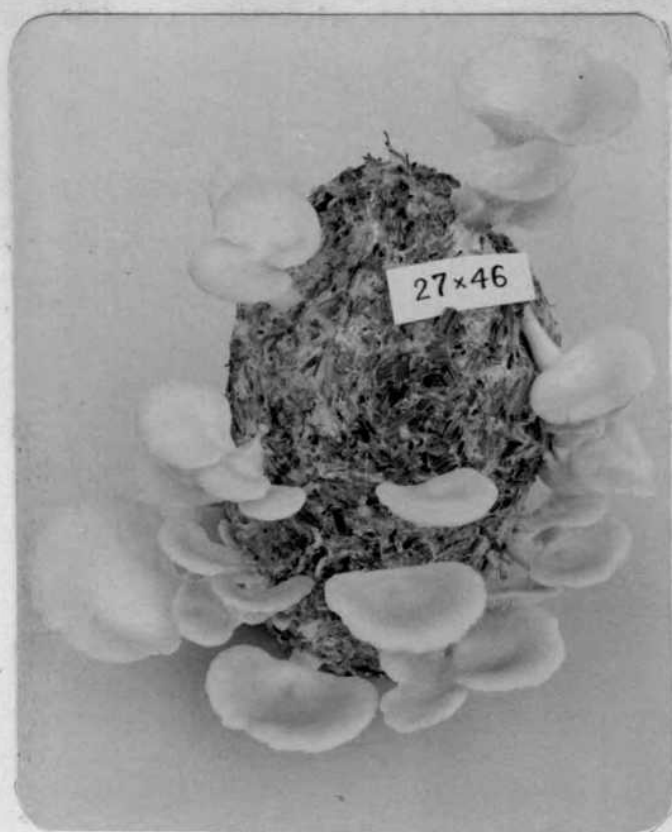


Fig.11. Variation in fruiting behaviour and quality characters of some recombined strains compared to wild culture of P.sajor-caju

(a) PS_{3 x 27}

(b) PS_{18 x 46}

(c)



(d)

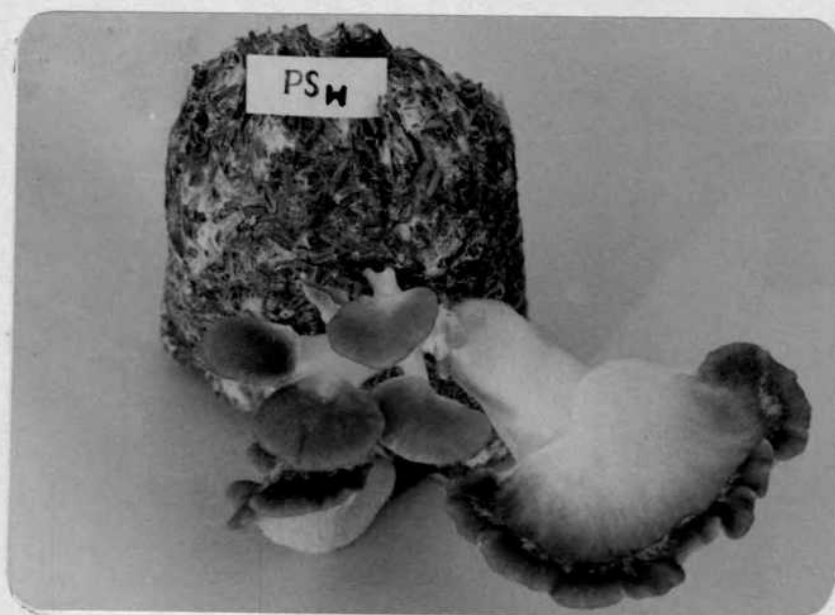
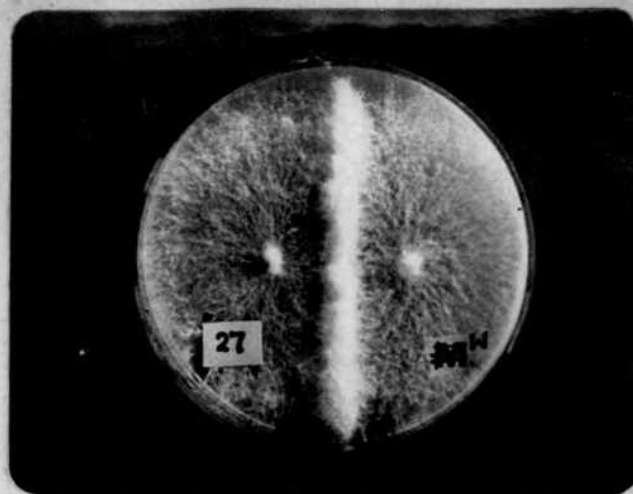


Fig.11. Variation in fruiting behaviour and quality characters of some recombined strains compared to wild culture of P.sajor-caju

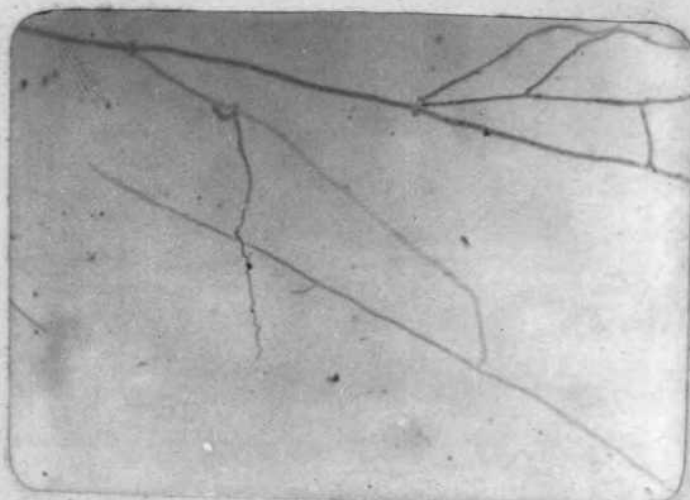
(c) PS_{27 x 46}

(d) PS_{Wild}

(a)



(b)



(c)

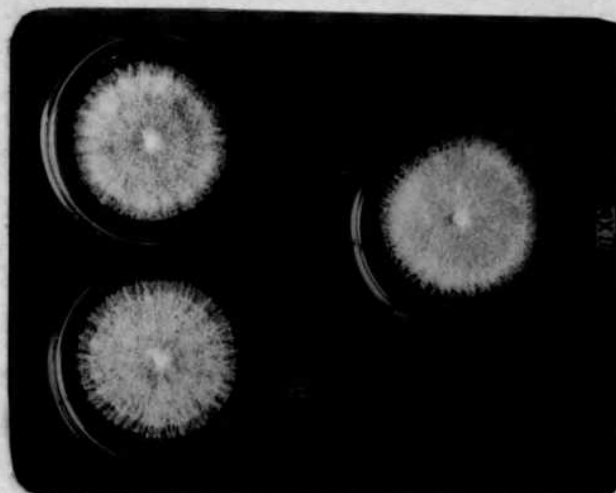


Fig.12. Positive response of di-mon mating (PS₂₇ x W) in Pleurotus sajor-caju

- (a) Formation of vigorous mycelia at the zone of contact
- (b) Hyphal anastomosis (magn. 241x)
- (c) Growth behaviour of recombined culture along with the recombining parents

N. Trials of cultivation of recombined cultures raised from di- mon mating :

Di- mon mating was induced in all the selected parent monosporous lines with that of the wild culture of Pleurotus sajor-caju. Recombination observed only in three lines out of the 9 parent lines tested (Fig.12). Recombined cultures of di- mon mating thus raised were subjected to the trials of cultivation to study the characters it received after recombination or any change in the basic characters of the original stock.

In this experiment wheat grain spawn of recombined cultures were inoculated in sterilized straw substrate and incubated at 25°C. Spawn run period and further development to fructification were thoroughly observed. Variation in spawn run period, bud mortality and yield is represented in Table 18. Yield data has been statistically analysed following "Randomised Block Design".

The result shows that spawn run period was found minimum of 14 days in $PS_{27} \times W$ while in $PS_9 \times W$ it was increased to 18 days surpassing even the spawn run period of wild culture (16 days). Number of bud was maximum in case of $PS_{27} \times W$. $PS_9 \times W$ governed reduced bud mortality of 55.81% compared to the standard (Wild) of 57.89%. $PS_{27} \times W$ and $PS_{59} \times W$ showed 64.28% and 59.09% bud mortality respectively. Yield behaviour of the recombined cultures were very much similar to the wild

culture. PS₂₇ x W, PS₅₉ x W and PS₉ x W produced 54.0 g, 53.67 g and 52.67 g of mushroom respectively compared to 54.33 g in wild culture. The variation in yield obtained was not statistically significant.

Table 18. Trials of cultivation of recombined cultures raised from di- mon mating.

Recombining parents Monokaryon x Dikaryon	Mating res- ponse	Spawn run period (days)	No. of buds	No. of fruit bodies	Bud morta- lity (%)	Yield (g)
PS ₃ x PS _W	-	-	-	-	-	-
PS ₉ x PS _W	+	18	43	19	55.81	52.67
PS ₁₈ x PS _W	-	-	-	-	-	-
PS ₂₃ x PS _W	-	-	-	-	-	-
PS ₂₇ x PS _W	+	14	56	20	64.28	54.00
PS ₃₅ x PS _W	-	-	-	-	-	-
PS ₄₆ x PS _W	-	-	-	-	-	-
PS ₅₉ x PS _W	+	16	44	18	59.09	53.67
PS ₆₄ x PS _W	-	-	-	-	-	-
PS _W (Wild culture)		16	38	16	57.89	54.33
CD(0.05)						NS

0. Study on temperature tolerance in recombined cultures.

(a) Temperature tolerance of recombined isolates raised from the crossing of monosporous isolates

During selection of parent monosporous lines to raise suitably modified recombined cultures, increased temperature tolerance was given special importance beside other desirable characters. After the crossing trials it was necessary to test the efficiency of temperature tolerance of recombined cultures it harboured. So an experiment was conducted to expose the cultures to the rising temperatures of 25°C to 38°C. Response to the increasing temperatures was estimated by comparing the area of growth of the cultures as against the area of growth covered by the same at optimum temperature of 25°C. Area of growth of recombined cultures at different temperatures has been analysed by following 'Factorial RBD method' and presented in Table 19, Fig. 13.

Recombined cultures, $PS_3 \times 27$, $PS_9 \times 35$, $PS_9 \times 59$, $PS_{18} \times 46$, $PS_{18} \times 64$ and $PS_{27} \times 64$ were found to be less temperature sensitive at least upto 30°C and maintained its optimum growth. But in rest of the cultures growth at 30°C was significantly reduced. With increase in the degrees of temperature above 30°C growth was sharply reduced in all the cases and stopped at 36° or 38°C. $PS_9 \times 59$ showed best performance of growth between 25° and 30°C. Comparing maximum temperature tolerance among the recombined cultures $PS_{23} \times 35$ and $PS_{23} \times 59$ were found to be

more temperature sensitive with maximum tolerance only upto 34°C whereas in other cultures tolerance limit increased upto 36°C.

Table 19. Study of temperature tolerance in recombined cultures.

Recombined cultures	Growth area after 10 days with increasing degrees of temperature (sq.cm.)						Maximum tempt. tolerance (°C)
	25°C	30°C	32°C	34°C	36°C	38°C	
PS ₃ x 18	109.36	104.59	76.66	16.57	2.43	Nil	36
PS ₃ x 27	107.14	105.68	78.53	48.27	6.07	Nil	36
PS ₉ x 35	99.40	96.77	64.47	15.90	1.49	Nil	36
PS ₉ x 59	113.09	110.10	71.18	36.32	2.11	Nil	36
PS ₁₈ x 46	102.07	99.93	68.80	24.45	3.08	Nil	36
PS ₁₈ x 64	105.68	106.41	59.99	28.27	2.89	Nil	36
PS ₂₃ x 35	92.63	74.20	38.70	2.21	Nil	Nil	34
PS ₂₃ x 59	81.71	61.65	43.00	8.04	Nil	Nil	34
PS ₂₇ x 46	109.73	103.87	71.78	26.79	4.37	Nil	36
PS ₂₇ x 64	93.31	96.42	65.32	22.56	1.17	Nil	36
PS _W	103.51	101.35	67.34	18.70	3.80	Nil	36
CD(0.05)	For temperatures				1.13		
CD(0.05)	For cultures				1.67		
CD(0.05)	For temperature x culture				3.74		

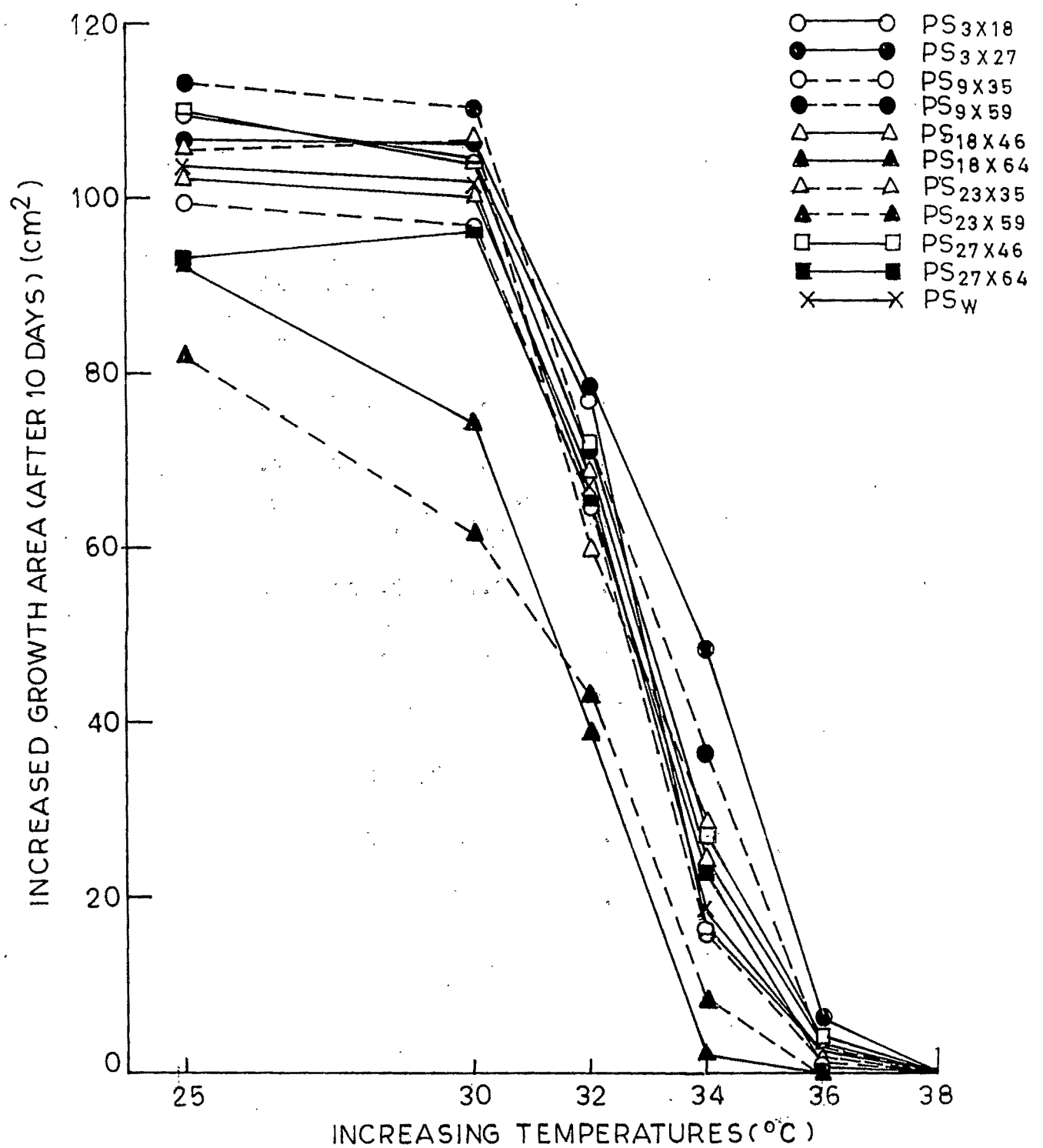


Fig.13: Study of temperature tolerance in recombined cultures.

