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Quantitative and Qualitative Parameters of Kabul Dhingri (*Pleurotus eryngii*) Mushroom Harvested from Different Substrates and Supplements

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ABSTRACT: *Pleurotus eryngii* is a widely emerging edible fungi and gaining immense popularity in world of mushrooms due to its specific properties like flavour and aroma, its low cost production and requirement of limited land resources. Being newly introduced to Rajasthan, standardization of its cultivation technology and biochemical analysis was done to explore more of the benefits of this mushroom. So, this study was conducted at All India Coordinated Research Project on Medicinal Mushroom Unit in Department of Plant Pathology, Rajasthan College of Agriculture, MPUAT, Udaipur to explore the quantitative parameters like yield and yield attributing characters as well as nutritional and health benefits of *Pleurotus eryngii* harvested from different substrates and supplements since this. The qualitative parameters including moisture contents, protein contents, carbohydrate contents, crude fiber and phenol contents were studied. Quantitative parameters were studied to find the substrate giving maximum yield and Biological efficiency (BE) in subtropical zones. Paddy straw gave the best results. On the other side, maize straw produced the quickest spawn run completion and initiation of pin heads emergence.

Moisture contents of fruit bodies grown over substrates and supplements, ranged from 82 to 90%. Fruit bodies harvested from wheat straw and wheat straw supplemented with 5 per cent wheat bran had maximum (90%) and minimum (82%) moisture contents, respectively. Percentages of total protein on dry weight of mushrooms were found to be the highest (33%) on maize straw and the lowest (15%) being recorded on wheat straw + 5 per cent wheat bran. The highest (67%) and the lowest (44%) total carbohydrate contents were obtained with the wheat straw + 5 per cent wheat bran and sorghum straw, respectively. Crude fiber was found maximum (31%) and minimum (11%) in fruit bodies harvested from wheat straw and wheat straw + 5 per cent wheat bran, respectively. Total phenol contents of *Pleurotus eryngii* grown on all experimental substrates and supplements were found to be 5.3-7 mg/g of dry weight, which was highest on wheat straw + 5 per cent wheat bran, followed by sorghum straw and wheat straw + 5 per cent rice bran and the lowest found on maize straw.

Keywords: carbohydrate, crude fiber, nutritional, phenol, *Pleurotus eryngii*, qualitative parameters, substrate.

INTRODUCTION

Healthy food is going to be a major challenge in this century with the population pressure. To meet the food demand for the rising population from the scarce land resources is a big challenge for our Indian democracy in this climate change era. Also, the spreading malnutrition and the diseases resulting due to this are severe in the economically weaker sections. Mushroom Farming, is one of the best options to face this challenge because mushroom grows on wastes and converts wastes into protein rich food without requiring extra land and has many nutritional and medicinal properties (Manikanandan, 2011). Mushrooms are supposed to be low calorific food (Ukwuru *et al.,* 2018). Since years, these fungi have been considered delight for their uncountable health benefits and have been extensively used in traditional medicines. Certain compounds in *Pleurotus eryngii* (DC.ex FR.) Quel. mushroom are responsible in bettering human health in different ways. Such bioactive compounds are tri-terpenoids, polysaccharides, antioxidants, proteins with low molecular weight, immunomodulating compounds and glycoproteins. Thus, this mushroom has been seen to enhance immune function; boost health; reduce cancer risk; prevent tumour growth; balance blood sugar; and help the detoxification mechanism of body. *Pleurotus eryngii* rendered its benefits in different pharmaceutical, medicinal and biotechnological studies (Couto and Herrera, 2006; Gregori *et al.,* 2007). It produces variety of biologically active compounds and

