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BIODEGRADATION OF VEGETABLE AND AGROWASTES BY *Pleurotus sapidus*: A NOVEL STRATEGY TO PRODUCE MUSHROOM WITH ENHANCED YIELD AND NUTRITION

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Abstract

Edible oyster mushroom *Pleurotus sapidus* was cultivated, on pea pod shell, cauliflower leaves, radish leaves, brassica straw in various combinations of paddy straw. The mushroom failed to grow on these vegetable wastes separately. However, it grew very well on these vegetable wastes when mixed with various combinations of paddy straw as substrate. Total yield and biological efficiency of the mushroom cultivated on substrate containing 20% and 30% vegetable wastes mixed with 70% and 80% (w/w) of paddy straw was found to be better, when compared with yield and efficiency obtained with paddy straw alone (100%). The protein content in the fruit bodies was found to be higher in the mushroom grown on paddy straw mixed with vegetable wastes than that obtained with paddy straw alone. Similarly, six amino acids (Leu, Ile, Val, Thr, Met and Phe) showed a significant increase when the mushroom was grown on a mixed substrate containing both vegetable wastes and paddy straw. On the contrary, the total sugar and reducing sugar content declined in the mushroom grown on the mixture of paddy straw and other wastes, when compared with the results obtained with paddy straw alone.

Key words: Biological efficiency, Proteins and amino acids, Nutritional analysis, *Pleurotus sapidus.*

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INTRODUCTION

India is the second major producer of vegetables in the world and contributes 14 percent of total world vegetable production (13). Taking estimated production of fruits and vegetables in India at 150 million tones, the total waste generation comes to about 50 million tons per annum (9). Fruits and vegetables wastes are more prone to spoilage than cereals due to their chemical composition. This creates unhygienic condition leading to spread of diseases and loss of resources. These wastes can be utilized for nutritionally rich food production through cultivation of oyster mushroom.

Of more than 2000 recorded species of the edible mushrooms, India accounts for nearly 300 species belonging to 70 genera (5). 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 mushrooms. The oyster mushrooms are botanically species of *Pleurotus* called as 'Dhingri' or 'Abalone'. They grow naturally in temperate and tropical forests 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 (14, 15, 16, 17, 18, 19, 20).

In recent times nutritional attributes of the oyster mushroom is being increasingly realized. 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 (12). The difference may be due to the variation in the genetic make-up, substrates, cultivation technologies and conditions at the stages of harvest as well as post harvest, which affect the composition.

Recycling of fruit and vegetable wastes can be one of the most important means of utilizing it in a number of innovative ways yielding new products, which may cater to essential requirements of human, animal and plant nutrition. Cultivation of mushroom on these residual wastes is one of the most ecofriendly practice to fight the malnutrition and environmental pollution caused by these agrowastes.

MATERIALS AND METHODS

In the present research work *Pleutotus sapidus* was grown on selected vegetable wastes viz. brassica straw, cauliflower leaves, radish leaves and pea pod shell in various combination with paddy straw.

The culture and their maintenance

The pure cultures of *P. sapidus* was procured from National Research Centre on Mushroom, 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 Sabji mandi (vegetable mar-

ket) of district Jaunpur (UP), India. Radish leaves and cauliflower leaves were collected from old Sabji mandi, 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 households. Old Sabjimandi and New Sabjimandi are 10 km away from the University Campus.

Cultivation

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 of Singh *et al* (14). Wheat grains were well washed in tap water and then it was half boiled in water. Thereafter, water from the 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 g $CaCO_3$ and 10 g $CaSO_4$ per kg of half boiled wheat grain). 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 30 minutes, then left for overnight. The process was followed by inoculation of bottles by transferring inoculums of *P. sapidus* from cultured plate. Then bottles were placed in incubation chamber at a temperature of 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 almost completely filled with white mycelia growth in 18-21 days.

