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Research Article

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Study on Lignolytic Activities and Degradation Profile of Agricultural Solid Wastes by Mushroom Production

Sadhana Chaurasia[1]*, Radheshyam Singh[2], Anand Dev Gupta[3], and Manjesh Kumar Yadav[4]

[1]Head, Dept. of Energy and Environment, MGCGV, Chitrakoot Satna MP, 485331, India

[2, 3]Research Scholar, Dept. of Energy and Environment, MGCGV, Chitrakoot Satna MP, 485331, India

[3]Mphil, Dept. of Energy and Environment, MGCGV, Chitrakoot Satna MP, 485331, India

*Corresponding Author E-mail: sadhanamgcgv@gmail.com

ABSTRACT

The two mushroom verities Pleurotus florida and P. sapidus was grown on two different substrates i.e. paddy and wheat straw. In this study it was observed that lingolytic enzyme LiP was appeared very less in both substrates with both the species during active colonization and primordia initiation on 6th day while highest recorded in each substrate with both species at the end 18th day.

MnP was found in higher concentration in compression to LiP. Highest Laccase activity with P. florida was found 8.2 U/ml with both the substrate. During early stage of spawn run cellulose degradation was found faster and maximum during 1st harvest along with elevated level of lignolytic enzymes indicated that fructification required more energy for its higher metabolic rate which was served by fungal enzyme attack. Laccase activities expression level was found higher at spawn run primordial initiation and was responsible for physical barrier of lignin so that more cellulosic and hemicellulosic content was exposed for further enzyme attack leading to extraction of energy for the growth of mushroom. The result obtained in the study is discussed in this paper.

Keywords: *Paddy straw, Wheat straw, Lignine, Laccase and Cellulose.*

INTRODUCTION

Everyone has seen umbrella like small structures white to various colored growing on plant remains mostly during rainy seasons, these are mushrooms. Mushrooms, besides being a nature's curiosity are also known to mankind from times immemorial for their nutritive and medicinal values. They are helpful in curing human diseases like diabetes, obesity, hyperglycemia, hypertension and tumor[6,7]. Mushrooms have very high nutritional value being rich in proteins, vitamins and minerals[5].

Pleurotus, a white rot basidiomycetous fungus grows mostly as a saprophyte on almost any uncomposted crop residue including many agro-industrial wastes to finally produce oyster mushroom under favorable conditions. Like other homobasidiomycetous fungi, *Pleurotus* species have two distinct phases in their life cycle: the vegetative phase, represented by the mycelium, and the reproductive phase, represented by fruiting bodies, the basidiocarps. However, paddy straw remains the best substrate[4] and is easily available even in semi-urban areas. Besides a broad substrate range *Pleurotus* spp have a broader growing temperature range (10-32ºC) compared to any other variety. Therefore, there is a need to divide oyster mushroom growing season based upon temperature regimes (12-18, 20-26 & 28-32ºC) and recommend best suited species/strains of *Pleurotus*, accordingly. Cultivation technology of *Pleurotus* is easy, simple and highly flexible, and various low cost production systems are well documented[2,4] and[3] to be used as rural technology for rural employment generation.

MATERIALS AND METHOD

Paddy and wheat straw were being one of the most suitable substrate for oyster mushroom production. Straws were chopped into 3-5 cm pieces and filled into polypropylene bags (35 x 50 cm, 100 gauge), and

pasteurized by dipping in 80-85°C hot water for about 20-25 min in a wide mouth container such as tub or drum. After cooling at room temperature bags were ready for inoculation. The spawned bags were closed and a few holes made at the top and bottom of the bags were finally stored for development of mycelia. The all spawn run bags were transferred into cropping room and polypropylene cover carefully removed using a sharp blade without disturbing the mycelia, the latter it was transferred on racks about 20 cm apart. The cropping room was made comparatively more lighted, humid and airy with rack 60 cm wide having a gap of 50-60 cm between two racks. The temperature of cropping room was maintained as per the experimental needs (16, 25 and 30°C).

