DOI : 10.5958/0974-0112.2019.00047.1

Indian J. Hort. 76(2), June 2019: 305-311

Correlating lignocellulose converting enzymes, substrate utilization and biological efficiency of *Pleurotus eryngii* strains grown on different agricultural residues

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ABSTRACT

In the present investigation, *P. eryngii* strain-substrate specificity, lignocellulolytic enzyme production and substrate utilization was correlated with the biological efficiency on different agricultural residues. Three strains of *P. eryngii* (DMR-P-120, DMR-P-135, DMR-P-257) were evaluated on low cost, easily available lignocellulosic agricultural wastes (wheat straw, paddy straw, maize stalks and soybean straw) in solid state fermentation for correlating the enzyme activity with yield potential of the strains. In the linear race tube growth studies, the mycelial extension rate increased with increase in days of incubation upto 28 days. Among all the substrates, maize stalks supported maximum mycelial extension rate (8.29 mm/day) for strain DMR-P-257 followed by wheat straw (7.86 mm/day) for strain DMR-P-257, soybean straw (6.71 mm/day) for strain DMR-P-120 and paddy straw (5.57 mm/day) for strain DMR-P-135 at 3rd week. A common trend was observed for the cellulolytic complex i.e. endoglucanase, exoglucanase, β-glucosidase, xylanase and laccase from the crude enzyme extracts and found that soybean straw showed the highest activity while paddy straw showed the least enzyme activity. Cultivation trials conducted for the three strains of *P. eryngii* on best performing substrates from race tube experiment viz. maize stalks and wheat straw for correlating lignocellulolytic enzymes at different morphological stages and biological efficiency. Among the growth stages, fruiting body stage showed highest lignocellulolytic enzyme activity. DMR-P-257 strain gave highest biological efficiency (36.18%) on wheat straw. Thus, the present study suggested that agricultural wastes could be used as a low-cost substrate for producing lignocellulosic enzyme activities which can be correlated for optimizing the best substrate for improving the yield potential of mushrooms.

Key words: Agricultural wastes, biological efficiency, lignocellulolytic enzymes, King oyster mushroom.

INTRODUCTION

Mushroom cultivation is considered as one of the most important agro-industrial business that can contribute to ecological recycling by reducing the environmental pollution in addition to the production of protein-rich food i.e., mushrooms having nutritional and medicinal properties (Zhang *et al*., 15). In India, huge amount of agricultural residues (including straw, stalks and bagasse) and agro industrial by-products (including waste paper, wood residue, groundnut cake, cottonseed cake) are available which could be exploited as a source of bioactive molecules (antioxidants) for improving the crop productivity as well as considered as a potential feedstock for production of useful industrial products (Ensuncho and Carriazo, 4). Most of these plants derived agro-industrial waste have been disposed off by burning or burying which leads to severe environmental and health problems. This problem can be mitigated by mushroom cultivation since most of the mushroom species have the capability to produce and secrete a wide spectrum of lignocellulosic enzymes which enabled them to grow on a wide range of lignocellulosic substrates along with a wider range of temperature.

Pleurotus eryngii also known as king oyster mushroom is one of the most valued, delicious mushroom due to its long shelf life, excellence in pileus and stalk consistency, culinary qualities and possess numerous range of bioactive compounds. *Pleurotus eryngii* is an exceptional choice for cultivation, as it is considered to have the best taste amongst all oyster species. It is relatively easy to cultivate, has a fairly long shelf life with reduced spore load and a quite high price in the market. However, its yield is generally lower in comparison to many other cultivated mushrooms.

