

## OPTIMIZATION OF DIFFERENT SPAWN TYPES OF *PLEUROTUS PULMONARIUS* AND BLENDING OF ITS FLOUR WITH LOCAL FLOURS BY USING MATLAB LINEAR PROGRAMMING

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### Abstract

Present work addressed SDG 2, SDG 3, SDG 12, and SDG 13 for improved spawn production on lab and commercial stage to increase overall production of *Pleurotus pulmonarius*. This study is aimed to produce three types of spawn i.e. mother spawn, planting spawn and commercial spawn. Mother spawn is the one that is cultured by the inoculation of pure culture media, while rest of both are produced from the inoculation of mother spawn. The planting spawn is produced in lab scale in relative lower quantity contrary to commercial spawn which is produced in polypropylene bags. Improved spawn technology is the key to produce mushroom on large scale globally. Optimization of mother spawn led to the cultivation of zinc fortified *Pleurotus pulmonarius* mushroom fruiting bodies. These fruiting bodies were dried and blended with wheat flours and maize flour by using MATLAB linear programming model. These blends were tested for minerals like sodium, potassium using flame photometer and zinc content was analyzed with atomic absorption spectroscopy. Freshness and quality of flour blends were analyzed by using colorimeter which gave value of  $a^*$ ,  $b^*$ ,  $c^*$ ,  $h^*$  and  $L^*$ . Minimum spawn run was noted at 100 g substrate concentration of wheat substrate (16 days), 5g dosage of planting spawn gave lowest spawn run of 12 days while optimization of commercial spawn 10 g/500 g dosage gave 14.1 days to completely cover the spawn bag with mycelium. Conventional flour blend of Government College University Lahore grown wheat gave maximum sodium concentration ( $2.09 \pm 0.082$  mg/2 g of flour blend) as compared to other blends. Maximum potassium concentration ( $6.74 \pm 0.249$  mg/2 g of flour blend) was noted in flour blended of NARC-11 wheat and zinc fortified *Pleurotus pulmonarius*. While, zinc quantity was highest ( $0.162 \pm 0.008$  mg/2 g) in university grown wheat flour blended with zinc fortified *Pleurotus pulmonarius* flour.

**Key words:** SDGs; MATLAB; Spawn; *Pleurotus pulmonarius*; Zinc; Sodium; Potassium; Linear Programming

### Introduction

Sustainable Development Goals (SDGs) are important for all the life on planet earth. These sustainable development goals are set by United Nations to provide quality of life to the humans equally regardless of their religion, ethnicity, profession, race or social status. In order to provide better future to our coming generation we must integrate all SDGs into our research and industrial practices. This work is related to the SDG 2 (Zero Hunger, Target 2.4) for sustainable food systems, optimization of *Pleurotus pulmonarius* spawn types, SDG 12 (Responsible Consumption and Production, Target 12.2) by adopting ways to utilize the resources in cultivation process of mushroom, SDG 13 (Climate Action, Target 13.1) by using agricultural waste as a lingo-cellulosic source to grow mushrooms, and SDG 3 (Good Health and Well-being, Target 3.9) by blending the zinc fortified mushroom flour with our frequently useable local flours to enhance the nutritional properties of our daily diet, contributing to public health and food security (Ifthikhar *et al.*, 2015; Majeed, *et al.*, 2024a; Majeed *et al.*, 2024b; Majeed, *et al.*, 2024c; Majeed, *et al.*, 2024d). Nowadays it is well known that mushrooms are famous for their taste, flavor and nutritive benefits. Mushrooms are unique in fungal kingdom system with macro fungal body bearing spore producing fleshy fruiting bodies. Genus *Pleurotus* known as oyster mushrooms which has 200

different species worldwide (Ritota & Manzi, 2023; Ali *et al.*, 2024). Oyster mushrooms are more easily cultivated than any other mushroom globally. It belongs to family Pleurotaceae, order Agaricales which are famous for exquisite taste, delicate texture and high nutritive properties (Jarial & Jarial, 2022; Kataria, 2023). *Pleurotus* mushroom has protein, dietary fibers and numerous vitamins like vitamin D, vitamin B<sub>1</sub> (Thiamine), vitamin B<sub>2</sub> (Riboflavin) (Kumar *et al.*, 2018; Johnnie *et al.*, 2023). Oyster mushrooms have pharmaceutical activities due to the presence of bioactive compounds like polysaccharides, polyphenols, terpenoids and lectins that have health benefits like antioxidants, anticancer, antiviral and immune-modulatory effects (Garcia *et al.*, 2022; Jarial, 2023). Moreover, they have properties related to bio-absorption, bioavailability and utilization of nutrients in a human body. Oyster mushrooms also contain antioxidants like ergothioneine and glutathione which are effective against oxidative stress and inflammation (Bhambri *et al.*, 2022). Edible mushrooms have high amount of protein which leads to self-sufficiency and help to fill the gap for most of vegetables, fruits and cereals. Fruiting phase of mushroom require lingo-cellulosic source for them to grow which is beneficial to reduce and reuse the agricultural waste (Adedokun, 2014; Jarial, 2023; Ifthikhar *et al.*, 2024; Kataria, 2023). Spawn is a pure mycelial culture that propagates on a solid substrate like grains. Improved spawn technology is the

