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ORIGINAL RESEARCH

King oyster mushroom production using various sources of agricultural wastes in Iran

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Abstract
Background King oyster mushroom (*Pleurotus eryngii*) contains many medicinal and nutritional values, excellent flavors, and a long shelf life. Cultivation of edible mushroom on agricultural and lignocellulosic waste like wheat straw and wood chips gives a high yield and nutritional contains. In present study, the effects of lignocellulosic organic wastes, such as wood chips, wheat and barley straw, sugar beet pulp, sawdust and maize stem residue as basal substrate and wheat and rice bran, soybean powder and their combinations were used as supplements. We analyzed mushroom production gap, mushroom fresh weight, moisture content, dry matter, and protein content of fruit body traits. A factorial experiment was conducted in a completely randomized design with four replications.
Results Supplementation of wheat bran in wood chips increased the mushroom production gap and dry matter content. The highest mushroom fresh weight and moisture content were achieved on barley straw and sugar beet pulp substrate complemented with rice bran, respectively. In addition, protein content ranged from 4.64 % (barley straw + wheat bran and wood chips + soybean powder + rice bran treatments) to 13.66 % (wheat straw + wheat bran + soybean powder treatment).
Conclusions The quality of *P. eryngii* was significantly affected by substrate ingredients. The type of substrate as well as the type and quantity of supplement appeared to have a substantial effect on prolonging the delayed-release nutrients.

Keywords Dry matter · *Pleurotus eryngii* · Protein content · Soybean powder · Wood chips

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Introduction

Pleurotus spp. is an important mushroom genus which contains many edible and medicinal species. Its ability to grow in a wide range of temperatures and utilize available lignocellulosic materials cause to be considered as a globally highly intriguing mushroom for production (Royse 2003; Baysal et al. 2003). *Pleurotus eryngii* is considered as the best species in the genus due to its excellence in cap and stem consistency, very long shelf life as well as acting as a massive source for a wide range of bioactive compounds than any other oyster mushroom. Therefore, it is widely used as a healthy food and has a high consumer demand in many countries (Yildiz et al. 2002; Estrada 2008; Lo 2008; Cheung 2008; Oke and Aslim 2011). The mushroom cultivation may be considered as the most efficient and economically viable biotechnology to convert agricultural and industrial wastes into high quality protein foods (Hussain 2001).

Lignocellulosic waste material composes of cellulose, hemicellulose, and lignin. These materials have been considered as noticeable source of nutrients for the mushroom growth. These wastes can be classified into different branches such as wood residues, waste paper, grasses, agricultural residues (including straw, stalks, and bagasse),

domestic wastes (lignocellulosic garbage and sewage), and municipal solid wastes (Rodriguez et al. 2008). The most extensively used waste for edible mushroom production has been wheat and rice straws, sawdust, hard wood chips, sugarcane bagasse, cotton seed hulls, corn cobs, rice, and wheat bran (Orts et al. 2008; Saber et al. 2010). Wheat straw has a large potential for plant nutrients in organic farming. The straw accounts for 35–40 % N, 10–15 % P, and 80–90 % K (Sharma and Sharma 2004; Davari et al. 2012). However, a scrutinized care should be undertaken to make sure that the waste is free of chemical components and toxic factors which could affect mycelium growth and subsequently the human health issue (Stemets 2000).

It has been shown that the type and amount of substrate and complement foods and their interaction with mushroom strains have a significant influence on mushroom productivity (Peng et al. 2000; Jafarpour and Eghbalsaeed 2012). Substrate supplementation to the nutrient can improve the nutrient requirements for an efficient production of mushroom. Both carbon concentration and the C/N ratios of the substrate have significant effect on mushroom production gap, whereas it has been well-documented that C/N ratio is more effective than carbon concentration (Gao et al. 2007). Substrate source is one of the important factors in mushroom production because it supports mycelia growth and development into mushroom fruit bodies.

