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Standardization of Cultivation Protocols of King Oyster (*Pleurotus eryngii*) Mushroom

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ABSTRACT

The word "Mushroom" has been used in different forms at different times and in different nations. Greeks believed mushroom as strength giving food to their warriors, while for Romans, it is "elixir of life". Chinese herbal medicines are mainly based on the Mushrooms and Japanese are fond of them because of its high medicinal values. Non Green Revolution or Mushroom Farming is one of the alternate ways to fulfill the food demand for the rising population from the scarce land resources for our Indian democracy in this vulnerable climate change era because mushroom grows on agro-wastes and converts them into protein rich valuable food without requiring additional land and has many nutritional and medicinal properties. *Pleurotus eryngii* has a very good shelf life and has little flavor or aroma in raw state and brings out typical flavors when cooked. Cultivation of this mushroom is a biotechnological process in which the agro-residues or waste plant materials can be converted into some valuable and beneficial food. Protein conversion efficiency and productivity of *P. eryngii* per unit land and per unit time is far better than animal and plant sources. This mushroom contains chemicals that boost up the immune system. Adding *P. eryngii* in diet may work as cholesterol-lowering dietary agent.

Keywords Agro-residues, Cholesterol-lowering, Mushroom, *Pleurotus eryngii*, Protein conversion efficiency.

INTRODUCTION

Commonly, mushroom refers to all higher fungi which possess stalks and caps or all the large and fleshy fungi (Chang and Miles 1992). They are rich in protein, dietary fiber, vitamins and minerals. The digestible carbohydrate profile of mushroom contains starch, pentose, hexose, disaccharides, amino sugars, sugar acids and sugar alcohols. Edible mushrooms have insignificant levels of lipid with higher proportion of polyunsaturated fatty acids. As a result, mushrooms are supposed to be low calorific food (Ukwuru *et al*. 2018). Specific biochemical compounds that have been found in mushrooms

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are responsible for improving and bettering human health in different ways. These bioactive compounds include polysaccharides, tri-terpenoids, antioxidants, low molecular weight proteins, glycoproteins and immunomodulating compounds (Manikanandan 2011). In recent years, cultivation of mushrooms in India has attained a tremendous growth with respect to the kind and productivity of mushroom. The most cultivated mushroom globally is *Agaricus bisporus*, followed by *Pleurotus* spp. that constitutes about 27% of the world's cultivated mushrooms (Atila 2017).

Pleurotus eryngii (DC.ex FR.) Quel, which is also called as Kabul Dhingri, King Oyster Mushroom, King Trumpet Mushroom, French Horn Mushroom, King Brown Mushroom, Trumpet royale, is an edible mushroom and is native to Mediterranean regions of Europe, the Middle East and North Africa. Its natural range extends from the Atlantic Ocean through the Mediterranean Basin and Central Europe into Western Asia and India. It is a weak parasite, and can survive on the roots or stem base of the live plants of the family *Apiaceae* (Zervakis *et al*. 2001, Lewinsohn *et al*. 2002). The advantage of *P. eryngii* is also taken in different medicinal, pharmaceutical and biotechnological studies (Couto and Herrera 2006, Gregori *et al*. 2007). It is capable of producing variety of biologically active compounds and has a well-developed ligninolytic enzymatic system which takes part in the degradation of lignin and many aromatic compounds (Cohen *et al*. 2002). In addition to this, because of its remarkable flavor and great nutritional value, the commercial production of *P. eryngii* has, in the last few years, increased tremendously at world level (Manzi *et al*. 1999).

It has potential to grow on variety of substrates. Cultivation of *P. eryngii* mushroom has been successfully done on various agricultural and agro-industrial wastes including different sawdust, wheat straw, millet straw, soybean straw, cotton waste, peanut shells, sugarcane bagasse, wheat and rice bran (Cangy and Peerally 1995, Torng *et al*. 2000, Zervakis *et al*. 2001, Philippoussis *et al*. 2001, Ohga and Royse 2004, Okano *et al*. 2007 and Kirbag and Akyuz 2008). According to Manzi *et al*. (1999, 2004), they are rich in dietary fibers, chitin, carbohydrates (9.6% of fresh weight) and polysaccharides (0.41% of fresh weight). The protein content is between 1.88 and 2.65% and the total nitrogen content is around 5.30%. The abundantly found amino acids are aspartic acid, glutamic acid and arginine. Significant amounts of vitamins and minerals (especially K, Mg, Na and Ca), very low concentration of lipid (0.8% of fresh weight), and high moisture (between 86.6% and 91.7%) are observed in the basidiomata (Manzi *et al*. 1999). The studies have reported various compounds derived from *P. eryngii* have led to the common belief that this fungus has anti-hypertensive, immunomodulating, antitumor, antibacterial, antioxidant, anti-hypercholesterolic, anti-hyperglycemic, antiviral, antifungal, anti-inflammatory and anti-osteoporotic effects (Wasser and Weis 1999).