Preparation of substrate

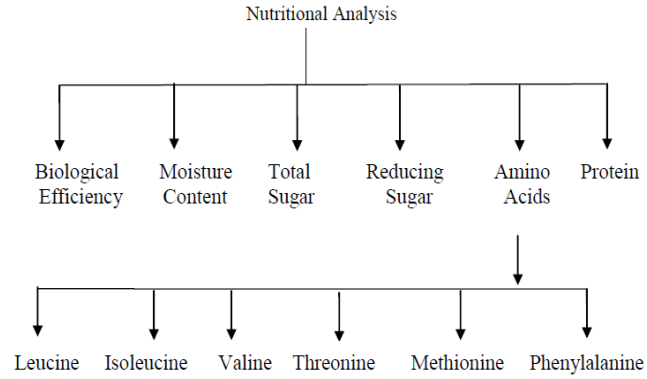
The collected vegetable wastes i.e. cauliflower leaves, radish leaves and pea pod shell were spread in open area for sun drying for 30-40 days. These dried substrates were autoclaved at the temperature 121˚C and pressure 15 psi for 40 minutes. Vegetable wastes were used separately as well as in various combinations with paddy straw 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, the paddy straw was again completely dipped in hot water (temperature 70-80˚C) for an hour. The excess 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. paddy straw and vegetable wastes (70%+30% and 80%+20% respectively, w/w).

Spawning

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 of 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 (equivalent to 300 g of dry substrate) spawned with *Pleurotus sapidus* were filled and kept in mushroom house on the iron racks on the bricks.

Nutritional analysis

The nutritional analysis of mushroom fruiting bodies was done after drying it 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 were used for evaluation of yield performance and biological efficiency of *P. sapidus* kept in mushroom house under *in vivo* condition. The yield was expressed as fresh fruit bodies produced per bag. Biological efficiency (B.E.) was calculated as the percentage conversion of dry substrates to fresh fruit bodies (6) i.e.

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

Moisture content

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

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

Sugar, amino acids and protein estimation

Total sugar estimation was done by using sulphuric acid phenol method (8) and the reducing sugar estimation was done by Dinitro salicylic acid method (21) with some modifications. Amino acids were estimated by following the method of Moore and Stein (11). Protein estimation was done by the method of Lowry *et al* (10).

Statistical analysis

The data were presented as mean ± standard error of mean (n=4). Statistical significance was analyzed by ANOVA following Duncan's multiple comparison test ($P<0.05$). In the given figures and tables the mean bar with star superscripts shows significant decrease or increase in comparison to control (100% PS)

RESULTS

When the *Pleurotus sapidus* 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. The mean yield of *Pleurotus sapidus* on different agrowastes in various combinations and their biological efficiency are given in Table 1 and Figure 1.

70% paddy straw and 30% other agrowastes supported better mushroom yield and bioefficiency than 80% paddy straw and 20% other agrowastes combination. Paddy straw and vegetable wastes combination gave better results 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. Maximum biological efficiency (96.88%) was recorded on 30 % pea pod and 70% paddy straw followed by the biological efficiency of 96.66% on 30% radish leaves and 70% paddy straw.

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 82% to 90%.

The protein content of mushroom fruit bodies ranged from 39.78 mg to 46.23 mg per 100 mg of dried fruit bodies. Maximum protein content was observed in the mushroom fruit bodies when it was grown on paddy straw and brassica straw combination. The mushroom grown on 70% paddy straw and 30% vegetable wastes showed more protein content than that grown on 80% paddy straw and 20% vegetable wastes combination. The mushroom fruit bodies produced on paddy straw and vegetable combinations showed more protein content than paddy straw alone.

Total sugar content recorded in the mushroom fruit bodies varied from 32 mg to 42 mg per 100 mg of dried mush-

Table1. Yield and Biological Efficiency of *Pleurotus sapidus* 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	152.33	76.33	29.00	12.34	270.00	90.00 ± 0.64
20%BS+80%PS	152.32	72.33	31.34	15.00	270.99	90.33 ± 0.49
20%PP+80%PS	157.33	76.30	30.00	21.70	285.33	*95.11 ± 0.85
20%CF+80%PS	155.00	75.00	30.00	16.67	276.67	*92.22 ± 0.78
20%RL+80%PS	157.33	75.00	29.33	21.67	283.33	*94.44 ± 0.43
30%BS+70%PS	152.25	74.25	31.50	26.00	284.00	*94.66 ± 0.79
30%PP+70%PS	159.00	78.33	28.33	25.00	290.67	*96.88 ± 0.67
30%CF+70%PS	162.00	75.00	26.64	16.69	280.33	*93.44 ± 0.83
30%RL+70%PS	165.00	75.00	30.00	20.00	290.00	*96.66 ± 0.68

