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EFFECT OF DIFFERENT SUBSTRATE ON GROWTH YIELD AND NUTRITIONAL COMPOSITION OF PLEUROTUS FLORIDA MUSHROOM AND EVALUATE THEIR ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY

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Abstract

In this study, oyster mushroom species Pleurotus florida was grown on three different substrates like wheat straw, paddy straw and mustard straw to find out what effect these three substrates have on growth yield, nutritional value, antimicrobial activity and antioxidant activity.

The main finding of this study is that when we grow Pleurotus florida species of oyster mushroom on different substrates, we find that we see effects on its growth yield, nutritional value, antimicrobial activity and antioxidant activity. The three substrates had different effects on yield, viz., stipe length, petiole diameter, pileus diameter, fruiting body diameter, early initiation day, pin head formation day, spore maturation time, substrate span run day, yield of first crop per field, yield of second crop per field, yield of third crop per field, total yield, biological efficiency, moisture content, dry matter content, effect on all aspects of growth and yield.

The oyster Pleurotus Florida mushroom genus is the third largest commercially produced and second most important cultivated mushroom genus in the world, comprising about 539 species. It comprises 22% - 25% of the total world production of cultivated mushrooms. Pleurotus mushroom has various nutritional and medicinal values. The fruit bodies of Pleurotus have unique flavor and are rich in nutrients such as carbohydrates, proteins, vitamins, minerals and dietary fiber. The properties of anti-bacterial, antioxidant activity present in Pleurotus florida are found. The present research discusses the important nutritional and medicinal benefits of Pleurotus florida by running it on different substrates to see whether differences in nutritional value, antimicrobial activity and antioxidant activity are observed on different substrates. From the result we came to know that out of three substrates of wheat straw, paddy straw and mustard straw two most important substrates are wheat straw and paddy straw which showed significant result in all aspects being highest in all aspects. Less important is mustard straw.

Keywords: Pleurotus Florida Mushroom, Substrate, Nutritional Value, Antimicrobial, Antioxidant

INTRODUCTION

Among the white-rot fungi, genus *Pleurotus* as the oyster mushrooms are famous for conversion of substrate into edible mushrooms and known as 'dhingri in India well known edible fungi. described the properties of *Pleurotus* spp. in relation to their biotechnological applications and its multitude potential. The cultivation of mushroom is recognized as worthwhile agribusiness and popular white vegetable having excellent flavour and taste. Moreover, they are easiest and least expensive commercial mushroom to grow artificially. Consequently, now oyster mushrooms are the second largest produced mushrooms in the world. The cultivation of *Pleurotus* spp. is an economically important food industry worldwide, which has vastly expanded in past few years and become the second most cultivated mushroom for food purposes. This is the most economic conversion system of lignocellulosic waste into food products. The genus Pleurotus comprise of edible lignocellulolyic mushrooms with medicinal properties and important biotechnological and environmental applications. Nutritionally it has unique flavour and aromatic properties, which is considered rich in protein, fibre, carbohydrates, vitamins and minerals. Pleurotus spp. is promising as medicinal mushrooms, exhibiting antibacterial, hypochlosterolemic and immunomodulation activities. Mushrooms, also known as 'white vegetables' or 'boneless vegetarian meat contains adequate level of proteins, vitamins and fiber apart from having certain valuable properties. Mushroom substrates defined as any kind of lingnocellulosic material which supports and enhance the growth







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of fruiting bodies. *Pleurotus florida* white oyster mushroom is white in color from primordial head formation to maturity and this mushroom also grows in bunches.