Biological Efficiency (BE %)

Biological efficiency was assessed by following formula-

$$\text{Biological Efficiency } \textbf{(BE \%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

The 20.0 gm of substrate (chopped paddy straw) were collected from poly bags at suitable time interval containing the colonize mycelia for the assay of enzymes (Lip, MnP & laccase).

Substrates were mixed with 50 ml of 0.01 M Phosphate buffer (pH 7.0) then agitated on orbital shaker for 20 minutes at 121 rpm at 4^0C. The mixture was squeezed gently through a fine cloth and centrifuged at 5000 rpm for 30 minute. Supernatant was used as crude enzyme source and filtrate was used for quantification of lignin, cellulose and hemicelluloses.

Lignolytic enzyme assays and Lignocllulosic estimation

Extracellular laccase (EC 1.10.3.2) production was measured by assaying the oxidation of 5mm 0l 1-1 ABTS in 100 mmol 1^{-1} Glycine-HCL (pH 3.0) at 420nm and using an extinction coefficient of $3600M^{-1}$ cm^{-1}.

Lignin peroxidase activity was evaluated UV spectrometry of the verity aldehyde produced during verity alcohol oxidation. The reaction mixture contained 375 ml sodium tartrate buffer 0.33M pH 3.0, 125 ml verity alcohol 4mM, 50 ml hydrogen peroxide 10mM, 450 ml distilled water and 250 ml culture medium for a final volume of 1250 ml.

Manganese peroxidase activity was measured by 2, 6-dimethoxyphenol (2, 6-DMP $426=27500M^{-1}$ cm^{-1}) oxidation for all enzymes under evaluation, one activity unit was defined as the amount of enzyme.

RESULT AND DISCUSSION

Growth experiment with *Pleurotus florida* and *Pleurotus sapidus* on paddy and wheat straw was performed. *P. florida and P. sapidus* showed highest growth with both the substrates at 25°C at the end of 21 days. Vertical growth study set up is depicted results are shown in Table-1.

Table 1: Comparative vertical growth of *P. florida and P.sapidus* on paddy and wheat straw at different temperatures regimes

Culture No./Name	Substrates	Temp. (±2°C)	*Growth in cm after days of inoculation						
			3rd	6th	9th	12th	15th	18th	21st
P.florida	Paddy straw	**20**	2.0	3.0	5.1	7.4	9.6	11.5	13.3
		25	2.1	3.3	5.6	7.7	10.1	12.5	14.6
		30	2.1	3.2	5.3	7.5	9.9	12.1	14.1
	Wheat straw	**20**	1.9	2.8	4.9	6.9	9.1	11.0	12.9
		25	2.1	3.1	5.1	7.3	9.5	12.1	14.1
		30	2.0	2.9	5.0	7.2	9.3	11.4	13.6
P.sapidus	Paddy straw	**20**	2.1	3.3	5.5	7.6	10.1	12.2	14.4
		25	2.5	4.9	7.2	9.7	12.4	14.8	14.8
		30	2.3	4.6	6.8	9.1	11.0	14.0	14.0
	Wheat straw	**20**	2.0	3.1	5.2	7.5	9.8	11.9	13.7
		25	2.3	4.1	6.7	9.3	12.0	14.3	14.7
		30	2.1	3.6	5.8	7.8	10.2	12.4	14.5

*Chopped paddy straw filled glass tubes (16 x1.5 cm) were used.

Paddy straw favors more vertical growth of both species as compared to wheat straw. 14.6 cm growth is recorded with *P. florida* while 14.8 cm growth is recorded with *P.sapidus* on paddy straw. 14.1 cm and 14.7 cm growth was recorded with wheat straw by *P. florida* and *P. sapidus* respectively.

Lowest values were observed at 20^0C with both substrates at the end of 21 days. 12.9 cm growth is recorded by *P. sapidus* on wheat straw. It is evident from result as shown in Table-1 that paddy straw favors higher growth of both the species. It might be due to lignocellulosic material is exposed enough for enzymatic attack in comparison to wheat straw. Higher is the growth, higher is the biological efficiency with any substrate. It reduces the optimization time which in turn make the process more beneficial. Further studies were carried out at 25^oC throughout entire investigation as it is the optimum temperature obtained by vertical growth studies.

