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Antioxidant properties of different edible mushroom species and increased bioconversion efficiency of *Pleurotus eryngii* using locally available casing materials

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ABSTRACT

Total phenolics, radical scavenging activity (RSA) on DPPH, ascorbic acid content and chelating activity on Fe^{2+} of *Pleurotus citrinopileatus*, *Pleurotus djamor*, *Pleurotus eryngii*, *Pleurotus flabellatus*, *Pleurotus florida*, *Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Hypsizygus ulmarius* have been evaluated. The assayed mushrooms contained 3.94–21.67 mg TAE of phenolics, 13.63–69.67% DPPH scavenging activity, 3.76–6.76 mg ascorbic acid and 60.25–82.7% chelating activity. Principal Component Analysis (PCA) revealed that significantly higher total phenolics, RSA on DPPH and growth/day was present in *P. eryngii* whereas *P. citrinopileatus* showed higher ascorbic acid and chelating activity. Agglomerative hierarchical clustering analysis revealed that studied mushroom species fall into two clusters; Cluster I included *P. djamor*, *P. eryngii* and *P. flabellatus*, while Cluster II included *H. ulmarius*, *P. sajor-caju*, *P. citrinopileatus*, *P. ostreatus* and *P. florida*. Enhanced yield of *P. eryngii* was achieved on spent compost casing material. Use of casing materials enhanced yield by 21–107% over non-cased substrate.

1. Introduction

In recent times, amounts of consumed mushrooms have risen greatly, involving a large number of species, due to continuous developments in cultivation, harvest, post-harvest processing and storage treatments, which facilitate the consumption throughout the year (Palacios et al., 2011). Besides nutritional properties, mushrooms have been demonstrated to possess medicinal properties (Barros, Baptista, Estevinho, & Ferreira, 2007; Manjunathan & Kaviyasaran, 2011). Their pharmacological action and therapeutic potential in promoting human health are well known (Barros et al., 2007; Guillamon et al., 2010). Mushrooms contain various polyphenolic compounds recognised as excellent antioxidants due to their ability to scavenge free radicals by single-electron transfer (Hung & Nhi, 2012).

Among edible mushrooms, *Pleurotus* (oyster mushroom) species have been used by human cultures all over the world for their nutritional value, medicinal properties and other beneficial effects. Oyster mushrooms are a good source of dietary fibre and other valuable nutrients. They also contain a number of biologically active compounds with therapeutic activities. They modulate the immune system, inhibit tumor growth and inflammation, have hypoglycemic and antithrombotic activities, lower blood lipid concentrations, prevent high blood pressure and atherosclerosis and have antimicrobial and other activities (Gunde-Cimerman, 1999). Cultivation of the *Pleurotus* species has increased greatly throughout the world during the last few decades. It is the 3rd largest cultivated mushroom in the world. Its popularity has been increasing due to its ease of cultivation, high yield potential and, high nutritional and medicinal values (Upadhyay, 2011). *Pleurotus eryngii* (King oyster mushroom) is by far the best tasting oyster mushroom. It contains various compounds e.g. polysaccharides, polyphenols and flavonoides which have antioxidative activities (Dubost, Ou, & Beelman, 2007). This mushroom can be successfully cultivated on many agricultural and agro-industrial wastes (Kirbag & Akyuz, 2008). Growers that produce *P. eryngii* typically harvest only one flush before cleanout. Development of cost-efficient production methods to improve yield and biological efficiency without sacrificing mushroom quality is a major focus of many researchers and growers. Therefore, attempt has been made to increase biological efficiency and yield of *P. eryngii* using locally available materials as casing overlays in North-Western Indian Himalayan growing conditions.

In the present study, we describe total phenolics, DPPH-scavenging activity, ascorbic acid content and metal chelating activity of some oyster mushroom species and explore the possibility of growing *P. eryngii* as a newly introduced mushroom species in North-Western Indian Himalayas as well as enhance its bio-conversion efficiency using locally available casing overlay.

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2. Materials and methods

2.1. Culture and culture maintenance

The mother cultures of *Pleurotus citrinopileatus*, *P. djamor*, *P. eryngii*, *P. flabellatus*, *P. florida*, *P. ostreatus*, *P. sajor-caju* and *Hypsizygus ulmarius* (blue oyster mushroom) were obtained from Mushroom Research and Training Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. For the propagation of the mother culture, 2.0% Malt-Extract Agar (MEA) medium was used. MEA plates (90 mm dia) were inoculated with a mycelium/agar plug (6 mm dia) of a young, actively growing margin of the colony. A mycelium/agar plug was inoculated at the centre of the plate and incubated at 20 °C for further study. For each mushroom species three replications were maintained. Diametric growth of mycelium of each replication was calculated. Mushroom mycelium (5 g) was homogenised in pestal and mortar at 4 °C and homogenate (100% conc.) was filtered through Whatman filter paper No. 1 and filtrate was stored at 4 °C for biochemical studies.

