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Original Research Article

Effect of Different Substrates on Yield and Nutritional Value of *Pleurotus ostreatus* and Evaluation of its Phyto-chemical and Antibacterial Properties

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ABSTRACT

Mushrooms are fleshy, spore bearing fruiting bodies or fungus. They belong to the class Basidiomycetes, order; Agaricales. According to Chang and Miles mushroom is "a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogeous. The study was conducted to observe the effect of different substrates on growth, yield and nutritional composition of oyster mushroom (*Pleurotus ostreatus*). Four different substrate Wheat straw, paddy straw, rice bran, sugarcane baggase supplemented with gram husk and wheat bran were observed in different ratio. The results indicated that different substrates showed significant different in growth, yield and nutritional composition of *Pleurotus ostreatus*. Wheat straw supplemented with gram husk and wheat bran gave maximum yield as compared with other substrates, but with increase in supplemented substrates showed adverse effect on growth and yield of *Pleurotus ostreatus*.

1. INTRODUCTION

Mushrooms are fleshy, spore bearing fruiting bodies or fungus. They belong to the class *Basidiomycetes*, order; Agaricales. According to Chang and Miles mushroom is "a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogeous. They are characterized by the presence of a stem, cap and gills on the underside of the cap". Initially, more than 200 species of mushrooms were used as functional foods around the world [1], but now only about 35 species have been commercially cultivated [2]. They are rich source of nutrients, particularly proteins, minerals as well as vitamins B, C and D [3]. On exposure to UV-light, mushrooms can also produce large amounts of vitamin D, which is normally difficult to obtain from a regular diet intake. Oyster mushrooms (*Pleurotus* species) are the third most cultivated mushrooms after white button and shiitake [4]. They are the easiest and least expensive commercial mushrooms to grow because they convert crop residues into food protein. The ligno-cellulosic substrates used for the cultivation of *Pleurotus* species are coffee pulp, cotton seed, sugarcane bagasse, wheat straw, wheat husk, rice bran, gram husk, cotton seed hulls, cassava peels, paddy straw, saw dust, agricultural waste, corncobs, water hyacinth, water lily bean, oil-palm fiber, paper and cardboard, etc. [5]. The use of these substrates depends on the capacity of the fungus to produce a ligno-cellulolytic enzyme complex [6]. This complex includes the oxidative enzymes laccase and manganese peroxidase (MnP), which are involved in lignin degradation [7], and the hydrolytic enzymes xylanase and cellulase [8], which are involved in hemi-cellulose and cellulose degradation, respectively. The genus Pleurotus produces many other bio-active substances that are of medicinal importance. Such compounds include dietary fiber, terpenoids, steroids, phenols, alkaloids, phlobatannins and anthraquinones [9]. The methanolic extract of mushroom contains a steroid (glycosylated form of ergosterol peroxide), which is an inhibitor of proliferation of tumor cell lines [10]. Mushrooms have anti-tumor, immuno-modulatory, anti-oxidant, hypo-cholesterolaemic, anti-hyperglycemic, anti-microbial, anti-viral activities [11]. *Pleurotus* species is one of the most

efficient ligno-celluloses decomposing types of white rot fungi [12]. Mycelium can produce a group of complex extra-cellular enzymes which can degrade wastes materials and helps in reducing pollution.

2. MATERIALS AND METHODS

2.1. Collection of Mushroom Spawn

Pleurotus ostreatus variety florida seeds or spawn were collected from the Government Nursery, Horticulture department, Patiala. Substrates were collected from the local farmers.

2.2. Substrates

Wheat straw, paddy straw, saw dust, rice husk, sugarcane baggase, wheat bran, gram husk.

2.3. Preparation of Substrates

Five substrates (Wheat straw, Paddy straw, saw dust, rice husk, and sugarcane bagasse) were sun dried and chopped or cut in small pieces. They were weighed and mixed except gram husk and wheat bran. The substrates were soaked overnight in water and allowed to ferment. The soaked substrates was allowed to drain excess water to maintain 60-75% of moisture content. Wheat bran and gram husk was added to the substrate after fermenting.

