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LOW COST TECHNOLOGY FOR OYSTER MUSHROOM PRODUCTION USING WASTE LIGNOCELLULOSIC MATERIALS, AND THEIR FUNCTIONAL CHARACTERISTICS

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ABSTRACT

Cultivation of Oyster mushroom (*Pleurotus ostreatus*) on plastic and lignocellulosic wastes could be one of the most economically sustainable biological recycling progressions. In the current study, *Pleurotus ostreatus* was cultivated in composts prepared from 100 to 50% mixtures of different waste materials including plastic waste, newspaper waste, and wheat straw with varying concentrations in triplicates at an average temperature of 24-28°C and relative humidity ranging from 70 to 90%. Various morphological parameters including spawn running time (days), emergence of primordia, time period from primordial initiation to harvesting, number of fruiting bodies were recorded. The complete spawn running was in 18 days which was significantly earliest with substrate S2 (newspaper), followed by 21 days with substrate S3 (wheat straw) while, the longest period 28 days was taken in Substrate S1 (Plastic + wheat straw). Similarly, the highest yield obtained with substrate S2, while, the least with substrate S1 bags. However, mushrooms grown on substrate S1 were found to have highest alkaloid and phenolic contents, while terpenes content was highest in those obtained from substrate S3. The protein content of the three fruiting bodies was also estimated and the highest level observed in mushrooms obtained using S3. Higher antioxidant activity and fiber level observed in fruiting bodies grown on plastic substrate. The changes in the functional groups of Oyster mushroom were analysed by Fourier Transform Infrared Spectrometry (FTIR). The results of FTIR spectroscopy revealed that hydroxyl group, Aliphatic organohalogen compound, and Olefinic compounds stretches were most common among the three fruiting bodies, while significant variations occurred in the wavelengths related to Aliphatic bromo compounds, Acetylenic Silicon-oxy compounds, Ether and oxy compound, Thiols and thio-substituted compounds and several others.

KEY WORDS: Oyster mushroom, substrates, waste lignocellulosic materials, FTIR

1. INTRODUCTION

Pleurotus ostreatus, commonly known as oyster mushroom, belongs family Tricholomataceae and has been cultivated worldwide commercially since decades due to its nutritional and economic values. The simple and low-cost production technique of Oyster mushroom males it second most popularly cultivated mushroom across world (Sánchez, 2010). Moreover, owing to its taste, medicinal properties and availability, the demand of this mushroom is increasing exponentially day by day (Garcha *et al.,* 1993). Furthermore, *Pleurotus* species can proficiently use agricultural wastes to grow upon and promote its degradation at the same time (Sánchez, 2010). Oyster mushroom are reported to grow on a widespread variety of substrates like sawdust, rice and wheat straw, leaves, cotton seed hulls, and numerous others. Nevertheless, the yield and the composition of mushroom largely rest on the biochemical and nutritional content of substrates (Badu *et al.,* 2011).

2. MATERIALS AND METHODS

2.1 Preparation of Substrates

All the substrate materials were soaked separately in water with 2% formalin overnight. Thereafter, draining excess water from soaked materials, they were air dried in clean place to reduce the moisture content up to 70% and

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Table 1: Hydroalcoholic Extraction of three mushroom fruiting bodies samples.

Sample	Weight of Powder (g)	Weight of Extract (mg)	% Recovery
S1	1	335	33.5
S2	1	282	28.2
S3	1	297	29.7

Table 2: Analysis of Antioxidant activity (DPPH Assay results)

Sample code	IC_{50} value (µg/ml)
Ascorbic Acid	19.74
Mushrooms grown on substrate S1	1009
Mushrooms grown on substrate S2	2857
Mushrooms grown on substrate S3	1276

calcium carbonate (10%) was mixed individually. Various combinations of substrates were prepared by using these materials but only the ones with best results are mentioned here. The first combination of substrate (S1) was plastic waste + wheat straw (50:50), the second combination of substrate (S2) was newspaper + wheat straw (80:20), and the third substrate (S3) was wheat straw only.

2.2 Spawning and bagging

All the substrates were weighed and mixed with spawn (10%) evenly, and finally bagged in set of triplicates. Subsequently, the bags were knotted at the mouth and few holes were made for aeration around the plastic bag with a sterilised wooden stick, and were marked accordingly with a permanent marker.