Nutritional properties of oyster mushroom:

In terms of the amount of crude protein, mushrooms rankbelow animal meats, but well above most other foods, including milk, which is an animal product. Furthermore mushroom protein containsall the nine essential amino acids required by man. Mushrooms are very nutritious products that can begenerated from lignocellulosic waste materials; and arerich in crude fibre and protein (between 19 percent and35 percent); contain low fat, low calories and no starch. Mushrooms are a good source of vitamin B, C and D, including niacin, riboflavin, thiamine, and folate, andvarious minerals including potassium, phosphorus, calcium, magnesium, iron and copper. They also provide carbohydrates, with an additional vegetable of highquality, and enrich the diet with high quality proteins, minerals and vitamins which can be of direct benefit tothe human health and fitness. The consumption of mushrooms can make a valuable addition to the oftenunbalanced diets of people in developing countries. Freshmushrooms have a high water content, around 90 percent, so drying them is an effective way to both prolong their shelf-life and preserve their flavourand nutrients.

The chemical composition of edible mushrooms determines their nutritional value and sensory properties. It differs according to species but also depends on the substratum, atmospheric conditions, age and part of the fructification. The perception of mushrooms as a highly nutritional food-stuff is well founded. Compositional analyses of the main cultivated varieties have revealed that on a dry weight basis, mushrooms normally contain 19 to 35% protein. Moreover, mushroom proteins contain all the essential amino acids and are especially rich in lysine and leucine which are lacking in most staple cereal foods. The low total fat content, and the high proportion of polyunsaturated fatty acids (72 to 85%) relative to total fatty acids, is considered a significant contributor to the health value of mushrooms. Mushrooms are considered to be a good source of digestible proteins with protein content above most vegetables and somewhat less than most meats and milk. Protein content can vary from 10-40% on a dry weight basis. Mushrooms contain all the essential amino acids, but can be limiting in the sulphur-containing amino acids, cystine and methionine (Breene, 1990; Chang, 1991). Mushrooms contain 3-21% carbohydrates and 3-35% fibre on a dry weight basis. A considerable proportion of the carbohydrate of mushrooms consists of dietary fibre which cannot easily be digested by humans and which function essentially as dietary fibre; in this way the calorific value of most mushrooms is low. Mushrooms contain 20-35% of high quality protein in dry weight which is higher than in vegetables and fruits. Mushrooms are very rich in lysine and tryptophan, the two essential amino acids deficient in cereals. Mushrooms contain good amount of vitamin C and vitamins of B complex group (thiamine, riboflavin and niacin). They are rich in K, P, and Na and contain low but available form of iron. Potassium: Sodium ratio is very high which is desirable for patients of hyper tension. Mushrooms are low calorie food with very little fat. They have no starch and are very low in sugars. Storage lipids as Cholestrol are absent and ergosterol is present. Carbohydrates are the main components of mushrooms apart from water. In Pleurotus species, carbohydrate content is reported in the range of 46.6 to 81.8%, while for Volvariella volvacea it is 40-50% and 67.5% in Lentinula edodes. In fresh mushrooms carbohydrate content varies from 3 to 28%.

Anti-microbial properties of oyster mushroom:

Fungi, with an estimated 1.5 million species, are the source of approximately one quarter of known natural bioactive compounds. According to Chang the value of world production of edible mushroom and mushroom derived medicinal products was estimated to be worth approximately 14 billion US dollars in 1994 and 2944 metric tones during the year 2009. Antimicrobial drugs have long been used for prophylactic and therapeutic purposes. Unfortunately the recent increase in the occurrences of drug-resistant bacterial strains is creating serious treatment problems. Consequently, the antimicrobial activity of various anti-tumour polysaccharides from medicinal mushrooms is being re-evaluated in terms of their clinical efficacy. Such compounds would be expected to function by mobilising the body's humoral immunity to ward off viral, bacterial, fungal and protozoal infections resistant to current antibiotics.