2.4. Sterilization

The substrates were filled in the container; added boiling water and covered the container for 2 hours for steam sterilization to remove the contamination. 2% formaldehyde, 1% lime and 1% urea was added to the substrates to decrease the chance of contamination and providing the nitrogen source. The substrates were allowed to cool down to room temperature after sterilization process as elevated temperature of substrate inhibits the growth of the mycelium.

2.5. Bag Preparation

The substrate was added to the bags in layers, starting from the base of the bags and spawn was added in between these layers and at the sides of the bag. The 3/4th portion of the bag was filled. The tiny holes were made in the bags to maintain the level of air and openings for the fruiting bodies. Bags were incubated for spawn running in the dark place or room at 21-25°C.

2.6. Cropping

12-15 days after the spawn run, the bags were torn apart to open the substrate. The substrates were irrigated twice a day by sprinkling the fresh water to maintain the moisture content. After 4-5 days, the pin heads were start appearing on all sides of the bags. These pins heads developed into fruiting bodies within 3-4 days.

2.7. Yield and Biological Efficiency

Total weight of the all the fruiting bodies harvested from all the three pickings were measured as the total yield of the mushroom. The biological efficiency (B.E.) (yield of the mushroom per kg substrate on dry weight basis) was calculated by the following formula –

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

3. PHYTOCHEMICAL ANALYSIS

3.1. Extract Preparation

5 gram mushroom powder was soaked in 60% methanol. Kept it in shaker for 3 days at room temperature for proper mixing. Liquid extract was filtered twice using funnel and muslin cloth and then filtered with whatman No.1 filter paper. The filtrates were stored in the refrigerator at 4°C.

3.2. Phyto-chemical Testing

The standard methods and protocols were followed for phyto-chemical analysis.

3.3. Test for tannins

Few drops of 5% ferric chloride were added to 1ml extract. Formation of dark blue or greenish black indicates the presence of tannins.

3.4. Test for saponins

1ml of distilled water was added to 1ml extract and then shaken for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.

3.5. Test for quinones

1ml of concentrated sulphuric acid was added to 1ml extract. Formation of red colour indicates the presence of quinones.

3.6. Test for flavonoids
1ml 2N sodium hydroxide was added to 1ml extract. Formation of yellow colour indicates the presence of flavonoids.

3.7. Tests for alkaloids
2ml concentrated hydrochloric acid was added to 1ml extract. Then by adding few drops of Mayer's reagent, green colour or white precipitate indicates the presence of alkaloids.

3.8. Tests for glycosides
3ml chloroform and 10% ammonia solution was added to 1ml extract. Formation of pink colour indicates the presence of glycosides.

3.9. Test for cardiac glycosides
2ml glacial acetic acid and few drops of 5% ferric chloride were added to 1ml extract. This was under layered with 2ml concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

3.10. Test for terpenoids
2ml chloroform and 2ml concentrated sulphuric acid was added to 1ml extract along the sides of the tube. Formation of red brown colour at the interface indicates the presence of terpenoids.

3.11. Test for phenols
2ml distilled water and few drops of 10% ferric chloride were added to 1ml extract. Formation of blue/green or red colour indicates the presence of phenols.

3.12. Test for steroids
2ml chloroform and 2ml concentrated sulphuric acid was added to 1ml extract along the sides of the tube. Reddish-brown ring formed at interface indicates the presence of steroids.

3.13. Test for coumarins
1ml of 10% sodium hydroxide was added to 1ml extract. Formation of yellow colour indicates the presence of coumarins.

3.14. Test for anthocyanins and betacyanins
1ml of 2N sodium hydroxide was added to 1ml extract and the mixture was heated for 5 minutes at 100°C in water bath. Appearance of bluish- green colour indicates the presence of anthocyanins and of yellow colour indicates the presence of betacyanins.

3.15. Quantitative Analysis
Quantitative analysis of total sugars, total proteins, total Phenols and Anti-oxidant activity was performed by Anthrone method, Lowery Method, Follin's Method and DPPH method respectively.

3.16. Antibacterial Activity
Antibacterial activity of *Pleurotus ostreatus* was observed by Kirby's agar well diffusion assay and zone of inhibition was observed.

