# Evaluation of white oyster mushroom production under different substrate types: a completely randomized design vs. simulation experiment

## Evaluación de la producción de hongos ostra blanco bajo diferentes tipos de sustrato: un diseño completamente aleatorizado vs. simulación del experimento

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**Abstract:** The cultivation of white oyster mushrooms has gained relevance for its economic, nutritional and ecological benefits. This study aims to identify the most efficient substrate to maximize mushroom production using a Completely Randomized Design. Three substrates (straw, sawdust and peanut shells) were evaluated with two replicates per treatment. Data analysis included analysis of variance, testing of assumptions, post-hoc tests for significant differences and analyzed the possibility of simulating this experiment under a certain distribution. The results of the exploratory analysis indicated that straw presented the highest average yield of 212.33 g, while peanut shells showed the lowest variability, ensuring greater stability in production. Statistical tests confirmed compliance with the assumptions of normality, constant variance and independence. Tukey's test revealed significant differences between substrates, highlighting that straw and sawdust maximize the production of white oyster mushrooms. This study highlights the importance of substrate selection in the optimization of mushroom cultivation and suggests simulation under normal distribution as a tool to reduce experimental costs and improve efficiency.

**Key words:** Fungi, simulation, designs, assumptions, Tukey, statistics.

**Resumen:** El cultivo de hongos Ostra blanco ha cobrado relevancia por sus beneficios económicos, nutricionales y ecológicos. Este estudio tiene como objetivo identificar el sustrato más eficiente para maximizar la producción de hongos mediante un Diseño Completamente Aleatorizado. Se evaluaron tres sustratos (paja, aserrín y cáscara de maní) con dos réplicas por tratamiento. El análisis de datos incluyó análisis de varianza, comprobación de supuestos, pruebas post-hoc para determinar diferencias significativas y analizó la posibilidad de simular este experimento bajo cierta distribución. Los resultados del análisis exploratorio indicaron que la paja presentó el mayor rendimiento promedio de 212.33 g, mientras que la cáscara de maní mostró la menor variabilidad, asegurando mayor estabilidad en la producción. Las pruebas estadísticas confirmaron el cumplimiento de los supuestos de normalidad, varianza constante e independencia. La prueba de Tukey reveló diferencias significativas entre los sustratos, destacando que la paja y el aserrín permiten maximizar la producción de hongos Ostra blanco. Este estudio resalta la importancia de la selección del sustrato en la optimización del cultivo de hongos y sugiere la simulación bajo la distribución normal como una herramienta para reducir costos experimentales y mejorar la eficiencia.

**Palabras Clave:** Hongos, simulación, diseños, supuestos, Tukey, estadística.

## Introduction

The cultivation of edible mushrooms, such as Pleurotus ostreatus or better known as white oyster mushrooms, has gained relevance for its contribution to food security, environmental sustainability and the generation of value in agricultural production chains. The optimization of cultivation conditions is key to maximize the productivity and quality of these mushrooms (Ramírez, 2019).

According to Castañeda and Martínez, (2019), increasing the inoculation rate significantly improves the yield of white Oyster mushrooms and the different culture media influence in vitro mycelial growth, providing fundamental information for industrialized processes. Mushrooms of the genus White Oyster mushrooms, known as mushrooms, are valued for their economic, nutritional and ecological relevance; their ability to grow on low-cost substrates, such as agricultural waste, positions them as a sustainable alternative in modern agriculture, promoting the circular economy by reducing waste (Castañeda and Martínez, 2019); also, their high protein content and richness in vitamins and essential minerals make them a strategic food to improve food security (Gómez et al, 2021).

In Paraguay, the journal of Agrarian Sciences has investigated and published about the cultivation of Pleurotus ostreatus and Ganoderma


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lucidum using wood and agricultural residues. The results showed that these substrates favor the growth and production of both fungi (De Madrignac, 2019); in Spain, research published by International Journal of Molecular Sciences has analyzed the impact of different lignocellulosic substrates on the production of white oyster mushrooms, determining that the combination of wheat straw and vine residues significantly improves the biological efficiency of the fungus (Pineda, 2020).