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Antioxidant properties of oyster mushroom:

Antioxidants work to neutralize free radicals before they do harm to our bodies. Free radicals are atoms that cause damage to our cells. Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Mushrooms that contain antioxidants or increase antioxidant enzyme activity may be used to reduce oxidative damage in humans. Various crude extracts of Pleurotus species have been shown to possess relatively strong antioxidant and antimicrobial activities. The antioxidant and antimicrobial activity of the ethanolic extract from the mycelium of P. florida, P. sajor-caju and P. aureovillosus were studied previously. Among all the species, P. florida showed a potent antioxidant activity and narrow antibacterial activity. The non enzymatic antioxidant capacity of P. florida was evaluated in two stages such as fresh and dried form of mushroom. Both fresh and dried samples of Pleurotus mushroom possess non-enzymatic activity. The antioxidant activity was positively correlated with total polyphenol content. Methanol extract of P. florida fruiting bodies possessed more effective antioxidant activity than the synthetic antioxidant agent, catechin due to a higher amount of total phenols.

MATERIAL AND METHODS

The present investigation entitled "Effect Of Different Substrate On Growth Yield And Nutritional Composition Of Pleurotus Florida Mushroom And Evaluate Their Antimicrobial And Antioxidant Activity". The experiment was carried out in the Department of Botany, Mahila Mahavidhyalay (MMV) Banaras Hindu University, Varanasi Uttar Pradesh India-221005 from June 20222 to December 2022. The information of methodology adopted in this experiment has been presented below:

Location and site of experiment

The present investigation was carried out the Department of Botany, Mahila Mahavidhyalay (MMV) Banaras Hindu University, Varanasi Uttar Pradesh India-221005 from June 20222 to December 2022.

1. Sample preparation:

Dry mushroom were procured from the Place. These are grown in the different solid substratum like mustard straw (A), wheat straw (B), and paddy straw (C). The grown samples were shade dried and grind it in mixture grinder for fine powder. 10gm of each samples (A, B, C) and 200ml of double distilled water (DDW) were mixed and put it at hot plate for heating at 50° C for 30min with random shaking. After 30 min, samples were filtered and the filtrate were dried in petri plate at 40° C, known as crude extract. Stored the crude extract in refrigerator for further analysis like Phytochemical, antioxidant, antibacterial, carbohydrate estimation and protein estimation.

2. Phytochemical Screening:

- (i) Test for Saponins: Took 2ml of extract A, B, and C separately and add 5ml of DDW. Shake the solutions vigorously for 2min. Formation of foam occurs in the solution is the indication of presence of saponins.
- (ii) Test for Steroids (LibermannBuchard Test): Took 1ml of samples and add 5ml of chloroform to it. Add concentrated sulphuric acid by side wall of test tube. Upper layer becomes red while lower layer becomes yellow with green fluorescence, indicated the presence of steroids in the extract.
- (iii) Test for Quinones: Took 1ml of extracts in test tube and add 2ml of diluted sodium hydroxide in it. Formation of blue-green color shows the presence of quinones.
- (iv) Test for Tannins (Braymer's Test): 2ml extracts were fixed with the 10% alcoholic ferric chloride solution. Formation of blue or green color indicates the indicated the presence of Tannins.
- (v) Test for Phenolic (Ferric chloride Test): Few drops of extracts were mixed with the 5% aqueous ferric chloride solution. Formation of dark blue or black color indicates the presence of phenol.
- (vi) Test for Alkaloids (Mayer's Test): Took 2ml of extracts in test tube and add 2 drops of Mayer's reagent. Formation of white creamy precipitate occurs, indicated the presence of alkaloids.







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- (vii) Saponification Test: 0.5 ml of extracts were taken in test tube, add 1ml sodium hydroxide (20% w/v) and 0.5ml ethanol to it. Then put the test tube in boiling water bath for 15 min. Then add 5ml DDW and shake it vigorously. Formation of froth indicates the presence of lipids and fats.
- (viii) Test for Amino acids (Ninhydrin test): Took 2ml of extracts and add 2 drops of Ninhydrin to it. Heat the test tube. Formation of purple or blue color indicates the presence of Amino acids.
- (ix) Test for Coumarins: 2ml of extracts were taken in test tubes and add 3ml of sodium hydroxide (10%). Yellow color indicates the presence of coumarins.
- (x) vvTest for Carbohydrate (Fehling's Test): Firstly mixed the Fehling solution A with Fehling solution B in equal amount (1ml each) in a test tube. This is known as Fehling solution. Now add 2ml of extracts in Fehling solution. Put this solution in water bath for 30 min at 90°C. Formation of red color precipitate occurs indicates presence of Carbohydrates.