primary requisite for enhanced production of mushrooms (Abdullah *et al.*, 2013). To produce maximum mushroom spawn a lot of knowledge, skills, expertise, equipment and controlled sterilized environmental parameters are required (Akçay *et al.*, 2023; Falahzadah *et al.*, 2023). Linear programming is a technique that can replace conventional food formulation processes. It is a mathematical modeling technique to optimize the objective function either maximization or minimization which is subject to linear equality and inequality constraints (Varghese & Srivastav, 2022). This work aimed the optimization of process conditions to produce *Pleurotus pulmonarius* mother spawn, planting spawn and commercial spawn. Moreover, zinc fortified *Pleurotus pulmonarius* flour was produced and blended with wheat and maize flours using linear programming model and conventional formulation model. Mineralization of these flour blends was done with color analysis to check the freshness of flours. Results of this work were unique from previous studies as characterization of *Pleurotus pulmonarius* mother spawn, planting spawn and commercial spawn has not been done yet.

## Materials and Methods

**Procurement:** Oyster mushroom spawn was obtained from a small enterprise (House of mushroom) working on mushroom cultivation in Lahore, Pakistan. This spawn bag was used to grow the fruiting bodies on wheat straw substrate.

**Culturing:** Pure culture was prepared after following the already reported method by Besufekad *et al.*, (2020) with minor modification. For better dissolution solution was placed on hot plate for half an hour. After complete dissolution, flask containing the agar solution was autoclaved for 15 minutes at 12°C temperature and 15 (Psi) pressure. Then, the agar was poured in petri dishes and kept for cooling at room temperature for overnight. Inoculation was done using small inside tissue of pileus. The inoculated petri plates were incubated at 25°C temperatures (Niazi & Ghafoor, 2022; Islam *et al.*, 2025).

**Molecular identification of organism:** The pure mycelium was grown on slant having the above reported medium (Besufekad *et al.*, 2020) at 25°C and sent for molecular identification to Macrogen, Korea.

**Optimization of substrate quantity:** Wheat was used as a lingo-cellulosic substrate to prepare mother spawn of *Pleurotus pulmonarius* mycelia. Grains were washed with tap water and soaked overnight. After that, they were boiled to make them soft but remained firm, then the water was drained and spread on a cheesecloth. Four jars were filled with 80 g,

90 g, 100 g and 110 g of these grains. These jars were autoclaved for 15 minutes at 121°C temperature and 15 Pa (Pascal). Inoculation was carefully done in total aseptic conditions using pure mother culture prepared on culture media. Jars were incubated at 25°C temperature and observed the spawn run or incubation time (days) (Borah *et al.*, 2019).

**Optimization of planting spawn:** Five doses (1g, 2g, 3g, 4g and 5g per 100g of wheat substrate) of mother spawn were used to evaluate the minimum spawn run (days) for planting spawn production. Keeping the rest of the parameters constant i.e. selected substrate wheat, temperature 25°C, Substrate quantity 100 g, 57.5 % moisture level, 0.5% calcium carbonate content, 2% calcium sulphate content and 0.5% glucose content.

**Optimization of commercial spawn:** Three doses of mother spawn (10g, 20g and 30g per 500g of wheat substrate) were used to optimize the commercial spawn. Other parameters were kept constant as above.

**Cultivation of zinc fortified *Pleurotus pulmonarius*:** Optimized mother spawn was used to produce zinc fortified *Pleurotus pulmonarius* as reported by Ali *et al.*, (2024). After cultivation fruiting bodies were dried in drying oven at 30°C and finely grounded to make flour.

**Preparation of Flour blends:** Various flour blends were prepared by using different varieties of wheat flour from National Agricultural Research Centre, Islamabad (NARC-11 and PAK13) and Agriculture Department, Government College University, Lahore (University grown wheat). These three varieties were blended with zinc fortified *Pleurotus pulmonarius* flour (Ali *et al.*, 2024) using random, conventional and LP based compositions. Furthermore, blends prepared from conventional and LP approach were fortified with Maize flour (MF). To prepare these blends linear programming model was applied using MATLAB software (MathWorks, USA, 2018). The flour blends prepared through linear programming model was compared with conventional formulation method. Then these formulation was assigned codes as mentioned in Table 1.

**Color analysis of flour blends:** Color analysis of flour blends was done by using colorimeter (Model: NR110) which was done in Food Science and technology lab, University of Lahore, Lahore. Colorimeter gave five values: L\*, a\*, b\*, c\* and h\*. L\* showed the lightness of flour from black to white on the scale of 0 to 100, a\*(greenness of flour blends) and b\* (yellowness of flour blends) represented the chromaticity, c\* determined the colorfulness of color. c\* also showed the distance of a color from the neutral axis in the lab color space and h\* showed the hue of color in degrees.

Table 1. Codes and composition of various testing samples.