Substrate and supplements for suitability of cultivation of *Pleurotus eryngii*

Torng *et al*. (2000) concluded that, with the increasing supplementation of the substrate with rice bran (0.0-47.95%), the average yield, biological efficiency (BE) of *P.eryngii* ATCC 36047 increased significantly in Holland 150 strain, the highest BE was observed with 38.08% rice bran but these decreased significantly when supplementation of rice bran increased to 47.95%. The best mycelial (spawn) run period was recorded in *P. flabellatus* on paddy straw (15 days) followed by wheat straw which took 16 days (Ram and Pant 2004). Cotton stalks and leaves gave the maximum number of *P. sajor-caju* sporophores (178.33), average weight of sporophore (5.12g) and yield (914.032 g) out of wheat straw, rice straw, bajra straw and leaves, cotton stalks and leaves, soybean straw, groundnut creepers + wheat straw, soybean straw, wheat straw and groundnut creepers (Tupatkar and Jadhao 2006). Akyuz and Yildiz (2007) reported that on cultivating *P. eryngii* on mixtures of wheat straw-cotton straw, wheat straw and millet straw supplemented with 15% rice bran, the highest BE being 73% was obtained with supplemented wheat-cotton straw. Paddy straw was the best agro-waste material which took 12.6 days for spawn run completion with yield of 648g/kg wet substrate as compared to wheat straw and sawdust (Maheshwari *et al*. 2007). Rodriguez Estrada and Royse (2007) stated that yields of *P. eryngii* were higher in substrates that contained manganese (50ug/l) and soybean in comparison to the basal cotton seed hull/sawdust substrate. Different *Pleurotus* spp. can be commonly cultivated on the pasteurized wheat or rice straw (Gregori *et al*. 2007). *P. eryngii* gave the highest yield of 23.2g/100g wet media and BE 77.2% on a mixture of wheat straw and cotton straw with 20% rice bran (Kribag and Akyuz 2008). Biological efficiency of *P. eryngii* was better on saw dust than on rice straw, although the fruiting bodies of mushroom were larger in size on rice straw (Moonmoon *et al*. 2010). Arathy and Das (2016) cultivated Pink Oyster Mushroom (*Pleurotus eous*) and reported that pinheads were first initiated by the coir pith compost (12 days) and the longest time was taken in case of saw dust. Maximum number of fruiting bodies was obtained with paddy straw. Banana pseudostem gave the highest total yield followed by the paddy straw. Dense mycelial growth was observed in coconut leaf stalk and sugarcane bagasse, but no fruiting body formation took place. Highest biological efficiency was obtained in banana pseudostem (105.8%), followed by paddy straw (92.6%) and thus, banana pseudostem was the best substrate in terms of yield.

Jeznabadi *et al*. (2016) used lignocellulosic organic wastes, such as wheat and barley straw, wood chips, sawdust, sugar beet pulp and maize stem residue as basal substrate and the wheat and rice bran, soybean powder and their combinations as supplements and observed that the mushroom production gap and dry matter content of *P. eryngii* was increased with the supplementation of wheat bran in wood chips. Barley straw and sugar beet pulp substrate complemented with rice bran gave the highest mushroom fresh weight.

Among five agro-substrates like date palm fibers (fibrillum), wheat straw, white sawdust and their combination, taking date palm wastes as substrate may be beneficial for successful cultivation of King Oyster Mushroom in farm (Oswaid *et al*. 2016). The best substrates for mycelial growth were found to be safflower hay and bean straw while oak sawdust gave the slower mycelial growth for all *Pleurotus* species examined Atila (2017).