In Ecuador, the province of Chimborazo has ideal conditions for mushroom production due to its climate and availability of agricultural residues such as sawdust and sugarcane bagasse. However, the lack of technological infrastructure and technical knowledge limits its expansion. In contrast, for Gómez et al, (2021), in Zamora Chinchipe, the commercialization of mushrooms has generated economic opportunities and logistical challenges that require solutions adapted to the regional context.

The use of fungi in the present experiment seeks to maximize their production according to different types of crops that use substrates rich in lignocellulose as the main source of nutrients. According to Hossain et al., (2021), sawdust is widely used due to its moisture retention capacity and its supply of key nutrients for mycelial growth. For Ali and Usman, (2020), straw contains cellulose and hemicellulose and, although its moisture retention is limited, its low cost and wide availability make it a popular substrate. Prasad and Singh (2023) mention that peanut shells are an agricultural by-product that provides a source of cellulose and lignin, in addition to favoring substrate aeration, which is crucial for fungal growth.

## Methodology

### Materials and methods

This study employed a Completely Randomized Design (CRD), an experimental approach that randomly assigns treatments to experimental units, widely used in research where experimental conditions are kept homogeneous, allowing to minimize sources of external variability and to clearly evaluate the effects of independent variables . Different types of substrate were evaluated in the production of white oyster mushrooms. For a more robust analysis and to simulate different production scenarios, 100 simulation replicates were generated based on specific probability distributions, which allowed modeling the variability in production and evaluating the behavior under controlled but stochastic conditions.

Substrate preparation began with cleaning and grinding, followed by pasteurization at 65-75°C for 4 hours to eliminate unwanted microorganisms, a fundamental technique to ensure culture viability and minimize contaminations (Prasad and Singh, 2023). The substrates were mixed with water to a humidity of 60-65%, tested by the fist method, and placed in heavy-duty polyethylene plastic bags as experimental units. The mycelium was inoculated uniformly in each experimental unit. The sealed bags were placed in a temperature-controlled incubation chamber (25 ± 2°C) and total darkness for 30 days, with weekly monitoring to record the progress of colonization (Sharma et al., 2022). After complete colonization, the bags were transferred to the fruiting area under specific conditions: temperature between 15-22°C according to Sharma et al., (2022), a humidity of 85-90% by manual irrigation with non-chlorinated water, natural diffuse light and constant ventilation to prevent CO2 accumulation. The period of each fruiting lasted approximately 21 days, with weighing and recording of the harvested mushrooms, also evaluating their visual quality. The accumulated production of each experimental unit was used as a response variable in the statistical analysis (Ali et al., 2020). Protocols of good agricultural practices were implemented, including the use of sterilized tools and strict control of environmental conditions, documenting the data in specific record sheets for each experimental unit. A Completely Randomized Design (CRD), an experimental approach that randomly assigns the different types of substrates to the experimental units, was used in this study. The substrates evaluated were: sawdust, straw and peanut shells, selected for their availability and potential to be used in sustainable crops according to Naseer et al., 2022). The sample consisted of 9 experimental units, distributed in three treatments with two replicates each.

**Response variable:** Y: =" Production of white oyster mushrooms" (grams).

**Factor of interest:** F: =" Substrate", which has three levels:

- 1: Straw
- 2: Peanut Shells


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- 3: Sawdust

In order to perform a more robust analysis and simulate different production scenarios, 100 simulation replicates were generated for each treatment. This simulation allowed modeling the variability in production and evaluating the behavior of the treatments under controlled but stochastic conditions.

Analysis techniques included:

**Descriptive analysis:** Measures of central tendency and dispersion of production were calculated for each treatment.

**Analysis of variance (ANOVA):** Analysis of variance was used to determine if there were significant differences in fungal production among the different treatments. This analysis is appropriate for experimental designs with only one factor of interest (Saini, 2023).

$$F_0 = \frac{CM_{Trat}}{CM_E}$$

**Validation of assumptions:** To ensure the validity of the analysis of variance, the following fundamental assumptions were checked for Gabriel, (2021).

1. **Normality:** The normality of the residuals was evaluated using the Shapiro-Wilk test and Q-Q plot.