In the present study, we aimed to investigate the effects of various readily available sources of Iranian agricultural residues and lignocellulosic wastes on production gap, mushroom fresh weight, moisture and dry matter content of fruiting body, as well as the protein content of *P. eryngii.*

Materials and methods

The *P. eryngii* spawn was purchased from the Mushroom Spawn Research Center in Karaj, Iran. To achieve suitable substrates and supplement formulation for the cultivation of *P. eryngii*, various combinations of waste materials were applied. In this study, wheat bran (WB), rice bran (RB), soybean powder (SP), and their combinations including (WB + RB = 1:1), (RB + SP = 1:1), (WB + SP = 1:1), and (WB + RB + SP = 1:1:1) were used as supplements. In addition, we used wheat straw, wood chips, sawdust, sugar beet pulp, barley straw and maize stem residue as substrate sources.

Pleurotus eryngii cultivation underwent several procedures, including culture medium confection, bagging, sterilization, spawn inoculation, incubation, mycelium maturation. In addition, all the packages were opened from the top side and induced the fruit body formation. Each supplement combination was filled in autoclavable plastic bag and sterilized at 15 psi pressure and 121 °C for 1 h. For pasteurization of the substrates, all the six substrates were soaked overnight in water following the tissue softening at 100 °C for 1.5 h, and finally were drained (Jafarpour and Eghbalsaeed 2012). To reach an optimal C/N ratio, all substrates were supplemented with nitrogen sources. One hundred grams of each supplement and 160 g of spawns (based on 10 and 16 % of substrates dry weight, respectively) were added to 1 kg of substrate based on the dry weight in each experimental unit (Zhang et al. 2002; Jafarpour and Eghbalsaeed 2012). All the environmental conditions in the culture hall were managed according to the growth requirements of *P. eryngii* as indicated in Table 1 (Stemets 2000).

After the incubation period, the main environmental factors were managed to promote fruit body formation. In this study, total mushroom production gap was classified into two basic phases; from pin head stage to primary fruiting body and from primary fruiting body to complete fruiting body phases. The differentiation started with formation of primordia and the fruit body development was completed during 4–7 days. The cropping room was watered intermittently to maintain the moisture during the cropping time. Mushroom fruit body was harvested when the mushroom cap surface was flattened and slightly uprolled at the cap margins. Mushroom dry weight was calculated following 24 h incubation. The mushroom fresh weight (g per bag) was calculated as total weight of harvested fresh mushrooms per bag divided to the total number of harvested mushrooms.

Total protein content was determined by the Kjeldahl method using a conversion factor of 4.38 (Guo et al. 2007). The moisture content of mushroom was also expressed in percentage and calculated by the following equation:

$$\text{Moisture content } (\%) = \frac{\text{Weight of fresh sample} - \text{weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

Biochemical analyses

Chemical compositions of all substrates were analyzed before starting the experiment (Table 2). Carbon and nitrogen content were estimated by loss of ignition and the Kjeldahl method, respectively. Then, the carbon/nitrogen ratio and organic matter of each substrate were calculated.

Statistical analysis

A factorial experiment comprising 42 treatment combinations was conducted in a completely randomized design with four replicates. Data analysis was carried out using SAS software. Differences among the means of groups were assessed using the Tukey post hoc test ($p < 0.05$).

Table 1 Environmental condition based on *P. eryngii* requirements

Parameter	Spawn run phase	Pin head	Fruiting body
Temperature (°C)	24–25	10–15	15–21
Relative humidity (%)	90–95	95–100	85–90
CO_2 (ppm)	5000–20,000	500–1000	≤2000
Air replacement	1	4–8	4–5
Light (lux day^{-1})	–	500–1000	500–1000

Table 2 Substrate and complement compositions (based on the dry weight)

