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*Original Article*

# Application of statistical methods to optimize medium for increased yield of Oyster Mushroom (*Pleurotus ostreatus*)

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**Abstract**

The aim of this study is investigating sustainable alternatives to grow Oyster mushroom (*Pleurotus ostreatus*) using paddy straw, bagasse, wheat bran, urea and humic acid at varying composition. The study optimizes the composition of the substrates, other than the paddy which is the main raw material, for the maximum yield of *Pleurotus ostreatus* and analyses the nutrient content of the biomass produced. Bagasse composition (17.5g/l - 32.5g/l), Wheat bran composition (3.5g/l – 6.5 g/l), Urea composition (3.0 g/l -7.0 g/l) and Humic acid (2% - 6%) were chosen as the process variables for optimization. A 5 level 4 variable central composite design was used to evaluate the effects of these parameters on the Yield of *Pleurotus ostreatus*. After the process of optimization, the significant interaction among the process variables studied. Humic acid is the key role in the determination of the yield. Depending on the different process parameters the yield of mushroom varied from 74 – 204g. Optimum process parameters for maximum yield of *Pleurotus ostreatus* were found to be Bagasse 21.25g/l, Wheat bran 3.5g/l, Urea 5.0g/l and Humic acid 4%. The process parameters also shows significant effect on yield, productivity and biological efficiency. Mycelial colonization of compost bags and subsequent growth of oyster mushroom was faster in high Humic acid-based substrates. Hence they produced larger and firmer fruiting bodies. The response surface methodology provided here can be used as a strategy to grow Oyster mushrooms under adverse conditions and limited resources.

***Key words***: Oyster mushroom*; Pleurotus ostreatus;* Humic acid; Wheat bran

## Introduction

The Oyster mushroom, or *Pleurotus ostreatus*, is a common edible mushroom, long cultivated in Asia, it is now cultivated around the world for food. They are commonly grown in mushroom houses but require a more humidity and fresh air than other variety. They grow well on a range of agricultural and wood waste products including hardwood chips, chopped cereal straws or corn cobs. The usage of substrate as fertilizer after mushroom production is more commonly seen if straw is used as a key ingredient in growth medium. Oyster mushrooms can also be used industrially for mycoremediation purposes. Fasidi and kadiri (1993) reported the growth of mushroom to be affected by moisture content, temperature, pH and light intensity when it was grown on lignocelluloses wastes. Ragini Bisaria *et al.* (1983) suggested the usage of Lignocelluloses for microbial conversions. The intra and extracellular contents of vitamins in *Pleurotus ostreatus* were studied in the course of submerged cultivation by Solomko and Eliseeva,(1988). The dry mycelium of *P. ostreatus* obtained after the cultivation revealed the production of thiamin (vitamin $B_1$), riboflavin (vitamin $B_2$), niacin (vitamin $B_5$), pyridoxine (vitamin $B_6$) and biotin (vitamin $B_7$). *Pleurotus* sp are medicinal mushrooms, exhibiting hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulation activities (Cohen *et al*., 2002).

Humic acid is a condensed, refractive mixture of aromatic organic acids which contains sulfur, nitrogen, phosphorus and metals such as Cu, Mg, Cu, Zn. Humus is reported to be a chemical complex, which is resistant mixture of brown or dark brown amorphous, hydrophilic, acidic, partly aromatic organic substances modified from the original tissues or synthesized by the various soil organisms that range in molecular weight from a few hundred to several thousand (Kononova *et al*., 1966). Identification of complex structure of soil humus consisting of humic materials such as humic acid, fulvic acid and humin aided its application in many field (Schnitzer,1978). *Pleurotus ostreatus* was cultured on a synthetic medium with growth regulators (Vinklarkova and Sladky,1978).

Humic acid was found to be insoluble at greater pH values. Hence the extraction and separation of various humic substances are carried out using 0.1-0.5N NaOH (Stevenson and Schnitzer, 1982). The negatively charged carboxylic and phenolic groups in the colloidal surfaces of humus are pH dependent (Schnitzer, 1986). The efficiency of humic acid in improving the N and P contents thereby aiding the increase in crop yield was revealed by Brannon and Somers, (1985).