owns a distinct ligninolytic enzymatic system (Cohen *et al.,* 2002). According to the reports of Manzi *et al.* (1999, 2004), they have enormous dietary fibres, carbohydrates (9.6 per cent of fresh weight), chitin and polysaccharides (0.41 per cent of fresh weight). The protein ranged between 1.88 and 2.65% and the total nitrogen content is 5.30% approximately. The abundantly found amino acids are glutamic acid, aspartic acid and arginine. Very low concentration lipid (0.8% of fresh weight), significant amounts of vitamins and minerals (especially Mg, K, Na and Ca) and high moisture content (86.6% to 91.7%) have been observed in the fruit bodies (Manzi *et al.,* 1999). This fungus is enriched with the ability to propagate over numerous kinds of substrates (Cangy and Peerally, 1995). *P. eryngii* has been cultivated successfully on several agro-industrial as well as agricultural wastes like wheat straw, sawdust, soybean straw, millet straw, peanut shells, cotton waste, wheat bran, sugarcane bagasse and rice bran (Torng *et al.,* 2000; Zervakis *et al.,* 2001; Philippoussis *et al.,* 2001; Ohga and Royse, 2004; Okano *et al.,* 2007; Kirbag and Akyuz, 2008).

MATERIAL AND METHODS

A. Mother Culture and master culture

Cultures procured from Directorate of Mushroom Research, Solan, Mushroom Research and Training Centre, HAU, Haryana and Mushroom Research Laboratory, IIHR, Bengluru were multiplied on malt extract agar medium and preserved in test tubes. Six combinations were formed from these cultures through mycelial anastomosis, out of which, one strain was observed suitable for subtropical climate, which was named as Pratap King Oyster-1 (P.K.O-1) and this strain was used in our experiment.

Wheat grains were used as substrate for spawn preparation. For this, overnight presoaked wheat grains were boiled and 1% $CaCO_3$ was added and then sterilization was done after filling them in milk glass bottles at 20 lbs psi pressure (126.5°C) for 2 hrs. Then each bag after cooling was inoculated with mycelial agar bits of P.K.O.-1 culture in Laminar Air Flow and incubated at 20±1°C for 20-25 days till the grains were fully impregnated with the mycelium (Monmoon *et al.,* 2010).

B. Substrate preparation and spawning

The experiment was laid out by chopping straws of different crops to have a comparison of important nutritional contents in the fruit bodies and yield attributes grown in its climate and their relation with the basal substrate, such as straws of wheat, paddy, maize, sorghum, wheat straw supplemented with 5 per cent rice bran and wheat straw supplemented with 5 per cent wheat bran (Plate 1). Straws chopped into 3-5 cm pieces were water soaked for 24 hours and chemically treated for sterilization. The sterilized substrate after draining excess water got ready for filling and spawning in polybags when it cools down to room temperature and the substrate moisture content remains about 65%. 10-12 small holes were made at the equal distances in the Polybags by using scissors before filling them with substrate and spawn. Spawning was done in layers at the spawn rate of 3 per cent. After spawning, bags were closed (Peng, 1996; Kumar, 2005). Wet weight and dry weight of substrate was 3kg and 1 kg, respectively.

C. Crop management and harvesting

Bags turned white due to spawn or mycelial run completion after 20 to 22 days. At this stage, poly bags were teared and removed off. Pin heads emerged out after 2-3 days which finally became mature fruit bodies in 48-72 hrs. These mature fruiting bodies were harvested by plucking off in clock wise direction (Kirbag and Akyuz, 2008).

D. Biological efficiency (B.E.)

B.E. was also computed according to the formula of Chang and Miles (1992).

$$\text{B.E.\%} = \frac{\text{Fresh weight of fruiting bodies (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

