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Research paper

Agro-industrial residues influence mineral elements accumulation and nutritional composition of king oyster mushroom (*Pleurotus eryngii*)

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ABSTRACT

The utilization of low value agro-industrial residues for improving nutritional quality of mushrooms has become a key research priority in the recent past. The article reports the effects of various agro-industrial waste materials such as cotton waste (mill droppings), wheat straw, rice straw, corn cobs, sugarcane bagasse and sawdust on nutritional composition of king oyster mushroom (*Pleurotus eryngii*). It was noted that the mycelium growth (vegetative growth) was the highest on cotton waste compared to other lignocellulosic wastes. Moreover, increased biological efficiency, proteins, carbohydrates, fats, total phenolic contents, accumulation of macro (P and K) and micronutrients (Zn, Fe, Na, Mg, Mn and Ca) and reduced DPPH activity were recorded in *P. eryngii* cultivated on cotton waste. The results suggest that the cotton waste can be utilized as an efficient, cost-effective substrate for the cultivation of *P. eryngii*, and the substrate could be exploited post-cultivation as animal feed, due to its upgraded properties.

1. Introduction

Edible mushroom cultivation has increased globally in recent years due to its appreciated culinary value as well as health benefits (Patel et al., 2012). Among the edible mushrooms, *Pleurotus eryngii*, commonly known as 'king oyster', has increasingly been preferred among consumers, owing to its good taste and nutritional content (Akyuz and Yildiz, 2008). On a global scale, the oyster mushroom is ranked second among commercially cultivated mushrooms, following *Agaricus bisporus* and constitutes about one-fourth of total mushroom production (Patel et al., 2012; Hoa and Wang, 2015).

The role of oxidation as a key physiological process in living systems has been the cornerstone of modern health sciences and research. Deficiency in endogenous antioxidant defense may result in oxidative stress, which might be associated with various health problems, including coronary heart diseases, neural disorders, diabetes, arthritis and cancer (Gogavekar et al., 2014). Therefore, exogenous dietary antioxidants are a key factor to assist in maintaining good health, as well as in preventing various diseases (Augustyniak et al., 2010). Edible mushroom species such as *P. eryngii* have been recognized as sources of antioxidants as they contain beneficial components and secondary metabolites that can protect against oxidative damage. Detection of high nutritive content of these edible mushrooms in terms of proteins, carbohydrates, vitamins, calcium and iron, with a combination of physiologically bioactive compounds beneficial to human health, has enhanced their therapeutic value in recent years (Venturella et al., 2015).

Unlike other protein-rich foods, oyster mushroom can be grown in various agro-industrial wastes, such as crop waste, soybean straw and cotton stalks due to its extensive enzyme systems which are capable of degrading complex organic compounds in biological wastes (De Silva et al., 2012). Maintaining optimum growth conditions and the provision of suitable substrate material are crucial to attain optimal growth and higher yield of *P. eryngii* (Hassan et al., 2010). Irregular mycelium growth due to inappropriate substrate selection often results in low yield and reduced nutritive contents of mushroom (Khan et al., 2008). As substrates serve as a source of nutrition and lignocellulose material to support growth, development and fruiting of mushrooms (Chang and Miles, 2004), the choice of a suitable waste material as substrate is essential for the cultivation of *Pleurotus* spp. to obtain maximum yield. Some previous studies showed that variation in chemical composition of growing substrates significantly affects the oyster mushroom yield,

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biological efficiency and nutritional value (Khan et al., 2008).

There is a need for the development and optimization of a detailed substrate formula for practical, industrial-scale production of king oyster mushroom. The objectives of the present study were to investigate suitability of different agro-waste feed stock as substrate materials for achieving maximal growth and yield of *P. eryngii*. Moreover, the effect of degradation pattern in selected feed stock on the overall antioxidant potential gain in the fruiting body of *P. eryngii* was investigated in addition to the estimation of the influence of substrates on mineral elements accumulation and nutritional composition.

2. Materials and methods

2.1. Agro-industrial wastes obtention

Six different types of agro-industrial wastes (cotton waste, wheat straw, sugarcane bagasse, corn cobs, rice straw, and *Acacia* sawdust) used in this study were collected from agricultural fields and agro-based industrial units in the vicinity of Faisalabad city (Lat. 31° 25′ N, Long 73° 4′ E). All samples (agro-industrial wastes) were collected (50 kg each) within 2 months prior to the study and stored in a cold dark room (temperature 10 ± 5 °C, relative humidity 20–30%)

2.2. Substrate preparation

Collected sun-dried agro-waste materials were cut in pieces (5–6 cm) with a hand cutter and later oven-dried at 60 °C. These substrates were then soaked separately in water overnight to ensure they absorbed sufficient moisture and were allowed to dry to obtain an average moisture content of 65 ± 1%, calculated by drying 100 g of substrate in an oven at 70 °C until constant weight was achieved. The polypropylene bags of 20 × 30 cm size were filled with 800 g prepared substrates and packed tightly. Substrate filled bags were autoclaved at 121 °C and 15 psi for 20 min, prior to inoculation of spawn.

