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Production and Evaluation of *Pleurotus* spp. Hybrids Cultivated on Ecuadorian Agro-Industrial Wastes: Using Multivariate Statistical Methods

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Abstract: The sustainable management of agricultural residues is a pivotal element in ensuring the sustainable development of agriculture. This is based on strategies that include the reutilization of residues as a substrate for the cultivation of economically significant mushroom species. The primary aim of this investigation is to assess the viability of utilizing two of the most prevalent agricultural residues in Ecuador as a substrate for the cultivation of hybrids within the *Pleurotus* genus. This assessment includes an evaluation of the nutritional and productivity parameters exhibited by the resulting mushrooms, employing multivariate statistical methodologies. The hybrid strains were developed by crossing compatible neohaplonts obtained through chemical dedikaryotization. A total of five neohaplonts of *Pleurotus ostreatus* as parental strain P1 and five monokaryons of *Pleurotus djamor* as parental strain P2 were randomly crossed in all possible combinations. Two parental hybrid strains, H1 and H2, were produced. These hybrids were cultivated using agricultural waste substrates, specifically, green banana leaves (GBL) and sugarcane bagasse (SB). Two distinct treatments or mixtures were tested: M1 (composed of 80% SB and 20% GBL) and M2 (composed of 20% SB and 80% GBL). It was found that the M1 blend promotes mushroom growth, yielding superior properties attributable to the higher proportion of nutritional content derived from sugarcane bagasse.

Keywords: agricultural residues management; green banana leaves; sugar cane bagasse; *Pleurotus*; hybrids; biplot

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1. Introduction

Management of agricultural residues encompasses a comprehensive approach that involves the planning, collection, categorization, processing, and appropriate final disposition of byproducts arising from agricultural operations. These bioproducts originate both during the cultivation phase and following harvest but prior to industrial processing. They include various components such as stems, leaves, branches, husks, corncobs, cereal straws, and others [1]. During the processing phase, these agricultural residues are repurposed through methods such as composting, utilization as livestock feed, conversion into organic fertilizers, energy generation, or the production of bioproducts. However, a significant portion of these residues is incinerated to clear post-harvest fields, thereby facilitating the commencement of new cultivation cycles [2]. This incineration practice can lead to adverse environmental consequences. As a result, there is a growing motivation to explore alternative measures that not only mitigate environmental impact but also contribute to the

long-term sustainability of agricultural practices. One such measure involves harnessing agricultural residues for mushroom cultivation [3].

Among the fungi commonly employed for this purpose, the *Pleurotus* genus, also known as oyster mushrooms, is the predominant choice. These mushrooms are renowned worldwide for their remarkable nutritional and medicinal value, and notably for their efficient ability to degrade lignocellulosic substrates such as agricultural residues. The fungi effectively harness the abundant fermentable carbohydrates within these residues as a primary energy source to facilitate their growth and development [4,5].

The demands of contemporary industries and the competitive nature of the market drive an ongoing quest for strategies to enhance mushroom traits, thereby boosting production yields. This pursuit encompasses traditional engineering methods as well as chemical dikaryotization for hybridization [6]. Chemical dedikaryotization is one of the successful methods for recovering the two monokaryotic components (mononuclear cells) of a dikaryon (binucleated cell) using toxic substances such as sodium taurocholate, colic acid, peptone, or glucose [7,8]. New fungal strains are created by matching compatible monokaryotic components that break the incompatibility barrier [9].

Nonetheless, the combination of various agricultural residues with supplements has proven effective in enhancing fungal performance. This makes the incorporation of alternative supplements a promising strategy in developing nations. Recent studies have demonstrated a notable 34.4% improvement in performance [10–12].

Sugarcane and bananas are two of the predominant food crops in the country's agricultural landscape. As of 2018, the total sugarcane production in Ecuador was estimated to be approximately 7,502,251 metric tons, with a significant concentration in the province of Guayas along the Ecuadorian coast [13]. From this total, approximately 1,300,000 tons of bagasse are generated as a bioproduct of the sugar and alcohol industry, along with ash, cachaza, and vinaza [14]. Bagasse exhibits distinct chemical properties, primarily characterized by its ash content (41%) and fiber content (11%), followed by crude protein (3%) and fat (2%) [15].

Conversely, Ecuador stands as one of the globe's foremost banana-producing nations. Production reached approximately 6.68 million metric tons by 2021, primarily concentrated in regions such as Guayas, Los Ríos, and El Oro along the Ecuadorian coast [16]. Among the agricultural residues generated by banana companies, banana leaves are the most abundant, followed by pseudostems, inflorescences, and peels [17]. Banana leaves exhibit a substantial composition of carbohydrates (37%), crude fiber (32%), and crude protein (18%), with relatively lower levels of ash (10%) and fat content (4%) [18].