An improvement in biological efficiency of this mushroom becomes essential to make this species economically attractive. The biological efficiency can be improved by enhancing the lignocellulolytic enzyme production of the indigenous *P. eryngii* strain with locally available substrates and /or through the addition of nutritional supplements. The strong

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dependence between mycelial growth and medium composition was observed by Skočaj *et al*., (10) on paper mill as a lignocellulosic substrate for *Pleurotus* cultivation. Keeping in view, the present study has been focused for the lignocellulolytic enzyme production from different cheap and locally available lignocellulosic agricultural wastes (wheat straw, paddy straw, maize stalks and soybean straw) by three strains of *P. eryngii* (DMR-P-120, DMR-P-135, DMR-P-257) for improving the yield potential of *P. eryngii* by correlating the enzyme activity of these *Pleurotus* strains.

MATERIALS AND METHODS

The *Pleurotus eryngii* strains (DMR-P-120, DMR-P-135, DMR-P-257) were procured from Directorate of Mushroom Research, Solan and sub-cultured on Potato Dextrose (PD) agar using standard microbiological procedures. Four different agricultural residues *viz.* wheat straw, paddy straw, maize stalks and soybean straw were collected locally. These residues were oven-dried in a hot air oven at 60^0C, ground and then sieved to 1mm particle size. For further use, these samples were stored in plastic containers under appropriate conditions. The comparative assessment of these agricultural residues was carried out in race tubes (25 x 198 mm). These tubes were filled to a height of approximately 130 mm and plugged with non-absorbent cotton, sterilized at 20 lbs for 90 minutes in an autoclave. After cooling, each tube was surface inoculated aseptically with the circular mycelial bits of *P. eryngii* strains (DMR-P-120, DMR-P-135, DMR-P-257), incubated at 25˚C for four weeks. The linear growth measurements were recorded in order to test the ability of these substrates for supporting the growth of mushroom strains under study. The mycelium linear growth was measured at weekly intervals for the 28 days along the length of race tube.

Lignocelluloytic enzymes were extracted from mycelium impregnated substrate after 28th day of incubation by adding 20 mL of 0.1 M citrate buffer to each culture flask and kept on a shaker at 30°C for 1 hour. The mixture was centrifuged at 12,000 rpm for 15 min at 4°C. The extract obtained was analyzed for measuring enzyme activities and extracellular proteins at weekly intervals. Effect of different agricultural residues on endoglucanase (Mandels *et al*., 7), filter paper activity (FPase; Mandels *et al*., 7), β-glucosidase (Toyama and Ogawa, 12), xylanase, laccase (Turner, 13) produced by *P. eryngii* strains was determined at weekly intervals. Enzyme activities were expressed in International Units (IU) where one unit corresponded to the amount of enzyme that oxidized one micromole of substrate per minute and results are expressed in terms of unit per gram of solid substrate (U/g).

Wheat straw and maize straw were selected (based on race tube experiment) for conducting cultivation trials, linear growth studies, and enzyme assays at different morphological stages of growth. The substrates were soaked with water overnight to attain a moisture content of 65-70 percent. Substrates were filled in autoclavable bags (40 × 25 cm) @ 1.0 kg substrate/bag. After putting ring and cotton plug, the bags were sterilized at 20 psi for 20 minutes. Before sterilization, by using test tubes a centre hole was made in each bag for later inoculation so that mycelium had enough time to reach the edges of the substrate before pinning initiated. After cooling, the bags were inoculated aseptically with wheat grain spawn @ 2-3% (wet weight basis). Then the bags were kept in growing rooms at an ambient conditions (temperature; 18-24°C and humidity; 70-80%). The spawned bags were incubated in dark. After complete colonization of the substrate, the bags were removed to expose the outer surface. At the time of pinning, fresh air is introduced to decrease the carbon dioxide (CO_2) levels below 100 ppm. The temperature (18±1°C), relative humidity (80-85%) and light (200-300 lux) were maintained respectively. During fruiting, 2-4 hours of light were provided to the inoculated bags. The yield of mushrooms and their different quality parameters were recorded regularly. The measurement of linear growth and enzyme assays were also recorded as previously described.

