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MORPHOLOGICAL AND BIOCHEMICAL VARIABILITY AMONGST PARENTS AND HYBRIDS OF OYSTER MUSHROOM

Thesis

Submitted to the

G.B. Pant University of Agriculture & Technology,

PANTNAGAR-263 145 (U.S. Nagar), Uttarakhand, INDIA

by

MEENAKSHI PRASAD

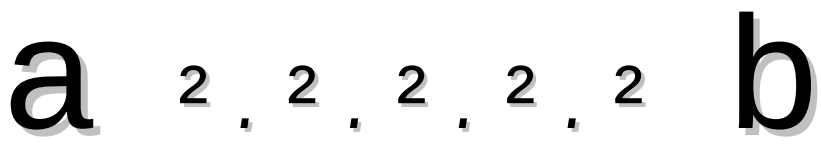
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science in Agriculture

(PLANT PATHOLOGY)

June, 2008

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Dedicated to

My

Maa Papa

Whose love, Care and Sacrifice

Makes my present



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ACKNOWLEDGEMENT

At first outset, my heart kneels to pray and thank almighty GOD, keep me, guide me and go with me today, with whose benediction it was possible for me to reach this phase of my life and preparation of this manuscript would have been possible.

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Time can never erase the immortal memories of golden times and blue moods shared with the Smita, Kamia, Jyoti, Pooja, Shivani, whose love and care, mischief and kind support did not ever let me feel out of my family and all the golden moments shared with them made me stay at Pantnagar a memorable one.

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June, 2008

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CERTIFICATE

This is to certify that the thesis **"**MORPHOLOGICAL AND BIOCHEMICAL VARIABILITY AMONGST PARENTS AND HYBRIDS OF OYSTER MUSHROOM**"**, submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN AGRICULTURE with major in Plant Pathology of the College of Post Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by Ms. Meenakshi Prasad, Id. No. 34049, under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

(R. P. Singh)
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Certificate

We, the undersigned, members of the Advisory Committee of Ms. Meenakshi Prasad, Id. No. 34049, a candidate for the degree of Master of Science in Agriculture with major in Plant Pathology agree that the thesis entitled **"**MORPHOLOGICAL AND BIOCHEMICAL VARIABILITY AMONGST PARENTS AND HYBRIDS OF OYSTER MUSHROOM**"**, may be submitted in partial fulfillment of the requirement for the degree.

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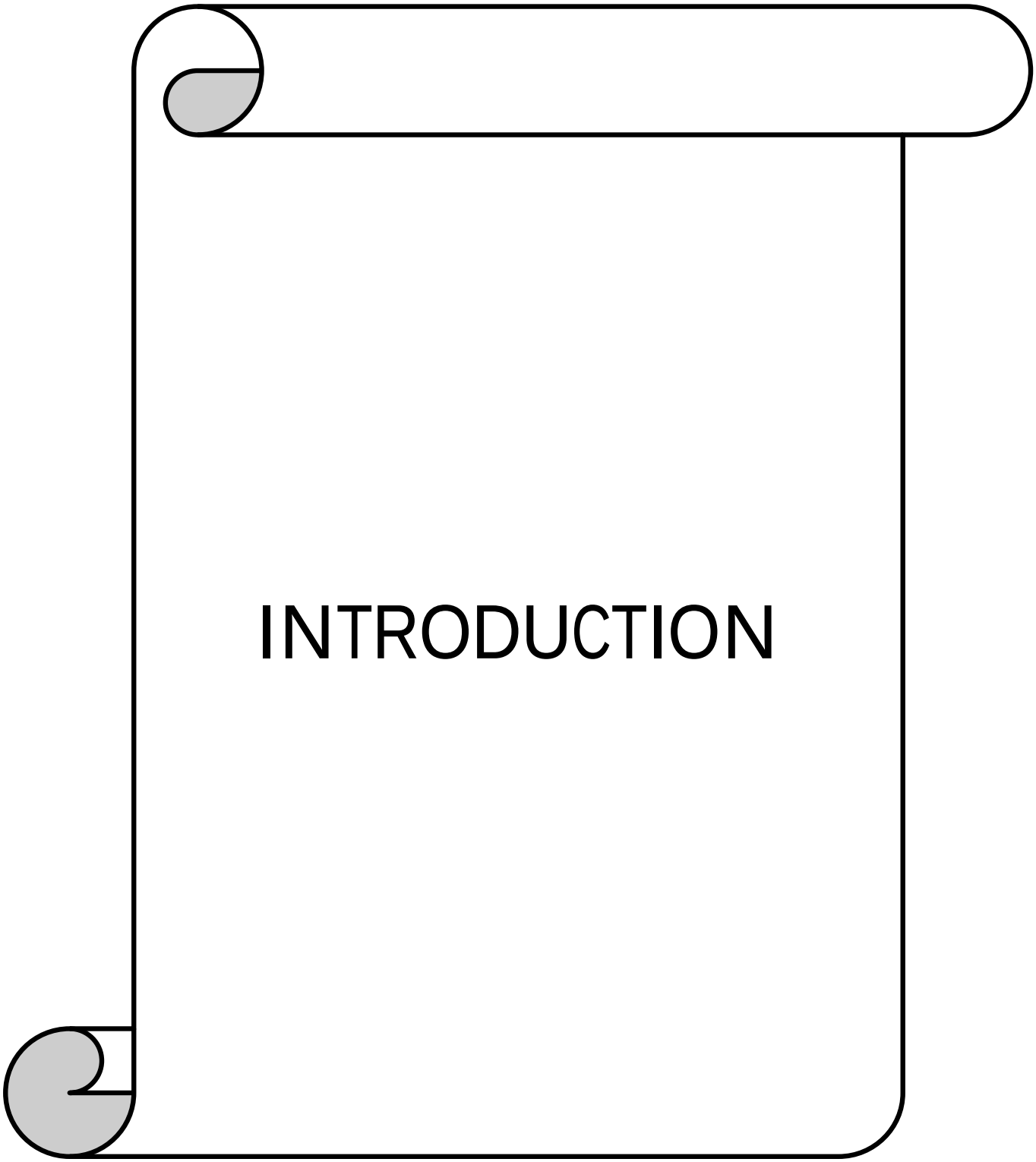
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INTRODUCTION

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Chapter 1 INTRODUCTION

The history of the man is the record of hungry creature in search of food on this planet and the use of edible fungi amongst his menus is as old as civilization itself. Mushrooms have been part of fungal diversity for around 300 million years ago.

Mushrooms are the member of higher fungi, which lack chlorophyll i.e., they can't utilize solar energy to manufacture their own food as green plants. However, mushroom can produce a wide range of enzymes that degrade the complex substrate on which they grow, following which they absorb the soluble substrate for their own nutrition.

The term mushroom is broadly defined as "a macro fungus with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with the naked eye and to be picked by hand" (Chang and Miles, 1993). Taxonomically Mushrooms belong to phylum Basidiomycota and Ascomycota (Alexopolous *et al*., 1996). They occur naturally and seasonally in various habitats and nicks all over the world.

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The Mushrooms comprise a large heterogeneous group having various shapes, sizes, colours, all quite different in character, appearance and edibility. Out of this large group, with more than 2000 edible species, about 300 species belonging to 70 genera are reported from India. However, only a few have been brought under cultivation on commercial scale. Out of 2000 species of prime edible mushrooms, about 80 have been grown experimentally, 20 cultivated commercially and six namely *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus*, *Auricularia*, *Volvariella* and *Flammulina* produced on industrial scales (Chang and Miles, 1991) and thus called as big Six mushrooms.

Of these, the *Pleurotus* mushroom is generally referred to as 'oyster mushroom' all over the world. In India, it is commonly known by 'Dhingri mushroom'. It is one of the choice edible mushrooms which can be cultivated in the tropics. It has gained importance only in the last decade and is now cultivated in many countries in the subtropical and temperate zones. *Pleurotus* has gained its name from the Greek word "Pleuro" which means formed laterally or in a side way position, referring to the lateral position of the stipe in relation to pileus (Jandaik, 1997). The word 'oyster' refers to the oyster-like appearance of the fruit bodies. According to the systemic position, *Pleurotus* belongs to the phylum Basidiomycota, class Basidiomycetes,

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order Agaricales and family Pleurotaceae (Tricholomataceae) (Kirk *et al*., 2001).

In nature species of *Pleurotus* are generally found as saprophytes, growing on dead and decaying parts of wood. However, the first attempt to grow this fungus for human consumption was made by Falck (1917) in Germany. The process of cultivation on readily available substrates was taken up earlier in India on paddy straw (Bano and Srivastava, 1962) and in Japan on saw dust (Schanel *et al*., 1966). This attracted attention of a number of researchers all over the world and today various species of *Pleurotus* are appreciated for their culinary attributes and broad adaptability under varied agro- climatic conditions.

Pleurotus species ranks third in global production contributing 14.2 per cent to the total annual world figures of 6160.8 thousand MT as against 31.81 per cent of *Agaricus bisporus* and 25.2 per cent of *Lentinula edodes* (Chang, 1999).

China has become an enormous producer and consumer of cultivated edible mushrooms and also a major producer of medicinal mushrooms. Total production of China in 1997 was 3.91 million tones which amount to 63.6 % of the total world output.

However, in India, *Agaricus bisporus* comprises over 90 per cent of total output of 70,000 tones, the remaining 10 per cent of the total

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production is that of oyster (*Pleurotus* spp.) and paddy straw (*Volvariella volvacea*) mushroom.

Mushrooms have been consumed as a part of normal diet from thousand of years due to their nutri-ceutical and pharmaceutical nature. Mushroom relatively higher in good quality protein, low in total fat, higher in polyunsaturated fatty acid, enriched with large amount of carbohydrates, valuable amount of fibers, significant amount of water, soluble vitamins such as thiamine B_1, riboflavin B_2, niacin, biotin, ascorbic acid vit- C and minerals such as potassium, phosphorus, sodium, calcium and magnesium (Manning, 1985 and Rai, 1997). Protein content in *Pleurotus* species has been documented in range between 8.9 and 38.7 per cent on dry weight basis (Bano and Rajarathnam, 1982) and 3.5 to 4 per cent on fresh weight basis (Chang and miles, 1989). Which shows that the protein content of mushrooms was much higher than cereals (wheat and maize) and comparable to that of food legumes (peas and lentil) (Rai, 1997; Shah *et al*., 1997).

Unlike other cultivated species of mushroom, genus *Pleurotus* exhibit much diversity in their adaptability to varying agro climates and this flexible nature provides much more cultivated species in this genus than any other cultivated mushroom genus, Out of 28 species collected and reported from India, more than a dozen are

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under cultivation in different parts of the country with different shades of the colour of the sporophores white, cream, grey, yellow, pink, or light brown depending upon the species. (Bal Krishnan and Nair, 1995; Verma, 1997; Wani and Sawant, 1998).

In recent years, industrial use of oyster mushroom for conversion of agriculture and industrial wastes into feed and food has gaining popularity. It has high gastronomic value. The species of *Pleurotus* can be grown on a wide variety of lignocellulolytic substrates with good productivity (Singh and Dwivedi, 1991; Ragunathan *et al*., 1996; Bonatti *et al*., 2004; Namdev *et al*., 2006; Ponmurugan *et al*., 2007). On an average 50-70% conversion on dry wet basis of straw is obtained in just 4 weeks of cropping. Besides, it takes shorter growth time as compared to other edible mushrooms, demands a limited environmental requirements, their fruiting bodies are not very often attacked by diseases and pests and they can be cultivated in a simple and cheap way without composting (Jwanny *et al.*, 1995; Patrabansh and Madan, 1997).

Commercialization of mushroom in India is hardly three decades old and with initial lag phase it has started showing upward trend. From a meager 4,000 tones production during 1985, India's total mushroom production reached 30,000 tones in 1995, which has further increased to 40,000 tones in 1996-97, 52,620 tones in 2000-01 and 70,000 tones

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in 2003-04 (Singh, 2002) (Table 1.1). Button mushroom dominated Indian mushroom market. However, its share in world mushroom production has dropped from 71.6 per cent in 1981 to 31.8 per cent in 1997 (Table 1.2). During the same period world production of oyster mushroom increased significantly from 2.8 per cent to 14.2 per cent (Chang, 1999). The trend is expected to continue and *Pleurotus spp*. may have a very good position in world mushroom market in future.

Thus in spite of excellent climatic conditions, multiple cropping possibilities, low cost of production and better shelf life *Pleurotus* species are cultivated on a limited scale. This is mainly due to non-availability of suitable strains, with attractive colour, fleshy sporophore, economic yields and temperature tolerance. Also by using the same culture for successive generation, the yield and quality potential of the strain goes on reducing.

The use of quality spawn of the better strain is one of the important aspects of the commercial cultivation of mushroom. The yield and quality of the mushroom produced is determined by the three factors viz., genetic make up of the strain used, the environmental conditions in which the crop is grown and the physiological and nutritional requirement of strains. Genetic manipulation of fungi is vital to meet the demand of the industrial world for fast growing and high yielding strains of edible mushrooms (Castillo *et al*., 1998). Genetic potential of *Pleurotus* strains can be improved through intra and inter-specific hybridization (Mithilesh Table 1.1. World Production (MT) of Cultivated Edible and Medicinal Mushrooms in 1997 (Chang, 1999b)

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Table 1.1. World Production (MT) of Cultivated Edible and Medicinal Mushrooms in 1997

Sl. No.	Species	China	Japan	Rest of Asia	North America	Letin America	E.U.	Rest of Europe	Africa	Total	% age
1.	Agaricus bisporus	384400	--	68400	425300	51600	875000	115200	36000	1955900	31.8
2.	*Lentinula edodes*	1397000	115300	47400	3600	300	500	300	--		25.4
3.	Pleurotus sp.	760000	13300	88400	1500	200	6200	5800	200	875600	14.2
4.	*Auricularia* sp.	480000	--	5300	--	--	--	--	--	485300	7.9
5.	*Volvariella volvacea*	120000	--	60800	--	--	--	--	--	180800	3.0
6.	*Flamulina* sp.	150000	109000	25700	--	--	--	--	--	284700	4.6
7.	*Tremella* spp.	130000	--	500	--	--	--	--	--	130500	2.1
8.	*Hypsizygus marmoreous*	2100	72000	100	--	--	--	--	--	74200	1.2
9.	*Pholioto nameko*	31000	24500	--	--	--	--	--	--	55500	0.9
10.	*Grifola frondosa*	2000	31100	--	--	--	--	--	--	33100	0.5
11.	Other	516400	2900	800	400	--	200	100	--	--	--
	Total	397290	368100	297400	430800	52100	881900	121400	36200	6160800	100
	Percentage	64.5%	6.0%	4.8%	7.0%	0.8%	14.3%	2.0%	0.6%		

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Table 1.2. State-wise production of cultivated mushrooms in India

Name of the state	Production (MT)			Type of mushrooms
	1996-97	2000-01	2003-04	
Jammu & Kashmir	400	400	500	*Agaricus*
Punjab	4000	13000	22500	*Agaricus*
Himanchal	500	600	2500	*Agaricus*
Haryana	4000	5000	6000	*Agaricus*
Uttar Pradesh	4000	2000	3000	*Agaricus/Pleurotus*
Uttaranchal	-----	2640	4050	*Agaricus*
Madhya Pradesh	2000	2500	2800	*Agaricus/Pleurotus*
Rajasthan	50	80	150	*Pleurotus/Calocybe*
Maharastra	7000	7000	7500	*Agaricus/Pleurotus*
Goa	1000	1000	1000	*Agaricus/Pleurotus*
Karnataka	600	700	750	*Agaricus/Pleurotus/ Volvariella*
Kerala	100	300	500	*Pleurotus/Volvariella*
Tamil Nadu	10000	11000	11500	*Agaricus/Pleurotus /Volvariella/ Calocybe*
Andhra Pradesh	2500	3000	3500	*Agaricus/Pleurotus*
Orissa	700	700	800	*Pleurotus/Volvariella*
West Bengal	250	300	400	*Agaricus/Pleurotus*
North Eastern States (Including Sikkim)	1000	1200	1250	*Agaricus/Pleurotus/ Volvariella/ Lentinula*
Delhi/Pondichery / Chandigarh/ Others	1000	1200	1300	*Agaricus/Pleurotus*
Total	40,000 *	52,620 **	70,000 ***	

Source: Mushroom conference Solan, 2000*, Coimbatore, 2002** and AICMIP workshop 2004***

and Harsh, 2007). Intra-specific hybridization yield very less percentage of improved strains (Ghosh and Chakravarty, 1991;

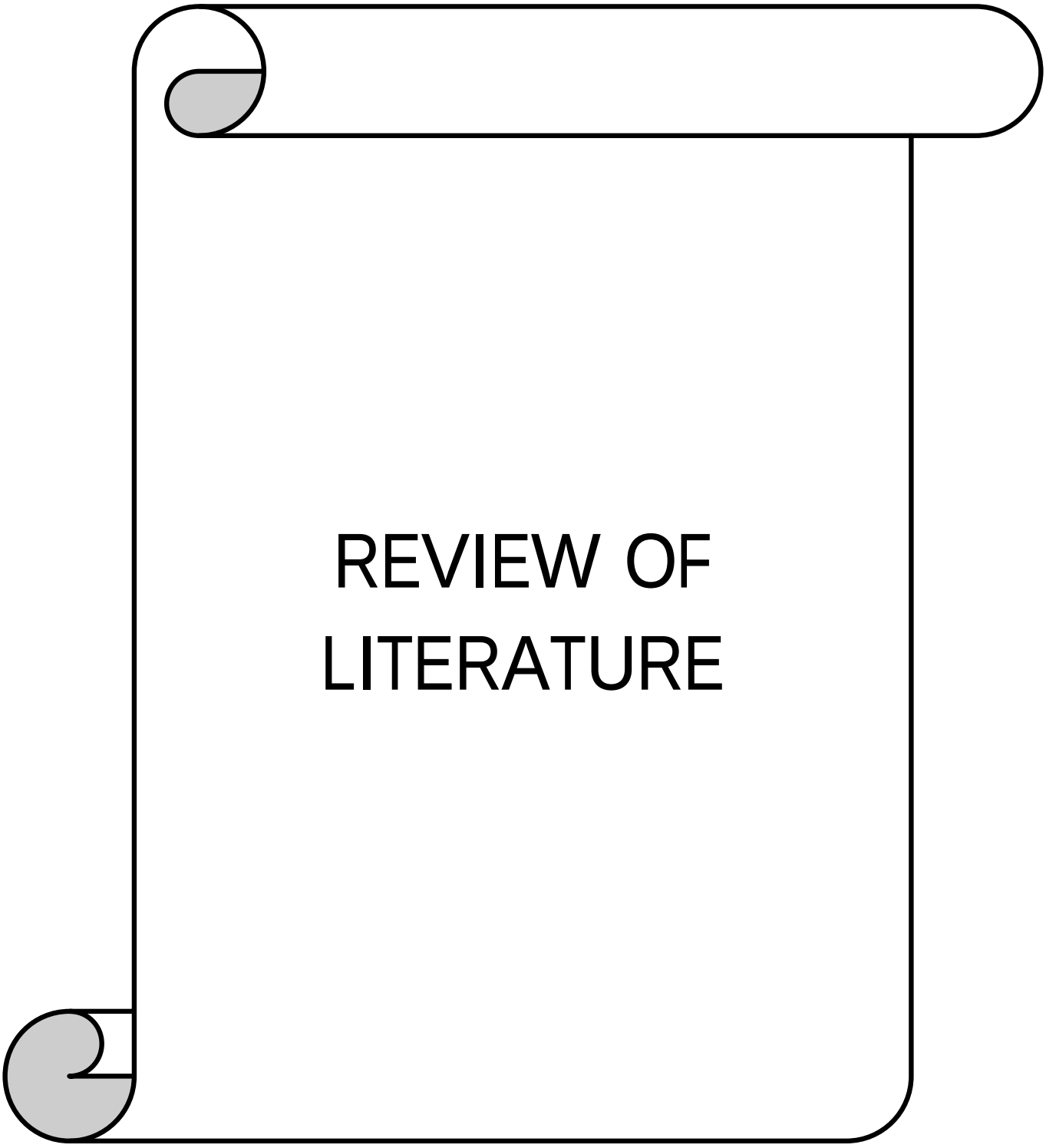
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Thakur and Bhandal, 1993), while inter-specific hybridization produces comparatively more strains with improved genetic potential (Bahukhandi and Sharma, 2002). Thus the introduction of new strain through hybridization is required to maintain the production of quality mushroom.