$H_0$ : The residuals have a normal distribution.

$H_1$ : The residuals do not have a normal distribution.

The Shapiro-Wilk test statistic is defined as:

$$W = \frac{1}{(n-1)S^2}[\sum_{i=1}^{a} a_i(X_{(n-i+1)} - X_i)]$$

2. **Constant variance (homoscedasticity):** A plot of residuals versus fitted values was visually inspected. In addition,

equality of variances between treatments was tested using Bartlett's test.

$$H_0: \sigma^2 = \sigma^2 = \cdots = \sigma^2$$

$$H_1: \sigma^2 \neq \sigma^2, \qquad para\ algún\ i = j$$

The test statistic is given by:

$$x_0^2 = 2.3026 * \frac{q}{c}$$

Where:

$$q = (N-k)\log_{10} S_p^2 - \sum_{i=1}^{k}(n_i-1)^{-1}\log_{10} S_i^2$$

$$c = 1 + \frac{1}{3(k-1)}\left(\sum_{i=1}^{k}(n_i-1)^{-1} - (N-k)^{-1}\right)$$

$$S_p^2 = \frac{\sum_{i=1}^{k}(n_i-1)S_i^2}{N-k}$$

3. **Independence of the errors:** The independence of the residuals was verified through the residuals plot as a function of the order of data collection and the semiparametric test of streak counting.

$H_0$ : The residuals are independent.

$H_1$ : There is correlation between the residuals.

The statistic for the gust test is calculated as:

$$\mu_R = \frac{2n_1n_2}{n_1+n_2} + 1$$

$$\sigma_R^2 = \frac{2n_1n_2(2n_1n_2-n_1-n_2)}{\left(n_1+n_2\right)^2(n_1+n_2'-1)}$$

$$Z = \frac{R - \mu_R}{\sigma_R}$$



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**Post-hoc tests:** If significant differences were found in the ANOVA, a Tukey mean comparison test was analyzed to identify specific differences between factor levels as recommended by Perez, (2020).

$$H_0: u_i = u_j$$

$$H_0: u_i \neq u_j$$

They were used to compare the differences between sample means with the critical value determined by:

$$T_{\propto} = q_{\propto}(k, N-k)\sqrt{\frac{MS_E}{n}}$$

**Data simulation and comparison with distributions:** Once the experiment was completed, the response variable was modeled based on three simulation models such as normal, uniform and exponential distribution under the following equations.

- **Normal Distribution**

$$X = \mu + \sigma * \left(\sum_{i=1}^{R} R_i\right)$$

- **Uniform Distribution**

$$X = a + (b - a) * R_i$$

- **Exponential Distribution**

$$X = \mu + \sigma \sum (R_i - 6)$$

It is important to note that 100 simulation replicates were run for each model and then, through numerical indicators , the model with the best fit was chosen to replicate the data obtained in the experiment.

## Results

This section presents the results obtained from the analysis of the completely randomized design, focused on evaluating the effect of different types of substrate on the production of white oyster mushrooms.

Table 1 shows the descriptive statistical analysis of the three substrates evaluated for the production of white oyster mushrooms. The results showed significant differences in the average yield of the treatments.

**Table 1.** *Comparative statistical analysis of substrates for white oyster mushroom production.*

| Measure | Straw | Peanut shells | Sawdust |
|---|---|---|---|
| Media | 212.33 | 71.83 | 109.17 |
| Standard error | 46.81 | 19.22 | 41.56 |
| Median | 215.00 | 61.00 | 94.50 |
| Standard deviation | 114.67 | 47.07 | 101.80 |
| Sample variance | 13149.07 | 2215.77 | 10363.77 |
| Kurtosis | -2.35 | -2.55 | -2.46 |
| Asymmetry coefficient | 0.06 | 0.29 | 0.27 |
| Range | 279.00 | 104.00 | 229.00 |
| Minimum | 78.00 | 24.00 | 16.00 |
| Maximum | 357.00 | 128.00 | 245.00 |
| Sum | 1274.00 | 431.00 | 655.00 |

Straw presented the highest average yield, with an average of 212.33 g, far surpassing the other treatments. Sawdust obtained an average yield of 109.17 g, while peanut shells showed the lowest yield, with a mean of 71.83 g. These differences highlighted the potential of straw



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as a more suitable substrate for white oyster mushroom production under the conditions evaluated.

