

Comparative Study on the Growth and Yield of *Pleurotus Ostreatus* Mushroom on Lignocellulosic by- Products

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Abstract: The edible mushroom *Pleurotus ostreatus* has been studied as a potential crop to reduce agricultural solid wastes and increase mushroom production. *P. ostreatus* cultivated on various agricultural wastes singly i.e. rice straw (RS), rice husk (RH) wheat straw (WH), barley straw (BS), and cotton wastes (CW). The biological efficiency (B.E) was 25.61, 9.51, 22.6, 21.628 and 25.78 % while rounding up the total weight of fruit yield 1.0 kg substrate was 258.04, 125.0, 226.0, 198.0 and 257.0 gms (on fresh wt. Basis), when grown on RS, RH, WS, BS and CW. The percentage of biomass loss from each substrate was 29.79, 16.89, 38.023, 30.0 and 33.40 %. Respectively, all the aforementioned wastes treated with *P. ostreatus* exhibited losses primarily in cellulose, hemicellulose, lignin, and fiber components after the harvest of *P. ostreatus*. The crude and soluble protein contents were enhanced by the incubation of the mushroom probably due to the addition of microbial protein. The losses of lignin and cellulose were higher in RS followed by CW, WS, and BS. Treated agro wastes with 1.25 % acid and base enhanced biodegradation ability and mycelial growth of the mushroom. Comparatively, loss of lignin, cellulose, hemicellulose and crude fiber is higher in treated then the untreated substrates. Similarly by the addition of chickpea powder as nitrogen source improved the growth pattern and metabolic activity of the *P. ostreatus*.

Keyword: Agricultural wastes, *Pleurotus ostreatus*, nitrogen source, Biodegradation.

1. INTRODUCTION

Mushrooms are fleshy saprophyte fungi and it found growing on damp rotten log of wood trunk of trees, decaying organic matter and in damp soil rich in organic substances. Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat (Mata *et al* 2005). The content of essential amino acids in mushroom is high and close to the need of the human body. Mushroom is easily digestible and it has no cholesterol content.

The Oyster mushroom belongs to the genus *Pleurotus*. They have a high saprophytic colonizing ability and can grow on virtually and agricultural waste. They rank among the top six mushrooms produced in the world. The consumption and production of edible mushrooms in developing countries have occurred for many years, but there has been an up surge of interest in cultivation of Oyster mushroom in the last decade as it is relatively easy to grow. Because of their spicy flavor and their medical effects in reducing plasma cholesterol these are widely consumed in Europe, China USA and Japan.

Cultivation of *Pleurotus* sp. reaches to the second largest in amount after *Agaricus bisporus*. Recently then being a growing interest in Pakistan to cultivate them on different readily available agricultural wastes (Hassan, *et al* 2011). Although, almost every kind of lignocelluloses are likely to be used as substrate for the cultivation of *Pleurotus* sp., the main and co- substrate differ among countries and even regions based on available abundance and lower prizes (Belewu, 2003).

However, the cultivation of mushroom is still very limited and the industry is still at its infancy in Pakistan (Kausar and Bajwa, 2005; Randive, 2012). The major problems associated with the transfer of technology for mushroom cultivation is the lack of technical know-how for its cultivation. The cultivation of edible mushroom using agricultural residues such as rice straw, rice husk, wheat straw, barley straw, banana leaves, cotton waste, corn cob, ground nut shell, saw dust and cassava peel is a process to convert these materials, which are otherwise considered to be wastes, into value added human foods (Abena, *et al* 2015). The present study describes utilization of abundantly available agricultural wastes i.e. rice straw, rice husk, wheat straw, barley straw and cotton wastes for the

cultivation of oyster mushroom. *P. ostreatus* mycelia grew very well on wide range of these cellulose wastes. RS, RH, WS, BS as well as CW all supported good growth and fast mycelia extension of the mushroom.

During an investigation for the cultivation of mushroom on agricultural residues, it was found that rice straw, wheat straw, sawdust, cotton waste, barley straw, and bagasse were suitable substrates for the cultivation of edible mushrooms (Wenjie, *et al* 2013).