Keeping above points in view, the present investigations were undertaken to identify and evaluate the parents and hybrids of Oyster mushroom in terms of qualitative (morphological and bio-chemical) and quantitative (yield) variability with the following objectives:

1. Selection of parents and their hybrids.
2. Cultural and Morphological variability of selected parents and their hybrids.
3. Yield performance of selected parents and their hybrids.
4. Biochemical variability of selected parents and their hybrids.

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REVIEW OF LITERATURE

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2.1 TAXONOMY

The genus *Pleurotus* is a tetrapolar heterothallic Basidiomycetes belonging to the order Agaricales and family Pleurotaceae (Kirk *et al*., 2001).

Pleurotus was first named by Fries (1821) with two species namely *P. corticatus* Fr. and *P. albertinii* Fr. Which had earlier been named as *Agaricus lepiota* Alb. and Schw. and collected from trunk of *Abies species*.

He described the genus: “Stipe eccentric, lateral or absent, epiphytic (rarely terrestrial), irregular, fleshy, and occasionally somewhat tough”.

Singer (1951) placed *Pleurotus* in the order Agaricales and family Polyporaceae. Singer (1962) made further changes classified *Pleurotus* under Agaricales. He divided the order Agaricales into four families namely Agaricaceae, Strophoriaceae, Pleurotaceae and Tricholomataceae.

Ainsworth *et al*., (1973) placed the genus *Pleurotus* in family Tricholomataceae under the order Agaricales.

Singer (1975) grouped the genus into five sections. Each of the section has some apparently distinct characteristics. The characteristics of each section may be of more use to distinguish species with certainty.

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Section 1: *Lepiotarii*

Type species: *P. dryinus* (Pers. Ex. Fr.). Kummer.

Internal tissue of the gills is thin walled at first, becoming sclerified with age, stipe and veil present.

Species reported as cultivated: *P. corticatus* (Fr. Ex. Fr.) Quel (Kaufert, 1936).

Section -2: *Pleurotus*

Type species: *P. ostreatus* (Jacqc. Ex. Fr.) Kummer.

Internal tissue of the gills is sclerified early. Gill edges entire (smooth). The cap is generally coloured. No veil present. Species reported as cultivated: *P. ostreatus* (Kurtzman, 1979), *P. sapidus* (Schulzer) Kalch. (Kurtzman, 1979), *P. eryngii* EC ex Fr. (Zadrazil, 1976).

Section -3: *Calyptrati*

Type species: *P. calyptratus* (Lindb. In Fr.) Sacc. Veil very thin, not completely covering cap, very short stem. Internal tissue of gills irregular, many hyphae are thick walled. Species reported as cultivated: apparently none.

Section -4: *Lentodiellum*

Type species: *Panus concavus* Berk.

Basidiocarp tissues particularly in older specimens, primarily formed by thick walled hyphae. Cap with a flush of cinnamon brown, buff or rust colour or with no pigment. Veil and a very thick annulus

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may be present. Species reported as cultivated: *Pleurotus sajor-caju* (*Lentinus*) (Fr.) Sing (Jandaik and Kapoor, 1976).

Section -5: *Tuber regium*

Type species: *P. tuber regium* (Fr.) Sing.

Much like section 4, but more coloured than most mushrooms in section 4. Produces a very large sclerotium. Species reported as cultivated: *P. tuber-regium* (Oso, 1977).

Pegler (1976) classified the *Pleurotus* species of the Indian sub-continent on the basis of size of spores and cystidia. According to him, *Pleurotus* species may be divided into two groups. First group, in which the spore size ranges from 6-9µm and characterized as tropical or sub-tropical species and second with spores more than 10µm in length and are more typical of the north temperate flora confined to the Himalayan localities. In the first group *P. ninquidus, P. flabellatus, P. membranaceus, P. eous, P. anserinus, P. placentodes, P. platypus* are included. In the second group *P. ostreatus, P. fossulatus, P. gemellarii* etc. are included.

Quimio (1978) first reported the fruiting of *P. flabellatus,* a locally collected pink isolate of *Pleurotus* from Philippines. However, Anonymous (1998-99) reported that *P. djamor* (Fr.) Boedijn was characterized by the pink colour fruiting bodies.

In a report of NRCM, Solan, *Pleurotus euosmus* was characterized by white fruit body but it produces pinkish fruit bodies in culture tube. The pileus of *P. ulmarius* was white in colour and

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conspicuously raised at the base of attachment with stipe. *P. porrigenus* was medium size and brownish white with a short stipe (Anonymous, 1999).

In the present investigation, we have selected a section of *Pleurotus* species, occurring around the world over quite different agro-industrial waste substrates and produce fruit bodies within an acceptable time.

2.2 CULTIVATION

2.2.1. Substrate

Pleurotus mushroom can degrade any kind of agricultural or forest wastes, which contain lignin, cellulose and hemicellulose. In nature species of *Pleurotus* are generally found as saprophytes, growing on dead or decaying part of wood. Parasitisms by a few species have been reported e.g. *P. eryngii* (Singer, 1961). Cultivation of *Pleurotus* species (*P. ostreatus*) is known for a long time in European countries. Growing it on tree stumps and logs was first described in the beginning of 20th century (Falck, 1917, 1919; Passecker, 1959; Singer, 1961; Luthard, 1969; Vessey and Olah *et al*., 1979).

Later cultivation of *Pleurotus* species has also been reported on other substrates. Etter (1929) produced fruiting bodies of *P. ostreatus* in culture. On saw dust medium, production of sexual spores of *P. corticatus* Fr. was reported by Kaufert (1936). Block *et*

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al., (1958) cultivated *P. ostreatus* for the first time under laboratory conditions on sawdust medium. They used a mixture of Oatmeal and sawdust for cultivation and found best results on Eucalyptus followed by pine sawdust.

In India successful cultivation of *P. flabellatus* on paddy straw was reported by Bano and Srivastava (1962). Later, Jandaik and Kapoor (1974) reported cultivation of *P. sajor-caju* on Banana pseudostem and chopped paddy straw. Rangaswamy *et al*., (1975) reported successful cultivation of *P. sajor-caju* using different waste material like paddy straw, sawdust and wood shaving.

Different plant waste materials such as wheat straw and ragi straw were used by Bano *et al*., (1979) for growing *P. flabellatus*. Das *et al*., (1987) successfully used different agricultural wastes for cultivation of two *Pleurotus* spp. The best results (1650g for *P. flabellatus* and 1970g for *P. sajor-caju* from 3 kg dry substrate) were obtained from wheat straw using spawn multiplied on wheat straw.

Upadhyay and Vijay (1991) tried five selected species of *Pleurotus* i.e. *P. ostreatus, P. florida, P. fossulatus, P. eryngii* and *P. cornucopiae* for yield. Highest yield of 94 per cent biological efficiency was obtained with *P. florida* followed by 32 per cent biological efficiency for *P. ostreatus* on wheat straw at low temperature ranging 12-16^{0}C and relative humidity 65-75 per cent.

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Salmones *et al*., (1995) have evaluated barley straw for five semi industrial taxa of *Pleurotus* i.e. *P. columbinus, P. djamor var. djamor. P. djamor var. roseus, P. djamor* var*. salmoeostramieus* and *P. ostreatus*. Biological efficiencies for above five respective taxa were 70.4 – 133.4 per cent, 83.3 per cent, 56.3 per cent, 85.1 per cent and 59 – 72.6 per cent.

Mathew *et al*., (1996) evaluated five species of *Pleurotus* for their yield performance on various substrates, both for spawn production and cultivation, in the plains and in the high altitudes of Kerala in studies conducted in the summer and rainy seasons. *P. sajor-caju, P. citrinopileatus and P. florida* were most suitable species for cultivation in both the plains and high altitudes.

Three species of *Pleurotus* namely *P. ulmarius*, *P. porrigenus* and *P. euosmus* were grown on wheat straw at NRCM, Solan during the year 1998-99. The resultant yield of 360g, 450g and 470g fresh mushroom/kg of dry substrate was obtained from *P. ulmarius*, *P. porrigenus* and *P. euosmus* respectively. *P. djamor* var. *roseus* was also cultivated on wheat straw during the same year (Anonymous, 1998-99).

Five agricultural wastes such as soybean straw, corn straw, corn cobs and wheat straw were evaluated for the yield of *P. florida* at NRCM, Solan during 1998-99. Highest biological efficiency was obtained on wheat straw (85%) followed by soybean straw (65%) and corn straw (64%) (Anonymous, 1998-99).

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Sisto *et al*., (1998) cultivated *Pleurotus* mushroom on new media by mixing traditional constituents. Such as wheat straw and dried root beet residues with residues of tomato industrial processing, grapevine pruning, olive waste water, dregs of pressed grapes, triturated carobs and hay from a palyphytic pasture field, compared with traditional compost in controlled environment conditions and glass containers. *P. eryngii* grew and produced well shaped basidiocarp on all tested substrate.

Ayyappan *et al*., (2000) utilized sugarcane trash and coir waste alone and in combination with paddy straw (3:1, 1:1 and 1:3 w/w) for sporophore production of two species of *Pleurotus*. The highest yield of *P. florida* (1395 g) and *P. citrinopileatus* (1365 g) were recorded in mixture of sugarcane trash and paddy straw in equal proportion.

Zhang *et al*., (2002) cultivated *P. sajor-caju* on rice and wheat straw without nutrient supplementation. The highest yield of *P. sajor-caju* was obtained on cotton straw (993g/kg of dry straw) followed by soybean, paddy, wheat and jowar straw with yield of 935.25g/Kg, 816g/kg, 708g/kg and 445.50g/kg of dry straw respectively (Chavan *et al*., 2003). Biological efficiency of *P. djamor* was found to be 52 per cent on paddy straw (Sharma, 2003).

Rathore and Thakore (2004) reported the maximum number of sporophores and yield (106 and 468g/kg of dried substrate, respectively) on wheat straw among the substrate tested.

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Tupatkar and Jadhao (2006) cultivated *P. sajor-caju* on different agro-substrates (wheat straw, rice straw, bajra straw and leaves, cotton stalks and leaves, soybean straw, groundnut creepers + wheat straw, soybean straw + wheat straw and groundnut creepers) to correlate yield performance. They reported substrate comprised of cotton stalks and leaves gave the highest number of sporophore (178.33), average weight of sporophore (5.12g) and yield (914.032).

Zape *et al*., (2006) used wheat straw as a substrate to grow *P. florida, P. eous* and *P. flabellatus*. Highest yield and biological efficiency were obtained in *P. florida* than *P. flabellatus* and *P. eous*.

Maheshwari *et al*., (2007) reported effect of paddy straw, wheat straw and sawdust on spawn run, pinhead formation and yield of *P. flabellatus*. He found paddy straw as the best agro-waste material, took 12.6 days for spawn run and mushroom yield of 648g/kg wet substrate as compared to wheat straw and sawdust.

2.3. SPAWN PRODUCTION

Little information is available on researches done on spawn production. Generally methods for spawn production of all cultivated mushrooms are almost similar these days. Spawn making was once a highly selective procedure. In seventeenth century, the mushroom growers used to plant the horse droppings containing living mycelium which they called spawn. In 1890, the French for the first time succeeded in germinating spores of *Agaricus* for the purpose of

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obtaining sterile spawn, which found great commercial acceptability in the market (Atkinson, 1961).

The first pure culture spawn was produced by Constantin (1894) in France on horse manure compost.

Duggar (1905) gave the technique of making pure culture spawn from mushroom tissue. Until this time, preparation of brick spawn in England becomes popular. These bricks were made from mixture of horse dung, cow dung and loam soil in which mycelial growth was multiplied for using them as spawn.

Sinden (1932) introduced rye grain spawn with addition of calcium salts.

Kligman (1943) defined that “spawn is merely the vegetative mycelium from a selected mushroom grown on a convenient medium”.

Terrier (1945) used wheat grains as the basal medium for spawn production.

Sinden (1972) stated that vegetative growth is called the mycelium and the material used for seeding the beds (compost) is called spawn.

Later on, a wide variety of carbohydrates were found suitable for spawn preparation, when adjusted to a slightly acidic condition and supplied with organic nitrogen and mineral salts. This established the fact that the spawn could be prepared not necessarily on natural substrate but also on other substrate, which could provide suitable

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nutrients for these fungi (Munjal, 1973). According to him, a good quality spawn should have silky creeping mycelium, which is considered better over the fluffy type growth. The substrate on which spawn is made also affects the yield of mushroom. Now the spawn is prepared on a variety of substrate under sterile conditions (Lambert, 1959; Stoller, 1962; Kumar *et al.,* 1975).

Bahukhandi and Munjal (1989) suggested use of sterilized presoaked wheat grains for Oyster mushroom spawn while, Lee, S.S. (1991) concluded that rice bran in sawdust spawn medium provides three kinds of nutrients (starch, nitrogen and minerals) supporting fast growth, prevent contamination by other microorganisms.

Rathaiah *et al*., (1999) reported parboiled paddy gave 7.5 per cent greater yield over conventionally cooked paddy spawn. The moisture content in parboiled paddy was less than that of cooked wheat.

Shah *et al*., (1999) recorded maximum yield of *P. sajor-caju* on Trifolium straw when used as a substrate for spawn production, Other four substrate i.e. wheat grain, ragi grain, wheat straw and a combination of wheat straw and Trifolium (1:1) gave downward mycelial growth and the time taken for spawn substrate colonization was recorded. The time required for complete spawn run was 11 days for Trifolium followed by wheat grain (11 days) with the longest spawn run for wheat straw (22 days).

Jhune-chang-sung (2000) observed that 10 to 20 per cent supplementation with rice bran resulted in a good mycelial growth

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and density. Field test showed that 15 to 20 per cent addition of the supplement resulted in the highest yield, the shortest time period from spawning to pinhead appearance and the lowest infection rate.

Sharma, B.B. (2003) tried different grains viz. jowar (*Sorghum bicolor*), kutki (*Panicum miliare*), kodo (*Paspalum scrobiculatum*), maize (*Zea mays*) and wheat (*Triticum aestivum*). The shortest period for spawn development (8 days) was observed with kutki grains indicating its suitability for efficient spawn production. However, Kumar *et al*., (2004) recorded highest biological efficiency (81.00) and yield (810g) of *Pleurotus sajor-caju* with wheat grain spawn.

2.4. PHYSICAL FACTORS:

Temperature, humidity and light have a significant effect on growth and formation of fruiting bodies of *Pleurotus* spp. (Chang and Han, 1977). The growth of mycelium and formation of fruit bodies of *Pleurotus* spp. depends on a number of physical, chemical and biochemical parameters (Zadrazil, 1978).

2.4.1. Temperature

The temperature requirement for mycelial growth and fruit body formation diffuse with different species. Kurtzman and Zadrazil (1982) mentioned different temperature ranges worked out by different workers in different *Pleurotus* species. The temperature mentioned, which was optimum for mycelial run and maximum fruit

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body production as 27^{0}C and 20^{0}C, respectively in *P. corticatus* (Kaufert, 1936).

Block *et al*., (1958) reported that in *P. ostreatus* (*florida*) 32^{0}C is optimum temperature for mycelial run and 21-26^{0}C for fruit body formation. *P. cystidiosus* needed 28^{0}C for mycelial growth and 25^{0}C for fruit body formation (Miller, 1969).While for *P. eryngii*, 25^{0}C has been found optimum for mycelial run and below 20^{0}C for fruiting (Zadrazil, 1975). Jandaik and Kapoor (1976) recorded 25-30^{0}C for mycelial run and 20-25^{0}C for fruiting of *P. sajor-caju*. Temperature for *P. tuber-regium* requires being 40-45^{0}C for mycelial run and 35^{0}C for fruiting body formation (Oso, 1977). Development intensity of mycelium decreased at 10^{0}C and tolerance reached maximum at 30-35^{0}C. The spread of mycelial growth is related to the temperature of the substrate. The temperature is highly important since it affects the growth and adaptability as well as quantity and quality of fruiting bodies (Zadrazil, 1978). *P. abalones* and *P. ostreatus* var. *florida* need 30^{0}C for mycelial growth and 25^{0}C for fruiting (Huang, 1979; Zadrazil and Kurtzman, 1982).

P. sajor-caju, P. ostreatus, P. sapidus, P. flabellatus, P. florida, P. membranaceous and *P. eryngii* showed maximum mycelium growth between temperature range of 25-30^{0}C (Zadrazil, 1976; Balazs and Szabo, 1979; Sohi and Upadhyay, 1989).With regard to sporophore production, most *Pleurotus* species cultivated in the tropics required

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a slightly lower temperature ranges from 21-25^{0}C (Jandaik and Kapoor, 1976; Bano *et al*., 1979).

Kalita *et al*., (2000) observed different species of *Pleurotus* can be deployed successfully to obtain better yield during the different growing season. *P. citrinopileatus* was suitable for cultivation during March-April which had slightly higher environmental temperature while *P. sajor-caju* performed better in the month of October and January.

Kushwaha *et al*., (2000) observed that spawn run and fruiting body of all the tested species of *Pleurotus* were greatly influenced by temperature of different months. The low temperature of December and January resulted in maximum period for spawn run and fruiting of various species of *Pleurotus*.

Five species of *Pleurotus* namely *P. flabellatus, P. sajor-caju, P. florida, P. ostreatus* and *P. cystidiosus* were evaluated using paddy straw. He tried to work out possibilities of their cultivation in different months and reported that mycelial growth was good and the time required for initiation of pinhead and production of first flush was less when grown from March to November (except during May). The highest biological efficiency was recorded in October cultivated *P. flabellatus* followed by *P. sajor-caju* Dubey (2003).

