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A multifaceted analysis of spent mushroom substrate of selected oyster mushrooms for enzymatic activity, proximate composition, and antimicrobial activity

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Abstract

The global market for mushrooms is growing due to its nutritional enrichment, potential usage as a bioremediation, enzyme production, and functional food development. However, the leftover post-harvest mushroom substrate (SMS) generates certain environmental concerns. This study aimed to investigate the potential of SMS obtained from two oyster mushroom species—*Pleurotus ostreatus* and *Pleurotus djamor*. These were examined regarding sustainability by analyzing their lignocellulosic enzyme production, cellulose yield, antimicrobial properties, and proximate composition. The findings for both *P. ostreatus and P. djamor* showed higher activity of amylase, that is, 0.3 U (μmol/min) and 0.7 U (μmol/min), respectively, compared to activity of cellulase, which showed 0.3 U (μmol/min) and 0.5 U (μmol/min), respectively. SMS showed the highest activity of lignocellulosic enzymes, compared to non-SMCs and controls at $p \leq 0.00$ and ≤0.01), proving fungual mycelia as the precursor of enzymes activity, as no mushroom is cultivated due to least enzymatic activity. The results for proximate analysis of SMCs showed a significant difference from non-SMCs. The findings for *P. djmor* revealed protein (1.23%), fats (1.3%), and ash (8.11), which were significantly higher than in *P. ostreatus.* A positive co-relation of 52% was established between SMCs with amylase, while a correlation of 20% was observed with cellulase, depicting an impact of mycelia in the breakdown of protein for amylase production. The SMC samples were also subjected to antibacterial analysis against *Staphylococcus aureus, E. coli,* and *Xanthomonas.* A higher minimum inhibition concentration (MIC) was recorded for *P. djamor*, that is, 8.80 mm, 11.66 mm, and 9.04 mm, compared to *P. ostreatus*, which showed its highest MIC as 9.18 mm, 9.30 mm, and 9.28 mm for *S. aureus, E. coli,* and *Xanthomonas*, respectively. It was evident from the study that SMC has a potential of being utilized for bioremediation, as it is therapeutically active against pathogens. Additionally, *Pleurotus* spp. is of great interest because of its ability to produce high nutritive value, cellulose yield, and a vast amount of lignocellulosic enzymes. The current experiment recommends the use of distilled water for mushroom farming, as enzymatic activities can significantly be affected by pH and buffers. Furthermore, the spent compost, being rich nutritionally, can be used for soil enrichment or as a biofertilizer.

Keywords: biofertilizer; lignocellulosic enzymes; spent mushroom substrate; *P. djamor*; *P. ostreatus*

Introduction

Globally, mushroom farming is booming because of their rich nutritional status (Singh and Sohrab, 2024). The projected global market for mushroom would be 24.05 million tons by the year 2028 (Arshadi *et al.*, 2023). *Pleurotus* genus is ranked second with respect to mushroom farming after *Agaricus* genus. This is due to its adaptability (Melanouri *et al.*, 2022; Prokisch *et al.*, 2021). There are numerous attributes associated with Oyster mushrooms, a common name for Pleurotus mushrooms. Different studies have shown their capacity to lower the ecological issues because of their low-cost production. Pleurotus mushrooms are characterized by their medical, biotechnological, and nutritional attributes. Numerous studies have reported the many relevant features of Pleurotus genus, which confirm their being an attractive low-cost industrial tool that resolves the pressure of ecological issues (Guo *et al.*, 2022; Leong *et al.*, 2022; Rajavat *et al.*, 2022). These key issues involve different enzymatic attributes, such as oxidases and hydrolases along with biomass from various fruit residues, production of biofuels, bioremediation, and medicinal attributes (Rauf *et al.,* 2023; Aziz *et al.*, 2023; Sana *et al.*, 2023; Ahmad *et al.,* 2023; Ejaz *et al.,* 2023; Mwangi *et al.*, 2022; Ranjithkumar *et al.*, 2022; Wang *et al.*, 2022). The substrates of oyster mushrooms have the potential to produce certain bioactive substances for functional foods (Caldas *et al.*, 2022). The biochemical characteristics of mushroom substrates are often affected at the time of preparation, resulting in their lower biological efficiency (Zhang *et al.*, 2023; Dedousi *et al.*, 2023; Lu *et al.*, 2023; Torres-Martínez *et al.*, 2022).