RESULTS AND DISCUSSION

Three *Pleurotus eryngii* strains (DMR-P-120, DMR-P-135, and DMR-P-257) were used for the study. The linear growth measurement experiment in race tubes showed the ability of wheat straw, maize stalks, paddy straw and soybean stalks to serve as an alternate substrate for *P. eryngii* strains by increasing their valorization. Among all the substrates, maize stalks supported maximum mycelial extension rate (8.29 mm/day) for strain DMR-P-257 followed by wheat straw (7.86 mm/day) with strain DMR-P-257, soybean straw (6.71 mm/day) with strain DMR-P-120 and paddy straw (5.57 mm/day) with strain DMR-P-135 at 3rd week (Table 1). In the case of maize stalks, DMR-P-257strain (8.29 mm/day) showed maximum linear growth followed by strain DMR-P-120 (7.57 mm/day) and DMR-P-135 (7.14 mm/day) at 3rd week. The results revealed that the maximum linear growth was pronounced on maize stalks while paddy straw showed inferior linear growth results. DMR-P-257 strain proved to be the fastest colonizer on different substrates. Results

indicated that DMR-P-257 strain had broad spectrum adaptability and hence may result in enhanced yield under cultivation trials. Zervakis *et al*. (14) observed that linear growth and colonization rate of *P. eryngii* strains differed from supplemented substrate to another and found highest extension rate on the wheat straw of *Agrocybe aegerita* (wild strain- SIEF 0834) and *Pleurotus* spp (wild strains LGAM P69, LGAM P26, LGAM P101).

Cellulases are a group of hydrolytic enzymes that catalyze cellulolysis and possess numerous industrial applications such as food, fuel, pulp, and textile. A common trend was observed for the cellulolytic complex i.e., endoglucanase, exoglucanase, and β-glucosidase enzymes in soybean straw that showed the highest activity while paddy straw showed the least cellulolytic activity. The results (Table 2) showed that maximum endoglucanase enzyme activity was obtained on soybean straw (1.13 U/mg of protein) by strain DMR-P-135 followed by wheat straw (0.55 U/mg of protein) for strain DMR-P-120, maize stalks (0.39 U/mg of protein) by strain DMR-P-257, paddy straw (0.30 U/mg of protein) by strain DMR-P-135 respectively. Paddy straw showed the least endoglucanase activity among all the substrates.

The results showed maximum exoglucanase enzyme activity on soybean straw (0.28 U/mg of protein) with strain DMR-P-120 followed by maize stalks (0.26 U/mg of protein) with strain DMR-P-257, wheat straw (0.24 U/mg of protein) with strain DMR-P-257 and paddy straw (0.20 U/mg of protein) with strain DMR-P-257. In this, paddy straw showed the minimum exoglucanase activity as compared to all other substrates. Maximum β-glucosidase

Table 1. Mycelial extension rate of different strains of *P. eryngii* on different substrates in 'race tubes'.

Powdered substrate	Strains	Mycelial extension rate (mm/day)			
		1st week	2nd week	3rd week	4th week
Wheat straw	DMR-P-120	2.00	4.85	7.43	5.00
	DMR-P-135	2.14	4.71	7.31	5.12
	DMR-P-257	2.57	4.71	7.86	4.14
Paddy straw	DMR-P-120	1.71	4.14	5.14	4.57
	DMR-P-135	1.71	4.14	5.57	4.71
	DMR-P-257	1.57	4.14	5.00	4.29
Maize stalks	DMR-P-120	2.57	5.28	7.57	3.86
	DMR-P-135	2.29	5.14	7.14	4.71
	DMR-P-257	2.29	6.00	8.29	2.71
Soybean straw	DMR-P-120	1.57	4.71	6.71	5.71
	DMR-P-135	1.71	4.14	5.13	5.00
	DMR-P-257	1.86	4.29	5.14	4.57

Data are mean of three replicates
Incubation temperature: 25±2°C

CD at 5% level
Substrates- 0.10
Strains- 0.89
Incubation days- 0.10

Table 2. Cellulases specific enzyme activity of different strains of *P. eryngii* on different substrates by solid state fermentation.

