YIELD PERFORMANCE AND NUTRITIONAL ANALYSIS OF *PLEUROTUS CITRINOPILEATUS* ON DIFFERENT AGROWASTES AND VEGETABLE WASTES

MOHAN P. SINGH [1], VINAY KUMAR SINGH[2]
[1]Department of Biotechnology, VBS Purvanchal University, Jaunpur – 222001 (UP)
India
[2]Department of Zoology, Banaras Hindu University, Varanasi,
India
* mpsingh.16@gmail.com

ABSTRACT

Pleurotus citrinopileatus was cultivated on paddy straw, brassica straw, pea pod shell, cauliflower leaves and radish leaves separately and on various combinations of paddy straw and aforementioned wastes. The mushroom failed to grow on pea pod shell, cauliflower leaves and radish leaves when it was cultivated separately on these vegetable wastes. However, it grew very well on paddy straw in combination with other substrates. Total yield and biological efficiency of the mushroom was found to be lower on paddy straw than paddy straw and other wastes combinations. 70% paddy straw and 30% other wastes combination supported maximum biological efficiency of mushroom followed by 80% paddy straw and 20% other wastes combination. The mushroom cultivated on paddy straw mixed with other lignocellulosic wastes i.e. brassica straw, pea pod shell, cauliflower leaves and radish leaves contained better nutrient content than the mushroom cultivated on paddy straw alone. The protein content, total sugar and nonreducing sugar content was found to be higher in the mushrooms grown on paddy straw and other agrowastes combination than on paddy straw alone. Similarly, six essential amino acids i.e. leucine, isoleucine, valine, threonine, methionine and phenylalanine content was higher in the mushrooms cultivated on paddy straw and other agrowastes combination than on paddy straw alone.

Keywords: Biological efficiency; Nutritional analysis; *Pleurotus citrinopileatus*

INTRODUCTION

The problem of malnutrition with ever-increasing ‘protein gap’ is quite obvious in Asian, African and many developing countries, since, the traditional source of protein has not kept pace with population growth. It is desired, therefore, to explore and exploit the possible source of protein production to help the country to overcome the malnutrition. Malnutrition posses a continuing constrain to India’s development. Mushroom, one of the highest protein producers per unit area and time from agrowastes can be very effective weapon to fight malnutrition.

Of more than 2000 recorded species of the edible mushrooms, India accounts for nearly 300 species belongs to 70 genera [1]. Out of 2000 species 100 are widely picked, 15-30 species are commonly eaten, 80 species are experimentally cultivated and 5 – 6 are produced on large scale [2]. Among these *Agaricus* and *Pleurotus* contribute maximum to total world production of cultivated edible mushroom. The oyster mushrooms are botanically species of *Pleurotus* called as ‘Dhingri’ or ‘Abalone’. They grow naturally in temperate or tropical forest on dead and decaying wooden logs or sometimes on outer bark of living trees. The fruit bodies of this mushroom are distinctly shelly or oyster shaped with different shades of white, cream, grey, yellow, pink or light brown depending upon the species. The oyster mushroom confers many advantages over other

mushroom in terms of its ease for cultivation, role in biodegradation and bioremediation, extracellular enzymes production and nutriceuticals production [3-9].

Nutritional attributes of the oyster mushroom is being increasingly realized in recent times. Low in calories and high in protein as compare to rice, wheat, cabbage and milk, they are good sources of several vitamins including thiamine, riboflavin, niacin, biotin and ascorbic acids. The oyster mushrooms are good source of minerals and rich in carbohydrate and fibres.

Various workers have reported nutritional and medicinal attributes of mushroom. But there is wide variation in the values reported for the same species by different workers [10]. The difference may be due to the variation in the genetic make-up, substrates, cultivation technology and conditions at the stage of harvests as well as post harvest, which affect the composition.