In terms of data variability, high standard deviations were observed in all treatments, indicating a high dispersion in individual yields. Straw had the highest standard deviation, followed by sawdust, while peanut shells had the lowest dispersion.

On the other hand, the kurtosis results indicated that the distributions of the three substrates are platykurtic, with negative kurtosis values. This implies that the data are less concentrated around the mean compared to a normal distribution. In addition, the skewness coefficients revealed a slight positive skewness in all treatments, suggesting a tendency toward higher values than the mean.

In terms of variable length, it was observed that straw presented the highest range of values, while sawdust and peanut shells had ranges of 229.00 g and 104.00 g, respectively.

**Table 2.** *Experiment data matrix*

| **Substrates** | **Production (grams)** | | | | | |
|---|---|---|---|---|---|---|
| 1: Straw | 297 | 284 | 357 | 146 | 78 | 112 |
| 2: Peanut Shells | 123 | 87 | 128 | 34 | 35 | 24 |
| 3: Sawdust | 245 | 186 | 166 | 23 | 16 | 19 |

The initial exploratory analysis showed that production means varied among treatments, with the "Straw" substrate showing the highest values, while treatment 2 showed the greatest variability.

The analysis of variance of the experiment is shown below.

**Table 3.** *Analysis of Variance (ANOVA)*

| FV | SS | GL | MS | Fo | p-value |
|---|---|---|---|---|---|
| Substrate | 63554,77778 | 2 | 31777,38889 | 3,7053 | 0,0492 |
| Error | 128643 | 15 | 8576,2 | - | - |
| Total | 192197,7778 | 17 | 11305,75163 | - | - |

Since the observed value was greater than the critical value, H0 was rejected and it was concluded that the substrate factor has an effect on white oyster mushroom production. This means that at least one pair of substrate types generated a different average mushroom production.

**Table 4.** *Summary of ANOVA*

| | |
|---|---|
| Significance level | 0,05 |
| Observed value | 3,705 |
| Critical value | 3,682 |
| Critical region | (3.68; )∞ |
| Decision | Reject $H_0$ |

**Verification of assumptions**

A graphical procedure to verify compliance with the assumption of normality is the QQ Plot of Probability of Normality on the model residuals.


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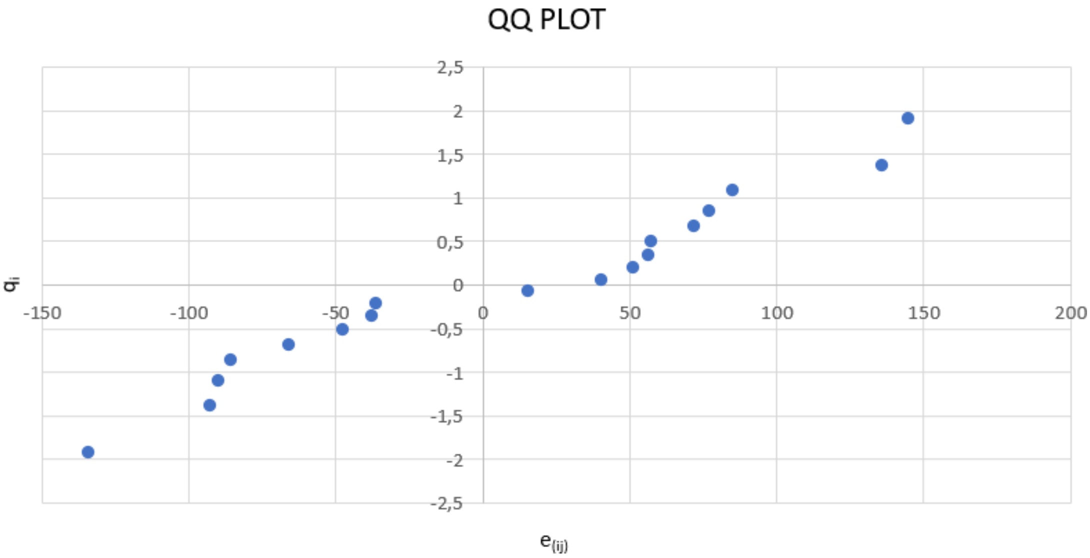
**Figure 1.** *Summary of ANOVA*




In Figure 1, it was observed that the points are approximately on the bisector, that is, the sample quantiles are very similar to the population quantiles. Therefore, it is graphically concluded that the sample of residuals comes from a normal distribution.