2.2. Estimation of total phenolics

Total phenolics in the mushroom extract were estimated by means of the Folin–Ciocalteau assay (Singleton & Rossi, 1965) with minor modifications. Each mushroom mycelial extract (200 μL and 100% pure) was mixed with 500 μL of 50% Folin–Ciocalteu reagent (1 N), incubated at room temperature for 2 min and then 2.5 ml of saturated sodium carbonate (20% aqueous solution) was added to it. The mixture was kept in dark for 30 min before absorbance was taken at 725 nm. A calibration curve using tannic acid (2–10 μg/mL) was prepared. Total phenolic content of the mushroom extract was expressed as Tannic Acid Equivalents (TAEs), which reflect the phenolic content as the amount of tannic acid (mg/g) of mushroom mycelium.

2.3. Radical scavenging activity on DPPH (2,2-diphenyl-1-picryl-hydrazyl)

The antioxidant activity in mycelial extracts of different mushroom species was determined by measuring the DPPH scavenging activity (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998). The scavenging effects on DPPH radicals were determined measuring the decrease in absorbance at 517 nm due to the DPPH radical reduction, indicating the antioxidant activity of the compounds in a short time. Mushroom mycelial filtrates (0.1 ml) at various concentrations (25, 50, 75 and 100% v/v) were added to 2.9 ml of DPPH (100 μM) solution. When DPPH reacts with an antioxidant, that can donate hydrogen, it gets reduced and the resulting decrease in absorbance at 517 nm was recorded using a UV–Vis spectrophotometer (Thermo-Scientific, UV–Vis spectrophotometer). Vitamin C (100 μM, purity ⩾99%) and vitamin E (D,L-α-tocopherol; 100 μM, purity ⩾98%) were kept as antioxidant standards. Radical scavenging activity (RSA) was calculated using the formula:

$$\text{RSA} = [(A_o - A_s)/A_o] \times 100$$

where A_o is the absorbance of 100 μM methanolic DPPH only and A_s is the absorbance of the reaction mixture.

2.4. Determination of ascorbic acid

Ascorbic acid was determined according to the method of Klein and Perry (1982). Mushroom mycelia (1 g) was extracted with 10 ml of 1% metaphosphoric acid for 45 min and filtered through Whatman filter paper No. 1. The filtrate (1 ml) was mixed with 9 ml of 2,6-dichloroindophenol and the absorbance was measured at 515 nm. Ascorbic acid content was calculated on the basis of the calibration curve of authentic L-ascorbic acid (calibration range was 10–100 μg/ml).

2.5. Chelating activity on ferrous ions

The chelating of ferrous ions by mushroom mycelia was determined by the modified method of Dinis, Madeira, and Almeida (1994). Mushroom mycelial extract (0.5 ml) was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the resulting solution was measured at 562 nm. The chelating activity was calculated by the given formula:

$$\text{Metal chelating activity } (\%) = [(A_o - A_s)/A_o] \times 100$$

where A_s is the absorbance of the solution when the extract/reference has been added at a particular level and A_o is the absorbance of the $FeCl_2$ and ferrozine solution without addition of extract. All analyses were run in triplicate. EDTA (purity ⩾98%) was used as standard.

2.6. Cultivation of P. eryngii using different casing materials

Spawn of *P. eryngii* was prepared in 500 ml clean glass bottles using a mixture of wheat grains (200 g), $CaCO_3$ (1.5 g) and $CaSO_4$ (6 g) as per methodology of Sharma and Kumar (2011). Bottles were filled with the wheat grains, plugged with non-absorbent cotton and autoclaved at 121 °C for 90 min. After cooling, five mycelial-agar plugs (5 mm dia) were placed into the grain mixture and then incubated for one week at 20 °C. Spawn was then shaken in order to evenly distribute mycelia on the grains. Spawn was incubated for an additional week, shaken again and then stored at 4 °C until used.

Wheat straw was used as a substrate. It was soaked in water for 6–8 h and then removed to drain out excess water. The moisture content was kept at 60% in the substrate. Moistened substrate (1.0 kg wet substrate or 300 g dry substrate) was packed in polypropylene bags (40 × 30 cm size) and then autoclaved at 121 °C for 90 min. Cooled substrate was inoculated with 40 ± 0.1 g of grain spawn. Bags were vigorously shaken to uniformly distribute spawn and incubated at ambient temperature of 20 ± 1 °C with a cycle of 8 h light/16 h dark using cool-white fluorescent bulbs.

The bags were kept for a week for maturation of the mycelium. Five different local casing materials viz. spent compost (SC), farm yard manure (FYM), SC + FYM (1:1 w/w), FYM + sandy soil (1:1 w/w) and SC + FYM + sandy soil (1:1:1 w/w) were overlaid on the surface of exposed substrate (3 cm thick), however, an uncased set of control for comparison was also kept. Bags were transferred to a production room set at 90% relative humidity (RH), 16 °C and 8 h light/16 h dark cycle. Cased substrate was watered at intervals of 3–4 d. Mushrooms were harvested manually when fully matured (pileus and margins become flat). Yield, biological efficiency (BE) and number of mushrooms were determined for all the treatments. Yield was expressed as fresh mushroom weight (g)/300 g dry substrate; BE is the ratio of fruiting body fresh weight to dry substrate weight expressed in percentage.