The aim of this study is to cultivate the *P. ostreatus* on different agricultural wastes which would there after minimize the pollution problems created due to the agricultural wastes. Utilization of low cost agro wastes for valuable end product will lead to develop low cost cultivation technology for rural community, which in turn would be a step forward to elevate poverty in Pakistan. Bhatti *et al* (2007).

2. MATERIAL AND METHOD

Establishing mycelial culture and spawn: To establish the mycelial culture of the mushroom Potato dextrose agar (PDA) medium was employed. The mycelium from PDA slant was used for spawn production. Sorghum and barley grains were washed in water and boiled 15 minutes. The boiled grains then placed on a sieve to drain, after which they were spread on a clean plastic sheet to dry. These grains were impregnated by calcium carbonate; these coated grains were kept in a well cleaned and drained jam jar. The jars were covered with cotton plugged and latter autoclaved at 121°C for 20 minutes after which jars were cooled at room temperature. The cooled spawn of *P.ostreatus* was distributed evenly over the surface. The jars were then incubated at $25 \pm 1^\circ\text{C}$ in a ventilated incubator for 8 to 12 days. Each jar was shaken thoroughly by hand periodically to distribute the mycelia to the grains.

I. Experiment

Preparation of the substrates and their spawning: The substrates included wheat straw, barley straw, paddy straw and rice husk and cotton waste. The wheat straw, barley and paddy straw and rice husk were obtained from the agricultural farm of Seihkupura, cotton waste was procured from local market. Every fresh substrate was dried before any degradation process throughout the study. Paddy, barley and wheat straw were chopped into 5 to 8 cm long pieces, paddy husk and cotton wastes were used as such. Each substrate was then soaked in water for 24 h separately, for moisture absorption and tendering. The substrates were then placed on wire sieves to drain.

The moist substrate were fortified with nitrogen supplement i.e. chick pea powder at 4 % (w/w) of the dry weight of the respective substrate. After fortification, the substrates were divided in to 200g, 300g, and 1kg lots. These substrates were packed in separate transparent polyethylene bags (heat resistant). 10 bags of each substrate were prepared. The substrates were autoclaved at 121°C for 20 minutes. After autoclaving, the substrates were cooled to room temperature. The spawn of *P. ostreatus* was distributed consistently over the surface of the substrates. The spawn was added at 2% (w/w) of the substrate in each case.

II. Experiment

All the aforementioned agro wastes were treated with 1.25 % sodium hydroxides or sulphuric acid. Clean and dried agro wastes were soaked in 1.25 % alkali or acid overnight separately, after soaking each material was washed thoroughly with tap water followed by double distilled water until it become neutral (pH 7). 150g, 200g and 300g of each substrate was filled in polythene bags (8 x 12 and 9.5 x 7 inches) after adjusting the moisture contents. All the bags were autoclaved at 121°C for 20 minutes. Each bag was aseptically inoculated with 2% (w/w) spawn. A plastic pipe with cotton plugs was introduced in all bags for ventilation. All bags were incubated in the incubator at $25 \pm 1^\circ\text{C}$ for 22 days.

Spawn running and fructification: The bags containing spawned substrates were placed on shelves in a disinfected spawn running / fructification room had a concrete floor. The spawn running room was kept humid by pouring / sprinkling of water every day on the floor. The humidity, temperature and light of the spawn running room were monitored daily. Substrates were subjected to fructification conditions when time taken to reach this stage was the mycelium had sufficiently colonized, 18d on paddy straw and cotton waste, 19d on barley straw and 20 d on wheat straw and bagasse. The spawn running longer then 22d resulted in mycelial degeneration (i.e. hyphae collapsed and patches of

substrates become visible again. Fructification conditions included opening of bags to provide more light and ventilation). Under these condition two days were allowed for pin head formation. Temperature in the spawn running room was monitored 24-26 oC along with relative humidity of 73-78% and light at only 1.01 m/ sqf provided by cool white fluorescent lights.

Harvesting of fruiting bodies and comparison of biological efficiencies on different substrates: Fruiting bodies were harvested when it completely mature (2-3 days of emerging). The substrates were incubated under the same conditions for another 7 days after each harvesting for second and third flushes. Mushroom from different substrates and treatments were kept separately for fresh weight measurements. The biological efficiencies (B.E) were calculated following (Khare, et al 2010).