Assessment of Biological efficiency

The yield data profile (represent g/kg fresh weight of substrate) was evaluated in terms of cumulative yield which in turn raise the biological efficiency. The yield profile of *P. florida* and *P. sapidus* on two different substrate are shown Table-2 and 3.

Table 2: Biological Efficiency (B.E. %) profile of two selected culture on paddy straw

Culture/isolates	Spawn running (Days)	Primordial initiation (Days)	Sporophores harvested, g/kg paddy straw						
			1st flush		2nd flush		3rd flush		Yield
			Days	Yield & Yield (%)	Days	Yield & Yield (%)	Days	Yield & Yield (%)	Total Yield & BE (%)
P.florida	12	16	21	455 (45.27)	29	345 (34.32)	39	205 (20.39)	1005 (100.5)
P.sapidus	14	18	22	405 (45)	32	300 (33.33)	45	195 (21.66)	900 (90.0)

In case of *P. florida* and *P. sapidus* primordial initiation was observed on end of16 and 18 days on paddy straw while 17 and 20 days on wheat straw. First flush of mushrooms were harvested within a week after primordial initiation. It is clearly evident from the table that cumulative yield of *P. florida* and *P. sapidus* are 1005 and 900 with paddy straw while 965 and 855 are with wheat straw on the basis of gm fresh wt/kg dry wt. of substrate. Similarly, biological efficiency 100.5 and 90 % was recorded on paddy straw with *P. florida* and *P. sapidus* swhile 96.5 and 85.5 was recorded with wheat straw.

Table 3: Biological Efficiency (B.E. %) profile of two selected culture on wheat straw

Culture/isolates	Spawn running (Days)	Primordial initiation (Days)	Sporophores harvested, g/kg wheat straw						
			1st flush		2nd flush		3rd flush		Yield
			Days	Yield & Yield (%)	Days	Yield & Yield (%)	Days	Yield & Yield (%)	Total Yield & BE (%)
P.florida	13	17	22	435 (45.07)	30	330 (34.19)	41	200 (20.72)	965 (96.5)
P.sapidus	15	20	24	390 (45.61)	34	285 (33.33)	46	180 (21.05)	855 (85.5)

Quantification of Lignolytic Activities and Degradation Profiles

The substrate (chopped paddy and wheat straw) was inoculated with *P. florida* and *P. sapidus* species and the sample of colonized substrates were taken at fixed time interval i.e. every six hours in between first to

third flush harvesting of mushrooms. Samples were used to determine the percent degradation of cellulose, hemicelluloses and lignin along with extracellular enzyme titers of LiP, MnP and laccase.

Table 4: Degradation profile of different component (cellulose, hemi celluloselignin) by *P. Sapidus & P. florida*

S.N.	Straw	Species	Component	Day				
				6	12	18	24	30
1.	**Paddy**	***P.florida***	Cellulose	1.8	4.5	9.5	10.1	6.0
			Hemi cellulose	1.0	3.5	5.5	16.1	9.0
			Lignin	1.5	5.5	9.8	4.0	2.0
2.	**Wheat**	***P.florida***	Cellulose	1.9	3.5	8.0	9.8	5.0
			Hemi cellulose	1.0	4.0	6.0	15.8	6.5
			Lignin	1.0	5.2	8.9	5.0	3.0
S.N.	**Straw**	**Species**	**Component**					
1.	**Paddy**	***P.Sapidus***	Cellulose	2.0	4.5	8.5	10.0	5.5
			Hemi cellulose	1.0	4.0	6.2	16.1	8.5
			Lignin	1.5	5.5	8.8	5.0	3.0
2.	**Wheat**	***P.Sapidus***	Cellulose	2.0	4.0	8.1	8.2	4.5
			Hemi cellulose	1.0	4.0	6.0	16.0	8.0
			Lignin	1.5	5.5	8.9	4.0	2.0