MATERIALS AND METHODS

In the present research work the oyster mushroom *Pleurotus citrinopileatus* was cultivated on different vegetable and agricultural wastes viz. brassica straw, cauliflower leaves, radish leaves and pea pod shell alone and in combination of different proportion with paddy straw. The pure cultures of *P. citrinopileatus* were procured from NRCM, Solan (HP), India and maintained on malt extract agar (MEA) medium at temperature 25 ± 2°C and pH 6 - 6.5 and subcultured at periodic interval of three weeks.

Collection of agricultural and vegetable wastes. Five different agrowastes were collected from the different agricultural fields and Sabjimandi of district Jaunpur (UP), India. Radish leaves and cauliflower leaves were collected from old Sabjimandi, Kotawali, Jaunpur and New Sabjimandi, Chaukiya, Jaunpur. Brassica straw and paddy straw were collected from agricultural field of village Dewkali and Kukuripur just behind the V.B.S. Purvanchal University and pea pod shell from different house holds. Old Sabjimandi and New Sabjimandi are 10 km away from the University Campus.

Preparation of spawn. Spawn is referred to as the vegetative mycelium of the fungus, which is grown on cereal grains. Wheat grain spawn was prepared by the following method. Wheat grains were well washed in tap water and then half boiled in water. After that water from wheat grains was drained out. To remove excess water, wheat grains were spread over a tilted platform. This was followed by mixing of buffers $CaCO_3$ and $CaSO_4$ in 3:1 ratio (30 gm $CaCO_3$ and 10 gm $CaSO_4$ per kg of half boiled wheat grains). The wheat grains were now half filled in bottles and plugged by cotton. The half filled bottles were autoclaved at the temperature 121°C and pressure 15 psi for 40 minutes then left for overnight followed by inoculation of bottles by transferring inoculums *P. citrinopileatus* from cultured plate. Then bottles were incubated in BOD incubator at temperature 25 ± 2°C.

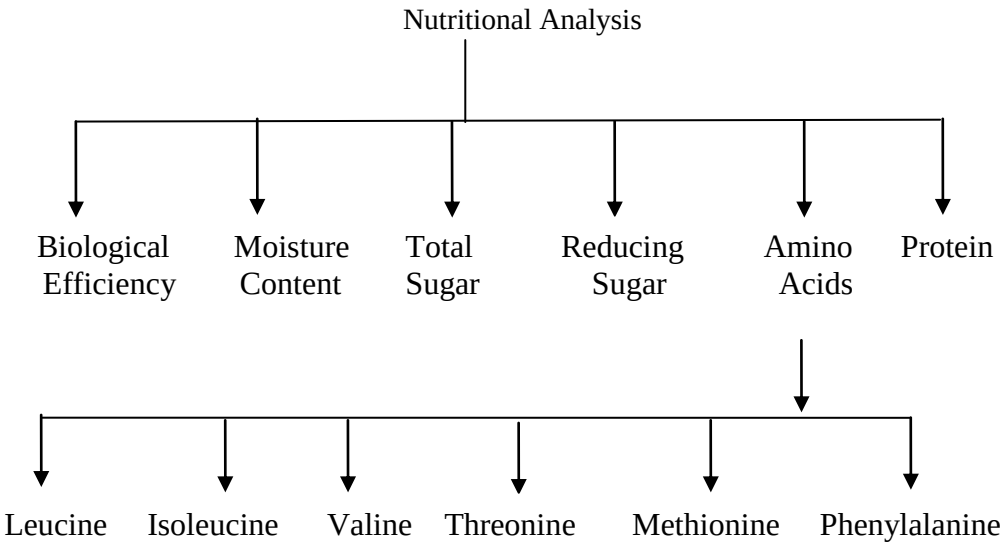
After 3-4 days of inoculation fungal mycelium started spreading on the grains. The mycelium is white net web like in appearance. The bottles were nearly half filled in 10-12 days and in 18-21 days these were completely filled with white mycelial growth.

