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ORIGINAL PAPER

Evaluation of Korshinsk Peashrub (*Caragana korshinskii* Kom.) as a Substrate for the Cultivation of *Pleurotus eryngii*

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Abstract

The cultivation of *Pleurotus eryngii* is increasing rapidly in China due to its nutritional and medicinal importance, excellent flavor, and long shelf life; therefore, cheaper and locally available alternative substrates are urgently needed. Experiments were performed to investigate the use of alternative substrates for *P. eryngii* cultivation. Korshinsk peashrub (*Caragana korshinskii* Kom.), a perennial shrub, was included in the substrate at varying rates to substitute for the sawdust and sugarcane bagasse (21/38 and 21/35%, respectively) in the typical substrate. The cultivation substrate including 38% Korshinsk peashrub did not significantly affect linear mycelial growth. The fruit body yield (247.3 g/bag) and biological efficiency (70.66%) achieved by using this substrate were significantly higher than those achieved using the control substrate (229.6 g/bag and 65.59%). Crude polysaccharide content was highest (6.12%) in the mushroom grown on 38% Korshinsk peashrub substrate; in this mushroom, crude polysaccharide content was increased by 70.47% compared with that of the mushroom grown on the control substrate (3.59%). These results reveal that supplementing the substrate in which *P. eryngii* is grown with Korshinsk peashrub can improve polysaccharose accumulation by *P. eryngii*. The findings described above reveal that Korshinsk peashrub is an efficient, cost-effective, and promising substrate additive that can improve *P. eryngii* quality and yield while largely substituting for sawdust and sugarcane bagasse.

Keywords *Pleurotus eryngii* · Korshinsk peashrub · Substrate · Biological efficiency · Nutritional value

Introduction

Pleurotus eryngii, also known as the king oyster mushroom for its remarkable flavor, high nutritional value and numerous medicinal features, is cultivated and consumed worldwide [1, 2]. This mushroom was originally cultivated in northern Italy and Switzerland, where it is known locally as cardoncello [3]. *P. eryngii* has recently become the most commonly cultivated mushroom in China, Korea, and Japan [4]. Production of *P. eryngii* in China was estimated at 1,364,835 tons in 2015 (data from the China Edible Fungi Association). Current demand for *P. eryngii* indicates that production will continue to increase rapidly for the foreseeable future.

P. eryngii can be cultivated on a wide variety of substrates containing sawdust, cotton seed hulls, soybean meal, wheat bran [5], sugarcane bagasse [6], chopped rice straw [7], umbrella plant [3], rice husks, corn stover, wheat straw, peanut meal, and other materials [8–10]. In China, the substrate used for commercial production of *P. eryngii* consists mainly of sawdust and sugarcane bagasse supplemented with wheat bran and other materials. However, as the prices of sawdust and sugarcane bagasse have increased or fluctuated substantially with the rapid expansion of commercial production of mushrooms, increasing substrate cost has become a major concern for commercial mushroom producers. Now, potential shortages of sawdust and sugarcane bagasse have highlighted the need to identify alternatives substrates that may be used for sustainable cultivation of *P. eryngii* in the future.

Korshinsk peashrub (*Caragana korshinskii* Kom.), a drought-tolerant perennial mesquite shrub, is widely distributed in northeastern, northwestern and northern China [11, 12]. Korshinsk peashrubs generally live for decades, and some may grow for more than a century. In the year of 2004, there are 7.73 million hm^2 of Korshinsk peashrub in the Inner Mongolia and Ningxia Hui Autonomous Region

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[13, 14]. The ability of Korshinsk peashrub to branch and regenerate is considerable, and its branches must be chopped every 4–5 years. If the rejuvenation cycle is calculated in 5 years, 1.55 million hm^2 of Korshinsk peashrub is chopped every year. The 3–4 tons of branches can be produced per hm^2, and 550,000 tons of branches will be produced [13]. Korshinsk peashrub grows vigorously when its branches are chopped. Therefore, because Korshinsk peashrub is an abundant resource that is easy to obtain, harvested branches should be fully utilized to avoid wasting significant energy and protect the environment during economic development. Every year, Korshinsk peashrub produces a large number of new branches that are rich in crude protein, crude fiber and minerals [11]. This lignocellulosic waste is one of the largest global carbon sources considered to be a potential substrate for the production of mushrooms, and it has been used to cultivate *Pleurotus ostreatus, Flammulina velutipe*s [15], *Pleurotus nebrodensis* var. *tuoliensis* [16] and *Pleurotus citrinipileatus* [14]; however, *P. eryngii* cultivation on substrates containing Korshinsk peashrub has not been evaluated comprehensively.