Table 3: Effect of different substrates on phytochemical and nutritional components of mushrooms

Quantitative/Qualitative parameter	Mushroom grown on Substrate S1	Mushroom grown on Substrate S2	Mushroom grown on Substrate S3
Protein content (mg/gm Fungal Powder)	20.179	32.157	41.786
% Fibre Content	23.2	2.4	8.4
Alkaloid Content (µg Atropine Equivalents/ mg Extract)	104.068	75.593	33.559
Terpenoids Content (µg/mg)	267.167	158.167	609
Phenolic Content (µg/mg Extract)	19.96	11.38	17.48

Table 4: Source Spectra Results- Mushrooms grown on Substrate S1

Peak Number	Wave No. (cm-1)	% T	Assignments	Possible Molecule Type
1	3914.97	80.48	Hydroxy group, O-H stretch	Alcohol
2	3779.63	79.93	Hydroxy group, O-H stretch	Alcohol
3	3425.47	52.04	Normal ‘‘polymeric’’ / Hydroxy group	O-H stretch
4	2063.96	79.82	Transition metal carbonyls / Isothiocyanate	(-NCS)
5	1637.16	71.69	Alkenyl / Primary amine/Amide /Quinone or conjugated ketone / Open-chain imino /Organic nitrates	C=C stretch/ NH bend / (C=N-)
6	1440.52	78.94	Methyl C-H	asym /sym. Bend
7	1231.12	79.30	Aromatic phosphates/Aromatic ethers	(P-O-C stretch)/aryl -O stretch
8	1151.91	79.37	Tertiary alcohol /Aromatic C-H in-plane bend / Secondary amine, /Tertiary amine	C-O stretch/ CN stretch
9	1069.64	77.93	Organic siloxane or silicone /Primary amine / Alkyl-substituted ether / Aliphatic fluoro compounds / Aromatic C-H in-plane bend/ Aliphatic bromo compounds	(Si-O-Si)/CN stretch /C-O stretch /C-F stretch / C-Br stretch
10	697.82	78.61	Thiol or thioether,/ Alcohol, OH out-of-plane bend / Aliphatic bromo compounds	CH2-S-(C-S stretch) / C-Br stretch

2.3 Incubation

The prepared mushroom bags were placed on racks in a sterilized dark room for incubation. The average room temperature around 26-30°C and relative humidity ranging from 70 to 80%, maintained by sprinkling clean water on bags when required. All the bags were incubated until mushroom mycelium had completely colonized the substrate.

2.4 Fruiting and harvest

After mycelium run completely covers the substrate in bags, they were shifted to the fruiting room with dispersed light and proper ventilation. Watering of bags is continued to maintain high relative humidity for the development of fruiting body. Once the fruiting was completed, i.e., the in-rolled borders of the mushroom caps commence to flatten; the mushrooms were plugged out of the bags by moving the stalk right and left sides carefully. For all tested substrate, 3 flushes of oyster mushrooms harvested from each culture bags.

2.5 Data collection

Various morphological parameters including spawn running time (days), emergence of primordia (days), period from primordial initiation to harvesting (days), and number of fruiting bodies observed and recorded. After complete harvesting, the accumulated data were used to find out total yield and the biological efficiency (BE). BE is calculated as the fraction of fresh mushroom weight (g) per dry weight of the substrates (g), and is expressed as percentage.

2.6 Qualitative and quantitative analysis of mushroom fruiting bodies

2.6.1 Extraction of samples with Hydroalcoholic Extraction : Dried mushroom powder 1 gm, collected from all the three substrates were separately assorted with 10 ml of solvent mixture (80% Methanol, 1% HCL in distilled water). Sample mixtures were incubated on a rocker shaker for 24 hours. The obtained extracts were individually filtered over Whatman filter paper 1 and oven dried completely at 40°C (Table 1). The prepared extracts were collected in micro centrifuge tube and stored at 4°C.

2.6.2 Estimation of Total Alkaloid Content : Total alkaloid content of mushroom extracts was analyzed by following modified protocols of Ajanal *et al.* (2012) and Trease and Evans, (2002). Extract 100µl was dissolved in 400µl 2N HCl, in 1.5 ml tube and incubated for 30 mins. Further, centrifuged at 10000 rpm and the collected 250µl of the supernatant was mixed with 1500 µl Chloroform and mixed well. After the two phases separated, the chloroform was removed and the pH was adjusted to 7. Thereafter, 500µl of BCG solution (0.1 mM) and 500µl of phosphate Citrate buffer were added to the supernatant and the final extraction was completed by adding 500µl chloroform and pooled in the fresh tube. All the three samples were dehydrated and liquified in 500µl DMSO. Lastly, 100µl of each sample was transferred to micro-test plate and read at 470nm.