Preparation of substrate, spawning and cultivation. The collected vegetable wastes i.e. cauliflower leaves, radish leaves and pea pod shell were spread in open area to sun dry for 30 to 40 days. These dried substrates were autoclaved at the temperature 121°C and pressure 15 psi for 40 minutes. Vegetative substrates separately and in various combinations with paddy straw were used for cultivation experiment. The paddy straw before mixing of vegetable wastes was completely dipped in water (50 litres for every 10 kg dry chopped paddy straw) in a drum or big bucket and was allowed to stay in water for 12 hours. After that excessive water was drained out. After draining, the paddy straw was again completely dipped in hot water (temperature 70-80°C)

for an hour. Then water was drained out and paddy straw was evenly spread on platform and mixed with dried autoclaved vegetable wastes (radish leaves, cauliflower leaves, brassica straw and pea pod shell) in two combinations i.e. 70% paddy straw and 30% other wastes and 80% paddy straw and 20% other wastes.

Spawning is the process of mixing spawn in the sterilized substrates. 3% wet weight basis spawn grain was mixed with the substrate and filled into polythene bags. The mouth of the bag was tied with rubber band and 12 holes of about 1cm diameter were made two at each corner at the base, four each on the broader area and one each on the narrow, rectangular side to drain out extra water and for proper aeration. Five bags of each combination of substrates (equiv. 300 g of dry substrate) spawned with *Pleurotus citrinopileatus* were filled and kept in mushroom house on the iron racks on the bricks.

Nutritional analysis. The nutritional analysis of mushroom fruit bodies were done after drying the mushroom samples taken separately from each bag in hot oven at a constant temperature of 40°C. The parameters selected for nutritional analysis is depicted in the flow diagram given below.



Biological efficiency

The four bags for each substrate and *P. citrinopileatus* were kept for evaluation of yield performance and biological efficiency in mushroom house under *in vivo* condition. The yield was expressed as of fresh fruit bodies produced per bag. Biological efficiency (B.E.) was calculated as the percentage conversion of dry substrates to fresh fruit bodies [11] i.e.

$$\text{Biological Efficiency} = \frac{\text{Fresh weight of mushrooms per bag (x)}}{\text{Dry weight of substrate per bag (y)}} \text{ X } 100$$

*Moisture content***.** It was done by picking fresh fruit body of the *P. citrinopileatus* and dried in hot air oven at 60°C for 15 hours.

$$\text{Moisture content} = \frac{\{\text{Fresh weight of mushroom (A)} - \text{Dry weight of mushroom (B)}\}}{\text{Fresh weight of mushroom (A)}} \text{X } 100$$

Sugar, amino acids, protein estimation. Total sugar estimation was done by using sulphuric acid phenol method [12] and the reducing sugar estimation was done by Dinitrosalicylic acid method. Amino acids were estimated by following the method of Moore and Stein [13]. Protein estimation was done by Lowry et al. method [14].

Data analysis. All the experiments were carried out in quadruplicates and the results are expressed as mean with standard deviation. Statistical significance was analyzed by ANOVA following Duncan's multiple comparison test ($P<0.05$) and student t-test. Each bar represents mean ± standard deviation (n=4). Asterisks show significant difference from control statistically at $P<0.05$.

RESULTS AND DISCUSSION

When the *Pleurotus citrinopileatus* was cultivated separately on radish leaves, pea pod shell and cauliflower leaves, the mushroom failed to grow on these three vegetable wastes. On the other hand when the mushroom was cultivated on paddy straw alone and paddy straw in combination with vegetable wastes, the fructification took place. Brassica straw alone as well as in combination with paddy straw supported better growth of the mushroom. The mean yield of *Pleurotus citrinopileatus* on different agrowastes in various combinations and their biological efficiency are given in Table 1 and Figure 1.