4. RESULTS AND DISCUSSION
Mushrooms were cultivated on different substrates. Some supplements such as gram husk, wheat bran and saw dust were added to check their effect on growth of oyster mushroom (*Pleurotus ostreatus*). Substrates were mixed in different quantities, partially fermented, pasteurized and filled into bags. Bags were incubated at 25-28°C in a dark room. Mycelial growth is a preliminary step that creates suitable internal conditions for fruiting. After 15-18 days, the complete mycelium run occurred. The fruiting bodies were formed within 25-30 days of inoculation. The cultivation of mushroom (*P. ostreatus*) has various stages of mushroom (Figure 1).

4.1. Yield
The yield of the mushroom (*Pleurotus ostreaus*) gets decreased followed by flushes. Various substrates shows a huge variation in the yield of mushroom. (Figure 2).

4.2. Phyto-chemical Analysis
Methanolic extract of dried mushroom powder was subjected to phytochemical analysis (Figure 3). The mushroom extract contains alkaloids, glycosides, phenols, steroids, saponins and terpenoids where as tannins, quinones, flavonoids, cardiac glycosides, coumarins and anthrocyanins seems to be absent.

4.3. Total Sugar Content
The concentration of sugar content increased with successive flushes. sugarcane bagasse and paddy straw influences the sugar content as compared to rice bran. Sugarcane baggase is good source of

sugar hence it increased the sugar concentration when used as a substrate. (Figure 4)

4.4. Total Protein Content

Protein content decreased with the successive flushes in all substrate combinations. Wheat straw and sugarcane bagasse supplemented with gram husk (nitrogen source) showed maximum variation in the protein content among the flushes. However, rice bran as the major substrate did not have much impact on the protein content. (Figure 5)

4.5. Total Phenol Content

Substrates did not have any major effect on the phenol content of mushroom. Phenol content decreased with every successive flush. Sugarcane baggase and saw dust had a positive effect on the phenolic content of mushrooms. (Figure 6)

4.6. Antioxidant Activity

No much variation was seen due to substrates or among the flushes.

The combination of wheat straw and paddy straw along with saw dust influences the antioxidant activity; it decreased in the successive flushes. (Figure 7)

4.7. Anti- bacterial activity

The methanolic extract of *Pleurotus ostreatus* shows antibacterial activity against various gram positive and gram negative bacteria. Staphylococcus aureus is gram positive bacteria and senstivite against mushroom extract. It inhibits the growth of S. aureus. Simialry, E. aerogenes is gram negative bacteria and sensitive against the crude mushroom extract. Gram positive bacteria were more susceptible than gram negative bacteria. Antibacterial activity depends upon the proteins present in the mushroom extracts.

5. CONCLUSION

From the above discussion it was observed that Pleurotus species degrade agricultural wastes at wide range of temperature. Different substrates were used for the cultivation of mushroom to observe the effect of different substrates on their growth and nutritional value. Different substrates (wheat straw, paddy straw, sugarcane baggasse and saw dust) were used. *Pleurotus ostreatus* showed increase in yield and enhance nutritional value when supplemented with external sugar and protein sources. *Pleurotus ostreatus* showed low anti-bacterial activity against different strains.

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(a) (b)

(c) (d)

Figure 1: Various stages of mushroom cultivation; (a) substrate preparation (b) Bag preparation (c) complete mycelium run (d) Formation of fruiting bodies.

Figure 2: Yield of mushroom per bag.