2.7. Statistical analysis

The data was statistically analysed in completely randomised design. Statistically significant differences ($p < 0.05$) among means of experimental results were evaluated by analysis of variance and means compared by Duncan's Multiple Range Tests. The analysis of phenolic content, DPPH scavenging capacity (%), ascorbic acid

content and metal chelating activity of each mushroom species was based on three replications and the results expressed as mean values ± standard error (SE). For multifactorial comparison, principal component analyses (PCA) and agglomerative hierarchical clustering (AHC) were used to display the correlation between the various parameters and their relationship with the different mushroom species. Varimax rotation was performed to produce orthogonal transformations to the reduced factors to identify the high and low correlations better. Multifactorial analysis was carried out using the XLStat-Pro 7.5 (Addinsoft, New York, USA) software.

3. Results and discussion

3.1. Total phenolic content

The total phenolic contents for the analysed edible mushrooms evaluated by the Folin–Ciocalteu method are shown in Fig. 1. Results were expressed as mg of tannic acid equivalents per gram of mushroom mycelium. Among the studied species, *P. eryngii* had the highest contents of phenolics (21.67 mg TAE/g of mycelium), followed by *P. djamor* (18.88 mg TAE/g of mycelium) while other studied species had lower phenolic contents, with *H. ulmarius* showing the lowest amount (3.94 mg TAE/g of mycelium). In the present study, total phenolics of different oyster mushroom species varied from 3.94 to 21.67 mg TAE/g (Fig. 1) compared to the reported values in other mushrooms such as *Coriolus versicolor* (23.28 mg/g), *Ganoderma lucidum* (47.25 mg/g), *Ganoderma tsugae* (51.28 mg/g), *G. lucidum* (antler) (55.96 mg/g) (Mau, Lin, & Chen, 2002) and *P. ostreatus* (1.8 mg/g) (Palacios et al., 2011).

Mushroom species contained different types of phenolic compound in varying numbers ranging from 3 to 15. The content of phenolic compounds could be used as an important indicator of antioxidant capacity. Several reports have convincingly shown a close relationship between antioxidant activity and phenolic contents (Pan et al., 2008). Mushroom extracts have high levels of phenolic compounds, composed of one or more aromatic rings bearing one or more hydroxyl groups, which can exhibit extensive free radical-scavenging activities as hydrogen donors or electron-donating agents and metal ion-chelating properties. The greater numbers of hydroxyl groups in the phenolics could exhibit higher antioxidant activity (Rangkadilok et al., 2007).

3.2. Radical scavenging activity (RSA) on DPPH

Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity. The radical scavenging activity (RSA) of mushroom mycelial extracts was tested against the DPPH. RSA of different edible mushroom species varied from 13.63% to 69.67% (Fig. 2) and increased with increase in concentration. It was higher for *P. eryngii*, *P. djamor* and *P. flabellatus* (67.4–69.67% at 100% mycelial extract concentration) in comparison to standards i.e. vitamins C and E while *H. ulmarius*, *P. sajor-caju* and *P. florida* showed comparatively lower RSA (41.99–43.88% at 100% mycelial extract concentration). The RSA results were consistent with the total phenolics of oyster mushrooms, the higher phenolic content the better RSA (Figs. 1 and 2). Scavenging effects of the acetonic, methanolic and hot water extracts from the fruiting bodies of *Pleurotus salmoneostramineus* (Alam et al., 2011a) and *P. citrinopileatus* (Alam et al., 2011b) on DPPH radicals increased with increasing concentrations. Lo (2005) found that *Agaricus bisporus*, *P. eryngii*, *Pleurotus ferulae* and *P. ostreatus* fruit bodies scavenged DPPH radicals by 46.6–68.4% at 5 mg/ml. Overall, it could be concluded that between these oyster mushrooms, the *P. eryngii* had a better antioxidant activity in terms of RSA on the DPPH free radical. With the presence of radical scavenging activity, consumption of *P. eryngii* might be beneficial to protect human body against oxidative damage, which can be further developed into health related degenerative illnesses.

3.3. Ascorbic acid content and metal chelating activity on ferrous ions

Mushroom mycelial extracts from different species showed varied amount of ascorbic acid (Table 1). The ascorbic acid content was significantly higher in *P. citrinopileatus* (6.76 mg/g) and *P. ostreatus* (6.55 mg/g) followed by *P. eryngii* (5.88 mg/g) while *H. ulmarius*, *P. sajor-caju*, *P. flabellatus* and *P. florida* showed significantly lower ascorbic acid content i.e. 4.20, 4.15, 3.89 and 3.76 mg/g, respectively. In contrast to the present findings, ascorbic acid was not detected in ethanolic and hot water extracts of *P. ferulae* and *P. ostreatus* fruit bodies (Tsai et al., 2009), however, it was found in the ethanolic extract from *Hypsizigus marmoreus* (Lee, Yen, & Mau, 2007). Ascorbate is also a powerful secondary