Powdered substrate	Strains	Cellulases specific enzyme activity (U*/mg of protein)		
		Endoglucanase	Exoglucanase	β-glucosidase
Wheat straw	DMR-P-120	0.55	0.12	3.37
	DMR-P-135	0.35	0.15	3.1
	DMR-P-257	0.42	0.24	3.42
Paddy straw	DMR-P-120	0.29	0.13	1.1
	DMR-P-135	0.3	0.18	1.06
	DMR-P-257	0.18	0.2	1.21
Maize stalks	DMR-P-120	0.36	0.17	2.06
	DMR-P-135	0.32	0.14	0.89
	DMR-P-257	0.39	0.26	2.86
Soybean straw	DMR-P-120	0.42	0.28	3.31
	DMR-P-135	1.13	0.26	3.45
	DMR-P-257	1.07	0.24	4.59

*U: µg produced/min/ml of enzyme extract;
Data is mean of three replicates;
Incubation temperature: 25±2°C

CD at 5% level
Substrates- 0.41;
Strains- 0.35;
Enzymes- 0.35

enzyme activity was observed on soybean straw (4.59 U/mg of protein) with strain DMR-P-257 followed by wheat straw (3.42 U/mg of protein) on strain DMR-P-257, maize stalks (2.86 U/mg of protein) with strain DMR-P-257 and paddy straw (1.21 U/mg of protein) with strain DMR-P-257 respectively. Among all the substrates, soybean showed the maximum β-glucosidase enzyme activity with strain DMR-P-257 (4.59 U/mg of protein) followed by strain DMR-P-135 (3.45 U/mg of protein) and DMR-P-120 (3.31 U/mg of protein) and paddy straw showed the least activity (Table 2). Similar to our study, Téllez-Téllez *et al*., (11) observed that *P. ostreatus* exhibited prominent oxidative with insignificant hydrolytic enzyme activities. Further, Ashger *et al.,* (2) recorded 0.198, 0.098 and 0.128 U ml^{-1} of an endoglucanase, exoglucanase and β-glucosidase activities in *P. eryngii.*

Among the substrates evaluated, the solid-state fermentation of soybean straw favored higher xylanase activity with strain DMR-P-257 (3.02 U/mg of protein) and paddy straw showed the least xylanase activity (1.03 U/mg of protein) with strain DMR-P-257 (Table 3).These findings are in agreement with earlier studies that *Pleurotus* sp. possess high xylanase activity and *P. ostreatus* possess 9~10-times higher xylanase activity (91.68 U/g) in comparison to *P. cornucopiae* (8.06 U/g) and *P. eryngii* (12.85 U/g) and concluded that variation in enzyme productivity depends on the unique characteristics of the mushroom species (Lim *et al.,* 6).

Laccase plays a dynamic role in polymerizing lignin by coupling with the phenoxy radicals produced by oxidation of lignin phenolic groups and have widespread applications, ranging from effluent decolorization and detoxification to pulp bleaching, removal of phenolics from wines, organic synthesis, manufacture of biosensors, synthesis of complex medical compounds, and dye-transfer blocking functions in detergents and washing powders. The laccase activity on soybean straw was found to be maximum with strain DMR-P-257 (62.81 U/mg of protein) followed by maize stalks (46.25 U/mg of protein) with strain DMR-P-120, wheat straw (35.27 U/mg of protein) with strain DMR-P-120 and paddy straw (26.67 U/mg of protein) with strain DMR-P-135. Among the strains, DMR-P-257 on soybean straw showed higher laccase activity while strain DMR-P-120 on paddy straw showed lowest laccase activity (Table 4). These results are in agreement with earlier studies that *Pleurotus* species possess high manganese peroxidase (MnP) and laccase activities (Patel *et al*., 9). Chang and Chang, (3) observed that higher laccase and MnP leads to increase in the degradation rate of nonylphenol toxic chemical, therefore, *P. eryngii* plays an important role in the degradation of xenobiotics. In *P. eryngii*, Akpinar and Urek, (1) recorded maximum laccase activity (1618.5 ± 25 U L^{-1}) in the medium containing apricot as a substrate after 12 days of incubation followed by pomegranate (373.5 ± 12 U L^{-1}, day 17). Lim *et al*., (6) observed highest laccase activity (8.01 U/g) in spent mushroom compost (SMC) extract of *P. eryngii* while enzymatic activity was 3.77 U/g and 2.97 U/g in SMC extracts for *P. cornucopiae* and *P. ostreatus*.