The aim of this study was to assess the possibility of using Korshinsk peashrub as a new supplement for substrate mixtures used to produce *P. eryngii*. The influence of the raw material composition of the substrate on the nutritional value of the fruit bodies produced by *P. eryngii* was also investigated.

Materials and Methods

Pleurotus eryngii Strain

Pleurotus eryngii (CCMSSC 003898) was provided by the China Center for Mushroom Spawn Standards and Control, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences. For all experiments, mycelia were grown on potato-dextrose agar (PDA) medium at 25 °C in the dark for 7 days before use.

Preparation of Korshinsk Peashrub Powder

The Korshinsk peashrub used in this study was provided by Ningxia Hui Autonomous Region Yanchi County Yuanfeng Grass Industry Co., Ltd. (Yanchi, China). The Korshinsk peashrub branches were chopped into small pieces (1–2 cm) with pruning shears and ground with a grinding machine to pass through an inner screen with 2 cm holes [15].

Proximate Carbon Analysis of Korshinsk peashrub

The total carbon content of Korshinsk Peashrub was determined by the potassium bichromate dilution-heat method (carried out in a boiling water bath to increase the oxidation rate of organic carbon). Total nitrogen content was determined by the standard Kjeldahl procedure. Determinations of cellulose, hemicellulose and lignin content were performed following the procedures of Goering and Van Soest [17].

Substrate Preparation

The compositions of the substrates used in this study are provided in Table 1. The control substrate formula comprises sawdust 21% and sugarcane bagasse 21%. For substrate T1 and T2, sawdust was replaced with Korshinsk peashrub (38 and 21%, respectively). For substrate T3 and T4, sugarcane bagasse was replaced with Korshinsk peashrub (35 and 21%, respectively). In all formulas, cottonseed hull 4.2%, ground corncobs 18.4%, maize powder 6.8%, CaO 0.8% and $CaSO_4$ 1% were comprised. In addition, wheat bran and soybean meal were added to adjust nitrogen content in formulas, wherein, the nitrogen content of substrates in T1, T3 and CK is 1.63%, while that of substrates in T2 and T4 is 2%. The preparation steps were as follows. The dry ingredients were mixed thoroughly, after which tap water was added to the mixture to reach a moisture content of 65% [18]. The final moisture content was determined in triplicate using the oven-dry method. The moistened substrate (1000 g) was packed into polypropylene bags (17 cm in width and

Table 1 Ingredient composition of substrates with different inclusion rates for *P. eryngii* cultivation (dry matter) (g 100 g^{-1})

Substrate	N	Sawdust	Sugarcane bagasse	Korshinsk peashrub powder	Cottonseed hull	Ground corncobs	Wheat bran	Maize powder	Soybean meal	CaO	$CaSO_4$
CK	1.63	21	21	–	4.2	18.4	18.4	6.8	8.4	0.8	1
T1	1.63	–	21	38	4.2	18.4	8.3	6.8	1.5	0.8	1
T2	2.00	–	21	21	4.2	18.4	18.4	6.8	8.4	0.8	1
T3	1.63	21	–	35	4.2	18.4	11.3	6.8	1.5	0.8	1
T4	2.00	21	–	21	4.2	18.4	18.4	6.8	8.4	0.8	1

35 cm in length) that is, the dry weight of substrate loaded in each bag is 350 g. A single vertical hole (2 cm in diameter and 18 cm in depth) was made in the center of the bag for the spawn inoculum and aeration. The bags were sealed with plastic rings and vent caps, after which they were autoclaved at 121 °C for 90 min. Sawdust was subjected to weathering and composting in open air for 6 months before it was incorporated into the substrates. All other ingredients, including Korshinsk peashrub, were mixed into the substrate fresh without any pretreatment. Carbon (C) and nitrogen (N) content were estimated by loss of ignition and the Kjeldahl method, respectively. The C/N ratio is the ratio of total carbon content in substrate to total nitrogen content in substrate, and it was set for each substrate based on a previous study [10].