The highest yield of fruiting bodies was obtained using a mixture of date waste and rice straw as substrates (Jwanny *et al*.,1995).The protein quality of edible mushrooms and Amino acid evaluation of *Pleurotus* sp. cultivated in banana leaves and a mixture of banana leaf and bagasse was studied by Ranzani and Sturion, (1998). Saw dust as substrate produced highest yield, biological efficiency and number of fruiting bodies for oyster mushroom cultivation as shown by Shah *et al*.(2004). Utilization of whey permeate and application of Response surface analysis (RSA) was investigated to determine the combination of substrate concentration, temperature and pH producing maximal mycelial extension rate under solid state cultivation (Bhak *et al*., 2005). Determination of nutritive value and yield performance of three types of oyster mushroom *P.eryngii , P.ostreatus, P.Sajor-caju* cultivated on wheat stalk revealed the production of ligninolytic enzymes which finds significant importance in the biodegradation of organopollutants, xenobiotics and industrial contaminants (Dundar *et al*., 2008). Although mushroom culture is one of the oldest microbial foods of man and the first solid-state fermentation product, the basic research of microbial technology has not been applied to significant extent. The present study investigates the production of Oyster mushroom by providing various combination of substrates and optimizing the composition of the substrate for maximum yield by Response Surface Methodology.

## Materials and Methods

### *Inoculum preparation*

The main culture of *Pleurotus ostreatus* was obtained from Thirukalukundram mushroom farm.

For the propagation of the main culture, 2.0% Malt-Extract Agar (MEA) was used. MEA plates were inoculated with a mycelium/agar plug (5-mm-diameter) of a young, actively growing margin of the colony. Prior to its use as an inoculum for grain spawn, a mycelium/agar plug was inoculated at the center of the plate and incubated at 25°C in the dark for seven days.

### *Spawn preparation*

200g sorghum was used as substrate for spawn production. The grain was soaked in 500 ml water. Excess water was drained and grains were shredded into tiny pieces. 50 g grain was placed in polythene bags and held in place by rubber bands, it is sterilized 121ºC for 20 min. After cooling, each bag was inoculated with spawns and incubated at 25ºC in full darkness for two weeks to enable the mycelia to permeate.

### *Conditions of cultivation*

Paddy straw was used as a main material in this study for cultivation of oyster mushrooms. Paddy straw was soaked in water filled plastic buckets for 16 h. After which, it is crushed and dried. The crushed straw is treated with different composition of bagasse, wheat bran, urea and humic acid. pH of the substrate mixtures were maintained at 6.0. Compost medium was mixed manually. The mixture of varying compositions were packed in polythene bags and sterilized. After sterilization, the substrate is semi dried, inoculated with the spawns in alternate layers and incubated at 25°C in dark for 2 weeks for the ramnification of mushroom mycelia. The culture rooms were damped by spraying the top of compost with water once a day. This maintains the relative humidity of 80 %. After the development of mycelium on compost bags, they were torn and maintained at 28 °C with adequate aeration, watering and high humidity to allow the fruiting bodies to emerge. The harvesting was done in 3 flushes of 1 week intervals. After the 2$^{nd}$ flush, the substrate was turned upside down and regularly watered to harvest the 3$^{rd}$ flush.

### *Moisture content*

The fresh weight of each mushroom sample was determined by chemical balance. The sample with 5 g initial weight was oven dried separately at 95°C for 24 h till constant weight is attained. The loss in weight obtained after drying was regarded as the moisture content in percentage (Manzi *et al*., 1999), which is calculated as follows using

$$Moisture\ \% = \frac{(W_0 - W_1)}{W_0} \times 100 \quad (1)$$

***Lipid***

3.0g of dried mushroom sample was extracted with 25 $cm^3$ of petroleum ether in a soxhlet extractor for 16 h. The extract was evaporated to dryness in a weighed flask using a vacuum evaporator. The weighed flask was dried in the oven at 105°C for 30 min. The weight of the extract was recorded after cooling in desiccator. The difference between the initial and final weights was regarded as the lipid content of the sample (Parent and Thoen,1977). Crude fat was calculated by