Extend of utilization of substrate was determined by the following methods: Analysing of the substrates before spawning and after harvesting. The parameters of analysis included: Moisture, dry matter, ash content, fat, protein, soluble protein, crude fiber, lignin, cellulose and hemi cellulose of the substrates.

All samples were dried for 24 h in an oven at 105 oC. They were then ground and sieved through 5 mm mesh. Each sample was stored separately in dry and clean bottle with airtight lid in a refrigerator until analyzed.

Substrates fiber was analyzed according to Goering and Van Soest (1970). Fiber content was determined by analyzing the acid- detergent fraction (ADF), which is the lignocellulosic fraction of the substrate, followed by analyzing the neutral detergent fraction (NDF), which includes lignin, cellulose and hemi cellulose fractions of the substrate. The hemi cellulose content of the substrate then is obtained by subtracting ADF from NDF. Lignin and cellulose content of the substrate were determined according the methods of Kurschner (1930) and ASTM (1961). The total substrate nitrogen content was determined according to Markham (1942).

Statistical analysis. Mean values of parameters studied were analyzed by the Duncan Multiple Range Test (DMR) (Steel and Torrie 1980).

3. RESULTS AND DISCUSSION

Of the five substrates used, the highest mushroom fresh weights were produced on rice straw followed by cotton waste supplement with 4 % organic nitrogen i.e. chickpea powdered (CP) as nitrogen source (Fig. 1 and Table III).

RS and CW gave appreciable yield of the mushroom mycelium. This result agrees with the report of Fasidi (1996). He reported that rice straw, wheat straw, cotton waste and rice husk as the natural substrate on which *Volvariella esculenta* grew very well. RS and CW are good for the production of *P. ostreatus* because of this richness in vitamins which are good stimulants for high mushroom yield. WS and BS were also best for *P. ostreatus* growth (Rana, et al 2007). Similarly; the highest number of fruiting bodies per bag was recorded on rice straw and cotton wastes (Fig 2). An average of 258.04-320.92, 257-326.64 g fresh weight mushrooms were harvested per 1 kg of dry weight substrates (RS & CW). The number of fruit bodies was 74-113, 72-108 per bag. (RS & CW) (Table-I & Fig-II) The biological efficiency (B.E) was 25.61-32.69 and 25.78-32.05% (Table-I & Fig III).

Wheat straw supplement with 4 % chick pea powder was the second best harvest (Table- III). An average of 226-286 g fresh weight mushroom was harvested per kg dry weight substrate in this treatment and the B.E was 22.6 -28.60 % and no. of fruit bodies was 59-78 (Fig 1, II & III). Whereas the third best harvest was obtained on barley straw with 4 % chick pea powder as the nitrogen supplement (198-226.932 g; B.E 21.628 -26.69%) (Fig. 1 II & III, Table I and III). Likewise, in case of rice husk waste the average fresh weight of the mushrooms was 125-15039 g per kg dry weight substrates (B.E 9.51-15.04 %),(Table-I & Fig III). The number of fruit bodies was 37-51. It was also noted that the no. of fruit bodies, mycelial growth, B.E was less in unfortified substrates as compared to fortified and treated substrates in each case.

Mycelia covered the RS in about 15 days while full colonization was observed within 20 days in both RS and CW. On the other hand complete colonization of mycelia occurred after 22-24 days in case of WS, BS and RH. Maximum growth was estimated from the rice straw and cotton waste (Table- I). The higher yield on RS and CW appeared to be due comparatively better availability of nitrogen and carbon from these substrates. So, RS and CW are recommended as best substrates for the cultivation

of Oyster mushroom which is in agreement with the finding of Hami (1990), who studied the Oyster mushroom cultivation on sawdust and found that *P. ostreatus* gave the maximum yield on these wastes. It also observed that when *P. ostreatus* grow on rice husk its mycelia yield is comparatively lower than other wastes (Table II, III IV & V). The lower yield on RH was due to the presence of silica compounds is another reason which makes the husk more resistant to fungal attack through their enzymic system (Chang, 1988). The lower yield on RH was also due to the growth of saprophytic moulds.

The crop of *P. ostreatus* was harvested in three flushes. The maximum yield was obtained in first flush than the second and the third flush. This study agreed with the observation of Oei (2003) for *Volvariella esculenta*.