Table1: Yield and Biological Efficiency of *Pleurotus citrinopileatus* on different combinations of agrowastes

Substrates	Flush I (g/ bag)	Flush II (g/ bag)	Flush III (g/ bag)	Flush IV (g/ bag)	Total ((g/ bag)	B.E. (%)
100% PS	150.00	76.67	45.00	xx	271.67	90.55
30%BS+70%PS	148.75	65.50	55.00	13.75	283.00	94.33
30%PP+70%PS	148.75	65.50	55.00	13.75	283.00	94.33
30%CF+70%PS	138.75	70.00	56.25	12.5	277.5	92.50
30%RL+70%PS	136.25	16.75	57.50	12.50	273.00	91.00
20%BS+80%PS	136.25	62.50	58.75	12.50	270.00	90.00
20%PP+80%PS	145.00	66.25	57.50	18.33	282.50	94.16
20%CF+80%PS	160.00	63.33	48.33	xx	271.67	90.56
20%RL+80%PS	150.00	78.30	45.00	xx	275.00	92.78

PS = Paddy Straw, BS = Brassica Straw, PP = Pea Pod, CF = Cauliflower Leaves, RL=Radish Leaves. BE = Biological Efficiency.

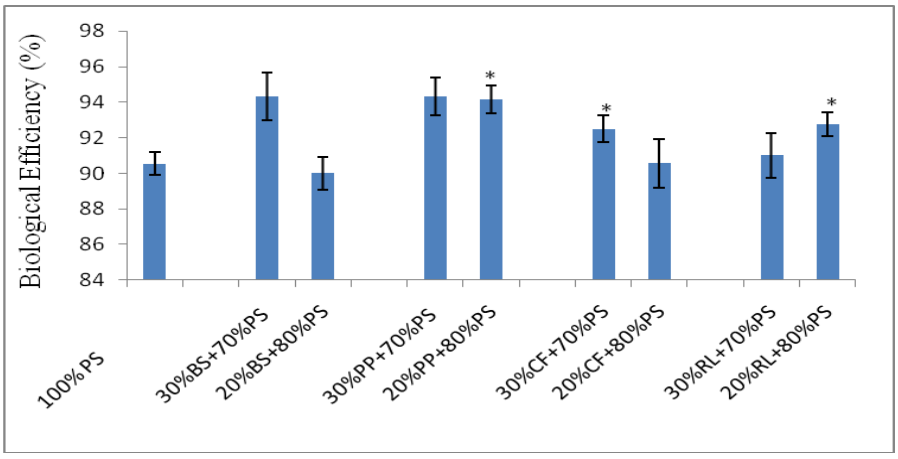


Figure1: Biological efficiency of *Pleurotus citrinopileatus* on different combinations of agrowastes.

PS = Paddy Straw, BS = Brassica Straw, PP= Pea Pod, CF = Cauliflower Leaves, RL=Radish Leaves.

70% paddy straw and 30% other agrowastes supported significant mushroom yield and bioefficiency than 80% paddy straw and 20% other agrowastes combination. Paddy straw and vegetable wastes combination gave better result in terms of total yield and bioefficiency than paddy straw alone. In all the cases first flush fruit bodies gave much more yield than second and subsequent flushes. There was decrease in the mushroom yield in the subsequent flushes.

The result of moisture content, protein and sugar content is given in Table 2 and Figure 2. The moisture content of fresh mushroom fruit bodies grown on various substrates ranged from 87.84 to 90%. Brassica Straw and Pea Pod with paddy straw in all combination in this experiment showed similar moisture retention capacity i.e. 89%.

Table 2: Nutritional content of *Pleurotus citrinopileatus* on different combinations of agrowastes

Substrates	Moisture (%)	Protein (mg/100g)	TS (mg/100g)	RS (mg/100g)	NRS (mg/100g)
100% PS	90.00	42.00	45.55	18.35	27.20
30%BS+70%PS	89.00	50.00	40.00	15.35	24.65
30%PP+70%PS	89.00	52.35	32.00	17.35	14.65
30%CF+70%PS	87.84	46.00	35.00	18.35	16.65
30%RL+70%PS	87.84	42.00	35.00	18.00	17.00
20%BS+80%PS	89.00	45.00	40.00	15.35	24.65
20%PP+80%PS	89.04	52.35	32.00	17.35	14.65
20%CF+80%PS	87.84	46.00	35.00	18.35	16.65
20%RL+80%PS	87.84	42.00	43.00	18.00	25.00