Treatment[a]	pH	EC (dS m^{-1})	Carbon (%)	C/N	Organic mater (%)
BP + RB	4.27	2.00	54.52	92.40	94.0
WS + RB	4.49	1.47	52.78	56.75	91.0
BP + WB	4.35	2.06	54.81	70.26	94.5
WC + RB	4.76	1.22	55.97	159.91	96.5
MS + WB	5.20	1.77	54.52	33.86	94.0
MS + RB	5.27	1.41	51.91	77.47	89.5
WS + SP	5.93	2.67	55.68	99.42	96.0
BS + RB	5.39	1.85	52.78	46.29	91.0
BS + WB	4.90	1.58	54.52	81.37	94.0
WC + WB	4.60	1.25	56.84	111.45	98.0
WC + SP	5.19	1.15	56.84	123.56	98.0
S + WB	4.83	1.42	54.52	87.93	94.0
WS + WB	4.76	1.79	53.65	93.65	92.5
BS + SP	5.50	1.67	52.49	34.53	90.5
BP + SP	4.35	2.35	53.65	60.28	92.5
S + RB	5.22	1.88	53.94	89.90	93.0
MS + SP	5.16	2.85	53.36	37.31	92.0
S + SP	5.30	1.43	1.55	86.10	95.0
BP + WB + RB	4.36	2.67	54.81	88.40	94.5
WC + SP + RB	4.81	1.53	55.10	122.44	95.0
WS + SP + RB	4.65	2.76	53.07	41.13	91.5
WS + WB + RB	4.57	3.27	54.81	119.15	94.5
BS + SP + RB	5.60	2.56	53.36	136.82	92.0
WC + WB + SP	4.80	1.50	56.84	145.74	98.0
MS + SP + RB	5.65	3.78	53.36	86.07	92.0
MS + RB + WB	5.13	2.07	54.23	95.14	93.5
WS + WB + SP	4.47	2.86	51.62	33.51	89.0
BP + SP + WB	4.09	2.81	53.65	55.88	92.5
BS + WB + SP	4.90	4.06	51.04	76.17	88.0
BS + RB +WB	4.45	2.55	53.07	123.41	91.5
WC + SP + RB + WB	4.74	2.55	55.68	94.37	96.0
BP + SP + WB + RB	4.33	3.16	54.23	55.33	93.5
BS + WB +SP + RB	5.16	2.98	53.36	31.57	92.0
S + WB + SP + RB	4.60	2.38	56.09	64.47	96.7

[a] *WS* wheat straw, *BS* barley straw, *MS* maize stem residue, *S* sawdust, *WC* wood chips, *BP* sugar beet pulp, *WB* wheat bran, *RB* rice bran, and *SP* soybean powder

Since substrate and complement interaction effect were significant for all traits ($p < 0.05$), we analyzed the combined factors as a single factor rather than the single main effects.

Results

In this study, during spawn run phases ink caps mushroom (*Coprinus* spp.) propagated on some of the substrates and caused contamination. Therefore, this experiment was repeated again. Among all the substrate combinations, the following combinations were more susceptible to the contamination (WS + WB + SP + RB, MS + SP + RB + WB, S + WB + SP, S + RB + WB, S + RB + SP, MS + SP + WB, BP + SP + RB, and WC + WB + RB) and subsequently were removed from the analysis.

Pleurotus eryngii mushroom production gap on different substrate compositions

The effects of various substrate and supplement combinations on mushroom production gap were assessed. The mushroom production gap of *P. eryngii* was significantly ($p \leq 0.05$) influenced by the substrate compositions (Fig. 1). Mushroom production gap varied from 9 (WS + RB treatment) to 15 days (WC + WB treatment).

Mushroom fresh weight

Mean comparisons of combined substrate and supplement factors indicated that the highest and the lowest mushroom fresh weight were attributed to BS + RB and S + RB treatments, respectively (Fig. 2). The most suitable combination for high fresh weight was BS + RB (83.49 g kg^{-1} substrate), followed by BP + RB (79.27 g kg^{-1} substrate) treatment. *P. eryngii* growth on substrates S + RB associated with the lowest fresh weight (61.44 g kg^{-1} substrate).

Moister content and dry matter content of fruit body

There were significant variations ($p \leq 0.05$) among different treatments on moisture content of the fruit body. Moisture content of the fruit body varied from 90.40 to 92.17 % for BP + RB treatment and S + SP treatment, respectively (Fig. 4). Moreover, there was a significant difference for the dry matter contents of *P. eryngii* growth in different treatments (Fig. 3). The mushrooms growth on WC + WB treatment had significantly higher dry matter content (210.85 g) than that of S + WB treatment (59.11 g) (Fig. 3).

Protein content of fruit body

The most suitable substrate and complement combination in terms of high protein content in fruit body belonged to WS + WB + SP treatment (13.66 %) while the lowest protein content (4.64 %) attributed in both WC + SP + RB and BS + WB treatments (Table 3) (Fig. 4).