Table 5: Enzyme activity (laccase Mnp & Lip) in paddy and wheat straw by *P. florida& P. Sapidus*

S.N.	Straw	Species	Component	Day				
				6	12	18	24	30
1.	**Paddy**	***P. florida***	Lip	0.1	0.4	1.5	3.0	0.2
			Mnp	0.8	1.5	7.5	8.5	0.2
			Laccase	4.2	8.5	8.2	3.5	3.0
2.	**Wheat**	***P.florida***	Lip	0.1	0.3	1.5	3.0	0.2
			Mnp	0.5	1.2	7.0	8.5	0.2
			Laccase	4.0	8.5	8.0	3.5	2.0
S.N.	**Straw**	**Species**	**Component**	**Day**				
1.	**Paddy**	***P.Sapidus***	Lip	4.0	8.5	8.0	3.5	2.5
			Mnp	0.2	0.4	1.5	2.5	0.2
			Laccase	0.5	1.5	7.0	8.5	0.2
2.	**Wheat**	***P.Sapidus***	Lip	0.1	0.2	1.5	2.5	0.1
			Mnp	0.5	1.5	7.0	8.0	0.2
			Laccase	4.0	8.5	7.5	3.5	2.5

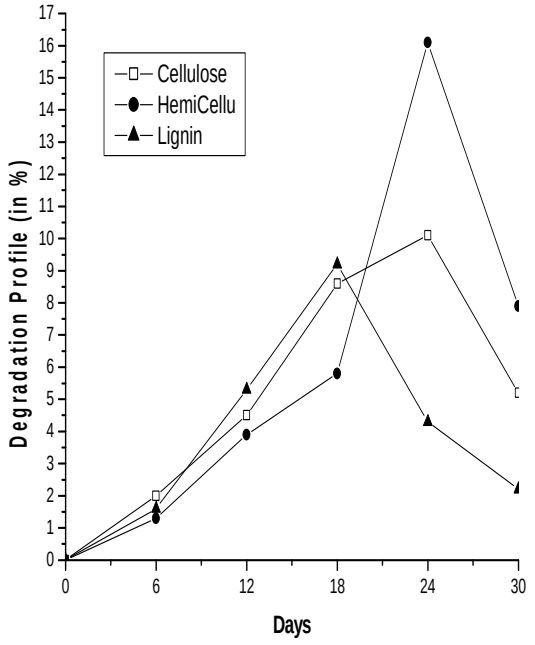

Fig 1. Degradation Profile of Paddy straw by *Pleurotusflorida*

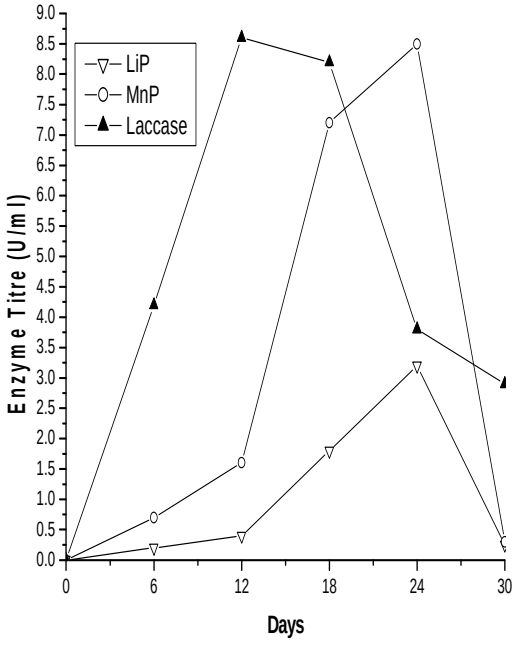

Fig 2. Extracellular Enzyme Titre (U/ml) during Mushroom Cultivation on Paddy Straw by *Pleurotusflorida*