(b) Temperature tolerance of recombined isolates raised from di- mon matings.

To determine the temperature tolerance of recombined cultures raised from di- mon matings of selected monosporous parent lines with that of wild culture, they were exposed to the rising temperatures of 25°, 30°, 32°, 34°, 36° and 38°C. Response to increasing degrees of temperatures was estimated by comparing its area of growth against the growth area of the same at optimum temperature of 25°C. Growth response of recombined cultures along with the wild culture has been statistically analysed following 'Factorial RBD method' and represented in Table 20, Fig. 14.

The result shows that all the recombined culture, except PS₅₉ x W, maintained more or less optimum growing ability upto 30°C beyond which area of growth was gradually decreased with increase in temperature. At 36°C all the cultures were seriously affected and growth became very insignificant. Further increase in temperature to 38°C led to the total blockage of growth. PS₂₇ x W showed significantly higher growth at 25°C compared to the other cultures. Temperature tolerance in PS₉ x W and PS₂₇ x W seemed parallel to the wild culture while PS₅₉ x W behaved as more temperature sensitive.

Table 20. Study on temperature tolerance of recombined cultures raised from di- mon matings.

Recombined cultures	Growth area after 10 days with increasing degrees of temperature (sq.cm.)						Maximum tempt. tolerance(°C)
	25°C	30°C	32°C	34°C	36°C	38°C	
PS ₉ x W	94.34	95.03	62.77	16.61	2.66	Nil	36
PS ₂₇ x W	102.43	99.58	71.78	29.60	3.20	Nil	36
PS ₅₉ x W	92.97	86.59	72.38	18.86	0.75	Nil	36
PS _W	98.87	96.76	68.51	22.39	4.90	Nil	36
CD(0.05)	For temperatures					1.63	
CD(0.05)	For cultures					1.46	
CD(0.05)	For temperature x culture					3.27	

P. Study on comparative spawn run and yield behaviour of recombined cultures in two flushes.

It has been observed that mushrooms occasionally exhibit comparatively poor performance of yield in the first flush but in the second flush it comes with a higher yield. So a cumulative yield of first and second flushes may give a clear picture about the yield potentiality of a mushroom fungus. To determine the yield behaviour of recombined cultures, sterilized

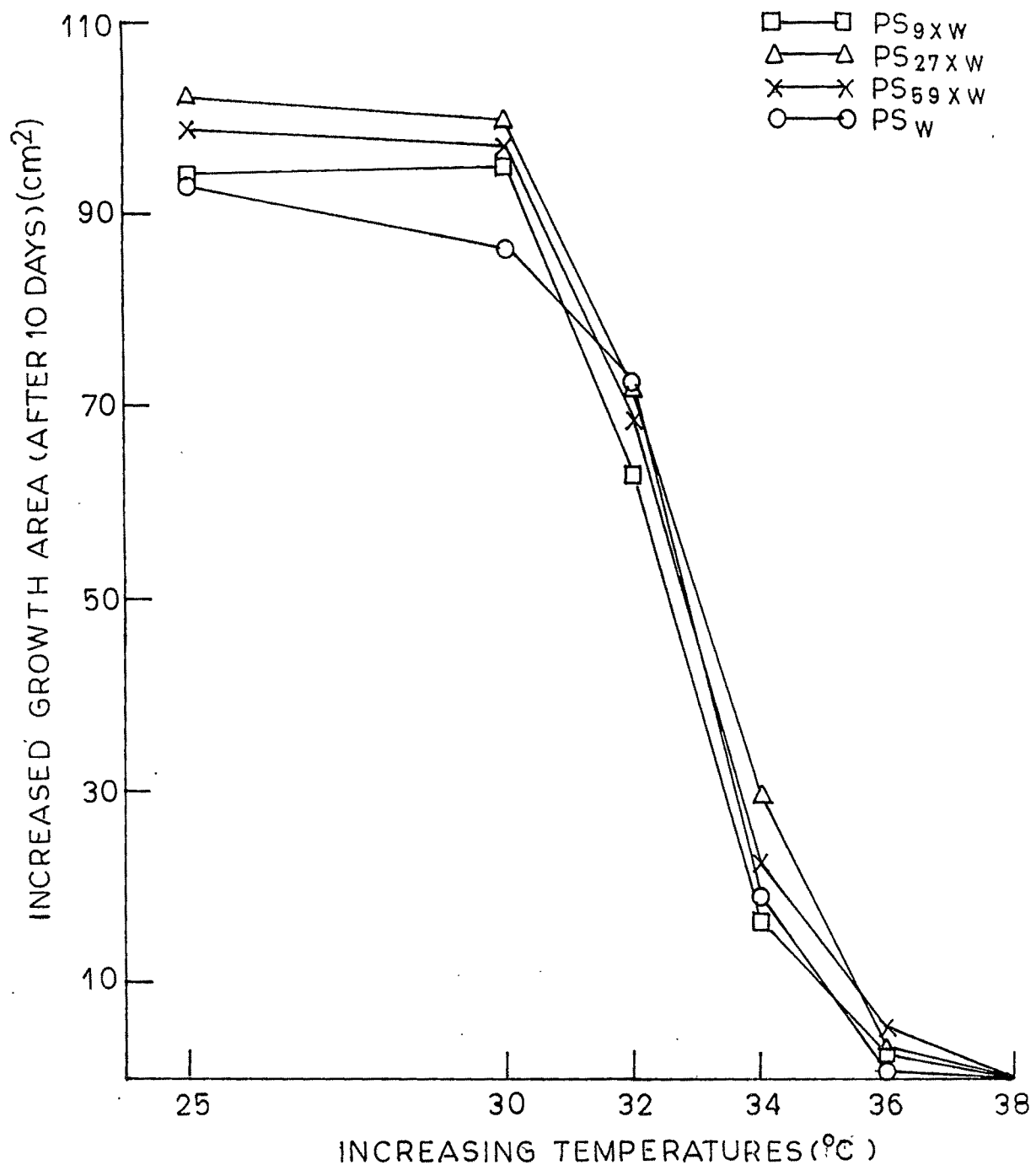


Fig.14: Study of temperature tolerance of recombined cultures raised from di-mon mating.

straw substrate was inoculated with 3% wheat grain spawn for each culture. Spawn run period, number of fruitbodies and yield in two consecutive flushes along with total number of fruitbodies and total yield of each culture are represented in Table 21. The total yield in two flushes has been statistically analysed following 'Randomised Block Design'.

Presented data shows that $PS_3 \times 27$ and $PS_{27} \times W$ were good colonisers with minimum spawn run period of 14 days, whereas in other cultures but $PS_{23} \times 59$, spawn run period varied between 15 to 19 days. $PS_{23} \times 59$ taken 21 days to complete its colonisation phase. Regarding number of fruitbodies $PS_3 \times 27$ produced maximum in both the flushes followed by $PS_{27} \times 46$, $PS_{23} \times 35$, $PS_{27} \times W$ and $PS_3 \times 18$ respectively. In rest of the cases the picture looked more or less similar. Cumulative yield of mushroom in most of the recombined cultures including the wild culture were found to lie closely between 63.67 and 70.0 g. $PS_3 \times 27$ gave the maximum yield of 70.0 g. Significant reduction in yield was obtained in $PS_{27} \times 64$, $PS_3 \times 18$, $PS_{18} \times 64$, $PS_9 \times 35$ and $PS_{23} \times 35$ respectively with the least value of 30.33 g obtained in case of $PS_{23} \times 59$.

Q. Determination of moisture content of the fruitbodies of recombined varieties of mushrooms.

Moisture content in fresh mushroom though found to lie around 90%, variation may takes place depending on the species and stages of harvest etc. To find out the variation in moisture content in the mushrooms of recombined cultures it were

Table 21. Study on comparative spawn run and yield behaviour of recombined cultures in two flushes (per 75 g dry straw).

Recombined cultures	FIRST FLUSH			SECOND FLUSH			TOTAL	
	Spawn run period (days)	No. of fruit-bodies	Yield (g)	Spawn run period (days)	No. of fruit-bodies	Yield (g)	No. of fruit-bodies	Yield (g)
PS ₃ x 18	18	24	48.33	10	5	9.00	29	57.33
PS ₃ x 27	14	35	53.00	8	12	17.00	47	70.00
PS ₉ x 35	17	18	41.50	8	5	12.75	23	54.25
PS ₉ x 59	15	18	53.67	8	4	10.00	22	63.67
PS ₁₈ x 46	16	16	51.67	9	8	16.33	24	68.00
PS ₁₈ x 64	17	21	48.83	10	5	8.00	26	56.83
PS ₂₃ x 35	19	23	37.67	10	8	13.00	31	50.67
PS ₂₃ x 59	21	19	23.33	9	6	7.00	25	30.33
PS ₂₇ x 46	15	25	54.83	10	9	14.67	34	69.50
PS ₂₇ x 64	18	20	49.50	10	5	10.83	25	60.33
PS ₉ x W	18	18	50.67	8	7	15.50	25	66.17
PS ₂₇ x W	14	21	52.00	9	9	14.67	30	66.67
PS ₅₉ x W	16	16	55.33	10	6	11.83	22	67.16
PS _W	16	17	51.50	9	5	16.50	22	68.00

CD(0.05)

4.99

grown in same environmental condition and collected at the identical stage of harvest (right stage of harvest i.e. just before spore discharge). Mushrooms were dried in hot air oven at 70-80°C for 72 hours or more till constant weight. Moisture content was calculated by subtracting the value of dry mass from the fresh weight of the samples and finally percentage moisture content was calculated therefrom. Percentage value of moisture content in the mushrooms of recombined cultures are presented in Table 22.

According to the presented data moisture content in mushrooms of different cultures varied from 87.60% to 92.82% with the minimum of 87.6% moisture observed in case of PS₉ x W. PS₂₇ x 64 contained 92.82% moisture and was considered maximum.

R. Analysis of protein content in the mushroom of recombined cultures.

To determine the variation in protein percentage in the mushroom of different recombined cultures they were grown on the same substrate of paddy straw and harvested at the right stage of harvest. Dried mushroom samples were subjected to the method of estimation of total nitrogen following micro-Kjeldahl method. From the value of estimated total nitrogen, crude protein fraction was calculated by multiplying percentage nitrogen content with the conversion factor of 6.25. Percentage protein content in the dry matter along with initial moisture content of mushroom of different cultures are presented in

Table 22. Determination of moisture content of the fruitbodies of recombined varieties of mushroom.

Recombined cultures	Fresh wt. of mushroom(g)	Moisture content(g)	% of moisture
PS ₃ x 18	72.0	65.65	91.18
PS ₃ x 27	96.0	86.46	90.06
PS ₉ x 35	65.0	59.65	91.77
PS ₉ x 59	97.0	89.37	92.13
PS ₁₈ x 46	85.0	76.60	90.12
PS ₁₈ x 64	40.0	36.20	90.50
PS ₂₃ x 35	50.0	44.46	88.92
PS ₂₃ x 59	50.0	46.20	92.40
PS ₂₇ x 46	60.0	53.89	89.82
PS ₂₇ x 64	71.0	65.90	92.82
PS ₉ x W	50.0	43.80	87.60
PS ₂₇ x W	69.0	61.80	89.56
PS ₅₉ x W	70.0	63.60	90.86
PS _(Wild)	60.0	54.52	90.87

Table 23, Fig. 15. Values of protein percentage were transformed into angular values and statistically analysed following CRD method.

The result shows that protein content in four of the recombined cultures, $PS_{27 \times 46}$, $PS_{18 \times 46}$, $PS_9 \times 59$ and $PS_{18 \times 64}$, were significantly higher than that of the other cultures and the wild culture as well. $PS_{27 \times 46}$ (37.5%) proved superior to all other cultures followed by $PS_{18 \times 46}$ (30.56%), $PS_9 \times 59$ (29.25%) and $PS_{18 \times 64}$ (28.81%). The remainders of the list were with protein value running between 24.31% to 26.25% and very close to the value obtained in case of wild culture.

Table 23. Analysis of protein content in the mushroom of recombined cultures.

Recombined cultures	Initial moisture content (%)	N % in mushroom samples (D.W.)	Crude protein content (%) (N x 6.25)
PS ₃ x 18	91.18	3.85	24.06 (29.37)
PS ₃ x 27	90.06	4.20	26.25 (30.82)
PS ₉ x 35	91.77	3.89	24.31 (29.54)
PS ₉ x 59	92.13	4.68	29.25 (32.74)
PS ₁₈ x 46	90.12	4.89	30.56 (33.56)
PS ₁₈ x 64	90.50	4.61	28.81 (32.46)
PS ₂₃ x 35	88.92	4.02	25.12 (30.08)
PS ₂₃ x 59	91.40	3.90	24.37 (29.58)
PS ₂₇ x 46	89.82	6.00	37.50 (37.76)
PS ₂₇ x 64	92.82	3.95	24.69 (29.79)
PS ₉ x W	87.60	3.99	24.94 (29.96)
PS ₂₇ x W	89.56	4.00	25.00 (30.00)
PS ₅₉ x W	90.86	4.09	25.56 (30.37)
PS _W	90.87	4.05	25.31 (30.20)
CD(0.05)			0.03 (0.95)

* Values in parentheses indicates angular transformed values.