To contrast this result, the Shapiro-Wilks statistical test was applied.

**Table 5.** *Summary of the Shapiro-Wilks Test*

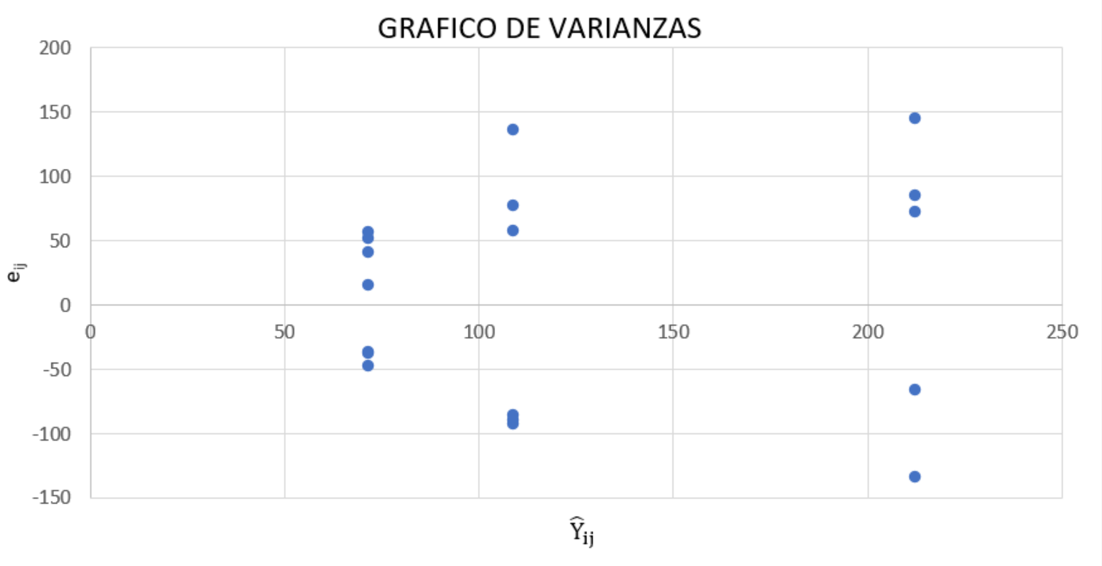
| | |
|---|---|
| Significance level | 0,05 |
| Observed value | 0,9012 |
| Critical value | 0,982 |
| Critical region | (0,98; ∞) |
| Decision | Do not reject $H_0$ |

Since the observed value was less than the critical value, $H_0$ was not rejected and it was concluded that the data come from a normal distribution F (x) is normal. And this agrees with what was observed in the QQ plot

### Homocedasticity

Two techniques were used for variance testing in relation to the treatments used, the graphical procedure used as base information the predicted data against the residuals.

**Figure 2**. *Graph of averages*




In Figure 2, it was observed that the points are randomly distributed in a horizontal band. Therefore, graphically it was concluded that the assumption of constant variance is fulfilled, the second technique applied Bartlett's statistical test for homogeneity of variancesAnd it can be seen that in the variables weight of 100 g mature, weight of production g/plant, and yield of gold coffee with and without adjustment, statistical differences were found between treatments; not so in the variables Weight 100 g of dry parchment coffee/plant and the variable Conversion cherry coffee to gold coffee.