The primary aim of this study is to assess the viability of employing two prevalent agricultural residues in Ecuador, banana leaves and sugarcane bagasse, as a substrate for the cultivation of hybrids of *Pleurotus ostreatus* and *Pleurotus djamor*. This assessment will include an evaluation of the productivity and nutritional parameters of the cultivated mushrooms, employing multivariate statistical methodologies. The study will focus on two distinct substrate mixtures, denoted as M1 (comprising 80% sugarcane bagasse and 20% banana leaves) and M2 (comprising 20% sugarcane bagasse and 80% banana leaves).

2. Materials and Methods

2.1. Collection and Activation of Fungal Strains

In this research, two parental strains of *Pleurotus* spp. were used: *Pleurotus ostreatus* (P1) and *Pleurotus djamor* (P2). The strains were obtained from the fungal collection of the Microbiology Laboratory of the Universidad Estatal de Milagro (UNEMI). The strains were identified as P1 (CC060) and P2 (CC051) and were preserved in a liquid medium. Their reactivation was carried out by inoculating 10 µL of the liquid medium containing the strains in stationary phase into Petri dishes containing malt extract agar (MEA) medium. Subsequently, the strains were incubated for 5 days at a temperature of 30 °C, under low light and dark conditions, until the respective mycelial growth was observed.

2.2. Recovery of Neohaplonts by Dedikaryotization of Fungal Strains

The process of inducing dikaryotization in fungal strains was accomplished using a dikaryotic solution known as the peptone-glucose solution (PGS). To prepare PGS, 20% peptone and 20% anhydrous glucose were meticulously dissolved in one liter of distilled water. Subsequently, precisely 50 mL of this solution was dispensed into two separate glass bottles and subjected to sterilization in an autoclave at 15 psi and 121 °C for 24 h. This prolonged incubation served to ensure the sterility of the solution [19].

Following the sterilization period, the monokaryotic components, also known as neohaplonts, were retrieved. The mycelia from the strains cultivated in the Petri dishes on MEA were harvested and divided into four equal portions. These segments were then homogenized in a volume of 50 mL of water using a blender. Distinct homogenization time intervals were utilized: 60 s and 90 s.

The samples were subjected to incubation at a controlled temperature of 28 °C until mycelial growth was confirmed. Following this incubation, the resultant liquid was homogenized in 50 mL of sterile distilled water for a duration of 20 s.

For subsequent steps, Petri dishes were used, and inoculation was carried out with volumes of 15 and 20 µL of the final homogenate. These inoculated Petri dishes were then placed in an incubator set at 28 °C until colonies appeared. Subsequently, microscopic examination at 10X magnification was conducted on the Petri dishes to discern the presence or absence of fibulae in the hyphae (neohaplonts). Neohaplonts are identified as hyphae in which cells have undergone multiple divisions originating from a single pair of nuclei within a dikaryotic hypha, resulting in the absence of protrusion formation [20]. Ultimately, the monokaryotic constituents were individually cultured in separate Petri dishes, each containing 10 mL of MEA, to confirm the absence of fibulae under the examination of a microscope set at 10X magnification [21].

2.3. Identification of the Compatibility Types of Neohaplonts and Pleurotus spp. Hybrid Strains Production

In the case of each parental strain, all monokaryotic components derived from the same strain were systematically paired with one another in Petri dishes containing MEA. Subsequently, these Petri dishes were incubated at a temperature of 28 °C and examined under a microscope at 10X magnification on a daily basis. The objective of this scrutiny was to identify mycelial growth exhibiting the presence of fibulae. In instances where such mycelium was confirmed, the dikaryon was then cultured in a separate Petri dish containing MEA to further validate the presence of fibulae, as per reference [7]. The fibula is an outward projection that emerges from the hypha during mitosis, housing one of the individual nuclei from the pre-mitosis binucleate cell. This nucleus subsequently amalgamates with the subapical cell of the primary hyphal axis, giving rise to a novel binucleate cell that retains duplicates of both nuclei [20].

In a controlled experimental setup, neohaplonts isolated from *Pleurotus ostreatus* were systematically paired with monokaryotic constituents sourced from *Pleurotus djamor*. Each combination was cultivated in a separate Petri dish containing 10 mL of MEA. The Petri dishes were then subjected to a consistent incubation regimen at 28 °C. Thorough microscopic examinations were conducted at 24 h intervals under a 10X magnification lens to ascertain the emergence of fibular structures. Ultimately, the resulting novel strain was cultured on dedicated Petri dishes containing MEA to conclusively confirm the presence of fibulae, as documented in references [22,23].