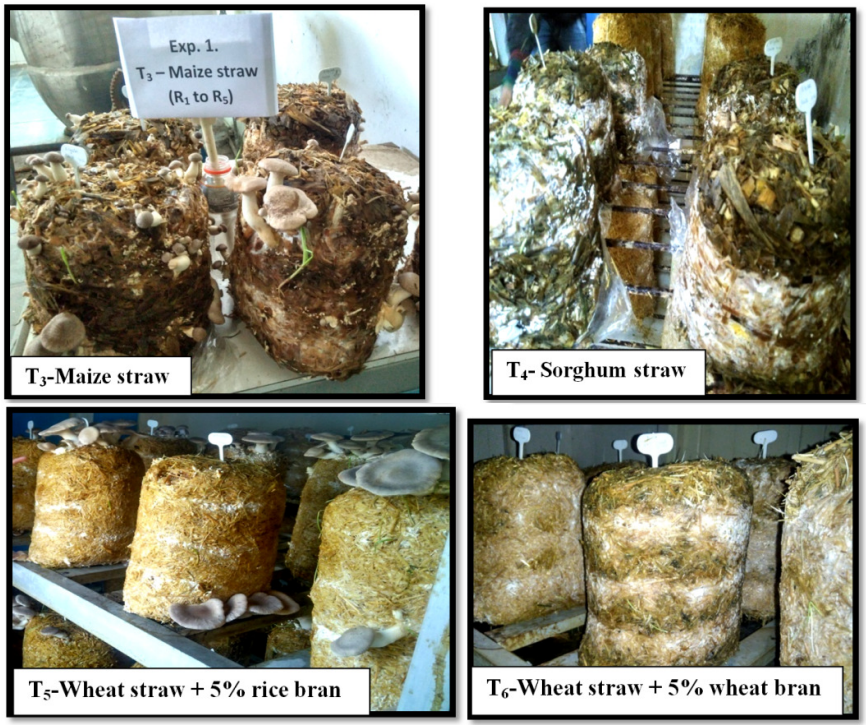

Plate 1. Photographs of *P. eryngii* on various substrates and supplements.

E. Qualitative parameters of Pleurotus eryngii on various substrates

Powdered samples were used to assay total moisture content, total protein content, total carbohydrate content, total crude fibre and total phenols. For this, fruit bodies grown on given substrates and supplements were harvested, dried and grounded to form fine powder (Plate 2).

a. Moisture content

Percent moisture was determined by sun-drying the fruit bodies and calculating the difference between fresh weights and dry weights of the test samples, by using the formula:

$$\text{Moisture \%} = \frac{\text{Weight of fresh fruiting bodies (g)} - \text{ weight of sun dried fruiting bodies (g)}}{\text{Weight of fresh fruiting bodies (g)}} \times 100$$

F. Protein content

Protein content was determined as per standard method and protocols (Lowry *et al.,* 1951). Sol A (2 per cent anhydrous sodium carbonate in 0.1 N sodium hydroxide), Sol B (0.5 per cent copper sulphate in 1 per cent sodium potassium tartarate), Sol C (Mix sol A and sol B in the ratio of 50:1) and Sol D (Folin-Ciocalteau reagent diluted up to 50 per cent with distilled water) were prepared as reagents.

Five ml of Solution C was mixed in 1 ml sample. Solution D (0.5 ml) was added rapidly after 10 minutes and mixed thoroughly. Sample absorbances were recorded after 30 minutes at 620 nm. One ml distilled water was taken for the blank. The protein content was estimated by drawing the standard curve using Bovine Serum Albumin (BSA) in the concentration ranging from 20 to100 µg/ ml.

G. Carbohydrate content

The total sugar contents (TSC) were determined by Anthrone's method taking glucose standard (Sadasivam and Manickam, 1992). Hundred mg sample was pipette out in a boiling tube and mixed with boil water for 3h for hydrolysis to occur with 5ml of 2.5 N Hydrochloric acid. After cooling the sample, it was neutralized with solid sodium carbonate till the ceasing of effervescences. Volume was made to 100 ml and centrifugation done. After collecting supernatant, half and one ml aliquots were used for further analyses. Volume again was made up to one ml with distilled water in tubes. Four ml anthrone reagent was poured in the test tubes and heated up to 8 minutes in boiling water bath. The sample tubes were cooled down and the green to dark green colour was measured at 630 nm.