PS = Paddy Straw, BS = Brassica Straw, PP = Pea Pod, CF = Cauliflower Leaves, RL=Radish Leaves. TS = Total Sugar, RS = Reducing Sugar, NRS = Non-reducing Sugar.
Values of protein and sugar are expressed in mg/100g wet weight of dry mushroom

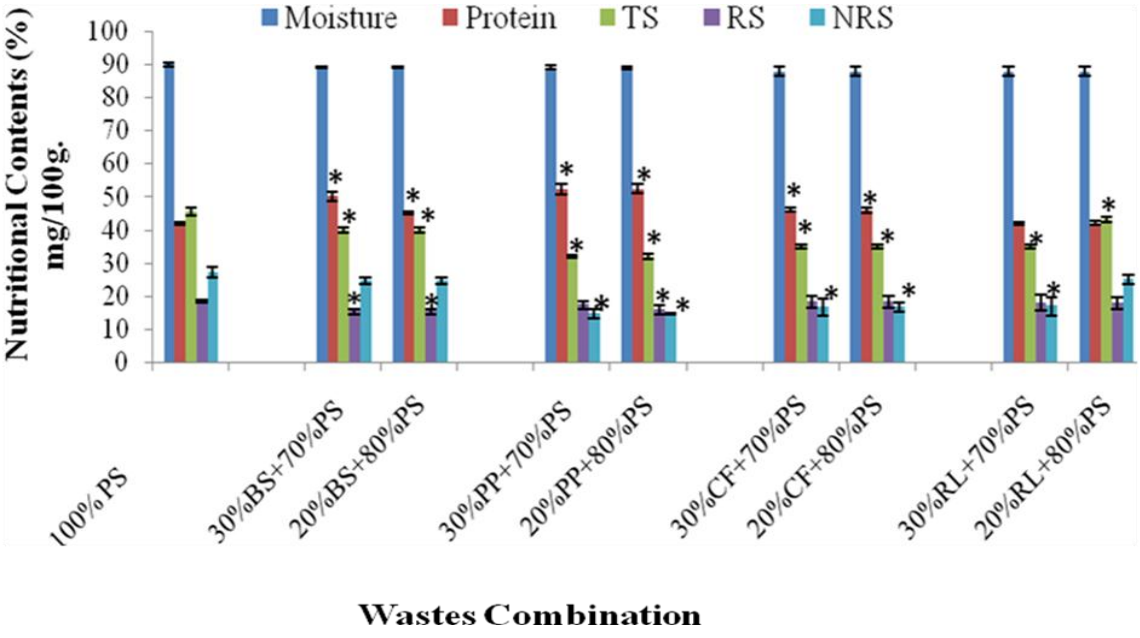


Figure2: Nutritional content of *Pleurotus citrinopileatus* on different combinations of agrowastes.

PS = Paddy Straw, BS = Brassica Straw, PP = Pea Pod, CF = Cauliflower Leaves, RL=Radish Leaves. TS = Total Sugar, RS = Reducing Sugar, NRS = Non-reducing Sugar.
Values of protein and sugar are expressed in mg/100g wet weight of dry mushroom

The protein content of mushroom fruit bodies ranged from 42 mg to 52.35 mg per 100 g of dried fruit bodies. Maximum protein content was observed in the mushroom fruit bodies when it was grown on paddy straw and pea pod combination. The mushroom grown on 70% paddy straw and 30% vegetable wastes had more protein content than 80% paddy straw and 20% vegetable wastes combination. The mushroom fruit bodies produced on paddy straw and vegetable combination showed more protein content than paddy straw alone.

