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Research Article

Cultivation of nutrient rich Oyster mushroom (*Pleurotus ostreatus*) from the agricultural wastes

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Abstract
The aim of the research was to produce nutritional rich mushroom using agricultural waste as substrate. The usage of these agricultural waste into useful products is a great challenge in agricultural waste management. Two different substrate such as rice straw and banana pseudostem and their combinations were used as substrate for the cultivation of oyster mushroom and its impact on growth and yield of mushroom was analyzed. The result of the study revealed that the type and chemical composition of substrate such as C, N, C/N ratio has significant effect on the yield and growth of mushroom. It is evident from the study that banana pseudostem substrate produces better yield and quality mushrooms. The proximate and phytochemical composition was also found to be high in mushroom grown in banana pseudostem waste. The study also suggests that agricultural wastes can be well managed by using it for mushroom cultivation.

Keywords: *Mushroom, rice straw, banana pseudostem, Oyster mushroom, spawn.*

1. Introduction

Mushrooms belong to the family fungi and it is used as food since ancient times (Rahi *et al.*, 2004). They are one of the excellent sources of protein. They are heterotrophic organisms and so it needs external nutrients to grow. Mushrooms grow using the nutrients from the substrate and also produces a number of enzymes to facilitate the degradation of substrates such as laccases, lignin per- oxidases, manganese peroxidases, aryl alcohol oxidase, aryl-alcohol dehydrogenases hemicellulose and cellulases etc. (Kabel *et al.*, 2017; Vos *et al.*, 2017). Nutritional properties of mushrooms are well applied in the fields of medicine and therapeutics such as, anti-tumor, antibiotic, antifungal, antiviral, anti-inflammatory effects, antioxidant, antihypertensive, antiplatelet-aggregating and antihyperglycemic effects (Anusiysa, 2021).

Oyster mushrooms are consumed for their medicinal and nutritional values. They are used traditionally as medicine for a wide variety of diseases. Oyster mushroom can be grown on a wide variety of substrate. However, the yield and the quality of oyster mushroom depends on the chemical and nutritional content of substrates (Badu *et al.*, 2011; Patil *et al.*, 2010). The major nutrients that promote the growth of mushroom are more carbon and less nitrogen. The materials containing cellulose, hemicellulose, lignin rice and wheat straw, cotton seed hulls, sawdust, wastepaper, leaves, and sugarcane residue can be used as mushroom substrates (Chang, *et al.*, 1989).

Banana pseudostem is a bulk agricultural waste which is widely cultivated in South India. Rice straw is a common agricultural waste and is widely applied in several sectors as feed for cattle and also it is a widely used and a well-known substrate for mushroom production. Mushroom cultivation using agricultural waste is one of the most ecofriendly methods to reduce pollution. In this regard a study was conducted to produce nutritionally rich oyster mushroom from agricultural waste. In this study, a comparative study was performed using different substrates such as rice straw, banana pseudostem and the combination of both to produce Oyster mushrooms. Therefore, the pollution arising from such agricultural wastes can also be well managed.

2. Materials & Methods

2.1 Collection of mushroom spawn

The oyster mushroom spawns were procured from Kerala Agricultural University, Thiruvananthapuram, India.

2.2 Mushroom spawning in different substrate

The different substrates (i) rice straw (RS), (ii) banana pseudostem (BPS) and (iii) the combination of rice straw and banana pseudostem (RS+BPS) were packed in a polypropelene bag with uniform substrate and spawn weight and the bags were perforated evenly for aeration and kept in a dark room at room temperature of 28±2 ^{o} C with a relative humidity. The inoculated substrates were kept in an incubation room at 28^{o} C and 60~70% relative humidity under dark condition. Water was sprayed on the bags after when the surface was entirely covered with mycelium. The total harvest time from initial harvest was recorded and each bunch weight was determined and also the cap dm was noted. The Biological efficiency (BE) was determined using the following formula (Mutetwa *et al.*, 2019).

BE % = weight of fresh fruiting body weight (g)/ dry weight of substrates (g) X 100

2.3 Phytochemical analysis

The harvested mushrooms were analyzed qualitatively for the presence of phytochemicals using standard procedures. The mushroom was dried and made into powder and extracted using the solvents such as methanol and petroleum ether. The phytochemicals such as alkaloids (Evans, 2002), carbohydrates (Harborne, 1998), glycosides (Siddiqui *et al.*, 1997), proteins (Lowry *et al.*, 1951), flavonoids (Harborne, 1973), triterpenoids (Ayoola *et al.*, 2008), phenols (Sofawora, 1993) and tannins (Trease & Evans, 1989), were analyzed qualitatively. Phytochemicals such as flavonoid (Ordonez *et al.*, 2006), phenols (Siddhuraju, 2007), and tannin were analyzed quantitatively.

2.4 Analysis of Proximate and chemical composition

Proximate composition of mushroom was analyzed using AOAC method. Substrate samples were dried at 40^{o} C in an oven to a constant weight and ground to powder samples. Total carbon (C) content was determined according to the method of Nelson and Sommers,1982 and total nitrogen (N) content was determined by the Kjehldal method after 96% H_2SO_4 hot digestion. Then the C/N ratio of each substrate was calculated. Electrolyte conductivity (EC) and pH were determined according to the methods of Cavins *et al.*, 2000 by using a pH meter.

