



Yield and proximate composition of *Pleurotus florida* cultivated on wheat straw supplemented with perennial grasses

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ABSTRACT

Pleurotus spp. are the most commonly cultivated and consumed mushrooms in India. This mushroom grows on almost all kinds of traditional as well as the non-traditional biomass sources giving good yield. In the present study, *Panicum virgatum* and *Panicum maximum* grasses were utilized for the cultivation of *Pleurotus florida*. The highest biological efficiency was obtained in case of 50% WS + 50% PM (68.45%) followed by the 100% WS (66.43%). The harvested fruiting bodies analysed for the nutritional parameters revealed that significant difference was found among the fruiting bodies produced on the selected agrowastes studied for the different proximates analyzed especially in protein content. Based on the biological efficiency of the substrates tested, utilization of *Panicum maximum* biomass seems to be a promising supplement with wheat straw for growing *P. florida*.

Key words: Grass, *Pleurotus*, Proximate, Yield

Mushrooms are untapped resources of nutritious and palatable food. They own extensive enzyme complexes which enables them to degrade almost all types of lignocellulosic waste materials (Prasad *et al.* 2017). They are utilized as major bioactive component in manufacturing various dietary supplements (Rathore *et al.* 2017) and also have prominent role in preventing and treating specific diseases (Prasad *et al.* 2015). *Pleurotus* spp. is the third largest cultivated mushroom species in India and abroad (Narain *et al.* 2016, Holkar and Chandra 2015) and is quite popular for its delicious taste, high vitamin, protein, carbohydrate, mineral but low fat content. Popularity of this mushroom is increasing rapidly due to its easy and economical cultivation technology (Gupta *et al.* 2016). Though, wheat straw is the commonly used substrate for the cultivation of *Pleurotus* spp. (Gupta *et al.* 2013), availability of sufficient straw round the year and in all parts of the country is uncertain. As a result, mushroom growers persistently look for alternatives that are readily available or cost effective, or the ones that can provide better yield and better mushroom quality (Royse *et al.* 2004). Recently, utilization of grasses like lemon, sabai, thatch, goose grass etc (Manimuthu and Rajendran 2015) in mushroom cultivation has been successful. Screening of other grasses as alternative substrates for higher yield capacity and quality mushroom production becomes imperative. This would not

only lower the cost of production of oyster mushrooms but might also lower the cost to the consumers.

Switch grass and Guinea grass are lignocellulosic in nature, besides their uses for energy/ as fodder, these can also be utilized for mushroom cultivation. Keeping the facts above in mind, the present study was an attempt to screen the potential of both the grasses to partially replace wheat straw for the cultivation of *Pleurotus florida* and also to study their nutritional composition.

MATERIALS AND METHODS

The wheat grain spawn of *P. florida* was obtained from Haryana Agro Industries Corporation, Murthal, Haryana, India.

The grass stalk tested in this study was *P. maximum* (Guinea grass) and *P. virgatum* (Switch grass). The stalks of *P. maximum* and *P. virgatum* were collected from Micromodel Complex, CRDT IIT Delhi. The collected stalks were completely dried under the sun, grounded and soaked in formalin water for about 10–12 h to ensure sufficient moisture and sterilization (Gothwal *et al.* 2012). The control substrate formulation (all ingredients based on dry substrate weight, w/w) consisted of 100% WS (wheat straw). Five ratios from the two grasses and wheat straw were used: 0:100%, 25%:75%, 50%:50%, 75%:25% and 100%:0% (Grass: Wheat Straw). Water content of the final mixture was adjusted to 65% (w/w).

Wet substrates of 1.5 kg in different combinations (equivalent to 700 gms dry substrate as per the ratios; moisture content 60%) was packed in perforated polythene bags of size 28×20 cm and subsequently inoculated with

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10% grain spawn of *P. florida*. WS containing 10% grain spawn was taken as the control. In all the substrates (control and combinations), 8% gram flour powder was added as nitrogen source. Each treatment was maintained in triplicate. The polythene bags were kept in a sterilized room and spawn run was allowed in a dark closed room at 20–22 °C for about 3–4 weeks. After completion of the spawn run, bags were punctured using clean knife from sides to facilitate primordial initiation. Water was sprinkled regularly, at morning and afternoon, to maintain a relative humidity of 80–90%. The windows of the room were opened for 1 h for sufficient light to enter. Fruit bodies developed after a period of about 3 weeks and thereafter, were harvested in 3 flushes, each separated by a time interval of 4–6 days.