Kumar (2017) studied the effect of different agro-wastes on spawn running, fruit bodies formation, pinhead formation, yield and biological efficiency of Oyster (*P. sajor-caju*) by cultivating it on wheat straw, black gram straw, sesame straw, sarson and soybean straw. Highest yield (86% B.E.) was obtained with wheat straw and same took lesser time for spawn run completion (16.66 days) and pin head initiation (24.33 days). More spawn run completion time (23.66 days) and pin head initiation time (30.66 days) was required by sarson straw and gave less yield (2.40kg/5.0 kg substrate) with 48.0% biological efficiency. Pal *et al*. (2017) investigated the suitability of substrate supplementation on yield of *P. pulmonarius*. Results revealed that maximum mycelial growth (10.50 days of inoculation) and minimum time for pinheads initiation (13.67 days) were obtained with wheat straw supplemented with cotton seed meal. Similarly, it produced maximum yield (155.3g) with biological efficiency of 77.65% followed by supplementation of saw dust, wheat straw (control), calcium ammonium nitrate (CAN), ammonium nitrate and urea, respectively.

Substrate sterilization methods

Mejía and Albertó (2012) reported that when treating substrate with IHW (immersion in hot water) treatment, yields get reduced at least 20% when compared with other straw treatments such as steam treatment, chemical treatment or untreated wheat straw. Sugars, proteins, minerals N, P, K and Ca were observed in residual water of IHW treatment. The loss of these nutrients would be the reason of decrease in yield. Carbendazim treatment was found to be giving highest yields (BE: 106.93%) while the lowest BE of 75.83% was obtained with IHW.

Even after autoclaving being the best method for substrate pre-treatment, hot water pasteurizing at 60^{o}C for 3 hrs of sugarcane bagasse proved to be a viable and promising method for substrate pre-treatment, which is worth adopting to obtain a good yield especially in rural areas, where autoclave sterilization is economically not feasible (Oseni *et al*. 2012). Hot water treatment and steaming of substrate significantly reduced contamination of substrate and improved the yield of mushroom as compared to the untreated control. Significantly higher yield (BE: 69.4%) was observed with steamed substrate than those grown on substrates treated with hot water (BE: 53.3%) (Jongman *et al*. 2013). The biological efficiency, economic yield and other yield attributes of oyster mushroom at 80°C were recorded more than to treatment at 60°C or 100°C, whereas, hot water treatment for 3 hrs also gave better results and the prevalence of contaminants were low (Akhter *et al*. 2017).

Optimum spawn rate

Cangy and Peerally (1995) cultivated 10 species of *Pleurotus* using spawning rates @0.75, 1.5, 3.0 and 6.0% of fresh weight of substrate and recommended 1% spawn rate to be sufficient while using the smaller bags (yields>16% of spawned substrate weight) at mean temperature 18°C (range 13-23°C). Fan *et al*. (2000) used spawn rates ranging 2.5-25% and found that 25% spawn rate appeared superior but the recommendations supported 10% spawn rate keeping in view of the process economics. The first fructification took place after 20-23 days of inoculation and the BE reached about 90-97% after 50-60 days. While Balakrishna *et al*. (2001) cultivated mushroom *Pleurotus sajor-caju*, taking spawn rates 2, 5, 10 and 15% with 2 kg substrate and observed 10% spawn rate giving the highest yield out of all. Royse (2002) observed maximum yield of *Pleurotus cornucopiae* at 3.75-5% spawn rate. Mycelial run was faster at higher spawn dose according to Ram and Pant (2004). It was very fast at 5% spawn in both species *P. sajor-caju* and *P. flabellatus* in all the three substrates like wheat straw, rice straw and sugarcane bagasse followed by 4 and 3%. Chauhan (2013) investigated different spawn rates viz., 1, 2, 3, 4 and 5% for evaluating better yield and biological efficiency of *Pleurotus djamor*. The minimum days for spawn run, pinhead initiation and maximum biological efficiency were in case of 5% spawn rate (12.75 days, 14.25 days and 78.29% respectively) followed by 4, 3, 2 and 1%. Among different quantities of wet substrate weight (2, 3 and 4kg) and spawn rates (50, 100 and 150g), the treatment with the combination of 3kg of substrate and 150g of spawn gave maximum yield on fresh weight basis and the highest biological efficiency, followed by the treatment with combination of 150g spawn and 4kg of substrate (Patel and Trivedi 2014).