2.6.3 Estimation of total Terpenoids content : The protocols of Ghorai *et al.* (2012) were followed with slight variations, where 500mg each of the three samples was taken in screw capped tubes and freezed. 1.5 ml of Hexane Acetone Mixture (1:1) was added and the tissue were homogenized for 5 minutes in bead beater. After centrifugation, the supernatant was collected and reduced to 1 ml using nitrogen gas and 2 ml of chloroform was thoroughly mixed. 200 µl Conc. H_2S0_4 was added to precipitate the extracts and incubated in dark at room temperature. Thereafter, the supernatant was removed and 3ml of absolute methanol was used to completely dissolve the precipitate. Spectrometric absorbance was read at 538 nm.

2.6.4 Estimation of Total Phenolic Content : The method described by McDonald et al. (2001) was used to estimate the phenolic compound in different test samples. The hydroalcoholic extract prepared were diluted with aqueous 40 µl Na_2CO_3 (1.0 M) and Folin Ciocalteu reagent. The absorbance was read at 760 nm by spectrophotometer. The preparation of standard curve of Gallic Acid was done in Methanol: Water (50:50 v/v) at concentrations 25 µg/mL to 300µg/mL.

2.6.5 Estimation of antioxidant activity : DPPH Assay was conducted using protocols described by Kenar and Kaladhar (2021). The free radicles scavenging activity was represented as '% inhibition' with respect to control (Ascorbic acid) and calculated using following formula;

$$\text{DPPH Scavenging activity} = ((Abs_{Control} - Abs_{Sample})/Abs_{Control}) \times 100$$

2.6.6 Estimation of crude fiber content : Coarse mushroom powder 2000 mg was extracted with Petroleum Ether. Then, 500 mg of dried sample was boiled with 25 ml H_2SO_4 (1.25%) for 30 minutes, filtered and washed. The obtained sample was boiled with 50 ml Sodium Hydroxide solution (1.25%) and filtered. The filtrate was again washed with a mixture of 5 ml of boiling sulfuric acid, 5ml alcohol and 30ml water. The residues obtained were transferred to ashing dish and dried as recommended.

2.6.7 Estimation of isolated Protein content : Mushroom tissue 100 mg was homogenized in 1000 µl Radioimmunoprecipitation assay cell lysis buffer. Lysate was centrifuged and supernatant was collected in an Eppendorf tube on ice, containing soluble protein.

Table 5: Source Spectra Results: Mushrooms grown on Substrate S2

Peak Number	Wave No. (cm-1)	% T	Assignments	Possible Molecule Type
1	3914.99	81.54	Hydroxy group, O-H stretch	Alcohol
2	3780.37	80.95	Hydroxy group, O-H stretch	Alcohol
3	3693.47	80.81	Hydroxy group, O-H Stretch	Alcohol
4	3402.16	68.43	Hydroxy group, H-bonded OH stretch	Alcohol and hydroxy compound
5	2927.08	76.63	Methylene C-H asym./sym. Stretch	Methylene(>CH2)
6	2299.63	80.44	Alkanes, Alkyl groups	C-H
7	1734.08	78.15	Aldehyde	Carbonyl compound
8	1638.39	78.11	Aromatic combination bands /Alkenyl C=C stretch / Quinone or conjugated ketone / Secondary amine, > N-H bend	Aromatic ring (aryl) /Olefinic (alkene) / Carbonyl compound/ Secondary amino
9	1440.90	79.73	Methyl C-H asym./sym. Bend	Methyl (-CH3)
10	1234.98	79.74	Aromatic ethers, aryl -O stretch	Ether and oxy compound
11	1150.98	79.95	Tertiary alcohol, C-O stretch/ Secondary amine, CN stretch / Sulfonates Organic Sulphates	Alcohol and hydroxy compound/ Ether and oxy compound (Secondary amino)/Sulfur-oxy compounds
12	1029.64	78.31	Cyclohexane ring vibrations/Skeletal C-C vibrations/ Aliphatic fluoro compounds, C-F stretch/Primary amine, CN stretch / Organic siloxane or silicone (Si-O-Si)	Methylene (>CH2) /Aliphatic organohalogen compound /Ether and oxy compound (Primary amino)/ Silicon-oxy compounds
13	702.55	81.33	Aromatic C-H out-of-plane bend/ Thiol or thioether, CH2-S-(C-S stretch)	Olefinic (alkene)/ Thiols and thio-substituted compounds
14	648.71	81.37	Aliphatic chloro compounds, C-Cl stretch/Alkyne C-H bend / Thioethers, CH3-S-(C-S stretch)	Aliphatic organohalogen compound / Acetylenic(alkyne) /Thiols and thio-substituted compounds