Discussion

The mushroom cultivation should be carried out in a carefully controlled biological system. However, contamination with microorganisms, which are in ways, is inevitable. Only a very small amount of the total agricultural

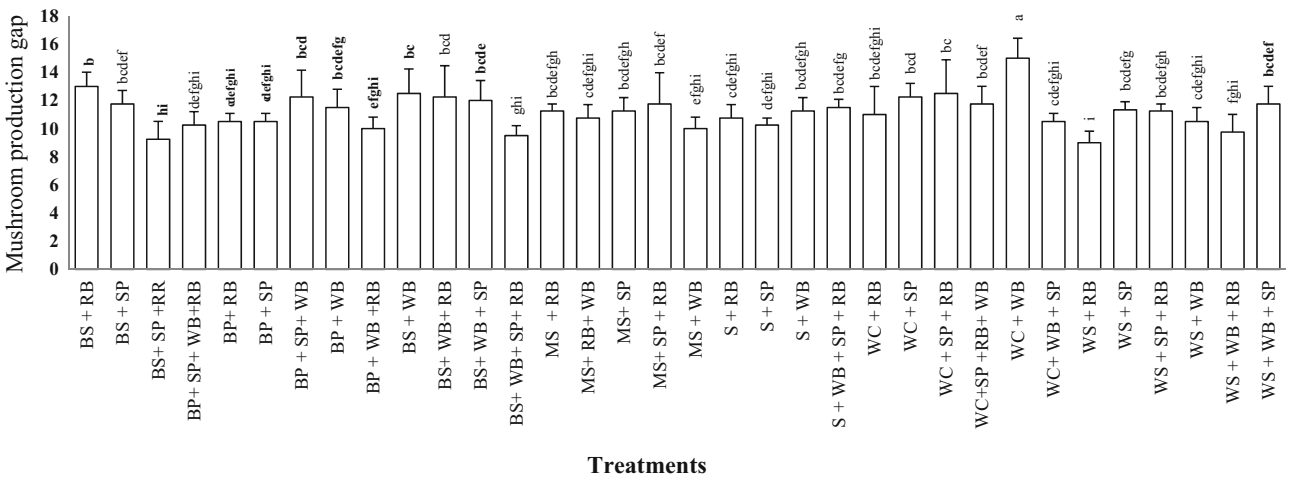

Fig. 1 Mushroom production gap of *P. eryngii* on wheat straw (*WS*), barley straw (*BS*), maize stem residue (*MS*), sawdust (*S*), wood chips (*WC*), sugar beet pulp (*BP*) as substrates supplement with wheat bran (*WB*), rice bran (*RB*), and Soybean powder (*SP*). *Bars* with *different letters* for each substrate are significantly different at $p \leq 0.05$ by Tukey test