On the basis of results of race tube experiment, two lignocellulosic substrates were evaluated for further cultivation and enzyme assay at different morphological stages of growth. Soybean straw, maize straw, and wheat straw showed the best results of linear growth in race tubes and enzyme activity of different enzymes after 28 days of incubation. The endoglucanase enzyme activity was observed maximum at fruiting stage on maize stalks (1.00 U/mg of protein) with strain DMR-P-120 followed by wheat straw (0.93 U/mg of protein) on strain

Table 3. Xylanase specific enzyme activity of different strains of *P. eryngii* on different substrates by solid state fermentation.

Strains	Xylanase specific enzyme activity (U/mg of protein)			
	Wheat straw	Paddy straw	Maize stalks	Soybean straw
DMR-P-120	1.45	1.30	1.78	1.79
DMR-P-135	1.34	1.11	1.93	2.8
DMR-P-257	1.81	1.03	2.47	3.02

U: µg produced/min/ml of enzyme extract — CD at 5% level
Data is mean of three replicates — Substrates- 0.85
Incubation temperature: 25±2^{0}C — Strains- 0.74

Table 4. Laccase specific enzyme activity of different strains of *P. eryngii* on different substrates by solid state fermentation.

Strains	Laccase specific enzyme activity Units/min/mg of protein			
	Wheat straw	Paddy straw	Maize stalks	Soybean straw
DMR-P-120	35.27	14.99	46.25	58.5
DMR-P-135	28.5	26.67	43.8	51.89
DMR-P-257	33.39	22.29	38.84	62.81

U: Units produced/min/ml of enzyme extract — CD at 5% level
Data is mean of three replicates — Substrates- 1.83
Incubation temperature: 25±2^{0}C — Strains- 1.59

DMR-P-257 (Fig. 1). Maize stalks showed the higher endoglucanase activity with strain DMR-P-120 (1.00 U/mg of protein) in comparison to wheat straw. Wheat straw showed higher exoglucanase activity (0.59 U/mg of protein) with strain DMR-P-257 followed by strain DMR-P-135 (0.55 U/mg of protein) and DMR-P-120 (0.53 U/mg of protein). Maize stalks showed lower exoglucanase activity compared to wheat straw at fruiting stage (Fig. 2). The results in Fig 3. showed that wheat straw possessed higher β-glucosidase activity with strain DMR-P-257 (8.14 U/mg of protein) followed by strain DMR-P-135 (6.96 U/mg of protein) and DMR-P-120 (6.90 U/mg of protein), respectively as compared to maize stalks.

At fruiting stage, wheat straw showed maximum xylanase activity with strain DMR-P-257 (2.52 U/mg of protein) while the xylanase activity in maize stalks was found to be (2.19 U/mg of protein) with strain DMR-P-120 (Fig. 4) Wheat straw showed higher xylanase enzyme activity on strain DMR-P-257 (2.52 U/mg of protein) followed by strain DMR-P-135 (2.50 U/mg of protein) and DMR-P-120 (1.56 U/mg of protein) in comparison to maize stalks. Maximum laccase enzyme activity was recorded on maize stalks (97.12 U/mg of protein) by strain DMR-P-120 in comparison to wheat straw (37.91 U/mg of protein) with strain DMR-P-257 at fruiting stage (Fig. 5). In maize stalks, strain DMR-P-120 showed higher laccase activity (97.12 U/mg of protein) followed by strain DMR-P-135 (90.44 U/mg of protein) and DMR-P-120 (12.16 U/mg of protein). Thus, maize stalks showed higher laccase activity in comparison to wheat straw.