Several workers reported that optimum temperature for the hyphal growth of *Pleurotus* spp. was 25^{0}C (El Fallal *et al*., 2003 ; Moorthy and Chandramohanan, 2003 and Han *et al*., 2004). However, Yao-Fangjie

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et al., (2004) reported that the optimal temperatures for hyphal growth of hybrid strains were lower than those of their parents.

2.4.2. Humidity

Humidity is the second cardinal factor which has very important role in development and growth of fruit bodies of mushroom (Zadrazil and Kurtzman, 1982). A moisture content of about 50-75 per cent was found to be optimum for the growth of mushroom mycelium and the maintenance of a high relative humidity of the air in the mushroom houses. It also reduces evaporation from the substrate surface (Flegg, 1962; Zadrazil and Brunnert, 1980).

Block *et al*., (1958), Han *et al*., (1974); Cailleus and Diop (1976) and Bano *et al*., (1979) worked out relative humidity by 90-95, 85-95, 70-80 and above 85 per cent for production of *P. abalones, P. eryngii, P. flabellatus* and *P. sajor-caju*, respectively.

Jang *et al*., (2003) and Han *et al*., (2004) reported that the most suitable relative humidity for *Pleurotus* spp. ranged from 60-80 per cent with an optimum relative humidity of 80 per cent.

2.4.3. Light

The effect of light promote fruiting of *Pleurotus* was apparently first noticed by Kaufert (1936). However, Gyurko (1972) observed that light is obligatory for sporophore development in *Pleurotus*. Stipe remains thin and cap rudimentary in complete darkness. It is inhibitory for mycelial growth in *P. ostreatus* (Eger *et al*., 1974) but according to Chang and

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Han (1977) light has no significant role in cultivation of *Pleurotus*. Branched growth and continuous segmentation of abnormal fruit bodies have been observed due to lack of light in *Pleurotus ostreatus* (Tschierpe, 1972 ; Zadrazil, 1975) while, *P. sajor-caju, P. eryngii, P. florida,* and *P. ostreatus* grew best in total darkness (Rawal and Singh, 1980).

Datta and Chakraborty (2002) observed that darkness favoured the spawn run in both *P. sajor-caju* and *V. volvacea*. But for primordial formation and their further development low intensity of light was essential.

Sharma (2004) reported that the highest growth (83.12mm) of *P. djamor* was observed in complete darkness. The lowest radial growth was recorded when light and darkness duration was same (12h; 50mm).

Jhang *et al*., (2005) recorded that the mycelia of *Pleurotus eryngii* grew better under darkness than under light (i.e. light inhibited growth of the mycelia).

2.6. RADIAL MYCELIAL GROWTH AND COLONY CHARACTERS

Kligman (1943) referred two types of colonies as “appressed”, a greyish white colony with little aerial hyphae and “fluffy” which produced an abundance of extensive aerial growth giving the appearance of cotton wool. However, many intermediate variations were encountered from “appressed” growth to “fluffy” and vice-versa.

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Goltapeh and Kapoor (1989) studied the extent of variation of six locally available strains of *A. bisporus* by observing the colony characters including colony diameter, appearance of colony, colour, shape, zonation, margin, sectoring, pigmentation and exudates formation. The colony appearance varied from appressed to fluffy, colour as white to grey-white, shape as irregular to circular, zonation not distinct, faintly zonated and conspicuous, margin as even, sub-merged and uneven.

Dutta and chakravarty (1989) reported whitish, linear colony growth of *P. flabellatus* and *P. florida* in culture. Jin and Xu, (1990) and Bahukhandi *et al*., (1991) observed variation in colony characters of strain of *A. bisporus* and *A. bitorquis* respectively. They suggested for spawn production, appressed mycelial growth is preferred to fluffy growth.

Stockton and Hargen (1993) analyzed radial growth in selected pedigrees of *A. bitorquis* (field collected, commercial and inter-specific hybrids). Out of these, the inter-specific hybrid gave greater radial growth measurements.

Maldonado (1998) reported the best growth of *Pleurotus djamor* on Malt Extract Agar medium.

Pahil *et al*., (1999) worked cytological studies of homokaryotic and dikaryotic stocks of tropical *A. bitorquis* isolates and indicated that homokaryons and dikaryons of the *A. bitorquis* can normally be distinguished on the basis of simple colony morphology. Homokaryons,

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generally have a slow and appressed mycelial growth while dikaryons exhibit a faster and stranded mycelial growth.

The mycelial growth of *P. ostreatus*, *P. sajor caju*, *P. citydeosus* and *Volvariella volvacea* were maximum on Malt Extract Agar medium (Nasim *et al*., 2001).

Yadav (2001) observed maximum growth of *P. sajor-caju* was on potato dextrose agar medium. A temperature of 25^{0}C and pH 6.0 were found to support the maximum growth.

Singh *et al*., (2000) studied the effect of different nutrient medium on all the commercially important mushrooms and reported that wheat extract agar (WEA) nutrient medium is best for all the tested species except *V. volvacea.*

Singh *et al*., (2000) and Yadav (2001) studied effect of different initial pH value of nutrient medium on *P. flabellatus, P. ostreatus, P. sapidus* and *P. sajor-caju* and found pH 5-8 most suitable to *Pleurotus* spp.

Bugarski *et al*., (2002) tested different pH values 5.0 to 6.0 of the medium for two strains of *P. ostreatus* and reported that different strains required different pH of the medium for growth.

Sanchez (2004) studied the mycelial growth of *P. pulmonarius* on potato extract agar medium (PEA) and wheat straw extract agar medium (WSEA) and found that the average hyphal length was

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11.80µm on WSEA while it was 11.75µm on PEA which indicates that WSEA is better medium than PEA.

Sawashe and Sawant (2005) carried out hybridization between *P. florida* and *P. eous*. They found that on solid medium, the hybrid cultures showed maximum mycelial growth compared to their parents while on liquid medium the hybrid cultures showed maximum average air dry weight.

2.7. HYBRIDIZATION

Optimization of industrial mushroom production depends on improving the culture process and breeding new strains with higher yield and productivities. However, not much work has been done on *Pleurotus* spp. on these aspects as compared to *Agaricus bisporus*. The major aim of hybridization is to combine desirable characteristics from different strains and create variation in the existing germplasm (Gharehaghaji *et al*., 2007). Testing the fertility of the strain is most time consuming and most important procedure in breeding of mushroom (Eger *et al*., 1976).

Chandrashekhar *et al*., (1981) investigated the mating system of *P. flabellatus* using twenty monokaryons obtained from a single sporophore. The selected strains of derived dikaryons were better performing as compared to parental dikaryons when brought under cultivation. Cultivation trials of derived dikaryons to select better strains over the parental dikaryons were found out to be promising.

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Bahukhandi and Munjal (1990) reported that the inter-stock hybrid of *P. sajor-caju* gave higher yield and in a shorter time than intra-stock hybrids. Inter-stock hybrids produced a yield of 630g per bag, while the intra-stock ones gave only 500g and 460g per bag. Earlier, similar observations were recorded by Eugenio and Anderson (1968); Wang and Anderson (1972) and Eger *et al*., (1976) in *P. ostreatus* and Anderson *et al*., (1973) in *P. sapidus.*

Genetic potential of *Pleurotus* strains can be improved through intra and inter-specific hybridization. Intra-specific hybridization yield very less percentage of improved strains (Ghosh and Chakravarty, 1991; Thakur and Bhandal, 1993), while inter-specific hybridization produces comparatively more strains with improved genetic potential (Bahukhandi and Sharma, 2002).

Nattaya and Wichain (1997) carried out hybridization between *P. ostreatus* and hybrid strain of KDCMU by means of mono-mono crossing. When the yield of hybrids and *P. ostreatus* were compared, *P. ostreatus* yielded of only 134.2g/bag while 4 strains yielded even higher with 168.2, 144.2, 148.2, 144.1g/bag, respectively.

Peng *et al*., (2001) studied breeding of three strains i.e. ATCC36047, ATCC90212 and Holland150 of *P. eryngii.* Monokaryon from ATCC6047 and Holland150 were isolated and their mating factors also determined by crossing between monokaryons derived from the two strains and screening of the hybrids thus obtained was conducted. Four highly productive hybrids i.e. B112, B012, B030 and B011 were obtained; they were different in their morphological

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characteristics and also the composition of substrate on which they grew. B012 seemed to prefer a substrate containing less nitrogen or protein and more starch with B112 and one of its parents Holland150 preferred a substrate containing more nitrogen or protein and less starch. These productive hybrids of the fungus took 54.4 to 60.0 days from inoculation to the end of the crop.

Bahukhandi and Sharma (2002) studied inter-specific hybridization between *P. sajor-caju*, *P. sapidus* and *P. cornucopiae* for obtaining quality strains. The resultant hybrids were categorized on phenotypic basis into two categories. In first group, hybrids obtained were similar in appearance, yield and colour etc. with their parents. The second group hybrids have blending of characters of their parents and possessed yield potential more or less similar to them. A specific hybrid (hybrid No. 3) obtained by mating between *P. sajor-caju* and *P. cornucopiae* in which the shape and size of the sporophore was similar to *P. sajor-caju* and white colour resembling with *P. cornucopiae*. The total average yield of this hybrid was found to be 23.3 per cent more than the parent.

Periasamy and Natarajan (2003) used conventional breeding technique for the hybridization of different monokaryons of *P. djamor* (Fr.) Boedijn var. *roseus*. The different dikaryons showed more biological efficiency, better spawn run period, fast growth, temperature tolerance and different colour of the Basidiomata than the parents. Out of twenty strains, four *Pleurotus* strains (2x10, 5x10, 10x24 and 30x24) were found superior to that of wild strain of *Pleurotus djamor* var. *roseus.*

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Maszlaver and Gyorfi (2003) compared the yield of mushroom hybrid HK 35 with *Pleurotus sajor-caju*. The yield of HK 35 was higher than that of *P. sajor-caju*.

Salmones *et al*., (2004) obtained monosporic cultures of three strains of *Pleurotus djamor* vars. *djamor* (IE-116), *roseus* (IE-116) and *salmoneostramineus* (IBUG-75). Fast and good mycelium growth was selected to make inter-specific crosses. Parental strains and six crosses were cultivated on barley straw. Results obtained were as follows, Primordial initiations in parental strains were observed between 9 to 11 days and 8 to 13 days in the crosses. On average 3 to 5 flushes were obtained, reaching biological efficiencies of 53.6 to 86.4% in the parental strains and 73.6 to 114.4% in the crosses, while rates of production were 0.89 to 1.44% and 1.22 to 1.9% and yields of 11.5 to 17.4% and 17.5 to 26.9%, respectively.

Sawashe and Sawant (2005) carried out hybridization between *P. florida* and *P. eous*. They found that on solid medium, the hybrid cultures showed maximum mycelial growth compared to their parents while on liquid medium the hybrid cultures showed maximum average air dry weight. Most of the hybrids cultures required minimum days to cover all the wheat grains compared to their parents. In general the mycelial growth of the hybrid cultures was faster as compared to their parents.

2.8. MORPHOLOGICAL CHARACTERS

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Morphological characters of the fungus which includes spore size, shape and average diameter of the sporocarp, average stipe length, width, pileus width and margin of the sporocarp were studied by various workers and are reviewed in the following pages.

2.8.1. Macromorphological characters

Pileus and stipe characteristics

Pileus length and width are morphological characters for assessment of basidiomata qualities.

Vooticumpee (1996) studied the average size of pileus and length of *P. sajor-caju*, *Pleurotus* spp. (Bhutanese oyster mushroom) and *P. ostreatus*. The pileus diameter and stipe length was 5.95-7.38cm, 1.30-3.95cm in *P. sajor-caju*, 6.23-7.50cm, 1.0-6.21cm in *Pleurotus* spp. and 5.68-6.18cm, 1.11-5.66cm in *Pleurotus ostreatus*, respectively.

Das *et al*., (2000) found that the sporophore diameter of *P. sajor-caju* ranges from 9.08-11.62cm while sporophore diameter of *P. florida* ranged from 5.11-8.24cm when grown on the wild grasses in winter season.

Chandrashekhar and Savalgi (2003) reported that the average sporophore diameter of *P. sajor-caju* ranged from 5.17-8.55cm on different sugarcane by-products. They also reported that the maximum stipe length (3.29cm) of *P. sajor-caju* was found on paddy straw. They observed that the length and width of pileus vary greatly in different substrates, species and different hybrids.

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Periasamy and Natarajan (2003) observed that the hybrids of *Pleurotus djamor* were different with each other in pileus morphology. The pileus length and width ranges from 5.2-8.4cm and 6.0-12.1cm, respectively. They also reported that the stipe length and width varied among the recombinant strains of *P. djamor*. The maximum length and width was 2.8 and 1.3cm and minimum was 0.7 and 0.6cm, respectively. Average size of stipe length and width also varied species to species.

Margin of *P. sajor-caju, P. florida* and *P. cornucopiae* were irregular, entire and dentate, respectively. Margins of *Pleurotus* spp. was found irregular, entire, dentate, incurved and lobed at maturity (Bahukhandi, 1986).

Periasamy and Natarajan (2003) also reported that the margin of wild and recombinant strains of *P. djamor* was recorded as entire, wavy, petaloid and multiwavy.

Tupatkar and Jadhao (2006) cultivated *P. sajor-caju* on different agro-substrates (Wheat straw, rice straw, bajra straw and leaves, cotton stalks and leaves, soybean straw, groundnut creepers + wheat straw, soybean straw + wheat straw and groundnut creepers) to correlate morphological parameters. The highest stipe length (3.56cm) and pileus size (29.40 cm^2) was obtained with soybean straw + wheat straw combination.

Mandhare *et al*., (2007) cultivated different *Pleurotus* spp. viz. (*P. sajor-caju*, *P. eous*, *P. flabellatus*, *P. florida, P. sapidus*) on paddy

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straw. On an average, stipe length (cm) and pileus size (cm^2) were assessed. Highest reported in *P. florida* (4.06, 54.25) followed by *P. flabellatus* (3.05, 38.17) and *P. sapidus* (2.06, 30.11) which was at par with *P. sajor-caju* (2.02, 27.50) and *P. eous* (1.97, 25.55).

2.9 BIOCHEMICAL CHARACTERS

Biochemical character of the fungus which include moisture, carbohydrate and fiber, protein, fat, vitamins and minerals. These parameters show wide variation from species to species, flush to flush, substrate, cultivation technology and the conditions at the stage of harvest as well as post harvest (Rai and Saxena, 1989; Crison and Sands, 1978).

Proximate composition of *Pleurotus* mushroom:

Component	:	Percentage by dry weight
Moisture	:	90.2-93.3
Carbohydrate	:	46.6-81.8
Crude protein	:	25.2-36.4
Crude Fat	:	1.5-1.9
Ash	:	9.5-12.3
Crude fibre	:	10.7-12.4 (Kausar *et al*., 1994)

Dry oyster mushrooms have been well known for good quality of protein due to presence of all essential amino acids in them and are especially rich in lysine required by the human being. Protein content in *Pleurotus* spp. has been documented to range between 8.9 and 38.7 per cent on dry weight basis (Bano and Rajarathnam, 1982) and 3.5 to 4 per cent on fresh weight basis (Chang and miles, 1989). Which shows

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that the protein content of mushrooms was much higher than cereals (wheat and maize) and comparable to that of food legumes (peas and lentil) (Rai, 1997; Shah *et al*., 1997).

The high protein content associated with low sugar/starch. Bano and Rajarathnam, 1982 reported carbohydrate content in the range of 46.6 to 81.8 per cent in *Pleurotus* spp on dry weight basis. Rai *et al*.,(1988) evaluated total carbohydrate content on a fresh weight basis range between 2.98 and 6.04 in seven *Pleurotus* spp (*P. eryngii, P. flabellatus, P. florida, P. membranaceus, P. ostreatus, P. sajor-caju, P. sapidus)*. Lowest carbohydrate content was in *P. eryngii* while *P. membranaceus* had the highest carbohydrate content.

Gupta *et al*., (1982) studied nutritive value of *Pleurotus eous* grown on chopped rice straw. The fruiting bodies contained 15.50 per cent reducing sugar, 0.31 per cent non reducing sugar and 27.4 per cent protein on dry weight basis.

Park and Kim (1988) studied effects of different substrates on chemical constituents of the fruit bodies in *P. ostreatus*. Out of three substrates, sawdust and logwood gave higher contents of total sugar and non-reducing sugar in the fruit bodies; while the reducing sugar content was higher in the rice straw substrate.

Yosida *et al*., (1987) reported total free sugars in hiratake mushroom (*P. ostreatus*) were 6.8-15.6% in pilei; 10.1-27.2% in stipes and 9.3-24.9% in bases of stipes on a dry weight basis.

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El-Kattan *et al*., (1991) calculated proximate composition of protein (g/100 g dry wt. basis) of *P. florida* and *P. sajor-caju* was 13.2 and 14.3 respectively.

Dhanda *et al*., (1996) cultivated four *Pleurotus* species, namely *P. flabellatus*, *P. florida, P. sajor-caju* and *P. ostreatus* on wheat straw. *P. ostreatus* and *P. sajor-caju* were found to have high protein content 27.4 and 26.9 per cent dry weight, respectively.

Zhang *et al*., (1998) cultivated *P. sajor-caju* on rice and wheat straw. The protein content of mushroom produced was 27.2 per cent on average on dry matter basis.

Gupta, S., (1998) found that fruiting bodies of *P. sajor-caju* are rich in potassium, sodium, iron, calcium and magnesium.

Ekanem and Ubengama (2002) collected oyster mushroom (*P. ostreatus*) at the button (day-old) and full-grown (mature) stages from dead mango tree stumps. Results showed that *P. ostreatus* contains significantly higher levels of protein at the full-grown stage than at the button stage.

Rios-Hurtado *et al*., (2003) cultivated *P. sajor-caju* on sawdust, maize tops, banana leaves and rice waste. The protein contents were 29.94, 31.94, 38.13 and 14.69 per cent respectively.

Gupta and Sarmah (2004) cultivated *P. sajor-caju* on paddy straw, banana leaves, sugarcane baggase, water hyacinth and beetle nut husk. Considerable variation in the composition of fruit bodies grown on different substrates was observed. Protein content varies from 18.46 to 27.78 per cent respectively on the dry matter basis.

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Oyetayo and Akindahunsi (2004) analysed two varieties of the *P. sajor-caju* (those obtained from the wild and those cultivated on shredded corn cobs). The caps of cultivated fungi accumulated higher concentration of crude protein than caps of the wild one.

Bonatti *et al*., (2004) evaluated protein content of *Pleurotus* on the fresh weight basis and found protein values to be similar to, even higher than, the values observed in various vegetables but lower than the protein contents of eggs, meat and cheese.

Alencia *et al*., (2006) analyzed biological quality of proteins from three strains of *Pleurotus* spp., were grown on a commercial substrate. Fruit bodies of all strains (IE136, INI8, PORO) presented high protein content, around 27 per cent with very high protein in vitro digestibility (98%).