$$Crude\ fat\ (\%) = \frac{W_{fat,S} \times 100}{Ws} \quad (2)$$

***Crude fibre***

3g dried and fat free mushroom sample was taken in a 1000 ml beaker and 200 ml of 1.25% H□SO□ were added. The level of beaker was marked. The contents of the beaker were boiled for 30 min with constant stirring; also the level of the water was supplemented. Contents were given 3 washings with hot water (150 ml) until it was acid free. The procedure was repeated with 1.25% NaOH. The alkali free residue was carefully transferred to a crucible and dried in an oven at 100°C for 4 hours until constant weight was obtained. The contents were heated on oxidizing (blue) flame until smoke ceased to come out of sample. The sample was placed in a muffle furnace at 550°c for 4 hours until a grey ash was obtained, cooled in desiccators and weighed (AOAC, 1990). The difference in weight gives crude fibre as calculated by

$$Crude\ fibre\ (\%) = \frac{\Delta W_{\text{after ignition}}\ (g)}{W_{\text{sample}}\ (g)} \times 100 \quad (3)$$

***Ash content***

3.0 g of dried mushroom sample were taken in a crucible and headed on oxidizing flame till smoke subsided. The crucible was transferred to muffle furnace at 550°C for 6 hours. The sample was cooled in desiccators and weighed (Manzi *et al*., 2001). The ash content in the sample was calculated by

$$Ash\ (\%) = \frac{W_{\text{Ash,sample}}\ (g)}{W_{\text{sample}}\ (g)} \times 100$$

***Protein content***

0.5 g of the powdered and mushroom sample was extracted with 50.0 $cm^3$ of 2.5% NaCl in a water-bath at 60°C for 1 h. The extract was filtered out and treated with 3% copper acetate to precipitate the protein. The precipitated protein was centrifuged and dissolved in 0.1 M NaOH. The quantity of protein in the alkaline solution was determined using the folins-phenol method (Kadiri and Fasidi,1990).

## Results and Discussion

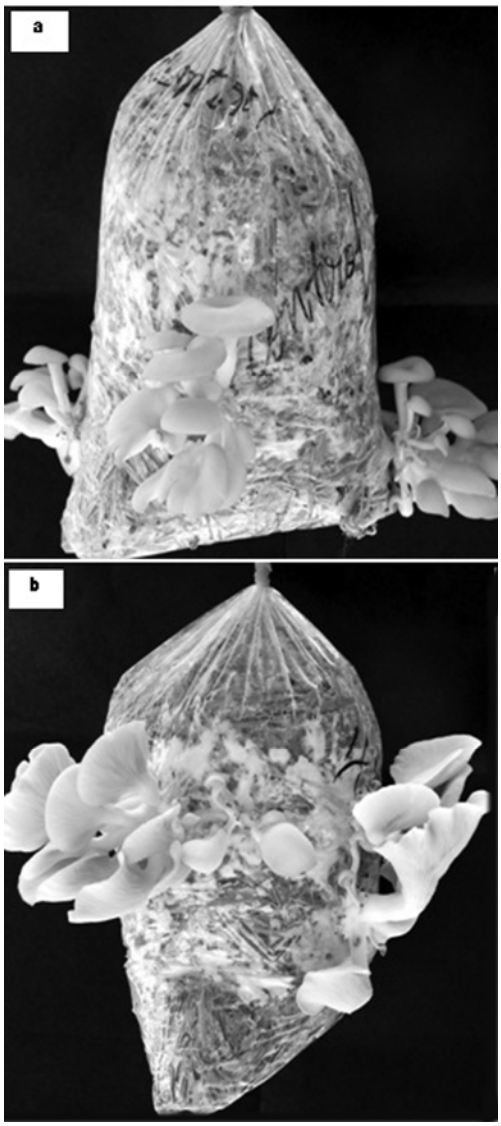
***Mushroom growth***

It was seen from the study spawn running, pinhead formation and fruiting body formation took place in about 2 -3 weeks after inoculation, 7-8 days after spawn running and 3-5 weeks after pin head formation. Similar findings were reported by (Tan *et al*., 1981; Ahmed *et al*., 1986; Quimio *et al*., 1976,1978). The mushroom growth in control and the substrate with 4 % humic acid is shown in Figure 1.a and Figure 1.b.