2.4. Substrate Preparation and Induction of Fungal Fruiting

Parental and hybrid strains were cultivated using two distinct substrate compositions, each formulated from agricultural residues: a mixture denoted as M1 comprising 80% sugarcane bagasse (SB) and 20% green banana leaf (GBL), and another mixture labeled as M2, consisting of 80% green banana leaf and 20% sugarcane bagasse. The moisture content of these substrates was adjusted to 80%, as determined through the oven method.

Subsequently, 0.5 kg of the prepared substrates, measured by wet weight, were placed in plastic bags and subjected to sterilization at 15 psi and 121 °C for a duration of 2 h. Following the sterilization process, the bags were allowed to cool and were then inoculated with 10% (*w*/*w*) of wheat grain. The inoculated bags were subsequently placed in a light-protected environment and incubated at a temperature of 28 ± 2 °C.

Upon full colonization of the substrates by the mycelium of the strains, the substrate-containing bags were promptly relocated to a dedicated fruiting chamber. The fruiting chamber was characterized by optimized environmental parameters, including a relative humidity range of 85–90%, a constant temperature of 18 ± 1 °C, a 12 h illumination period, and controlled air recirculation.

2.5. Mushroom Productivity Parameters

Fungal productivity was evaluated based on biological efficiency (BE; fresh weight of collected mushrooms/dry weight of substrate × 100), performance (Y; fresh weight of col-lected mushrooms/fresh weight of substrate × 100) and production rate (PR; ratio of BE/total number of production days since inoculation) [24].

2.6. Nutritional Composition of Mushrooms

Fungi were dried at 60 °C for 24 h and then ground for proximal analysis using standard methods. Ash content, crude fiber, and crude fat were determined according to the methods of Horwitz and Latimer [25]. Carbohydrates were calculated using the formula: 100 − (% protein + % fat + % ash content) [23]. Total nitrogen was evaluated with the micro-Kjeldahl method; crude protein was calculated from the total nitrogen content using the conversion factor 4.38. The energetic value of mushrooms should be estimated based on the content of crude protein (N × 4.38), fats, and carbohydrates using modified specific factors of 3.75, 8.37, and 4.2 kcal g^{-1} of each component, respectively [26].

2.7. Color Determination of Carpophores

The color of the carpophores was determined using the Konica Minolta Color Reader CR-10 colorimeter. CIE-Lab system values (L*, a*, and b*) were obtained and converted to an RGB (red, green, and blue) system using the ColorMine.org converter. The RGB values were placed in Adobe Color software to obtain a hexadecimal value, and from this value, the Munsell code was obtained to determine the color [27].

2.8. Statistical Analysis

In all of the experiments, a completely randomized design was carried out. The results were verified assuming the normality of the data. Subsequently, the results were examined using a one-way analysis of variance (ANOVA) to determine the significant difference at a $p < 0.05$ level, of the productivity parameters and the chemical composition of the fungi [28]. Values were expressed as the mean ± standard deviation (SD) of ten repetitions of each measurement. Once statistical differences were found, the Duncan's test with $\alpha = 0.05$ was used. The analyzes were carried out applying statistical software (Statgraph-ic ver.16). A principal component analysis (PCA) was also developed to obtain a factorial graph of plane 1-2 (PCA Biplot), one for the productivity parameters (biological efficiency, production rate and performance) and another for the chemical parameters (fat, crude protein, ash, crude fiber and carbohydrates) to establish the relationship between the parameters (R software version 4.3.1).

Biplot Graph

A biplot is a graphical representation of an N × M matrix, where N is the number of individuals [29] and M is the number of variables [30]. Its graphic structure allows for the analysis of data with more than one variable. Multivariate analysis reduces the dimensionality of a data set by projecting the data onto a lower-dimensional space called Euclidean space. Biplots provide a functional tool that can represent the results of a

principal component analysis (PCA), displaying values and statistical products such as the distances between data units, their corresponding groupings, variance, and correlations between variables or between individuals [31].

For example, in a 2-dimensional representation in Figure 1, the individuals or rows are shown with orange circles, and the lines starting from the origin and ending in an arrowhead represent the variables or blue columns, which are called vectors.