There was a reduction in the weight of the wastes used as substrate and this shows that the *P. ostreatus* has the ability to degrade lignocellulosic material during the idiophase stage followed by severe nitrogen and carbon depletion (Manson, et al 1989). Biomass losses in rice straw, rice husk, wheat straw, barley straw, and cotton waste were (29.79-51.7, 16.89-24.07, 38.023-48.86, 30.0-35.34, and 33.40-50.79%) respectively, (Fig-1) which showed that degradation and solubilization of biomass was intense in case of each agricultural waste (Table II, III, IV & V). The crude and soluble protein, crude fiber, cellulose, hemicellulose and lignin contents of the untreated waste were different from the treated once, which is in agree with the report of Mata (2005). The crude and soluble protein contents of the substrate treated and fortified agro wastes were significantly higher than untreated sample, due probably to the addition of fungal protein during solubilization and degradation (Table II III & IV). This agrees with the report of Jacqueline and Visser, (1996) who reported that the extra cellular enzymes secreted by the fungus contain amorphous homo and hetro polysaccharides which are often in association with fungal protein. The protein contents of the fungus untreated, treated and nitrogen supplemented samples increased from RS 7.07-9.418,8.46 and 13.38, RH 5.61-5.96,5.73-5.904, WS 7.32- 8.67,8.44 and 11.7, BS 6.73-9.04,9.67 and 12.81 and CW, 7.64-9.01,9.04 and 12.55 %. The soluble protein contents were in RS 0.57-0.896, 0.714-1.696, RH 0.42-0.46, 0.37 and 0.52, WS 0.57-0.58, 0.64 and 0.714, BS 0.58-0.68, 0.69 and 1.09 and CW 0.54 - 0.642,0.611 and 0.971%. The high crude and S. protein will likely increase the importance of the wastes as ruminant diet. But the fat content decreases constantly 1.13- 1.62,1.5 and 0.713 in RS, 0.483-0.650,0.55 and 0.34 in RH, 4.003-3.69,3.85 and 1.75 WS, reduction of fat in BS 1.63-1.63,1.55 and 0.99, and in CW 4.15-3.66,3.58 and 2.43 % (Table II ,III & V) (Shah, et al, 2004).

The fiber friction decreased significantly after the fungus growth as compared to the raw wastes. The decrease in fiber fraction could be due to the production of various enzymes during the vegetative and reproductive phases with lignocellulose degrading properties. The decreased in fiber content of the wastes were in RS 24.23, 21.67, 22.61 and 16.22, RH 34.86 -33.59, 32.313 and 30.95, in WS 30.51-28.52, 29.35 and 27.067, BS 39.29- 34.87 and 33.99-29.9, and in CW 65.02-63.55,61.64 and 61.36 % (Table II, III & V). Similarly, the % loss of lignin contents was in RS 34.38-32.57,32.93 and 47.86 , RH 2.39-3.91, 2.39 and 14.79 %, WS 18.44-27.25, 37.33 and 45.72, BS 5.34-16.10, 24.36 and 38.82 and CW 29.53-33.77,32.15 and 41.49%. The solubilization of the lignin occurs during the vegetative phase and enzymes like laccase, manganese peroxidase and lignin peroxidase are secreted while cellulose degrading enzymes is secreted during reproductive phase (Table IV& VI) (Tamara, et al, 1995).

The reduction of hemicellulose content recorded for the fungus treated different agro wastes were, RS, 21.39-24.82, 21.48 and 33.21, RH 8.15-10.01,9.03 and 16.04, WS,13.74-16.29,18.05 and 27.66, BS 14.66-19.35, 21.453 and 26.91, CW 9.14 -16.93, 13.74 and 37.82 %.(Table IV & VI) The % of cellulose loss was in RS, 15.71-27.33,17.38 and 37.85, RH, 4.20- 6.54,7.14 and 10.65, WS, 15.99-22.19, 23.27 and 29.03%, in BS, 9.95-13.36, 11.00 and 30.52, and in CW 10.37- 15.68, 15. 31 and 31.95 (Table IV & VI).

To investigates the effects of acid and basic treatment of agro wastes on the growth of *P. ostreatus* mycelia are shown in Table V & VI. Higher mycelial growth and degradation activities observed in treated substrates as compared to untreated agro waste. Appreciable degradation was recorded in acid and base treated substrate (Table V & VI).