Mushrooms were harvested from the substrate when the caps got fully open up and before the margin of fruiting bodies started curling. The harvested fruiting bodies were then counted and weighed. At the end of the harvest period, the accumulated data were used to calculate the biological efficiency and fresh weight of mushroom.

Biological efficiency (%) = Weight of fresh mushrooms harvested per bag/weight of dry substrate per bag × 100

Mushroom weight (g) = Total weight of freshly harvested mushrooms per bag/total number of mushrooms harvested per bag.

Moisture and ash were determined according to AOAC, 1995. pH was measured using a pH metre. Carbon and nitrogen contents were estimated using a CHN analyzer. The fruit bodies were analysed for the proximate composition, i.e. crude protein, fat, energy, carbohydrate, fibre and energy (Gupta *et al.* 2013). Reported values are an average of three determinations.

All the analysis was subjected to one-way ANOVA with Duncan's multiple range test (DMRT) and interpreted at 5% level of probability.

RESULTS AND DISCUSSION

Carbon and nitrogen ratio plays an important role in spawn running (Narayan *et al.* 2009) as it facilitates the mycelium colonization and maturity of the fruiting bodies. Highest C/N ratio was obtained in 50% PM + 50% WS

combination. It was observed that the pH of almost all the substrates was in the range of 6.02 to 6.83 which was optimum for mycelial growth and subsequent fruiting body development except for 100% *Panicum virgatum* where the pH was 5.98.

The mycelium of the fungus completely colonized the substrates within 28 days of spawning. The biological efficiency was high during the first flush as compared to the other flushes (Table 2). Highest biological efficiency was obtained in case of 50% WS + 50% PM, i.e. 68.45% as compared to that of the control (66.43%) indicating that high C/N ratio of the substrate (Table 1) facilitated the increase in the yield. This was followed by the combinations 100 WS control (66.43%), 75% WS + 25% PM (62.50%) > 75% WS + 25% PV (53.96%) > 25% WS + 75% PM (53.80%). It had also been reported that the C/N ratio of substrate formulas has close correlation with total colonization period, mushroom weight, yield and B E (Hoa *et al.*, 2015). Supplementation with nitrogen sources increases crop productivity, but to a certain level, as high nitrogen can inhibit fruiting of mushrooms (Silva *et al.* 2007). More than 50% yield was obtained in almost all the substrate combinations indicating the excellent ability of *Pleurotus* mushroom to degrade all kinds of lignocelluloses (Gogavekar *et al.* 2012). In the present study, individual grasses were also used as substrates for cultivation of *Pleurotus*. However, low biological efficiency of 26.35% and 32.12% for *P. virgatum* and *P. maximum* were obtained respectively. The mycelial growth was improper and slow as compared to other substrates tested. One possible reason could be that due to the lack of enough pores and compactness in the substrates, it retarded the growth of the mycelium there by influencing the overall yield. It has been mentioned in the previous studies that small air spaces slow down gas exchange, which restricts the yield in mushrooms (Royse and Sanchez-Vazquez 2001). The grass plants alone did not supported the mycelia run of *P. florida* that might be due to the limited gas exchange inside the substrate. Similar to our present findings, Obodai *et al.* (2003) also could not get any mycelial growth as well as mushroom yield when 100% grasses were utilized for the cultivation.