Total sugar content recorded in the mushroom fruit bodies varied from 32 mg to 45.55 mg per 100 g of dried mushroom. Maximum total sugar content was observed in the mushroom fruit bodies produced on paddy straw alone. Total sugar content in the fruit bodies produced on various combinations of paddy straw and vegetable wastes was found to be lower than paddy straw. This was contrary to the protein content of fruit bodies. Non-reducing sugar in *P. citrinopileatus* cultivated on various substrates ranged from 14.65 to 27.25 mg/100mg weight of dry mushroom.

The amino acid content of *P. citrinopileatus* cultivated on paddy straw alone and combination of paddy straw and vegetable wastes is given in Table 3 and Figure 3. Six amino acids i.e. leucine, isoleucine, valine, threonine, methionine and phenylalanine determined from the fruit bodies of *P. citrinopileatus* grown on paddy straw had lower amount than on paddy straw and other agrowastes combination. Among the six amino acids amount of valine was observed as the maximum followed by threonine and other amino acids.

In the present investigation *P. citrinopileatus* failed to grow when cultivated separately on radish leaves, pea pod shell and cauliflower leaves. The probable reason for this is that these three vegetable wastes when processed for cultivation and dipped into and taken out from water they hold large amount of water. Hence due to presence of excess water in the substrate and lack of proper aeration *Pleurotus* mycelia does not grow and spread adequately and spawn run and fructification fail to occur. On the other hand when these vegetable wastes are mixed with paddy straw, these shortcomings are overcome and spawn run and fructification take place. The results of yield performance (Table 1 and Figure1) indicate that the first flush of fruiting bodies gave maximum yield in comparison to second and subsequent flushes. The lowest yield was recorded in the last flush. Block *et al.* also reported higher yield of *P. ostreatus* in first flush while yield of second flush was two third of first flush and yield of third flush was two third of second flush [15]. However, Chang *et al* observed uniform distribution of fruit bodies of *P. sajor-caju* in all the four flushes on cotton wastes while they observed higher yield of first flush (46%) than second flush (29%), third flush (15%) and fourth flush (9%) on paddy straw [11]. Bisaria *et al* reported higher yield of *P. florida* in first flush than subsequent flushes on paddy and wheat straw [16].

In the present work better yield and bioefficiency of *P. citrinopileatus* was seen when they were cultivated on paddy straw mixed with other agrowastes than paddy straw alone. This may be due to presence of various macro and microelements in the brassica straw, radish leaves, pea pod shell and cauliflower leaves which could have promoted the growth of the mushroom and ultimately yield and biological efficiency.

Moisture content *per se* may not be of any nutritional significance but it considerably influences the nutritional value of mushroom fruit bodies. In the present work the moisture content of the mushroom have been found to be 87.84% to 90%. Crisan and Sands, and Bano and Rajarathnam reported moisture content of fresh cultivated mushroom between 90 to 94% [17, 18]. The oyster mushroom *P. sajor-caju* when cultivated on paddy straw mixed with vegetable wastes had better nutrient contents than paddy straw alone. This is because of the fact that vegetable wastes are rich in minerals and vitamins. The composition of these substrates affects the nutritional value of mushroom fruit bodies. The mushroom mycelia secret extracellular enzymes which play key role in the degradation of substrates and which in turn affect the growth, development and nutritional value of fruiting bodies. In the present work it was observed that mushroom cultivated on paddy straw and other agrowastes mixed substrates are rich in qualitative

and quantitative protein. This is reflected from the higher amount of six amino acids i.e. leucine, isoleucine, valine, threonine, methionine and phenylalanine and higher amount of protein contents.

Table 3: Amino Acid content of *Pleurotus citrinopileatus* on different combinations of agrowastes.