It was also found that acid and base treatment was most effective for the production of fruiting bodies. 1.25% acid and basic wash of the agro waste improved the fermentation process.

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Table I. Differences between the biomass losses, number of fruit bodies and biological efficiencies of agricultural wastes.

Treatment	Biomass loss	No. of fruit bodies	Biological efficiencies
Rice straw	%	Mean	%
Control	29.79d	74cde	25.61abc
With chick pea	51.70a	113a	32.69a
Acid treated	49.79a	82 bcd	26.85abc
Basic treated	49.077a	81cde	26.66abc
Rice husk			
Control	16.89e	37g	9.51d
With chick pea	30.00d	51 fg	15.04cd
Acid treated	24.07e	39g	12.33d
Basic treated	22.63e	46 fg	10.81d
Wheat straw			
Control	38.023bc	59ef	22.6ab
With chick pea	48.86 a	78cde	28.60ab
Acid treated	46.86 a	68 de	25.81ab
Basic treated	47.423a	66 def	26.09bcd
Barley straw			
Control	30.00d	57 ef	21.63abc
With chick pea	35.34 cd	77 cde	26.69abc
Acid treated	33.00 cd	63ef	23.97abc
Basic treated	33.33 cd	64 def	18.06abc
Cotton waste			
Control	33.40d	72de	25.78abc
With chick pea	50.79 a	113a	32.05a
Acid treated	48.26 a	82 b	26.04abc

Mean with different letters in a column show significant difference ($P=0.05$) as determined by DMR test.

Table II. Biodegradation of agricultural wastes by *P.ostreatus*.

<i>P.ostreatus</i> /Waste used	DM %	MC %	Ash %	Fat %	CP %	SP %	CF %	NFE %
Rice straw	**	**	***	*	*		***	***
Non deg.Value	85.13a	7.53b	19.00b	1.59a	1.70b	0.55a	25.64c	52.07a
Deg value	81.31bc	49.43a	15.97c	1.13b	7.07a	0.572a	24.22d	51.62a
Rice Husk	**	***	*		**		***	***
Non deg.Value	83.07ab	10.87b	25.30a	0.54b	3.19b	0.347a	36.18a	34.78c
Deg value	77.83c	39.93b	20.51a	0.48a	5.61b	0.418a	34.86b	38.47c
Wheat straw	*	***					*	*
Non deg.Value	88.16a	6.24b	13.79a	6.11a	4.123a	0.432a	36.133a	39.82b
Deg value	83.53b	59.43a	11.41a	3.67a	7.324a	0.567a	30.513b	47.09a
Barley straw	**	***	*	*	*	*	***	***
Non deg. value	89.37b	2.85 c	3.20ab	2.45ab	3.16b	0.29ab	43.683b	47.48a
Degrade. Value	85.39b	55.74b	2.62b	1.63a	6.734a	0.58a	39.293c	49.06a
Cotton waste	**	***						
Non deg.Value	93.21a	3.09c	5.71a	4.11a	3.65b	0.301b	67.35a	17.86b
Deg value	90.82b	56.16a	3.68ab	4.15a	7.64a	0.54ab	65.02a	19.51b

Mean with different letters in a column show significant difference ($P=0.05$) as determined by DMR test.

Table III. Effect of nitrogen (Chick pea powder) on the mycelial growth and degradation capacity of *P.ostreatus*.

Agricultural Wastes	DM %	MC %	Ash %	Fat %	CP %	SP %	CF %	NFE %
	***	**	***	**	**	*	***	***
Rice straw	61.86d	60.74a	13.64a	0.71c	12.47a	1.69a	16.22e	57.41b
Rice husk	71.87c	49.08b	14.29a	0.34c	5.95b	0.52b	30.95c	48.52b
Wheat straw	78.40b	61.49a	12.02b	1.75ab	11.65a	0.72ab	27.07d	47.51a
Barley straw	79.51b	61.94a	1.19c	0.99bc	12.81a	1.09ab	29.9b	54.71b
Cotton waste	82.25a	63.11a	1.71c	2.43a	12.53a	0.97ab	61.36 a	21.94c

Mean with different letters in a column show significant difference ($P=0.05$) as determined by DMR test.