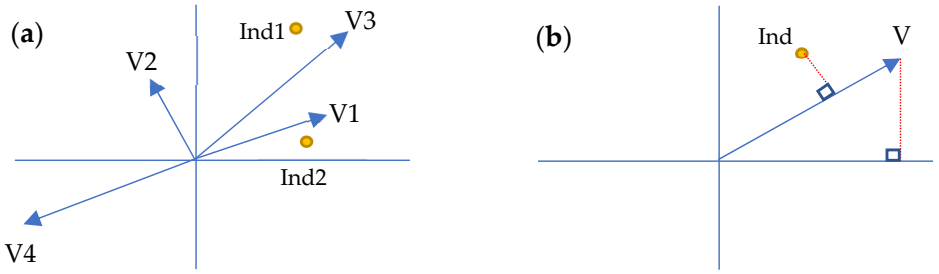

Figure 1. General representation of a biplot graph. (**a**) Representation of the variables of study as vectors (V1, V2, V3 and V4) and the interpretation of the degree of relationship according to the angle formed between them (**b**) Representation of the degree of relationship between the vector (V) with the axes of the plane and the vector (V) with the individual (Ind).

In graphs Figure 1a,b the axes of the planes represent the components that have the greatest representativeness of the individuals (Ind) and variables from the results of a PCA. (a) each vector represents a variable within the study and its graphical interpretation shows the degree of relationship, for V1 and V3 the relationship of the variables is strong because their angle is acute and the more the angle is shortened the greater the relationship, If the angle they form is 90°, in the case of V1 and V2, their relationship is zero. While the angle is obtuse, as in the case of V4 and V1, their relationship is inverse. (b) The angle formed by the vector projected towards the axes provides the information with which component there is a greater relationship. The angle formed by the projection from the individual (Ind) to a vector shows that there is a greater relationship with one variable or the other.

We apply the multivariate method through principal component analysis and its result is interpreted by means of the biplot tool using the multibiplotR package [32], of the R software [33].

3. Results and Discussion

The parental strains underwent dedikaryotization, resulting in the isolation of nine monokaryotic components. The optimization of conditions to enhance the yield of neohaplonts for the *Pleurotus ostreatus* strain involved varying homogenization durations (60 and 90 s) and inoculation volumes (50 µL and 20 µL) in the dedikaryotization solution within Petri dishes containing MEA.

On the other hand, the conditions employed for achieving the highest recovery of monokaryons from the *Pleurotus djamor* strain involved a homogenization time of 90 s and inoculation volumes in the dedikaryotization solution of 100 µL and 15–20 µL for the Petri dish with MEA. In a similar study, homogenization times ranging from 55 to 65 s were utilized, along with inoculation volumes in the dedikaryotization solution of 50–100 µL, and 30, 60, and 90 µL of the homogenized inoculum on Petri dishes with MEA to recover fifteen neohaplonts from two *Pleurotus* strains [21].

In another study, extended homogenization times (300 s) were employed, along with inoculation volumes of 50 microliters in the dedikaryotization solution and 25–50 microliters on the Petri dish containing malt extract agar (MEA). This methodology yielded the recovery of a total of thirty-nine neohaplonts, with eighteen and twenty-one neohaplonts isolated from two distinct *Pleurotus* strains, respectively [34].

The generation of neohaplonts through fungal dedikaryotization in a laboratory setting is contingent upon a multitude of factors. These factors encompass culture conditions,

including parameters such as the composition of the growth medium, temperature, lighting conditions, and humidity levels. Furthermore, the duration of contact between reproductive structures, the utilization of chemical stimulants to facilitate dedikaryotization, the genetic characteristics of each parental strain, and their compatibility with the strain targeted for crossbreeding all play pivotal roles in this process [35].

3.1. Formation of Hybrid Strains

The neohaplonts obtained from the two parental strains, P1 and P2, were utilized to create two hybrid strains through the pairing of five *Pleurotus ostreatus* neohaplonts and five *Pleurotus djamor* monokaryons. This resulted in a total of 25 potential crossbreeding combinations.

Table 1 illustrates the mating of neohaplonts to form hybrid strains. Comparable results have been reported by other researchers. For example, Tagavi et al. [36] documented the creation of four hybrid strains (dikaryons) resulting from the crossbreeding of *Pleurotus ostreatus* and *Pleurotus eryngii* monokaryons. This process yielded four hybrids out of 700 possible combinations. Similarly, Rosnina et al. [37] reported the generation of eleven hybrids from 100 potential crosses involving *Pleurotus citrinopileatus* monokaryons and *Pleurotus pulmonarius* monokaryons. This was evidenced by the observation of fibula formation within the hyphae of the resulting hybrids.

Table 1. Formation of hybrid strains by mating compatible neohaplonts.