Table 6: Source Spectra Results: Mushrooms grown on Substrate S3

Peak Number	Wave No. (cm-1)	% T	Assignments	Possible Molecule Type
1	3914.75	82.52	Hydroxy group, O-H stretch	Alcohol
2	3780.13	81.97	Hydroxy group, O-H stretch	Alcohol
3	3423.15	62.79	Hydroxy group, H-bonded OH stretch	Alcohol and hydroxy compound
4	2113.80	82.17	C=C Terminal alkyne (monosubstituted	Acetylenic (alkyne)
5	1637.21	77.58	Alkenyl C=C stretch	Olefinic (alkene)
6	1437.07	81.46	Methyl C-H asym./sym. Bend /Methylene C-H bend	Saturated Aliphatic (alkene/alkyl), Methyl (-CH3)
7	1069.12	80.68	Alkyl-substituted ether, C-O stretch / Aliphatic fluoro compounds, C-F stretch	Ether and oxy compound/ Aliphatic organohalogen compound
8	649.15	81.66	Aliphatic bromo compounds, C-Br stretch/Alkyne C-H bend	Aliphatic organohalogen compound/Acetylenic(alkyne)

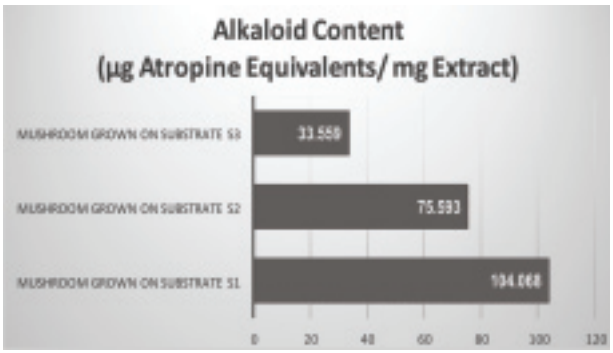

Fig 1: Graphical representation of Alkaloid content in mushrooms grown on different substrates: Comparative analysis

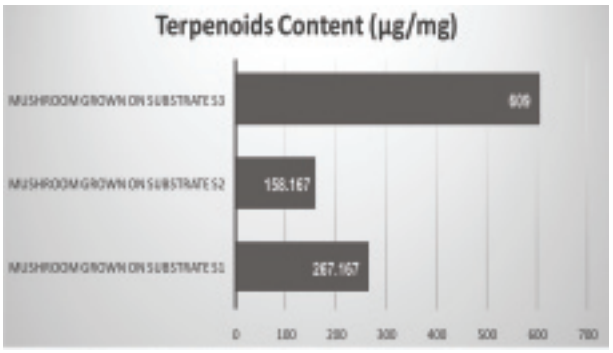

Fig 2: Graphical representation of Terpenoids content in mushrooms grown on different substrates: Comparative analysis

