

ORIGINAL RESEARCH ARTICLE

Efficiency of food wastes and wetland plants as originating materials for the growth and cultivation of *Pleurotus ostreatus*

Awatef Slama^{1*}, **Faten Mezni¹**, **Marwa Khammassi¹**, **Salima Bahri²**, and **Abdelhamid Khaldi¹**

¹Laboratory of Management and Valorization of Forest Resources, University of Carthage/The National Research Institute of Rural Engineering, Water, and Forestry, Ariana, Tunisia

²Laboratory of Forest Ecology, University of Carthage/The National Research Institute of Rural Engineering, Water, and Forestry, Ariana, Tunisia

Abstract

The valorization of lignocellulosic residues and unconventional plant biomass offers a promising strategy for sustainable mushroom production within a circular bioeconomy framework. This study evaluated the suitability of four substrates, namely wheat straw, *Typha latifolia* (cattail) biomass, *Ampelodesmos mauritanicus* biomass, and coffee waste, for the cultivation of *Pleurotus ostreatus*. The effects of two concentrations of glucose and potassium oxide on fungal colonization and fruiting performance were investigated under laboratory-scale conditions. The hypothesis was that substrate composition and nutrient supplementation would differentially influence mycelial development and mushroom production. Mycelial growth was evaluated through daily measurements of colony expansion, whereas fruiting performance was assessed through mushroom yield. Substrate type significantly affected both mycelial growth and yield. *A. mauritanicus* showed high potential for mycelial colonization, particularly after supplementation with glucose (20 g/L) or potassium (10 g/L). For fruiting body production, potassium supplementation improved cattail performance, with the highest yield obtained with 20 g/L potassium. Glucose and potassium supplementation also enhanced the productivity of *A. mauritanicus*. All tested fertilization concentrations increased *P. ostreatus* fructification from 1.5 to 8%, except when glucose was added to the coffee waste substrate. The tested supplementation rates had substrate-dependent effects, confirming that nutrient addition did not produce a universal response. Mineral analysis of harvested mushrooms revealed differences in nutritional composition among treatments. These results highlight the potential of cattail and *A. mauritanicus* biomass as alternative substrates for *P. ostreatus* cultivation. Further economic feasibility studies are required before scale-up applications.

Keywords: Oyster mushroom; Yield; Plants; Substrate; Fertilizers; Glucose

*Corresponding author:

Awatef Slama
(awatef.slama@ingref.ucar.tn)

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1. Introduction

The rise in global nutritional demand driven by the world's rapidly increasing population has accelerated in recent years. Among food products, mushrooms are used worldwide as a food source.¹ Edible mushrooms are known for their important

nutritional, nutraceutical, and pharmaceutical properties.² Numerous mushroom species, such as oyster mushroom (*Pleurotus ostreatus*), contain protein, fiber, vitamins, and antioxidants, which certify their high nutritional quality and dietetic virtues.³

In fact, more than 70% of agricultural and forest products are underutilized and discarded as waste.⁴ Currently, the efficient use of the added value of these agro-industrial residues has risen since the recovery of the massive biomass waste in human food produces significant products.⁵ This biomass comprises high amounts of lignin, cellulose, hemicellulose, and other mineral elements, which can provide the required nutrients for mushroom growth.⁶

Plant species play an important role in the functioning of forestry and wetland ecosystems. Several plant species contribute to environmental invasion through their rapid growth and prolific biomass production, often leading to the local extinction of native species within affected ecosystems. Consequently, the accumulation of biomass from forestry residues and semi-aquatic macrophytes in forests and wetlands alters nutrient cycling and contributes to increased greenhouse gas emissions.⁷ Many invasive or underutilized plant species, including the wetland macrophyte *Typha latifolia* L. and the terrestrial Mediterranean grass *Ampelodesmos mauritanicus* (Poir.), generate large quantities of lignocellulosic biomass that could instead be valorized.⁸ One promising approach is the biological utilization of these plant residues as cultivation substrates for edible mushrooms, an application that has been extensively investigated.⁹ Indeed, mushroom cultivation is considered a sustainable process to exploit forest and wetland plant biomass and agro-industrial by-products to preserve the value and nutritional basic substrates formerly known and to generate foods.¹⁰ Among these substrates, numerous agricultural and agro-forestry wastes, such as wood sawdust, sugar cane bagasse, and straw, have been shown to provide valuable materials for oyster mushroom growth.¹¹ Other plant species have also been used in oyster mushroom cultivation, such as haulms, rice straw, wheat straw, or sorghum straw, corn cobs, cottonseed hulls, palm and fruit fibers, while the most commonly used are beans/soybeans and sawdust. Likewise, compost has also been demonstrated as a favorable material for oyster mushroom cultivation.¹² The most commonly used plant biomass substrates in mushroom cultivation are wheat straw and cereals that constitute, concurrently, nutritional products for humans and animals.