2.3. Inoculum source and spawn preparation

The culture of king oyster mushroom (P-9) was revived from the culture collection bank of the Mushroom Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan and further maintained on malt agar medium at 4 °C following the method described by Ragunathan and Swaminathan (2003).

2.4. Experimental conditions

The mushroom cultivation process was accomplished in the experimental mushroom growing room of the Institute of Horticultural Sciences, University of Agriculture, Faisalabad (Pakistan) in which the temperature, ventilation and relative humidity were controlled. Six different substrates were used for the growth of *P. eryngii* viz. cotton waste, wheat straw, rice straw, corn cobs, sugarcane bagasse and saw dust. The bags were arranged randomly in the growing room on iron racks to maximize the utility of available space for the cultivation of mushroom. The experiment was laid out in completely randomized design with three replications in each treatment. Each experiment was repeated three times and the data presented is the mean of values obtained from these experiments.

2.5. Mushroom cultivation and harvesting

Cultivation of king oyster mushroom was carried out following the reports published by Ashraf et al. (2013) with some modifications. After sterilization, substrate-filled bags were inoculated by spreading spawn grains on the surface of the substrate with a weight percentage of about 1% of the wet weight of substrate and the bag opening was plugged with cotton wool and tied with a rubber band. These bags were shifted in a sterilized room for spawn running where temperature was maintained at 25 ± 2 °C with a thermostat. When the bags became white due to colonization by fungal mycelium, visible beneath the transparent polythene sheets, the bags were cut open to induce the fruiting body formation. After opening the mouths of the bags, the temperature (20–22 °C) and humidity (80–90%) of the growing room were maintained by sprinkling water regularly in the morning and afternoon. Fruiting bodies developed after a period of 5 weeks and thereafter were harvested in 3 flushes from each bag.

2.6. Mycelial growth, yield and biological efficiency determination

Number of days required for the completion of mycelial growth (days to colonize substrate) on different substrates was recorded. Total yield was calculated as the sum of three flushes grown on different substrates and was expressed in grams per bag. The biological efficiency was calculated as the percentage conversion of dry substrates to fresh fruiting bodies using the formula.

$$\text{B.E}\ (\%) = \left(\frac{\text{Fresh weight of mushroom per bag}}{\text{dry weight of substract per bag}} \right) \times 100$$

2.7. Post-harvest biochemical analyses (mushroom + substrates)

The fruiting body samples were harvested at 2nd flush and later oven dried at 65 °C until constant weight was attained. The dried materials were grounded into powdered form using food processor (Moulinex, France). The obtained powder was used for proximate analysis i.e. total proteins (%), total carbohydrates (%), fats (%), crude fiber (%), and ash (%) estimated by following standard procedure (AOAC, 1990). The Mcro Kjeldhal method was used for the estimation of total protein (N × 6.24), whereas the Soxhlet apparatus was used for the determination of crude fat from powdered sample extracted with petroleum ether. The ash content was estimated from the sample incinerated at 600 ± 15 °C. The difference 100-(% Moisture +% Crude protein +% Crude fat +% Crude fiber +%Ash) was used for the calculation of total carbohydrate. Energy value of fruiting bodies was analyzed by using the method of Ragunathan & Swaminathan, (2003) and was calculated according to the following equation: Energy (kcal/100 g) = 4 × (g protein) + 3.75 × (g carbohydrate) + 9 × (g fat). The moisture contents (%) of mushrooms was calculated according to the method of AOAC (1990).

2.7.1. Sample preparation for elements determination

The dried samples (2 g) were acid digested by soaking them in concentrated HNO_3 (20 ml) for 18–20 h and later heated on a hot plate at 70 °C till orange brown coloured residue obtained. The samples were cooled before adding 5 ml of perchloric acid and heated again till the residue became white. The residual samples were dissolved in deionized water and the volume was made up to 100 ml. Whatman No. 4 filter paper was used for the filtration of residual sample from this solution.

2.7.2. Elements analysis

Phosphorus was determined by the method proposed by Gasim et al. (2008) using a spectrophotometer. Minerals (Fe, Mn, Zn, Ca, and Mg) were analyzed by atomic absorption spectrometry (Perkin Elmer, A Analyst 300). All mineral elements were estimated by following the method proposed by Kaneez et al. (2001) and expressed as mg/kg.

For determining the chemical composition of the substrates prior to and after mushroom cultivation, samples of raw and spent cultivation media were analyzed for their content in N, P K and protein (N × 6.25) as previously described. The procedure published by Gothwal et al. (2012) was used for the estimation of cellulose by acetolysis followed by hydrolysis to form glucose units. These glucose units were then