Idowu *et al*. (2016) investigated effect of different spawn rates (3, 5, 7, 9, 11 and 13%) on *P. ostreatus* and observed 13% spawn level producing the maximum number of fruit bodies (11.33), fruit body weight (65.69g), widest pileus (6.57cm) and longest stipe (5.53cm). Nine per cent spawn levels produce the densest mycelia; the mean fruit body weight was highest (7.56g) at 9%. Thirteen per cent spawn level took significantly shortest days for substrate colonization and primordial initiation and 3% took the longest. Although, the spawn run was faster at higher spawn rate but there was no significant difference in yield when spawn rate was increased from 4 to 8%. Thus, taking cost of spawn and performance shown by different doses into consideration, the optimum dose was reported to be 2-4% out of 0.5, 1, 2, 4, 6, and 8% spawn rates tested for the cultivation of *P. pulmonarius* (Pal *et al*. 2017).

Kumar *et al*. (2018) cultivated the Oyster mushroom (*Pleurotus florida*) taking wheat straw as substrate. Different grain spawn (maize, bajra, paddy and wheat spawn) at different spawn rates (5 and 6%) were used. The results revealed that, 6% spawn rate of paddy produced maximum yield (550.0 g/kg dry substrates with 55.0% biological efficiency) which was statistically more than all other spawns rates, followed with 6% spawn rate of bajra (540.0 g/kg dry substrates with 54.0% biological efficiency). The minimum yield was produced by using 5% spawn rate of maize (503.33 g/kg dry substrates with 50.33% biological efficiency).

Climatic parameters

Temperature: Zadrazil (1976) observed 30°C to be the optimum temperature for the mycelial growth of *Pleurotus ostreatus*, *P. florida*, and 25°C be the optimum for *P. eryngii*. Theochari *et al*. (2002) stated that around 26°C and around 20°C are best suited temperatures for *Pleurotus ostreatus* and *Pleurotus eryngii*, respectively which are preferably important for cultivation of *Pleurotus* mushroom in hot climates during summer. Miles and Chang (2004) reported 20-30°C to be the temperature range which supports mycelial growth of all the Oyster mushrooms. However, different species require different temperatures for fruiting. *Pleurotus* spp. grows in wide temperature range from 15 to 30°C. The optimal temperatures for the fruiting body development for *P. sajor-caju* are 20-24°C. Rout *et al*. (2015) observed 25°C be the suitable incubation temperature in their experiment in which they studied the influence of incubation temperature on the linear mycelial growth of oyster species and revealed that the superior growth occurred at 25°C. Kumar and Sarathi (2017) in their experiment observed that a good harvest of mushroom (107% Biological Efficiency) was obtained during the month of October. A range of average maximum temperature (23.5-34.6°C) and minimum temperature (13.4-24.2°C) was found most appropriate for the cultivation of oyster mushroom.

Relative humidity: Kumar (2000) reported that the maximum mycelial growth of *Pleurotus flabellatus* was obtained at 25-90% relative humidity. Jang *et al*. (2003) and Han *et al*. (2004) observed that the optimum relative humidity for the growth and development of *Pleurotus* spp. is 80 % while the most suitable relative humidity ranged from 60-80. Uddin *et al*. (2011) investigated the cultivation of different species of oyster mushroom: *Pleurotus ostreatus*, *P. florida* and *P. sajor-caju*. They reported that the best supporting months for the production were during December to February (14-27°C, 70-80% RH) in which minimum days were required for the primordial initiation, and the maximum number of fruiting bodies took place, biological yield and biological efficiency were also obtained highest. Patel and Trivedi (2014 and 2015) used different types of substrate and successfully cultivated *Pleurotus sajor-caju* under 20-30°C temperature and 60-95% humidity.

Tariqul Islam *et al*. (2016) cultivated the mushroom (*Pleurotus pulmonarius*) in three different environmental conditions including natural indoor environment (NIE), humidifying 75-90% till primordial initiation (HTPI) environmental condition and in continuous 75-90% humidity (CH). The highest average fruit body weight, number of fruit bodies, cap diameter, stalk height, percentage of primordial initiation, mature fruit body formation and the lowest dead primordials were found in continuous 75-90% indoor humidity condition. The lowest morphology and yield percentages were found in natural indoor environment and humidifying 75-90% till primordial initiation condition. In HTPI condition, the lowest morphology and yields were recorded although the primordial initiation was higher than NIE.