Lalani and Patel (2007) cultivated *P. florida* on different substrates viz wheat straw, paddy straw, maize straw, cotton stalks, and sugarcane bagasse. Highest protein was found in mushroom obtained from sugarcane bagasse (12.3%) followed by maize straw (11.2%) and cotton stalks (9.1%) which was at par with wheat straw (8.9%) and paddy straw (8.5%).

Mandhare *et al*., (2007) studied nutritive value of *Pleurotus* species grown on paddy straw. *P. florida* contained highest protein (28.9%) followed by *P. flabellatus* (27.7%) and *P. eous* (25.0%). All the *Pleurotus* species contained low reducing sugar (1.59-1.76%).

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MATERIALS AND METHODS

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3.1 SELECTION OF PARENTS AND THEIR HYBRIDS

The culture of ten available species of *Pleurotus* (parents) viz. *P. sajor-caju* (Fries) Singer, *P. djamor* (Fr.) Boedjin var. roseus, *P. florida* (Eger), *P. flabellatus* (Berk & Br.) Sacc, *P. platypus* (Cook & Massee.), *P. fossulatus* (Fr.), Singer, *P. citrinopileatus* (Fr.) Singer, *P. ostreatus* (Fr.) Jacq., *P. sapidus* (Schulzer) kalchbr and *H. ulmarius* (Singer) and a total of thirteen available species of *Pleurotus* hybrids i.e. A_1B_5, A_4B_4, A_6B_{14}, $A_{12}B_{12}$, $A_{13}B_{13}$ (hybrids of first sets of cross *P. sajor-caju* x *P. djamor*), A_1C_1, A_1C_2 , A_5C_6 (hybrids of second sets of cross *P. sajor-caju* x *P. florida*), A_2D_4, A_7D_7, $A_{11}D_6$ (hybrids of third sets of cross *P. sajor-caju* x *P. flabellatus*) and F_1H_1, F_4H_4 (hybrids of fourth sets of cross *P. florida* x *H. ulmarius*) were obtained from Mushroom Research and Training Center, Pantnagar. Each culture was multiplied on Malt Extract Agar medium and maintained in culture tubes at 3 to 7°C for further studies. All the species of *Pleurotus* were cultivated using wheat straw as substrate employing standard practices. At last selection of the species having outstanding characters were made on the basis of their yield and quality (morphological) parameters of the sporophores.

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3.2 CULTURES AND THEIR MAINTENANCE

Each selected culture species were revived fresh on Malt Extract Agar medium using hyphal tip method on the Petriplates and were incubated at 24 ± 2°C temperatures for about a week for their growth. The MEA medium was used to maintain stock culture in refrigerator for further studies.

3.2.1 COMPOSITION OF CULTURE MEDIA USED

Malt Extract Agar

Malt Extract	:	20 g
Agar	:	20 g
Distilled water	:	1000 ml

The media was autoclaved at 121°C in 15 lbs psi for 20 minutes.

3.3 CULTURAL VARIABILITY OF SELECTED PARENTS AND THEIR HYBRIDS

3.3.1 Radial growth of selected parents and their hybrids

The selected parents and their hybrids were grown on Malt Extract Agar medium for morphological variations and colony characteristics. Petriplates containing 25 ml of the medium were inoculated at the center with precut 3-4mm diameter discs of actively growing mycelium under aseptic conditions. Three replicates were maintained and these plates were incubated at 24 ± 2 ^{o}C temperature in a BOD incubator. Observations on radial growth were taken from 2nd day of inoculation till the colony covered the plate fully.

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3.3.2 Comparative Colony characters on MEA

The growth of parents and their hybrids were studied for comparative colony characters using MEA medium. A 7 mm mycelial disc was placed at the center of petriplate containing 25 ml of solidified media. The treatments were replicated thrice and incubated at 24 ± 2 ºC temperature in a BOD incubator. After 7 days these plates were observed for Colony characters viz., appearance, and colour, shape, zonation, margin, pigment and exudates formations.

3.4 CULTIVATION TECHNOLOGY

3.4.1 Spawn preparation

The spawn was prepared on wheat grains in half-liter capacity glass bottles. The grains were soaked in clean water and then washed. They were boiled (wheat grain and water 1:25 w/v) in water for 20-25 minutes taking care that the grains do not split during boiling. The boiled grains were spread over a wire net for overnight to remove excessive water and also to enable them to cool down to about 20-25^{0}C. The cooled grains were then mixed with 1.2 per cent commercial grade gypsum ($CaSO_4$) and 0.3 per cent calcium carbonate ($CaCO_3$) in the ratio of 4:1 (w/w). Gypsum prevents the sticking of wheat grains together and lime maintains the p^H 5.5-7.5. The grains were then filled in clean glass bottles up to 2/3rd of its capacity. The bottles were plugged with non-absorbent cotton and covered with butter paper. These bottles were then sterilized at 22 lbs steam pressure (121^{0}C) for 90 minutes. Sterilized bottles were taken

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out from the autoclave, while still hot and were shaken to avoid clumping of grains.

Next day, the bottles were inoculated with bits of a vigorously growing culture of the fungus. Inoculated bottles were incubated at 25±1^{0}C and shaking is done after 7 days. Entire grains were covered with fine mycelial growth after 15 days. This spawn is known as mother or master spawn. The spawn prepared for commercial use was on sterilized grains in polypropylene bags inoculated with mother spawn.

3.4.2 Substrate preparation

The substrate used for the experiment was wheat straw. It was soaked in a tank of water chemically treated with bavistin 7.0g + formalin 100ml/100 liters of water for 18 hours. The tank was covered with polythene sheets to prevent the evaporation of formalin. Thereafter, straw was taken out from the tank and kept for 2-3 hours to drain out excess of water and spread on cemented floor treated with 2 per cent formalin for two hours and then spawning is done.

3.4.3 Spawning

Through method was employed for spawning. The freshly prepared grain spawn was used @ 2 per cent of the wet weight of substrate and filled up in polythene bags @ 5.00 kg/bag. The upper surface of the polythene bags was folded and stapled. Thereafter, 8-10

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small holes were made with the help of nail pin. Then the bags were kept in crop room for spawn run.

3.4.4 Spawn run

The bags were kept in the crop room at relative humidity of 60-80 per cent and temperature ranging 22-25 ^{0}C. After the spawn run in straw, it becomes a compact mass within a period of 15-18 days. Then the polythene bags were removed making cut and kept for sporophore formation.

3.4.5 Harvesting

Fruit bodies were harvested after maturity. The yield data for four flushes in a period of 30 days from first flush were recorded in terms of number, weight, weight per fruit body and Biological efficiency.

$$\text{Biological efficiency (B.E.\%)} = \frac{\text{Total fresh weight of fruit bodies}}{\text{Dry weight of substrate}} x100$$

3.5 MORPHOLOGICAL VARIABILITY OF SELECTED PARENTS AND THEIR HYBRIDS

Morphological and qualitative differences among the fruiting bodies of different parents and their hybrids were studied selecting ten fruit bodies randomly starting from first flush. Morphological variations and quality characters among fruit bodies were studied by measuring the fruit body weight, stipe length and width, pileus length and width, colour and margin of sporophores (Lodge *et al*., 2004).

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3.6 BIOCHEMICAL VARIABILITY OF SELECTED PARENTS AND THEIR HYBRIDS

3.6.1 Estimation of total soluble sugars

The amount of total soluble sugars was estimated using the "Anthrone method" colorimetrically (Hedge and Hofriter, 1962). Reagents required were 2.5N HCL, anthrone reagent (200mg anthrone was dissolved in 100 ml ice cold 95 per cent sulphuric acid) and standard glucose (stock-100mg of glucose was dissolved in 100 ml water, working standard 10 ml of stock was diluted to 100 ml with distilled water).

Sample (100 mg) was weighed into boiling tube. It was hydrolyzed by keeping it in boiling water bath for 3 hours with 5 ml of 2.5 N HCL and then cooled to room temperature. The solution was neutralizing with solid sodium carbonate until the effervescence ceased. The volume of solution was made to 100 ml with distilled water and centrifuged at 5000 rpm for 10 minutes. Supernatant was collected and 0.5 ml aliquot was taken for analysis. The standard was prepared by taking 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard. The volume was made to 1 ml in all the tubes including the sample tubes by adding distilled water. Then 4 ml of anthrone reagent was added. The solution was heated in a boiling water bath for 8 minutes and cooled down rapidly and the green to dark green colour was read at 630 nm. A standard graph was drawn by plotting concentration of the standard of X- axis versus absorbance on the Y-

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axis. From the graph, the amount of carbohydrates present in the sample tube was calculated using the following formula:

$$\text{Amount of carbohydrate present in sample (\%)} =$$

$$= \frac{\text{Sugar volume from graph}}{\text{Aliquot sample used (0.5)}} \text{ x } \frac{\text{Total volume of extract}}{\text{Weight of sample}} \text{ x100}$$

3.6.2 Estimation of reducing sugars

Reducing sugars were estimated following the “Nelson Somogyi method” (Somogyi, 1945 and 1952). Reagents required were: Alkaline copper arsenomolybdate reagent and standard stock glucose solution.

The sample (100 mg) was weighed and sugar was extracted with hot 80 per cent ethanol twice (5 ml each time). Supernatant was collected and evaporated on water bath. Distilled water (10 ml) was added to dissolve the sugars. Aliquots of 0.1 ml of alcohol-free extract were pipette out to separate test tubes. The working standard solution (0.2, 0.4, 0.6, 0.8 and 1.0 ml) was pipetted out into a series of test tubes. The volume was made to 2 ml in all samples and standard tubes with distilled water. Alkaline copper tartarate reagent (1 ml was added to each test tube) the tubes were placed in boiling water for 10 minutes. Tubes were cooled down and 1 ml of arsenomolybdic acid reagent was added to all the tubes. The volume was made to 10 ml with distilled water and absorbance of blue colour at 620 nm was read after 10 minutes. From the graph, the amount of

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reducing sugars present in the sample was calculated using the following formula:

$$\text{Reducing sugars in sample } (\%) = = \frac{\text{Sugar volume from graph}}{\text{Aliquot sample used (0.5)}} \text{ x } \frac{\text{Total volume of alcohol free extract}}{\text{Weight of sample}} \text{ x100}$$

3.6.3 Estimation of non-reducing sugars

The contents of non-reducing sugars were calculated by subtracting the reducing sugar from total carbohydrate content.

Non-reducing sugars

= Total carbohydrate Content – Amount of reducing sugars in samples (%)

3.6.4 Estimation of proteins:

The amounts of protein were calculated using the method of Lowry (Lowry *et al*., 1951).

The reagents required were:

(i) Solution A: 2 per cent sodium carbonate in 0.1 N NaOH.

(ii) Solution B: 0.5 per cent copper sulphate in 1% sodium potassium tartarate.

(iii) Solution C: Solution A (50 ml) was mixed with 1 ml of Solution B just prior to use.

(iv) Folin- ciocalteu reagent (FCR): The commercial reagent (2N) was diluted with an equal volume of water.

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(v) Stock standard protein solution: 50 mg of bovine serum albumin/50 ml of water.

(vi) Working standard solution: Stock solution (10 ml) was diluted to 50 ml with distilled water to obtain 200 µg protein/ml.

The sample (0.5) was ground with a phosphate buffer (pH 7.0) in the mortar with the help of pestle. It was centrifuged and the supernatant was used for protein estimation. The Working standard solution (0.2, 0.4, 0.6, 0.8 and 1.0 ml) was pipetted out into a series of test tubes. The sample extract (0.1) was also pipetted out into other test tubes. The volume was made to 1 ml with distilled water in all the tubes. A tube with 1 ml of water served as the blank. In it, 5 ml of Solution C was added. It was mixed well and incubated at room temperature for 10 minutes. After it, Folin-ciocalteu reagent (0.5 ml) was added and mixed and the solution was incubated at room temperature in dark for 30 minutes. The absorbance at 660 nm was taken. A standard graph was drawn and the amount of protein in the sample was calculated.

3.7 STATISTICAL ANALYSIS OF DATA

Statistical analysis of the data was done at the Computer Centre of G.B. Pant University of Agriculture & Technology, Pantnagar, and using appropriate programme as per the requirement of the experiment. Critical difference (CD) calculated at 5 per cent level of significance were used for comparison of difference between the treatment means.

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RESULTS AND DISCUSSION

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4.1. SELECTION OF PARENTS

A total of ten species of *Pleurotus* i.e. *P. sajor-caju* (Fries) Singer, *P. djamor* (Fr.) Boedjin var. roseus, *P. florida* (Eger), *P. flabellatus* (Berk & Br.) Sacc, *P. platypus* (Cook & Massee.), *P. fossulatus* (Fr.), Singer, *P. citrinopileatus* (Fr.) Singer, *P. ostreatus* (Fr.) Jacq., *P. sapidus* (Schulzer) kalchbr and *H. ulmarius* (Singer) were screened for their yield and quality, and finally five *Pleurotus* spp.(parents) viz. *P. sajor-caju* (Fries) Singer, *P. djamor* (Fr.) Boedjin var. roseus, *P. florida* (Eger), *P. flabellatus* (Berk & Br.) Sacc, and *H. ulmarius* (Singer) were selected on the basis of the yield and quality parameters. The selected *Pleurotus* spp. were further evaluated and variability amongst them observed in terms of culture & colony characters, morphological, yield performance and bio-chemical make up.

4.1.1. Yield Parameters for Parents

Among the ten parents studied for spawn run period, *P. sajor-caju* took significantly less period (10.50 days) while other spp. of the *Pleurotus*, *P. djamor*, *P. florida*, *P. flabellatus* and *H. ulmarius* completed spawn run in a period of 13.50, 13.00, 13.50 and 14.00 days respectively (Table 4.1 and Fig. 4.1). The number of sporophores varied significantly between the species. Maximum, 91.25 numbers of sporophores were produced by *P. platypus* followed by *P. sajor-caju* producing 89.55

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Table 4.1. Selection of the *Pleurotus* spp (parents) on the basis of yield parameters

Sl. No.	Parents	Spawn run (days)	No of sporophores	Av. Yield g/kg dry substrate	Av.wt./fruit body	B.E.(%)
1.	*P. sajor-caju*	10.50	89.55	824.83	9.21	82.48
2.	*P. djamor*	13.50	85.38	473.31	5.54	47.33
3.	*P. florida*	13.00	75.40	653.53	8.66	65.35
4.	*P. flabellatus*	13.50	56.55	763.04	13.50	76.30
5.	*P. platypus*	16.50	91.25	635.20	6.96	63.52
6.	*P. fossulatus*	14.75	81.01	410.34	5.06	41.03
7.	*P. citrinopileatus*	17.00	76.56	594.67	7.76	59.46
8.	*P. ostreatus*	18.00	83.24	490.15	5.88	49.01
9.	*P. sapidus*	16.00	74.67	544.33	7.30	54.43
10.	*H. ulmarius*	14.00	61.53	631.36	10.26	63.13
	CD at 5%		4.35	7.66		

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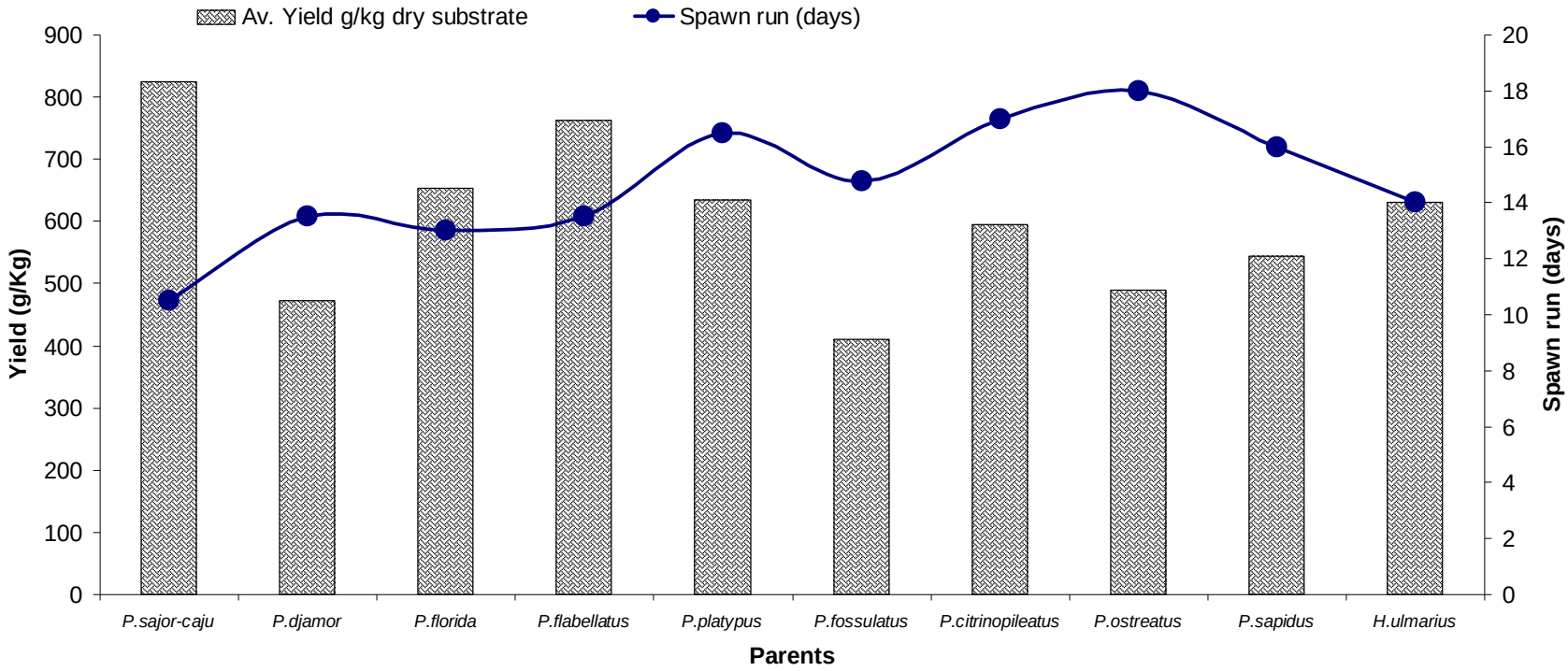


Fig. 4.1. Selection of the *Pleurotus* spp (parents) on the basis of yield parameters

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numbers of sporophores. The maximum average yield of 824.83g per kg dry substrate was recorded from *P. sajor-caju*. It was followed by *P. flabellatus* (763.04g) and *P. florida* (653.53g) respectively. Among the ten species of oyster mushroom, maximum average weight (13.50g) per sporophore was recorded in case of *P. flabellatus* followed by *H. ulmarius* (10.26g) and *P. sajor-caju* (9.21g) respectively while average weight of remaining spp. was considerably lower (Table 4.1).

4.1.2. Quality Parameters for Parents

The smallest stipe length was observed in *P. djamor* (1.05cm) followed by *P. flabellatus* (3.37cm) and *P. sajor-caju* (3.53cm) respectively. However, stipe width was maximum in *H. ulmarius* (1.42cm) followed by *P. florida* (1.27cm), *P. ostreatus* (1.25cm), *P. sajor-caju* (1.14cm) and *P. djamor* (1.03cm). Significantly maximum average pileus length (7.85cm) was measured in *P. flabellatus* followed by *P. florida* (7.12cm) and *P. sajor-caju* (7.06cm). In view of width of pileus, *H. ulmarius* (8.08cm) proved to be more valuable compared to others (Table 4.2).