Formation of Hybrid Strains by Mating of Compatible Neohaplonts						
	Strains			**P2**		
Strains	**Neohaplonts**	**1**	**2**	**3**	**4**	**5**
P1	**1**	—	—	—	—	—
	2	—	**+**	—	—	—
	3	—	—	—	—	—
	4	—	—	—	—	—
	5	—	—	**+**	—	—
+	*Positive pairing = dikaryon*					
—	*Negative mating = incompatible neohaplonts*					

Note: The positive intersection between rows and columns corresponds to the formation of hybrids between the two *Pleurotus* strains.

Protoplast fusion represents an alternative method for obtaining interspecific hybrid strains through the somatic pathway. In one study, the fusion of protoplasts from *Pleurotus ostreatus* var. *florida* and *Pleurotus djamor* var. *roseus* resulted in the generation of two hybrid strains among 11 regenerated somatic lines [38]. Similarly, in another research endeavor, two hybrid strains were acquired through protoplast fusion between *Pleurotus ostreatus* and *Pleurotus djamor* within a pool of 412 regenerated colonies [39].

The restricted yield of hybrids arising from interspecific hybridization primarily hinges on genetic factors, such as genetic compatibility and genome similarity. When species have a closer evolutionary history and share common characteristics, their gametangia can fuse and surpass prezygotic barriers that may be inherent in specific species [40].

3.2. Productivity Parameters

Table 2 presents the productivity parameters of the parental, hybrid, and reconstituted strains cultivated in two distinct substrates: 80% sugarcane bagasse-20% green banana leaf (referred to as mixture M1) and 80% green banana leaf-20% sugarcane bagasse (referred to as mixture M2).

Based on the results, it was observed that, within the parameter of biological efficiency, strain P2 developed in mixture M1 showed a superior biological efficiency profile compared to the other strains evaluated; similarly, the production rate parameter showed differences in strain H2 developed in mixture M1. Mixture M2, on the other hand, did not show significant differences in biological efficiency or production rate. From a performance perspective,

no statistically significant differences were reflected between the two types of mixtures (M1 and M2), so both offered a similar performance depending on the biological context.

Table 2. Productivity parameters of parental and hybrid strains produced in mixture M1 and M2.

Strains	Type of Strains	Substrates	Biological Efficiency (%)	Production Rate (%)	Performance (%)
P1	Parental 1	M1	74.93 ± 4.85 ^{a}	2.19 ± 0.02 ^{a}	23.87 ± 4.39 ^{a}
		M2	83.85 ± 8.71 ^{A}	2.74 ± 0.18 ^{B}	27.98 ± 5.087 ^{A}
P2	Parental 2	M1	95.66 ± 2.92 ^{b}	2.53 ± 0.40 ^{a}	30.40 ± 8.25 ^{a}
		M2	72.95 ± 14.71 ^{A}	2.60 ± 0.23 ^{A}	33.16 ± 4.19 ^{A}
H1	Hybrid 1	M1	88.35 ± 3.97 ^{a}	2.52 ± 0.40 ^{a}	28.33 ± 7.25 ^{a}
		M2	87.50 ± 10.82 ^{A}	2.68 ± 0.37 ^{B}	26.92 ± 5.82 ^{A}
H2	Hybrid 2	M1	86.78 ± 13.72 ^{a}	2.84 ± 0.15 ^{b}	24.60 ± 2.61 ^{a}
		M2	85.93 ± 14.75 ^{A}	2.18 ± 0.08 ^{A}	26.93 ± 8.68 ^{A}

Note: In each column (parameter), the different letters (A, a, B, b) indicate significant differences between the values ($p < 0.05$, Duncan, n = 10).

This aligns with findings from previous studies, where the cultivation of *Pleurotus ostreatus* in mixtures predominantly composed of sugarcane bagasse in combination with other organic substrates, such as sawdust, wheat bran, or cassava peels, has yielded favorable results. For instance, in a study involving a substrate formulation of 600 g of sugarcane bagasse, 400 g of sawdust, and 10 g of wheat bran, the highest recorded biological efficiency reached 86.28%, a value in close proximity to the maximum value reported in the present investigation (95.66%) [41].

Similarly, in other research endeavors, it was observed that treatments utilizing a 50% sugarcane bagasse and 50% sawdust proportion yielded slightly lower biological efficiency, while an increase in the bagasse proportion to 100% corresponded to biological efficiency values of approximately 65.65% [42,43]. Furthermore, treatments primarily based on sugarcane bagasse demonstrated total yield values ranging from 207.96 to 250.51 g per bag [44].