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

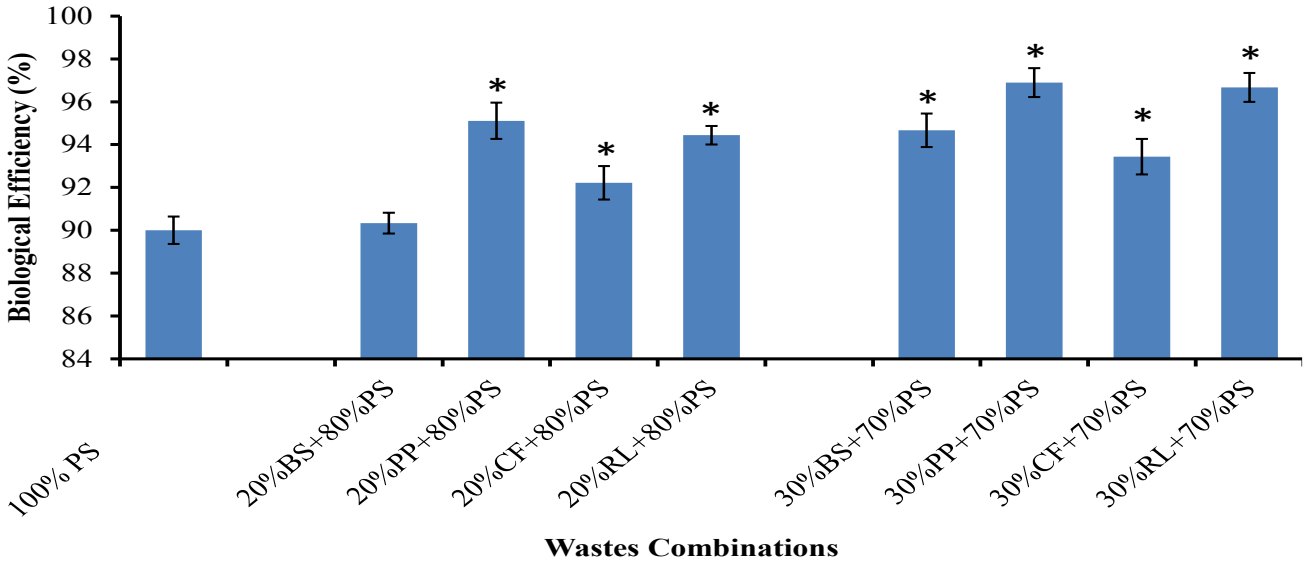


Figure1. Biological efficiency of *Pleurotus sapidus* on different combinations of agrowastes.

Table 2. Nutritional content of *Pleurotus sapidus* on different combinations of agrowastes.

Substrates	Moisture (%)	Protein (mg/100mg)	TS (mg/100mg)	RS (mg/100mg)
100% PS	90.00± 0.41	39.78± 0.52	42.00 ± 0.31	25.15 ± 0.17
20%BS+80%PS	*83.00 ± 0.61	*42.25 ± 0.53	*38.35 ± 0.31	*20.15 ± 0.12
20%PP+80%PS	*83.00 ± 0.71	*44.80 ± 0.47	*35.25 ± 0.30	*18.00 ± 0.42
20%CF+80%PS	*84.00 ± 0.78	*44.61 ± 1.26	*35.75 ± 0.32	*20.00 ± 0.16
20%RL+80%PS	*87.00 ± 0.81	*44.23 ± 0.57	*34.15 ± 0.23	*20.65 ± 0.18
30%BS+70%PS,	*82.00 ± 1.33	*46.11± 0.83	*36.30 ± 0.38	*22.00 ± 0.16
30%PP+70%PS,	*83.00 ± 1.12	*47.15 ± 0.47	*32.80 ± 0.12	*14.35 ± 0.15
30%CF+70%PS,	*88.00 ± 0.80	*46.23 ± 0.74	*33.65 ± 0.30	*18.25 ± 0.17
30%RL+70%PS,	*86.00 ± 0.73	*46.23 ± 1.27	*32.00 ± 0.13	*17.33 ± 0.20