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**Table 6**. *Summary of Bartlett's Test*

| | |
|---|---|
| Significance level | 0,05 |
| Observed value | 0,3207 |
| Critical value | 5,991 |
| Critical region | (5, 991; ∞) |
| Decision | Do not reject $H_0$ |

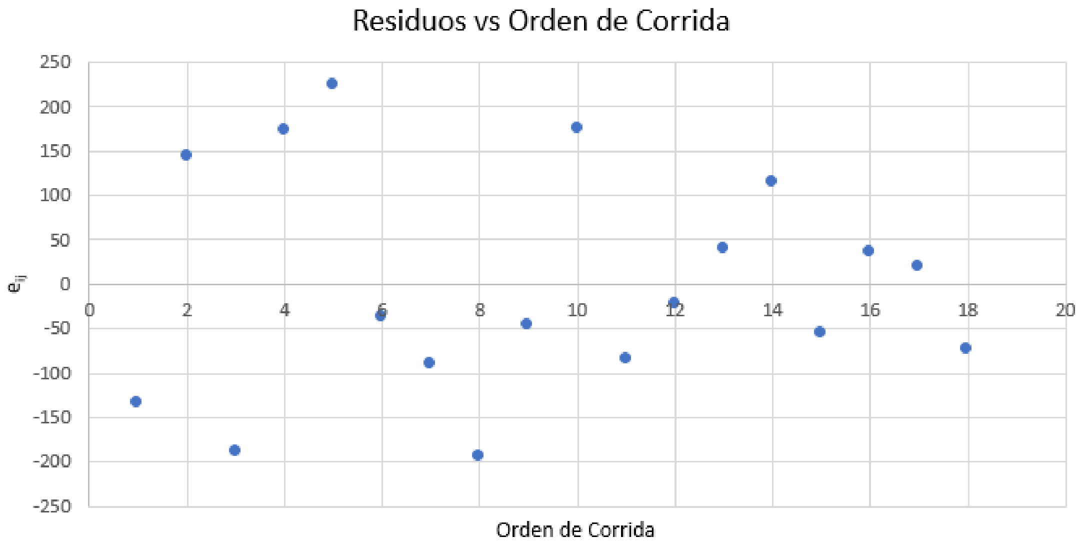
Since the observed value was less than the critical value, $H_0$ was not rejected and it was concluded that the assumption of constant variance was met. And this agrees with what was observed in the graphical technique.

**Independence**

The assumption of independence in the residuals was verified by plotting the order

The weight of humus treatment D1 was 1.6 kg, followed by agricultural gypsum with 1.4 kg and in last place the control treatment with 0.15, well below the other treatments.

**Figure 3.** *Independence Graph*




In Figure 3, a clearly defined trend or random pattern in the points was observed. Therefore, graphically it was concluded that it complies with the independence assumption; on the other hand, the statistical test of Rachas was applied with the following findings.

**Table 7.** *Summary of the Gust Test*

| | |
|---|---|
| Significance level | 0,05 |
| Observed value | -1,915 |
| Critical value | 1,96 |
| Critical region | (−∞;-1.96) y (1.96; +∞) |
| Decision | Do not reject $H_0$ |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Since the observed value was less than the critical value, H0 was not rejected and it was concluded that the assumption of independence is fulfilled. And this is in agreement with what was observed in the graphical technique.

The following is the comparison of means test for the choice of treatment to maximize mushroom production in the sense that the assumptions of the ANOVA model were fully met with a significance of .

**Post-Hoc Testing**

**Table 8.** *Comparison of Tukey's Test*

| Difference Population | Sample Difference | Tukey | Decision | Interpretation |
|---|---|---|---|---|
| u1 - u2 | 140,5 | 138,7516 | Reject $H_0$: Significant | Substrates 1 and 2 generate different average levels of oyster mushroom production. |
| u1 - u3 | 103,1667 | 138,7516 | Do Not Reject $H_0$: Not Significant | Substrates 1 and 3 generate equal average levels of oyster mushroom production. |
| u3 - u2 | 37,3333 | 138,7516 | Do Not Reject $H_0$: Not Significant | Substrates 3 and 2 generate equal average levels of oyster mushroom production. |

Substrate 1 (straw) and substrate 3 (sawdust) generated similar average levels, which guarantees the maximization of the production of white oyster mushrooms. However, to determine the best substrate option, it is necessary to consider other factors, such as cost, availability, decomposition time and management conditions, among other economic, operational and other aspects.