Cultivation protocols of *Pleurotus eryngii*

Kumar (2005) investigated that *P. eryngii* showed the maximum mycelial growth at 20±1°C temperature, 6.0 pH and 138 lux light intensity. Maximum spawn run and sporophore production was recorded at spawn rates of 3 and 3.5%. On quantification of substrate, 3 and 4 kg wet weight among substrate weights 2, 3, 4, and 5kg, gave maximum spawn run and fruit body production. Out of four different spawning methods (thorough mixing, three layering, two layering and top dressing), the three layer of spawning in between the substrate gave the best results. The Starch and Urea out of the four carbon sources (maltose, glucose, sucrose and starch) and nitrogen sources [KNO_3, $(NH)_4SO_3$, urea and soybean powder], respectively were recorded to be the best for the maximum mycelial growth of *P. eryngii*.

Hassan *et al*. (2010) examined the suitability of local lignocellulosic wastes for growing media. Sawdust, soybean straw and rice straw media supplemented with wheat bran at the supplementation level of 25% gave the maximum mycelium linear growth being 8.9, 8.4 and 7.6 mm/day. While, the maximum mycelium linear growth on sugarcane bagasse 6.7 mm/day was achieved at 30% wheat bran level. Incubation period for the different tested media ranged from 30-41 days (sawdust recorded the shortest period while sugarcane bagasse recorded the longest one), the maximum yield being 201 g/kg wet media and biological efficiency (BE) 65.22% was obtained when grown on sawdust. Sugarcane bagasse gave the lowest yield 139 g/ kg wet media with biological efficiency of 45.71%. *P. eryngii* grown on different tested media contained 87.63-90.26% moisture, 21.33- 24.08 % crude protein, 2.96-3.78% ether extract and 6.54- 8.02% ash.

Chauhan (2013) reported that Kodo grain spawn was observed to be the best substrate for spawn production of *P. djamor*, but wheat grain spawn gave the highest biological efficiency. Five per cent spawn dose on wheat straw substrate produced the best results. Lantana gave good yield when used as a substrate for the cultivation of *P. djamor*. Wheat straw supplemented with wheat bran in the ratio of 9:1 also gave good yield and biological efficiency. An optimum temperature of 25°C and pH in acidic (5.5) and slightly acidic (6.0) range were preferred by this fungus. Different solid media were tried for maintaining the pure cultures, among which MEA and PDA, and in case of various liquid media, Glucose-asparagine solution supported maximum radial growth of *P. djamor*. Maltose, Potassium Nitrate and Giberrellic acid were recorded being good carbon source, nitrogen source and growth regulator, respectively that supported the best mycelial growth of the test fungus. An exposure of 6 hrs light and 18 hrs dark period resulted in maximum radial growth.

Patel and Trivedi (2014) cultivated the oyster mushroom, *P. sajor-caju* on paddy straw where they examined for the best spawn rate using wheat grain spawn and the optimum quantity of substrate to be taken for obtaining maximum yields. The substrate was given pre-treatment. The temperature and humidity of the growing room was maintained 20-30°C and 60-95%, respectively. The fast completion of spawn run (14.33 days), primordial formation (17.33) and pinhead formation (20.33) was first observed with substrate of 3 kg on wet weight basis at spawn rate of 150 g that also resulted in giving maximum stem length and cap diameter, which was followed by spawn run (15.67 days), primordial formation (18.33 days) and pinhead formation (20.67 days) in treatment with 150 g spawn and 4 kg of substrate. The treatment of 3 kg of substrate and 150 g of spawn gave the maximum yield on fresh weight basis and biological efficiency. Total soluble protein (16.5%) and crude fiber (11.9%) was also in considerable amount in *P. sajor-caju* when cultivated on paddy straw as substrate.

Saha (2017) in her investigation reported that the winter season was found to be promising for obtaining maximum yields and B.E.s of different strains of *P*. species. Wheat straw while taking as substrate for growing *P*. spp. (strain - PL-16-04), resulted in the highest yield and B.E. Steam pasteurization was found to be the best method for sterilization of wheat straw substrate. Maximum radial growth was observed at temperature 28°C with 75% relative humidity. Different straw substrate showed loss in major nutrient (NPK) content in fresh and spent mushroom substrate. Maximum growth was reported at pH 7 and best suited media was PDA. Green wavelength shows maximum radial growth. Fast mycelial run was seen in sorghum grain spawn and maximum yield was recorded when spawn was raised on wheat grain and used spawn rate was @ l2% (60 g) of spawn.