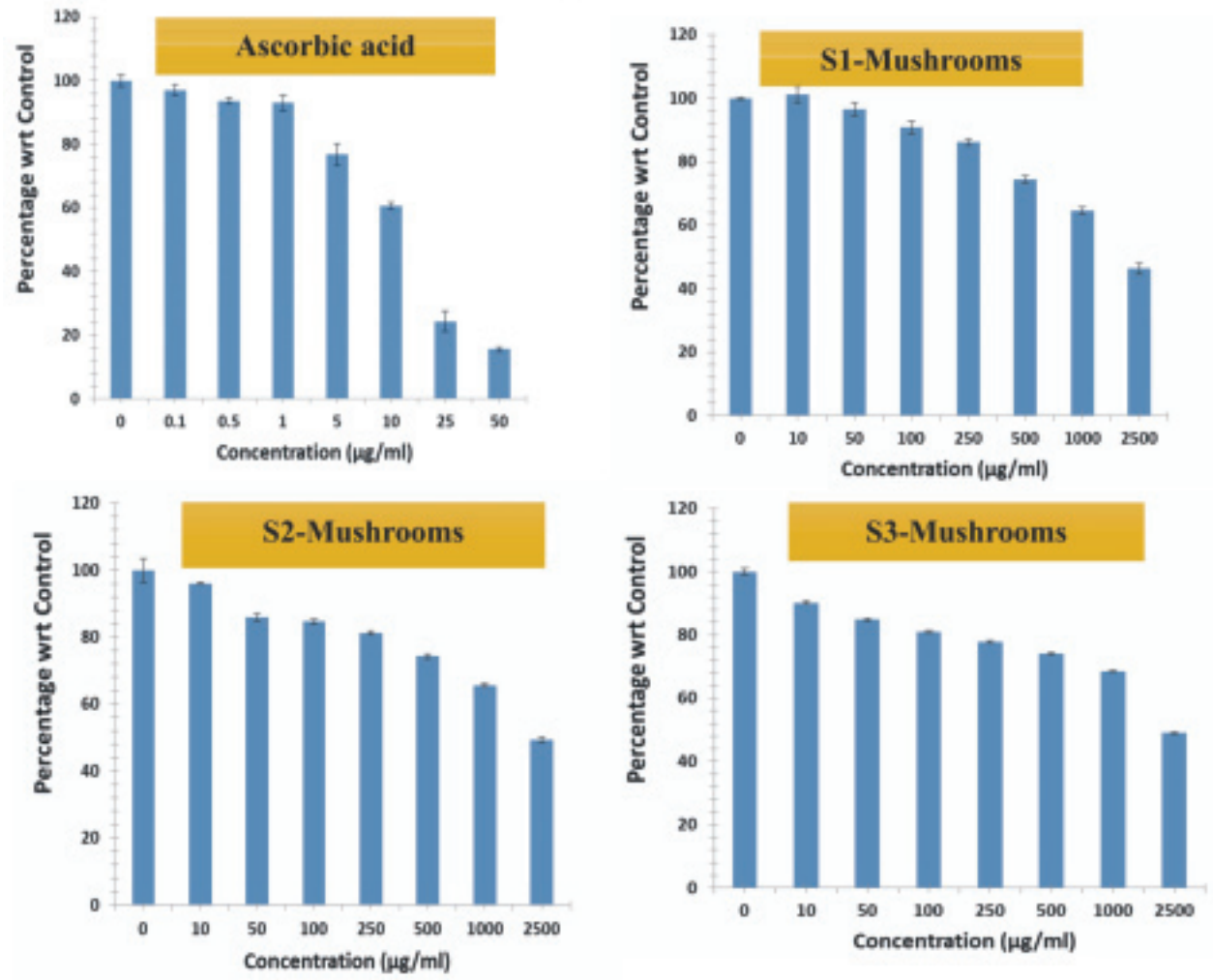

Fig 3: Graphical representation of antioxidant activity of mushrooms grown on different substrates S1, S2 and, S3; and ascorbic acid as control.

Bradfoard reagent was added with standard Bovine Serum Albumin in different concentration for standard curve preparation and that of with prepared sample for protein estimation in plates and incubated. Finally, the absorbance was taken at 595 nm using ELISA Microplate Reader.

3. RESULT AND DISCUSSION

3.1 Effect of different substrates on growth, yield and morphological parameters of mushrooms

3.1.1 Spawn running time (days): One of the important aspects of mushrooms cultivation is the growth of fungal mycelium in the substrate. Among the tested substrates, the earliest one to complete spawn run in compost was substrate S2 (18 days), followed by substrate S3 (21 days) and, longest period of time was taken in Substrate S1(28 days). This high mycelial running rate can be accredited to the high lignocellulosic content of newspaper. Kopiñski *et al.* (2012), also reported similar findings where best growth of Oyster mushroom was reported in combination of waste newspaper with wheat straw in 3:1 ratio while no fruiting in only newspaper as substrate. Albores´ *et al.* (2006), attributed mycelium running rate to be positively correlated to C : N ratio of the substrate.