Code	Control flours	Code	Control flour with fortified Mushroom flour	Code	Flour blend using Conventional method	Code	Flour blend using LP method
A	(Control of NARC-11 100g)	B	(W.F 98g + F.M.F 2g)	C	(W.F 73g + M.F 25g + F.M.F 2g)	D	(W.F 29.2g + M.F 68.8g + F.M.F 2g)
E	(Control of PAK-13 100g)	F	(W.F 98g + F.M.F 2g)	G	(W.F 73g + M.F 25g + F.M.F 2g)	H	(W.F 29.2g + M.F 68.8g + F.M.F 2g)
I	(Control of University wheat 100g)	J	(W.F 98g + F.M.F 2g)	K	(W.F 73g + M.F 25g + F.M.F 2g)	L	(W.F 29.2g + M.F 68.8g + F.M.F 2g)
M	(Control of zinc fortified <i>Pleurotus pulmonarius</i> flour 100g)	-	-	-	-	-	-

WF (wheat flour), FMF (Fortified mushroom flour), MF (Maize flour)

**Estimation of Minerals in flour blends:** All samples encoded from A to M were tested for mineral contents *i.e.*, Na, K and Zn. Flour blend samples for mineralization (mineral estimation) were prepared by using method of acid digestion as shown in Fig. 2 (Luvitaa *et al.*, 2023).

### Statistical analysis

Data was analyzed using SPSS software version 21. The data was expressed as the mean  $\pm$  standard deviation of measurements from triplicate experiments. ANOVA was used to determine levels of significance ( $P < 0.05$ ).

### Results

**Molecular identification of organism:** Based on partial sequence of internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2 partial sequence analysis, it is found that the tested sample has 100% evolutionary relations to *Pleurotus pulmonarius* as shown in Fig. 1. *Pleurotus pulmonarius* commonly known as the Indian oyster, Italian oyster, phoenix mushroom, or the lung oyster. Accession number issued by Gen bank is PV789582. It was assigned the name “*Pleurotus pulmonarius* MBA”.

**Scientific classification:** Kingdom-Fungi, phylum-Basidiomycota, class-Agaricomycetes, order-Agaricales, phylum-Basidiomycota, family-Pleurotaceae and genus-*Pleurotus*.

### Optimization of Cultural Conditions

**Optimization of quantity of substrate:** Quantity of substrate plays crucial role towards spawn production. Mushroom mycelium required a certain space to grow because it has to do gaseous exchange. The quantity of wheat was optimized to maximize the spawn run. The jars were incubated in previously optimized temperature at 25°C. Different concentrations *i.e.*, 80 g, 90 g, 100 g and 110 g were evaluated in this context. Using 80g substrate, the number of day was 19 days while at 90 g of substrate quantity the number of days was 18 days and at 110 g concentration of wheat, the number of days was highest. It was observed that mycelium gave minimum spawn run time (16 days) at 100 g substrate as shown in Figs. 3 and Fig. 4. Therefore, 100g substrate was optimized for further studies.

**Optimization of different spawn doses on planting spawn:** Effect of different spawn doses plays significant role in the production of spawns. Figs. 5 and Fig. 6 showing the effect of five doses of mother spawn (1g, 2g, 3g, 4g and 5g) on the mycelial incubation time of planting spawn. Previously optimized parameters were kept constant *i.e.*, selected substrate wheat (S1), temperature 25°C (T2), Substrate quantity 100 g, 57.5 % moisture level, 0.5% calcium carbonate, 2% calcium sulphate and 0.5% glucose content. Therefore, minimum incubation time optimized was taken at 5g dose of mother spawn (12 days).

**Optimization of mother spawn doses on commercial spawn:** Doses of mother spawn plays crucial role towards

commercial spawn production. Commercial spawn is the one which is grown in polypropylene bags. Bags were filled with 500 g of selected substrate wheat. Three doses of mother spawn 10g/ 500g of substrate, 20g/ 500g of substrate and 30g/ 500 g of substrate were inoculated. Previously optimized parameters like temperature 25°C (T2), Substrate quantity 100 g, 57.5 % moisture level, 0.5% calcium carbonate, 2% calcium sulphate and 0.5% glucose content were kept constant. Minimum incubation time for mycelial completion was observed at 10 g/ 500 g dosage (14.1 days) as shown in Figs. 7 and 8. Therefore, maximum spawn run optimized was given by 30g/ 500g of dosage (18.7 days).

**Mineralization of flour blends:** Complete range of flour blends was tested for selected minerals. In table 2, three minerals (sodium, potassium and zinc) were estimated in the flour blends. Highest amount ( $2.09 \pm 0.082$  mg/ 2 g of flour blend) of sodium was present in conventional formulation of university wheat flour. On the other hand, lowest amount of sodium was quantified in control of Pak-13 wheat flour. Zinc fortified *Pleurotus Pulmonarius* flour showed  $6.91 \pm 0.432$  mg/ 2 g of flour. Potassium concentration ( $6.74 \pm 0.249$  mg/ 2 g of flour) was highest in zinc fortified mushroom flour and NARC-11 wheat flour. The lowest amount ( $4.40 \pm 0.216$  mg/2 g of flour) was calculated in control of university wheat flour. Zinc fortified *Pleurotus pulmonarius* gave  $27.39 \pm 0.571$  mg/ 2 g of flour of potassium content. Zinc quantity was highest in university wheat flour formulations, maximum amount was estimated in sample “J” blend ( $0.162 \pm 0.008$  mg/ 2 g of flour) which is a lower than control of university wheat flour “I” ( $0.149 \pm 0.005$  mg/ 2g of flour).