A = PS ₃ x18	B = PS ₃ x27	C = PS ₉ x35	D = PS ₉ x59	E = PS ₁₈ x46
F = PS ₁₈ x64	G = PS ₂₃ x35	H = PS ₂₃ x59	I = PS ₂₇ x46	J = PS ₂₇ x64
K = PS ₉ xW	L = PS ₂₇ xW	M = PS ₅₉ xW	W = PS WILD	

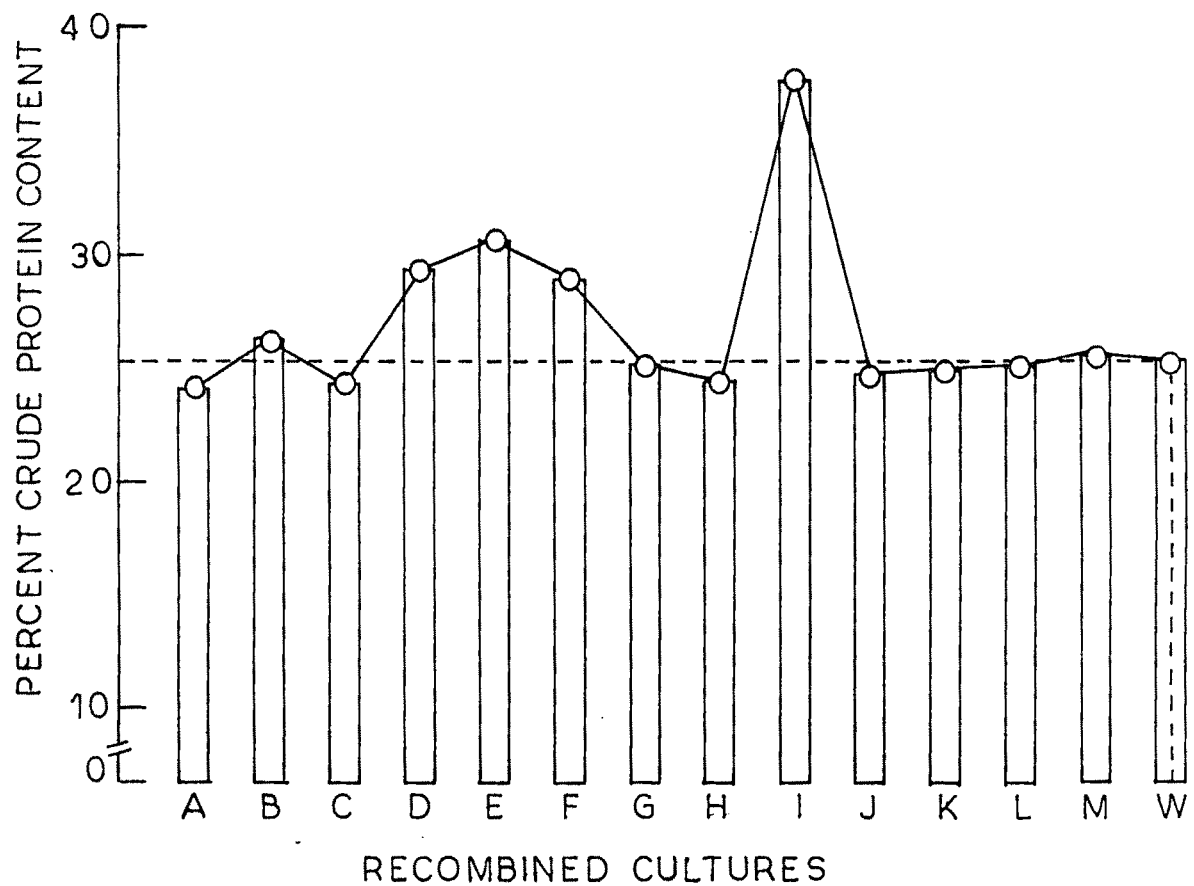


Fig.15: Analysis of protein content in the mushroom of recombined cultures.

R E S U L T S - II

A. Selection of best ecological variant of *Pleurotus citrinopileatus* through cultivation trial.

In course of a recent survey of mushroom in different localities at Kalimpong sub-division of Darjeeling district of West Bengal during the summer months (May-October) of 1984 and 1985, a species of *Pleurotus* was found to grow wildly on the decaying wood logs of alder (*Alnus nepalensis*) and walnut (*Juglans regia*). Occasional rain and swampy weather favoured the mushroom to come up for a long period of time. Fruitbodies collected in different times and from different localities were isolated and mycelial cultures were raised so as to get the best ecological variants of that particular species. The isolates thus obtained were domesticated, identified and found to belong to the same species of *Pleurotus citrinopileatus*. Major agronomic characters of the isolates were challenged by growing condition. A comparative data on spawn run and yield along with pileus colour and maximum possible temperature of fructification is presented in Table 24.

From the comparative data, no remarkable variation among them was traced out although minor deviation observed in some of the isolates. Quality characters particularly pileus colour was uniformly creamy white. Spawn run period in all the isolates found more or less similar but yield in isolates 1, 6 and 7

was slightly lower than that of the others. Isolates 3 and 5 were comparatively more temperature sensitive than the rests. Considering all the parameters, isolate 4 was sorted out as the best cultural type of the newly introduced species. This isolate was used in the succeeding experiments side by side with the well established tropical oyster mushroom Pleurotus sajor-caju, which was used as a check.

Table 24. Selection of best ecological variant of P.citrinopileatus through cultivation trial.

Isolates	Spawn run period (days)	Yield per 75 g dry straw (g)	Pileus colour	Maximum tempt.of fructifi- cation(°C)
Isolate-1	15	68	Creamy white	32
Isolate-2	16	72	"	32
Isolate-3	16	70	"	30
Isolate-4	14	78	"	32
Isolate-5	16	75	"	30
Isolate-6	15	65	"	32
Isolate-7	15	68	"	32

B. Studies on the comparative radial growth of P.citrinopileatus on semisolid media.

Fungal growth behaviour on semisolid media gives an idea about the mode of growth of the fungi in culture and to some extent the efficiency of colonisation in organic substrate. To

find out the growth pattern in Pleurotus citrinopileatus, a newly introduced mushroom fungus, poured petriplates with Potato-Dextrose-Agar (PDA) medium were inoculated with mycelial culture of the same. To compare the growth pattern of P.citrinopileatus with the well established tropical variety of mushroom Pleurotus sajor-caju, the later was considered as a check in the following experiment. After ten days of incubation at 25°C diameter of growth of the fungi was measured and the area of the region occupied by the growth was calculated. Growth response in the two fungi is represented in Table 25. Areas of growth covered by the fungi was statistically analysed following RBD method.

Table 25. Studies on the comparative radial growth of P.citrinopileatus on semisolid media.

Mushroom species	Diameter of growth (cm)	Area of growth(πr^2) (cm ²)	Culture morphology
<u>P.citrinopileatus</u>	11.60	105.68	Fungus with less white, less fluffy mycelial growth
<u>P.sajor-caju</u>	11.48	103.51	Fungus with densely clustered, white, fluffy mycelial growth
CD(0.05)		NS	

The result shows that P.citrinopileatus is a fast growing mushroom fungus with growth behaviour very akin to P.sajor-caju.

Regarding the area of radial growth covered by the mycelia in 10 days, P.citrinopileatus with 105.68 sq.cm area though slightly emerged over the value of 103.51 sq.cm in P.sajor-caju still the variation is not statistically significant.

C. Studies on the temperature tolerance of P.citrinopileatus compared to P. sajor-caju.

(a) Effect of temperature on radial growth :

Effect of temperature on the growth of fungi can be determined by comparing its growth at a particular temperature in relation to its optimum growth level. To determine the maximum temperature tolerance of Pleurotus citrinopileatus, young mycelial cultures were exposed to the rising temperatures from 20°C to as high as 38°C till increase in radial growth became totally ceased out. The experiment was divided into the 'initiation phase' followed by 'treatment phase'. At the initiation phase poured petriplates with PDA medium were inoculated with the mycelial cultures and were incubated at 25°C for 5 days. Mycelial growth after the initiation phase was measured and demarkated from the lower surface of petriplates with glass marker along the extreme margin of growth area. Petriplates with young mycelial growth were kept separately at the prefixed temperatures of 20°, 25°, 30°, 34°, 36° and 38°C, for 5 days. After the treatment phase total area of growth was measured and net increase in growth at different temperatures was calculated by subtracting initial growth area from the total area occupied and represented in Table 26, Fig. 16. Growth response in terms

of area of growth at the increasing temperatures has been statistically analysed by "Factorial RBD" method.

Table 26. Effect of temperature on radial growth of P.citrinopileatus and P.sajor-caju

Mush-room species	'INITIA-TION PHASE' (5 days)	'TREATMENT PHASE' (5 days after initiation phase)					
		Mean area of growth at different degrees of temperature					
	Mean area of growth (cm ²)	20°C	25°C	30°C	34°C	36°C	38°C
		Net area (cm ²)	Net area (cm ²)	Net area (cm ²)	Net area (cm ²)	Net area (cm ²)	Net area (cm ²)
<u>P.citrinopileatus</u>	33.18	60.13	71.41	73.05	55.07	9.11	Nil
<u>P.sajor-caju</u>	32.77	61.22	69.30	65.75	42.97	3.97	Nil
CD(0.05)	For Temperatures						4.07
CD(0.05)	For Mushroom species						2.57
CD(0.05)	For Temperature x Mushroom species						5.75

Increase or decrease in temperature from an optimum level was found to provide adverse effect on the growth of fungi. In P.citrinopileatus optimum growth (71.41 and 73.05 sq.cm) was observed between 25° and 30°C. Although, growth at 20°C was marginally reduced, significant reduction took place at 34°C and 36°C. In P.sajor-caju similar adversity observed in low or high temperature treatments. Here also optimum growth observed

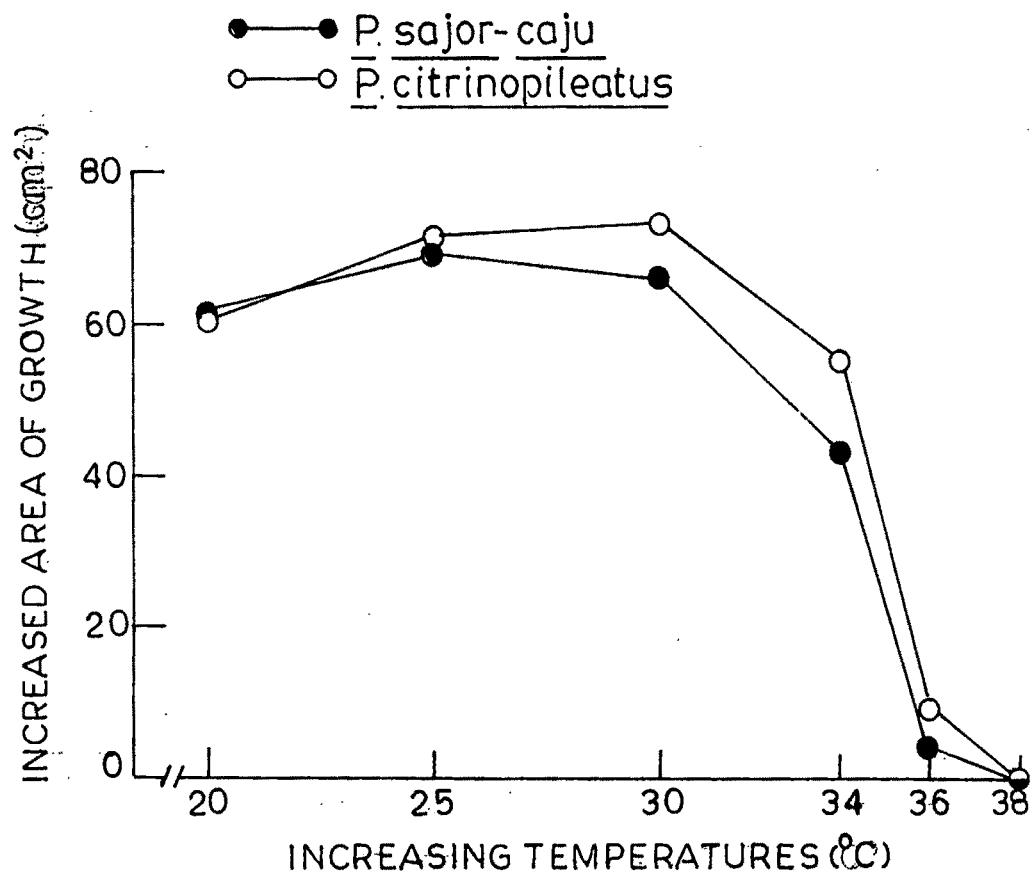


Fig.16: Effect of temperature on radial growth of P. citrinopileatus and P. sajor-caju.

between 25° and 30°C. Regarding variation of growth among the two species no significant difference observed at 20° and 25°C but above that temperature P.citrinopileatus showed significantly higher growth than that of P.sajor-caju. As the interaction effect of species with temperature, P.citrinopileatus was found to exert its best performance at 30°C (73.05 sq.cm), a similar effect was exhibited in both the species at 25°C. Maximum adversity showed by P.sajor-caju at 36°C temperature.

(b) Effect of temperature on the growth in liquid culture :

To determine the temperature tolerance of Pleurotus citrinopileatus in terms of production of mycelial mass in liquid culture, inoculated Potato-Dextrose (PD) broth media were incubated at the increasing temperatures of 20° to 36°C. The experiment was divided into (i) initiation phase, and (ii) treatment phase. At the initiation phase inoculated cultures were incubated at the optimum temperature of 25°C for 5 days. One set of replicates after the initiation phase was harvested and the weight of dry mycelial mass was recorded. Remainder of the replicates were exposed to the temperatures of 20°, 25°, 30°, 34° and 36°C for 5 days. After the treatment phase mycelial mass for each culture was harvested and recorded as in Table 27, Fig. 17. Production of net mycelial mass was calculated by subtracting the value of initiation phase from total production at different temperatures. The values were analysed following "Factorial RBD" method.

Table 27. Effect of temperature on the growth in liquid culture of P.citrinopileatus and P.sajor-caju.