The margin of fruit bodies were observed, entire in *P. sajor-caju*, *P. flabellatus, P. platypus*, *P. fossulatus*, *P. citrinopileatus* and *H. ulmarius*, wavy in *P. djamor*, dentate in *P. florida,* entire inrolled (*P. ostreatus* and *P. sapidus*) and inrolled in *H. ulmarius.* Colour of fruit bodies of different species of oyster mushroom varied significantly. It was pink (*P. djamor*), grey (*P. sajor-caju* and *P. ostreatus*), creamy white (*P. flabellatus* and *P. fossulatus*), blue (*H. ulmarius*), white (*P. florida*) and dull white (*P. sapidus*) (Table 4.2).

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Table 4.2. Selection of the *Pleurotus* spp (parents) on the basis of quality parameters

Sl. No.	Parents	Stipe Length (cm)	Stipe Width (cm)	Pileus Length (cm)	Pileus Width (cm)	Colour of Fruit body	Margin of pileus
1.	*P. sajor-caju*	3.53	1.14	7.06	7.96	Grey	Entire
2.	*P. djamor*	1.05	1.03	6.51	7.11	Pink	Wavy
3.	*P. florida*	4.62	1.27	7.12	7.40	White	Dentate
4.	*P. flabellatus*	3.37	1.02	7.85	7.94	Creamy White	Entire
5.	*P. platypus*	4.25	0.90	6.02	4.90	White	Entire
6.	*P. fossulatus*	4.28	0.96	6.50	6.97	Creamy White	Entire
7.	*P. citrinopileatus*	3.89	1.00	6.22	7.67	White	Entire
8.	*P. ostreatus*	3.78	1.25	6.33	7.21	Grey	Entire
9.	*P. sapidus*	4.30	0.86	6.90	7.60	Dull White	Entire
10.	*H. ulmarius*	4.76	1.42	6.83	8.08	Blue	Inrolled
	CD at 5%	0.29	0.16	0.14	0.19		

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4.2. SELECTION OF HYBRIDS

A total of thirteen species of *Pleurotus* hybrids i.e. A_1B_5, A_4B_4, A_6B_{14}, $A_{12}B_{12}$, $A_{13}B_{13}$ (hybrids of first sets of cross *P. sajor-caju* x *P. djamor*), A_1C_1, A_1C_2 , A_5C_6 (hybrids of second sets of cross *P. sajor-caju* x *P. florida*), A_2D_4, A_7D_7, $A_{11}D_6$ (hybrids of third sets of cross *P. sajor-caju* x *P. flabellatus*) and F_1H_1, F_4H_4 (hybrids of fourth sets of cross *P. florida* x *H. ulmarius*) were screened for their yield and quality, and finally four *Pleurotus* hybrids viz. A_1B_5 (*P. sajor-caju* × *P. djamor*), A_1C_2 (*P. sajor-caju* × *P. florida*), $A_{11}D_6$ (*P. sajor-caju* × *P. flabellatus*) and F_4H_4 (*P. florida* × *H. ulmarius*) were selected on the basis of the yield and quality parameters. The selected *Pleurotus* hybrids were further evaluated and variability amongst them observed in terms of culture & colony characters, morphological, yield performance and bio-chemical make up.

4.2.1. Yield Parameters for Hybrids

Among the four different sets of cross, thirteen hybrids were screened. For spawn run period, A_1B_5 took significantly less period (12.50 days) while other hybrids among different sets of cross A_1C_2, $A_{11}D_6$ and F_4H_4 completed spawn run in a period of 13.00, 13.00 and 13.50 days respectively (Table 4.3 and Fig. 4.2). The number of sporophores varied significantly between the hybrids. Maximum, 138.65 numbers of sporophores were produced by A_1C_2 followed by $A_{11}D_6$ (107.21) and A_1B_5 (110.05) respectively among different sets of cross. The maximum average yield of 1534.55g per kg dry substrate was recorded from A_1C_2. It was followed by $A_{11}D_6$ (1168.25g), A_1B_5 (976.75g) and F_4H_4 (907.33g)

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Table 4.3. Selection of the *Pleurotus* spp (hybrids) on the basis of yield parameters

Sl. No.	Hybrids	Spawn run (days)	No of sporophores	Av. Yield g/kg dry substrate	Av. wt./fruit body	B.E.(%)
1.	A_1B_5	12.50	110.05	976.75	8.87	97.67
2.	A_4B_4	12.75	97.87	920.40	9.40	92.04
3.	A_6B_{14}	13.50	92.40	933.46	10.10	93.34
4.	$A_{12}B_{12}$	11.00	40.04	613.16	15.31	61.31
5.	$A_{13}B_{13}$	14.25	116.83	935.71	8.00	93.57
6.	A_1C_1	13.50	105.30	1158.86	11.00	115.88
7.	A_1C_2	13.00	138.65	1534.55	11.06	153.45
8.	A_5C_6	12.00	114.95	858.40	7.46	85.84
9.	A_2D_4	13.00	93.93	899.96	9.58	89.99
10.	A_7D_7	14.50	72.88	708.52	9.72	70.85
11.	$A_{11}D_6$	13.00	107.21	1168.25	10.90	116.82
12.	F_1H_1	13.50	101.03	925.50	9.16	92.55
13.	F_4H_4	13.50	97.53	907.33	9.30	90.73
	CD at 5%		4.37	59.50		

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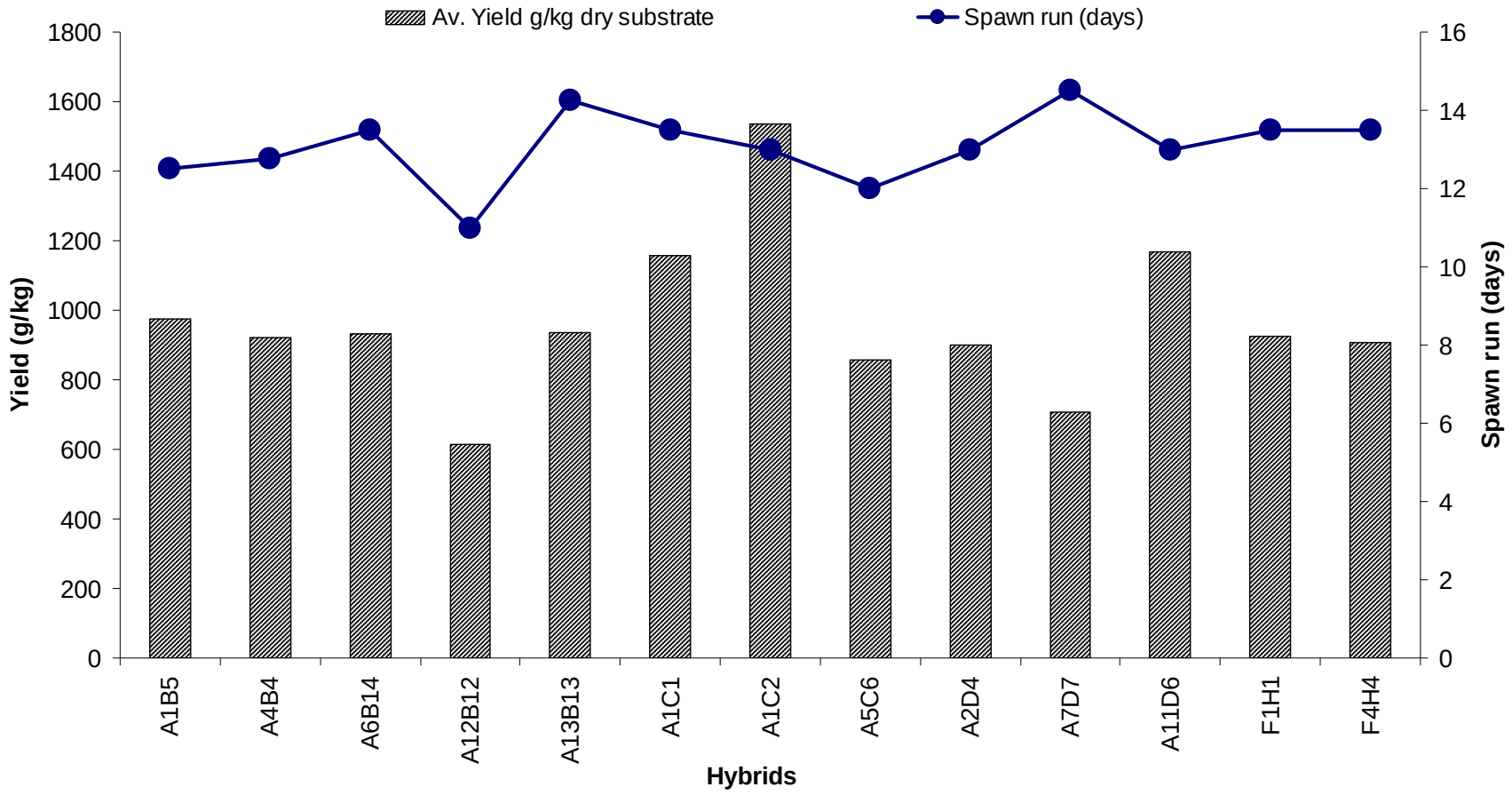


Fig.4.2. Selection of the *Pleurotus* spp (hybrids) on the basis of yield parameters

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respectively among different sets of cross. Among the four different sets of cross, maximum average weight 11.06g per sporophore was recorded in case of A_1C_2 followed by $A_{11}D_6$ (10.90g) while average weight of remaining hybrids was considerably lower (Table 4.3).

4.2.2. Quality Parameters for Hybrids

The maximum stipe length was observed in A_1B_5 (4.47cm) followed by F_4H_4 (4.26cm). While stipe width was maximum in A_4B_4 (1.39cm) followed by A_1B_5 (1.35cm), F_4H_4 (1.23cm) and A_1C_2 (1.20cm). Significantly maximum average pileus length (7.85cm) was measured in A_2D_4 followed by $A_{11}D_6$ (7.78cm) and A_6B_{14} (7.31cm). In view of width of pileus, A_1C_2 (8.26cm) proved to be more valuable compared to others (Table 4.4).

The margin of fruit bodies were observed, entire in A_1B_5, A_1C_1, A_1C_2, A_5C_6, A_2D_4, A_7D_7 and $A_{11}D_6$, wavy in A_6B_{14}, irregular in A_4B_4, $A_{13}B_{13}$, F_1H_1 and F_4H_4, and inrolled in $A_{12}B_{12}$. Colour of fruit bodies of different hybrids varied significantly. It was grey (A_1B_5, A_4B_4, A_6B_{14}, $A_{12}B_{12}$, $A_{13}B_{13}$, A_1C_1, A_1C_2), white (A_5C_6, F_1H_1 and F_4H_4) and creamy white (A_2D_4, A_7D_7 and $A_{11}D_6$) (Table 4.4).

4.3. CULTURAL VARIABILITY OF SELECTED PARENTS AND THEIR HYBRIDS

4.3.1. Radial Growth of Selected Parents and Their Hybrids

The results showed that among parents, maximum growth and growth rate was found in *P. florida* (65.00mm and 8.12mm/day) respectively which was at par with the growth & growth rate of *P. sajor-*

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Table 4.4. Selection of the *Pleurotus* spp (hybrids) on the basis of quality parameters

Sl. No.	Hybrids	Stipe length (cm)	Stipe width (cm)	Pileus length (cm)	Pileus width (cm)	Colour of fruit body	Margin of pileus
1.	A_1B_5	4.47	1.35	6.05	7.45	Grey	Entire
2.	A_4B_4	3.04	1.39	5.95	7.65	Grey	Irregular
3.	A_6B_{14}	3.99	1.11	7.31	7.78	Grey	Wavy
4.	$A_{12}B_{12}$	4.12	0.87	6.62	7.74	Grey	Inrolled
5.	$A_{13}B_{13}$	3.05	0.77	7.03	7.86	Grey	Irregular
6.	A_1C_1	3.31	1.08	6.55	6.87	Grey	Entire
7.	A_1C_2	3.12	1.20	6.81	8.26	Grey	Entire
8.	A_5C_6	1.47	1.18	7.02	8.10	White	Entire
9.	A_2D_4	3.08	1.04	7.85	7.72	Creamy White	Entire
10.	A_7D_7	2.08	1.10	7.12	7.80	Creamy White	Entire
11.	$A_{11}D_6$	4.05	1.08	7.78	7.05	Creamy White	Entire
12.	F_1H_1	4.15	1.16	6.25	7.55	White	Irregular
13.	F_4H_4	4.26	1.23	6.32	7.95	White	Irregular
	CD at 5%	0.14	0.11	0.11	0.15		

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Table 4.5. Radial growth of selected parents & their hybrids on Malt Extract Agar Medium (mm)

Sl. No.	Parents/Hybrids	Observation (days)				Average growth rate (mm/day)
		2nd	4th	6th	8th	
1.	*P. sajor-caju* (A)	15.63	28.30	44.66	64.00	8.00
2.	*P. djamor* (B)	13.63	25.33	41.33	61.00	7.62
3.	*P. florida* (C or F)	15.93	29.66	45.50	65.00	8.12
4.	*P. flabellatus* (D)	14.33	27.00	43.00	62.33	7.80
5.	*H. ulmarius* (H)	13.60	26.00	42.33	62.00	7.75
6.	A_1B_5	15.50	24.50	42.66	63.00	7.87
7.	A_1C_2	15.75	26.80	43.63	64.33	8.04
8.	$A_{11}D_6$	15.23	25.66	42.00	63.66	7.95
9.	F_4H_4	14.75	24.66	41.33	62.66	7.83
	CD at 5%					
	Parents/Hybrids		0.815			
	Days		0.543			
	Parents/Hybrids × Days		1.630			

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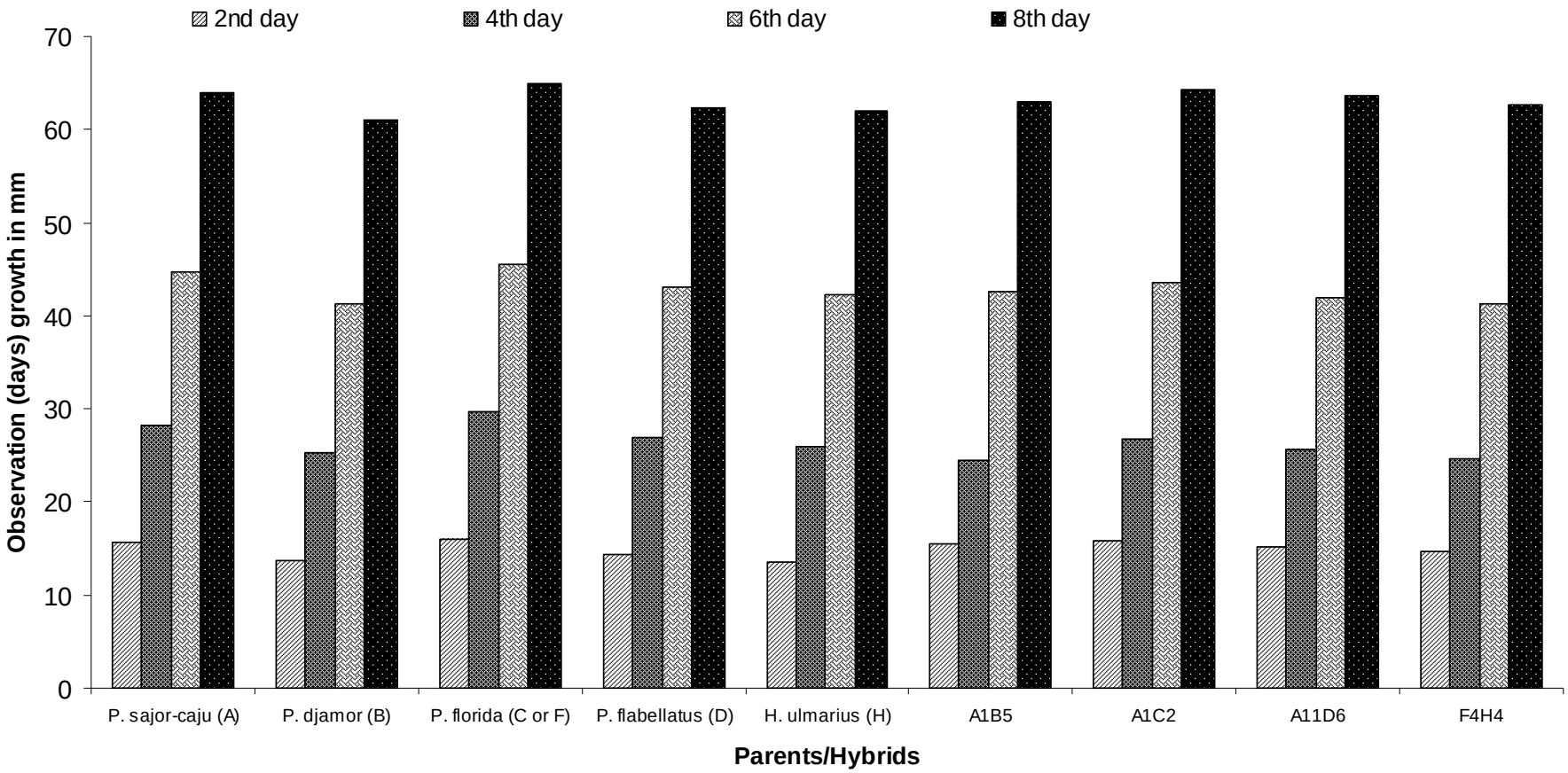
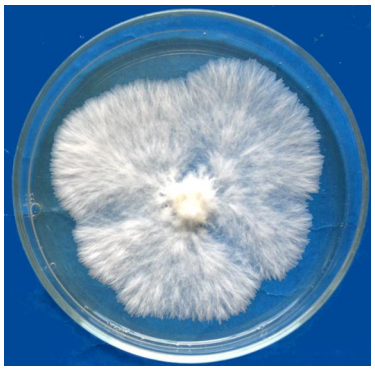


Fig. 4.3. Radial growth of selected parents & their hybrids on Malt Extract Agar Medium (mm)

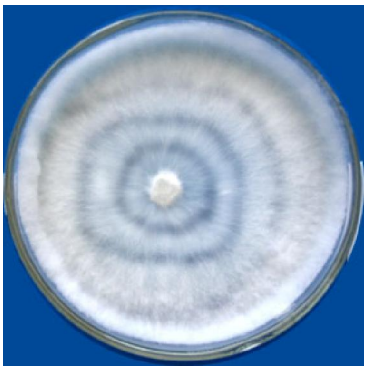
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P. sajor-caju



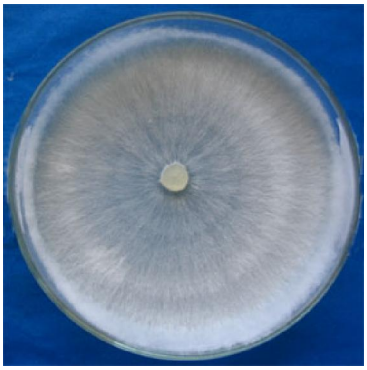
P. djamor



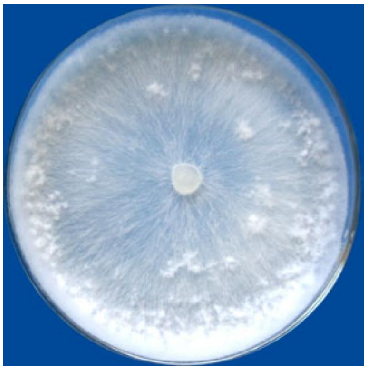
P. florida



P. flabellatus



H. ulmarius



A_1B_5



A_1C_2



$A_{11}D_6$



F_4H_4

Plate-1. Radial growth of selected parents and their hybrids on MEA media.