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

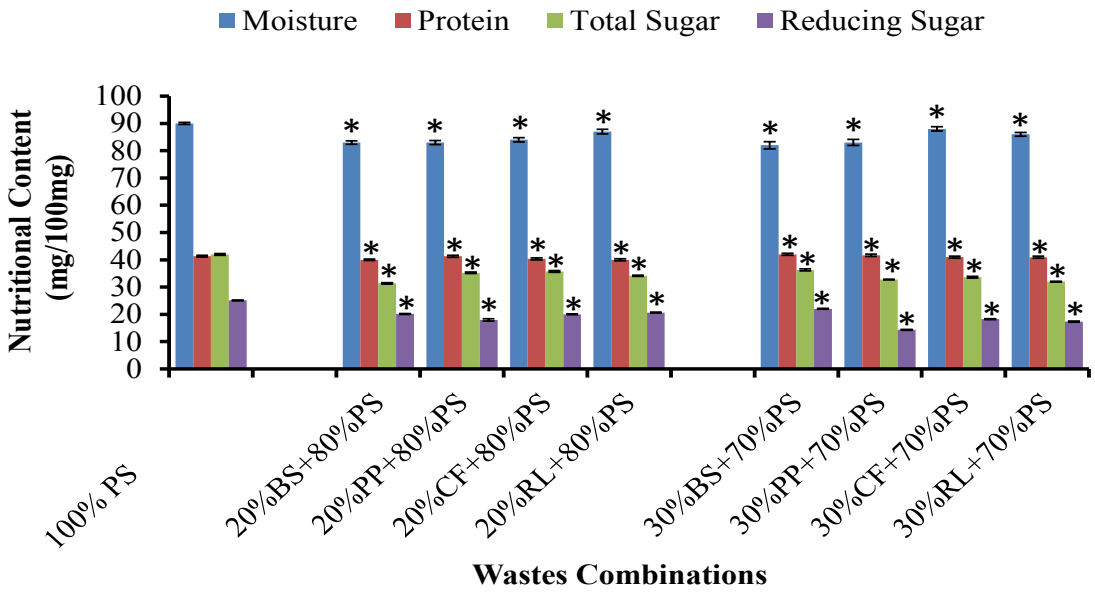


Figure2. Nutritional contents of *Pleurotus sapidus* on different combinations of agrowastes.

room. 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 that on the paddy straw alone. This was contrary to the protein content of fruit bodies. Non-reducing sugar in *P. sapidus* cultivated on various substrates ranged from 14.35 to 25.15 mg/100 mg of dry mushroom.

The amino acid content of *P. sapidus* cultivated on paddy straw alone and combination of paddy straw and vegetable wastes are 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. sapidus* grown on paddy straw alone exhibited lower amount than that on the paddy straw and other agrowastes combination. Among the six amino acids amount of valine was found to be the maximum, followed by threonine and other amino acids.

DISCUSSION

In the present investigation *P. sapidus* 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 retained large amount of water, which prevented the proper aeration of *Pleurotus* mycelia and fructification failed to occur. On the other hand when these vegetable wastes were mixed with paddy straw, these shortcomings were overcome and adequate spawn run and fructification took 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* (4) have also reported higher yield of *P. ostreatus* in first flush, while yield of second flush was reduced to two third of the first flush. Similarly the yield of third flush was again reduced to two third of the second flush (4). Contrary to this, Chang *et al* (6) observed uniform distribution of fruit bodies of *P. sajor-caju* in all the four flushes on cotton wastes. However, the yield of first flush (46%) was higher than the second flush (29%), third flush (15%) and fourth flush (9%) on paddy straw (6). Bisaria *et al* (3) have also reported higher yield of *P. florida* in first flush than subsequent flushes on paddy and wheat straw.