Spent mushroom substrate (SMS) refers to the leftover substrate from oyster mushrooms. During mushroom farming, two types of substrates, composted and non-composted substrates, are often used. Composted substrates are fermented substrates, while the later lacks fermentation. SMS is a complex mixture of lignocellulose by-products and a rich source of organic matter and mycelium (Economou *et al.*, 2020; Guo *et al.*, 2022). Studies have shown that 1 kg of fresh mushroom causes 5 kg of SMS (Lin *et al.*, 2014; Zisopoulos *et al.*, 2016). The biochemical composition of SMS consists of lignocellulose and its derived enzymes, various organic compounds, such as carbohydrates, fats, and proteins, and inorganic compounds, such as ammonium nitrates. SMS mostly comprises wheat, sawdust, rice straw, and corncobs, which are highly lignocellulosic, and nutrients such as nitrogen (N), phosphorus (P), and potassium (K) (NPK). Apart from this, different heavy metals, such as copper (Cu), zinc (Zn), and cadmium (Cd), are also found in SMS (Nureen *et al.,* 2023; Gul *et al.*, 2023; Wajid *et al.*, 2023). The substrates are often disposed of by burning, spreading on land, or compositing (Ahlawat and Sagar, 2007; Diamantopoulou and Philippoussis, 2015). This leads to pollution in the environment if not disposed of with a sustainable approach (Zhang S *et al.*, 2023). Current studies have shown that SMS is of great value and numerous high value-aided products, such as enzymes (laccase, peroxidases, etc.) can be synthesized. SMS are highly nutritious in terms of proteins and hydrocarbons; hence, it can be an effectively used in different industries, such as food and beverages (Aggelis *et al.*, 2003; Chowdhary *et al.*, 2019; Economou *et al.*, 2017; Ghorai *et al.*, 2009; Lavelli *et al.*, 2018). Research demonstrated that SMS comprised lignin (11–15%), cellulose (11–15%), hemicellulose (29–35%), and protein (7%).

Different challenges and exciting opportunities exist for the SMS of oyster mushrooms to contribute to sustainability. The current study was conducted keeping in mind the multifaceted potential of SMS (fermented and non-fermented) of two oyster mushroom species—*Pleurotus ostreatus* and *Pleurotus djamor*. The study aimed to analyze lignocellulosic enzyme production, cellulose yield, antimicrobial properties, and proximate composition of these two species. The study was effective regarding its wide range of application in bioremediation, biofuel production, and animal feed. A sustainable and value-added agricultural approach was achieved by optimizing mushroom selection.

Methodology

For the current study, two different types of mushroom substrates, spent and non-spent, of *P. ostreatus* and *P. djamor* species along with control were collected from Mushroom House, AUP, Peshawar, Pakistan. The substrates were subjected to refrigeration at 20°C till further analysis. All analyses were conducted in triplicate. Furthermore, the experiments were conducted in a completely randomized design (CRD).

Cellulose extraction

In order to extract cellulose from the samples, the protocol of Sun and Tomkinson (2003) was followed: 10 g of each sample was taken and treated in acidic medium, that is, 70% nitric acid and 80% acetic acid, followed by heating at 120°C. The mixture was diluted, filtrated, and rinsed with ethanol later to remove acidic residues and extraction breakdowns. The final residues were oven-dried at 120°C. In order to remove hemi-cellulose and lignin from the obtained residues, the protocol of (Lohmousavi *et al.* 2020) was used, where NaOH was used. Residues were then washed with distilled water, followed by filtration and oven-drying at 80°C. Later on,

bleaching of the samples was done in hydrogen peroxide. The bleached straw cellulose powder was oven-dried after filtration and removal of bleaching residues.

Preparation of standard solutions and substrates

The following standard solutions and substrates were prepared during the experiment.

Preparation of maltose, glucose, and starch solution
Maltose and glucose standards were prepared in 1:5 ratio (w/v). A stock solution of 2000 ppm was prepared afterward for each standard. Additionally, 1% starch solution was prepared for enzyme detection.