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caju (64.00mm and 8.00mm/day), *P. flabellatus* (62.33mm and 7.80mm/day) and *H. ulmarius* (62.00mm and 7.75mm/day) (Table 4.5, Plate 1 and Fig 4.3). Among hybrids, A_1C_2 grew significantly faster (64.33mm and 8.05mm/day) which was at par with $A_{11}D_6$ (63.66mm and 7.95mm/day) (Table 4.5, Fig 4.3 and Plate 1). In general it could be concluded that mycelial growth rate of hybrids were significantly higher or at par with their respective parents.

Thus, the results are almost in accordance with the findings of Sawashe and Sawant (2005) who also found on solid medium, the hybrid culture showed maximum or at par mycelial growth as compared to their parents.

4.4 COMPARATIVE COLONY CHARACTERS

All the nine selected parents and their hybrids were grown on MEA medium to study their colony characters viz. colony appearance, colour, shape, zonation, margin and exudates produced or not. The observations are summarized in Table 4.6 and Plate 2 & 3.

4.4.1. Colony appearance

Three types of colony appearance viz. appressed, fluffy, and cottony were observed (Plate 2). Appressed colony appeared in one of the parent *P. djamor* and among hybrids A_1B_5 and A_1C_2. Fluffy colony appeared in the parents' *P. sajor-caju*, *P. florida* and *P. flabellatus* and among hybrids $A_{11}D_6$ and F_4H_4. *H. ulmarius* showed cottony type appearance. (Table 4.6 and Plate 2).

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4.4.2. Colony colour

The colour of colonies of parents and their hybrids varied from white, creamy white, dull white to grayish white. Among the parents grayish white and dull white colony colour was observed in *P. sajor-caju* and *P. djamor* respectively. Creamy white colony colour was observed in hybrid $A_{11}D_6$ while rest of the parents and their hybrids form white colony colour (Table 4.6).

4.4.3. Colony Shape

Shape of the colonies varied from circular to irregular. All the parents and their hybrids showed circular colony except *P. djamor*, which showed irregular colony (Table 4.6 and Plate 2 & 3).

4.4.4 Zonation

Zonation in the colony categorized as conspicuous and not distinct. Zone formation was not distinct in the colonies of *Pleurotus spp.* and their hybrids, but it was conspicuous type in case of *P. florida*, *P. flabellatus* and in hybrid $A_{11}D_6$ (Table 4.6 and Plate 3).

4.4.5. Colony margin

Margins of the colonies were of even type in all cases of parents and their hybrids, except *P. djamor* which produced colony with uneven margin (Table 4.6 and Plate 3).

4.4.6. Exudation

Exudation was observed in case of parent *P. florida* and in hybrid $A_{11}D_6$ only (Table 4.6 and Plate 3).

Kligman (1943) referred two types of colonies as “appressed”, a grayish white colony and “fluffy” giving the appearance of cotton

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Table 4.6. Colony characters of selected parents and their hybrids (*in vitro*)

Sl. No	Parents/hybrids	Appearance	Colour	Shape	Zonation	Margin	Exudation
1.	*P. sajor-caju* (A)	Fluffy	Greyish White	Circular	Not Distinct	Even	-
2.	*P. djamor* (B)	Appressed	Dull White	Irregular	Not Distinct	Uneven	-
3.	*P. florida* (C or F)	Fluffy	White	Circular	Conspicuous	Even	+
4.	*P. flabellatus* (D)	Fluffy	White	Circular	Conspicuous	Even	-
5.	*H. ulmarius* (H)	Cottony	White	Circular	Not Distinct	Even	-
6.	A_1B_5	Appressed	White	Circular	Not Distinct	Even	-
7.	A_1C_2	Appressed	White	Circular	Not Distinct	Even	-
8.	$A_{11}D_6$	Fluffy	Creamy White	Circular	Conspicuous	Even	+
9.	F_4H_4	Fluffy	White	Circular	Not Distinct	Even	-

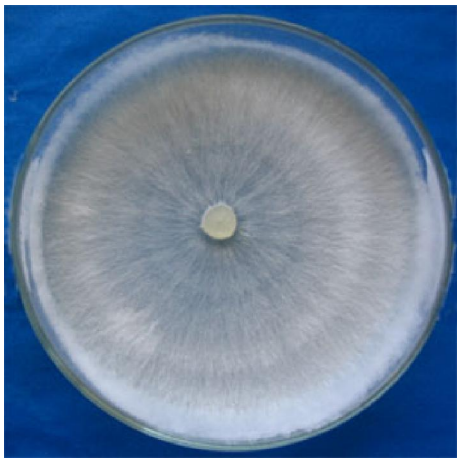
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Appressed



Fluffy



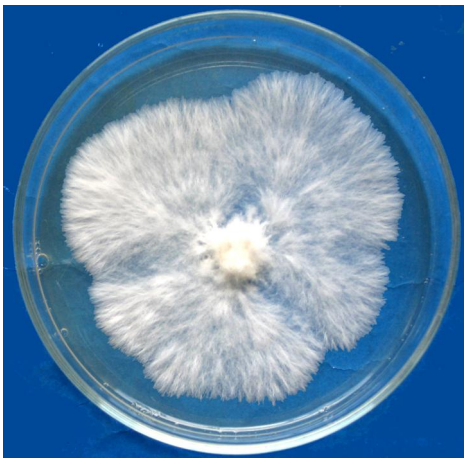
Cottony



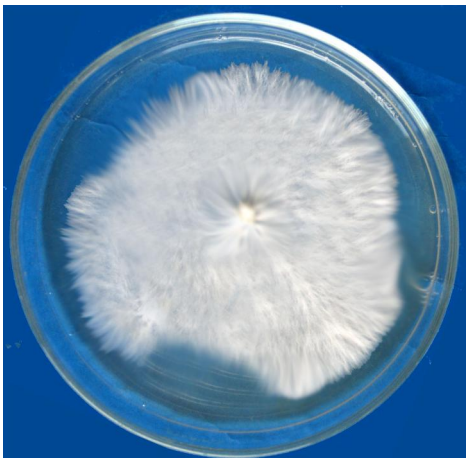
Circular

Plate-2. Colony characters of selected parents and their hybrids.

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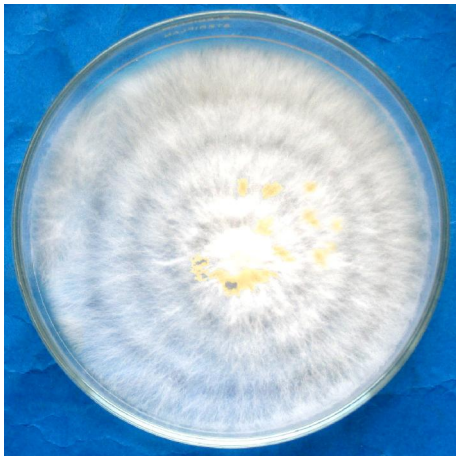
Irregular



Uneven



Zonation



Exudation

Plate-3. Colony characters of selected parents and their hybrids.

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wool. Goltapeh and Kapoor (1989) studied variation in the strains of *A. bisporus* by observing the colony characters including colony diameter, appearance of colony, colour, shape, zonation, margin, sectoring, pigmentation and exudates formation. Jin and Xu (1990) and Bahukhandi *et al.,* (1991) also observed variation in colony characters of strain of *A. bisporus* and *A. bitorquis* respectively.

Verma (2005) studied the colony characters of different strains of *A.bisporus*. He found appressed, intermediate and fluffy type of Colony appearance, grayish white to milky white coloration, circular to irregular shape and zonation in some strains.

The observation of the present study also indicates variation among the colony characters of the parents and their hybrids and is in agreement with the findings of earlier workers. However, genetic make up of the *Pleurotus* spp. environmental & nutritional factors for the studies might be also the reason for variation in results.

4.5. MACROMORPHOLOGICAL VARIABILITY OF SELECTED PARENTS AND THEIR HYBRIDS

Macromorphological characters varied among the selected *Pleurotus* species and their hybrids also differ in their macromorphological characters as depicted in Table 4.7, Fig 4.4 and Plate 4 & 5.

4.5.1. Stipe length

Among the five selected parents, *H. ulmarius* produced fruit bodies with significantly longer stipe (4.78cm) which was at par with

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P. florida (4.65cm). Hybrid A_1B_5 produced fruit bodies having 4.50cm long stipes, which was significantly longer than the parents, and it was at par with hybrid F_4H_4 (4.25cm) and $A_{11}D_6$ (4.02cm). However, significantly shorter stipes was produced by *P. djamor* (0.98cm) and hybrid A_1C_2 (3.10cm) (Table 4.7, Fig 4.4 and Plate 4 & 5).

4.5.2. Stipe width

Hybrid A_1B_5 produced fruit bodies having wider stipe (1.36cm) which was at par with F_4H_4 (1.24cm) and A_1C_2 (1.17cm). Among parents, the maximum width 1.40cm recorded for the parent *H. ulmarius* followed by *P. florida* (1.27cm) and *P. sajor-caju* (1.16cm) (Table 4.7, Fig 4.4 and Plate 4 & 5).

4.5.3. Pileus length

Pileus length was recorded maximum in case of *P. flabellatus* (7.88cm) and this character was inherited to its hybrid $A_{11}D_6$ (7.77cm) which was at par with *P. flabellatus* in terms of pileus length (Table 4.7, Fig 4.4 and Plate 4 & 5).

4.5.4. Pileus width

Hybrid A_1C_2 produced fruit bodies with maximum pileus width (8.20cm) followed by F_4H_4 (7.73cm), A_1B_5 (7.46cm) and $A_{11}D_6$ (7.03cm) which were significantly wider than their parents *P. sajor-caju* (7.94cm), *P. florida* (7.40cm), *P. djamor* (7.09cm), *P. flabellatus* (7.93cm) and *H. ulmarius* (7.9cm) (Table 4.7, Fig 4.4 and Plate 4 & 5).

4.5.5. Colour of fruit body

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Table 4.7 showed that all the five parents varied in terms of colour of the fruit bodies viz. *P. sajor-caju* produced grey colour fruit bodies, *P. djamor* produced pink coloured fruit bodies, *H. ulmarius* produced blue colour fruit bodies while fruit bodies of *P. florida* and *P. flabellatus* were white and creamy white in colour. However, the hybrids did not vary much in colour of fruit body viz. hybrid A_1B_5 and A_1C_2 produced grey coloured fruit bodies while hybrid $A_{11}D_6$ and F_4H_4 produced creamy white and white colour of fruit bodies respectively (Table 4.7 and Plate 4 & 5).

4.5.6. Margin of pileus

P. sajor-caju and *P. flabellatus* produced fruit bodies with entire and smooth type of pileus margins, and this character was inherited to their hybrids except F_4H_4 producing fruit bodies with irregular type pileus margin. Wavy and dentate type pileus margins were produced by *P. djamor* and *P. florida* respectively. *H. ulmarius* produced fruit bodies with inrolled type of pileus margin (Table 4.7 and Plate 4 & 5).

These findings are in accordance with Periasamy and Natarajan (2003) who reported that the hybrids of *P. djamor* were different with each other in pileus morphology. The pileus length and width ranges from 5.2-8.4 and 6.0-12.1 cm respectively. Average size of stipe length and width also varied species to species. Similar observations were also recorded by several workers (Vooticumpee, 1996; Das *et al.*, 2000; Chandrashekhar and Savalgi, 2003 and Gioia *et al.*, 2005).

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Table 4.7. Macromorphological variability of selected parents & their hybrids

Sl. No.	Parents/Hybrids	Stipe length (cm)	Stipe width (cm)	Pileus length (cm)	Pileus width (cm)	Colour of fruit body	Margin of pileus
1.	*P. sajor-caju* (A)	3.57	1.16	7.05	7.94	Grey	Entire
2.	*P. djamor* (B)	0.98	1.01	6.50	7.09	Pink	Wavy
3.	*P. florida* (C or F)	4.65	1.27	7.13	7.40	White	Dentate
4.	*P. flabellatus* (D)	3.38	1.05	7.88	7.94	Creamy White	Entire
5.	*H. ulmarius* (H)	4.78	1.40	6.82	8.09	Blue	Inrolled
6.	A_1B_5	4.50	1.36	6.03	7.46	Grey	Entire
7.	A_1C_2	3.10	1.17	6.83	8.20	Grey	Entire
8.	$A_{11}D_6$	4.02	1.06	7.77	7.03	Creamy White	Entire
9.	F_4H_4	4.25	1.24	6.33	7.73	White	Irregular
	CD at 5%	0.11	0.17	0.19	0.88		

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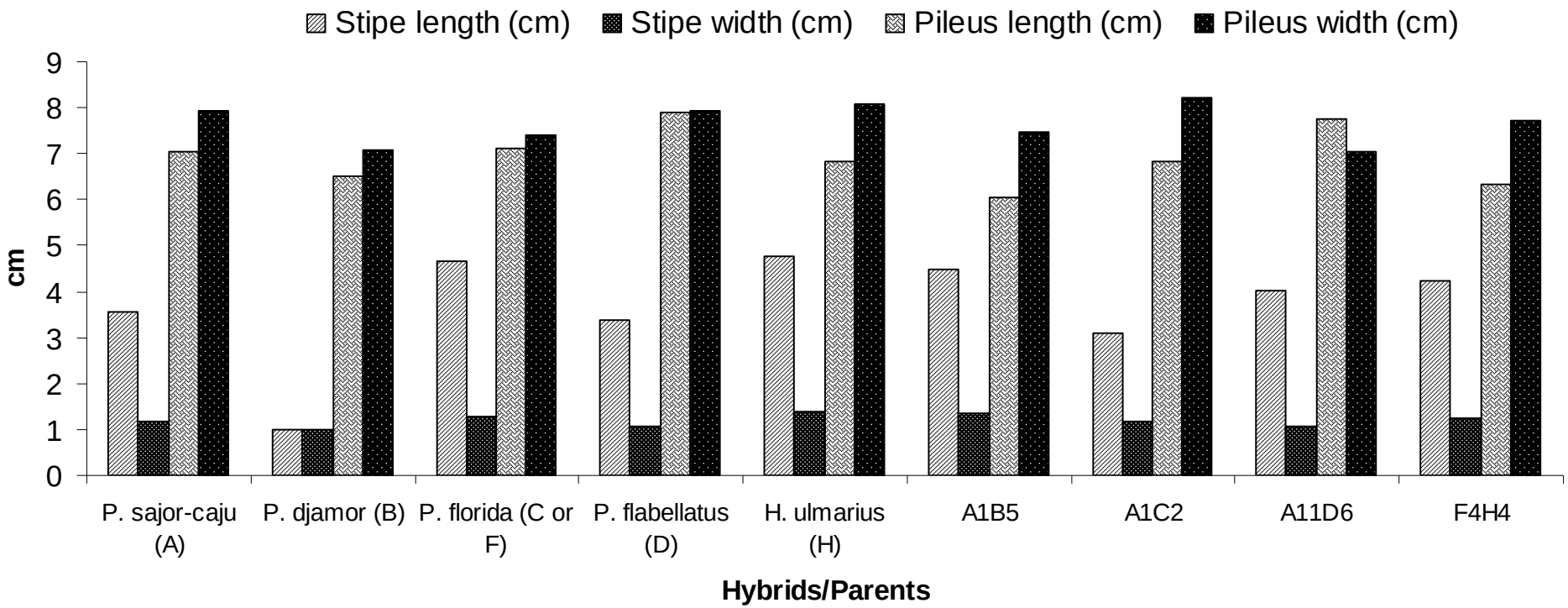
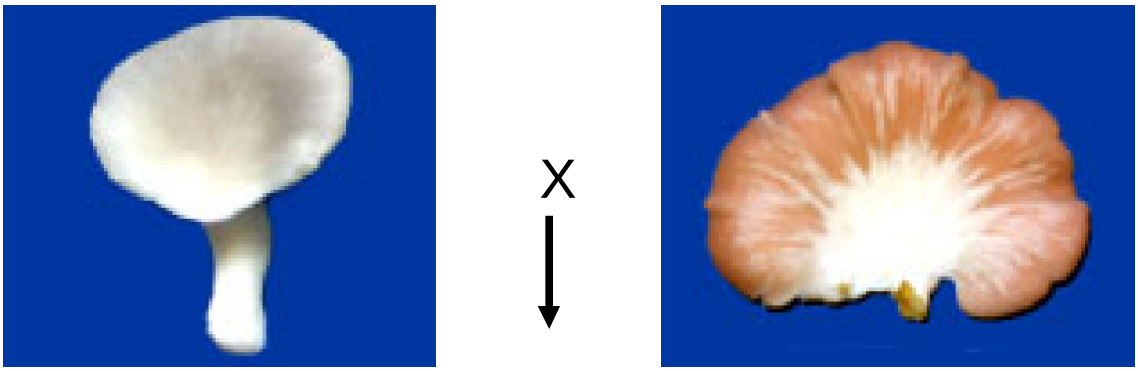


Fig. 4.4. Macromorphological variability of selected parents & their hybrids

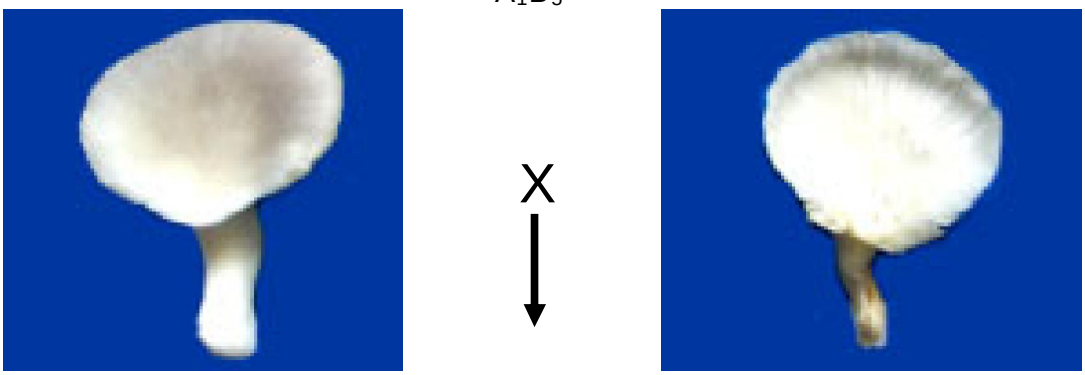
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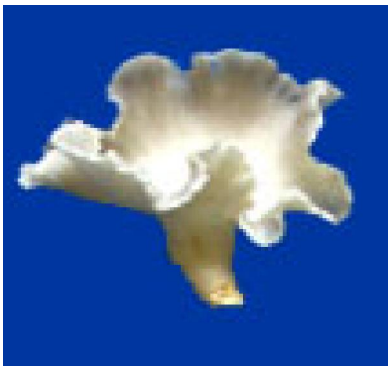
P. sajor-caju X *P. djamor*



A_1B_5



P. sajor-caju X *P. florida*



A_1C_2

Plate-4. Macromorphological variability of selected parents and their hybrids.