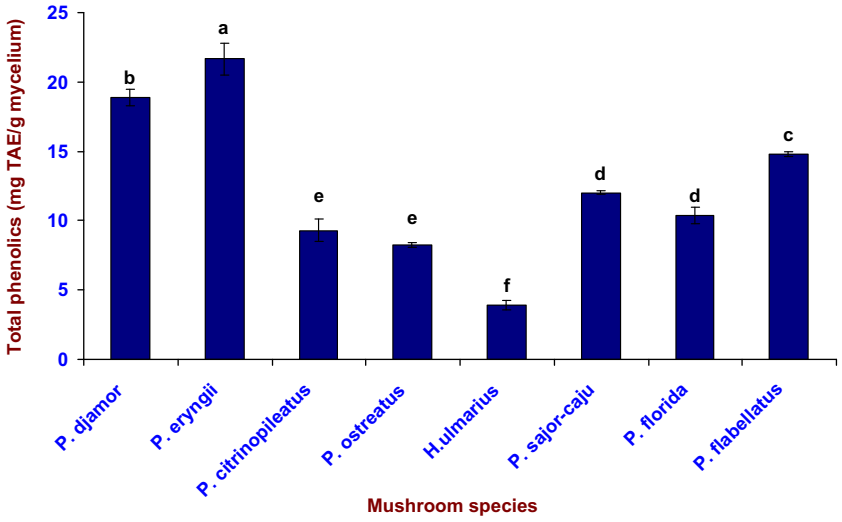

Fig. 1. Total phenolics in eight edible mushrooms, *P. djamor*, *P. eryngii*, *P. citrinopileatus*, *P. ostreatus*, *H. ulmarius*, *P. sajor-caju*, *P. florida*, and *P. flabellatus*. Results are expressed as mg of tannic acid equivalents/gram of mycelium. Vertical bars indicate the standard error ($n = 3$). Values with different letters (a–f) were significantly different ($P < 0.05$).

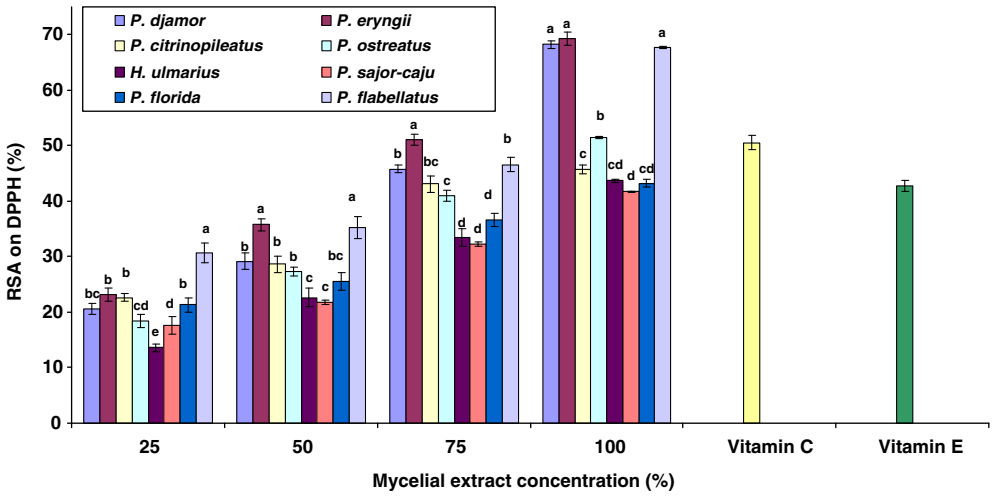

Fig. 2. Radical scavenging activity (%) on DPPH of eight edible mushrooms, *P. djamor*, *P. eryngii*, *P. citrinopileatus*, *P. ostreatus*, *H. ulmarius*, *P. sajor-caju*, *P. florida*, and *P. flabellatus* at four mycelial extract concentration. Each value is expressed as mean ± standard error ($n = 3$). Values with different letters (a–e) were significantly different ($P < 0.05$).

Table 1
Ascorbic acid content, metal chelating activity and growth of different species.

Mushroom spp.	Ascorbic acid content[A]	Metal chelating activity[B]	Diametric growth[C]	Growth/day[D]
P. djamor	4.89 ± 0.04c	71.79 ± 0.81b	79.93 ± 0.98c	7.27 ± 0.09c
P. eryngii	5.88 ± 0.12b	70.82 ± 0.15b	89.80 ± 0.12a	8.16 ± 0.01a
P. citrinopileatus	6.76 ± 0.08a	82.71 ± 0.25a	79.17 ± 0.61c	7.19 ± 0.05c
P. ostreatus	6.55 ± 0.08a	60.94 ± 0.31ed	89.83 ± 0.08a	8.16 ± 0.01a
H. ulmarius	4.20 ± 0.05d	65.42 ± 0.25c	61.87 ± 0.63d	5.63 ± 0.06e
P. sajor-caju	4.15 ± 0.06d	62.12 ± 0.06d	60.17 ± 0.52e	5.46 ± 0.06b
P. florida	3.76 ± 0.24e	61.56 ± 0.25d	85.17 ± 0.44b	7.76 ± 0.03b
P. flabellatus	3.89 ± 0.06ed	60.25 ± 0.46e	86.23 ± 0.45b	7.84 ± 0.04d
EDTA (100 ppm)	–	53.46		