Table 1 Characteristics of the substrate combinations used for cultivation of *Pleurotus florida*

Substrate ^a combination	Treatment symbol	pH	Carbon (%)	Nitrogen (%)	C/N ratio
100% wheat straw control	100 WSC	6.61±0.05	48.10±0.08	1.14±0.02	42.08±0.84
100% <i>Panicum virgatum</i> control	100PVC	5.98±0.08	49.12±0.05	1.06±0.01	46.05±0.62
100% <i>Panicum maximum</i> control	100 PMC	6.02±0.04	47.10±0.01	1.41±0.03	33.84±0.76
25% wheat straw + 75% <i>Panicum maximum</i>	25 WS + 75 PM	6.56±0.04	48.09±0.02	1.14±0.02	42.19±0.73
50% wheat straw + 50% <i>Panicum maximum</i>	50 WS + 50 PM	6.47±0.03	49.62±0.03	1.00±0.01	49.30±0.72
75% wheat straw + 25% <i>Panicum maximum</i>	75 WS + 25 PM	6.23±0.02	49.07±0.05	1.14±0.02	42.92±0.74
25% wheat straw + 75% <i>Panicum virgatum</i>	25 WS + 75 PV	6.83±0.04	49.19±0.02	1.08±0.01	45.27±0.61
50% wheat straw + 50% <i>Panicum virgatum</i>	50 WS + 50 PV	6.16±0.03	49.06±0.04	1.06±0.03	46.02±1.30
75% wheat straw + 25% <i>Panicum virgatum</i>	75 WS + 25 PV	6.13±0.05	48.94±0.03	1.08±0.01	45.18±0.60

^aAll the substrates contained 8% gram flour and 1% CaCO₃. Average of three replicates. WS: Wheat straw; PV: *Panicum virgatum*; PM: *Panicum maximum*

Table 2 Mushroom yield in different flushes (g) and biological efficiency (%) of *Pleurotus florida* grown on different substrate combinations

Substrate combination	I Flush	II Flush	III Flush	IV Flush	V Flush	Total yield	BE
100 WS	190.73 ^{fg} (1.46)	140.51 ^f (2.02)	72.77 ^f (1.16)	52.38 ^f (1.25)	41.89 ^e (0.72)	498.30 ^f (2.29)	66.43 ^f (0.30)
100 PV	100.12 ^a (1.09)	67.12 ^a (2.54)	30.45 ^a (1.88)	NY*	NY*	197.69 ^a (3.34)	26.35 ^a (0.56)
100 PM	121.56 ^b (2.12)	70.23 ^a (1.56)	38.88 ^b (1.14)	10.23 ^a (1.03)	NY*	240.90 ^b (2.45)	32.12 ^b (0.68)
25% WS + 75% PM	167.15 ^c (3.01)	118.78 ^{cd} (4.55)	54.92 ^d (1.57)	37.66 ^{cd} (1.26)	25.05 ^b (2.70)	403.58 ^d (3.61)	53.80 ^d (0.48)
50% WS + 50% PM	195.44 ^h (2.91)	146.14 ^g (3.47)	73.23 ^f (2.62)	55.01 ^f (2.37)	43.60 ^e (2.13)	513.43 ^g (8.04)	68.45 ^g (1.07)
75% WS + 25% PM	186.19 ^f (4.6)	133.45 ^e (2.96)	63.92 ^e (0.93)	47.08 ^e (2.35)	38.17 ^d (1.28)	468.82 ^e (5.27)	62.50 ^e (0.70)
25% WS + 75% PV	137.66 ^c (2.68)	114.19 ^c (3.72)	44.52 ^c (0.59)	33.89 ^b (1.77)	19.37 ^a (1.03)	349.64 ^c (2.19)	46.61 ^c (0.29)
50% WS + 50% PV	143.85 ^d (1.64)	106.11 ^b (3.30)	42.40 ^c (0.96)	37.02 ^c (0.35)	23.61 ^b (1.28)	353.01 ^c (1.9)	47.06 ^c (0.25)
75% WS + 25% PV	162.00 ^e (0.24)	121.55 ^d (0.57)	51.82 ^d (0.61)	40.03 ^d (0.78)	29.38 ^c (0.65)	404.79 ^d (1.09)	53.96 ^d (0.14)

NY*- No yield obtained; Numbers in parentheses depict SD. Data were analyzed by Duncan's multiple range test.