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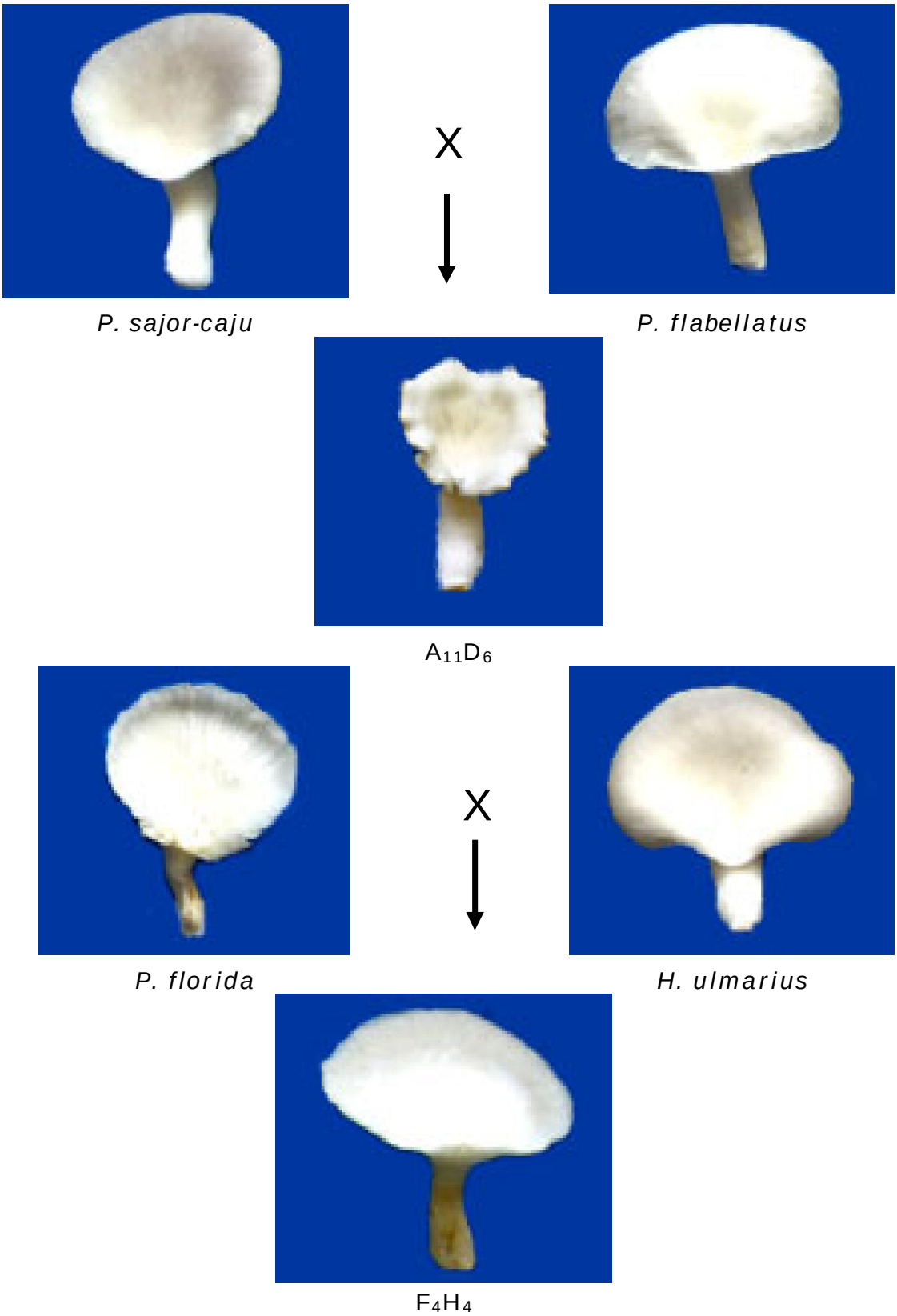


Plate-5. Macromorphological variability of selected parents and their hybrids.

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Bahukhandi (1986) also recorded irregular, entire and dentate margins of *Pleurotus* spp. while Periasamy and Natarajan (2003) observed wavy, petaloid and multiwavy type margins of fruit bodies at maturity.

4.6 YIELD PERFORMANCE OF SELECTED PARENTS AND THEIR HYBRIDS

4.6.1 Spawn run period

The mean of spawn run period was recorded minimum in case of *P. sajor-caju* (10.50days), which was at par with hybrid A_1B_5 (12.75 days), A_1C_2 (13.25 days), $A_{11}D_6$ (13.37 days), *P. florida* (13.50 days), *P. djamor*, *P. flabellatus* and F_4H_4 (13.75 days) respectively (Table 4.8).

4.6.2 Pin head appearance

After spawning, *P. sajor-caju* and hybrid $A_{11}D_6$ took minimum time, on an average 15.50 days and 16.37 days respectively in producing pin heads which was significantly earlier than others parents and hybrids. It was followed by A_1B_5 (17.25 days), A_1C_2 (17.62 days), F_4H_4 and *P. florida* (18.25 days), *P. djamor* and *H. ulmarius* (18.37 days), and *P. flabellatus* (18.50 days) respectively during the two cropping year (Table 4.8).

4.6.3 Fruit body maturation

P. sajor-caju (18.25 days) and hybrid $A_{11}D_6$ (20.00 days) matured significantly earlier than others parents and hybrids. It was followed by A_1B_5 (20.25 days), A_1C_2 (21.25 days), F_4H_4, *P. djamor* and

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Table 4.8. Spawn run, appearance of pin head and first harvest period of selected parents and their hybrids

Sl. No.	Parents/ Hybrids	Yield g/kg dry weight of substrate								
		Crop I			Crop II			Mean of Crop I and Crop II		
		Spawn run (days)	Pin head appearance (days)	First harvest (days)	Spawn run (days)	Pin head appearance (days)	First harvest (days)	Spawn run (days)	Pin head appearance (days)	First harvest (days)
1.	*P. sajor-caju* (A)	10.50	15.00	18.00	11.00	16.00	18.50	10.75	15.50	18.25
2.	*P. djamor* (B)	14.00	18.50	22.00	13.50	18.25	21.00	13.75	18.38	21.50
3.	*P. florida* (C or F)	13.00	18.00	21.00	14.00	18.50	22.00	13.5	18.25	21.50
4.	*P. flabellatus* (D)	13.50	18.25	21.75	14.00	18.75	23.00	13.75	18.50	22.38
5.	*H. ulmarius* (H)	14.50	18.50	22.75	14.00	18.25	22.50	14.25	18.38	22.63
6.	A_1B_5	13.00	17.50	20.50	12.50	17.00	20.00	12.75	17.25	20.25
7.	A_1C_2	13.00	16.00	19.75	13.75	16.75	20.25	13.25	16.38	20.00
8.	$A_{11}D_6$	13.50	17.75	21.50	13.00	17.50	21.00	13.38	17.63	21.25
9.	F_4H_4	13.50	18.00	21.00	14.00	18.50	22.00	13.75	18.25	21.50
	CD at 5%	1.73	1.74	1.76	1.72	1.73	1.71	-	-	-

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P. florida (21.50 days), *P. flabellatus* (22.37 days) and *H. ulmarius* (22.62 days) respectively during both the cropping year (Table 4.8).

4.6.4 Number of fruit bodies harvested

During crop I and crop II four flushes were harvested. The number of sporophore produced in each flush was reduced during successive flushes. Hybrids significantly produced maximum number of sporophores than their parents in both the cropping year. Maximum recorded for A_1C_2 (140.60) followed by $A_{11}D_6$ (113.51) which were at par with A_1B_5 (111.00) and F_4H_4 (102.35) respectively While among parents, *P. sajor-caju* produced maximum number of sporophores 95.16 in number followed by *P. djamor* (91.70) and *P. florida* (75.26) respectively (Table 4.9, Fig 4.5 and Plate 6-9).

4.6.5 Average yield

All the hybrids were significantly superior in terms of yield as compared to their respective high yielding parents. The data revealed that the hybrid A_1C_2 gave the maximum yield (1536.90 g/kg dry substrate) followed by $A_{11}D_6$ (1150.84 g/kg dry substrate), A_1B_5 (1017.26 g/kg dry substrate) and F_4H_4 (950.98 g/kg dry substrate) in both the cropping year (Table 4.9, Fig 4.5 and Plate 6-9).

4.6.6 Average weight per fruit body

Maximum average weight per fruit body was recorded for hybrid A_1C_2 (10.94g) followed by $A_{11}D_6$ (10.15g) which were at par with F_4H_4 (9.30g) and A_1B_5 (9.19g) respectively While among parents, maximum

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Table 4.9. Yield Performance of selected parents & their hybrids during the year 2006-2008

Sl. No.	Parents/Hybrids	Yield g/kg dry weight of substrate											
		Crop I				Crop II				Mean of crop I and crop II			
		No.	Wt (g)	Wt/fb (g)	B.E (%)	No.	Wt (g)	Wt/fb (g)	B.E (%)	No.	Wt (g)	Wt/fb (g)	B.E (%)
1.	*P. sajor-caju* (A)	91.63	825.00	9.00	82.50	98.70	843.51	8.54	84.35	95.16	834.25	8.77	83.425
2.	*P. djamor* (B)	95.76	486.02	5.07	48.60	87.63	478.47	5.46	47.84	91.69	482.24	5.265	48.22
3.	*P .florida* (C or F)	69.92	650.83	9.30	65.08	80.60	662.25	8.21	66.22	75.26	656.54	8.755	65.65
4.	*P. flabellatus* (D)	61.17	773.28	12.64	77.32	52.23	765.30	14.65	76.53	56.7	769.29	13.64	76.925
5.	*H. ulmarius* (H)	59.35	628.30	10.60	62.83	64.15	645.06	10.05	64.50	61.75	636.68	10.32	63.665
6.	A_1B_5	118.33	1025.40	8.66	102.54	103.67	1009.12	9.73	100.91	111.00	1017.2	9.19	101.72
7.	A_1C_2	136.47	1526.25	11.18	152.62	144.73	1547.53	10.70	154.75	140.6	1536.8	10.94	153.68
8.	$A_{11}D_6$	108.71	1145.25	10.53	114.52	118.32	1156.44	9.77	115.64	113.51	1150.84	10.15	115.08
9.	F_4H_4	105.35	975.81	9.26	97.58	99.36	926.15	9.32	92.61	102.36	950.98	9.29	95.095
	Mean	93.52	892.90			94.93	892.65						

CD at 5%		CD at 5%	
Year	1.40	Year	1.01
Treatments	2.97	Treatments	2.15
Year x treatment	4.20	Year x treatment	3.04

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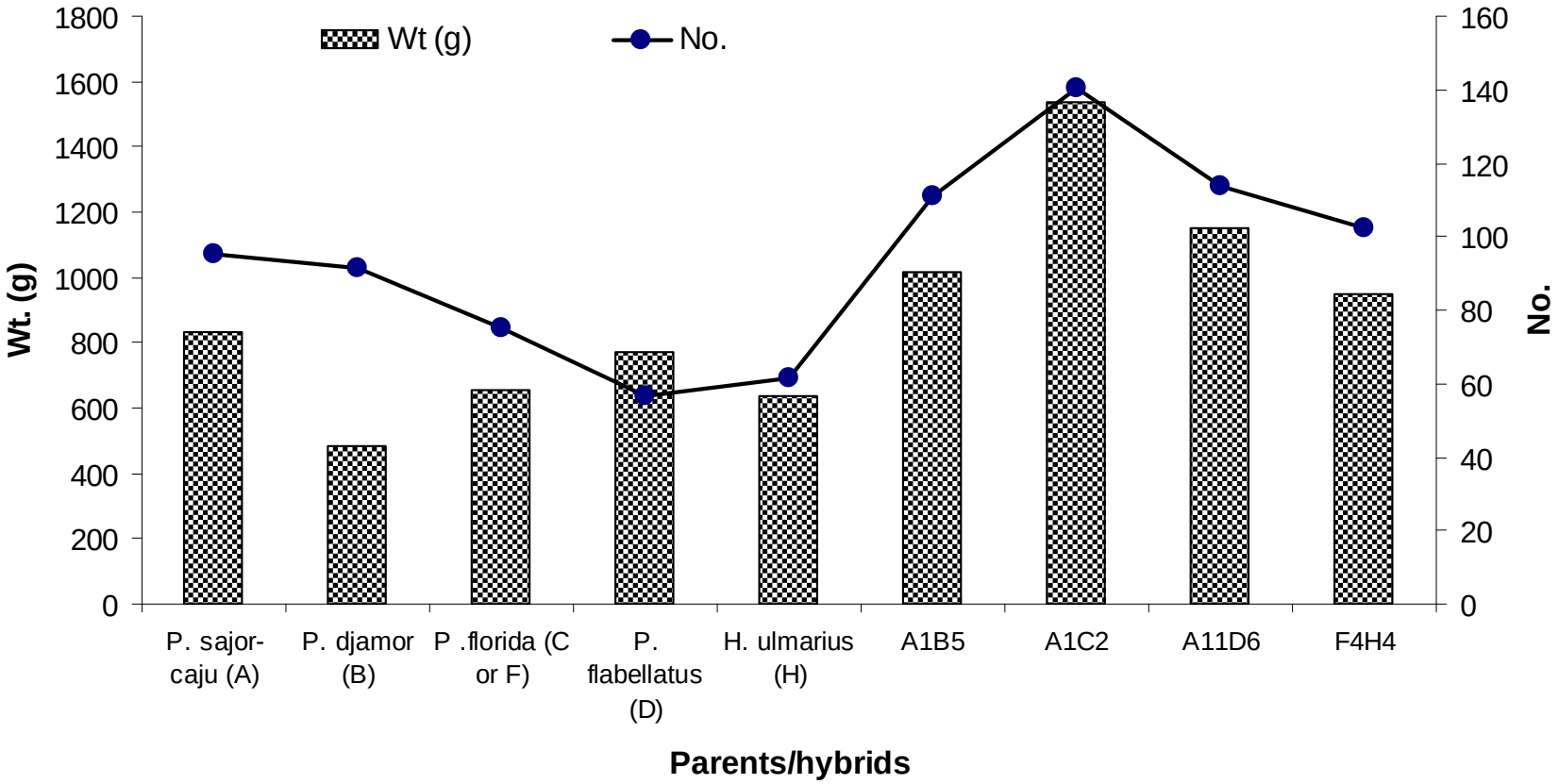


Fig. 4.5. Yield Performance of selected parents & their hybrids during the year 2006-08

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P. sajor-caju *P. djamor*



A_1B_5

Plate-6. Yield performance of selected parents and their hybrids.

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P. sajor-caju *P. florida*



A_1C_2

Plate-7. Yield performance of selected parents and their hybrids.

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P. sajor-caju *P. flabellatus*



$A_{11}D_6$

Plate-8. Yield performance of selected parents and their hybrids.

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P. florida *H. ulmarius*



F_4H_4

Plate-9. Yield performance of selected parents and their hybrids

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recorded for *P. flabellatus* (13.64g) followed by *H. ulmarius* (10.32g), *P. sajor-caju* (8.77g) and *P. florida* (8.75g) respectively (Table 4.9 and Plate 6-9).

4.6.7 Biological Efficiency

Biological Efficiency of selected *Pleurotus* spp. (parents and hybrids) is summarized in Table 4.9. Hybrid A_1C_2 showed maximum biological efficiency (153.70) followed by $A_{11}D_6$ (115.08), A_1B_5 (101.72) and F_4H_4 (95.09) respectively which was significantly higher than their respective parents.

Thus, our findings were in accordance with Periasamy and Natarajan (2003) who reported the maximum biological efficiency (128%) from the hybrid strain 5/6 of *P. djamor* grown on wheat straw.

Bahukhandi and Sharma (2002) also reported that the hybrid produced by crossing between *P. sajor-caju* and *P. cornucopiae* resulted 23.3 per cent more yield as compared to its parents. This hybrid also took lesser time (15 days) for spawn run and shorter cropping period of thirty-one days. The present findings, in general are in support to shorter cropping period & higher yield of hybrids comparing to their parents.

4.7 BIOCHEMICAL VARIABILITY OF SELECTED PARENTS AND THEIR HYBRIDS

The biochemical components like total soluble sugars, reducing sugars, non reducing sugars and crude protein were estimated from

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selected *Pleurotus* spp. (parents and hybrids) and results are given in Table 4.10 and Fig 4.6.

The data revealed that the total soluble sugars varied between 15.25-17.57 per cent on dry weight basis. There were significant differences among parents and hybrids. Among parents, the maximum total soluble sugars was estimated from *H. ulmarius* (17.81 per cent) followed by *P. sajor-caju* (17.57 per cent), *P. djamor* (16.73 per cent), and *P. florida* (15.67 per cent) respectively. Significantly lower total soluble sugars was estimated from *P. flabellatus* (15.81 per cent), while among hybrid, the maximum total soluble sugars was estimated from A_1B_5 (17.23 per cent) followed by F_4H_4 (17.03 per cent) and $A_{11}D_6$ (15.54 per cent). Significantly a lower total soluble sugar was estimated from hybrid A_1C_2 (15.25 per cent).

Reducing sugars content varied between 17.53-14.94 per cent on dry weight basis. Among parents, the maximum reducing sugar content (17.53 per cent) was estimated from *H. ulmarius* followed by *P. sajor-caju* (17.26 per cent) and *P. djamor* (16.44 per cent). However significantly poor reducing sugar content was estimated from *P. florida* (15.67 per cent) which were at par with the *P. flabellatus* (15.81 per cent) While among hybrids, maximum reducing sugar content (17.08 per cent) was estimated from F_4H_4 followed by A_1B_5 (16.93 per cent). However significantly poor reducing sugar content was estimated from A_1C_2 (14.94 per cent) which were at par with the $A_{11}D_6$ (15.23 per cent).