Table IV. Biodegradation of agricultural wastes and % age losses of organic matters (% differences of carbohydrates of different wastes after biodegradation)

<i>P. Ostreatus</i> Wastes Used. (% Value of degraded contents).	Cellulose	Cellulose loss%	Hemi-cellulose	Hemi Cellulose loss%	Lignin	Lignin loss%
Rice Straw						
Without N2	28.91	15.71d	19.20	21.39d	9.19	34.38d
With N2	21.32	37.85b	16.31	33.21c	7.31	47.86a
Rice husk						
Without N2	35.81	4.20g	13.16	8.15f	34.01	2.38f
With N2	32.88	10.65e	11.99	16.04e	30.04	14.79e
Wheat straw						
Without N2	33.18	15.99d	33.16	13.74d	11.51	18.44.e
With N2	28.03	29.03bc	27.73	27.66b	7.66	45.72e
Barley straw						
Without N2	36.69	9.95f	20.45	14.66e	11.03	5.34e
With N2	28.32	30.51c	17.52	26.91b	7.12	38.82d
Cotton wastes						
Without N2	49.11	10.37f	11.15	9.14d	12.54	29.53e
With N2	37.29	31.96a	7.63	37.83a	10.41	41.49b

Mean with different letters in a column show significant difference ($P=0.05$) as determined by DMR test.

Table V. Agricultural wastes treated with 1.25% acid and base.

Agricultural Wastes	DM %	MC %	Ash %	Fat %	CP %	SP %	CF %	NFE %
	***	**	***	*			***	***
Rice straw (Acid)	79.21ab	55.00abc	16.42a	1.62b	9.42ab	0.89a	21.67de	50.87a
(Basic)	79.827c	58.23a	17.24a	1.49b	8.46ab	0.71a	21.61e	50.19a
Rice husk (Acid)	76.05bc	42.12bc	18.63a	0.65b	5.96b	0.46a	33.59cd	41.17b
(Basic)	74.73bc	48.26c	18.03a	0.55b	5.73b	0.36a	32.31cd	43.06b
Wheat straw (Acid)	81.51ab	62.093a	10.63bc	4.49a	8.67ab	0.58a	28.52de	47.69a
(Basic)	82.26ab	58.94abc	9.073b	4.19ab	8.443ab	0.64a	29.35de	48.943a
Barley straw (Acid)	84.08ab	57.08abc	1.82c	1.63ab	9.04ab	0.68a	34.87b	52.65a
(Basic)	83.23a	61.24abc	1.84c	1.55ab	9.67ab	0.69a	33.99bc	52.88a
Cotton waste (Acid)	87.70a	57.94abc	3.84c	3.66ab	9.01ab	0.64a	63.55b	17.99a
(Basic)	87.73	62.36ab	4.12c	3.58ab	9.04a	0.611a	61.64b	22.29a

Mean with different letters in a column show significant difference ($P=0.05$) as determined by DMR test.

Table VI. Biodegradation of acid and basic treated agro wastes. (% differences of carbohydrates of different wastes after biodegradation)

Agricultural waste	Cellulose	Cellulose loss%	Hemi-cellulose	Hemi Cellulose loss%	Lignin	Lignin loss%
Rice straw Acid	24.93	27.34a	18.36	24.82a	9.45	32.57a
Basic	28.34	17.38abc	19.17	21.48a	9.39	32.93a
Rice husk Acid	34.95	6.54c	13.03	10.02a	33.483	3.91b
Basic	34.49	7.14c	12.99	9.03a	33.66	2.39b
Wheat straw Acid	30.73	22.19ab	32.18	16.29a	10.27	27.25a
Basic	30.31	23.27ab	31.50	18.05a	8.85	37.33a
Barley straw Acid	35.31	13.36bc	19.33	19.35a	9.76	16.10ab
Basic	36.27	11.01bc	18.82	21.45a	8.79	24.36a
Cotton waste Acid	46.19	15.68abc	9.65	16.93a	11.78	33.78a
Basic	46.40	15.32ab	10.58	13.74a	12.07	32.15a

Mean with different letters in a column show significant difference ($P=0.05$) as determined by DMR test.