***Experimental design***

Adoption of Central Composite Design employed here requires prior knowledge on the upper and lower limits of the parameters, awareness of the cultivation process and its factors. The preliminary trails indicated that amount of Bagasse, Wheat bran, Urea and volume of Humic acid were significant variables for the cultivation process. Hence, these four variables were chosen to obtain the optimum levels. The effect of composition trials on the yield of *Pleurotus ostreatus* is given in Figure 2. A four-factor, three-level Central Composite design was used to determine the optimal values for the factors. The central composite design (CCD) with a quadratic model was employed. Four independent variables namely Bagasse (17.5 g/l -30.0 g/l), Wheat bran composition (3 g/l–6 g/l), Urea composition (4 g/l-6 g/l) and Humic acid composition (4 – 6 ml) was chosen. Each independent variable had 4 levels which were –2, – 1, 0 and +1, +2. A total 30 different combinations (including eight star points and six replicates of centre point) were chose in random order according to a CCD configuration. The levels of variables and experimental design

matrix were presented in Table 1.a and Table 1.b.




The coded values of independent variables were found from equation

$$x_i = \frac{X_i - X_0}{\Delta X}, \quad i = 1,2,\ldots,k \quad (5)$$

where $x_i$ is the dimensionless value of an independent variable, $X_i$ is the real value of an independent variable, $X_0$ is the value of $X_i$ at the center point and $\Delta X$ is the step change. A second-order polynomial model was used to fit the quadratic, resulting in the equation,

$$Y_{pre} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_1^2 + \beta_6 x_2^2 + \beta_7 x_3^2 + \beta_8 x_4^2 + \beta_9 x_1 x_2 + \beta_{10} x_1 x_3 + \beta_{11} x_1 x_4 + \beta_{12} x_2 x_3 + \beta_{13} x_2 x_4 + \beta_{14} x_3 x_4$$

where $Y_{pre}$ is the measured response, $x_1, x_2, x_3, x_4$ are the coded independent input variables, $\beta_0$ is the intercept term, $\beta_1, \beta_2, \beta_3, \beta_4$ are the linear coefficients showing the linear effects, $\beta_5, \beta_6, \beta_7, \beta_8$ are the quadratic coefficients showing the squared effects and $\beta_9, \beta_{10}, \beta_{11}, \beta_{12}, \beta_{13}, \beta_{14}$ are the cross product coefficients showing the interaction effects. Thirty experiments were performed in triplicate. The results obtained were submitted to analysis of variance on SAS package and the regression model was given as

$$Y_{pre} = 367.549 + 18.5x_1 - 286.778 x_2 + 64.292x_3 + 61.167 x_4 - 0.342 x_1^2 + 28.33 x_2^2 - 9.063x_3^2 + 2.188x_4^2 - 0.2667x_1x_2 + 0.8333x_1x_3 - 1.333x_1x_4 + 2.667x_2x_3 - 3.5x_2x_4 - 3.75x_3x_4 \quad (6)$$

where $Y_{pre}$ is the Yield of *Pleurotus ostreatus* in this study and $x_1, x_2, x_3, x_4$ are the coded levels of independent input variables, amount of Bagasse, Wheat bran, Urea and volume of Humic acid respectively. The significance of each coefficient was determined by Student's t-test and P-value, which is listed in Table 2.a. The larger the magnitude of t-test and smaller the P-value, the more significant is the corresponding coefficient (Du, 2003). The regression model could be used to predict the future observations on the response Y corresponding to particular values of the regressor variables. During the prediction of new observations and estimation of the mean response at a given point, care must be taken about extrapolating beyond the region containing the original observations. The regression coefficient for the second order polynomial equations and results for the linear, quadratic and interaction term are discussed in detail. The optimum amount of Bagasse, Wheat bran, Urea and volume of Humic acid were obtained by solving the regression equation.