Principal Component Analysis

Figure 2 presents the factorial graph of plane 1–2 (PCA Biplot) with three eigenvalues: Dim1, 0.428; Dim2, 0.343 and Dim3, 0.229. The eigenvalue is the variation in the distances along each principal component. The groups have been calculated using Biplot coordinates and the general description is based on three variables (biological efficiency, production rate and performance). The graph indicated that the parameters of biological efficiency and production rate are correlated, while the parameter of biological efficiency is independent of performance. The hybrid strains (H1 and H2) that grew in mixture M1 showed a close relationship with biological efficiency. The parental strain P2 that developed in mixture M1 also showed a relationship with the production rate. Some individuals that grew in mixture M2 showed an inverse relationship with performance.

3.3. Chemical Composition of Fruiting Bodies

The chemical composition of the fungi of the parental and hybrid strains produced in the mixture M1 and the mixture M2 is presented in Table 3.

Based on the results, it was observed that there were no statistically significant differences in the percentage of fat and crude protein between the conditions of mixtures M1 and M2, indicating that both offer similar parameters, regardless of whether they were hybrid or parental strains. With respect to ash content, the parental strain P2 developed in mixture M1 showed significant differences compared to the other groups. In the crude fiber content, the hybrid strain H2 and the parental strain P2 developed in mixture M2 showed significant differences in crude fiber content compared to the other strains evaluated, so variability was observed between the mixtures. From the perspective of the percentage of

carbohydrates, the hybrid strains developed in mixture M1 exhibited significant differences in their carbohydrate content compared to the other individuals.

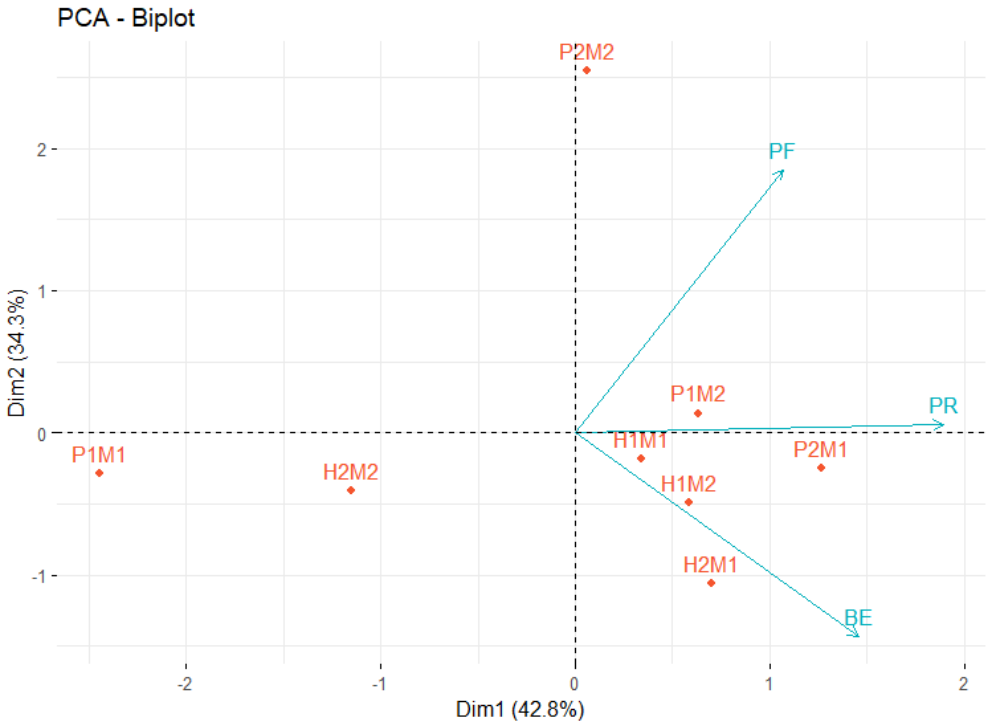

Figure 2. Biplot for productivity parameters of *Pleurotus* parental and hybrid strains grown in the mixture M1 and the mixture M2. The variables evaluated are biological efficiency (BE), production rate (PR) and performance (PF). The individuals are P1M1: parental 1 in mixture 1; P2M1: parental 2 in mixture 1; P1M2: parental 1 in mixture 2; P2M2: parental 2 in mixture 2; H1M1: hybrid 1 in mixture 1; H2M1: hybrid 2 in mixture 1; H1M2: hybrid 1 in mixture 2 and H2M2: hybrid 2 in mixture 2.

Table 3. Chemical composition of the fungi of the parental and hybrid strains cultivated in SB and GBL.