Growth Rate Test

The growth rate of mycelia in different substrate combinations was assessed by the linear growth assay as described previously [19]. Different substrate combinations were separately packed into 320 mm long (30 mm diameter) glass tubes to a density of approximately 0.8 g cm^{-3}. The substrate was then inoculated into one end of the tube using a 5 mm diameter mycelia disk. After inoculation, both ends of the tubes were plugged with sterile cotton plugs and incubated at 22 ± 1 °C in the dark for 7 days. Mycelial growth was measured in mm. The growth rate of each group was calculated after 7 days of incubation. Growth speed was measured in terms of apparent spawn running time (the number of days required for mycelia to fully colonize the substrate), which was calculated over the entire growth period, with no correction for the length of the lag period. The spawn running time was determined for 5 replicates.

Spawn and Fruiting

Cultivation of king oyster mushrooms was carried out following the methods reported by Zhang et al. [20] with some modifications. After the substrate was fully covered with mycelia, the bags were transferred to mushroom house. The environmental conditions were set as temperature at 16 ± 2 °C, a 12 photoperiod (1500–2000 lx), relative air humidity at 80–90%, and CO_2 concentration at 3000 ppm. Growth parameters including stipe length (cm), stipe diameter (cm) and pileus diameter (cm) were recorded with a slide caliper before each harvest. Only a single flush of fruit bodies was formed, harvested, and assessed in this study, which was similar to the commercial cultural practices for *P. eryngii* in China. Fructification lasted 18–20 days. The fresh weights of fruit body were determined for 15 replicates, and biological efficiency (BE, %) was calculated by dividing fresh weight of fruit body with dry substrate per bag [5].

Chemical Analysis

Fruit bodies of *P. eryngii* were collected randomly from the three different treatment groups in equal proportions after the first flush and dried in an oven at 60 °C to a constant weight, after which they were kept at 4 °C. The obtained powder was used for proximate analysis i.e. total proteins (%), fats (%) and ash (%) estimated by following standard procedure [21]. The total soluble sugar content of the samples was determined by the sulfuric acid-anthrone colorimetric method [22]. The crude protein content ($N \times 4.38$) of the samples was estimated by the macro Kjeldahl method [23]. The crude fat content of the samples was determined by extracting a known weight of powdered sample with petroleum ether using a Soxhlet apparatus. The ash content of the samples was determined by incineration at 600 ± 15 °C. These analyses were performed by the PONY Testing International Group (Beijing, China).

Statistical Analysis

The original data were processed using Microsoft Excel (Microsoft Inc., Redmond, WA, USA). Differences among the means of groups were assessed using Duncan's multiple range tests at 95% confidence level ($p < 0.05$). Statistical analyses were conducted using SPSS 19.0 (IBM Inc., Armonk, NY, USA).

Results and Discussion

Composition of Korshinsk Peashrub Powder

The Korshinsk peashrub branches had high hemicellulose content, with 16.63% cellulose, 26.43% hemicellulose and 11.20% lignin, confirming it as an ideal carbon source for *Pleurotus* production in terms of chemical composition. The lignocellulosic content of Korshinsk peashrub branches was lower than that of sawdust (42.12% cellulose, 34.55% hemicellulose and 15.12% lignin) and sugarcane bagasse (27.58% cellulose, 36.87% hemicellulose and 14.90% lignin). In the past, successful use of composted substrates including Burma reed for *P. eryngii* cultivation has been reported, and Burma reed is high lignocellulose content, with 40.22% cellulose, 16.63% hemicellulose and 10.86% lignin [1]. Moreover, Korshinsk peashrub branches had a total carbon content of $39.40 \pm 0.18\%$ and total nitrogen content of $2.45 \pm 0.05\%$; therefore, the C/N ratio of the branches was lower than that of sawdust and sugarcane bagasse (Table 2).

Table 2 Analysis of lignocellulosic waste materials used for *P. eryngii* cultivation (dry matter)

Material	C (%)	N (%)	C/N	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Sugarcane bagasse	44.54	0.33	134.97	27.58	36.87	14.90
Sawdust	46.68	0.39	119.69	42.12	34.55	15.12
Korshinsk peashrub	39.40	2.45	16.08	16.63	26.43	11.20

Table 3 Mycelial growth rate of *P. eryngii* cultivated in different substrates

Substrate combinations	Growth rate (mm day^{-1})
CK	4.03 ± 0.17^a
T1	3.94 ± 0.21^a
T2	3.98 ± 0.31^a
T3	3.45 ± 0.25^b
T4	3.51 ± 0.06^b