The results of the second-order response surface model in the form of analysis of variance (ANOVA) are given in Table 2.b. The Fisher's F-test ($F_{(14;15)} = S^2_m/S^2_s = 3804.614 > F_{t(14;15)} = 2.42$) with a very low probability value [ ($P_{model} > F$) < 5.37E-24 ] indicated the model was highly significant. The goodness of fit of the model was examined by determination coefficient ($R^2$ =0.9997), which implied that the sample variation of more than 99.97% was attributed to the variables and only 0.03 % of the total variance could not be explained by the model. A high value of correlation coefficient (R=99.99) signifies an excellent correlation between the independent variables. The adjusted determination coefficient (Adj $R^2$ = 0.9995) was also satisfactory to confirm the significance of the model. The residuals were examined to check the adequacy

of the model. The residuals were plotted against the predicted Y as shown in Figure 3. The ''horizontal band' indicated no abnormality, confirming the adequacy of the regression model (Draper *et al*., 1981). The amount of bagasse, urea and volume of humic acid added for the process of cultivation had a strong positive linear effect on the response. The amount of wheat bran and humic acid added showed a squared effect. However, humic acid addition was seen to be more significant compared to wheat bran. There was sufficient amount of interaction between bagasse- urea and wheat bran - urea, whereas the amount of wheat bran alone was less significant. The model predicted the maximum yield of 204.25 g, which appeared at the bagasse, Wheat bran, and Urea concentration of 21.25 g/l, 3.5 g/l, 5.0 g/l and Humic acid of 4 %, respectively. The subsequent experiments with the optimized conditions yielded consistent results with the prediction. The effects of four variables on the Yield of *Pleurotus ostreatus* were studied and the relevant conditions for the process were optimized.

***Mushroom yield(Y), productivity (P %) and biological efficiency (BE %)***

The maximum yield 204 g of Oyster mushroom was obtained on the nineteenth trial with 4 % humic acid. This finding is similar to Vaughan and Linehan, (1976) who reported enhancement of root growth and shoot growth in wheat plants by the application of humic acid. This was due to valuable byproducts released due to the microbial degradation of humic acid. The yield obtained was found to vary between the trials from 74 g to 204 g. Productivity was determined from the relation between mushroom fresh weight ($M_F$) and compost fresh weight ($C_F$).Biological efficiency was determined from the relation between mushroom fresh weight ($M_F$) and compost dry weight ($C_{D)}$ and is given by

$$P\% = \frac{M_F}{C_F} \times 100 \qquad (7)$$

$$BE\% = \frac{M_F}{C_D} \times 100 \qquad (8)$$

High productivity value of 18.47 % and biological efficiency of 27.16 % was observed for the nineteenth trial (Table 3). This was similar to the findings of Meire Cristina Nogueira de Andrade *et al*., (2007) who reported a productivity of 8.70 % and biological efficiency of 28.70 % in *Agaricus blazei* mushrooms grown in presence of *Trichoderma* sp.

***Analysis of Nutrients***

The protein content of the 19$^{th}$ trial was found to be 40 g, this was found to be more than the content of protein reported by Breene (1990) who illustrated the content of protein between 19 to 39 g in 100 g of dried matter. Fat value was 0.1 g in accordance with the value of 2.0 g in 100 g dry matter reported by Shah *et al*.(1997). 34.8 % dietary fibre was obtained from the cultivation of Pleurotus *ostreatus* by Justo *et al*. (1999), whereas our study reported a dietary content of 19.5 %.

## Conclusion

The data obtained from the experiments demonstrated the strategies for enhancing production of *Pleurotus ostreatus.* The results of the model for mixture design experiments showed that the amount of bagasse, urea and percentage of humic acid added for the process of cultivation had a strong positive linear effect. The model predicted the maximum yield at bagasse, Wheat bran, and Urea concentration of 21.25 g/l, 3.5 g/l, 5.0 g/l and Humic acid of 4 % respectively. Bagasse- urea and wheat bran - urea composition showed sufficient amount of interaction, whereas wheat bran alone was less significant. However, humic acid addition was seen to be more significant compared to wheat bran and it had a positive influence on the yield of *Pleurotus ostreatus*. According to these results, the yield of 204.25 g predicted by the model agrees well with the experimental value of 204 g. This indicates the adequacy of the generated model in predicting the yield of *Pleurotus ostreatus*. Substrate optimization studies revealed the application of 4% humic acid for maximum yield. The nutrient value of the mushroom was found to be 40 g protein, 0.1 g fat and 19.5 g fibre. The optimum substrate composition even showed a drastic effect on the productivity and biological efficiency. The increased productivity and biological efficiency was found to be 18.47 % and 27.16 % respectively.