**Simulation**

From the experimental design, a random variable was simulated in order to model the behavior of mushroom production under different substrates, without the need to perform the experiment again.

During the simulation process the response variable white oyster mushroom production was compared using three simulation models (uniform, exponential and normal), under the following evaluated parameters:

**Table 9.** *Simulation Process Comparison*

| **Experiment parameters** | | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Uniform Distribution** | | **Exponential Distribution** | | **Normal Distribution** | |
| | **A** | **B** | $\lambda$ | $\mu$ | $\mu$ | $\sigma$ |
| **Values** | 24 | 357 | 0,006 | 153,466 | 153,466 | 99,060 |
| **Simulation statistics** | | | | | | |
| **Statistics** | 139,411 | 251,320 | 161,505 | 0,006 | 204,268 | 7,590 |
| **Difference between parameters and statistics** | | | | | | |
| **Differences** | 115,411 | 105,079 | 161,498 | 153,460 | 50,802 | 91,470 |

Table 9 shows the comparison of the parameters obtained from the experimentation and the simulation statistics the results showed that it is possible to simulate the response variable mushroom weight without the need to experiment again under the normal distribution.

In the present project, the impact of different substrates on the production of white oyster mushrooms in the province of Chimborazo was evaluated, with the objective of identifying the substrate that maximizes the crop production and if there is any distribution with



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which it can be simulated for future applications avoiding costs, time, among others. The results obtained showed that mushroom production varied significantly according to the type of substrate used. A study carried out in Colombia used a completely randomized design with four treatments and four replications, using oak, conacaste and liquid amber mulch as substrates. It was observed that oak reached a fungal production of 70.57%, while the other substrates did not allow fungal growth, (Acevedo, 2017). In comparison, in our study, certain agricultural residues as in the straw substrate and sawdust substrate generate similar average levels and that maximize the production of white Oyster mushrooms. However, to decide the best substrate option, during the experimental process, two key aspects were identified to be considered when choosing between them: economic and time. which is in agreement with previous research highlighting the influence of the culture medium on mushroom performance.

## Conclusions

The exploratory analysis of the three substrates evaluated for the production of white oyster mushrooms showed that straw is the most efficient substrate, with an average yield significantly higher than the other treatments. However, this substrate also presented the greatest variability in its results, suggesting the need for more rigorous control of growing conditions to optimize its performance. Although peanut shells had the lowest average yield, they showed the least variability among the three substrates evaluated, which makes them a viable option for productions that prioritize consistency over quantity. Sawdust is an intermediate alternative in terms of both yield and variability. Regarding the application of the completely randomized design, it was confirmed that it complies with the assumptions of normality, constant variance and independence, which validates the results of the ANOVA. This analysis revealed that the type of substrate has a significant effect on the production of white oyster mushrooms. Given that at least one substrate type produces oyster mushrooms differently from another in terms of quantity, the question arises: which substrate types are significantly different from each other? Tukey's method allows us to answer this question, as it is more precise at this level of significance, since it keeps it constant in each comparison of means. That said, it is concluded that the straw substrate and the sawdust substrate generate similar mean levels and maximize the production of white oyster mushrooms. However, in order to decide the best substrate option, two key aspects were identified during the experimental process that should be considered when choosing between them: quantity and time. From a quantitative production perspective, the sawdust substrate is more favorable, while in terms of time, the straw substrate offers greater results in a short period. Therefore, the choice will depend on the specific priorities and needs of the reader or user interested in these results. From the simulation applied to the experimental variable, it was concluded that it is possible to model the production of white oyster mushrooms using a normal distribution. This finding represents a significant advance, since it allows predicting the production behavior without the need to repeat the experiment physically, optimizing time and resources. The simulation not only reinforces the results obtained in the experimental design, but also provides a valuable tool to predict and improve mushroom production. For future lines of experimentation with the objective of obtaining more accurate conclusions, it is recommended to implement a completely randomized block design (CRBD), also considering the time factor. This approach will allow obtaining more robust results.

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