3.1.2 Formation of pinhead, number of fruiting bodies and maturity : There was noteworthy difference in the number of fruiting bodies among all substrates. The maximum fruiting bodies of Oyster mushroom were recorded in bags with plastic + wheat straw i.e., 31 in the 1st flush while the lowest number was found in bags with wheat straw as substrate, i.e., 17, and the bags with newspaper + wheat straw, straw as substrate had 26 fruiting bodies. Haque (2004) reported maximum fruiting bodies in paddy straw as substrate while Nasiruddin *et al.* (2008) reported highest number of fruiting bodies of Oyster mushroom grown on sugarcane bagasse as substrate and that of lowest mustard straw as substrate. Substantially the formation of mushroom pinhead is altogether different phenomenon as related to the mycelial growth (Patrick *et al.,* 2014). Although it took longer for substrate S1 to completely colonise the substrate, but the duration of pinhead maturation was significantly shortest in contrast to other substrates. The similar findings were reported by Muswati *et al.* (2021), and reportedly the longest growing time of the mushroom was found in compost with baobab fruit shells + wheat straw however, maximum pins were formed in the same. As stated by Mkhize *et al.* (2016), the formation of primordia is unswervingly related to the accessibility of C : N from lignocellulose substrate and it has fundamental role in mycelium growth and development of the fruiting body. The growth rate o mycelium was fast in S2 and S3, but the pinheads or primordia were significantly less in numbers and this

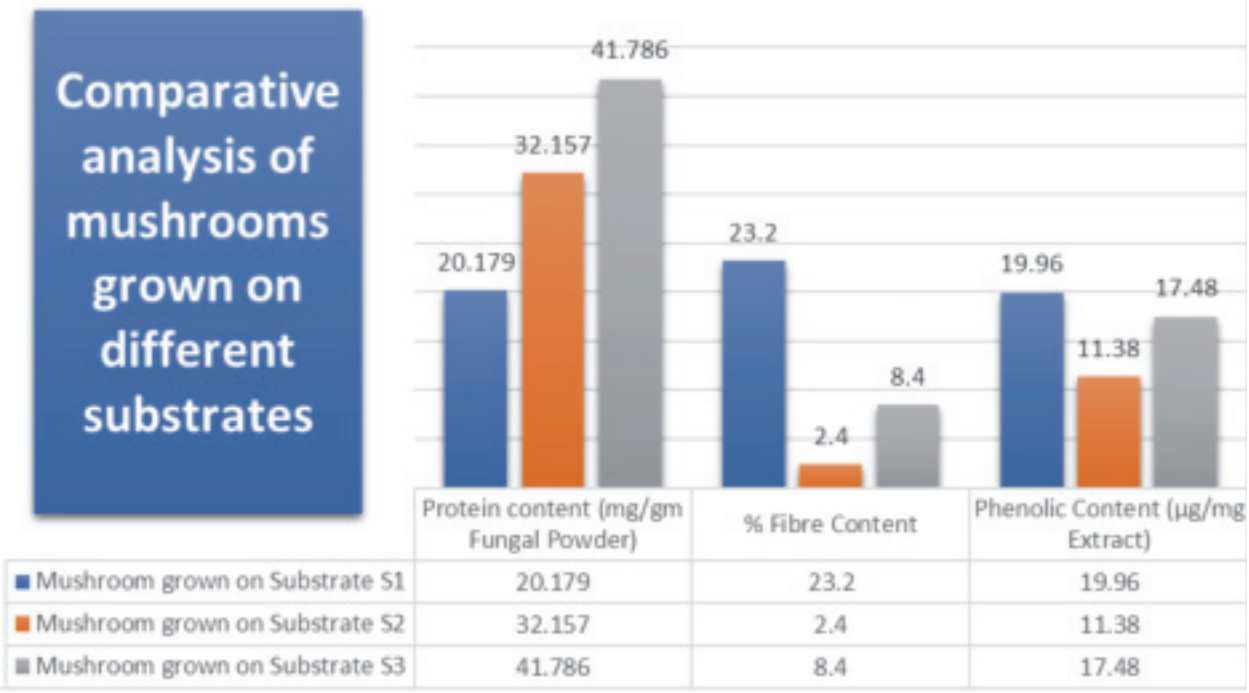

Fig 4: Comparative analysis of qualitative and quantitative parameters of the mushrooms grown on different substrates (S1, S2, and, S3)