**Table- 1a:** Levels of variables used in the Central composite design with four independent variables for optimization and their significance

| Variables | Range and levels | | | | |
|---|---|---|---|---|---|
| | **−2** | **−1** | **0** | **+1** | **+2** |
| $X_1$:Conc.of bagasse(g/l) | 17.5 | 21.25 | 25.0 | 28.75 | 32.50 |
| $X_2$:Conc.ofwheat bran(g/l) | 3.5 | 4.25 | 4.50 | 5.75 | 6.50 |
| $X_3$:Conc. of urea(g/l) | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 |
| $X_4$: Humic acid (%) | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 |

**Table -1b:** Central composite design matrix for optimization

| Trials | Bagasse(g/l) | Wheat bran (g/l) | urea(g/l) | Humic acid (%) | Yield of Pleurotus *ostreatus*(g) | |
|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | $X_4$ | Experimental | Predicted |
| 1 | -1 | -1 | -1 | -1 | 132 | 131.92 |
| 2 | +1 | -1 | -1 | -1 | 129 | 128.83 |
| 3 | -1 | +1 | -1 | -1 | 119 | 118.50 |
| 4 | +1 | +1 | -1 | -1 | 112 | 112.42 |
| 5 | -1 | -1 | +1 | -1 | 115 | 114.83 |
| 6 | +1 | -1 | +1 | -1 | 124 | 124.25 |
| 7 | -1 | +1 | +1 | -1 | 109 | 109.42 |
| 8 | +1 | +1 | +1 | -1 | 116 | 115.83 |
| 9 | -1 | -1 | -1 | +1 | 173 | 172.83 |
| 10 | +1 | -1 | -1 | +1 | 150 | 149.75 |
| 11 | -1 | +1 | -1 | +1 | 149 | 148.92 |
| 12 | +1 | +1 | -1 | +1 | 123 | 122.83 |
| 13 | -1 | -1 | +1 | +1 | 141 | 140.75 |
| 14 | +1 | -1 | +1 | +1 | 130 | 130.17 |
| 15 | -1 | +1 | +1 | +1 | 125 | 124.83 |
| 16 | +1 | +1 | +1 | +1 | 111 | 111.25 |
| 17 | -2 | 0 | 0 | 0 | 113 | 113.42 |
| 18 | 2 | 0 | 0 | 0 | 97 | 96.75 |
| 19 | 0 | -2 | 0 | 0 | 204 | 204.25 |
| 20 | 0 | +2 | 0 | 0 | 172 | 171.92 |
| 21 | 0 | 0 | -2 | 0 | 102 | 102.42 |
| 22 | 0 | 0 | +2 | 0 | 74 | 73.75 |
| 23 | 0 | 0 | 0 | -2 | 115 | 114.92 |
| 24 | 0 | 0 | 0 | +2 | 151 | 151.25 |
| 25 | 0 | 0 | 0 | 0 | 124 | 124.33 |
| 26 | 0 | 0 | 0 | 0 | 125 | 124.33 |
| 27 | 0 | 0 | 0 | 0 | 123 | 124.33 |
| 28 | 0 | 0 | 0 | 0 | 125 | 124.33 |
| 29 | 0 | 0 | 0 | 0 | 124 | 124.33 |
| 30 | 0 | 0 | 0 | 0 | 125 | 124.33 |