**Color analysis:** Color analysis was done to analyze the colors of different flour blends as shown in table 3. Different flour blends was tested for a\* [degree of redness to blueness], b\* [degree of yellowness], c\* [degree of saturation of colors], h\* [degree of hue of color] and L\* [degree of whiteness]. The highest a\* value ( $2.46 \pm 0.054$ ) shown by sample H, whereas lowest value was given by sample I ( $1.62 \pm 0.053$ ). b\*, lowest value was shown by zinc fortified mushroom flour (M)  $0.01 \pm 0.004$  and highest value was shown by sample L (linear programming model formulation of university wheat flour with maize and fortified mushroom flour). Highest c\* was shown by sample D (linear programming model of NARC-11 wheat flour formulation) whereas lowest degree of saturation was revealed by control of university wheat flour (I)  $1.70 \pm 0.038$ . The h\* value was highest ( $57.81 \pm 0.346$ ) shown by sample A (NARC-11 wheat flour) having no flour added and the lowest value was shown by zinc fortified mushroom flour (M) ( $10.24 \pm 0.029$ ) having zinc uptake added during growing phase of fruiting bodies. It was observed that h\* decreased as the shade diminished with more addition of more flours. L\* was decreased from control as the maize and fortified mushroom flour decreased the whiteness of wheat flour. The highest L\* value was shown by control of university wheat flour (I)  $28.09 \pm 0.331$  while lowest degree of whiteness ( $9.23 \pm 0.093$ ) was shown by sample M (zinc fortified mushroom flour).

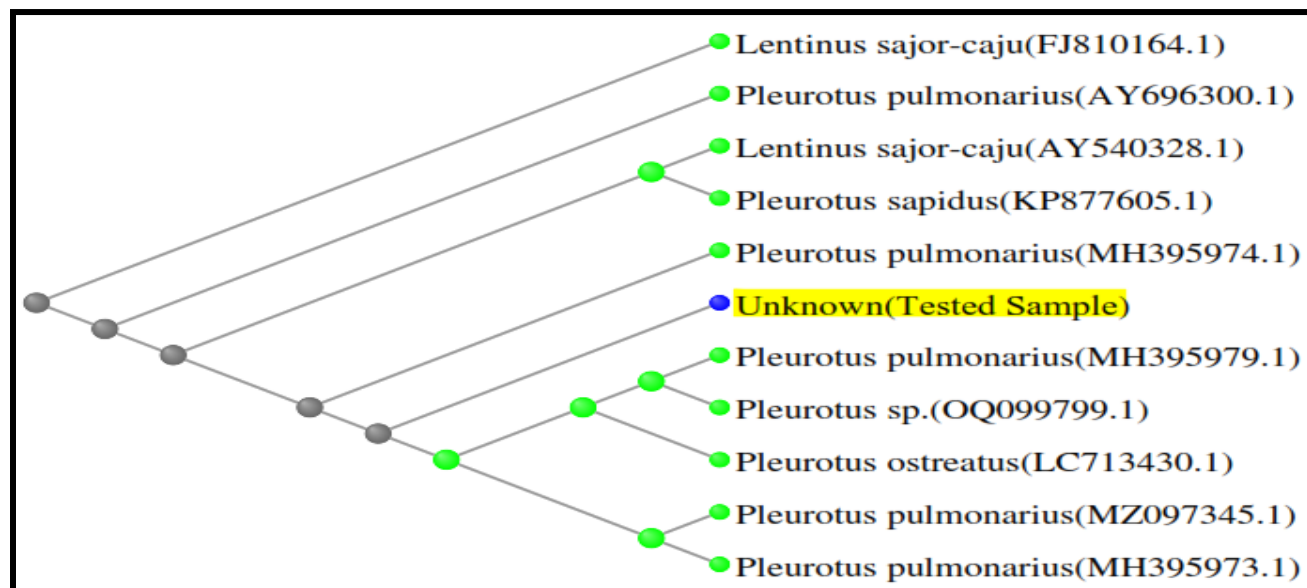
Fig 1. Phylogenetic tree of *Pleurotus pulmonarius* MBA [PV789582].

Table 2. Mineralization of flour blends.

Sample	Sodium concentration (mg/ 2g of flour)	Potassium concentration (mg/ 2g of flour)	Zinc concentration (mg/ 2g of flour)
A	1.68 ± 0.124	5.07 ± 0.123	0.051 ± 0.008
B	1.74 ± 0.062	6.74 ± 0.249	0.056 ± 0.005
C	1.85 ± 0.124	6.67 ± 0.748	0.054 ± 0.004
D	1.85 ± 0.326	4.97 ± 0.124	0.014 ± 0.004
E	1.46 ± 0.040	4.91 ± 0.041	0.022 ± 0.005
F	1.76 ± 0.041	6.68 ± 0.205	0.029 ± 0.008
G	1.66 ± 0.040	5.65 ± 0.169	0.126 ± 0.011
H	1.72 ± 0.081	5.09 ± 0.169	0.101 ± 0.083
I	1.78 ± 0.040	4.40 ± 0.216	0.149 ± 0.005
J	1.92 ± 0.081	5.43 ± 0.069	0.162 ± 0.008
K	2.09 ± 0.082	5.68 ± 0.163	0.141 ± 0.009
L	1.69 ± 0.081	4.63 ± 0.216	0.101 ± 0.011
M	6.91 ± 0.432	27.39 ± 0.571	4.76 ± 0.19

The values obtained are mean of triplicates, one way ANOVA ( $p < 0.05$ ), Tukey's HSD showing significant difference

Table 3. Color analysis of flour blends.