Figure 3: Phyto-chemicals of mushroom extract

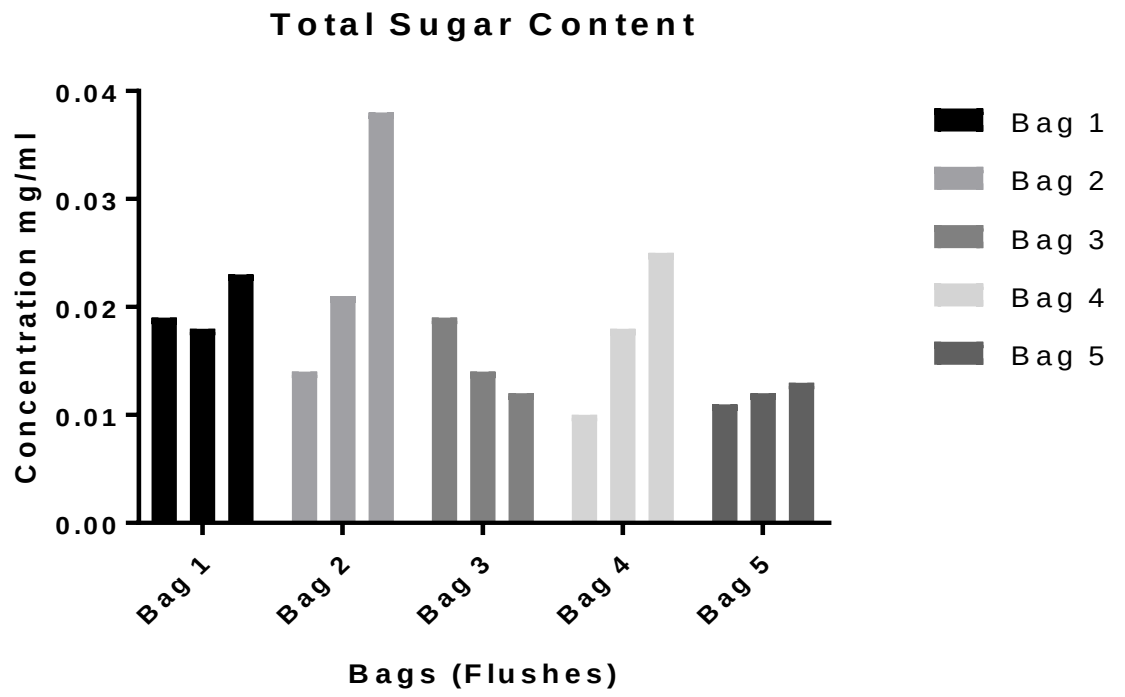

Figure 4: Total Sugar Content present in samples

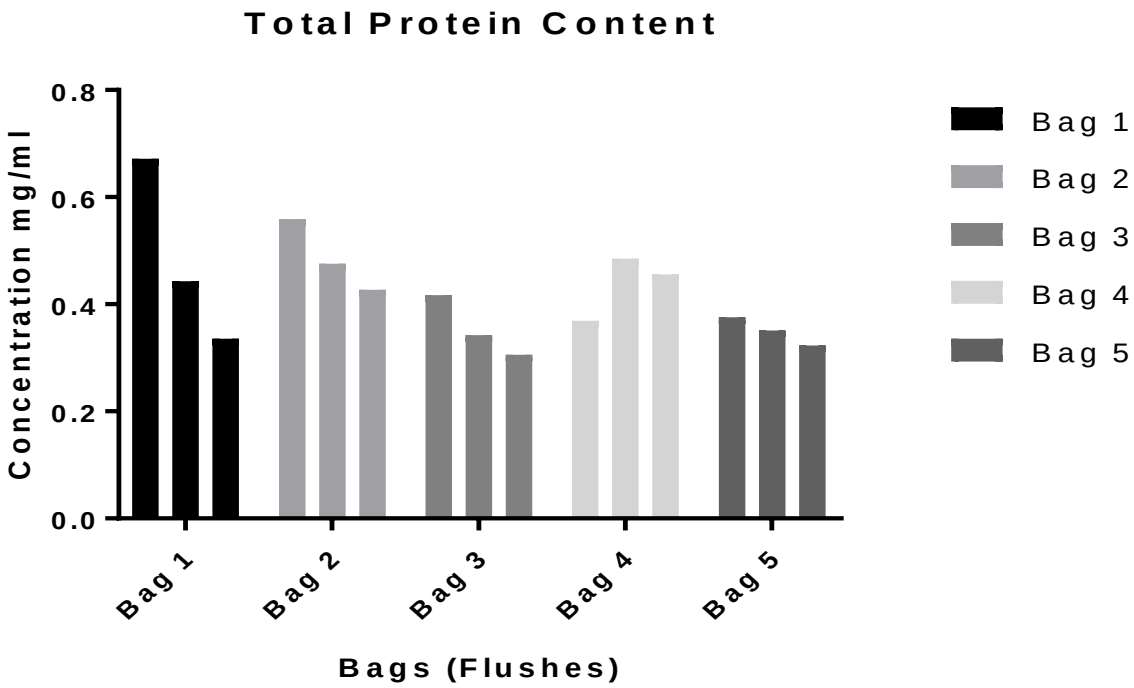

Figure 5: Total protein Content present in samples