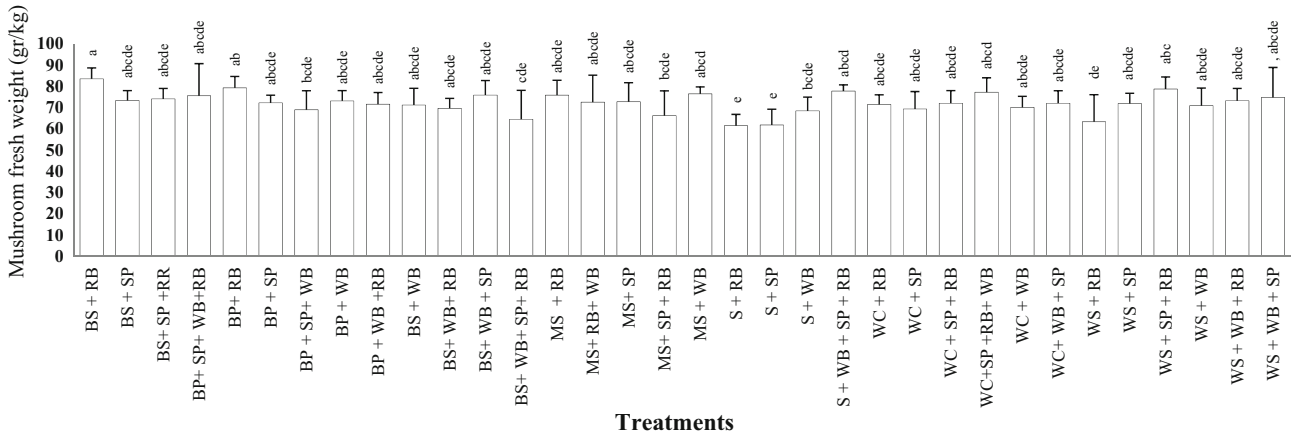

Fig. 2 Mushroom fresh weight of *P. eryngii* on wheat straw (*WS*), barley straw (*BS*), maize stem residue (*MS*), sawdust (*S*), wood chips (*WC*), sugar beet pulp (*BP*) as substrates supplement with wheat bran (*WB*), rice bran (*RB*), and soybean powder (*SP*). *Bars* with *different letters* for each substrate are significantly different at $p \leq 0.05$ by Tukey test

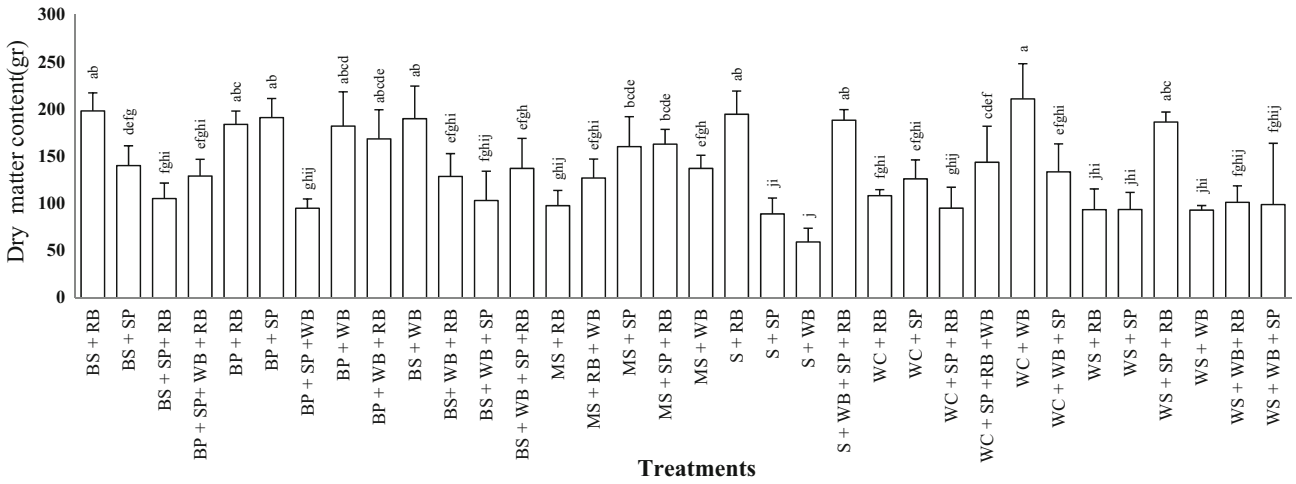

Fig. 3 Dry matter content of *P. eryngii* on wheat straw (*WS*), barley straw (*BS*), maize stem residue (*MS*), sawdust (*S*), wood chips (*WC*), sugar beet pulp (*BP*) as substrates supplement with wheat bran (*WB*), rice bran (*RB*), and soybean powder (*SP*). *Bars* with *different letters* for each substrate are significantly different at $p \leq 0.05$ by Tukey test

produced waste is utilized for useful applications while their large volumes are disposed causing environmental pollution. Substrates complemented with organic and/or inorganic substances often boost mushroom production (Renganathan et al. 2008). In this study, we estimated the mushroom production gap, fresh weight, moisture, dry matter and protein content of fruit body in *P. eryngii* mushroom on different substrate compositions. Unfortunately, the first run of experiment was totally associated with a massive bacterial contamination. We tried to reproduce the study with all combined factors. However, because of the above-mentioned inevitable contamination sources, some treatments were excluded from the analysis. We could not analysis the contaminated units in terms of ammonia content to assess any association with the disease incidence. Therefore, we would suggest conducting this assay for further analyses.