d. Crude Fiber

Fat free sample (5g) was placed in beaker (500 ml). 1.25% of H_2SO_4 was added to the sample and boiling up to 30 minutes was done. The residue was washed in running water after filtering the mixture for making it free of acid. 1.25 per cent sodium hydroxide (200 ml) was mixed in this resulting residue and boiled up to 30 minutes. Residue was again filtered and washing in hot water was done. A pre-weighed crucible was taken and residue was transferred to it. In a hot air oven, it was dried at 100°C to a constant weight. Residue was finally ashed in the furnace and drop in weight was being calculated (AOAC, 1980).

$$\text{Crude fibre \%} = \frac{\text{Residue weight(g)} - \text{Ash weight after ignition(g)}}{\text{Sample weight (g)}} \times 100$$

H. Total phenols

Total phenols were determined by Folin-Ciocalteu colorimetric method. Eighty per cent ethanol was added to the powdered sample (1g) and centrifuged at 10,000 rpm up to 20 minutes (step repeated twice). Supernatant was collected and dried by evaporation. Five ml distilled water was added to residue and one ml of this diluted sample was added to twenty fold diluted Folin-Ciocalteu reagent (3 ml) and twenty per cent (w/v) sodium carbonate (2 ml). This mixture was incubated at 50°C for one minute in water bath and made to cool. Total phenols were computed by the absorbance read at 650 nm by using a standard curve (Thimmiah, 1999).

Plate 2. Photograph of powdered samples of *P. eryngii* fruiting bodies harvested from different substrates and supplements

RESULTS AND DISCUSSION

A. Effect of different substrates and supplements on quantitative parameters

Substrate is crucial factor involved in cultivation of mushroom. It has a direct influence on the quality, yield and production of mushrooms. Thus, various substrates and supplements were tested to find out the best substrate in terms of yield, biological efficiency and nutritional content. Table 1 and Fig 1 shows that the least time for complete colonisation of substrate was seen on straw of maize followed by straw of sorghum and the longest duration to complete spawn run was seen over wheat straw with five per cent rice bran. Same was the trend observed for the pinheads initiation time length. Contrastingly, maximum BE was recorded over straw of paddy (88.4%), straw of wheat (76.7%) and straw of maize (75%) (Fig. 2). Significant differences in yields of fruit bodies harvested from these substrates and supplements were obtained.

In this study, substrates like paddy and wheat gave highest yields and maize straw also performed well under Udaipur climate. Also, paddy is at top among production of cereals in India, thus, straw of paddy is easily accessible at low costs for cultivation of mushroom. According to the Maheshwari *et al.* (2007), 12.6 days were taken by paddy straw for completion of mycelial run and same gave biological yield of 648 g/kg wet substrate. As per the findings of Gregori *et al.* (2007), several species of *Pleurotus* are commonly being cultivated over the pasteurized substrates like wheat or rice. According to reports of Akyuz and Yildiz (2007), maximum BE of 73% was observed from supplemented wheat-cotton straw among various substrate mixture like wheat straw-cotton straw, wheat straw and millet straw + 15 per cent rice bran. Maize is a major crop of Udaipur so it is fairly recommendable substrate and one can reap its benefits from his own field (Deora *et al.,* 2021).

Table 1: Effect of different substrates and supplements on quantitative parameters of *P. eryngii.*

Treatment	Spawn run completion duration (days)	Duration of pin head initiation (days)	Yield (g)	BE %
Wheat Straw	19.0	25.0	767.4	76.7
Paddy Straw	18.4	24.2	884.0	88.4
Maize Straw	8.0	12.4	750.0	75.0
Sorghum Straw	11.8	17.6	474.8	47.5
Wheat Straw + 5 per cent rice bran	24.0	28.0	721.0	72.1
Wheat Straw + 5 per cent wheat bran	19.0	25.0	534.8	53.5
SEm±	0.4	0.5	28.2	2.8
CD(p=0.05)	1.1	1.5	82.3	8.2

All the observations are average of five replications.