Strains	Types of Strains	Substrates	%Fat	% Crude Protein	% Ash	% Crude Fiber	% Carbohydrates
OS	Parental (P1)	M1	2.28 ± 0.13^{a}	28.59 ± 0.88^{a}	9.04 ± 0.40^{b}	10.21 ± 1.50^{a}	49.87 ± 1.32^{a}
		M2	1.38 ± 0.30^{A}	27.63 ± 2.19^{A}	4.72 ± 0.37^{A}	9.93 ± 1.66^{B}	56.34 ± 2.97^{A}
DJ	Parental (P2)	M1	2.46 ± 0.40^{b}	26.82 ± 2.01^{a}	7.72 ± 0.73^{b}	10.96 ± 0.50^{b}	52.04 ± 0.65^{b}
		M2	1.87 ± 0.09^{A}	25.77 ± 2.71^{A}	4.40 ± 0.12^{A}	10.59 ± 0.85^{C}	57.41 ± 3.42^{A}
OS_2 X DJ_2	Hybrid 1 (H1)	M1	2.67 ± 0.14^{b}	25.18 ± 3.46^{a}	8.37 ± 0.72^{b}	8.75 ± 0.09^{a}	55.03 ± 3.18^{b}
		M2	1.83 ± 0.44^{A}	24.22 ± 4.08^{A}	6.30 ± 1.21^{B}	7.54 ± 1.59^{B}	60.10 ± 3.96^{A}
OS_5 X DJ_3	Hybrid 2 (H2)	M1	1.77 ± 0.46^{a}	23.32 ± 3.91^{a}	5.70 ± 1.47^{a}	9.16 ± 1.05^{a}	60.05 ± 2.85^{c}
		M2	2.35 ± 0.33^{B}	26.07 ± 1.90^{A}	8.52 ± 0.24^{C}	5.01 ± 0.69^{A}	58.04 ± 2.52^{A}

Note: The means in a column with different superscripts are significantly different ($p < 0.05$, Duncan).

This coincided with the results obtained in other studies in which mushrooms of the genus *Pleurotus* spp. were cultivated in mixtures of residues at different proportions.

Zhou et al. [45] in their study compared the nutritional properties in different treatments, including one based on 21% sugarcane bagasse + 10.5% sawdust with corn stalks and another in the opposite direction 21% of sawdust + 10.5% sugarcane bagasse with sawdust; in the treatment with the highest proportion of sugarcane bagasse, a fat value of 2.14% was obtained, while in the other treatment 1.02%, values that were similar to the results in the present study with higher values in the mixture M1 with 80% of sugarcane bagasse.

In other studies respect to the percentage of crude protein, values of 20.6% were obtained in the treatment based on 50% sugarcane bagasse + 50% rubber tree sawdust and 23.2% in the treatment based on 25% sugarcane bagasse, 25% of and 50% rubber tree sawdust, with values that do not differ significantly from one treatment to another in a similar way to the present study [44].

In the case of the percentage of ash and carbohydrates, in other studies it has been observed that treatments with a higher content of sugarcane bagasse presented higher values of ash and carbohydrates, with 7.97% of ash and 35.57% of carbohydrates in the treatment based on sugarcane bagasse only, while 6.95% of ash and 34.50% of carbohydrates in the treatment based on sugarcane bagasse with elephant grass, similarly to the present study where M1 was more efficient [46].

In other studies, higher values of crude fiber were presented in treatments in which sugarcane bagasse has been mixed with sawdust, where the treatment with the highest proportion of bagasse presented the highest values (28.75%), similarly to the present study in which the highest value was reported in mixture M1 with 10.96% [47].

Principal Component Analysis

Figure 3 presents the factorial graph of plane 1–2 (PCA Biplot) with three eigenvalues: Dim1, 0.523; Dim2, 0.342 and Dim3, 0.118. The eigenvalue is the variation in the distances along each principal component. The groups have been calculated using Biplot coordinates and the general description is based on five variables (fat, crude protein, ash, crude fiber and carbohydrates). The graph indicated a close relationship between the parameters of ash content and fat content, while it presented an inverse relationship between the content of crude protein and carbohydrates. In turn, the parental strains (P1 and P2) that developed in mixture M1, presented a close relationship with the content of crude protein and the hybrid strain H1 in mixture M1 presented a close relationship with the content of ash and fat, while the hybrid strain H1 in mixture M1 presented a relationship with the content of carbohydrates.

3.4. Color of Fruiting Bodies

The fungal color of the parent and hybrid strains produced in the mixture M1 and M2 is presented in Table 4.

The coloration of the mushrooms produced by the two hybrid strains closely resembled that of the fruiting bodies of their respective parental strains. There was a higher phenotypic resemblance to the parental strain P1 in the case of hybrid H2, while hybrid H1 exhibited a higher phenotypic resemblance to the parental strain P2. However, it is noteworthy that within the M1 mixture, hybrid H1 displayed a distinct intermediate coloration, transitioning from the pale-yellow characteristic of the parental strains to a light yellowish-brown hue, indicative of a unique hybrid color phenotype.