Samples	a*	b*	c*	h*	L*
A	1.82 ± 0.108	0.41 ± 0.052	1.87 ± 0.074	57.81 ± 0.346	20.85 ± 0.054
B	1.93 ± 0.032	1.04 ± 0.063	2.40 ± 0.069	48.63 ± 0.243	15.76 ± 0.225
C	2.29 ± 0.096	2.59 ± 0.120	3.39 ± 0.044	26.09 ± 0.099	13.76 ± 0.225
D	2.21 ± 0.062	3.20 ± 0.040	3.79 ± 0.132	13.67 ± 0.140	11.45 ± 0.123
E	2.20 ± 0.100	0.62 ± 0.274	2.54 ± 0.085	39.60 ± 0.420	16.39 ± 0.123
F	2.17 ± 0.041	1.59 ± 0.101	2.60 ± 0.122	38.62 ± 0.050	15.87 ± 0.058
G	1.90 ± 0.104	1.64 ± 0.057	2.69 ± 0.033	37.87 ± 0.082	14.21 ± 0.155
H	2.46 ± 0.054	1.76 ± 0.041	2.78 ± 0.057	12.52 ± 0.227	10.40 ± 0.044
I	1.87 ± 0.100	0.47 ± 0.128	1.70 ± 0.038	56.54 ± 0.232	28.09 ± 0.331
J	2.36 ± 0.024	1.50 ± 0.046	1.82 ± 0.047	32.17 ± 1.223	18.5 ± 0.177
K	2.08 ± 0.024	1.96 ± 0.068	2.73 ± 0.048	15.8 ± 0.174	15.25 ± 0.148
L	1.62 ± 0.053	3.23 ± 0.131	3.72 ± 0.040	10.49 ± 0.401	10.77 ± 0.085
M	1.81 ± 0.020	0.01 ± 0.004	1.92 ± 0.134	10.24 ± 0.029	9.23 ± 0.093

Where, a\*: Degree of redness to blueness, b\*: Degree of yellowness, c\*: Degree of saturation, h\*: Degree of hue of color and L\*: Degree of whiteness. The values obtained are mean of triplicates, one way ANOVA, Tukey's HSD showing significant difference ( $p < 0.05$ )





Fig 2. Acid digestion of flour blends for mineralization analysis through Atomic Absorption Spectroscopy and Flame Photometer a. Charring, b. Ashing, c. Acid digestion, d. Filtration, e. Flour blend samples



Fig. 3. Optimization trials of selected substrate concentration.

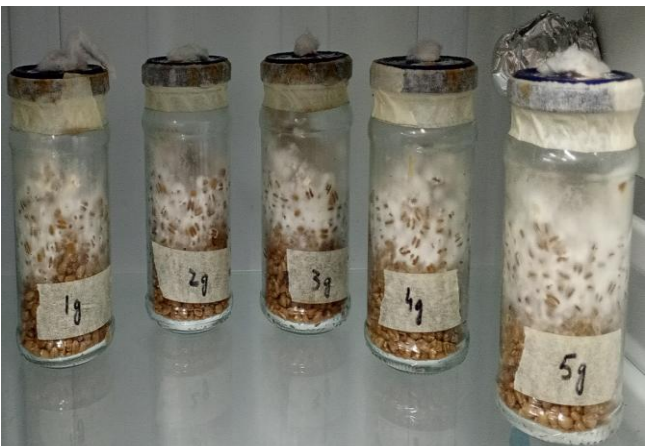


Fig. 5. Optimization trials of mycelial dosage for planting spawn.



Fig. 7. Optimization trials of spawn doses for commercial spawn.

Discussion

Mushroom cultivation is directly dependent on the production of good quality spawn. In this study, mother, planting and commercial spawn of *Pleurotus pulmonarius* were optimized to enhance the overall production of *Pleurotus* mushroom on lab and industrial scale.