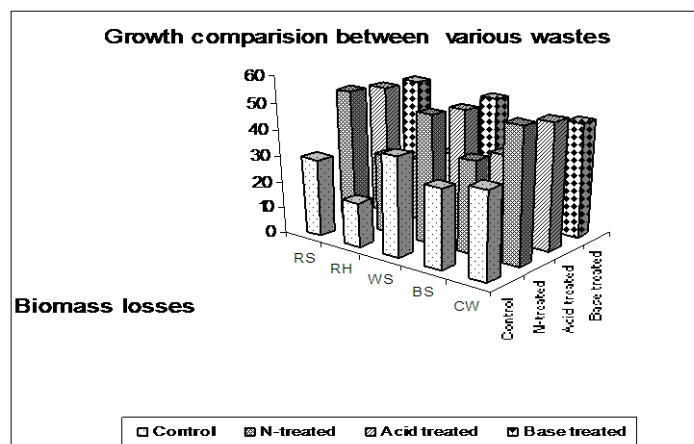


Fig1.

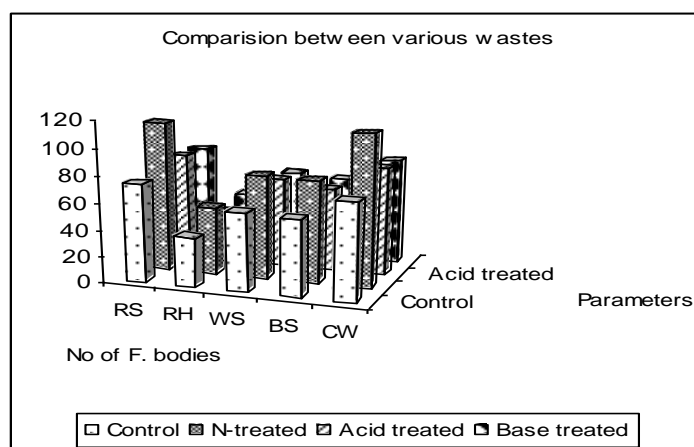


Fig2.

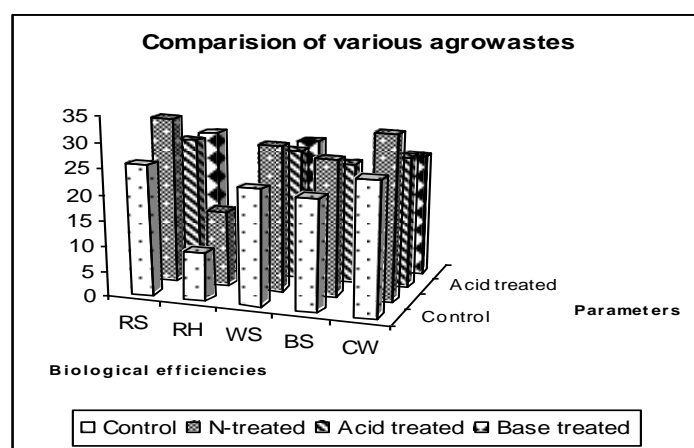


Fig3.

REFERENCES

- [1] Abena O, Adjapong, Kwane D, Ansah, Faustina A and Henry O 2015. Maize residue as a viable substrate for farm scale cultivation of Oyster mushroom (*Pleurotus ostreatus*) Dept. Gen. Agri. School Appl. Sci.Tech. vol. 2015 Article ID 213251 1-6.
- [2] ASTM Method 1961. American Society for Testing Materials and standard method of lignin in wood. 1106-56T pages 848.
- [3] Belew MA 2003. Nutritional qualities of corn cobs and waste paper incubated with edible mushroom (*Pleurotus sajor caju*). Nig. J. Anim. Prod. Vol. **30** (1): 20-25.
- [4] Bhatti MI, Jiskani MM, Wagan KH, Pathan MA and Magsi MR 2007. "Growth development and yield of oyster mushroom, *Pleurotus ostreatus* (jacq.Ex. Fr.) Kummer as affected by different spawn rates," Pak. J. Bot. 39: 2685-2692.