Values are expressed as means ± S.E. of triplicate measurements. Values with different letters indicate significant difference ($P < 0.05$, ANOVA).
[A] Values in mg/g mycelium.
[B] Values in%.
[C] Values in mm at 11 d after inoculation.
[D] Values in mm.

antioxidant, reducing the oxidised form of α-tocopherol, an important antioxidant in nonaqueous phases (Padh, 1990).

The metal chelating activity of different mushroom mycelia extracts on ferrous ions is shown in Table 1. The results clearly indicated that all the studied species had higher metal chelating activity in comparison to EDTA (53.46% at 100 ppm). The chelating activity of *P. citrinopileatus* (82.71%) was significantly higher followed by *P. djamor* (71.79%) and *P. eryngii* (70.82%) while lowest was shown by *P. ostreatus* (60.94%) and *P. flabellatus* (60.25%). However, it was 65.42% in case of *H. ulmarius*. Lee et al. (2007) also reported 75.6–92.6% chelating ability of *H. marmoreus* at 1–5 mg/mL. Chelating agent may serve as secondary anti-oxidants as they reduce redox potential and stabilize the oxidised forms of metal ions. Since ferrous ions are the most effective pro-oxidants in food system, the high ferrous ion chelating abilities of the extracts from *P. citrinopileatus*, *P. djamor* and *P. eryngii* would be beneficial. This study could provide valuable information to support oyster mushroom species as an excellent source of antioxidants in human diet especially to the low-income community.

3.4. Diametric growth of mushroom mycelium

Diametric growth of different mushroom species was observed at 20 °C (Table 1). The data revealed that the significantly higher diametric growth was exhibited by *P. ostreatus* (89.83 mm) and *P. eryngii* (89.80 mm) followed by *P. flabellatus* and *P. florida* (86.23 and 85.17 mm, respectively), however, minimum growth was shown by *P. sajor-caju* (60.17 mm). Upadhyay (2011) categorised *Pleurotus* spp. on the basis of temperature requirement of a species into two groups-winter or low temperature requiring species, which include *P. eryngii*, *P. ostreatus*, *P. florida*, and summer or moderate temperature requiring species which include *P. sajor-caju*, *P. citrinopileatus* etc.

3.5. Multivariate analysis

Principal Component Analysis (PCA) is a useful statistical technique which has found application in reduction of the original variables (total phenolics, RSA on DPPH, metal chelating activity and ascorbic acid content) in a smaller number of underlying variables (Principal Component) in order to reveal the interrelationships between the different variables and to find the optimum number of extracted Principal Components. The first Principal Component (PC) always describes sample variation and the following PC successively explains smaller parts of the original variance. This means that correlated variables are explained by the same PC and less correlated variables by different PC. The principal component analysis (PCA) and their correlation are shown in Fig. 3. The first factor F1 represents 57.28% of variability, while the second factor F2 represents 22.65% of variability among the data. All biochemical parameters were occupied on the right side of the biplot and among the parameters the ascorbic acid and chelating

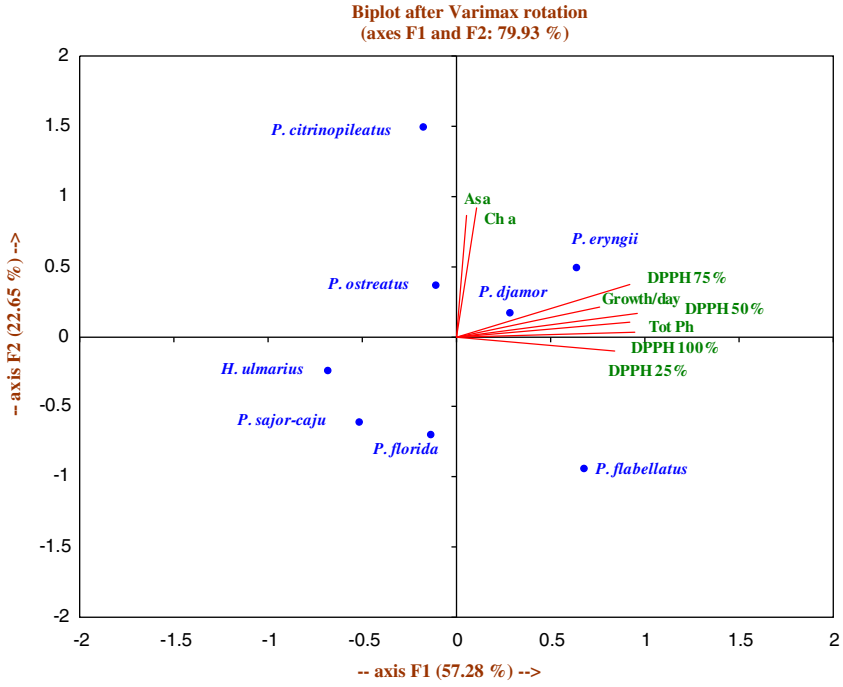

Fig. 3. Multifactorial comparison of different mushroom species and biochemical parameters using principal component analysis (PCA).