Preparation of 3,5-dinitrosalicylic acid (DNS) and carboxymethyl cellulose (CMC)
In order to make DNS reagent, 1 g of of 3,5-dinitrosalicylic acid was dissolved in 50 mL of distilled water, followed by addition of 30-g sodium potassium tartrate tetrahydrate. Later on, 20 mL of 2-N NaOH was added. A standard stock solution was prepared by making the final volume to 100 mL. For carboxymethyl cellulose, powdered CMC was purchased from distributor of Sigma Aldrich in Pakistan, of which 0.2 mg was dissolved in 50 mL of distilled water. It was later sonicated for 10 min for complete dissolution so that it could be used for cellulase enzyme detection.

Analysis of extracellular enzymes

The extracellular enzymes were analyzed as per the protocol (Kumar et al., 2020; Miller, 1959). All the collected samples were subjected to enzymatic analysis after extraction of extracellular enzymes in 1:50 ratio (w/v) from alkaline buffer kept for 24 h. The samples were centrifuged at 200 rpm for 12 h at 20°C. Later on, supernatants were collected in additional vials and stored at 575 nm for enzymatic analysis.

Measurement of amylase and cellulase activity
Amylase activity in spent and non-spent mushrooms, along with control, was measured using Miller's (1959) method. In brief, 1.5 mL of supernatant extract from each treatment was mixed with 1.5 mL of 1% starch solution. The mixture was incubated at 50°C for 1 h. To this, 1.5 mL of DNS was added. This was kept on water bath for 10 min. After boiling, the quantification was measured at 575 nm via spectrophotometer. Amylase activity was calculated with maltose as a standard.

For measuring cellulase activity in samples, a mixture of samples' extracts in CMC solution was incubated at 50°C for 1 h. DNS reagent was added to stop the reaction. The quantification was measured at 575 nm using a spectrophotometer, calculating cellulase activity using glucose as a standard.

Proximate analysis

The proximate analysis of all the collected samples was determined in the Department of Agricultural Chemistry and Biochemistry, University of Agriculture, Peshawar, as per the methods described by Association of Official Analytical Chemists (AOAC, 2004).

Moisture Content
All the samples were cut into small pieces and subjected to oven-drying. The samples were kept at 105°C for 24 h. After that, moisture content was determined by the following equation:

$$\text{Moisture Content } (\%) = \frac{A - B}{W} \times 100$$

where A = initial weight of crucible and sample, B = final weight of crucible and sample, and W = weight of the sample.

Ash content
Each sample, 1 g, was oven-dried overnight at 105°C. It was then ignited in muffle furnace at 600°C for 5 h. Ash content of the samples was calculated by the following equation:

$$\text{Ash Content } (\%) = \frac{A - B}{W} \times 100$$

where A = initial weight of crucible and sample, B = final weight of crucible and sample, and W = weight of the sample.

Determination of crude protein
Initially, percentage of N was calculated using the Kjeldhal procedure, in which all the samples were taken in digestion flasks. To this, digestion mixture and H_2SO_4 were added slowly. The mixture was kept on digestion heater till the appearance of green color. The digest was made alkaline in 40% NaOH solution, followed by distillation. Following the distillation, ammonia collected was titrated with 0.1-N HCl solution and titrated value was recorded as follows

$$\%N = \frac{(S - B) \times 0.1 \times 0.014 \times D \times 100}{W * V}$$

where S = sample titration reading and B = Blank titration reading.

The conversion factor of 6.25 was used for determination of protein.

Determination of crude fat

Soxhlet extraction procedure was followed in order to determine the crude fat as per AOAC (2005). Each sample was first heated and then crushed by using pestle and mortal. In a thimble, 1 g from each sample was taken and petroleum ether was added to it. Extraction was done by turning on water and heater. Clean glasswares were used to collect the extract, which was washed with ether and allowed to evaporate in a water bath. Then the glasswares were placed in an oven at 105°C for 2 h. These were allowed to cool down in a desiccator for 30 min,

$$\%\text{Fat} = \frac{W_2 - W_1}{W_s} \times 100$$

Determination of crude fiber

Fiber content was determined by acid digestion, followed by the basic digestion method. Samples were properly filtered after acid digestion. The samples were washed and subjected to basic digestion. The filtrate was also washed with hot water. The filtrate left from basic digestion was collected in a clean and dried crucible. This crucible was dried in an oven at 130°C for 2 h. Readings were taken after the crucible was cooled in a desiccator.

$$\%\text{Fiber} = \frac{W_A - W_B}{W_s} \times 100$$

Determination of nitrogen-free extract

The nitrogen-free extract (NFE) was calculated by differences after analysis of all other constituents of proximate analysis as per the equation given below:

NFE (%) = 100 – (moisture [%] + crude protein [%] + crude fat [%] + crude fiber [%] + ash [%]).