In the present study, the molecular identification of the isolate as *Pleurotus pulmonarius* MBA, confirmed through ITS region sequencing, is consistent with earlier reports that highlight ITS as a reliable barcode marker for distinguishing *Pleurotus* species. Niazi & Ghafoor (2025) reported the phylogenetic analysis of *Pleurotus* sp. as studied in present work revealing that the pure culture of mushroom gave 100% similarity with *Pleurotus pulmonarius*. A good quality spawn of mushroom ensures the quick colonization of substrate which leads to better

nutrients utilization and assimilation (Mishra *et al.*, 2019). The size of fruiting bodies also directly affected by the quality of spawn which enable the robust mycelial network of mushroom to increase the size ultimately enhancing the yield (Kumar *et al.*, 2018). In this work linear programming model was compared with conventional formulation of flour blends. This comparison aim to develop nutritionally optimized flour blends. Maize flour contains protein, dietary fiber and omega 6 fatty acids, which are important for better bowel functioning, fighting infection and heart health (Adesanmi *et al.*, 2020). Royse *et al.*, (2004) concluded that a greater concentration of nutrients in the initial mycelial growth material, known as spawn, can provide more energy for the growth and development of mycelium. However, it is important to note that using a lower amount of spawn may not be enough to start the growth process, while using too much can hinder growth through competitive inhibition, these result were similar to the present study showing minimum spawn run time (16 days) at 100 g substrate exceeding or lowering from this gave lesser growth of mycelium. In planting spawn optimization 5g dose of mother spawn gave minimum spawn run (12 days) which is according to the findings of (Eira, 2003) who found that the quantity of inoculum (spawn) should not surpass 10% of the substrate weight in order to prevent a substantial decline in biological efficiency (it refers to the ratio of the harvested mushroom's fresh weight to the dry substrate's weight used for cultivation). (Ram & Pant, 2004) observed that a higher spawn dose led to a rapid spawn run in *Pleurotus* species and reported 5.0 g/ 100g of substrate gave higher mycelial activity which is aligned with our results of optimization of planting spawn. For commercial spawn production *Pleurotus pulmonarius* gave minimum spawn run (14.1 days) for mycelial completion at 10 g/ 500 g dosage which was the recommended range of 7-10 % reported by (Oei & Nieuwenhuijzen, 2005).

Deviating from this range can result in financial losses. Moreover, increasing the size of the inoculum has been shown to enhance the utilization of the solid substrate and promote the activity of laccase, which is a crucial enzyme in the growth of mushrooms. A higher spawning rate results in a shorter time for mycelial colonization, primordial formation and the first flush of the mushroom crop, while also reducing the chance for competing organisms to invade the cultivation environment (Yang *et al.*, 2013). Nutritional analysis of sodium, potassium and zinc were done to optimize the flour blends. Highest amount of sodium was observed in sample K ( $2.09 \pm 0.082$  mg/2g of flour blend), which is higher than the results reported by Awofadeju & Olapade (2020) showing 62.68 mg/ 100 g of sodium in wheat flour. This might be due to the presence of maize and *Pleurotus pulmonarius* flours which contain higher amount of sodium than wheat flour. pHighest amount of potassium was observed in sample B ( $6.74 \pm 0.249$  mg/2g of flour blend) which is lower than results reported by Awofadeju & Olapade (2020) which might be due to the presence of *Pleurotus pulmonarius* flour. Sample J (university wheat flour blended with zinc fortified *Pleurotus pulmonarius* flour) contained highest amount of zinc ( $0.162 \pm 0.008$  mg/2g of flour blend) which is higher than as reported by Wang *et al.*, (2020) 2.89 mg/ 100 g of potassium in wheat flour, this might be due to the presence of higher amount of zinc in university wheat variety and fortification of zinc fortified *Pleurotus pulmonarius* flour.

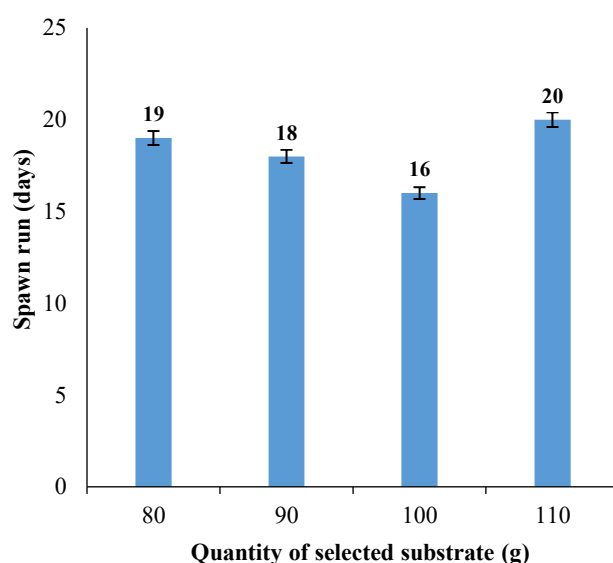


Fig. 4. Effect of quantity of selected substrate on mother spawn run (days).

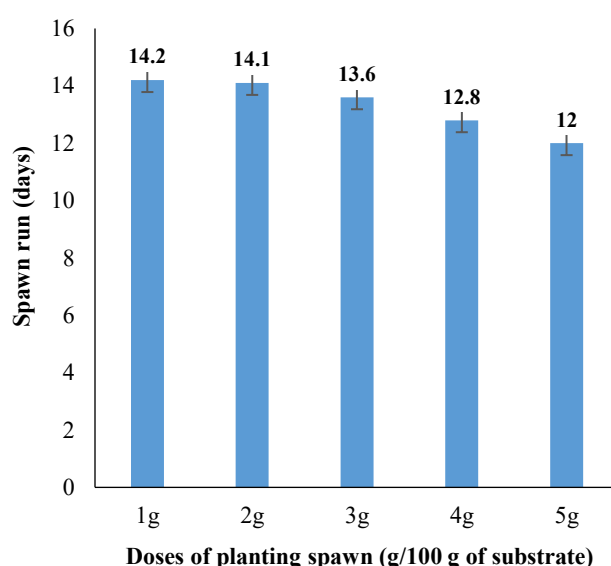


Fig. 6. Effect of spawn doses (g) on the planting spawn.