3. Results

3.1 Effect of various substrate combination on the growth and yield of Oyster mushroom

Oyster mushrooms were cultivated in two different substrates and their combinations were analyzed to determine the growth and yield. The proximate analysis of mushrooms was determined for its nutritive value. The results of the study showed that there was a remarkable difference in the growth and yield of mushrooms grown in different substrates combinations and is shown in Table. 1. The first sprouting day varied significantly with difference in substrate. It was found that the first sprout was observed in RS+BPS substrate followed by RS. The mushroom cap dm observed to be high (111.7±0.5) in mushroom grown in RS+BPS and it was 105.2±0.9 in RS which was followed by BPS (99.4±0.7). Also, the weight of mushroom bunch was high (75.3±0.3) in RS+BPS followed by RS and BPS. It was observed that the number of effective fruiting bodies was high in mushroom grown in banana pseudostem substrate. Biological efficiency was high in RS+ BPS (88.6±0.7%) followed by BPS.

Table. 1 Effect of various substrate combination on the growth and yield of Oyster mushroom

Substrate formula	First Pin head formation	Harvesting period(day)	Cap diameter (mm)	No. of effective fruiting bodies	Mushroom weight (g/bunch)	Biological Efficiency (BE) (%)
RS	23.6±0.5	42.7±0.9	105.2±0.9	12.8±0.6	58.9±0.7	80.4±0.5
BPS	17.6±0.7	46.4±0.6	99.4±0.7	16.3±0.9	62.7±0.2	87.5±0.8
RS+ BPS	15.6±0.8	49.6±0.7	111.7±0.5	14.4±0.3	75.3±0.3	88.6±0.7

3.2 Phytochemical analysis of mushroom grown in different substrate

The harvested mushrooms were dried, powdered and extracted in petroleum ether and methanol. It was found that most of the phytochemicals were soluble in methanol than petroleum ether. The phytochemical analysis showed the presence of alkaloid, flavonoid, phenol, triterpene, glycosides, tannin, protein, carbohydrate and lipid in mushrooms grown in all the different substrate combinations and is shown in the Table .2. The result revealed that the methanolic extract of mushroom grown in BPS waste showed the presence of all the screened phytochemicals and the quantitative analysis was performed in methanolic extract. The quantitative analysis revealed that mushroom grown in BPS possess high phenol, flavonoid and tannin of 3.9±0.54 mg GAE/g, 4.7±0.84 mg/100g and 3.8±0.89 CE mg/g respectively.

Table.2 Phytochemical composition of mushroom grown in different substrate

Phytochemicals	*mRS	**pRS	*mBPS	**pBPS	*mRS+mBPS	**pRS+ **pBPS
Alkaloid	+	-	++	-	+	-
Flavonoid	+	+	+++	+	++	+
Phenols	+	+	+++	+	++	+
Triterpene	+	-	+	-	+	-
Glycosides	++	-	+++	-	+	+
Tannin	+	+	+++	+	++	+
Protein	+	+	++	+	++	-
Carbohydrate	++	-	++	+	+	+
Lipid	+	+	+++	+	++	+

*methanolic extract of mushroom, ** petroleum ether extract of mushroom

Table: 3 Quantitative analyses of Phytochemicals

Contents	MRS	MBPS	MRS+MBPS
Phenol (mg GAE/g)	3.4±.02	3.9±0.54	3.5±0.21
Flavonoid (mg/100g)	2.1±0.15	4.7±0.84	2.5±0.54
Tannin (CE mg/g)	3.2±0.3	3.8±0.89	3.5±0.68

The proximate composition of mushrooms cultured in different substrate showed variation in the protein, carbohydrate, lipid, sodium, potassium and Vitamin C content. The protein (26.36±0.83g/100g) and lipid (6.08±0.31g/100g) content were high in mushroom grown in BPS followed by RS+BPS. The highest carbohydrate content was observed in BPS (17.28±0.89 %) grown mushroom and least carbohydrate content was found in mushroom cultivated in RS. The proximate and chemical composition of mushrooms were shown in Table .3 and 4 respectively.

Table.4 Proximate composition of Mushroom grown in different substrate

Contents(g/100g)	**RS**	**BPS**	**RS+BPS**
Protein	22.36±0.52	26.36±0.83	24.03±0.42
Lipid	4.89±0.09	6.08±0.31	5.98±0.51
Carbohydrate	14.52±0.24	17.28±0.89	16.60±0.97
Sodium	78.8±0.21	80.4±0.87	79.4±0.69
Potassium	250±0.84	248±0.25	204±0.31
Moisture	84.2±0.32	86.6±0.82	88.71±0.27
Vitamin C	19.8±0.27	23.1±0.84	2.05±0.64