In cultivation trials, the number of days for complete impregnation and pinning of substrate were minimum for wheat straw substrate (35-46 days) compared to maize stalks (42-48 days). For all the strains of *P. eryngii,* pinning was observed earlier on wheat straw as compared to maize stalks (Table 5). The maximum biological efficiency was shown by all the strains on wheat straw (Plate 1). The pattern of biological efficiency shown by the three strains was DMR-P-257 (36.18%) > DMR-P-120 (34.42%) > DMR-P-135 (24.42%).

In the present investigation, the activity of cellulolytic enzymes along with xylanase shows a gradual increase in pin head stage and a sharp increase during the fruiting body stage. These observations can be correlated with slow depletion of cellulose and hemicellulose component in the vegetative phase and their fast depletion in reproductive phase which further supports the view that cellulose serves as an energy source for the formation of fruit bodies in *Pleurotus* species (Pandey *et al*., 8). This study also revealed that laccase activity increased during spawn run and reached a maximum

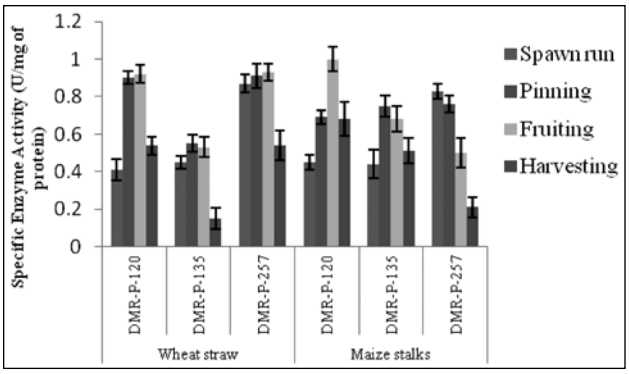

Fig. 1. Endoglucanase specific enzyme activity in U/mg of protein at different stages of growth.

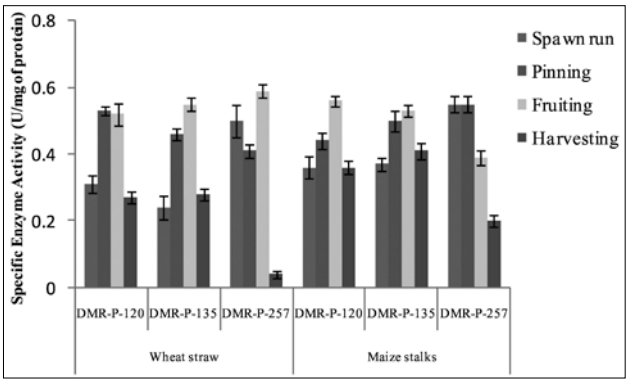

Fig. 2. Exoglucanase specific enzyme activity (U/mg of protein) at different stages of growth.

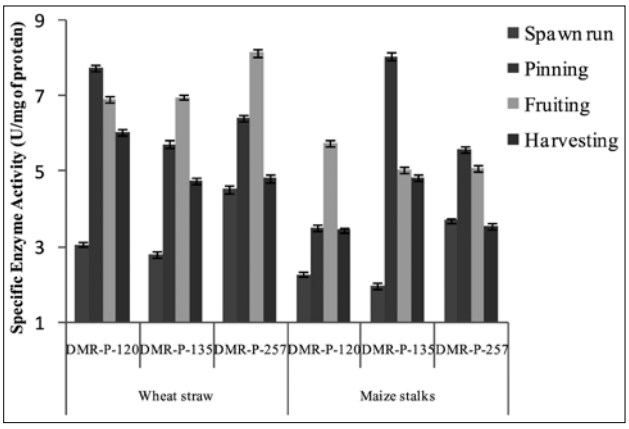

Fig. 3. β glucosidase specific enzyme activity in U/mg of protein at different stages of growth.