Different lowercase letters mean significant differences in each column ($p < 0.05$)

CK treatment with sawdust 21% and sugarcane bagasse 21%, *T1* treatment with Korshinsk peashrub 38% and sugarcane bagasse 21%, *T2* treatment with Korshinsk peashrub 21% and sugarcane bagasse 21%, *T3* treatment with sawdust 21% and Korshinsk peashrub 35%, *T4* treatment with sawdust 21% and Korshinsk peashrub 21%

Growth Rate of Mycelia

As shown in Table 3, the mycelial growth rates on the Korshinsk peashrub replacement sawdust substrates (T1 and T2) were 3.94 ± 0.21 and 3.98 ± 0.31 mm day^{-1}, respectively; these rates were not significantly different from that achieved on the control substrate (4.03 ± 0.17 mm day^{-1}). However, the mycelial growth rates on the Korshinsk peashrub replacement sugarcane bagasse substrates (T3 and T4) were 3.45 ± 0.25 and 3.51 ± 0.06 mm day^{-1}, respectively, which were significantly decreased compared with that achieved on the control substrate. It has been reported that the ability of *Pleurotus* species to use different carbon sources may be an expression of the physiological differences among the species [24, 25]. Philippoussis et al. showed that the mycelial growth rate is related to the bioavailability of nitrogen, and the formulation of the substrate simultaneously influences nutritional levels and porosity [26]. In addition, the nitrogen of lignocellulosic substrates is available only when it is enzymatically released, which is utilized during the development of edible fungi. Hence the main function of lignocellulosic substrates is to provide a reservoir of cellulose, hemicelluloses, and lignin [1]. This study showed that the mycelial growth rate was high when sugarcane bagasse was used in the substrate, but it remained lower than the rate achieved when sugarcane bagasse was not added to the substrates, indicating that the growth rate of *P. eryngii* mycelia was not affected by replacing the sawdust in typical substrate with Korshinsk peashrub powder. Based on a previous study, the main function of Korshinsk peashrub might be to provide a reservoir of cellulose, hemicelluloses, and lignin, which is utilized during the growth of spawn and fruit body formation.

Characteristics of *P. eryngii* Fruit Bodies on Different Substrates

The typical commercial cultivar was cultivated on five different media and evaluated for quality, weight, morphological characteristics, and cultivation properties (Table 4). The *P. eryngii* cultivated on the replaced with Korshinsk peashrub substrates (T1, T3 and T4) had the growth periods of 69.3, 72.1, 69.4, and 67.9 days, respectively, which are significantly different from the days required by control group (71.9 days). The *P. eryngii* cultivated on the replaced with Korshinsk peashrub substrates (T1, T3 and T4) resulted in a decrease in time to production of 2.5–4.0 days, The fruit body yields on substrates T1 and T4 were 247.3 and 206.2 g/bag, respectively, whereas the biological efficiencies of substrates T1 and T4 were 70.66 and 58.91%, respectively. Both of these measurements were significantly different from those of fruit bodies grown on the control substrate (229.6 g/bag and 65.59%). The fruit body yields on substrates T2 and T3 were 224.5 and 224.7 g/bag, respectively, whereas the biological efficiencies on substrates T2 and T3 were 64.16 and 64.19%, respectively; both of these measurements were not significantly different from those of fruit bodies grown on the control substrate. The morphological characteristics including stipe length (cm), stipe diameter (cm) and pileus diameter (cm). As shown in Fig. 1 and Table 4, the morphological characteristics of *P. eryngii* fruit bodies grown on different substrates were significantly different. The pileus diameter ranged from 4.7 to 8.0 cm, moreover significant differences were found. The lengths of fruit bodies harvested from treatment groups with Korshinsk peashrub are longer than that from control group, but only T1, T2 and T3 groups have significantly longer length, showing the addition of Korshinsk peashrubs are beneficial for the extension of fruit body. However, the thickness of