Mushroom species	'INITIA-TION PHASE' (5 days) Mean wt. of dry mycelial mass (mg)	'TREATMENT PHASE' (5 days after initiation phase)				
		Mean weight of dry mycelial mass (mg) at different degrees of temperature				
		20°C	25°C	30°C	34°C	36°C
		Net wt.	Net wt.	Net wt.	Net wt.	Net wt.
<u>P.citrino-pileatus</u>	62.16	178.20	209.88	205.5	149.00	-0.83
<u>P.sajor-caju</u>	63.25	169.42	212.50	186.9	123.25	-1.00
CD(0.05)	For Temperatures					6.06
CD(0.05)	For Mushroom species					4.28
CD(0.05)	For Temperature x Mushroom species					8.56

From the observations it is clear that both the species gave maximum growth at 25°C. At 36°C growth reached its negative value. Variation in growth and production of mycelial mass between 20° and 34°C was statistically analysed following "Factorial RBD" method. In P.citrinopileatus optimum growth was obtained between 25° and 30°C, below or above that temperature growth was significantly reduced. In P.sajor-caju 25°C temperature was proved most favourable with detrimental effect expressed in all other cases. Comparing temperature tolerance

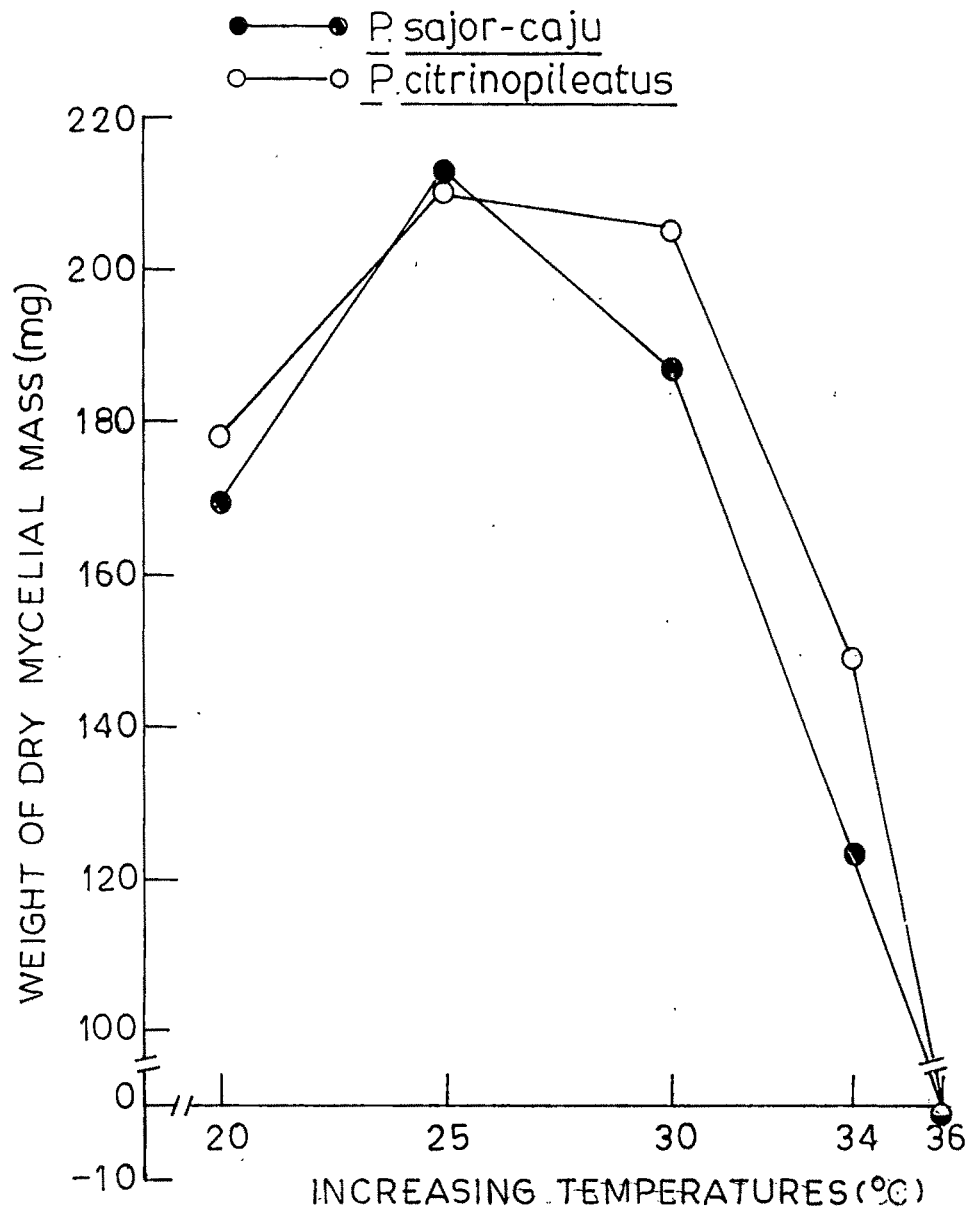


Fig.17: Effect of temperature on the growth in liquid culture of P. citrinopileatus and P. sajor-caju.

among the two species similar response found at 25°C but in all other temperatures between 20° and 34°C, P.citrinopileatus produced higher amount of mycelial mass than that of P.sajor-caju. Regarding best interaction effect of species with temperature, growth response of P.sajor-caju at 25°C and of P.citrinopileatus at 25° and 30°C were statistically at par. P.sajor-caju gave minimum growth at 34°C.

D. Studies on the effect of rising temperatures on the spawn run period of P.citrinopileatus :

Temperature may be considered as the most important and determining factor for growth and fructification of mushroom fungi. Period of spawn run and crop earliness varies largely with the changes in prevailing incubation temperature. To determine the effect of rising temperature on the spawn run of Pleurotus citrinopileatus, sterilized straw substrate was inoculated with 3% wheat grain spawn and the inoculated substrate was kept for incubation at different temperatures of 20°, 25°, 30°, 32° and 34°C. Time required for complete colonisation of the substrate at different temperatures was carefully observed and recorded in Table 28.

The represented data shows that optimum temperature for colonisation of P.citrinopileatus lay between 25°-30°C and minimum spawn run period obtained was 15 days. At 32°C spawn run period increased to 16 days but increase in temperature to 34°C further extended the spawn run period to 19 days. On the other hand decrease in temperature from the optimum level

(25 - 30°C) equally affects colonisation and as a result spawn run period became lengthened. In P.sajor-caju optimum spawn run period varied between 16-17 days. Colonisation of P.sajor-caju could not keep space with the increasing temperatures compared to P.citrinopileatus. It needed maximum of 21 days time for colonisation at 32°C^{but} at 34°C growth underwent degeneration. After all P.sajor-caju may be considered as less efficient coloniser and more temperature sensitive than P.citrinopileatus as it had taken much time to colonise at each temperature and temperature beyond 32°C severely affected its growth.

Table 28. Studies on the effect of rising temperatures on the spawn run period of P.citrinopileatus.

Mushroom species	Period of spawn run (days) at different temperatures				
	20°C	25°C	30°C	32°C	34°C
<u>P.citrinopileatus</u>	18	15	15	16	19
<u>P.sajor-caju</u>	20	16	17	21	-

E. Effect of temperature on growth and yield of P.citrinopileatus :

By a thorough review on the effect of temperature on growth of mushroom fungi it can be recommended that optimum temperature requirement for the vegetative growth and fructification may be different. Pleurotus citrinopileatus was found to

maintain its optimum mycelial growth between 25-30°C with a substantial amount of growth even at 34°C. To determine the range of temperature suitable for fructification, unsterilized straw substrate inoculated with 3% wheat grain spawn was placed for incubation at the temperatures of 20°, 25°, 30°, 32° and 34°C. For each temperature spawn run period, number of buds, number of fruitbodies and yield was observed for two consecutive flushes. Response of growth and fructification to different temperatures is presented in Table 29.

P.citrinopileatus produced optimum growth between 25-30°C with minimum spawn run period of 19 and 9 days before first and second flushes respectively. But it gave maximum yield (516 g) at 25°C with slight reduction at 30°C (490 g). Increase or decrease in temperature beyond the optimum range of 25°-30°C led to extend the spawn run period with substantial decrease in yield also. In case of P.sajor-caju optimum growth was observed at 25°C, with decrease in temperature to 20°C spawn run period was lengthened but increase in temperature to 30°C spawn run took place with optimum rapidity. Interestingly, when spawn run was done at 30°C it could not go to fructify. A maximum yield of 425 g was obtained at 25°C compared to 372 g at 20°C temperature.

F. Studies on the yield behaviour of P.citrinopileatus in different methods of cultivation :

Beside physical and nutritional factors, yield efficiency of a mushroom species largely depends on a suitable method of

Table 29. Effect of temperature on growth and yield of Pleurotus citrinopileatus

Incuba- tion tempera- ture(°C)	<u>P. citrinopileatus</u>				<u>P. sajor-caju</u>			
	Spawn run period (days)	No. of buds	No. of fruit- bodies	Yield (g)	Spawn run period (days)	No. of buds	No. of fruit- bodies	Yield (g)
20	21,10	146+105 = 251	109+88 = 197	240+144 = 384	22,11	109+56 = 165	62+29 = 91	268+104 = 372
25	19,9	215+95 = 310	198+89 = 287	362+154 = 516	20,10	151+52 = 203	79+28 = 107	320+105 = 425
30	19,9	265+76 = 341	247+60 = 307	372+118 = 490	19,-	-	-	-
32	22,11	219+82 = 301	191+69 = 260	316+102 = 418	-	-	-	-
34	24, -	-	-	-	-	-	-	-

cultivation. To determine the yield behaviour of Pleurotus citrinopileatus compared to Pleurotus sajor-caju and to find out a suitable method of cultivation it was subjected to the different methods of cultivation as - (i) cultivation in nylon net and (ii) cultivation in polythene bag using unsterilized straw as substrate. Optimum physical conditions were maintained throughout the experiment. Observations in response to spawn-run period, number of fruitbodies and yield in first and second flushes are represented in Table 30, Fig.18. Yield data has been statistically analysed following "Randomised Block Design".

Table 30. Studies on the yield behaviour of P.citrinopileatus in different methods of cultivation.

Methods of cultivation	Spawn run period (days)		No. of fruit bodies		Yield (g.)	
	P.C. ¹	P.S. ²	P.C.	P.S.	P.C.	P.S.
Cultivation in nylon net	18,10	20,10	152+87 = 239	69+32 = 101	326+176 = 502	293+112 = 405
Cultivation in polythene bag	19,10	20,9	153+77 = 230	63+31 = 94	321+175 = 496	304+91 = 395
CD(0.05)	For methods of cultivation					NS
CD(0.05)	For mushroom species					20.69
CD(0.05)	For method of cultivation x mushroom species.					NS

1 = P.C. = Pleurotus citrinopileatus

2 = P.S. = Pleurotus sajor-caju

(a)



(b)



Fig.18. Pleurotus citrinopileatus on flushes in different methods of cultivation.

(a) Cultivation in nylon net.

(b) Cultivation in polythene bag.

Result shows that spawn run period in both the species of mushroom was more or less similar with a marginal tendency of crop earliness observed in P.citrinopileatus. Also, number of fruitbodies obtained in the two methods of cultivation in P.citrinopileatus (239 and 230) were much higher compared to P.sajor-caju (101 and 94).

Regarding yield no remarkable variation was found in any of the species due to change in methods of cultivation. Considering yield efficiency, P.citrinopileatus produced substantially higher yield (502 g and 496 g) in both of the two methods of cultivation compared to P.sajor-caju with 405 g and 395 g of yield, respectively.

G. Yield behaviour of P.citrinopileatus in different flushes:

To study the efficiency of production of mushroom in Pleurotus citrinopileatus compared to Pleurotus sajor-caju, a fixed amount of unsterilized straw substrate (600 g dry wt.) was inoculated with the mushroom fungi separately and allowed to grow under optimum physical conditions. On completion of spawn run the fungi came to produce several flushes of mushroom in a definite sequential order till the substrate became totally exhausted. Behaviour of both the mushroom fungi in different flushes on points of number of buds, number of fruitbodies and yield was recorded separately. Calculated substrate : Yield ratio along with other information are presented in Table 31. Yield data was statistically analysed by "Randomised Block Design".

Table 31. Yield behaviour of P.citrinopileatus in different flushes

Flushes of mushroom	No. of buds		No. of fruitbodies		% bud mortality		Yield (g)	
	PC	PS	PC	PS	PC	PS	PC	PS
1st flush	195	149	172	72	11.79	51.67	292	270
2nd flush	130	74	114	39	12.30	47.29	181	119
3rd flush	63	32	57	18	9.52	43.75	86	57
4th flush	52	Nil	46	Nil	11.53	-	66	Nil
Total/Mean*	440	255	389	129	11.59*	49.41*	625	446
CD(0.05)								45.9
Substrate : Yield ratio, PC = 1 : 1.04								
PS = 1 : 0.74								

From the overall performance P.citrinopileatus may be considered as an efficient producer of mushroom because, the yield of this mushroom in each flush lay well above the value of P.sajor-caju. Moreover, it produced substantial amount of mushroom even in fourth flush as against mere three flushes of mushroom in P.sajor-caju. From a definite amount of substrate (600 g dry straw) P.citrinopileatus produced a total of 625 g of mushroom as against the yield of 446 g in P.sajor-caju and the difference is highly significant. Again, P.citrinopileatus produced sufficiently higher number of buds and due

to very low percentage of bud mortality (11.59%) most of them developed into fruitbodies. Conversely, P.sajor-caju produced lesser number of buds and due to high bud mortality (49.41%), the fewer that survived developed into large fruitbodies. Regarding substrate (D.W.) : Yield ratio, P.citrinopileatus governed the value of 1 : 1.04 as against the value of 1 : 0.74 in P.sajor-caju.

H. Assessment of fruiting behaviour and qualitative characters of mushroom of P.citrinopileatus

Commercial importance of a mushroom species largely depends on the qualitative characters of mushroom beside its yield behaviour. Qualitative characters of Pleurotus citrinopileatus, a newly reported mushroom species has been challenged side by side with well established tropical variety of mushroom Pleurotus sajour-caju. The experiment was done on the basis of observations taken in the foregoing experiments. A comparative account of findings of the two species of mushroom is presented in Table 32.

P.citrinopileatus produced large number of fruitbodies distributed throughout the open surface of the colonised substrate. But in P.sajor-caju lesser number of fruitbodies were formed and found to be sparsely distributed or localised in large patches. Average fruitbody weight in P.sajor-caju was greater than P.citrinopileatus. In the former species fruitbody weight varied from 3-5 g or more compared to 2-3 g in later species. Stipe length in both the species was found to

be more or less similar. Most striking qualitative difference in these two species was marked to be the fruitbody colour. In P.citrinopileatus it appeared creamy white in colour compared to whitish brown colour of the fruitbody in P.sajor-caju. Both the species were found to be similar on the characters of entire pilear margin and flavour of the fruitbodies. But thickness of the fruitbody was lesser in P.citrinopileatus than that of P.sajor-caju.