Table 3 Proximate analysis of mushroom fruiting bodies harvested from different substrate combination

Substrate combination	Moisture (%)	Ash (%)	Fibre (%)	Lipid (%)	Crude protein (%)	Carbohydrate (%)	Energy (kJ)
100 WS	89.23±0.10 ^a	8.69±0.09 ^{bc}	12.25 ±0.30 ^a	2.4 ± 0.40 ^g	27.89±0.23 ^d	51.23±0.45 ^e	1385.02±3.23 ^c
100 PV	89.44±0.09 ^a	8.52±0.10 ^b	12.42±0.21 ^c	1.45±0.32 ^b	27.22±0.32 ^a	49.12±0.23 ^a	1322.43±2.45 ^a
100 PM	89.35±0.12 ^a	8.67±0.12 ^{bc}	12.24±0.32 ^a	1.38±0.12 ^a	27.97±0.34 ^d	50.26±0.56 ^c	1380.34±4.23 ^b
25% WS + 75% PM	90.04±0.06 ^b	9.05±0.06 ^d	12.57±0.14 ^d	1.57 ± 0.39 ^c	27.45±0.24 ^b	50.56±0.77 ^d	1384.26±3.11 ^c
50% WS + 50% PM	89.48±0.33 ^a	8.93±0.10 ^{cd}	12.36 ±0.40 ^b	2.12± 0.22 ^h	28.56±0.13 ^f	51.61±0.84 ^f	1441.26±3.23 ^f
75% WS + 25% PM	89.37±0.34 ^a	8.71±0.17 ^{bc}	13.43± 1.66 ^f	1.93± 0.51 ^f	27.72±0.41 ^c	52.35±0.34 ^g	1432.90±4.34 ^c
25% WS + 75% PV	90.01±0.10 ^b	8.55±0.08 ^b	12.22 ± 0.28 ^a	1.45 ± 0.14 ^b	28.04±0.40 ^e	50.23±0.45 ^c	1384.71±2.33 ^c
50% WS + 50% PV	90.05±0.05 ^b	8.23±0.30 ^a	12.36± 0.46 ^b	1.78 ± 0.14 ^d	27.67±0.11 ^c	50.04±0.11 ^b	1386.37±5.23 ^c
75% WS + 25% PV	89.19±0.06 ^a	8.73±0.15 ^{bc}	13.19 ± 0.22 ^c	1.85±0.55 ^e	27.29±0.22 ^a	51.06±0.45 ^b	1395.16±3.23 ^d

Data are analyzed by Duncan's multiple range test.

The proximate analysis of the fruiting bodies was also done (Table 3). Moisture content and ash content varied between narrow limits of 89.19–90.04% and 8.23–9.05%, respectively. Since the substrates were supplemented with grasses that had high carbohydrate and fibre content, as a result the fruiting bodies were also found to be rich in carbohydrates (52.35%) and fibre (13.43%) indicating that the substrate nutritional composition largely affects the mushroom fruit bodies as the mycelium utilizes them for its growth. On the other hand, the protein content was found to be in the range of 27.22% to 28.56% while lipid values ranged from 1.38% to 2.12%. It was interesting to observe that the fat content was reduced after supplementation with both the grasses. Maximum protein content was obtained

from fruit bodies harvested from the substrate combination 50% WS + 50% PM (28.56%) giving a significant increase over the control. However, fruit bodies harvested from other combination of substrates were higher or at par with the control. It is recognized that the protein content of mushrooms varies with the kind of substrate chosen, due to differential nature of the nutrient supply (Gothwal *et al.* 2012). It is noteworthy that the protein content of the fruit bodies harvested from the combination of substrates is more than that of the control. The energy values varied between 1382.90 kJ to 1447.37 kJ, with maximum being obtained from the substrate combination 50% WS + 50% PV. This is greater than the reported values of *P. sajor caju* harvested from cotton stalks (Ragunathan and Swaminathan 2003).

Wheat straw and other lignocellulosic wastes have already been utilized in India for the cultivation of *Pleurotus* species. But the usage of grasses is also an useful approach for quality mushroom production. Two perennial grasses used in this study have been utilized very successfully on the cultivation of *Pleurotus* spp. first time in India. These grasses also contributed nutritive values in the oyster mushroom like protein and carbohydrate. However, further research on supplementation of the grasses with other substrates could be tested for quality mushroom production.

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