Substrates	Leu (mg/100g)	Ile (mg/100g)	Val (mg/100g)	Thr (mg/100g)	Met (mg/100g)	Phe (mg/100g)
100% PS	0.535	0.580	0.930	0.700	0.321	0.598
30%BS+70%PS	0.600	0.722	0.995	0.725	0.325	0.635
30%PP+70%PS	0.635	0.695	1.010	0.865	0.375	0.655
30%CF+70%PS	0.625	0.600	*1.033*	0.825	0.495	0.665
30%RL+70%PS	0.725	0.695	1.035	0.875	0.500	0.695
20%BS+80%PS	0.595	0.700	0.935	0.700	0.300	0.600
20%PP+80%PS	0.615	0.655	1.000	0.800	0.325	0.635
20%CF+80%PS	0.610	0.585	1.020	0.810	0.435	0.645
20%RL+80%PS	0.700	0.680	1.000	0.825	0.475	0.685

PS = Paddy Straw, BS = Brassica Straw, PP= Pea Pod, CF = Cauliflower Leaves, RL=Radish Leaves.
Leu = Leucine, Ile = Isoleucine, Val = Valine, Thr = Threonine, Met = Methionine,
Phe = Phenylalanine. Values are expressed in mg/100g weight of dry mushroom

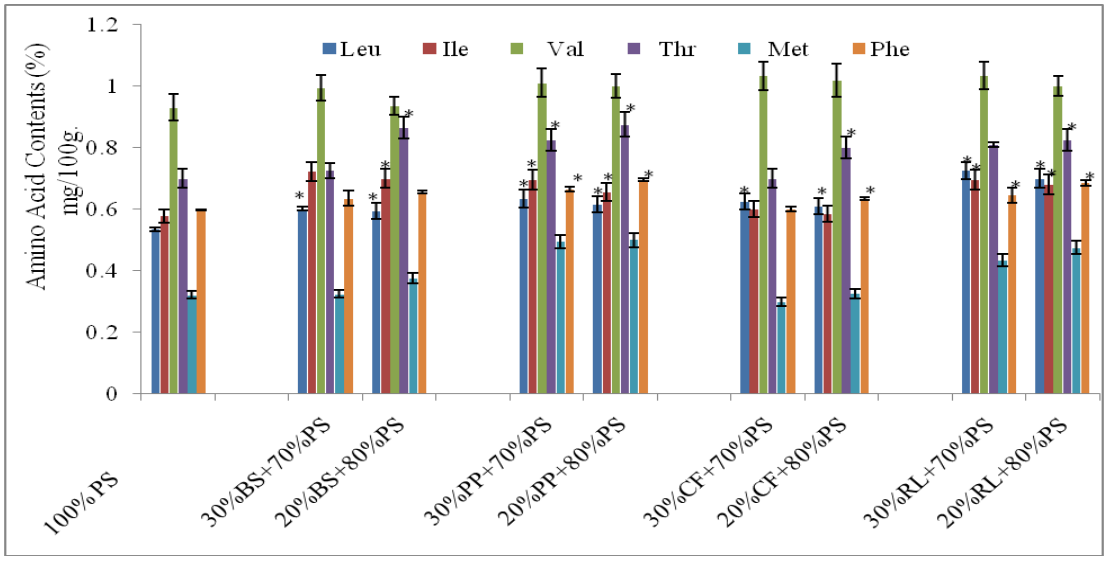


Figure3: Amino Acid content of *Pleurotus citrinopileatus* on different combinations of agrowastes.
PS = Paddy Straw, BS = Brassica Straw, PP= Pea Pod, CF = Cauliflower Leaves, RL=Radish Leaves.
Leu = Leucine, Ile = Isoleucine, Val = Valine, Thr = Threonine, Met = Methionine,
Phe = Phenylalanine. Values are expressed in mg/100g weight of dry mushroom

CONCLUSIONS

The observations of present investigation suggest that the edible mushroom *Pleurotus citrinopileatus* grown on paddy straw mixed with brassica straw, pea pod shell, cauliflower leaves and radish leaves gives fruit bodies with enhanced protein, sugar and amino acid content. Besides, these substrates also support better yield performance and biological efficiency. The three vegetable wastes i.e. pea pod shell, cauliflower leaves and radish leaves used in the present investigation are generated from every households and vegetable markets in large quantities. These wastes are generally left to rot in situ in many cities of India causing outbreak of many diseases. It pollutes the environment and causes environmental degradation. These wastes can be

utilized as resources for mushroom production with improved nutriceuticals. This can be used as an effective weapon against malnutrition particularly in those regions of the world where malnutrition related diseases and deaths are common.