Table 32. Assessment of fruiting behaviour and qualitative characters of mushroom of P.citrinopileatus

Mushroom species	Distribution of fruit-bodies	Average wt. of fruit bodies (g)	Stipe length (cm)	Pileus colour	Pilear margin	Flavour (F)	Thickness (T)
<u>P.citrinopileatus</u>	Uniformly distributed throughout the surface	2-3	1.0-3.5	Creamy white	Entire	F	T-
<u>P.sajor-caju</u>	Scattered in large patches	3-5	1.25-3.00	Whitish brown	Entire	F	T

I. Adaptive trial of cultivation of P.citrinopileatus in different districts of West Bengal in different seasons of the year

Beside other physical factors temperature may be considered as most important one to determine the feasibility of mushroom cultivation in natural condition during particular seasons of the year. Prevailing climatic temperatures in different seasons were

found to vary in different agro-climatic regions of West Bengal. Pleurotus citrinopileatus showed its ability to withstand considerably higher temperature of around 34°C. To determine the growing excellence in varying environmental conditions, it was subjected to an adaptive trial of cultivation in a selected area of each agro-climatic region. Total period of the year was divided conveniently into (i) Pre-Winter (July to October), (ii) Winter (November to mid-March) and (iii) Post-Winter (mid-March to June) Phases. Spawn run period of yield of mushroom grown at the different regions of West Bengal and in different phases of the year was recorded. The performance as observed is represented in Table 33.

The result shows that North-Gangetic plain is best suited for cultivation of P.citrinopileatus round the year with optimum performance of spawn run and yield during winter phase. The overall picture, in South Gangetic plain, Lateritic tracts and Coastal area, on the points of spawn run period and yield looked more or less similar. Here also shorter spawn run period and greater yield was obtained during winter phase rather than that of pre-winter phase. In all the three regions, cultivation during post-winter phase proved abortive. A somewhat different picture was witnessed in case of Hilly tracts where pre-winter and post-winter phases proved more or less equally effective for its cultivation with a spawn run period of 17 days and maximum yield of 634 g during post-winter phase compared to 18 days spawn run period and 592 g yield in pre-winter phase. But in winter phase remarkable fall in temperature severely affected the

mycelial growth leading to the total crop failure during this phase of cultivation.

Table 33. Adaptive trial of cultivation of P.citrinopileatus in different districts of West Bengal in different seasons of the year

Agro-climatic regions of West Bengal	Periods of cultivation					
	Pre-Winter (July-October)		Winter (Nov.-Mid-March)		Post-Winter (Mid.March-June)	
	Spawn	Yield	Spawn	Yield	Spawn	Yield
	run period (days)	(g)	run period (days)	(g)	run period (days)	(g)
Hilly tracts (Kalimpong)	18	592	-	-	17	634
North Gangetic Plain (Siliguri)	20	498	18	610	19	529
South Gangetic Plain (Kalyani)	20	449	17	625	-	-
Lateritic tracts (Jhargram)	21	456	18	584	-	-
Coastal area (Gosaba)	20	468	17	622	-	-

J. Variation in protein content of mushroom at different stages of harvest

For the estimation of protein content in the fruitbodies of Pleurotus citrinopileatus and Pleurotus sajor-caju and to determine its variation in different stages of development,

mushroom samples of respective stages were analysed. Mushrooms grown on straw substrate were collected at three developmental stages as (i) pre-mature (bud stage), (ii) right stage of harvest and (iii) over-mature stage. Samples dried to constant weight were analysed for its total nitrogen content by micro-Kjeldahl method. The estimated values of nitrogen percentage were multiplied by the conversion factor of 4.38 (i.e. $N \times 4.38$) to get the corrected protein fraction in the samples. Initial moisture content of the mushrooms along with analytical report of nitrogen percentage and protein fraction are represented in Table 34, Fig. 19. Values of protein percentage were converted to angular transformed values and statistically analysed by CRD method.

The result shows that moisture content at different stages of harvest in both the species lay around 90% with marginal reduction in bud stage. In P.sajor-caju percentage moisture at bud stage was 88.49% compared to 89.07% in P.citrinopileatus. According to the analytical report protein fraction varied significantly at different stages of development. Of both the species in common, protein content at the bud stage remained too high than those of the following stages. P.citrinopileatus with 24.88% protein was maximum at the bud stage which was reduced to 16.86% at the right stage of harvest and reached the minimum of 13.01% in over mature stage. Similarly, P.sajor-caju contained 26.45%, 17.74% and 13.58% protein in succeeding stages of development. Although protein content in both the species bore close

similarity still the values in P.sajor-caju at bud stage and right stage of harvest remained higher than those of P.citri-nopileatus. But at the over mature stage difference was not significant.

Table 34. Variation in protein content of mushroom at different stages of harvest

Stages of harvest	Moisture content (%)		Nitrogen (%)		Protein (%)	
	PC ¹	PS ²	PC	PS	PC	PS
Bud stage	89.07	88.49	5.68	6.04	24.88 (29.92)	26.45 (30.95)
Right stage of harvest	90.22	90.75	3.85	4.05	16.86 (24.24)	17.74 (24.90)
Over mature stage	89.88	90.76	2.97	3.10	13.01 (21.14)	13.58 (21.62)
CD(0.05)						0.02 (0.74)

1 = PC = Pleurotus citrinopileatus

2 = PS = Pleurotus sajor-caju

* Values in the parentheses are angular transformed values.

K. Proximate nutritive composition of the mushroom :

To determine the proximate composition of mushrooms of P.citrinopileatus and P.sajor-caju, fruitbodies at the right stage of harvest were collected and dried to constant weight. Protein, fat and carbohydrate contents were estimated and the

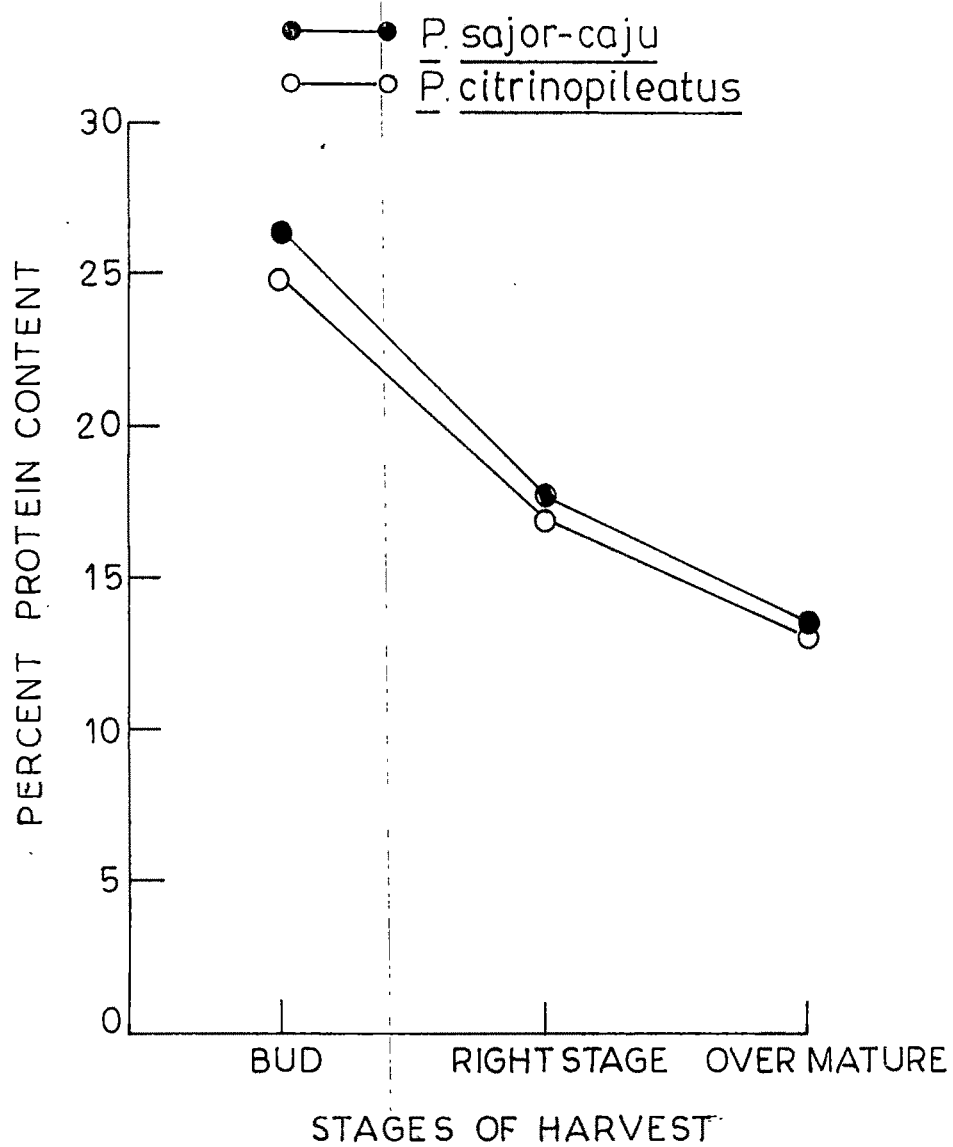


Fig.19: Variation in protein content of mushroom at different stages of harvest.

values obtained on dry weight basis were recorded. Carbohydrate, fat and protein, as a whole, acts as the source of energy that are interchangeable in terms of energy according to their specific chemical compositions. Atwater conversion factors (FAO, 1973) used to calculate the energy values of food from protein fat and carbohydrate have been modified to 2.62, 8.37 and 3.48 respectively to calculate the energy value of mushrooms (Crisan and Sands, 1978). Based on this principle energy values of P.citrinopileatus and P.sajor-caju have been calculated from its proximate composition and are presented in Table 35.

Table 35. Proximate nutritive composition of the mushroom.

Mushroom species	Initial moisture content (%)	Proximate composition (Dry wt.) (%)			Energy value (K cal)
		Protein	Fat	Carbohydrate	
<u>P.citrinopileatus</u>	90.22	16.86	5.8	50.55	268.63
<u>P.sajor-caju</u>	90.75	17.34	3.9	49.15	249.12

The result shows that proximate composition in both the species is more or less similar but the notable difference in fat content. P.citrinopileatus is with higher fat content (5.8%) than that of P.sajor-caju (3.9%). Calculated energy value of the former species is slightly higher (268.63 K cal) than the later one (249.12 K cal).

L. Estimation of mineral elements in the fruitbodies of *P.citrinopileatus*

Fruitbodies of mushroom fungi is considered as the rich source of mineral elements and is found to contain every minerals available in the substrate. To quantify the availability of essential elements in *Pleurotus citrinopileatus* as well as *Pleurotus sajor-caju*, ground dried samples of the mushroom, grown on paddy straw, were analysed using atomic absorption spectrophotometer. The calculated values of essential elements along with heavy metals in terms of milligram per 100 gram sample or in ppm, are produced in Table 36.

Table 36. Estimation of mineral elements in the fruitbodies of *P.citrinopileatus*

Mushroom species	MINERAL ELEMENTS								
	Essential elements (mg/100 g dry samples)							Heavy metals (ppm)	
	K	P	Na	Mg	Ca	Fe	Mn	Cu	Zn
<u><i>P.citri-</i></u> <u><i>nopilea-</i></u> <u><i>tus</i></u>	7020	1650	620	62	31	11.5	5.6	210	72
<u><i>P.sajor-</i></u> <u><i>caju</i></u>	6280	1490	290	65	28	8.9	6.1	210	66

From the available data it is apparent that potassium and phosphorus were the major minerals in both the mushrooms.

P.citrinopileatus contain 7020 and 1650 mg potassium and phosphorus respectively compared to 6280 and 1490 mg potassium and

phosphorus in P.sajor-caju. Sodium and magnesium contents in P.citrinopileatus were arranged in decreasing degrees of 620 and 62 mg in contrast to 290 and 65 mg in P.sajor-caju. Unlike other elements, calcium, iron and manganese were found to be present in similarly low concentrations in both the species of mushroom. Among the heavy metals copper and zinc were analysed and found to vary within the prescribed limits of FPO and PFA Act. Copper content in both the species were 210 ppm (dry wt. basis) as against maximum limit of 30 ppm on wet weight basis ($\equiv$ 300 ppm on dry wt. basis) prescribed for fruit products. Regarding zinc, both P.citrinopileatus and P.sajor-caju displayed far less concentration than the specific limits of PFA (1954), FPO (1955) and rules thereof for dried or dehydrated vegetables. The two species contained 72 and 66 ppm Zn (dry wt.basis) respectively against the maximum tolerance limit of 50 ppm (wet wt. basis $\equiv$ 500 ppm on dry wt. basis).

M. Animal feeding trial on mushroom diets

(a) Effect of mushroom diet on the body weight of test animals:

Mushrooms are well recognised as protein rich vegetables and appreciated as with protein based food items. Protein content in mushroom was found to vary with the growing condition, substrate utilized and species as well. By estimating the protein content alone it is not possible to get the idea of actual protein fraction and its digestive and nutritive properties. Beside protein fraction, overall digestibility and cause of

health hazard, if any, can be well justified through an animal feeding study. To evaluate the dietary significance of the newly introduced mushroom species, Pleurotus citrinopileatus, a feeding trial was conducted using young white mice of Swiss-strain as test animal. Increased body weight of test animals after the total diet phase, was statistically analysed following CRD method. An overall picture of the experimental trial is presented in Table 37, Fig. 20.