ability were observed on the right upper side of the biplot with high positive loading for both factors I and II, while total phenols, growth per day, DPPH scavenging activity were grouped together with slightly lesser positive loadings on the right side of the biplot. This suggests that DPPH, total phenols, ascorbic acid, and chelating ability are having positive correlation with growth per day of different mushroom species. Keles, Koca, and Genccelep (2011) also reported that the PCA analysis showed positive correlations among the total phenol, DPPH, FRAP and ascorbic acid in wild mushrooms.

PCA revealed that eight studied species were clustered into four clusters (Fig. 3). The first group consisting of *P. eryngii* and *P. djamor* showed strong positive correlation with both the factors (F1 and F2) while second group contained *P. flabellatus* was positively correlated with factor F1 and negatively correlated with factor F2. Third group was negatively correlated with both factors F1 and F2 having *P. sajor-caju*, *P. florida* and *H. ulmarius*, however, fourth group contained *P. citrinopileatus* and *P. ostreatus* indicating high positive loading with F2 and negative to F1.

The results also showed that *P. djamor* and *P. eryngii* contained higher total phenolics, DPPH activity, diametric growth and growth/day, while *P. citrinopileatus* shown with higher ascorbic acid and chelating ability. *H. ulmarius* showed significantly lesser antioxidant parameters compared to others *Pleurotus* species. This interpretation suggests that the *P. djamor* and *P. eryngii* may be considered for its higher antioxidant parameters.

Agglomerative hierarchical clustering (AHC) analysis revealed that eight species fall into two major clusters (Fig. 4). Cluster I included three mushrooms in two groups, group I included *P. djamor* and *P. eryngii* and group II included only *P. flabellatus*, while Cluster II included five mushrooms in two groups, *P. florida, P. sajor-caju*, *H. ulmarius* and *P. ostreatus* in one group and *P. citrinopileatus* in another group.

3.6. Yield and biological efficiency of *P. eryngii*

Five different casing materials viz. spent compost (SC), farm yard manure (FYM), SC + FYM (1:1 w/w), FYM + sandy soil (1:1 w/w) and SC + FYM + sandy soil (1:1:1 w/w) were evaluated for

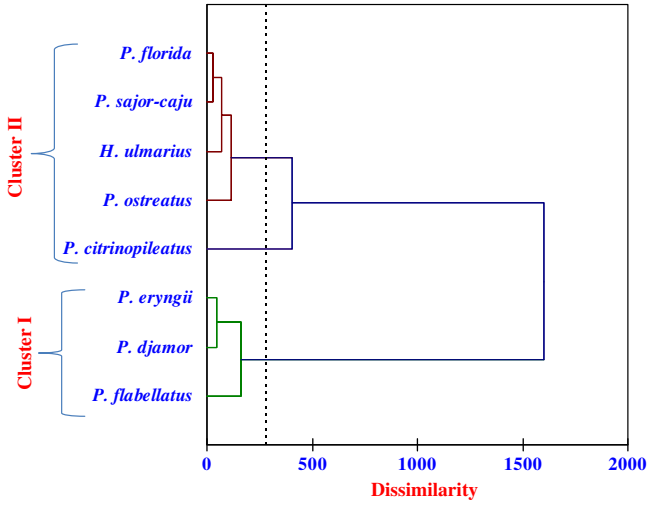

Fig. 4. Agglomerative hierarchical clustering (AHC) between eight mushroom species on the basis of seven measured parameters under lab condition.

yield and BE of *P. eryngii*. First mushroom harvest began 33 d after spawning and lasted for 5 d. Yield and biological efficiency were significantly affected by casing materials used (Table 2). The significantly higher yield and BE were observed for treatments SC and SC + FYM + SS (210.0/300 g dry substrate with BE 70% and 191.7/300 g dry substrate with BE 63.9%, respectively), while lowest yield (101.7/300 g dry substrate) and BE (33.9%) was observed for non-cased substrate. Number of fruiting bodies was also affected by use of casing layer. Number of mushrooms for cased treatments ranged from 11.66 to 13.00, considerably higher than the non-cased treatment (8.67). Weight/fruit body was highest in case of treatment SC (16.2 g) and SC + FYM + SS (15.2 g), however, it was lowest in case of treatments FYM + SS (10.6 g) and non-cased control (11.9 g).

Table 2
Number of fruiting bodies, weight/fruit body, yield and biological efficiency of *P. eryngii* as influenced by application of casing overlay.