Table.5 Chemical composition of Oyster mushroom grown in different substrate

Substrate	**C**	**N**	**C/N**	**pH**	**EC**
RS	50.5±0.4	0.95±0.7	53.16±0.6	6.71±0.9	3.1±0.2
BPS	49.1±0.6	0.99±0.2	49.6±0.7	6.9±0.8	3.4±0.5
RS+ BPS	51.2±0.8	1.02±0.3	50.1±0.4	6.8±0.5	3.3±0.6

2. Discussion:

The substrate composition and the chemical composition of substrate have significant influence on mushroom growth. In the present study it was found that mushroom grown in RS+ BPS showed fastest growth and higher weight in mushroom bunch and biological efficiency where the mushroom grown in BPS showed better protein, lipid and phytochemical composition. The result of present study lined with the study reported by Kamthan *et al.,* in 2017 where it was found that the combination of substrate has a significant effect on protein yield and, biological efficiency. In the present study the pin head formation was fastest in mushroom grown in RS when compared to the study reported by Bughio (2001), where oyster mushroom took 43.25 to 53.00 days for pinhead formation which uses wheat straw and sorghum leaves as substrate. The biological efficiency was found to be high in mushroom grown in BPS (87.5%) which is higher than the study reported by Muswati *et al.,* in 2021 where the maximum BE was 86.15%. Correspondingly Bugarski *et al.,* in 1994, found that the first fruiting body occurred on different days depending on substrates. In the current C/N ratio was low in mushroom cultivated in BPS followed by RS+BPS which was found to be inversely proportional to growth and yield of mushroom. The results of present study were in agreement with the finding of Yang *et al., in* 2000, who suggested that higher C/N ratio promotes the mycelium growth, and fruiting body growth is favored by lower C/N ratio. Hoa *et al.,* in 2015 reported that the C/N ratio of substrate formulas has close correlation with mushroom weight, yield, BE and protein content of mushroom and the total harvest period.

Shyni *et al.,* in 2019 reported that mushrooms showed the presence of alkaloids, carbohydrates, glycosides, proteins, flavonoids, triterpenoids, phenols, tannins, saponins and anthroquinones. Correspondingly, Mir *et al.,* in 2015 suggested that the ethyl acetate extract of oyster mushroom possess phytochemicals such phenol, steroid, alkaloid and flavonoids. Similarly, Shyni., *et al.,* in 2019 found phytochemicals such as alkaloids, carbohydrates, glycosides, proteins, flavonoids, triterpenoids, phenols, tannins, saponins and anthroquinones in *Calocybe indica.* From the present study it was also found that mushrooms grown in BPS showed higher protein, lipid, carbohydrate and vitamin C. Similarly, Khan *et al.,* (2008), reported the *Pleurotus sajor-caju* contains 87.2 ± 0.5% moisture and 24.5±2.9 g/100g protein and lipid content was high in *P. cystidiosus*. Correspondingly, Sahu *et al.,* (2018) observed highest flavonoids (5.41+0.15 mg/100g) in the oyster mushroom grown in paddy straw. The result of the present study revealed that mushroom grown in BPS showed better proximate and phytochemical composition. The pseudo-stem can also be converted into bio-fertilize. It also contains high amount of cellulose and starch, and thus it can be utilized as feed for cattle. Moreover, there have been numerous research studies that reported the use of banana pseudo-stem fiber in fabrication of polymer/fiber composites. Cellulosic cotton textile very easily catches flame, and it is very difficult to be extinguished. This problem of course poses a dangerous risk to life of human beings and textile products. Therefore, major efforts have been made in the past years to improve the flame retardancy of the cotton textile material by using many synthetic chemicals, which are available commercially. Phosphorous-based flame retardancy agents together with nitrogen- based compounds are the most effective combination that has been reported so far. However, there are some drawbacks such as high cost and not environmentally friendly. Hence, there is a growing trend that focuses on more cost-effective, environmentally friendly methods, and sustainable fire retardant products. Several literature studies have been reported on providing fire retardancy to the cotton textile material by using natural products. One of them is using the waste banana pseudo-stem sap (BPS). Banana pseudo-stem sap (BPS) is a liquid that is extracted from the banana pseudo-stem. Additionally, there are many more potential applications of banana pseudo-stem components (M.Singhal *et.al,* 2015).

3. Conclusion

Mushrooms are widely accepted and highly proteinaceous substances which have significant role in curing malnutrition, and it have well known medicinal property. In the present study it is evident that various agricultural wastes such as rice straw and banana pseudostem and their combinations can be used as substrate for mushroom cultivation. The current study revealed that mushroom grown in BPS+RS produces better growth and biological efficiency and the BPS grown mushroom was rich in proximate composition and phytochemicals. Numerous reports are available for the cultivation of mushroom using different substrates, but this is the first research report which utilized the combination of banana pseudostem and rice straw waste for the cultivation of nutrient rich Oyster mushroom. By adopting this mushroom cultivation method environment is protected by the complete utilization of waste resources such as banana pseudostem and rice straw and through this the farmers will get additional income for their livelihood. Hence banana pseudostem and rice straw waste can be well managed by using it in mushroom cultivation and thereafter it can be used as manure.

Declaration of conflict of Interest

The authors declare that they have no conflicts of interest.

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