Table 37. Effect of mushroom diet on the body weight of test animals

Diet group	Initial body weight per mouse (g)	ADAPTIVE PHASE	MIXED DIET PHASE	TOTAL DIET PHASE (20 days)		
		(10 days)	(10 days)	Increased body weight per mouse (g)		
		Increased body wt. per mouse (g)	Increased body wt. per mouse (g)	10 days	20 days	Total
PC-group	13.86	3.89	0.89	2.41	2.85	5.26
PS-group	14.11	3.93	1.07	3.00	2.40	5.40
Normal group	14.10	3.85	2.60	2.60	2.51	5.11
CD(0.05)						NS

The result shows that net increase in body weight of individual animal in each feed group after adaptive phase was uniform and lay between 3.85 to 3.93 g. A sudden fall in the body weight was observed in the succeeding mixed diet phase with a

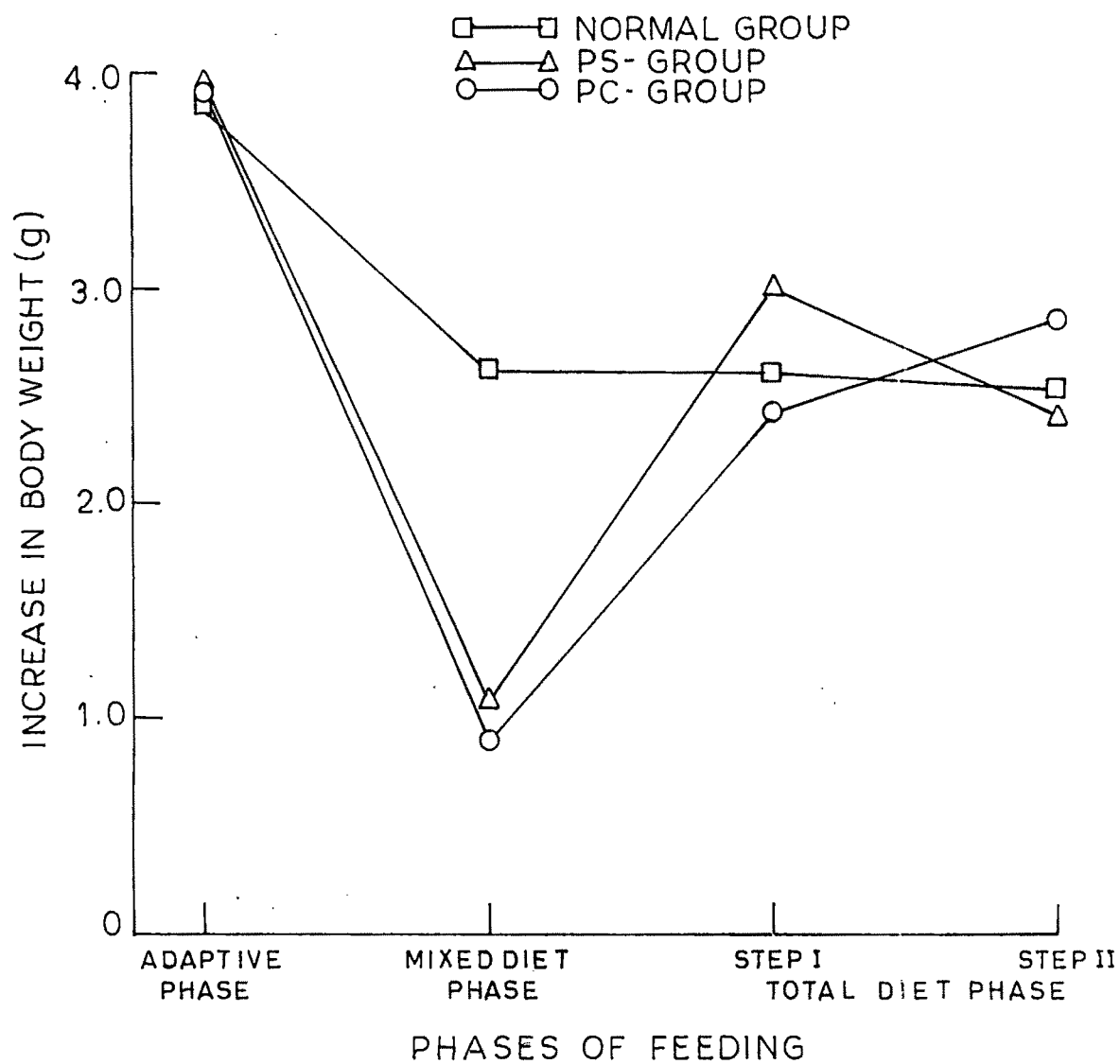


Fig.20:Effect of mushroom diet on the body weight of test animals.

mere 0.89 g and 1.07 g increase in body weight per mouse in PC and PS diet group respectively compared to 2.6 g in Normal diet group. After first 10 days of total diet phase increase in body weight was recovered with the maximum of 3 g per mouse in PS-group followed by 2.41 g in PC-group against 2.6 g in Normal group. In the next 10 days PC-group showed maximum increase in body weight of 2.85 g while Normal group and PS-group were with a decreasing order of 2.51 g and 2.40 g respectively. A sum of the increase in body weight after the 20 days of total diet phase it reached the maximum of 5.4 g per mouse in PS-group followed by marginal reduction to 5.26 g in PC-group and the minimum of 5.11 g in Normal group. The difference in body weight obtained in animals of different diet groups was statistically non-significant.

(b) Determination of Protein Efficiency Ratio (PER) in relation to growth of test animals fed on mushroom and normal diet :

In the conducted experiment of animal feeding study, the diets considered were with a constant of 10% protein irrespective of the other common additives. In case of Normal diet, protein was supplied in the form of spray dried milk powder. But in mushroom diets milk protein was replaced with equivalent amount of protein in ground dried mushroom powder. After the feeding trial, total gain in body weight per mouse against the total protein intake was determined. Protein Efficiency Ratio (PER) for each diet group was calculated therefrom following the method prescribed by Chapman et al. (1959). PER values obtained

in different diet groups were transformed into its angular values and statistically analysed following CRD method. The result obtained is furnished in Table 38.

Table 38. Determination of Protein Efficiency Ratio in relation to growth of test animals fed on mushroom and Normal diet

Diet group	% protein in diet supplied (D.W.)	Total food intake (g)	Total protein intake (g)	Gain/loss in body weight(g)	PER
PC-group	10	63.78	6.38	5.26	0.82 (64.90)
PS-group	10	75.35	7.53	5.40	0.72 (58.05)
Normal group	10	82.85	8.28	5.11	0.62 (51.94)
CD(0.05)					0.002 (2.55)

* Values in the parentheses are angular transformed values of the mean values.

An overview of the represented data indicates a good correlation between increase in body weight with protein intake by the test animals. PC-group showed the maximum PER of 0.82 and is statistically superior compared to 0.72 and 0.62 in PS-group and Normal group respectively. Increased PER in PC-group means relatively greater increase in body weight in relation to the amount of protein utilized. In PC-group gain in body

weight was 5.26 g against 6.38 g protein intake whereas in Normal group gain in body weight was 5.11 g at the cost of 8.28 g protein. In PS-group 7.53 g protein led to 5.4 g increase in body weight.

(c) Determination of overall digestibility and apparent digestibility of mushroom diets :

During the total diet phase of feeding trial net amount of food consumed by each animal along with the amount of excreta was accounted. Also, nitrogen content in the food supplied as well as in the dry mass of excreta was analysed to see the amount of nitrogen assimilated by the test animals in the stipulated time of experimentation. From the available information overall digestibility and apparent digestibility of the diet supplied were calculated following the formulae as advanced by Vijaya Khader (1979).

$$\text{Overall diges-} \\ \text{tibility (\%)} = \frac{\text{Food intake(g)} - \text{Faeces excreted(g)}}{\text{Food intake (g)}} \times 100$$

$$\text{Apparent diges-} \\ \text{tibility (\%)} = \frac{\text{Nitrogen intake (g)} - \text{Nitrogen excre-} \\ \text{ted in faeces(g)}}{\text{Nitrogen intake (g)}} \times 100$$

Calculated values of overall digestibility and apparent digestibility were transformed into its angular values and statistically analysed by CRD method and represented in Table 39 and Table 40 respectively.

According to the presented data PS-group with 82.93% overall digestibility proved to be maximum followed by the values of 82.86% and 80.93% in Normal group and PC-group respectively. The variation obtained is not significant. Again, apparent digestibility obtained in PS-diet was 76.16% compared to 71.43% and 71.23% digestibility of Normal diet and PC-diet in sequence. The value obtained in case of PS-diet was significantly superior to both of the other groups.

Table 39. Determination of overall digestibility of mushroom diets.

Diet group	Total food intake(g)	Total amount of excreta (g)	Overall digestibility (%)
PC-group	63.78	12.16	80.93 (64.11)
PS-group	75.35	12.86	82.93 (65.60)
Normal group	82.85	14.20	82.86 (65.54)
CD(0.05)			NS

* Values in parentheses are angular transformed values of the mean values.

Table 40. Determination of apparent digestibility of mushroom diets.

Diet group	Food intake (g)	N% in food	Total N intake (g)	Total ex-creta (g)	N% in ex-creta	Total N ex-creted (g)	Apparent digestibility (%)
PC-group	63.78	2.29	1.46	12.16	3.44	0.42	71.23 (57.56)
PS-group	75.35	2.29	1.72	12.86	3.17	0.41	76.16 (60.77)
Normal group	82.85	2.29	1.89	14.20	3.83	0.54	71.43 (57.69)
CD(0.05)							0.025 (0.91)

* Values in parentheses are angular transformed values of the mean values.

N. Determination of nutritive value of the mushroom of *P.citri-*
nopileatus and *P.sajor-caju*

(a) Quantitative estimation of amino acid composition :

It is well documented that nutritional quality of a proteinaceous food depends on its amino acid composition in relation to its protein content and its digestibility. Amino acid composition may serve as a good relative measure for comparison of mushrooms or other food stuffs of established nutritive value. With a view to determine the nutritive value of *Pleurotus citrinopileatus* along with the matching standard of *Pleurotus*

sajor-caju, mushroom samples were analysed with the help of Technicon Sequential Multipurpose Amino Acid Analyser. Amino acid profiles of the mushroom species with quantitative data of essential and non-essential amino acids obtained are represented in the Table 41. The contents were expressed in terms of milligram amino acid per gram of corrected crude protein nitrogen.

According to the available data it is clear that all the essential amino acids except phenylalanine and threonine in P.sajor-caju is greater in amount than that of P.citrinopileatus. Of course it bears a marginal difference only. On the other hand P.citrinopileatus shows remarkably higher amount (460 mg/g) of phenylalanine compared to 363 mg/g in P.sajor-caju. Regarding threonine it is 286 mg/g against 282 mg/g in P.sajor-caju. Considering non-essential amino acids P.sajor-caju contains higher amount of arginine, aspartic acid, glutamic acid, proline and serine. Conversely, histidine, alanine and glycine content remain higher in P.citrinopileatus. Remarkably higher difference observed in cases of aspartic acid and glutamic acid.

(b) Estimation of Essential Amino Acid (EAA) Index based on whole egg and FAO-reference protein :

In absence of animal feeding studies or as a supporting evidence to feeding studies essential amino acid composition can be implicated in determining the nutritional value of various food items. Oser (1951, 1959) advanced the view of

Table 41. Quantitative estimation of amino acid composition of P.citrinopileatus and P.sajor-caju

Amino acids	Concentration of amino acid(mg/g corrected crude protein nitrogen)	
	<u>P.citrino-</u> <u>pileatus</u>	<u>P.sajor-</u> <u>caju</u>
Isoleucine	184	204
Leucine	334	349
Lysine	319	335
Methionine	100	119
Cystine	65	71
Phenylalanine	460	363
Tyrosine	178	208
Threonine	286	282
Tryptophan	nd	nd
Valine	239	278
Argenine	300	303
Histidine	126	120
Alanine	390	366
Aspartic acid	456	617
Glutamic acid	1113	1231
Glycine	275	268
Proline	249	299
Serine	267	310
Nitrogen content (D.W.)	3.85%	4.05%

nd = Not determined.

using Essential Amino Acid Index (EAA-Index) to rate the dietary protein in terms of the ratio of essential amino acids contained in a food to the essential amino acid content in reference protein. He prescribed the use of whole egg as reference protein in EAA calculations. Based on detailed analytical report, FAO (1973) described whole egg as with unnecessarily high nutritional standard and thus formulated a charter of essential amino acid pattern based on adult human dietary needs. In the following EAA calculations of Pleurotus citrinopileatus and Pleurotus sajor-caju both whole egg and FAO reference pattern were considered. The EAA calculations for the two species of mushroom are represented separately in Table 42 and Table 43.

From the calculated values it seems pertinent to mention similar dietary standard of protein in both the species of mushroom. EAA-Index of P.citrinopileatus based on egg and FAO reference pattern were 68.41 and 92.55 respectively compared to the slightly higher values of 72.04 and 97.50 in P.sajor-caju.

(c) Determination of Amino Acid Score (AAS) and sequence of limiting amino acids

Amino acid score, also called chemical score is considered as a second alternative, after EAA-Index, to the animal feeding studies to determine the nutritional value of a protein food. It is based on the amount of most limiting amino acid present in the test protein in relation to the amount of same amino acid in reference protein. Amino acid score can be calculated following the formula as advanced by FAO (1973).

Table 42. Estimation of Essential Amino Acid (EAA). Index of P.citrinopileatus based on egg and FAO reference protein

	Egg-R/P		FAO-R/P		P.citrino- pileatus		EAA-calcula- tions (Egg)		EAA-calcula- tions (FAO)	
	(A) $\frac{\text{gm AA}}{\text{gm N}}$	(B) $\frac{1}{(A)}$	(C) $\frac{\text{gm AA}}{\text{gm N}}$	(D) $\frac{1}{(C)}$	(E) $\frac{\text{gm AA}}{\text{gm N}}$	(F) Egg- ratio 100 (BxE)	(G) Log Egg- ratio	(H) FAO- ratio 100 (DxE)	(I) Log FAO- ratio	
Isoleucine	0.34	2.94	0.25	4.00	0.184	54.10	1.7332	73.60	1.8669	
Leucine	0.54	1.85	0.44	2.27	0.334	61.79	1.7909	75.82	1.8798	
Lysine	0.44	2.27	0.34	2.94	0.319	72.41	1.8598	93.79	1.9721	
Methionine + Cystine	0.36	2.78	0.22	4.55	0.165	45.87	1.6615	75.07	1.8755	
Phenylalanine + Tyrosine	0.58	1.72	0.38	2.63	0.638	109.74	2.0404	167.79	2.2248	
Threonine	0.29	3.45	0.25	4.00	0.286	98.67	1.9942	114.40	2.0584	
Tryptophan	0.11	9.09	0.06	16.67	-	-	-	-	-	
Valine	0.41	2.44	0.31	3.23	0.239	58.32	1.7658	77.20	1.8876	

Sum of log Egg-ratio = $\Sigma G = 12.8458$ Sum of log FAO-ratio = $\Sigma I = 13.7651$
Mean log ratio = $\Sigma G/7 = 1.8351$ Mean log ratio = $\Sigma I/7 = 1.9664$
EAA-Index (=antilog of $\Sigma G/7$) = 68.41 EAA-Index (=antilog of $\Sigma I/7$) = 92.55

Table 43. Estimation of Essential Amino Acid (EAA) Index of P.sajor-caju based on whole egg and FAO-reference protein

	Egg-R/P		FAO-R/P		P.sajor- caju	EAA-calcula- tions (Egg)		EAA-calcula- tions	
	(A) $\frac{\text{gm AA}}{\text{gm N}}$	(B) $\frac{1}{(A)}$	(C) $\frac{\text{gm AA}}{\text{gm N}}$	(D) $\frac{1}{(C)}$	(E) $\frac{\text{gm AA}}{\text{gm N}}$	(F) Egg- ratio 100 (BxE)	(G) Log Egg ratio	(H) FAO- ratio 100 (DxE)	(I) Log FAO- ratio
Isoleucine	0.34	2.94	0.25	4.00	0.204	59.98	1.7780	81.60	1.9117
Leucine	0.54	1.85	0.44	2.27	0.349	64.56	1.8100	79.22	1.8988
Lysine	0.44	2.27	0.34	2.94	0.335	76.04	1.8810	98.49	1.9934
Methionine + Cystine	0.36	2.78	0.22	4.55	0.190	52.82	1.7228	86.45	1.9368
Phenylalanine + Tyrosine	0.58	1.72	0.38	2.63	0.571	98.21	1.9921	150.17	2.1766
Threonine	0.29	3.45	0.25	4.00	0.282	97.29	1.9881	112.80	2.0523
Tryptophan	0.11	9.09	0.06	16.67	-	-	-	-	-
Valine	0.41	2.44	0.31	3.23	0.278	67.83	1.8314	89.79	1.9532

Sum of log-Egg-ratio = ΣG = 13.0034 Sum of log FAO-ratio = ΣI = 13.9228
Mean log-ratio = $\Sigma G/7$ = 1.8576 Mean log ratio = $\Sigma I/7$ = 1.9890
EAA-Index (=antilog of $\Sigma G/7$) = 72.04 EAA-Index (=antilog of $\Sigma I/7$) = 97.50

$$\text{Amino Acid Score} = \frac{\text{mg amino acid/gm test protein}}{\text{mg amino acid/gm reference protein}} \times 100$$

Amino acid score reflects the "percentage of adequacy" of the protein and approximates the efficiency of utilization of the test protein in Children (FAO, 1973). By calculating a factor equal to 100 times the reciprocal of the Amino Acid Score, one can determine the quantity of test protein required to provide minimal amino acid pattern present in reference protein (Crisan and Sands, 1978). From the quantitative data of amino acids in Table 41. Amino acid score of Pleurotus citrinopileatus and Pleurotus sajor-caju were calculated on the basis of both egg and FAO reference protein. Amino acid score and sequence of limiting amino acids are represented in Table 44.