The comparison of substrate and supplement combinations indicated that supplementation of wheat bran in wood chips increased the mushroom production gap and dry matter content compared to that of the other treatments. In agreement with Mandeel et al. (2005), however, application of wheat bran with high protein and low C/N ratio caused a longer mushroom production gap. The wood chips from non-pretreated conifer wood are not always readily colonized due to the presence of inhibitory components (Croan 2004). This might indicate a very low disintegration rate of substrate compositions for the release of nutrients within this long period of 15 days. Therefore, nutrients in the supplements may have not been released and so not

Table 3 Nitrogen and protein content of mushroom fruiting body

Treatment	Nitrogen content of substrate (%)	Nitrogen content of fruit body (%)	Protein (%)[a]
BP + RB	0.59	1.57	6.87
WS + RB	0.93	1.54	6.74
BP + WB	0.78	1.87	8.19
WC + RB	0.35	1.29	5.65
MS + WB	1.61	2.26	9.89
MS + RB	0.67	1.34	5.86
WS + SP	0.56	2.09	9.15
BS + RB	1.14	2.00	8.76
BS + WB	0.67	1.06	4.64
WC + WB	0.51	1.65	7.22
WC + SP	0.46	1.92	8.40
S + WB	0.62	1.6	7.00
WS + WB	0.58	1.76	7.70
BS + SP	1.52	2.70	11.82
BP + SP	0.89	1.83	8.01
S + RB	0.60	1.57	6.87
MS + SP	1.43	2.34	10.24
S + SP	0.64	1.73	7.57
BP + WB + RB	0.62	1.40	6.13
WC + SP + RB	0.45	1.06	4.64
WS + SP + RB	1.29	3.09	13.53
WS + WB + RB	0.46	2.10	9.19
BS + SP + RB	0.39	1.85	8.10
WC + WB + SP	0.39	1.84	8.05
MS + SP + RB	0.62	1.83	8.01
MS + RB + WB	0.57	1.62	7.09
WS + WB + SP	1.54	3.12	13.66
BP + SP + WB	0.96	1.96	8.58
BS + WB + SP	0.67	1.84	8.05
BS + RB + WB	0.43	1.51	6.61
WC + SP + RB + WB	0.59	1.76	7.70
BP + SP + WB + RB	0.98	1.68	7.35
BS + WB + SP + RB	1.69	1.44	6.30
S + WB + SP + RB	0.87	1.35	5.91

[a] N × 4.38

WS wheat straw, *BS* barley straw, *MS* maize stem residue, *S* sawdust, *WC* wood chips, *BP* sugar beet pulp, *WB* wheat bran, *RB* rice bran, and *SP* soybean powder

accessible to the mycelium and hence had no influence on the initial growth of the mycelium. Philippoussis et al. (2003) showed that the mycelial growth rate is related to the bioavailability of nitrogen and that the formulation of the substrate influences nutritional levels and porosity.

The mixture of sawdust and wheat bran led to the lowest dry matter content with the relatively short mushroom production gap among all substrate combinations. Jonathan (2002) also suggested that mushroom mycelia grow very well, provided that there are good substrates with required nutrients and optimal physical factors (temperature, pH and relative humidity). The low mushroom production gap on wheat straw may be due to the inability of *P. eryngii* mushroom to produce hydrolyzing enzyme, which converts the wheat straw to utilizable amino acids, nitrogen, and carbon compounds necessary for the growth. Carbon is readily available from cellulose, hemicelluloses, and lignin of straws, but nitrogen mainly presents in a bound form which is not available until it is enzymatically released. The main function of wheat straw is to provide a reservoir of cellulose, hemicelluloses, and lignin, which is utilized during the growth of 'spawn' and during fruit body formation. Environmental conditions, type of lignocellulosic substances, as well as supplementation of substrates with various complement food including nitrogen and carbon sources have been reported to be important for the improvement of the growth period and the mushroom quality (Panjabrao et al. 2007; Onyango et al. 2011). Our results also indicated the highest mushroom fresh weigh was observed on barley straw substrate complemented with the rice bran. The result obtained for mushroom fresh weight was expectable since the fruit body yield is largely dependent on the performance of the fungus at mycelial stage and primordia formation (Baysal et al. 2003).