**Table -2a:** Regression coefficients and their significance

| Model term | Parameter estimate | Standard error | Computed t-value | P-value |
|---|---|---|---|---|
| Intercept | 367.5486 | 14.25772 | 25.77892 | 7.76E-14 |
| $X_1$ | 18.5 | 0.53029 | 34.88659 | 8.91E-16 |
| $X_2$ | -286.778 | 2.651448 | -108.159 | 4.1E-23 |
| $X_3$ | 64.29167 | 1.847796 | 34.79369 | 9.27E-16 |
| $X_4$ | 61.16667 | 1.778863 | 34.38527 | 1.1E-15 |
| $X_1*X_1$ | -0.34222 | 0.007839 | -43.6549 | 3.18E-17 |
| $X_2*X_2$ | 28.33333 | 0.195982 | 144.5714 | 5.3E-25 |
| $X_3*X_3$ | -9.0625 | 0.11024 | -82.2073 | 2.49E-21 |
| $X_4*X_4$ | 2.1875 | 0.11024 | 19.84313 | 3.54E-12 |
| $X_1*X_2$ | -0.26667 | 0.05132 | -5.19615 | 0.000109 |
| $X_1*X_3$ | 0.833333 | 0.03849 | 21.65064 | 9.97E-13 |
| $X_1*X_4$ | -1.33333 | 0.03849 | -34.641 | 9.89E-16 |
| $X_2*X_3$ | 2.666667 | 0.19245 | 13.85641 | 5.91E-10 |
| $X_2*X_4$ | -3.5 | 0.19245 | -18.1865 | 1.24E-11 |
| $X_3*X_4$ | -3.75 | 0.144338 | -25.9808 | 6.92E-14 |

**Table -2b:** Regression Statistics and ANOVA for the regression model

| Source | Degree of freedom | Sum of squares | Mean sum of squares | F | Significance F |
|---|---|---|---|---|---|
| Regression | 14 | 17754.87 | 1268.205 | 3804.614 | 5.37E-24 |
| Residual | 15 | 5 | 0.3333 | | |
| Total | 29 | 17759.87 | | | |

***Multiple R 0.999859 ; $R^2$ 0.999718 ; Adj $R^2$ 0.999456 ; Standard Error 0.57735***

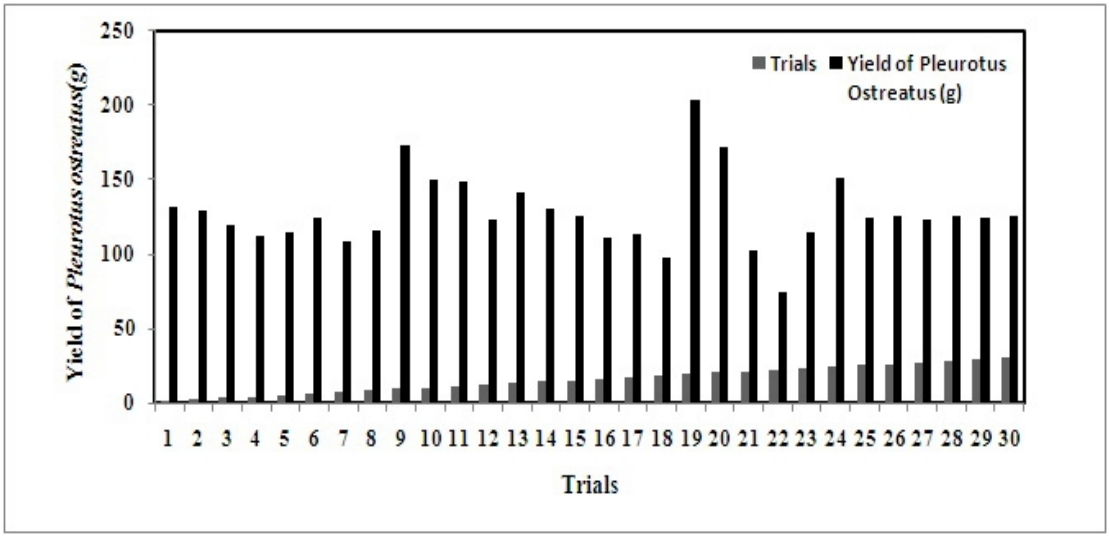


**Table- 3:** Protein, fat, Moisture, fibre, ash content in *Pleurotus ostreatus* and Productivity and Biological efficiency of substrates.