3. Determination of total Protein:

Protein content of the mushrooms were measured according to Khatun et al (2015) with some modifications. Dry (10 mg) mushrooms were crushed with a pinch of neutral sand and centrifuged at 5000 rpm for 15 min. The pellet was taken discarding the supernatant. The mushrooms were taken in different concentration like 5, 10, 15, 20 and 25µg/ml.Different concentrations of supernatant were mixed with 1 mL Bradford reagent. Bradford reagent were used formeasuring protein content. After 5 min, absorbance was recorded to 595nm wavelength in a UV-vis spectrophotometer (Simadzu UV- 1800 Spectrophotometer) againsta control sample. BSA (Bovine Serum Albumin) is taken as a standard for protein estimation.

4. Determination of Carbohydrate:

The total amount of carbohydrates was calculated using the Anthrone method (Dhakad, 2017) with some modifications. Where 200 mg of fresh mushrooms were placed in boiling tubes and hydrolyzed with 5 ml of 2.5N HCl over the course of three hours. It was allowed to cool and neutralized with sodium carbonate, till the effervescence stopped. Prepare a volume up to 100 ml with distilled water, then centrifuge it at 10,000 g for five minutes. Following that, 1 ml of supernatant was taken, 5 ml of anthrone reagent was added, and the mixture was boiled in a boiling water bath for 10 minutes before being quickly cooled. At 620 nm, the absorbance of the green to dark green color was measured. For the curve, a standard glucose solution was used to represent the sample's carbohydrate content in mg/g.

5. Free Radical Scavenging Assay:

Radical-scavenging activity of samples against stable DPPH (2, 2-diphenyl-1-picryl hydrazyl radical) were determined spectrophotometrically. The DPPH assay was carried out as described by Ravindra M. (2007). Stock solutions of crude extracts were prepared as 1 mg/ml in double distilled water (DDW). 300µl of different concentration samples were added to 3 ml of 0.002% methanolic solution of DPPH. After 30 min of incubation in the dark at room temperature, the absorbance was read against a blank at 517 nm. The assay was carried out in triplicate and percentage of inhibition was calculated using the following formula:

Percent inhibition= (Control-sample /control)*100------1

6. Antibacterial Activity:

The antimicrobial activity of the extracts was carried out by disc diffusion methodusing 10μ l of suspension containing 10^8 CFU/ml of bacteria spread on nutrient agar (NA) medium. Sterile 6 mm diameter filter paper discs were impregnated with 20μ g all the extract and placed onto nutrient agar. The plates were then incubated in shaking incubator (REMI CIS-24) at 37° C for 18-24 hfor bacterial pathogens. The antimicrobial activity was evaluated by measuringthe diameter of inhibition zone. The streptomycin is taken as a positive control and DMSO is taken as a negative control. The experiment wascarried out in triplicate and the mean of the diameter of the inhibition zones was calculated.







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RESULT AND DISCUSSION

The preliminary test for the presence of phytochemicals shows the many bioactive compounds in the mustard, wheat and paddy straw grown mushrooms. As shown in the Table 1 phytochemicals present like phenol, steroids, saponins, alkaloids and amino acids. According to Unekwu et al, some mushrooms also have cardiac glycosides, tannins, terpenes etc.