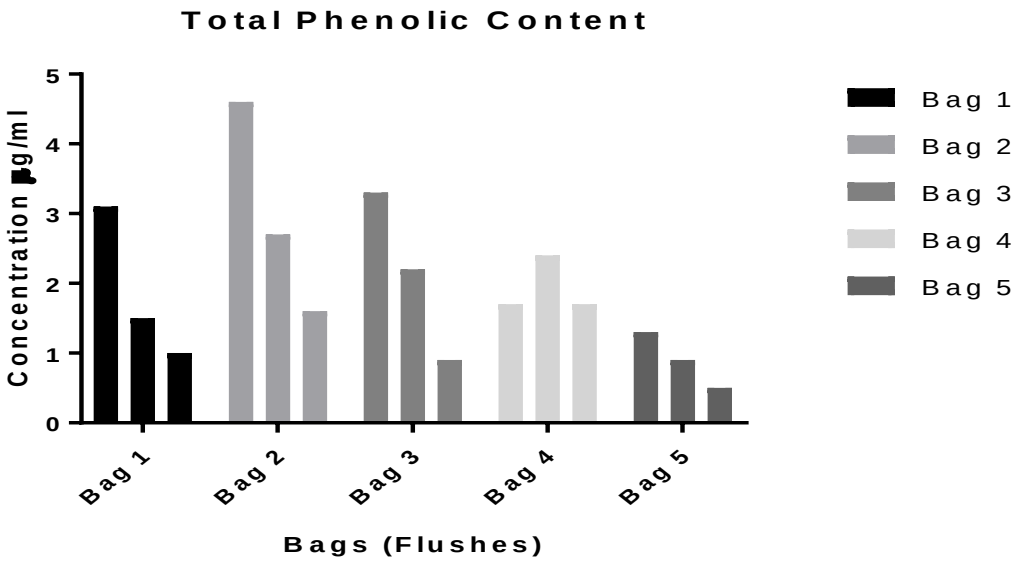

Figure 6: Total Phenol Content present in samples

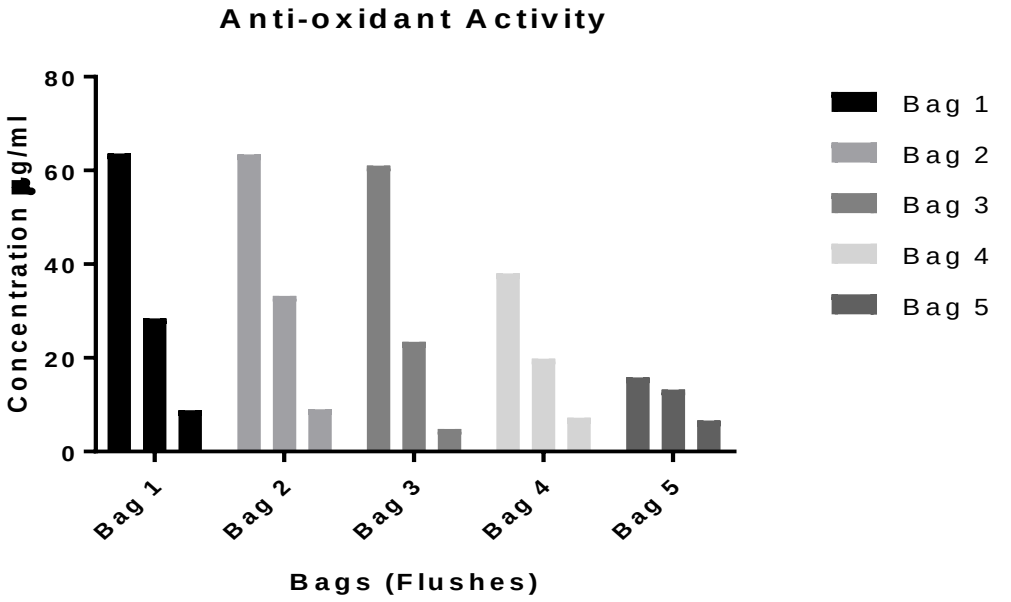

Figure 7: Anti-oxidant activity of mushroom.

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