- [5] Chang ST and Miles PG 1988. Edible Mushroom and their cultivation. CRC press, Inc. Boca Raton, Florida U.S.A.27:83-88. Chow.
- [6] Fasidi IO 1996. Studies on *Volvariella esculenta* (mass) singer: Cultivation on Agricultural wastes and proximate composition of stored mushrooms. Food Chem., 55 (2): 161-163.
- [7] Goering HK and Van Soest PJ 1970. Forage fibre analysis (Apparatus, Reagents, and Procedures and some application). Agricultural hand book No. 379. Agricultural Research Service, United state Department of Agriculture.
- [8] Hami H 1990. Cultivation of oyster mushroom. (*Pleurotus spp.*) On saw dust of different woods M.Sc thesis Department of plant pathology, University of Agriculture, Faisalabad, Pakistan.
- [9] Hassan S, Mohammad AY and Kiramat K 2011. "Cultivation of Oyster mushroom (*Pleurotus ostreatus*) Jacq. P Kumm) in two different agro ecological zones of Pakistan." Afri. J. Biotech. Vol. 10: 183-188.
- [10] Jacqueline E W, Visser B 1996. Biotechnology: Building on Farmers, knowledge: In Assessing the potential edited by joske Bunders, Biertus Haverkort and Wim Hiemstra. Published by Macmillan Education Ltd. London, Basingstroke.
- [11] Khare KB, Mutuku JM, Achwania OS, and. Otaye DO 2010. "Production of two oyster mushrooms *Pleurotus sajor caju* and *P. florida* on supplemented and un-supplemented substrates." Int.J.Agri.Appli.edSci.: 6, 4-11.
- [12] Kausar T, Bajwa R 2005. Incorporation of Button Mushrooms in Pakistani Dishes. w Pak. J. Sci. Ind. Res. 48 (6): 417-421.
- [13] Kurschner K and Hank A. 1930. Determination of cellulose. Z.Untersuch. Lebnsn; 59: 448- 485.
- [14] Manson JC, Sims PGG, Broad P 1989. Biological routes to improve digestibility of animal feeds In: Biotechnology in livestock in developing countries. Edited by Hunter, A.G. Proceeding of an International Conference on the Application of Biotechnology to livestock in developing countries. Univ. of Edinburgh. Organized by the staff of the Center for Tropical Veterinary Medicine.
- [15] Markham R 1942. A steam distillation apparatus suitable for Mikrokjeldahl analysis. Biochem. J. 36: 760-791.
- [16] Mata G, Hernandez DM, Andreu 2005. Changes in lignocellulolytic enzyme activities in six *Pleurotus* spp.strains cultivated on coffee pulp in confrontation with *Trichoderma* spp.World. J. Microb. Biotechnol. 21 (2): 143-150.
- [17] Muhammad SN, Muhammad A A, Sajid A, Hasan S, Rizwan L and Muhammad S 2014. Growth and yield performance of oyster mushroom on different substrates. Mycopath 12(1): 9-15
- [18] Oei P 2003. Mushroom cultivation, appropriate technology for mushroom growers. Backhugs Publishers, Leiden. The Netherlands. Sompson Ruktahi S, Uthai W, Chenkahia S (2004).
- [19] Rana I, Kanojiya SA, Sandhu SS, 2007. Effect of waste organic substrates supplemented with mango leaf aqueous extract on the mycelial growth of *Pleurotus sajor-caju* and *Pleurotus florida*. J. Pure Appl. Microbiol., 1: 307-312. Rossi
- [20] Shah ZA, Ashraf M, Ishtiaq M 2004. Comparative study on cultivation and yield performance of Oyster mushroom (*Pleurotus ostreatus*) on different substrates (Wheat straw, leaves, saw dust). Pak. J. Nut. 3(3): 158-160.
- [21] Steel RGD and Torrie JH 1980. Principle and Procedure of Statistics. McGraw- Hill Publishers, London, U.K. 481.
- [22] Tamara V, Mika K and Annele H 1995. Lignin peroxidase, Manganese peroxidases and other ligninolytic enzymes produced by *phlebia radiate* during solid state fermentation of wheat straw. Appl. Envi. Micro. 3515-3520.
- [23] Wenjie Y, Feng LG and Zhengjie W 2013. Yield and size of oyster mushroom grown on rice and wheat straw basal substrate supplemented with cotton seed hull. Saudi J. Biol. Sci. 20(4): 333-338.