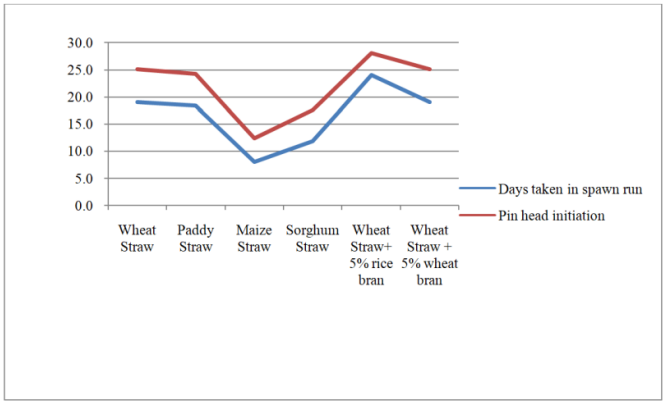

Fig. 1. Average number of days for completion of spawn run and initiation of pin heads over different substrates.

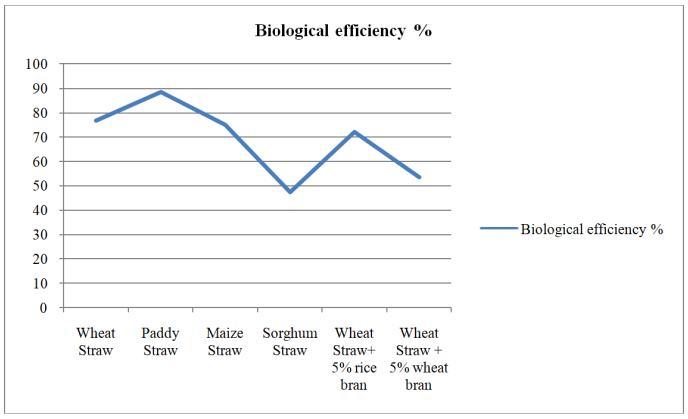

Fig. 2. Biological efficiency over various substrates.

B. Effect of different substrates and supplements on qualitative parameters

The mushroom has unique features like bulbous fruit body, excellent taste and having more polysaccharides as compared to other edible mushrooms. On growing *P. eryngii* on different substrates and supplements, the quality parameters of fruit bodies harvested from these showed variations accordingly. The results are presented in Table 2 and Fig 3. Moisture content of fruit bodies harvested from various substrate and supplement mixtures, ranging from 82 and 90% showed statistically significant deviations. Fruit bodies harvested from wheat straw contained the highest moisture content (90%) and the moisture contents in fruit bodies from straw of paddy, straw of sorghum and wheat straw supplemented with 5 per cent rice bran were observed at par (89%) and the lowest being recorded with wheat straw + 5% wheat bran in Udaipur's climate for growing Kabul Dhingri Mushroom. Likewise, other parameters related to the nutritional profiling of mushrooms gave significant variations with respect to the different substrate mixtures and supplements.

Total protein on dry weight basis was recorded highest (33%) on maize straw followed by 29% with wheat straw supplemented with 5 per cent rice bran and the lowest (15%) being recorded on wheat straw supplemented with 5 per cent wheat bran, respectively. Maximum (67%) and minimum (44%) total carbohydrate contents were obtained with the wheat straw supplemented with 5 per cent wheat bran and sorghum straw, respectively. Wheat straw produced fruiting bodies that contained 65% carbohydrate content.

The crude fiber content was recorded maximum (31%) and minimum (11%) with wheat straw and wheat straw + 5% wheat bran, respectively. The total phenol contents of *Pleurotus eryngii* grown on all experimental substrate and supplements were found to be 5.3 - 7 mg/g dry weight. The total phenol content was found highest on wheat straw + 5% wheat bran, followed by sorghum straw and wheat straw + 5% rice bran and the lowest found on maize straw. Our findings confirm the results and reports published by the researchers. The fluctuations from the results are due to the different climatic conditions in the Udaipur region. Khan (2005) found that the moisture content of the *Pleurotus sp.* ranged from 87-87.5%. The protein content, carbohydrate content (on dry weight basis) and fibers (in dry sample) of the mushroom were estimated 20-25, 39-43 and 22-23%, respectively. Mushrooms are a treasure of vitamins, minerals, proteins (20-25%), fibers (13-24%), polysaccharides (37-48%) (Sabaratnam *et al.,* 2011; Alam *et al.,* 2008).