ACKNOWLEDGEMENT

We would like to thank Council of Science and Technology, Uttar Pradesh (CSTUP), India for providing financial assistance through Major Research Project (CST/AAS/D-781). The project fellowship provided by CSTUP to Vinay Kumar Singh is gratefully acknowledged.

REFERENCES

[1] Chadha K.L. and Sharma S.R. (1995). Mushroom research in India. In: *Advances in Horticulture vol.13*. Chaddha K.L. and Sharma S.R. Eds. Malhotra Publishing House, New Delhi, pp 1-33.

[2] Benjamin D.R. (1995). Incidence of mushroom poisoning In: *Mushroom: Poision and Panacea.* S.W.H. Freeman and Company, New York,pp. 152-161.

[3] Singh M.P. and Pandey V.K. (2007). Biodegradation of brassica haulms by *P. eryngii*. In: *International conference of Mush. Biology and Biotechnology,* NRCM Solan, pp. 90.

[4] Singh M.P. and Sharma R. (2002). *Pleurotus florida* eger- an effective biodegrader of steam sterilized lignocellulosic wastes *Poll Res* 21 (1): 63- 67.

[5] Singh M.P. 2000. Biodegradation of lignocelluosic wastes through cultivation of *Pleurotus sajor-caju* In*: Science and Cultivation of Edible Fungi.* Van Griensven Ed. Balkema, Rotterdem, pp 517-521.

[6] Singh M.P. et al. (2007a). Extra cellular enzyme profiles by white rot fungi on lignocellulosic wastes. *Poll Res*.26: 163-167.

[7] Singh M.P. et al. (2007b). Extracellular enzymatic activities by *Pleurotus* species on vegetable wastes. *Mush. Res*. 93-97.

[8] Singh M.P. et al. (2008). Production of xylanase by white rot fungi on wheat straw. *Asian Jr. of Microbiol. Biotech. Env. Sc*. 10: 859-862.

[9] Singh M.P. et al. (2011). Biodegradation of Brassica haulms by white rot fungus *P. eryngii*. Cell. Mol. Biol. 57 (1): 47- 55.

[10] Rai R.D. (1995). Nutritional and medicinal value of mushrooms. In: *Advances in Horticulture vol.13*. Chaddha K.L. and Sharma S.R. Eds. Malhotra Publishing House, New Delhi, pp 49-52.

[11] Chang S. T. et al. (1981). The cultivation and nutritional value of *Pleurotus sajor-caju*. *Eur. J. Appl. Microbiol. Biotechnol.* 12: 58-62.

[12] Dubois M. et al. (1956). *Anal Chem.* 26: 350.

[13] Moore S. and Stein (1948). In: *Methods Enzymol.* Colowick S.P. and Kaplan N.D.Eds. Academic Press, New York, 3, 468.

[14] Lowry O.H. (1951). Protein measurement with folin-phenol reagent. *J. Biol. Chem.* 193: 265-275.

[15] Block S.S. et al. (1959). Experiments in the cultivation of *Pleurotus ostreatus*. *Mush. Sci*. 4: 309-325.

[16] Bisaria R. et al. (1987). Biological efficiency and nutritive value of *Pleurotus sajor-caju* cultivated in different agrowastes. *Biol. Wastes*. 19: 239-255.

[17] Bano Z. and Rajarathnam S. (1982). *Pleurotus* mushroom as nutritious food. In: *Tropical Mushroom – Biological Nature and Cultivation Methds*. Chang S.T. and Quimio T.H. Eds. The Chinease University Press, Hongkong. pp. 363-382.

[18] Crisan E.V. and Sands A. (1978). Nutritional value of edible mushroom. In: *The Biology and Cultivation of Edible Mushrooms*. Chang S.T. and Hayes W.A. Eds. Academic Press, New York. pp 137-168.