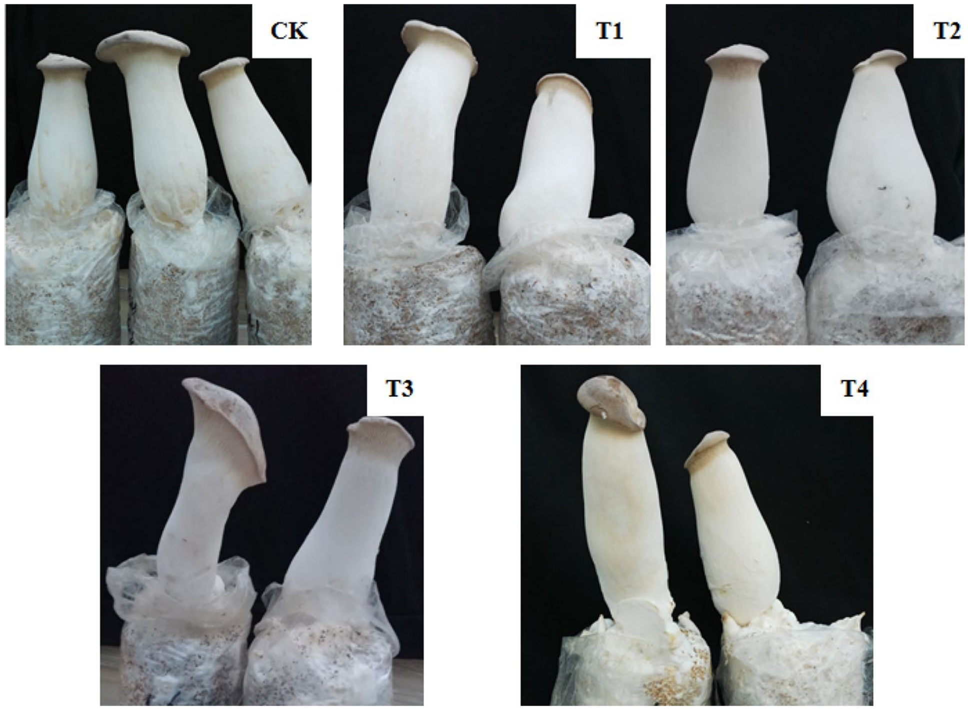

Fig. 1 Morphological characteristics of *P. eryngii* fruit bodies from mycelia grown on different substrates. *CK* treatment with sawdust 21% and sugarcane bagasse 21%, *T1* treatment with Korshinsk peashrub 38% and sugarcane bagasse 21%, *T2* treatment with Korshinsk peashrub 21% and sugarcane bagasse 21%, *T3* treatment with sawdust 21% and Korshinsk peashrub 35%, *T4* treatment with sawdust 21% and Korshinsk peashrub 21%

Table 4 Morphological and fruit characteristics of *P. eryngii* cultivated in different substrates

Substrate	Days to production (day)	Fruit body yield (g/bag)	BE (%)	Length of fruit body (cm)	Thickness of stipe (cm)	Diameter of pileus (cm)
CK	71.9 ± 2.6^{c}	229.6 ± 22.7^{b}	65.59 ± 6.47^{b}	12.8 ± 0.5^{a}	5.9 ± 0.4^{cd}	5.8 ± 1.1^{ab}
T1	69.3 ± 2.3^{b}	247.3 ± 16.9^{c}	70.66 ± 4.84^{c}	13.6 ± 1.2^{b}	4.4 ± 0.4^{a}	5.5 ± 0.8^{a}
T2	72.1 ± 2.4^{c}	224.7 ± 12.5^{b}	64.19 ± 3.58^{b}	14.6 ± 0.7^{c}	6.1 ± 0.4^{d}	5.5 ± 0.3^{a}
T3	69.4 ± 1.9^{b}	224.5 ± 15.1^{b}	64.16 ± 4.32^{b}	14.4 ± 0.9^{c}	4.7 ± 0.3^{b}	5.7 ± 0.6^{ab}
T4	67.9 ± 2.7^{a}	206.2 ± 14.3^{a}	58.91 ± 4.10^{a}	13.3 ± 0.4^{ab}	5.7 ± 0.4^{c}	6.2 ± 0.8^{b}

Different lowercase letters mean significant differences in each column ($p < 0.05$)

CK treatment with sawdust 21% and sugarcane bagasse 21%, *T1* treatment with Korshinsk peashrub 38% and sugarcane bagasse 21%, *T2* treatment with Korshinsk peashrub 21% and sugarcane bagasse 21%, *T3* treatment with sawdust 21% and Korshinsk peashrub 35%, *T4* treatment with sawdust 21% and Korshinsk peashrub 21%

stipes (i.e. the diameter of stipe) collected from T1 and T3 groups with 38 and 35% Korshinsk peashrub are significant lower than that from T2, T4 and control group, showing the percent of Korshinsk peashrub in substrate being excessive will influence the robust of stipe. Quality was evaluated based on the combination of shape, weight and color, so it is likely to be a good criterion for evaluating mushrooms. The findings mentioned above suggest that, in terms of time to production, BE, pileus diameter, stipe diameter and length, *P. eryngii* grows best on a typical substrate with 38% of the sawdust replaced with Korshinsk peashrub.