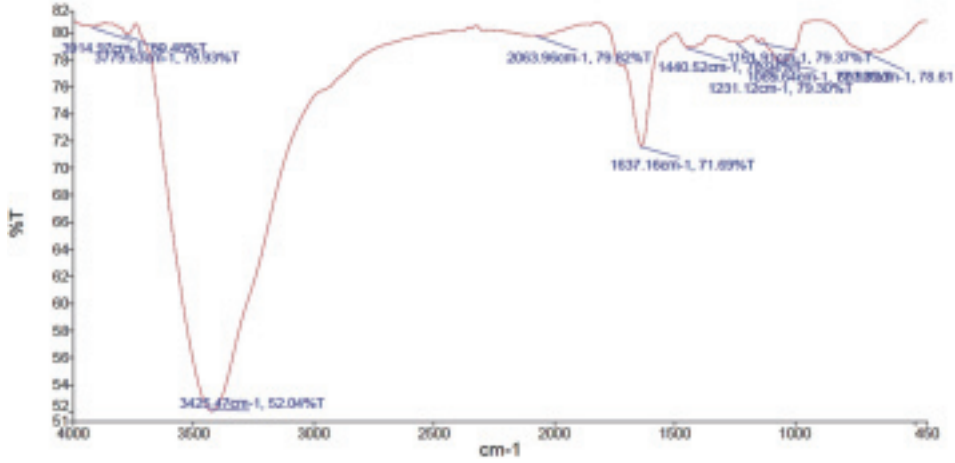

Fig 5: FTIR Analysis: Mushrooms grown on Substrate S1

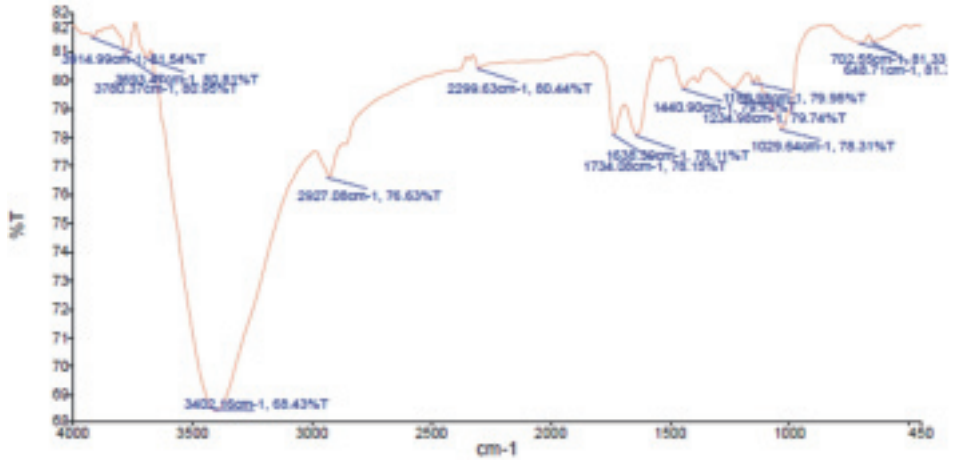

Fig 6: FTIR Analysis: Mushrooms grown on Substrate S2

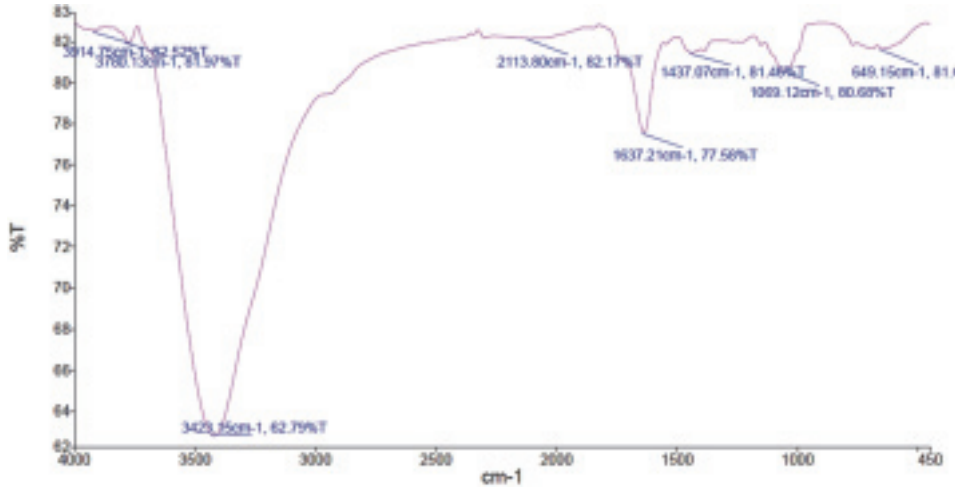

Fig 7. FTIR Analysis: Mushrooms grown on Substrate S3

majorly can be attributed to high Nitrogen content in wheat straw which is likely to inhibit mushroom growth. Therefore, our findings show a positive correlation amid the C:N ratio of substrates used and maturation of mushroom fruiting bodies.

3.2 Qualitative and Quantitative analysis of mushrooms grown on different substrates: comparative study:

3.2.1 Phytochemical analysis : *Pleurotus ostreatus* is an edible medicinal mushroom that has numerous active metabolites, functional as anti-inflammatory, antioxidants, cholesterol lowering agents and even as anti-cancerous. The phytochemical screening of the three types of mushroom fruiting bodies grown on different substrates showed the presence of the terpenoids, phenolics, alkaloids, but their concentrations varying significant among the three types. The alkaloid and phenolic content were detected at highest levels in fruiting bodies grown on plastic + wheat straw, as shown in Table 3. The high phenolic compounds are alleged to be able to inhibit TNF-α gene expression as well as their production (Paul *et al.,* 2006), moreover, hydroxyl content of phenols is the main compound that is believed to be responsible for the antioxidant possessions of oyster mushrooms (Sigh *et al.,* 2015). Meanwhile, the mushrooms grown on wheat straw possessed least amount of alkaloids and those on newspaper + wheat straw had least phenolic content as depicted in Fig. 1 and 4. However, highest terpenoid content was again found in mushrooms of substrate S1 (Fig. 2).

3.2.2 DPPH Assay (Antioxidant activity) : The antioxidant activities were evaluated against DPPH Assay where DPPH free radical compound examines the free radical scavenging ability of the test sample. The antioxidants present in samples neutralise the DPPH by transferring an electron or hydrogen atom and the colour changes during this process are detected by the decrease in its absorbance at 517 nm. In the present study, Ascorbic acid was used as control and it showed highest scavenging effects on DPPH assay, followed by oyster mushrooms grown on substrate S1 (1009µg/ml IC_{50}), while least antioxidant activity was observed from the substrate S2 (Table 2 Fig. 3).