during fruiting. Kirbag and Akyüz (5) reported that the use of agricultural waste as substrate for cultivation of *P. eryngii* increased the BE to 48-85%. Thus, all the three strains of *P. eryngii* were capable of producing extracellular enzymes required to degrade major components of the lignocellulosic materials and

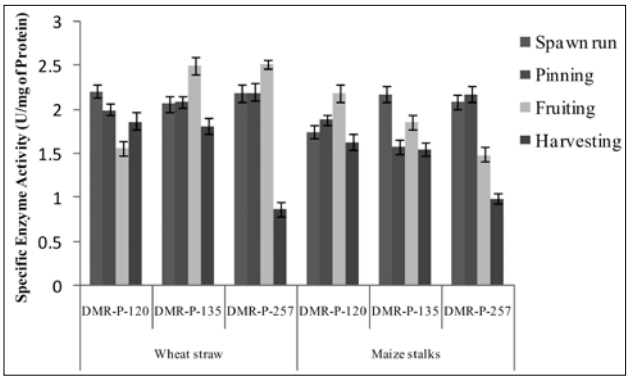

Fig. 4. Xylanase specific enzyme activity in U/mg of protein at different stages of growth.

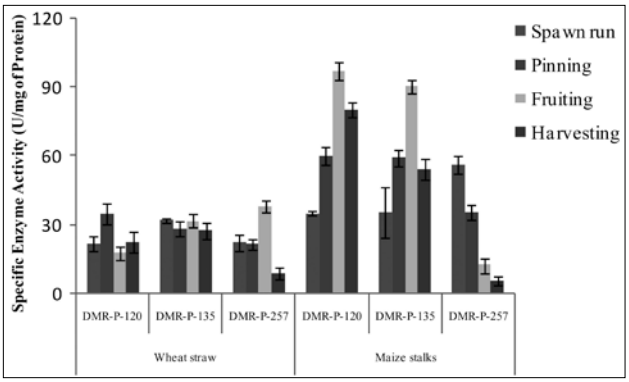

Fig. 5. Laccase specific enzyme activity (U/mg of protein) at different stages of growth.

Table 5. Cultivation data of *P. eryngii* on wheat straw and maize stalks.

Substrate	Strains	DFCI	DFP	DFH	Yield /100 kg	NFB	WFB	BE (%)
Wheat straw	120	42	46	56	11.46	275	41.69	34.42
	135	35	39	48	4.42	233	19.14	24.42
	257	35	38	46	12.05	425	28.35	36.18
Maize stalks	120	42	46	54	9.4	325	28.92	28.22
	135	42	48	57	6.87	275	25	20.64
	257	42	45	53	9.64	264	36.5	29.2

Data of 4 bags each
DFCI- Days for complete impregnation
DFP- Days for pinning
DFH- Days for harvesting
NFB- Number of fruiting bodies
WFB- Weight of fruiting bodies
Wet Substrate – 1kg/bag
BE (%)-Biological efficiency
CD at 5%
Substrates- 0.14
Strains-0.10

Impregnation stage | Pinning stage | Fruiting and Harvesting stage

Plate 1. Different growth stages of *Pleurotus eryngii.*

there was a direct correlation with the production of enzymes and yield potential of this mushroom.

The results concluded that there would be management of agricultural wastes as lignocellulosic substrate for systemic lignocellulolytic enzymes and enhanced yield potential of *P. eryngii.* The above mentioned, strains were deployed successfully and the results indicated that the selection of lignocellulosic substrate could be an important factor for the production of lignocellulolytic enzymes by *P. eryngii* and in this study; the mycelial growth rates and the enzyme activities were up-regulated by maize stalks and wheat straw. Therefore, these enzyme expressions could be used as a marker for selecting the better locally available substrate for commercial cultivation under Punjab conditions.

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Received : January, 2018; Revised : May, 2019;
Accepted : May, 2019