Antimicrobial assay

Standard disc diffusion method (Tassou *et al.,* 2000) was used to assess antibacterial activity of methanolic extracts from spent and non-spent compost *of P. ostreatus* and *P. djamor* mushrooms. Microbial inoculations on prepared media (Nutrient Agar Media) were made using Whatman Grade 6 filter paper discs (6 mm). The discs were soaked in three concentrations (500 mL, 1000 mL, and 1500 mL) of methanolic extract applied. Each plate had the following five discs: standard (positive control), dimethyl sulfoxide (DMSO) (negative control), and three discs for varying extract concentrations. Zone of inhibition (in mm) was measured after 24 h against *E. coli, S. aureus*, and *Xanthomonas.* Percentage of inhibition was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Zone of inhibition for extract}}{\text{Zone of inhibition for standard}} \times 100$$

Statistical analysis

The collected data were analyzed according to factorial combination in a completely randomized design and the mean was calculated using the least significant difference (LSD) test at 5% probability.

Results and Discussion

Comparative study of lignocellulosic enzymes in mushroom substrates

Comparative graphical results are depicted in Figure 1. Spent and non-spent mushroom compost of *P. djamor* (pink) exhibited the highest amylase activity of 1 U (μmol/min) and 0.81 U (μmol/min), respectively. This was significantly higher, compared to *P. ostreatus* (white), which exhibited an amylase activity of 0.74 U (μmol/min) and 0.59 U (μmol/min), respectively. In contrast, an activity of 0.08 U (μmol/min) was recorded for the control. In addition to amylase activity, cellulose content in *P. djamor* (pink) was observed as 0.586 U (μmol/min) and 0.35 U (μmol/min) in its spent and non-spent mushroom substrates, respectively. Cellulose content in *P. ostreatus* was calculated as 0.42 U (μmol/min) and 0.2 U (μmol/min) in its SMS and non-SMS, respectively. Compared to this, the control exhibited 0.02 U (μmol/min) of cellulose content. The current results demonstrated that amylase and cellulase activity could be increased by 70% and 80%, respectively, if the SMS was subjected to hot water fermentation. Hence, it was concluded that mushroom cultivation could enhance enzymatic production in both SMS and non-SMS. The lack of growth and enzyme activity in the control provided further support to obtained results.

Comparison of protein (%) with respect to enzymatic profiling of mushroom composts

The proximate analysis of samples demonstrated that the protein content was linked to the production of enzymes. The study showed a high respective protein content of 1.23% and 1.01% for spent *P. djamor* and spent *P. ostreatus*, followed by respective protein content of 1.05% and 0.92% in non-spent *P. djamor* and and *P. ostreatus*. The fermented substrate, however, resulted in 0.99%, while non-fermented substrate exhibited 0.88% of protein

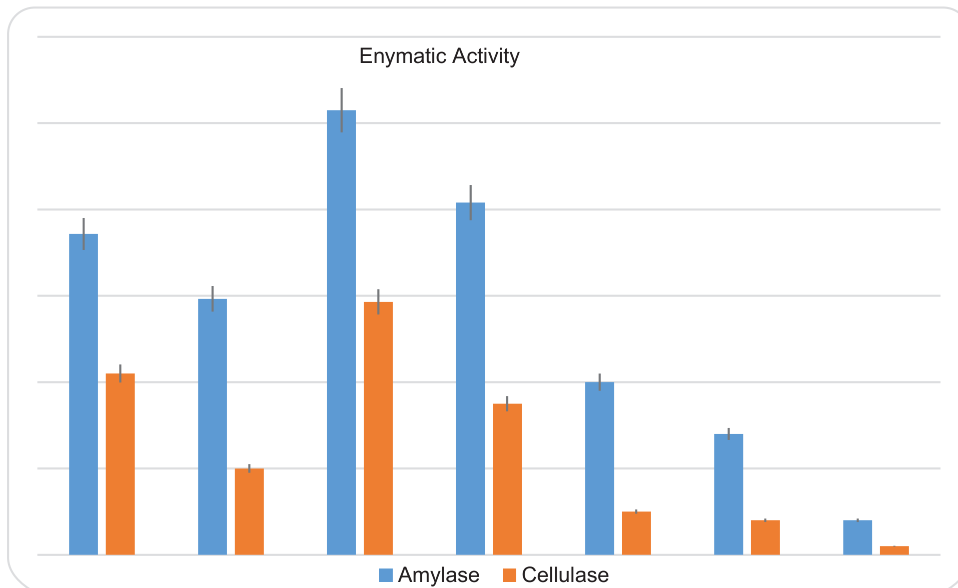

Figure 1. Comparative analysis of lignocellulosic enzymes.