The result shows that when egg was reference protein, sulphur containing amino acids were found to be most limiting amino acids in both the fungi. Amino acid score thus calculated were 45.83 (=46) and 52.78 (=53) in P.citrinopileatus and P.sajor-caju respectively. The sequence of limiting amino acids in former species was sulphur containing amino acids (Sc), Isoleucine (Ileu) and Valine (Val) respectively while sulphur containing amino acids (Sc), Isoleucine (Ileu) and Leucine (Leu) was the sequence in later species. But when FAO reference protein was concerned Isoleucine and Leucine were the most limiting amino acids with amino acid score of 73.6 (=74) and 79.32 (=79) respectively in the two species mentioned. The sequence of limiting amino acids in P.citrinopileatus was Isoleucine

(Ileu), sulphur containing amino acids (Sc) and Leucine (Leu) whereas P.sajor-caju bore the sequence of Leucine (Leu), Isoleucine (Ileu) and sulphur containing amino acids respectively.

Table 44. Determination of Amino Acid Score and sequence of limiting amino acids in P.citrinopileatus and P.sajor-caju

Amino acids	Amino Acid Score			
	<u>P.citrinopileatus</u>		<u>P.sajor-caju</u>	
	Egg	FAO	Egg	FAO
Isoleucine	54.12	73.60	60.00	81.60
Leucine	61.85	75.91	64.63	79.32
Lysine	72.50	93.82	76.14	98.53
Methionine + Cystine	45.83	75.00	52.78	86.36
Phenylalanine + Tyrosine	100.00	167.89	98.45	150.26
Threonine	98.62	114.40	97.24	112.80
Tryptophan	-	-	-	-
Valine	58.29	77.10	67.80	89.68
Sequence of limiting amino acids	Sc, Ileu, Val	Ileu, Sc, Leu	Sc, Ileu, Leu	Leu, Ileu, Sc

O. Tabulation of estimated nutritive value of P.citrinopileatus and P.sajor-caju

Biological value (BV), a calculated measure, helps to predict the nitrogen retention by test organisms, if fed with

a protein food. Based on the estimated values of Essential Amino Acid Index, Biological Value can be calculated following the regression equation as used by Oser (1959).

$$\text{Biological Value (BV)} = 1.09 (\text{EAA-Index}) - 11.7$$

From experimental results he observed the predicted BV to approximate the BV obtained in feeding studies. On the basis of available values of EAA-Index, BV for Pleurotus citrinopileatus and Pleurotus sajor-caju was calculated and represented in Table 45.

Evaluation of protein may not be guaranteed if it is solely based on compositional analysis. Again due to a major variation in quality and quantity of protein in different mushroom species it seems difficult to compare their nutritional status. Hence quality, quantity and its in-vivo availability should be considered with similar importance. To overcome this problem Crisan and Sands (1978) developed a solution by calculating Nutritional Index (NI) with the equation as -

$$\text{Nutritional Index} = \frac{(\text{EAA-Index} \times \text{Percentage protein})}{100}$$

Nutritional Index for P.citrinopileatus and P.sajor-caju ^{on} was calculated based on both egg and FAO-reference pattern and presented in Table 45. An overview on the presented data considering EAA-Index, BV and NI it may be recommended that the two mushroom species are nutritionally close to each other.

When egg is reference protein EAA-Index, BV and NI for P.citri-
nopileatus are 68.41, 62.87 and 11.53 respectively compared to
72.04, 66.82 and 12.78 in P.sajor-caju. Similarly, when FAO-
reference protein is concerned, 92.55, 89.78 and 15.60 are the
values of EAA-Index, BV and NI of P.citrinopileatus compared
to the values 97.50, 94.57 and 17.30 in P.sajor-caju. Predicted
values for all the parameters in the two species of mushrooms
though vary close to each other P.sajor-caju in every step over-
lapped P.citrinopileatus. So, P.sajor-caju may be placed above
P.citrinopileatus in the chronological order of nutritional
status.

Table 45. Tabulation of estimated nutritive value of P.citrino-
pileatus and P.sajor-caju

Mushroom species	Essential Amino Acid Index		Biological Value		Amino Acid Score		Nutritional Index	
	Egg	FAO	Egg	FAO	Egg	FAO	Egg	FAO
<u>P.citri-</u> <u>nopilea-</u> <u>tus</u>	68.41	92.55	62.87	89.18	46	74	11.53	15.60
<u>P.sajor-</u> <u>caju</u>	72.04	97.50	66.82	94.57	53	79	12.78	17.30

DISCUSSION

PART-I. Studies on *Pleurotus sajor-caju*, a cultivated mushroom

Over the centuries, far more attention has been paid to the methods of cultivation of mushroom than to the breeding in an attempt to improve crop production. With the progress made in understanding of sexuality in cultivated edible fungi, some work on breeding has started in recent years. The pioneer work on breeding of Oyster mushroom (*Pleurotus ostreatus*) was done by Eger and his associates (1976), who produced a sporeless dikaryon, "F42 x 11". Though the sexual behaviour of *P.sajor-caju* was reported to be heterothallic and tetrapolar by Jandaik (1983), no breeding work has been reported on this mushroom so far.

The primary step in the sequence of events in the breeding program of mushroom is the germination of basidiospores. A suitable technique for germination of basidiospore was developed. The basidiospores stored at 0°C were found to retain high level of germination even after one year. Sixty monosporous lines were raised and their comparative growth patterns (circular growth on semisolid medium and mycelial mass in liquid medium), temperature sensitivity and capacity for induction of primordia were studied.

For determination of sexuality of the mushroom, at first 12 monosporous lines were tested for mating reactions and were

classified into four distinct groups. Occurrence of such four distinct classes of monosporous lines proved tetrapolar incompatibility in this mushroom. PS₃, PS₉, PS₁₂ and PS₅ were considered as tester strains in the respective incompatibility classes and by their mating reactions with rest of the unclassified lines majority of them were separated into their respective classes.

For conducting breeding program, nine monosporous lines belonging to different incompatibility classes were selected from a mosaic of growth pattern, maximum temperature tolerance and primordia formation as presented in the Table 46.

After crossing of members belonging to different incompatibility groups, ten recombined cultures were obtained. Growth pattern and maximum temperature tolerance of these recombined cultures were studied. From cultivation trials of the recombined cultures, data on yield, colour, pilear margin, average fruitbody weight were determined. Also moisture content and protein content were estimated. A comparative account of the data is presented in Table 47.

It is a common experience that better spawn run leads to better yield. When a comparison was made between yield, circular growth on solid medium and mycelial mass in liquid medium of different recombined strains (Table 47), it was found that in cases of significant reduction of yield, there was corresponding significant reduction in circular growth. So circular growth is a better indicator of spawn run.

Table 46. Mosaic of growth pattern, temperature tolerance, primordia formation and incompatibility classes in selected monosporous lines

Growth pattern	Max. tempt. of tolerance (°C)	Circular growth at max. tempt.		Primordia formation				Incompatibility class		
		Lines	Growth (cm ²)	' + '	' - '	A ₁ B ₁	A ₁ B ₂	A ₂ B ₁	A ₂ B ₂	
Slow-	36°C-	36°C								
PS ₂₃ , PS ₃₅	PS ₃ , PS ₉ , PS ₂₇ , PS ₄₆ ,	PS ₃	4.50	PS ₃	PS ₉	PS ₃	PS ₉	PS ₃₅	PS ₁₈	
		PS ₉	0.51	PS ₁₈	PS ₂₃	PS ₄₆	PS ₂₃	PS ₅₉	PS ₂₇	
Medium slow-	PS ₅₉ , PS ₆₄	PS ₂₇	9.49	PS ₄₆	PS ₂₇	PS ₆₄				
PS ₉ , PS ₁₈		PS ₄₆	4.39	PS ₆₄	PS ₃₅					
PS ₄₆		PS ₅₉	3.67		PS ₅₉					
	34°C-	PS ₆₄	4.39							
Medium fast-	PS ₁₈ , PS ₂₃									
PS ₃ , PS ₅₉	PS ₃₅									
Fast-		34°C								
PS ₂₇ , PS ₆₄		PS ₁₈	10.52							
		PS ₂₃	0.19							
		PS ₃₅	2.35							

Table 47. Growth and cultivation parameters of different recombined cultures

Recom- bined strain	Circular growth at opti- mum temp. (cm ²)	Wt. of myce- lial mass at optimum temp. (mg)	Maximum temp. of toler- ance (°C)	Growth at maxi- mum temp. of toler- ance (cm)	Yield/ 75 g (total of dry straw in two flushes) (g)	Pileus colour of fruit- body	Pileus margin of fruit- body	Mois- ture content of fruit- body (%)	Average fruit- body weight (g)	Protein content of fruit- body (%) (D.W.)
PS ₃ x18	105.65	104.22	36	2.43	57.33	Normal	Slightly wavy	91.18	1.84	24.06
PS ₃ x27	103.51	100.57	36	6.07	70.00	White	Entire	90.06	1.63	26.25
PS ₉ x35	95.85	90.97	36	1.49	54.25	Normal	Entire	91.77	2.49	24.31
PS ₉ x59	109.26	102.74	36	2.11	63.67	Normal	Entire	92.13	2.63	29.25
PS ₁₈ x46	98.61	99.88	36	3.08	68.00	Creamy white	Entire	90.12	3.58	30.56
PS ₁₈ x64	102.10	96.93	36	2.89	56.83	Brown	Striated & wavy	90.50	2.57	28.81
PS ₂₃ x35	89.49	87.44	34	2.21	50.67	Less brown	Slightly wavy	88.92	1.57	25.12
PS ₂₃ x59	78.94	93.10	34	8.04	30.33	Less brown	Slightly wavy	92.40	1.12	24.37
PS ₂₇ x46	106.01	101.86	36	4.37	69.50	Creamy white	Entire	89.82	2.09	37.50
PS ₂₇ x64	90.14	100.21	36	1.17	60.33	Less brown	Wavy	92.82	2.37	24.69
PS _{Wild}	100.00	100.00	36	3.80	68.00	Normal (whitish brown)	Entire	90.87	3.39	25.31

Out of 10 recombined strains, the following 5 produced fruitbodies with protein content higher than the wild species:

- | | | |
|-------------------------|-------------------------|-------------------------|
| i) PS _{27x46} | ii) PS _{18x46} | iii) PS _{9x59} |
| iv) PS _{18x64} | v) PS _{3x27} | |

Three strains, PS_{3x27}, PS_{27x46} and PS_{18x46}, produced yield either marginally higher than or equal to the wild species. In other two strains (PS_{23x59}, PS_{23x35}) yield was significantly lower. Though all the three high yielding strains had a maximum temperature tolerance of 36°C, there was sharp variation in growth in these strains. On the basis of growth obtained at 36°C, the strains can be arranged in the following descending order of sequence as -

PS_{3x27} $\longrightarrow$ PS_{27x46} $\longrightarrow$ PS_{18x46}

Among them, PS_{3x27} and PS_{27x46} showed higher growth at 36°C than the wild species and possessed higher tolerance of temperature. Regarding other characters, PS_{3x27}, PS_{18x46} and PS_{27x46} produced white or creamy white sporophores all having desired entire margin. In comparison with the wild species, moisture content of the sporophores of these strains was also lower. Therefore, in this breeding program, it has been possible to develop three new strains with improved characteristics which are presented in Table 48.

Table 48. Characteristics of some improved recombined strains of P.sajor-caju

Strains	Vegetative phase			Reproductive phase		
	Max. temp. of tolerance (°C)	Growth at max. temp. of tolerance (cm ²)	Yield (per 75 g dry straw) (g)	Pileus colour	Pileus margin	Moisture content of fruitbodies (%)
PS _{3x27}	36	6.07	70.0	White	Entire	90.06
PS _{18x46}	36	3.08	68.0	Creamy white	Entire	90.12
PS _{27x46}	36	4.37	69.5	Creamy white	Entire	89.82
PS _{Wild}	36	3.80	68.0	Whitish brown	Entire	90.87

There was successful crossings in a few di-mon matings as PS_{9xW}, PS_{27xW}, and PS_{59xW}. But there was no significant difference in yield, temperature tolerance and protein content of the fruitbodies in these recombined cultures.

Though the recombined strains, PS_{3x27}, PS_{18x46} and PS_{27x46} need to pass through regorous adaptive trials to prove their acceptability, it is evident from the present investigation that there is tremendous scope for improvement of this species of mushroom using breeding methods.

PART-II. Studies on Pleurotus citrinopileatus, a domesticated mushroom :

Pleurotus citrinopileatus is a mushroom newly introduced in West Bengal with an attractive creamy white colour, whereas

the established oyster mushroom P.sajor-caju produces sporophores with greyish brown shade. Spawn run in this new mushroom occurred in a temperature range of 20-34°C (the optimum being 25-30°C) and fructification occurred upto 32°C. In P.sajor-caju, spawn run occurred from 20-30°C (the optimum being 25°C), but there was no fructification at 30°C. Pleurotus citrinopileatus was found to produce substantially higher yield, irrespective of the method of cultivation, than P.sajor-caju. Both the species were found to be similar on characters of entire pilear^l margin and flavour of the fruitbodies. But the thickness of the fruitbody was lesser in P.citrinopileatus than that of P.sajor-caju.