The nutritional value of *P. eryngii* cultivated on different substrates is shown in Table 5. The samples grown on 38 and 35% Korshinsk peashrub substrates (T1 and T3) had similar fat, ash, protein and polysaccharose contents. Crude polysaccharide content was highest (6.12%) in the

Table 5 Nutritional value of *P. eryngii* (g 100 g^{-1}*, mean ± SD, n = 3)

Substrate	Ash	Fat	Protein	Polysaccharide
CK	5.10 ± 0.07^{a}	1.87 ± 0.01^{d}	20.75 ± 0.01^{d}	3.59 ± 0.02^{a}
T1	3.95 ± 0.06^{d}	1.41 ± 0.01^{b}	15.11 ± 0.01^{a}	6.12 ± 0.03^{e}
T2	4.36 ± 0.03^{c}	1.77 ± 0.01^{c}	19.07 ± 0.01^{c}	5.30 ± 0.05^{c}
T3	4.04 ± 0.08^{d}	1.33 ± 0.01^{a}	15.88 ± 0.02^{b}	6.04 ± 0.02^{d}
T4	4.78 ± 0.07^{b}	2.11 ± 0.01^{e}	23.72 ± 0.02^{e}	3.66 ± 0.00^{b}

Different lowercase letters mean significant differences in each column ($p < 0.05$)

CK treatment with sawdust 21% and sugarcane bagasse 21%, *T1* treatment with Korshinsk peashrub 38% and sugarcane bagasse 21%, *T2* treatment with Korshinsk peashrub 21% and sugarcane bagasse 21%, *T3* treatment with sawdust 21% and Korshinsk peashrub 35%, *T4* treatment with sawdust 21% and Korshinsk peashrub 21%

*Dry matter

samples grown on substrate T1 (38% Korshinsk peashrub substrate lacking sawdust); it was increased by 70.47% compared with that of the control substrate (3.59%). According to Krystyna [27], the content of polysaccharides is different depending on the growth substrate. It is possible that supplementation with a high concentration of Korshinsk peashrub powder improved polysaccharose accumulation in *P. eryngii* compared with that of mushrooms grown on the control substrate, while reducing ash, fat and protein accumulation. The sample grown on substrate T2 (21% Korshinsk peashrub substrate lacking sawdust) had polysaccharose content higher than that of the control sample, as well as lower ash, fat and protein contents. No significant differences were found between the fat and protein levels of the samples obtained using substrate T4 (21% Korshinsk peashrub substrate lacking sugarcane bagasse) and those of the control sample, but the sample from substrate T4 had ash and polysaccharose contents lower than those of the control sample. In a previous study, the fruit bodies of a closely related species, *Pleurotus ostreatus*, showed high carbohydrate [25]. Overall, the results described above show that Korshinsk peashrub can significantly improve polysaccharose accumulation by *P. eryngii*. Many recent studies of mushroom chemical content analyses have reported that the nutritive value of cultivated mushrooms can be affected by cultivation in different substrates [9, 28]. The high polysaccharose content found in all samples in this study indicates that Korshinsk peashrub substrates can be used to produce high-quality *P. eryngii* to meet consumer demand.

Conclusion

We assessed the effects of different substrates on the growth period, fruit body weight, yield, BE and nutritional value of edible mushrooms *P. eryngii*. Our results show that supplementing the typical substrate with Korshinsk peashrub improved total yield, fresh weight and BE by influencing protein, fat, ash and polysaccharide accumulation. Our findings show that Korshinsk peashrub can supply nutrients to mushrooms to improve growth and yield; therefore, production of *P. eryngii* on Korshinsk peashrub substrate may offer commercial producers an alternative basal ingredient to mitigate the impact of diminishing supplies of sawdust and sugarcane bagasse, thus allowing them to cope with the decreasing availability and rising prices of substrate components. Nevertheless, different combinations and mixing ratios of Korshinsk peashrub, sawdust/sugarcane bagasse, and supplements should be investigated in more detail to discover the most effective methods of optimizing mushroom yield, increasing BE, and improving fruit body characteristics.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

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