Table 1. Comparative analysis of protein, amylase, and cellulose in various mushroom substrates.

Treatment	Protein (%)						
	WS	WNS	PS	PNS	FS	FNS	Control
Protein	1.01	0.92	1.23	1.05	0.99	0.88	0.44
Amylase U (µmol/min)	0.74	0.5	1.3	0.8	0.4	0.2	0.08
Cellulase U (µmol/min)	0.42	0.20	0.5	0.3	0.10	0.08	0.02

WS: White Spent; WNS: White Non-Spent; PS: Pink Spent; PNS: Pink Non-Spent FS: Fermented Sterile; FNS: Fermented Non-Sterile

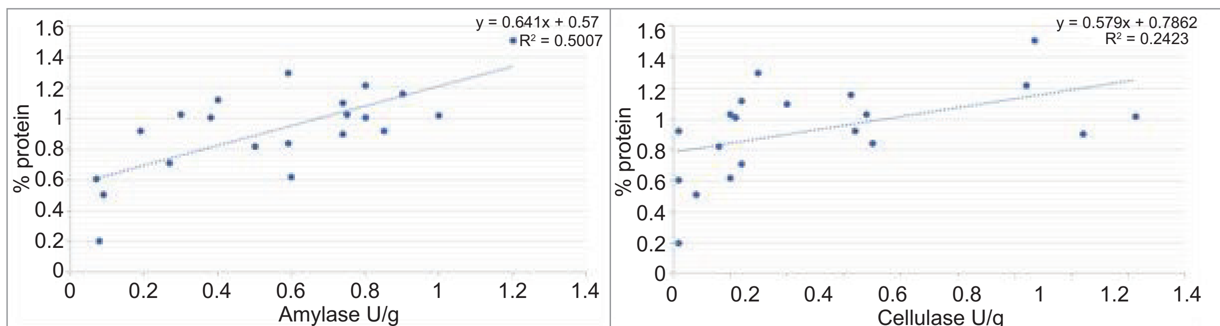

Figure 2. Corelation of amylase and protein contents.

content. Furthermore, a contrast result for the control revealed 0.44% of protein content (Figure 2). All the findings are given in Table 1.

A link was drawn between protein and enzymatic profiling. The wheat straw with no treatments (Control) showed the presence of 0.44% protein and hence secretes 0.08-U amylase and 0.02- U (µmol/min) cellulose. The fermentation of wheat straw leads to an increase in protein content by 55%, amylase content by 32%, and cellulose content by 8%. In *P. djamor* protein content increased by 79%, amylase content by 122%, and cellulose content by 48%. In *P. djamor*, protein content increased by 61%, amylase content by 72%, and cellulose content by 28%. In *P. ostreatus* (spent), protein content increased by 57%, amylase content by 66%, and cellulose content by 40%. In *P. ostreatus* (non-spent), protein content increased by 48%, amylase content by 42%, and cellulose content by 18%. These results emphasized significant differences between the two types of mushroom compost in terms of protein content and enzyme production. Enzymes are made up of amino acids and are essentially

proteins. Therefore, it was concluded that if one species had higher protein content, it inclined to have higher enzyme activity, compared to other species. A 24% co-relation was demonstrated between protein and cellulase enzyme in SPS whereas amylase enzyme showed a co-relation of 50%. Protein is present in spent mushroom compost, and its decomposition and utilization by microorganisms contribute to the improvement of compost quality and mushroom production. All the findings are shown in Table 2.

Variations in calculated enzymatic activities could be due to consumption or usage of substrates similar to spent compost, where mushrooms were cultivated and harvested while non-spent compost was analyzed as it as with no mushroom cultivation after inoculation of seeds. The literature shows that the levels of extracellular enzyme activity vary largely during the three flushes of oyster mushrooms after 6 months of harvesting prior to utilization as an animal feed or a fertilizer. According to the results obtained, it could be ascertained that these mushrooms grow well on all types of substrates, biosynthesizing active lignocelluloses, degrading enzymes.