Nutritional parameters of *Pleurotus eryngii* on various substrates

P. eryngii (Kabul Dhingri) has tremendous nutritional value and it has more polysaccharides when compared to other edible mushrooms. Khan (2005) investigated about the nutritive values of *Pleurotus* sp. (*P. sajor-caju* and *P. florida*) in Bangladesh and found that the moisture content of the mushrooms 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. The pileus and gills of the mushrooms were protein rich and stipe was fiber rich. Mushrooms are a great source of proteins (20–25%), polysaccharides (37-48%), fibers (13–24%), vitamins and minerals (Sabaratnam *et al*. 2011, Alam *et al*. 2008) and has some secondary metabolites, including phenolics, polyketides, terpenes and steroids (Cheung *et al*. 2003). Mushrooms are a good source of carbohydrates as well. Values ranging between 51.4 and 59.9 g/100g dry mushroom were reported by La Guardia *et al*. (2005) and by Guo *et al*. (2007) for *P. eryngii* var. *eryngii* and for *P. djamor*, respectively.

Akyub and Kirbag (2010) selected 2 different compost mediums viz., wheat straw (WS) and wheat straw + cotton stalk (CS) in the ratio of 1:1 and composts were supplemented with rice bran (RB) at a rate of 10 and 20% forming the substrates like WS + 10% RB, WS + 20% RB, WS-CS (1:1), WS-CS (1:1) + 10% RB and WS-CS (1:1) + 20% RB. They studied the effects of these compost mediums on the nutritive value of *P.eryngii* var. *ferulae*. On air-dried weight basis, the dry matter, moisture and protein content of the samples were in the range of 91.7-92.7, 7.3-8.3 and 8.5-19.7%, respectively. Hassan *et al*. (2010) examined the suitability of local lignocellulosic wastes for growing media. *P. eryngii* grown on different tested media contained 87.63-90.26% moisture, 21.33-24.08% crude protein, 2.96-3.78% ether extract and 6.54-8.02% ash. Higher phenolic content was reported by Yildirim *et al*. (2012) for wild-grown edible *P. eryngii* (29 to 32 mg of gallic acid equiv./g dry mushroom) collected from different regions of Tunceli (Turkey).

Nadir (2014) studied that how the quality of oyster mushroom (*P. ostreatus*) got affected by using different substrate mixtures. He mixed artichoke stalks with wheat straw in different ratios (A: 100% artichoke, B: 75% artichoke, C: 50% artichoke, D: 25% artichoke and E: 0% artichoke) and considered 100% wheat straw as the control. The composition of growth medium did not affect significantly the moisture, protein, carbohydrate and crude fiber contents of the fruit bodies. Samples grown on various substrates gave a moisture content range of 91.3 to 92.5%. Sample grown on substrate C was observed to be containing the highest percentage of total protein (20.6%) on dry weight of mushroom basis, followed by substrate E (19.9%), A (18%) and D (17.9%), and the lowest (16.4%) on substrate B (75% wheat straw + 25% artichoke stalks). The highest (58.9%) total carbohydrate content was obtained with substrate B (75% artichoke stalks + 25% wheat straw) and that the lowest (46.7%) with substrate C (50% artichoke stalks + 50% wheat straw). The highest (22.6%) crude fiber content was recorded with substrate.

Patel and Trivedi (2014) reported that the total soluble protein (16.5%) and crude fiber (11.9%) were in considerable amount in *Pleurotus sajor-caju* when cultivated on paddy straw as substrate. Rodrigues *et al*. (2015) studied the chemical composition and nutritive value of five mushrooms species. Protein, sugar and fat contents ranged between 16.2-26.6, 52.7-64.9 and 2.3-3.5 g/100 g dry mushroom, respectively. The highest total phenolic content was observed for *P. citrinopileatus* var. *cornucopiae* with 1140 µg catechol equiv./g dry mushroom. *P. eryngii* was characterized by the lowest total protein content (16.2 g/100 g dry mushroom) followed by the highest content in total sugar (64.9 g/100 g dry mushroom) and in total fat (3.4 g/100 g dry mushroom). The highest moisture content was obtained on barley straw and sugar beet pulp substrate complemented with rice bran. The protein content was observed in the range of 4.64% (barley straw + wheat bran and wood chips + soybean powder + rice bran treatments) to 13.66 (wheat straw + wheat bran + soybean powder treatment) (Jeznabadi *et al*. 2016).

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