Casing material	Number of fruiting bodies	Wt./fruit body[A]	Yield[B]	B.E.[C]	Yield increase[D]
SC	13.00 ± 0.57a	16.19 ± 0.72a	210.0 ± 8.6a	70.0 ± 2.9a	106.5
FYM	12.00 ± 0.57a	14.77 ± 0.79ba	176.7 ± 6.0b	58.8 ± 2.0b	73.8
SC ± FYM	11.67 ± 0.88a	12.77 ± 0.36cb	148.3 ± 7.3c	49.4 ± 2.4c	45.8
FYM ± SS	11.66 ± 0.33a	10.61 ± 0.65c	123.3 ± 4.4d	41.1 ± 1.5d	21.2
SC ± FYM ± SS	12.66 ± 0.88a	15.21 ± 0.61a	191.7 ± 6.0ba	63.9 ± 2.0ba	88.5
Non-cased	08.67 ± 0.88b	11.89 ± 0.84c	101.7 ± 4.4e	33.9 ± 1.5e	–

SC, spent compost; FYM, farm yard manure; SS, sandy soil.
Values are expressed as means ± S.E. of triplicate measurements. Values with different letters indicate significant difference ($P < 0.05$).
[A] Values in gm.
[B] Values in g/300 g dry substrate.
[C] Values in%.
[D] Values in%.

Biological efficiency of *P. eryngii* obtained from different casing treatments were significantly higher than values reported by other researchers who produced this mushroom on bagged/non-cased substrate and obtained mushrooms from only one flush. Peng (1996a) obtained a BE of 31% for mushrooms harvested from sawdust based substrate while Royse (1999) reported a BE as low as 7% for a sawdust based substrate supplemented with a commercial nutrient (SpawnMate IISE). Peng (1996b) applied a casing overlay to colonised substrate (rice straw, 70% moisture) and reported relatively low BE (47%). In contrary, we demonstrated that a casing overlay increased the yield and BE by 21–107% compared to non-cased control. Increases in yield were mainly due to the extended production cycle that occurred whenever a casing layer was applied. Casing layer application before the first flush resulted in the largest increase in yield in case of *P. eryngii* (Rodriguez Estrada, Jimenez-Gasco, & Royse, 2009). Moreover, mushrooms harvested from cased substrates had higher moisture content. So increases in yield were partially due to extra water absorbed by the mushrooms.

Use of a casing layer for *A. bisporus* and *Calocybe indica* cultivation is essential. Factors such as casing layer, its depth, microbial dynamics and physico-chemical properties play vital roles in yield and quality of mushrooms (Krisnamoorthy & Muthuswamy, 1997; Kumar, Beniwal, & Pahil, 2006). Limited information is available regarding application of casing soils for production of *P. eryngii*. Other studies regarding nutritional qualities and texture of the fruiting bodies obtained as a result of a casing overlay should be undertaken in order to obtain a more comprehensive evaluation of influence of a casing overlay on quality of fruit bodies.

4. Conclusion

With the results obtained, it can be concluded that out of eight mushroom species studied, *P. eryngii* recorded significantly higher amount of total phenolics, DPPH scavenging activity and mycelia growth/day while *P. citrinopileatus* possessed significantly higher ascorbic acid and chelating activity. Casing of substrate using locally available materials to enhance yield and maximise bioconversion efficiency of *P. eryngii* are relatively easy, feasible and low-cost practice. Commercial mushroom farms that produce *Pleurotus* in addition to *A. bisporus* and *C. indica* may adapt this technology in Indian conditions.

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References

Alam, N., Yoon, K. N., Cha, Y. J., Kim, J. H., Lee, K. R., & Lee, T. S. (2011a). Appraisal of the antioxidant, phenolic compounds concentration, xanthine oxidase and tyrosinase inhibitory activities of *Pleurotus salmoneostramineus*. *African Journal of Agricultural Research, 6*, 1555–1563.

Alam, N., Yoon, K. N., Lee, K. R., Kim, H. Y., Shin, P. G., Jong Cheong, C., et al. (2011b). Assessment of antioxidant and phenolic compound concentrations as well as xanthine oxidase and tyrosinase inhibitory properties of different extracts of *Pleurotus citrinopileatus* fruiting bodies. *Mycobiology, 39*, 12–19.

Barros, L., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2007). Effect of fruiting body maturity stage on chemical composition and antimicrobial activity of *Lactarius* sp. mushrooms. *Journal of Agricultural and Food Chemistry, 55*, 8766–8771.

Dinis, T. C., Madeira, V. M., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation as peroxyl radical scavenging effects. *Chemical and Pharmaceutical Bulletin, 36*, 2090–2097.

Dubost, N. J., Ou, B. X., & Beelman, R. B. (2007). Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chemistry, 105*, 727–735.

Guillamon, E., Garcia-Lafuente, A., Lozano, M., D'Arrigo, M., Rostagno, M. A., & Villares, A. (2010). Edible mushrooms: Their roles in the prevention of cardiovascular diseases. *Fitoterapia, 81*, 715–723.