Comparative analysis of cellulose yield for collected samples

A significant difference in cellulose yield was observed between SMS and non-SMS of both mushroom species. The highest cellulose yield of 10.89% and 10.69% was recorded for non-SMS of *P. djamor* and *P. ostreatus*. This showed the availability of cellulose for mushroom spores to be grown in the substrate. Compared to non-SMS, SMS showed the cellulose yield of 9.7% and 9.5% for *P. djamor* and *P. ostreatus*, respectively. This clearly indicated the release of cellulase enzymes, which eventually broke down the cellulose present in the substrate. Surprisingly, fermented and non-fermented straw yielded a high proportion of cellulose, that is, 28% and 27%, respectively, which indicated that hot water treatment could enhance cellulose production. The results of fermented and non-fermented straw were compared to the control, which exhibited a cellulose yield of 28%. All the results are shown in Table 3.

Studies have shown the presence of insoluble substances such as dry matter, lignins, glucan, xylan, and cellulose and hemi-cellulose given by (Karimi *et al.*, 2015). Studies have also shown the efficient biodegradation of cellulose and hemi-cellulose of wheat straw and coffee pulp by various mushroom species (Salmones *et al.*, 2005). Other studies have reported the biodegradation of lignocellulose by fungi at the time of fruiting (Geetha and Sivaprakasam, 1998; Thomas *et al.*, 1998) (Tables 4–6).

Antimicrobial assay

The antibacterial activity of selected samples was investigated for *S. auerous*, *E. coli*, and *Xanthomonas* using disc diffusion method for three concentrations of 500 ppm, 1000 ppm, and 1500 ppm.

Table 2. Comparison between amylase and cellulase enzymes on the basis of analysis of spent and non-spent mushroom compost of oyster species.

	Amylase (U (μmol/min))	Cellulase U (μmol/min)
P. ostreatus (spent) compost	0.74±0.26^{b}	0.42±0.2^{a,b}
P. ostreatus (non-spent compost)	0.5±0.1^{c}	0.20±0.1^{b,c,d}
P. djamor (spent compost)	1.3±0.1^{a}	0.5±0.2^{a}
P. djamor (non-spent compost)	0.8±0.02^{b}	0.3±02^{a,b,c}
Fermented straw	0.4±0.1^{d}	0.10±0.02^{c,d}
Non-fermented straw	0.2±0.09^{d}	0.08±0.06^{d}
Control	0.08±0.01^{e}	0.02±0.01^{d}

All values are expressed as mean±standard deviation; n = 3. The superscript alphabets show the significant variance among the mean value.

Table 3. Comparsion of cellulose in various substrates.

Treatment	Initial weight (g)	Final weight (g)	Yield (%)
White (spent)	10	0.95	9.5
White (non-spent)	10	1.069	10.69
Pink (spent)	10	0.97	9.7
Pink (non-spent)	10	1.089	10.89
Fermented straw	10	2.86	28
Non-fermented straw	10	2.77	27
Control	10	2.84	28

Table 4. Zone of inhibition for mushroom compost against against *S. aureus* ;selected strains (positive control = azthromycin standard disc 20 mm) in mean and standard deviation values (±).

Sample	Concentration (ppm)			Mean
	500	1000	1500	
White spent	8.47±0.25	9.40±0.44	9.67±0.21	9.18±0.6
White non spent	5.23±0.31	7.13±0.21	8.83±0.42	7.07±1.8
Pink spent	7.30±0.36	8.30±0.46	10.80±0.17	8.80±1.8
Pink non spent	6.80±0.10	7.43±0.15	9.20±0.70	7.81±1.2
Control	3.97±0.47	6.20±0.30	7.17±0.25	5.78±1.6

Table 5. Zone of inhibition for mushroom compost against E.coli (Positive control Azthromycin= standard disc 24.50mm) in mean values and standarad deviations (±).