Regarding adaptive trials of P.citrinopileatus conducted in different agroclimatic regions of West Bengal, it was found that North Gangetic plain was best suited for its cultivation round the year with optimum performance of spawn run and yield. The overall picture in South Gangetic plains, Lateritic tracts and Coastal areas, on the points of spawn run period and yield looked more or less similar. Here shorter spawn run period and higher yield were obtained during winter phase (November-Mid March) rather than in prewinter phase (July-October) and cultivation during post-winter phase (mid March-June) proved abortive. In the Hilly tracts, however, congenial condition prevailed for its cultivation during prewinter and post-winter phases, but due to excessive drop of temperature, there was no crop in winter months.

While the cultivation of P.sajor-caju is restricted in winter months only, this new oyster mushroom P.citrinopileatus, can be cultivated not only in winter months but in pre-winter months also in the plains of West Bengal. Its cultivation can be extended to post-winter months (mid March-June) in the South Gangetic plains, Lateritic tracts and Coastal areas taking recourse to low-cost cooling measures. Moreover, this mushroom produces significantly higher yield than P.sajor-caju. As a result, the mushroom has become popular among the mushroom growers in different parts of West Bengal. A number of private spawn production centres are distributing spawns of this species of mushroom to the mushroom growers.

This mushroom was originally isolated from Kalimpong in Darjeeling district. This mushroom known as 'Kanye cheo' was found to grow wildly on wood logs and was a very popular variety of mushroom in this region. As this mushroom has been screened through generations of human experience, it is naturally expected that the mushroom should not contain any toxic compound in it. Considering the acceptance of the mushroom by the growers in West Bengal, it is of utmost importance to determine its proximate composition and nutritive value to compare it with other established species of oyster mushroom.

When the proximate composition of mushroom of P.citrinopileatus was compared with the same of P.sajor-caju, it was found that the later contained higher protein, while mushroom of P.citrinopileatus contained higher fat and carbohydrates and hence, had higher energy value.

Determination of true nutritive value of edible mushrooms is a complex task. Properly designed feeding studies of the mushroom was conducted with young white mice of Swiss strain. The increase in body weight in mice fed with P.citrinopileatus diet was found to be marginally lower than in mice fed with P.sajor-caju. However protein efficiency ratio (gain in body weight in g. per g. of protein) was found to be higher in mice fed with P.citrinopileatus diet. Overall digestibility and apparent digestibility were also found to be lower with P.citrinopileatus diet. The feeding trial with mice established that the mushrooms of P.citrinopileatus is a nutritionally rich food and marginally inferior to P.sajor-caju. It formed a good, balanced diet when mixed with cereals (wheat flour) and served as a good source of protein of experimental animals.

Protein is the most critical component contributing to the nutritional value of a food. The protein contents of both the mushrooms were found to be highest in the bud stage, 24.88 per cent in P.citrinopileatus and 26.45 per cent in P.sajor-caju, and then decreased gradually with advancement of sporophore development. The results are in conformity with the findings of Miljkovic & Dzamic (1984) in Boletus edulis and Cantharellus cibarius. But according to Sivaprakasam (1983) and Kosson and Bakowski (1984), protein content increased with advancement of sporophore development.

Mushrooms contain a significant amount of non-protein nitrogen in the chitinous cell wall. Since sufficiently critical

methods for determining true protein content of mushroom in the presence of chitin have not been devised, it is not possible to get an idea of the nutritive value of mushroom by estimating its protein content alone. So other than feeding studies, the most reliable measure, presently available for determining nutritive value of mushroom appears to be their amino acid content, especially the contents of essential amino acids.

The amino acid constituents of protein play an important role in human nutrition. It is interesting to note that all the essential amino acids essential for human nutrition are present in adequate quantities in P.citrinopileatus. As lysine, threonine and tryptophan are present in appreciable amounts in this mushroom, it offers considerable scope for supplementation of cereal diet with the mushroom, as cereal diets are known to be deficient in these amino acids.

The nutritional standards based on amino acid contents, used in the present investigation are : Essential Amino Acid (EAA) Index, Predicted Biological Value (BV), Amino Acid Score (AAS) and Nutritional Index (NI). All these nutritional standards based on both whole egg protein and FAO standard protein were marginally higher in case of P.sajor-caju than ⁱⁿ P.citrinopileatus. When we compare the nutritive value of this mushroom with other food stuffs calculated against FAO reference protein (Crisan and Sands, 1978), we can rank this mushroom as shown in Table 49.

Table 49. Status of oyster mushrooms among other food stuffs

Essential Amino Acid Index	Amino Acid Score	Nutritional Index
100 Pork, Chicken, Beef	100 Pork	59 Chicken
99 Milk	98 Chicken, Beef	43 Beef
98 <u>P.sajor-caju</u>	91 Milk	35 Pork
91 Potato, Kidney bean	79 <u>P.sajor-caju</u>	31 Soybean
89 <u>P.citrinopileatus</u>	74 <u>P.citrinopi- leatus</u>	26 Spinach
88 Corn	63 Cabbage	25 Milk
86 Cucumber	59 Potato	21 Kidney bean
79 Peanut	53 Peanut	20 Peanut
76 Spinach	50 Corn	17 <u>P.sajor-caju</u>
69 Turnip	46 Kidney bean	17 Cabbage
44 Tomato	42 Cucumber	16 <u>P.citrinopi- leatus</u>
	33 Turnip	14 Cucumber
	31 Carrot	11 Corn
	28 Spinach	10 Turnip
	23 Soybean	9 Potato
	18 Tomato	6 Carrot

The mushroom contains comparatively higher amounts of mineral contents as compared to fresh fruits and vegetables. It is a better source of minerals than P.sajor-caju, in respect of essential elements like K, P, Na, Ca, Fe and Mn. This mushroom contains 72 ppm Zn and 210 ppm Cu, which are much below

the maximum tolerance limit of human beings and so it is quite safe to consume the mushroom from this point of view.

The present study clearly revealed that the sporophores of P.citrinopileatus, though nutritionally marginally inferior to P.sajor-caju, formed a good source of protein containing all essential amino acids and minerals in adequate proportions, making the sporophores not only delicious but also a nutritionally rich food. The manufacture of food products with well balanced contents of essential nutritional factors and dietary substances has been recently developed in advanced countries. As this mushroom can be grown in wider range of temperature (20-32°C) producing higher yields, this mushroom can be cultivated for longer duration in a year under natural climatic condition and can be produced at a lower cost than P.sajor-caju. So this mushroom bears the potentiality to play a significant role in the large scale production of artificial food based on mushroom in near future.

Chapter—5

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

PART-I. Studies on *Pleurotus sajor-caju*, a cultivated mushroom

- (a) (A suitable method for the germination of basidiospore in *Pleurotus sajor-caju* was developed.) Freshly collected spores or spores conveniently preserved at 0°C were stimulated to reach a higher enough percentage of germination. Higher percentage of germination led to increase the variability of characters in the population.
- (b) To ensure easy and effective means of isolation of single spore a suitably designed method was followed and a total of 60 monosporous lines were developed.
- (c) (Variation of characters on points of growth behaviour, temperature sensitivity and primordia formation among the monosporous lines was thoroughly studied with possible circumspection.)
- (i) Tremendous variation in growth behaviour was obtained. Percentage growth (radial) compared to wild culture was found to run within a range of 1.70% to as high as 102.91%. Based on its growth behaviour monosporous lines were separated in four different groups as slow, medium slow, medium fast and fast growing lines.
- ii) Different monosporous lines were found to harbour a wide variation in temperature sensitivity. Of the 60 monosporous lines PS₄₀, PS₅₅ and PS₅₇ were found to be very

temperature sensitive and were unable to withstand a temperature of 30°C. Another 12 members were comparatively better tolerant and reached the level nearing 34°C. Rest 45 lines survived at 34°C but could not overcome the level of 36°C. As a general tendency growth activity gradually dropped after 30°C and totally checked at 36°C.

- (iii) Primordia formation was tried in all the monosporous lines providing requisite physical and nutritional conditions. Only 7 members out of 60 were found to develop primordia. These were demarkated as cultures with good fruiting ability.
- (d) For the determination of sexuality tester strains of respective incompatibility groups were developed based on the crossing response of limited number of monosporous lines. Tester strains were subjected to the mating reactions with rest of the monosporous lines and the whole lot was found to cluster in four distinct sexual groups.

(Taking recourse of cultural behaviour and sexual response the wild culture of Pleurotus sajor-caju was demarkated as heterothallic in nature with tetrapolar incompatibility.)
- (e) Considering all the available characters, 9 monosporous lines taken from all the incompatibility groups were selected as parent lines. During selection special emphasis was given on growth and temperature sensitivity.
- (f) Selected parent monosporous lines were exposed to the mating reactions with compatible partners in all possible

combinations. Ten recombined cultures (heterokaryons) were isolated from the confronting zone and maintained.

- (g) Selected parent lines were also tested for di-mon mating taking wild culture as the dikaryotic partner. Three monosporous lines were found to respond in di-mon mating and thus 3 di-mon recombined cultures were also obtained.
- (h) Growth and temperature sensitivity of the recombined cultures were studied at the temperature range of 25-38°C and optimum growth was obtained upto the level of 30°C. Fast growing nature of the fungi although reinstated in the recombined cultures and some of the cultures (PS_{9x59}, PS_{27x46} and PS_{3x18}) slightly emerged over the wild culture, no remarkable change was noticed.
- (i) (Recombined cultures were subjected to the cultivation trials side by side with the wild culture. No improvement in spawn run and yield of mushroom could reach upto the expectation. But the quality characters particularly the colour of mushroom ensured a desirable change.) PS_{3x27}, PS_{18x46} and PS_{27x46} were with white and creamy-white fruitbodies compared to the fruitbodies of wild culture with greyish brown shade.
- (j) (Protein and moisture content in all the recombined cultures were determined. Spectacular variation in protein content of the mushroom was obtained in some of the cultures.) PS_{27x46} harboured maximum protein content of 37.5% (D.W.) against 25.19% in wild culture. PS_{18x46}, PS_{9x59} and PS_{18x46} were with 30.56%, 29.25% and 28.81% protein respectively.

PART-II. Studies on *Pleurotus citrinopileatus*, a domesticated mushroom

- (a) (Ecological variants of *Pleurotus citrinopileatus*, a newly introduced species of mushroom, were collected and isolated from its wild habitat of Kalimpong sub-division in Darjeeling district of West Bengal. The isolates were domesticated and best ecological variant of the species was obtained through selection, comparing its behaviour on growth, temperature tolerance and yield.)
- (b) (Growth and temperature tolerance of the species was studied side by side with *Pleurotus sajor-caju*. It was found to establish its fast growing ability and endured a long range of temperature.) Optimum growth was maintained between 25-30°C and produced substantial amount of growth even at 34°C.
- (c) Suitable method of cultivation of the species was developed. Optimum spawn run and yield was obtained between 25-30°C. At 32°C spawn run period was found to lengthen but there was a substantially higher yield.
- (d) Under favourable growing condition *P.citrinopileatus* gave much higher yield which came in four subsequent flushes against mere three flushes and lesser yield of *P.sajor-caju*.
- (e) Creamy white fruitbodies, higher number of buds and very less bud mortality in *P.citrinopileatus* is of worth mentioning.

- (f) (Adaptive trials of cultivation in different agroclimatic regions of West Bengal was conducted in different periods of the year and North Gangetic plain was found best suited to grow this mushroom round the year.)
- (g) (Protein content in mushroom collected at different stages of harvest and proximate composition of the mushroom was estimated. P.sajor-caju was found to bear slightly higher amount of protein than P.citrinopileatus but considering proximate composition (Protein, fat & carbohydrate) the later was found superior.)
- (h) Amino acid composition of protein and available mineral elements in the mushroom was analysed. It was found to bear all the essential amino acids necessary for human nutrition and in adequate concentrations. (Compared with P.sajor-caju, P.citrinopileatus was considered as a better source of minerals.)
- (i) (To evaluate the dietary significance and chance mammalian toxicity of the mushroom it was passed through a well designed feeding trial using male white mice of Swiss-strain as test animal.) Mushroom was supplied as the major source of protein.
- i) Overall development and increase in body weight of the test animals after the stipulated period of feeding was assessed. (PC- diet proved its good dietary composition compared to PS and Normal diets.)

- (ii) Protein efficiency ratio, overall digestibility and apparent digestibility of the mushroom diet was estimated and high nutritional standard of the PC- diet was established.
- (j) As a supporting evidence to feeding trial, some alternate predictive analytical methods were implicated using quantitative data of essential amino acid composition. Essential amino acid Index (EAA-Intex), Biological value (BV), Amino Acid Score (AAS) and Nutritional Index (NI) were calculated. From these predicted analytical data nutritional status of the mushroom was assigned in the list of human food items.

Chapter-6

**FUTURE SCOPE OF
RESEARCH**

FUTURE SCOPE OF RESEARCH

For centuries mushroom is being recommended as a choice food item advocating its delicacy and high nutritional standard. Still its taste is mainly restricted in the temperate countries with popular cultivar of mushroom Agaricus bisporus. The science of mushroom thus automatically centered around this temperate species A.bisporus. As it is a homothallic species with inherent reluctance to genetic recombination, far more attention has been paid to improve its quality and yield through cultivation trials and selection of ecological variants. Recently, mushroom is being cultivated in the rare pockets of tropical countries. A number of mushroom fungi has been tried for introduction and popularisation in tropical environment. The basic crisis dwells on the lack of suitable varieties of mushroom. Work on breeding with mushroom species scarcely struck to reports. Non-viability or rare germination of the basidiospores and homothallism are the major hindrances to undertake breeding programme for varietal improvement in most of the conventional mushroom species.

However, the sexual behaviour of Pleurotus sajor-caju, selected for the breeding programme in the present investigation, was found to be heterothallic and tetrapolar, and did not offer any such problem. Through suitable techniques, it was also possible to increase the rate of germination of basidiospores of the mushroom fungus. In the breeding programme of the mushroom

taken with limited number of monosporous cultures, it was possible to achieve new recombinants with increased number of fruit bodies, higher growth rate at maximum temperature of tolerance and improved quality characters such as pileus colour and protein content. So by using larger number of monosporous lines, it might be possible to develop one or more recombinant strains, with improved quality, well suited for cultivation in tropical environment.

Pleurotus citrinopileatus another new collection of oyster mushroom with some additional good characters of high yielding capacity, better temperature tolerance and desirous quality, of course indicates its resourcefulness. Increase in the variability of characters by collection of likely new wild species and by application of other breeding methods such as mutation in the recombining stock it is possible to make a break through in the field of mushroom research and to usher in a new era of mushroom science.

Chapter—7

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