Table 1. Phytochemicals of different mushrooms				
S.N.	Phytochemical Test	Mustard Straw (A)	Wheat Straw (B)	Paddy Straw (C)
i.	Coumarin	-	-	-
ii.	Saponins	+	+	+
iii.	Steroids (LibermannBuchard Test)	++	+	++
iv.	Quinones	-	-	-
v.	Tannins (Braymer's Test)	-	-	-
vi.	Phenol (Ferric chloride Test)	++	+	++
vii.	Alkaloids (Mayer's Test):	+	+	+
viii.	Saponification	-	-	-
ix.	Amino acids (Ninhydrin test)	+	++	+
х.	Carbohydrate (Fehling's Test)	+	+	+

- (++) Moderately present,
- (+) Faintly present,
- (-) Absent

The protein estimation of mushrooms were determined by Bradford method. With the increment of concentration of mushrooms the protein content is also increases from 0.448 to 0.492 for A, 0.488 to 0.525 for B and 0.498 to 0.561µg/ml for C. While in the standard BSA the protein content were 0.515 to 0.967µg/ml observed. In compare to BSA the protein content were less in all the samples. The highest content of protein were found in the Sample C that was grown on paddy straw substrate while the lowest contest were found in sample A that was grown on mustard straw.







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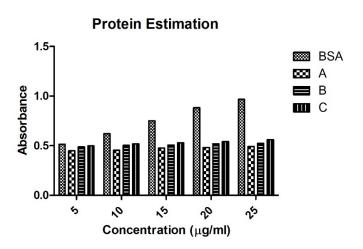
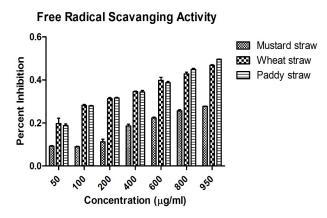


Fig. Determination of Protein of different mushrooms

For the determination of carbohydrate, anthrone method were used. The results for the amount of carbohydrates showed that sample C had the greatest total carbohydrate content, 2.25 mg/g of fresh mushroom sample, followed by sample A, which had 1.82 mg/g of fresh sample. Estimated lowest carbohydrate content for sample B was 1.24 mg/g. Sample C's carbohydrate findings are noticeably better than those of the other samples of mushrooms.

It is possible to quickly assess the antioxidant activity of particular substances or extracts using the DPPH free radical scavengingmethod (Cheung). DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) is a dark-colored crystalline powder that produces a violet solution when dissolved in ethanol or methanol and stable DPPH free radicals. Because of their strong absorption band around 517 nm, reduction of these DPPH free radicals gives rise to a colorless solution when neutralized by antioxidants present in the sample. The results of the DPPH free radical scavenging activity of different mushroom A, B and C were shown in the graph. Extract C has more antioxidant activity than B and A. The IC₅₀ value of Extract C was 587.87 μg/ml while the extract B and C were 605.82 and 1038.66 μg/ml respectively.



Graph: Antioxidant activity of different







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The effect of antibacterial activity of different mushrooms (A, B, C) were shown in the figure. The Graph showing the inhibition against Kp, Ec and Pseudo as 12mm, 18mm and 20mm respectively for antibiotics streptomycin. Only Ec and Pseudo exhibited the zone of inhibition from 8-14 and 7-10mm respectively for other samples. A has maximum zone of inhibition against Ec (14mm) B has 10 and C has 8mm of inhibition. For Pseudo A has 10 mm, B has 8 mm and C has 7 mm zone of inhibition. All the three extracts did not show any inhibition against MRSA, Kp and SB.

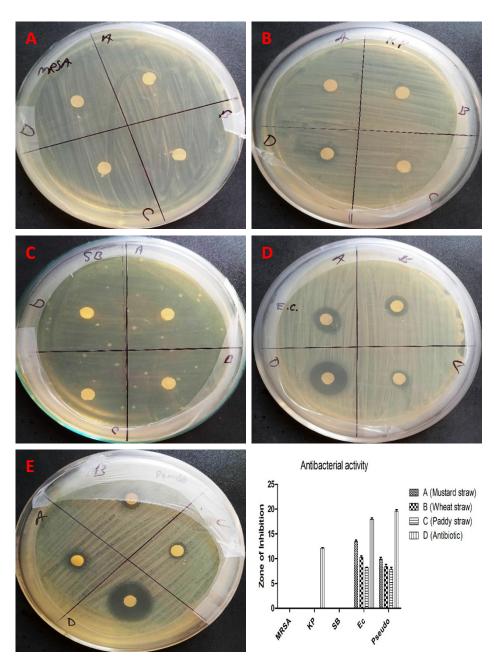


Fig. Antibacterial activity of Different mushroom







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SUMMARY & CONCLUSION

To consider different parameters of growth, yield and nutritional composition of oyster mushroom (Pleurotus florida) with different proportions of wheat straw, paddy straw and mustard straw. Based on the experimental findings, it is clear that wheat straw is more favorable for growth and yield than paddy straw and mustard compared to higher levels.