In the present work, the yield and bioefficiency of *P.*

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

Substrates	Leu mg/100mg	Ile mg/100mg	Val mg/100mg	Thr mg/100mg	Met mg/100mg	Phe mg/100mg
100% PS	0.485±0.003	0.473±0.002	0.901±0.002	0.800±0.004	0.370±0.002	0.590±0.006
20%BS+80%PS	0.635±0.01	0.535±0.004	0.965±0.006	0.875±0.007	0.375±0.003	0.600±0.004
20%PP+80%PS	0.625±0.002	0.575±0.006	0.935±0.006	0.900±0.007	0.395±0.003	0.700±0.006
20%CF+80%PS	0.565±0.015	0.525±0.003	1.000±0.000	0.800±0.004	0.425±0.003	0.675±0.010
20%RL+80%PS	0.675±0.003	0.650±0.004	1.020±0.012	0.885±0.007	0.465±0.003	0.740±0.004
30%BS+70%PS	0.675±0.005	0.595±0.005	1.000±0.008	0.895±0.005	0.400±0.002	0.700±0.003
30%PP+70%PS	0.665±0.017	0.600±0.002	1.000±0.010	0.925±0.006	0.475±0.002	0.735±0.007
30%CF+70%PS	0.600±0.005	0.610±0.005	1.010±0.005	0.925±0.008	0.475±0.003	0.735±0.013
30%RL+70%PS	0.695±0.003	0.700±0.004	1.085±0.006	0.935±0.006	0.495±0.003	0.755±0.007

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/100 mg weight of dry mushroom

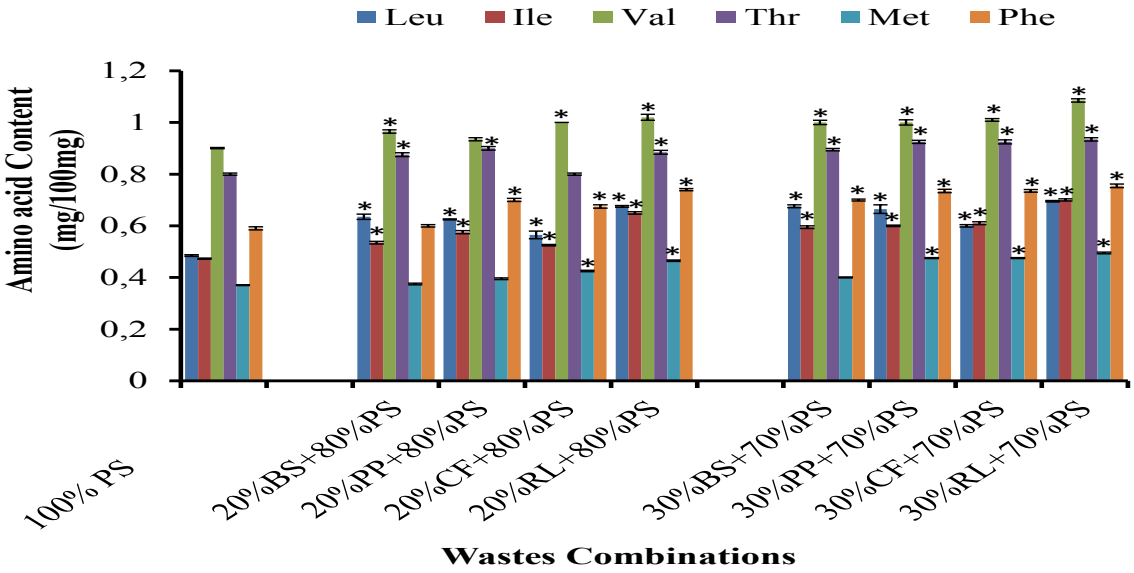


Figure3. Amino Acid content of *Pleurotus sapidus* on different combinations of agrowastes.

sapidus is found to be better when it is cultivated on paddy straw mixed with other agrowastes. The better yield and bioefficiency of *P. sapidus* on brassica straw, radish leaves, pea pod shell and cauliflower leaves in combination with paddy straw may be due to presence of various macro and microelements supplied by the vegetable wastes, which are not available in the paddy straw alone. The composition of substrates affects the nutritional value of mushroom fruit bodies. The mushroom mycelia secret extracellular enzymes (15) and may be helpful in enhancing the 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.

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 is found to be in the range of 82% to 90%. Earliar reports of Crisan and Sands, and Bano and Rajarathnam have also suggested that the moisture content of fresh cultivated mushroom ranges between 90 to 94% (1,7).

The observations of present investigation suggest that the edible mushroom *Pleurotus sapidus* grown on paddy straw mixed with brassica straw, pea pod shell, cauliflower leaves and radish leaves gives fruit bodies with enhanced protein and amino acid content. These wastes are generally left to rot *in situ* in many cities of India causing outbreak of many diseases. These wastes can be effectively utilized as a resource material for mushroom production with improved nutriceuticals.

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Other articles in this theme issue include references (22-49).

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