In a separate study focusing on intraspecific hybridization through protoplasts, it was observed that when a pink strain of *Pleurotus djamor* was hybridized with a brown strain, the resultant hybrids exhibited diverse coloration, including pink, pinkish grey, brown, and white. This observation indicated that the pink color was not entirely dominant, and the white color exhibited characteristics of a recessive allele. Consequently, it becomes evident that the coloration of fungal fruiting bodies represents a quantitative trait influenced by the interplay of multiple genes [48].

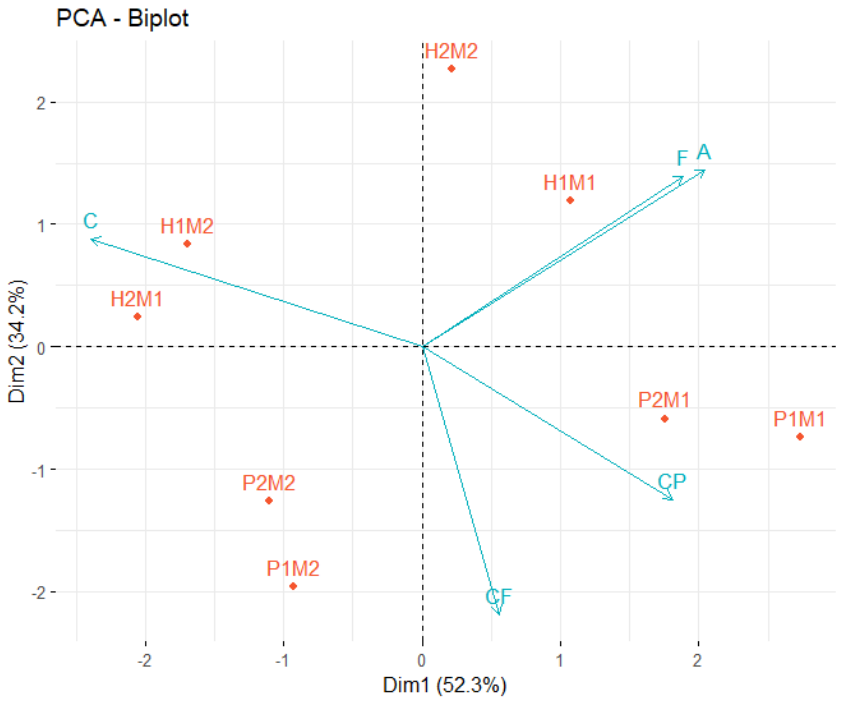

Figure 3. Biplot for nutritional parameters of *Pleurotus* parental and hybrid strains grown in the mixture M1 and the mixture M2. The variables evaluated are fat (F), crude protein (CP), ash (A), crude fiber (CF) and carbohydrates (C). The individuals are P1M1: parental 1 in mixture 1; P2M1: parental 2 in mixture 1; P1M2: parental 1 in mixture 2; P2M2: parental 2 in mixture 2; H1M1: hybrid 1 in mixture 1; H2M1: hybrid 2 in mixture 1; H1M2: hybrid 1 in mixture 2 and H2M2: hybrid 2 in mixture 2.

Table 4. Coloration of the fungi of the parental and hybrid strains of *Pleurotus* strains fruiting in the mixture M1 and M2.

Strains	Type of Strains	Substrates	Color
P1	Parental 1	M1	Pale yellow
		M2	Pale yellow
P2	Parental 2	M1	Pink
		M2	Pink
H1	Hybrid 1	M1	Light yellowish brown
		M2	Pale yellow
H2	Hybrid 2	M1	Pink
		M2	Pale pink

Note: Qualitative description of fruiting body colors of the resulting parental and hybrid strains.

4. Conclusions

In the present study, mixture M1 based on 80% sugarcane bagasse and 20% green banana leaf proved to be the mixture of growth substrates with the greatest potential due to its high values of biological efficiency and production rate, parameters that are closely related in view of the data recorded without significant differences between hybrid and parental strains. Similarly, it presented representative values in ash and carbohydrate content.

Mixture M2 based on 20% sugarcane bagasse and 80% green banana leaf presented similar yields with respect to mixture M1 and in nutritional parameters such as fat content, crude fiber and crude protein, highlighting the incidence of sugarcane bagasse in obtaining better results. This raises the initiative to use these mixtures of waste as potential sources for mushroom cultivation, reusing waste and promoting the circular economy.

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