Table 2: Effect of different substrates and supplements on qualitative parameters of *P. eryngii*.

Treatment	Moisture content %	Protein content %	Carbohydrate content %	Crude fibre content %	Phenol content mg/g
Wheat Straw	90.0	20.0	65.0	31.0	6.4
Paddy Straw	89.0	22.0	45.0	24.0	6.4
Maize Straw	85.0	33.0	51.0	27.0	5.3
Sorghum Straw	89.0	26.0	44.0	13.0	6.7
Wheat Straw+ 5 per cent rice bran	89.0	29.0	59.0	19.0	6.5
Wheat Straw + 5 per cent wheat bran	82.0	15.0	67.0	11.0	7.0
SEm±	**0.6**	**0.6**	**0.7**	**0.7**	**0.5**
CD(p=0.05)	**1.6**	**1.8**	**2.0**	**2.1**	**1.5**

All the observations are average of five replications.

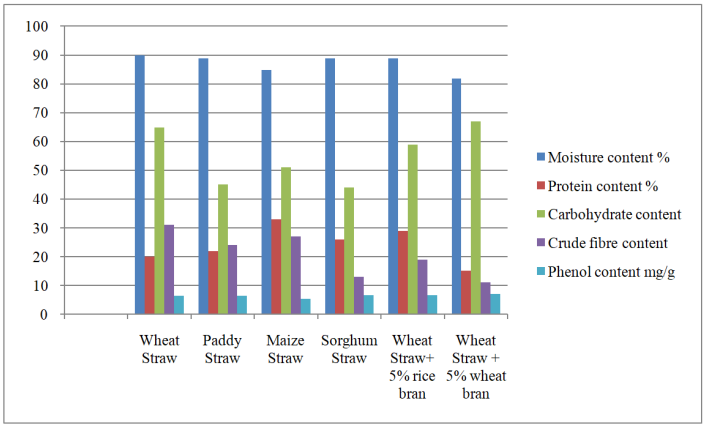

Fig. 3. Qualitative parameters of *P. eryngii* from different substrates and supplements

SUMMARY CONCLUSION AND FUTURE SCOPE

Among the six substrates and supplements tested (Wheat straw, Paddy straw, Maize straw, Sorghum straw**,** Wheat straw + 5 per cent rice bran, Wheat straw + 5 percent wheat bran), Paddy straw resulted in maximum B.E (88.4%) whereas Maize straw showed the minimum time for completion of spawn run (8 days) and pin head initiation (12.4 days). But, due to the unavailability of Paddy straw in this region, other substrates with good yields are recommended. The quality parameters like moisture, protein, carbohydrate, crude fiber and phenol contents were studied. Moisture contents of fruit bodies ranged from 82-90%. Total protein on dry weight basis and total carbohydrates were in the range of 15-33% and 44-67%, respectively. The crude fiber content was recorded 11-31% in our experiment. Total phenols of *P. eryngii* over experimental substrates and supplements were observed to be in the range of 5.3-7 mg/g dry weight.

In future aspect, *Pleurotus eryngii* is gaining importance and immense popularity in world of mushrooms in terms of their certain properties, low cost production, requirement of limited land and other resources and environment- friendliness. Many health benefits have been identified to control the malnutrition and human diseases. Presence of bio-active compounds and conversion of wastes into protein rich food are some of the features that have drawn attention from many researchers in recent few years. More work is expected to be done to explore more of the benefits of this mushroom.

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Conflict of Interest. None.

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