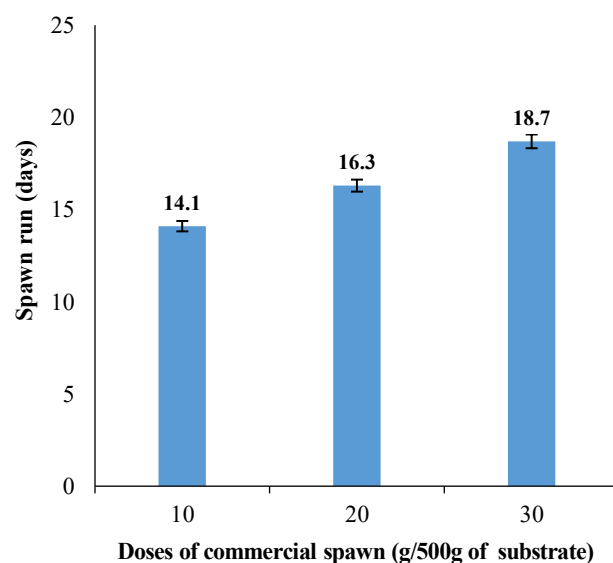


Fig. 8. Optimization of mother spawn dosage on commercial spawn.

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**Authors contribution:** All authors contributed equally to the conception, design, data collection, analysis, and writing of this paper. MBA has given extended experimental trials. This work is the part of the PhD Thesis of Muhammad Bin Ali.