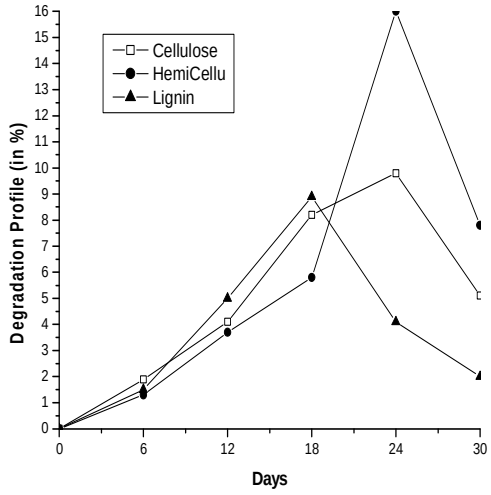

Fig 3. Degradation Profile of Paddy straw by Pleurotussapidus

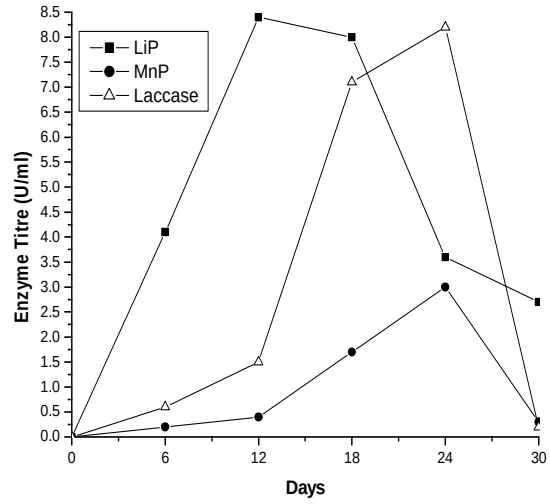

Fig 4. Extracellular Enzyme Titre (U/ml) during Mushroom Cultivation on Paddy Straw by Pleurotusspidus

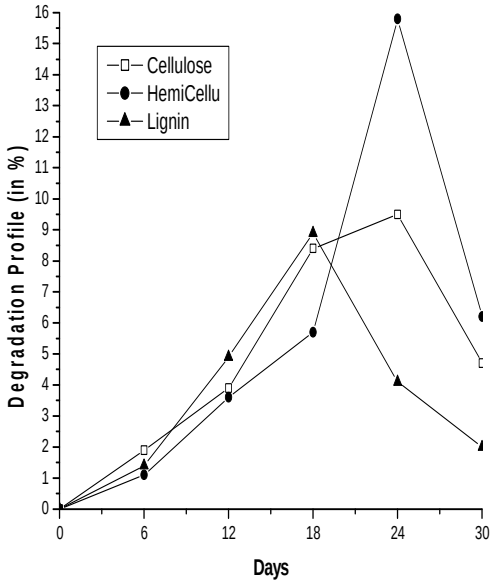

Fig 5. Degradation Profile of Wheat straw by *Pleurotusflorida*

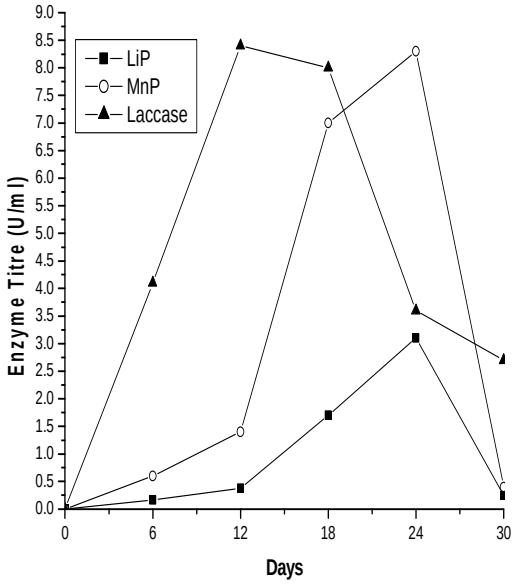

Fig 6. Extracellular Enzyme Titre (U/ml) during Mushroom Cultivation on Wheat Straw by *Pleurotusflorida*