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Table 4.10. Percentage of TSS, Reducing sugars, Non reducing sugars, Protein in *Pleurotus* spp. (parents & their hybrids)

Sl, No.	Parents/Hybrids	TSS %	Reducing sugars %	Non reducing sugars %	Protein %
1.	*P. sajor-caju* (A)	17.57	17.26	0.300	25.63
2.	*P. djamor* (B)	16.73	16.44	0.290	24.52
3.	*P. florida* (C or F)	15.67	15.35	0.320	28.43
4.	*P. flabellatus* (D)	15.81	15.50	0.310	27.35
5.	*H. ulmarius* (H)	17.81	17.53	0.280	23.42
6.	A_1B_5	17.23	16.93	0.300	25.82
7.	A_1C_2	15.25	14.94	0.310	28.75
8.	$A_{11}D_6$	15.54	15.23	0.310	27.60
9.	F_4H_4	17.03	17.08	0.280	24.73
	CD at 5%	0.30	0.33	0.17	0.57

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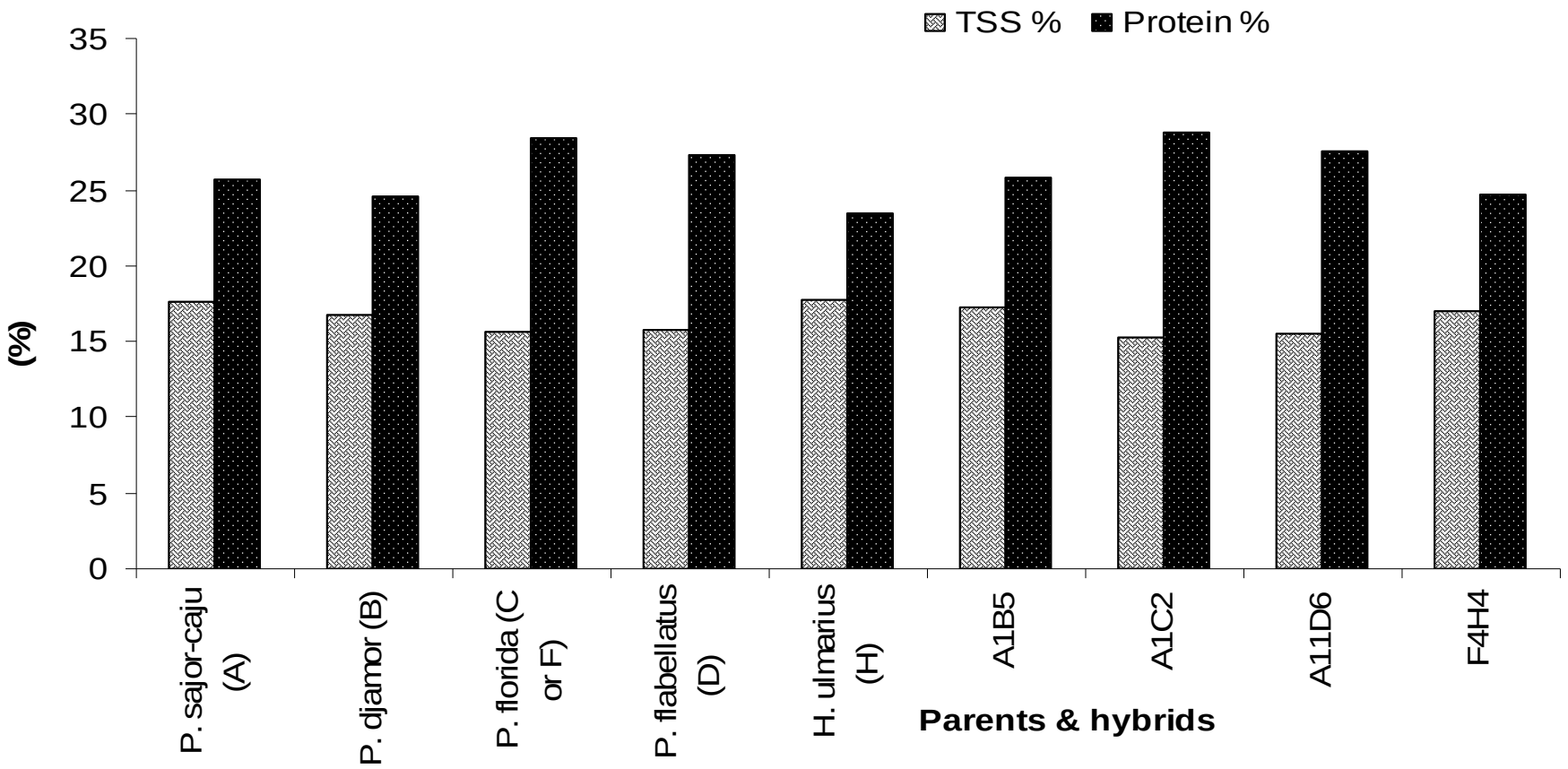


Fig. 4.6. Percentage of TSS and Protein in *Pleurotus* spp. (parents & their hybrids)

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The content of non reducing sugars was varied among parents and hybrids from 0.280-0.320 per cent on dry weight basis. The significantly higher content of non reducing sugars was estimated from parent *P. florida* (0.320 per cent) and among hybrid A_1C_2 and $A_{11}D_6$ (0.310 per cent) respectively.

The data revealed that protein content was found higher in hybrid A_1C_2 (28.75 per cent) and among parent *P. florida* (28.43 per cent) which was significantly higher than the protein content of other *Pleurotus spp* (parents and hybrids). It was followed by $A_{11}D_6$ (27.60 per cent), *P. flabellatus* (27.35 per cent), A_1B_5 (25.82 per cent) *P. sajor-caju* (25.63 per cent), and *P. djamor* (24.52 per cent) respectively.

However significantly lower protein content was found in hybrid F_4H_4 (24.73 per cent) and parent *H. ulmarius* (23.42 per cent) respectively.

Gupta *et al.,* (1982) studied biochemical constituent in *Pleurotus eous.* The fruiting bodies contained 15.50 per cent reducing sugar, 0.31 per cent non reducing sugar and 27.4 per cent protein.

Bano and Rajarathnam, 1982 documented Protein content in *Pleurotus* species has been in range between 8.9 to 38.7 per cent on dry weight basis while, Mandhare *et al*., (2007) were found highest protein content (28.9%) in *P. florida* followed by *P. flabellatus* (27.7%)

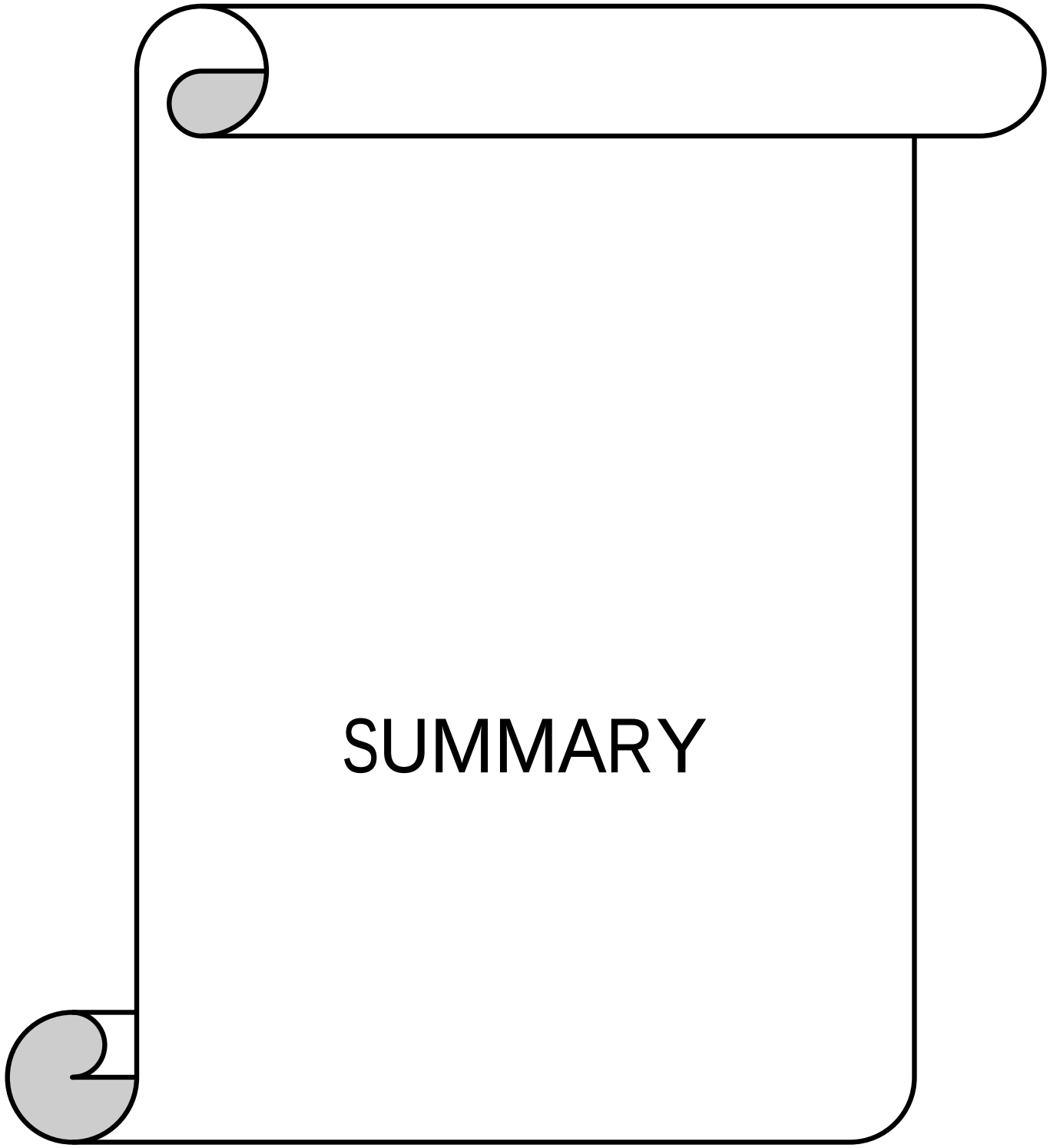
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and *P. eous* (25.0%) whereas they also reported low reducing sugar content (1.59-1.76%) in all the *Pleurotus* species.

However, nutritive value of hybrids were found in accordance with their parents, as no hybrids were registered so far, thus very little or no any literature available to this aspects of work.

The observation of the present study thus also indicates variation among the biochemical constituents of the parents and their hybrids and is in agreement with the findings of earlier workers. However, Total soluble sugars, reducing sugars, non reducing sugars and crude protein depend on used substrates, supplements, environmental conditions and genetic constitution of strains. These factors might be also the reason for variation in results.

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Considering the importance of strains in mushroom production, the present investigation was carried out to identify and evaluate variability amongst *Pleurotus* spp. (Parents and Hybrids) in terms of culture and colony characters, morphological, yield performance and bio-chemical make up. The research experiments were conducted in prevailing crop room condition during 2006-08 at Mushroom Research Training Center, Pantnagar. The findings observed under present investigation are summarized as follows.

1. Among the ten parents and thirteen hybrids, only five parents and their four hybrids were selected on the basis of yield & quality parameters for further studies.
2. Cultural variability among selected parents & hybrids were made on solid MEA medium. Mycelial growth rate of *Pleurotus* hybrids were recorded significantly higher or at par with their respective parents. Maximum growth (64.33mm) and growth rate (8.04mm/day) was recorded for the hybrid A_1C_2 followed by $A_{11}D_6$.
3. The *Pleurotus* spp. (parents and hybrids) selected for cultural studies varied in terms of one or the other colony character. All the selected high yielding hybrids and their parents were grown on MEA medium to study their colony characters viz., colony

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appearance, colour, shape, zonation, margin, exudates produced or not. The selected hybrids varied significantly among themselves in terms of their colony characters.

4. Macro-morphological & qualitative differences among the fruiting bodies of different dikaryon were studied selecting ten fruit bodies. Each of selected hybrids and their parents randomly starting from first flush. Stipe length was found minimum in hybrid A_1C_2 (3.10cm) followed by $A_{11}D_6$ (4.02cm). Pileus length was found maximum in hybrid $A_{11}D_6$ (7.77cm) followed by hybrid A_1C_2 (6.83cm). However, pileus width was found maximum in hybrid A_1C_2 (8.20cm) followed by hybrid F_4H_4 (7.73cm).

5. The yield data for four flushes in a period of 30 days from first flush were recorded in terms of number, weight & biological efficiency. Among the hybrids, hybrid A_1B_5 took minimum time for spawn run while hybrid A_1C_2 took minimum time for pin head appearance as well as for first harvest. All the hybrids were significantly superior in terms of yield as compared to their respective parents. A_1C_2 hybrid of *P. sajor-caju* and *P. florida* gave maximum yield i.e.140.60 in number, weigh 1536.90 g/kg dry substrate (B.E. 153.70) followed by $A_{11}D_6$.

6. The bio chemical constituents of the fruiting body of *Pleurotus* spp. (parents and hybrids) were estimated in terms of total

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soluble sugars, reducing sugar, non reducing sugars and protein. There were significant differences among parents and hybrids.

7. The significantly lower content of total soluble sugars (15.25 per cent) and reducing sugar (14.94 per cent) was estimated from hybrid A_1C_2 followed by $A_{11}D_6$ while among parents, lower total soluble sugars (15.67 per cent) and reducing sugar (15.35 per cent) content was estimated from *P. florida* followed by *P. flabellatus*.
8. The significantly lower non reducing sugars content (0.280 per cent) was estimated from hybrid F_4H_4 and from parent *H. ulmarius*.
9. The protein content in hybrids are significantly higher or at par with their respective parents. Higher protein content (28.75 per cent) was found in hybrid A_1C_2 followed by $A_{11}D_6$.

Finally it is concluded that the hybrid of *P. sajor-caju* × *P. florida* i.e. A_1C_2 found to be the best by producing maximum yield (1536.90g/kg dry substrate) and fruit bodies (140.60 in number) with higher protein content (28.75 per cent) & lower total soluble sugars content (15.25 per cent) followed by $A_{11}D_6$ respectively.

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APPENDICES

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APPENDIX -I

Standard Meteorological week's average weather data from August to November, 2006 at GBPUA&T, Pantnagar, Uttarakhand.

Months	Weeks	Date with duration	Temp. (^{0}C)		Relative humidity (%)	
			Max.	Min.	Max.	Min.
Aug. 2006	32	06-12	33.3	25.3	86	61
	33	13-19	33.7	26.4	85	61
	34	20-26	31.8	25.3	88	70
	35	27-02	29.9	24.1	93	81
Sept. 2006	36	03-09	32.8	24.4	91	62
	37	10-16	33.1	24.4	89	61
	38	17-23	32.0	23.8	89	66
	39	24-30	32.9	22.6	90	56
Oct. 2006	40	01-07	33.3	21.1	90	51
	41	08-14	32.1	20.5	91	56
	42	15-21	28.8	17.2	91	52
	43	22-28	28.6	14.4	90	57
	44	29- 04	29.1	14.3	88	44
Nov. 2006	45	05-11	28.3	14.4	90	52
	46	12-18	27.1	13.1	92	52
	47	19-25	25.9	10.6	91	39
	48	26-02	22.3	05.8	90	44

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APPENDIX -II

Standard Meteorological week's average weather data from August to November, 2007 at GBPUA&T, Pantnagar, Uttarakhand.

Months	Weeks	Date with duration	Temp. (°C)		Relative humidity (%)	
			Max.	Min.	Max.	Min.
Aug. 2007	32	06-12	33.3	26.6	88	65
	33	13-19	30.2	24.1	94	80
	34	20-26	32.3	25.9	88	67
	35	27-02	30.5	24.8	79	74
Sept. 2007	36	03-09	31.3	24.9	92	69
	37	10-16	32.7	23.2	88	59
	38	17-23	34.0	24.0	84	60
	39	24-30	28.1	22.2	94	73
Oct. 2007	40	01-07	32.2	19.9	89	51
	41	08-14	31.4	16.9	89	45
	42	15-21	29.1	17.6	87	51
	43	22-28	29.7	15.6	84	46
	44	29- 04	29.4	15.3	88	46
Nov. 2007	45	05-11	27.8	13.7	90	48
	46	12-18	26.8	10.9	88	43
	47	19-25	27.1	10.2	90	35
	48	26-02	25.0	10.5	88	43

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ABSTRACT

Oyster Mushroom (*Pleurotus* species) is one of the choice edible mushrooms grown in India as well as in World having high nutritive value and manifold uses. This mushroom contributes about 14.2 per cent of the total world mushroom production (6.16 million tones). However, share of this mushroom in mushroom production of India is hardly 6 per cent. Besides other factors, the strains play a most valuable role on yield and quality at diverse environmental conditions. Genetic manipulation of fungi is vital to meet the demand of the industrial world for fast growing and high yielding strains. The introduction of new strains through hybridization is thus required to maintain the production of quality mushroom. Considering the importance of strains in mushroom production, the present investigations were undertaken to identify and evaluate the parents and hybrids of Oyster mushroom in terms of qualitative (morphological and bio-chemical) and quantitative (yield) variability.

A total of available species of ten parents and thirteen *Pleurotus* hybrids, finally five parents viz. *P. sajor-caju* (Fries) Singer, *P. djamor* (Fr.) Boedjin var. roseus, *P. florida* (Eger), *P. flabellatus* (Berk & Br.) Sacc, and *H. ulmarius* (Singer) and their four hybrids A_1B_5 (*P. sajor-caju* × *P. djamor*), A_1C_2 (*P. sajor-caju* × *P. florida*), $A_{11}D_6$ (*P. sajor-caju* × *P. flabellatus*) and F_4H_4 (*P. florida* × *H. ulmarius*) were selected on the basis of their yield and quality parameters.

The selected parents and hybrids were studied for cultural variability on solid MEA medium. Mycelial growth rate of *Pleurotus* hybrids were recorded significantly higher or at par with their respective parents. Maximum growth (64.33 mm) and growth rate (8.04 mm/day) was recorded for the hybrid A_1C_2. Comparative colony characters were studied also on MEA medium viz. colony appearance, colour, shape, zonation, margin, exudates produced or not. The selected parents and hybrids varied significantly among themselves in terms of their colony characters. Macro-morphological & qualitative differences among the fruiting bodies of selected parents and hybrids were studied selecting ten fruit bodies randomly starting from first flush. Stipe length was found minimum in hybrid A_1C_2 (3.10 cm), Pileus length was found maximum in hybrid $A_{11}D_6$ (7.77cm) while Pileus width was found maximum in hybrid A_1C_2 (8.20 cm).

On the basis of yield performance, the hybrid of *P. sajor-caju* × *P. florida* i.e. A_1C_2 found to be best by producing maximum yield (1536.90g/kg dry substrate) and fruit bodies (140.60 in number) followed by $A_{11}D_6$ hybrid of *P. sajor-caju* × *P. flabellatus* yield (1150.84 g/kg dry substrate) and fruit bodies (113.51 in number).

The data of bio chemical constituents of the fruiting body of *Pleurotus* spp. (parents and hybrids) revealed that all the *Pleurotus* spp. contained low total soluble sugars (15.25-17.81 per cent on dry weight basis) and high protein content (23.42-28.75 per cent on dry weight basis). Amongst all, the hybrid of *P. sajor-caju* × *P. florida* i.e. A_1C_2 has lower total soluble sugars (15.25 per cent) and higher protein content (28.75 per cent).

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