## References

- Abdullah, N., R. Ismail, N.M.K. Johari and M. Annuar. 2013. Production of liquid spawn of an edible grey oyster mushroom, *Pleurotus pulmonarius* (Fr.) Quél by submerged fermentation and sporophore yield on rubber wood sawdust. *Sci. Hortic.*, 161:65-69.
- Adedokun, O.M. 2014. Oyster mushroom: Exploration of additional agro-waste substrates in Nigeria. *Int. J. Agric. Res.*, 9(1): 55-59.
- Adesanmi, A.R., S.A. Malomo and T.N. Fagbemi. 2020. Nutritional quality of formulated complementary diet from defatted almond seed, yellow maize and quality protein maize flours. *Food Prod. Proc. Nutr.*, 2(1): 2661-8974.
- Akcay, C., F. Ceylan and R. Arslan. 2023. Production of oyster mushroom (*Pleurotus ostreatus*) from some waste lignocellulosic materials and FTIR characterization of structural changes. *Sci. Rep.*, 13(1): 12897.
- Ali, M.B., T. Iftikhar and H. Majeed. 2024. Green synthesis of zinc oxide nanoparticles for the industrial biofortification of (*Pleurotus pulmonarius*) mushrooms. *Heliyon*, 10(19): 2405-8440.
- Awofadeju, J.O.F. and A.A. Olapade. 2020. Nutritional and sensory evaluation of wheat-maize cookies enriched with African walnut (*Tetracarpidium conophorum*) seed protein isolate. *HCPTBN*, 15(1-2): 54-64.
- Besufekad, Y., A. Mekonnen, B. Girma, R. Daniel, G. Tassema, J. Melkamu, M. Asefa, T. Fikiru and L. Denboba. 2020. Selection of appropriate substrate for production of oyster mushroom (*Pleurotus ostreatus*). *J. Yeast Fungal Res.*, 11(1): 15-25.
- Bhambri, A., M. Srivastava, V.G. Mahale, S. Mahale and S.K. Karn. 2022. Mushrooms as potential sources of active metabolites and medicines. *Front. Microbiol.*, 13:837266.
- Borah, T.R., A.R. Singh, P. Paul, H. Talang, B. Kumar and S. Hazarika. 2019. Spawn production and mushroom cultivation technology. *ICAR research complex for NEH region*, 46.
- Eira, A. 2003. Cultivo do cogumelo medicinal *Agaricus blazei* (Murrill) ss. In: Viçosa.
- Falahzadah, M., A. Jamil, S. Danishar, A. Osmani and G. Ajir. 2023. Comparison of low-cost substrates and spawn levels for oyster mushroom (*Pleurotus ostreatus*) cultivation. *J. Nutr. Food Sci.*, 13(3): 17.
- Garcia, J., F. Rodrigues, M.J. Saavedra, F.M. Nunes and G. Marques. 2022. Bioactive polysaccharides from medicinal mushrooms: A review on their isolation, structural characteristics and antitumor activity. *Food Biosci.*, 49: 101955.
- Iftikhar, T., H. Majeed, F. Altaf and A. Khalid. 2024. Upcycling of the industrial waste as a sustainable source of axenic fungal strain (*Aspergillus oryzae*) for scale up enzymatic production with kinetic analysis and Box–Behnken design application. *Z. Phys. Chem.*, 238(1): 115-131.
- Iftikhar, T., R. Abdullah, M. Iqtedar, A. Kaleem, M. Aftab, M. Niaz, Sidra, B. Tabassum and H. Majeed. 2015. Production of lipases by *Alternaria* sp.(mb1 2810) through optimization of environmental conditions using submerged fermentation technique. *Int. J. Biosci.*, 6655: 178-186.
- Islam, M.Z., S.S. Hassan, T.A. Shah, A.Z. Gaafar, S. Tahir, J. Zhao, K. Dinislam, G. Yang and B. Zhao. 2025. Innovative fungal strategies for sustainable forest management: the role of *pleurotus citrinopileatus* in wildfire risk mitigation. *Pak. J. Bot.*, 57(6): 2361-2369.
- Jarial, R. and K. Jarial. 2022. Commercial cultivation of the elm oyster mushroom *Hypsizygus ulmarius* (Agaricomycetes) on different substrates and its medicinal benefits. *Int. J. Med. Mushrooms*, 24(12): 1521-9437.
- Jarial, R.S. 2023. Utilization of agro-industrial wastes and organic supplements for cultivation of blue oyster mushroom. *Bangladesh J. Bot.*, 52(1): 179-185.
- Johnnie, C., D. Seecharan and A.A. Ansari. 2023. Comparative yield, yield related parameters and elemental composition of oyster mushroom (*Pleurotus ostreatus*) grown on different substrates: Mushroom culture. *Mushroom Res.*, 32(1): 27-33.
- Kataria, S. 2023. Underutilized nutrient rich millets: challenges and solutions for India's food and nutritional security: A review. *Int. J. Plant Soil Sci.*, 35(2): 45-56.
- Kumar, B., G. Singh, V. P. Singh, J. Patil, P. Mishra, D. Choudhury and S. Srivastava. 2018. Effect of different inorganic additives on spawn run, cropping period and yield performance of oyster mushroom (*Pleurotus* species).
- Luvitaa, K.S., M.A. Wambui, M. Fredrick and O.D. Otieno. 2023. Zinc bioaccessibility in finger millet porridge blended with zinc-dense mushroom. *Heliyon*, 9(8): 2405-8440.
- Majeed, H., T. Iftikhar and R. Abid. 2024. Green synthesis of insecticidal, bactericidal, UV absorbent, sustainable paint formulations using *Mentha piperita* (peppermint). *React. Chem. Eng.*, 9(9): 2358-2366.
- Majeed, H., T. Iftikhar and R. Manzoor. 2024. Extraction and characterization of novel alternative cellulosic fiber for sustainable textiles from *Aloe barbadensis* Miller stems (agricultural waste). *Heliyon*, 10(18): 2405-8440.
- Majeed, H., T. Iftikhar and S. Mustafa. 2024. Statistical approach for newly isolated and identified microbial lipases production. *Polym. Bull.*, 81(17): 15823-15840.
- Majeed, H., T. Iftikhar, M.A. Nadeem and M.A. Nazir. 2024. Green synthesis of *Eucalyptus globulus* zinc nanoparticles and its use in antimicrobial insect repellent paint formulation in bulk industrial production. *Heliyon*, 10(2): 2405-8440.
- Mishra, A., G. Singh, A. Kumar, A. Yadav and M. Mohit. 2019. Comparative studies of spawn growth on different grains substrate in three *Pleurotus* spp. (*Pleurotus florida*, *Pleurotus flabellatus* and *Pleurotus sapidus*).
- Niazi, A.R. and A. Ghafoor. 2022. Molecular phylogenetics and optimization of growth conditions of indigenous edible and therapeutically significant *Pleurotus floridanus* from Pakistan. *Pak. J. Bot.*, 54(5): 1919-1926.
- Oei, P. and B.V. Nieuwenhuijzen. 2005. *Small-scale mushroom cultivation*: Agromisa Foundation and CTA. ISBN CTA: 92-9081-303-2.
- Ram, R. and D. Pant. 2004. Effect of different amounts of spawn on spawn run period. *Ind. J. Plant Pathol.*, 22(1/2): 131.

- Ritota, M. and P. Manzi. 2023. Edible mushrooms: Functional foods or functional ingredients? A focus on *Pleurotus* spp. *AIMS Agric. Food*, 8(2): 391-439.
- Royse, D.J., T. Rhodes, S. Ohga and J. Sanchez. 2004. Yield, mushroom size and time to production of *Pleurotus cornucopiae* (oyster mushroom) grown on switch grass substrate spawned and supplemented at various rates. *Bioresour. Technol.*, 91(1): 85-91.
- Varghese, C. and P.P. Srivastav. 2022. Formulation and sensory characterization of low-cost, high-energy, nutritious cookies for undernourished adolescents: An approach using linear programming and fuzzy logic. *Innov. Food Sci. Emerg. Technol.*, 75: 102904.
- Wang, M., F. Kong, R. Liu, Q. Fan and X. Zhang. 2020. Zinc in Wheat Grain, Processing, and Food. *Front. Nutr.*, 7: 124.
- Yang, W., F. Guo and Z. Wan. 2013. Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. *Saudi J. Biol. Sci.*, 20(4): 333-338.