However, the yield of wheat straw (80%) treated with paddy and mustard

was equally effective in straw and mustard straw, Mustard and paddy straw respectively on different days of harvest. also indicated That up to 30% of wheat straw can be saved and can be suitably grown in proportion to paddy straw and mustard straw.

Preliminary testing for the presence of phytochemicals shows several bioactive compounds in the mushroom grown from mustard, wheat and paddy straw. Phytochemicals such as phenols, steroids, saponins, alkaloids and amino acids are present. According to Unekwu et al, some mushrooms also contain cardiac glycosides, tannins, terpenes, etc.

I came to know during the research that wheat straw and paddy straw have the highest amount of phytochemical present, the least amount of phytochemical is present in mustard straw.

The protein estimation of mushroom was determined by Bradford method. During my research it was observed that all the samples Wheat Straw(B), Paddy Straw(C) and Mustard Straw(A) had less protein content as compared to the given BSA (Bovine Albumin Protein) sample. The highest content of protein was found in sample C which was grown on paddy straw substrate while the lowest content was found in competition sample A which was grown on mustard straw.

Anthrone method was used for the determination of carbohydrates. The carbohydrate content results showed that sample C had the greatest total carbohydrate content, followed by fresh mushroom sample followed by fresh mushroom sample A. The estimated minimum carbohydrate content for fresh oyster mushroom sample B was . The carbohydrate extracts of sample C are significantly better than those of other mushroom samples.

I came to know during the research that in all the three substrates Wheat Straw (B), Paddy Straw (C) and Mustard Straw (A) the maximum carbohydrates were found in Paddy Straw (C) as compared to others.

It is possible to quickly assess the antioxidant activity of particular substances or extracts using the DPPH free radical scavenging method (Cheung). Due to their strong absorption band around 517 nm, the reduction of these DPPH free radicals gives rise to a colorless solution when neutralized by antioxidants present in the sample. The results of DPPH free radical scavenging activity of different oyster mushroom substrates mustard straw (A), wheat straw (B) and paddy straw (C) are shown in the graph. Extracted paddy straw (C) has higher antioxidant activity than wheat straw (B) and mustard straw (A). The IC50 value of extract paddy straw C was 587.87 μg/ml while that of extract wheat straw (B) and mustard straw (A) was 605.82 and 1038.66 μg/ml, respectively.

On finding, it was found that the highest antioxidant activity was found in the substrate of paddy straw(c) and the least antioxidant activity was found in the substrate of mustard straw(A).

The effect of antibacterial activity of different mushroom substrates (A, B, C) is shown in Fig. Showing inhibition against Kp, Ec and Pseudo for antibiotics streptomycin as 12mM, 18mM and 20mM respectively. Only Ec and Pseudo displayed zones of inhibition ranging from 8–14 and 7–10 mm, respectively, for the other samples. Mustard straw (A) has an interception area of Ec (14 mm), wheat straw (B) 10 and paddy straw (C) 8 mm. The inhibition zone is 10 mm for pseudomustard straw (A), 8 mm for wheat straw (B) and 7 mm for paddy straw (C). All the three oyster mushroom substrates showed no inhibition against MRSA, Kp and Sb.

On investigation we found that all the three substrates mustard straw, paddy straw and wheat straw lack antibiotic and showed antimicrobial activity against pseudomonas and streptomycin antibiotic Ec and psudo, but this substrate effect did not show effect against three bacteria mrsa and sb.







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