3.2.3 Protein and Fibre contents : Crude protein content in the mushrooms show significant difference from the substrate they obtained from. The highest amount of protein was present in mushroom obtained from substrate S3 (41.786 mg/gm mushroom powder). Tolera *et al.* (2017), reported that the procedure used for drying the mushroom prior to protein analysis has great effect on its value like, high protein content found in sundried mushrooms as compared to oven dried mushrooms. Correspondingly, crude fiber content was highest in mushrooms grown in plastic + wheat straw substrate (23.2%), whereas, lowest fibre levels observed in the mushrooms obtained from substrate S2 (Table 3).

3.3 FTIR Analysis

FTIR analysis perceives the occurrence of functional groups in binding of particles with biomass. The functional characteristics of oyster mushroom are mainly due to their chemical composition (Bernas et al., 2006). The regions of band obtained and peaks found in biomass of the three types of mushrooms are described in Figures 5 to 7. The results clearly show that hydroxyl group, Aliphatic organohalogen compound, and Olefinic compounds stretches were most common among the three fruiting bodies, however, significant variations occurred in the wavelengths related to Aliphatic bromo compounds, Acetylenic Silicon-oxy compounds, Ether and oxy compound, Thiols and thio-substituted compounds and several others.

4. CONCLUSION AND RECOMMENDATIONS

The findings of the current study concluded that composition of compost substrate has profound effects on the performance and growth of oyster mushroom. The presence of high phenolic contents as well as greatest antioxidant activity of mushrooms grown on waste plastic + wheat straw substrate, along with possession of highest fiber content in it shows that growing mushroom on such waste is highly feasible and recommended practice. Also, the cultivation of *P. ostreatus* on plastic waste and lignocellulosic wastes can be great initiative in utilization of non-biodegradable waste along with endorsing production of healthy food.

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