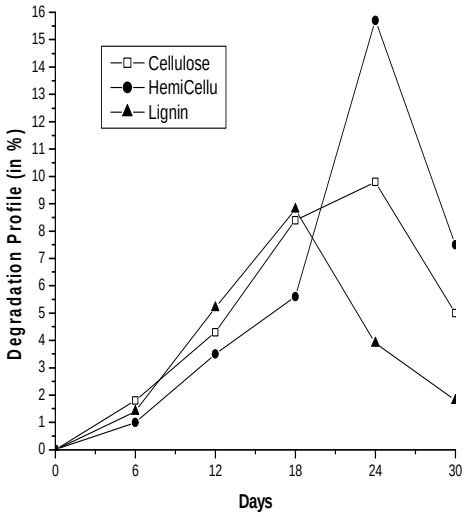

Fig 7. Degradation Profile of Wheat straw by Pleurotussapidus

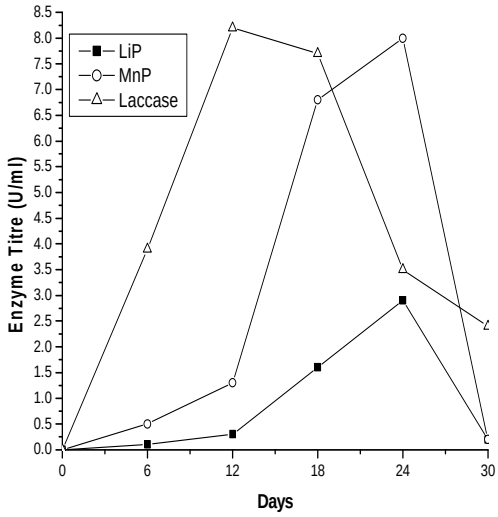

Fig 8. Extracellular Enzyme Titre (U/ml) during Mushroom Cultivation on Wheat Straw by Pleurotusspidus

Total cellulose, hemicelluloses and lignin content at 0 hrs. in paddy and wheat straw is presented in Table-4. Progressive decreases in the lignocellulosic content up to 30 days are presented in Fig-1 to 8 along with lignolytic activity.

Table 6: Lignocellulosic composition of paddy and wheat straw

Substrate	Lignocellulosic composition in %		
	Cellulose	**Hemicellulose**	**Lignin**
Paddy straw	30.4	35.1	22.6
Wheat straw	28.6	32.4	21.3

Lignocellulosic compositions were highly variable from species to species even in various parts of the plants within the same species. It is also affected by agro-climatic conditions, soil composition and other environmental factors.

Time of primordial initiation, 1st, 2nd and 3rd harvest of crop is presented in Table-2 and 3. Linear decrease in hemicellulosic and cellulosic content was observed during second and third harvest while reveres trend was observed with lignin during primordia initiation and first flushes of the crop.

A progressive decrease in cellulose content was observed and highest 10.1 and 9.5% with *P. florida* in case of paddy straw while 9.8% was observed with *P. sapidus* in both substrates at the end of 24th days like wise hemicellulosic content with *P. florida* in paddy and wheat straw was found to be 16.1 and 15.8% respectively. However, 16 and 15.8% was observed with *P. sapidus.* Similarly, trend was also observed in case of lignin content. It also exhibited linear decrease with *P. florida* was 9.2% and 8.9% respectively in paddy straw while 8.9% and 8.8% decrease in lignin content was observed by *P. sapidus* in paddy and wheat straw respectively at the end of 18th days.

The Fig-1 to 8 showed that on 18th day maximum lignin content was degraded while decrease in cellulose and hemicelluloses was observed at the end of 24th day suggested that lignin form a barrier to inner part of cellulosic content. If one can see the secondary growth structure of plants it was well established that cellulosic content is surrounded by hemicelluloses followed by lignin means lignin is the outer most part. Lignin is considered as physical barrier for the degradation of cellulosic and hemicellulosic content. Ruptures in physical barrier (lignin) allow to fungal lignolytic enzyme especially laccase to hydrolyzed cellulosic and hemicellulosic components. It was observed from the data that lignin was degraded first followed by hemicelluloses. Cellulose is the ultimate target for fungal enzyme attack in both the substrates with both the species. 9.5 and 9.8% decrease in cellulose content was observed with *P. florida* and *P. sapidus* respectively in case of paddy straw while hemicellulosic content was found to be 15.8 and 15.7% with *P. florida* and *P. sapidus* respectively.In case of wheat straw 8.9 and 8.8% respectively *P. florida* and *P. sapidus* was observed.