Sample	Concentration (ppm)			Mean
	500	1000	1500	
White spent	6.87±0.29	8.47±0.32	12.57±0.71	9.30±2.9
White non spent	7.93±0.35	9.73±0.31	6.90±0.53	8.19±1.4
Pink spent	7.10±0.36	11.03±0.25	16.83±0.50	11.66±4.8
Pink non spent	6.80±0.36	8.80±0.30	9.73±0.40	8.44±1.4
Control	4.83±0.25	5.50±0.26	5.40±0.79	5.24±0.3

Table 6. Zone of inhibition for mushroom compost against Xanthomonas(Positive control Azthromycin = standarad disc (21.0) negative control (DMSO) in mean values and standarad deviations (±).

Sample	Concentration (ppm)			Mean
	500	1000	1500	
White spent	7.33±0.40	9.23±0.74	11.27±0.45	9.28±1.9
White non spent	7.00±0.26	7.97±0.21	10.30±0.36	8.42±1.6
Pink spent	6.67±0.15	9.10±0.26	11.37±0.59	9.04±2.3
Pink non spent	6.30±0.10	8.37±0.42	10.27±0.51	8.31±1.9
Control	2.97±0.21	4.93±0.25	5.77±0.35	4.56±1.4

In case of *S. aureus*, two controls, positive (azthromycin) and negative (DMSO), were taken for selected concentrations. The results revealed a linear variation, that is, increase in concentration led to increase in zone of inhibition. Maximum MIC was recorded for white (spent) mushrooms, with a zone of inhibition being 9.67±0.21 mm, while minimum MIC was recorded for white (non-spent) mushrooms, with a zone of inhibition being 9.20±0.70 mm at 1500 ppm. In comparison, the control showed a low MIC of 7.17±0.25 mm at 1500 ppm.

Similar trend was also observed for *E. coli*, where maximum MIC of 7.93 mm was recorded for *P. ostreatus* (non-spent), followed by 7.10 mm for pink (spent) mushrooms, while the control showed an MIC of 4.83 mm. In the current study, an increasing trend in MIC was also observed when the samples were subjected to antimicrobial analysis for *Xanthomonas*. Maximum MIC of 7.93 mm was recorded for *P. ostreatus* (non-spent) mushrooms, followed by an MIC of 7.10 mm for pink (spent) mushrooms, while the control showed an MIC of 4.83 mm.

The following findings supported the above-mentioned results. The methanolic extract of *P. ostreatus* cultivated on sorghum grain residue substrate recorded the highest antibacterial activity for *E. coli* (19.8 mm), and *P. aeruginosa* recorded an MIC of 16.4 mm. The methanolic extract of *P. florida* cultivated on a wheat grain substrate recorded maximum antibacterial activity for *E. coli* (18.6 mm) and *S. faecalis* (14.8 mm). Therefore, the results showed that *P. ostreatus* and *P. florida* cultivated on coffee straw and sorghum grain substrate had maximum antimicrobial activity, compared to other substrates (Getachew *et al.*, 2020).

Conclusions

The study concluded that cellulase and amylase present in the SMS of two selected species of the same fungal genus varied. This was due to the type and carbohydrate quantity present in SMS that influenced enzymatic production. The study also showed the influence of extraction solution on the extraction of enzymes. The findings revealed a direct link between amylase and starch-filled compounds. Additionally, a direct link was also established between cellulose and cellulose-rich compounds. The spent mushroom compost of *P. osteratus* had the highest lignocellulosic enzymatic activity, compared to *P. djamor.* Amylase activity was higher, compared to cellulase activity, in compost of all mushrooms. The individual treatments as described earlier has showed an effect on the lignocellulotic activity compared to control Non-spent mushroom compost cellulose yield attributes were higher, compared to spent mushroom compost cellulose yield. Spent mushroom compost manifested antimicrobial performance, compared to non-spent, toward bacterial pathogens, indicating release of bioactive compounds from mushroom mycelium. The results are of great importance because *P. djamor* (pink) showed higher

antimicrobial potential, lignocellulotic enzymes activity, and improved nutritional composition with interactive effects when compost was used for mushroom cultivation (spent) than non-spent and controls.

Recommendations

The current study recommends the use of distilled water in the sterilization process in place of tap water, as distilled water enhances the net yield of mushrooms. Additionally, this study suggests finding out whether lignocellulosic enzymes change if pH and buffer are changed. The *P. djamor* (spent) compost can be used as a biofertilizer because it is rich nutritionally and can fertile the soil efficiently.

Conflict of Interest

The authors declare no competing interests.

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