Gunde-Cimerman, N. (1999). Medicinal value of the genus *Pleurotus* (Fr.) P. Karst. (Agaricales s.l., Basidiomycetes). *International Journal of Medicinal Mushrooms, 1*, 69–80.

Hung, P. V., & Nhi, N. N. Y. (2012). Nutritional composition and antioxidant capacity of several edible mushrooms grown in the Southern Vietnam. *International Food Research Journal, 19*, 611–615.

Keles, A., Koca, I., & Genccelep, H. (2011). Antioxidant properties of wild edible mushrooms. *Journal of Food Processing and Technology, 2*, 130–135.

Kirbag, S., & Akyuz, M. (2008). Effect of various agro-residues on growing periods, yield and biological efficiency of *Pleurotus eryngii*. *Journal of Food, Agriculture and Environment, 6*, 402–405.

Klein, B. P., & Perry, A. K. (1982). Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. *Journal of Food Science, 47*, 941–948.

Krisnamoorthy, A. S., & Muthuswamy, M. (1997). Yield performance of *Calocybe indica* (P & C) on different substrates. *Mushroom Research, 6*, 29–32.

Kumar, Y., Beniwal, J., & Pahil, V. S. (2006). Physiological and agronomical studies on *Agaricus bitorquis*. *Crop Research, 32*, 494–498.

Lee, Y. L., Yen, M. T., & Mau, J. L. (2007). Antioxidant properties of various extracts from *Hypsizigus marmoreus*. *Food Chemistry, 104*, 1–9.

Lo, S. H. (2005). Quality evaluation of *Agaricus bisporus*, *Pleurotus eryngii*, *Pleurotus ferulae* and *Pleurotus ostreatus* and their antioxidant properties during post harvest storage. Master's thesis. National Chung-Hsing University, Taichung, Taiwan.

Manjunathan, J., & Kaviyasaran, V. (2011). Nutrient composition in wild and cultivated edible mushroom, *Lentinus tuberregium* (Fr.) Tamilnadu, India. *International Food Research Journal, 18*, 784–786.

Mau, J. L., Lin, H. C., & Chen, C. C. (2002). Antioxidant properties of several medicinal mushrooms. *Journal of Agricultural and Food Chemistry, 50*, 6072–6077.

Padh, H. (1990). Cellular functions of ascorbic acid. *Biochemistry and Cell Biology, 68*, 1166–1173.

Palacios, I., Lozano, M., Moro, C., Arrigo, M. D., Rostagno, M. A., Martinez, J. A., et al. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chemistry, 128*, 674–678.

Pan, Y., Wang, K., Huang, S., Wang, H., Mu, X., He, C., et al. (2008). Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longan* Lour.) peel. *Food Chemistry, 106*, 1264–1270.

Peng, G. T. (1996a). Preliminary studies on the cultivation of *Pleurotus eryngii* (DC.: Fr.) Quel. on sawdust filled in polypropylene bags. *Journal of Agricultural Research China, 45*, 388–392.

Peng, G. T. (1996b). The cultivation of *Pleurotus eryngii* (DC.: Fr.) Quel. on rice straw substrate. *Journal of Agricultural Research China, 45*, 382–387.

Rangkadilok, N., Sitthimonchai, S., Worasuttayangkurn, L., Mahidol, C., Ruchirawat, M., & Satayavivad, J. (2007). Evaluation of free radical scavenging and antityrosinase activities of standardised longan fruit extract. *Food and Chemical Toxicology, 45*, 328–336.

Rodriguez Estrada, A. E., Jimenez-Gasco, M. M., & Royse, D. J. (2009). Improvement of yield of *Pleurotus eryngii* var. *eryngii* by substrate supplementation and use of a casing overlay. *Bioresource Technology, 100*, 5270–5276.

Royse, D. J. (1999). Yield stimulation of king oyster mushroom, *Pleurotus eryngii*, by brewer's grain and SpawnMate IISE supplementation of cottonseed hull and wood chip substrates. *Mushroom News, 47*, 4–8.

Sanchez-Moreno, C., Larrauri, J., & Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture, 76*, 270–276.

Sharma, V. P., Kumar, S. (2011). Spawn production technology. In M. Singh, B. Vijay, S. Kamal, G. C. Wakchaure (Eds.), *Mushrooms cultivation, marketing and consumption* (pp. 31–42). Solan, India: Directorate of Mushroom Research (ICAR).

Singleton, V. L., & Rossi, J. A. J. (1965). Colorimetric of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Tsai, S. Y., Huang, S. J., Lo, S. H., Wu, T. P., Lian, P. Y., & Mau, J. L. (2009). Flavour components and antioxidant properties of several cultivated mushrooms. *Food Chemistry, 113*, 578–584.

Upadhyay, R. C. (2011). Oyster mushroom cultivation. In M. Singh, B. Vijay, S. Kamal, G. C. Wakchaure (Eds.), *Mushrooms cultivation, marketing and consumption* (pp. 129–138). Solan, India: Directorate of Mushroom Research (ICAR).