| Trials | Protein (g) | Moisture (%) | Fat (g) | Fibre (g) | Ash (g) | $M_F$ (g) | $C_F$ (g) | $C_D$ (g) | P (%) | BE (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 22 | 79 | 0.7 | 2.3 | 4.9 | 132 | 1008 | 773 | 13.09 | 17.08 |
| 2 | 25 | 63 | 0.5 | 2.6 | 7.2 | 129 | 1009 | 775 | 12.70 | 16.65 |
| 3 | 29 | 73 | 0.9 | 18.6 | 3.2 | 119 | 1123 | 885 | 10.59 | 13.45 |
| 4 | 22 | 88 | 0.4 | 4.4 | 2.6 | 112 | 1158 | 884 | 9.67 | 12.68 |
| 5 | 21 | 82 | 0.9 | 3.3 | 3.1 | 115 | 1124 | 881 | 10.23 | 13.05 |
| 6 | 35 | 92 | 0.2 | 17.2 | 1.4 | 124 | 1135 | 880 | 10.92 | 14.09 |
| 7 | 18 | 95 | 0.8 | 2.9 | 2.5 | 109 | 1100 | 790 | 9.90 | 13.79 |
| 8 | 20 | 94 | 0.9 | 4.3 | 2.3 | 116 | 1105 | 780 | 10.49 | 14.87 |
| 9 | 21 | 89 | 0.4 | 5 | 3.2 | 173 | 1124 | 745 | 15.39 | 23.22 |
| 10 | 19 | 86 | 0.5 | 9.6 | 3.2 | 150 | 1120 | 769 | 13.39 | 19.51 |
| 11 | 22 | 93 | 0.9 | 7.9 | 2.6 | 149 | 1127 | 798 | 13.22 | 18.67 |
| 12 | 22 | 95 | 0.6 | 8.9 | 2.9 | 123 | 1121 | 783 | 10.97 | 15.71 |
| 13 | 25 | 96 | 0.4 | 11.0 | 4.9 | 141 | 1120 | 781 | 12.58 | 18.05 |
| 14 | 23 | 94 | 0.7 | 13.3 | 4.2 | 130 | 998 | 665 | 13.02 | 19.55 |
| 15 | 23 | 91 | 0.5 | 13.5 | 3.6 | 125 | 869 | 662 | 14.38 | 18.88 |
| 16 | 24 | 90 | 0.5 | 12.6 | 3.8 | 111 | 1001 | 783 | 11.08 | 14.18 |
| 17 | 32 | 89 | 0.6 | 4.6 | 3.4 | 113 | 1121 | 784 | 10.08 | 14.41 |
| 18 | 30 | 87 | 0.6 | 5.5 | 3.5 | 97 | 1124 | 754 | 8.62 | 12.86 |
| 19 | 40 | 82 | 0.1 | 19.5 | 3.1 | 204 | 1104 | 751 | 18.47 | 27.16 |
| 20 | 31 | 84 | 0.7 | 2.2 | 3.9 | 172 | 1127 | 854 | 15.26 | 20.14 |
| 21 | 29 | 91 | 0.8 | 3.5 | 4.1 | 102 | 1124 | 851 | 9.07 | 11.98 |
| 22 | 27 | 93 | 0.7 | 14.6 | 1.6 | 74 | 550 | 441 | 13.45 | 16.78 |
| 23 | 26 | 90 | 0.8 | 15.2 | 2.6 | 115 | 1125 | 952 | 10.22 | 12.08 |
| 24 | 20 | 97 | 0.5 | 7.9 | 2.6 | 151 | 1121 | 774 | 13.47 | 19.50 |
| 25 | 22 | 92 | 0.5 | 6.2 | 2.9 | 124 | 1112 | 752 | 11.15 | 16.49 |
| 26 | 23 | 74 | 0.4 | 5.9 | 2.8 | 125 | 1100 | 750 | 11.36 | 16.67 |
| 27 | 22 | 86 | 0.4 | 2.6 | 3.2 | 123 | 1132 | 852 | 10.86 | 14.44 |
| 28 | 25 | 82 | 0.3 | 5.2 | 4.2 | 125 | 1104 | 802 | 11.32 | 15.59 |
| 29 | 21 | 84 | 0.3 | 6.9 | 4.3 | 124 | 1105 | 802 | 11.22 | 15.46 |
| 30 | 26 | 91 | 0.3 | 5.2 | 4.2 | 125 | 1109 | 821 | 11.27 | 15.23 |

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