A significant difference was detected in the protein contents of mushrooms grown on different substrate compositions. The highest nitrogen content (3.12 %) and the highest protein content (13.66 %) in fruit body were found in wheat straw substrate complemented with wheat bran and soybean powder. This indicates that soybean powder provided a good source of protein for the mushroom growth. The protein content of mushrooms depends on several factors, such as substrate chemical composition, pileus size, cultivation time and strain, as well as the type and amount of additive nutrients in the substrate (Bernaś et al. 2006). This result may be due to the biological and chemical differences and C/N ratio of substrates (Ragunathan and Swaminathan 2003). In addition, complements and vitamins can influence on the mushroom growth and addition of these supplements aims mainly to increase the levels of nitrogen and carbohydrates available.

In the present study, some treatments were missed due to the pollution in substrate such as ink cap growth. Although applying a pasteurization step assumes that the material used was not already heavily contaminated with microorganisms before it was pasteurized, the pasteurization condition can be revised in further studies. Ink cap (*Coprinus* spp.) fungi appeared on the substrate before mushroom fruiting. *Coprinus* indicates the presence of free ammonia or conversely a high content of nitrogen in the compost. On the other hand, the appearance of ink cap

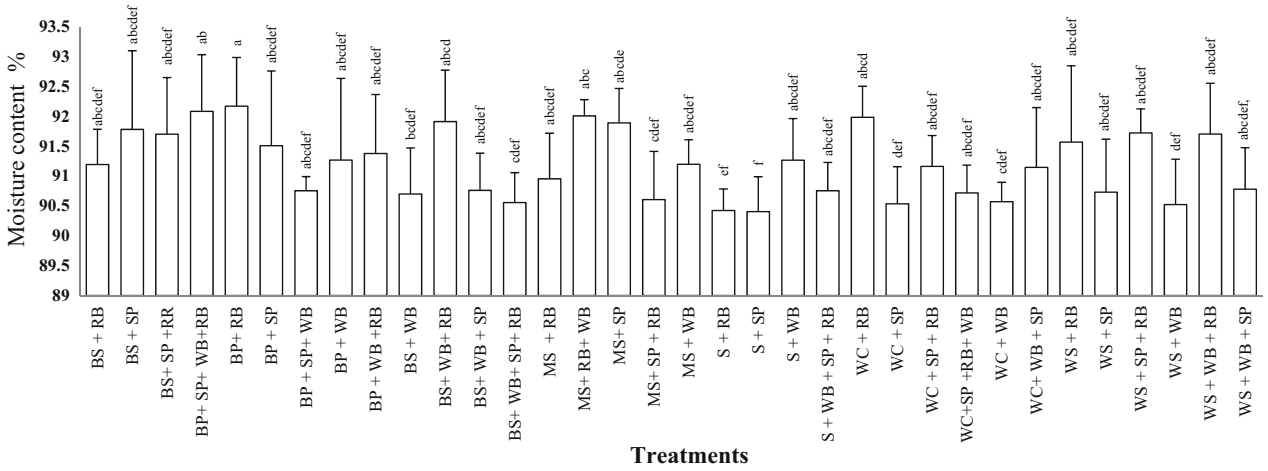

Fig. 4 Moisture content of *P. eryngii* on wheat straw (*WS*), barley straw (*BS*), maize stem residue (*MS*), sawdust (*S*), wood chips (*WC*), sugar beet pulp (*BP*) as substrates supplement with wheat bran (*WB*), rice bran (*RB*), and soybean powder (*SP*). *Bars* with *different letters* for each substrate are significantly different at $p \leq 0.05$ by Tukey test

fungi in the growing room indicates the low quality of substrate due to the disturbance of the fermentation and the substrate's pasteurization process (Sharma et al. 2007). Sterilized substrate production and containers and use of plant products could considerably minimize the nematode damage to mushrooms and maximize the yields. Therefore, the need for substrate supplementation to complement is necessary to afford the nutrient requirement of the fungi in terms of both organic (carbon and nitrogen) and inorganic components.

Conclusions

In this study, we assessed different substrates and supplement combinations on mushroom production gap, dry matter content, mushroom fresh weight, total protein content and moisture content of *P. eryngii* fruit body. Supplement combinations and substrate type are beneficial for mushroom production because of enhancing the physical and enzymatical accessibility of the ingredients for fungus growth and development. This study booted this idea that utilization of some agricultural waste for the production of cultured mushroom can solve one of the most environment problems and provide a human nutritious food source such as mushrooms.

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