The growth and fruiting of *P. florida* and *P. sapidus* on two different substrates depend on the abilities of two selected species to utilized the lignocellulosic material which in turn will depend on species ability to synthesize lignolytic (hydrolytic) enzymes necessary to degrade the components of complex lingocellulosic materials to simple compounds, which can be easily assimilated. It was observed that lingolytic enzyme LiP was appeared to very less (0.2 U/ml in both substrates with both the species) during active colonization and primordia initiation on 6th day while highest (approx. 3.0 U/ml) were recorded in each substrates with both species at the end 18th day i.e. period crop harvesting .

MnP was found to be appeared in higher concentration in compression to LiP and it was found that *P. florida* exhibited 8.5 and 8.0 U/ml with paddy and wheat straw respectively while 8.2 and 6.8 U/ml was observed *P. sapidus* on paddy and wheat straw respectively at the end of 18th day i.e. period of harvesting

Similar trend of laccase activities was observed with both the species on both substrates. Highest Laccase activity with *P. florida* was found to be 8.2 U/ml with both the substrate while *P. sapidus* gave 8.4U/ml and 8.2 U/ml with paddy and wheat straw respectively.

Correlation between Lignocellulosic Degradation Profile and Extracellular lignotytic Activity

On the observation of data, graphically presented in Fig-1 to 8 it was observed that a good positive correlation is exist between complex lignocellulosic degradation and production lignolytic enzymes as reported by [1,8,9] and [10]. During early stage on spawn run cellulose degradation was found to be faster and higher rate, and was found maximum during 1st harvest along with elevated level of lignolytic enzymes indicated that fructification required more energy for its higher metabolic rate which is served by fungal enzyme attack[11] Result presented in Fig-1 to 8 together with the correlation between lignolytic activities and lingocellulosic degradation profile suggested that it play in important could play important role in lignocellulosic degradation by selected isolate. Laccase activities expression level was found to higher spawn run primordial initiation and is responsible for physical barrier of lignin so that more cellulosic and hemicellulosic content is exposed for further enzyme attack leading to extraction of energy for the

growth of mushroom fungus. The results obtained and presented in this dissertation are well in accordance of pervious researchers[12,13].

CONCLUSION

The major problems in front of developing countries are environmental clean-up, limited supply of food and energy, crisis of unemployment. When analyzed properly in terms of proper development and economic order, in simplified terms main aspect emerge: population, agriculture, industrial production, energy management and search for new pharmaceuticals. The problem can be overcome by searching or developing new biotechnological processes through suitable approaches. Huge amount of agricultural waste generates through agricultural practices and their disposal is problematic. Mushrooms may play an important role in this content; mushroom belongs to class Basidiomycetes and known to mankind from ancient times. Out of thousands of varieties of mushrooms a dozen of them are commercially cultivated and are used as food supplement. Among these groups *Pleurotus* species is important because of ease of cultivation in comparison to *Agaricus*. *Pleurotus* species is capable to degrade lignocellulosic wastes because of ability to produce nonspecific oxygenases and can be used for degradation of xenobiotics, phenolics and dyes. The paddy and wheat straw was used in the present investigation for production of Oyster mushroom. The paddy straw was found to favor more growth in comparison to wheat straw. Growth, productivity and enzymatic activitieswere measured in two species of *Pleurotus* such as *P.sapidus* and *P.florida.* These parameters depend on the ability of species to utilize lignocellulosic material which depends on capability of organism to produce enzymes for degradation of these complex wastes. Results reveal that lignolytic enzymes produced in minimum amount by both species during active colonization while maximum production was observed during period of harvesting. Further improved strains are required for the maximum ability degradation.

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