On the other hand, forested areas are generally wetlands where multiple, untapped plant species coexist

and where economic development is globally affected by the complexity of this natural environment, particularly its geographical features and climate change. The cultivation of edible mushrooms could offer new opportunities to develop forest clearings and help the local population grow mushrooms using dead wood and unexploited invasive biomass plant species.¹³

The present study investigates the potential of forestry biomass from two plant species and a food by-product (coffee waste), supplemented with glucose and potassium (K), as substrates for oyster mushroom (*P. ostreatus*) cultivation. The hypothesis is that the use of natural crude lignocellulosic plant-based substrates, combined with nutrient supplementation, could enhance mushroom production and provide a sustainable alternative for cultivation in resource-limited forestry areas.

2. Materials and methods

2.1. Substrate preparation

A. mauritanicus Poir. (Figure 1A) and cattails—*T. latifolia* L. (Figure 1B)—were harvested from the Nefza region (Beja, Tunisia) in January 2024. Coffee wastes (Figure 1C) were collected from the Tunis region and washed, spin-dried, and desiccated at 45 °C for 48 h before use. Stems and leaves of *A. mauritanicus* Poir., *T. latifolia*, and wheat straw (Figure 1D) were sun-dried for 15 days, then milled with an electric grinder (HM/5304mm, Ceramic instruments S.R.L., Italy). Sieving was performed using an electric strainer (Retsch AS400, Retsch GmbH, Germany) at 250 rpm for 5 min for all substrates, maintaining the substrate particles at a size of ≤ 4 mm (sieve diameter).

2.2. Fungal strain and supplementation essay

P. ostreatus (Jacq.) P. Kumm. (ASI 2504) was provided by the Korea International Cooperation Agency as part of a cooperation project with the National Research Institute of Rural Engineering, Water, and Forestry (number 14/2012). Pure cultures were routinely established by subculturing mycelium isolated from fresh fruiting bodies onto potato dextrose agar (PDA) medium. Two supplement fertilizers were tested: glucose ($C_6H_{12}O_6$) and potassium oxide (K_2O). Supplements were dissolved in a sterile water solution in two concentrations: 10 g/L and 20 g/L. Treatments were designated C1 and C2 for glucose (10 and 20 g/L, respectively) and K1 and K2 for K_2O (10 and 20 g/L, respectively). The control treatment consisted of only sterile distilled water on the substrates. The volume of solution added to each substrate to obtain 65% of moisture was calculated according to Miles and Chang (Equation 1)¹⁴:



Figure 1. Plant-based substrates. (A) *Ampelodesmos mauritanicus*. (B) *Typha latifolia*. (C) Coffee wastes. (D) Wheat straw.

$$v = \frac{65}{100 - 65} \times P_s \quad (1)$$

where:

v = volume of water added (mL);

P_s = dry substrate weight (g);

65 = moisture content (%).

2.3. Inoculation

2.3.1. Mycelial growth

All substrates were sterilized (121 °C, 15 psi, 90 min), humidified at 65% (using sterile water or supplement at the appropriate volume and concentration), and then filled into glass Petri dishes (2303-1090, CITOTEST Scientific Co., Ltd, China). Three replicates were prepared for each treatment. Mycelial plugs (8 mm in diameter) excised from

actively growing *P. ostreatus* cultures in PDA medium were used to inoculate all Petri dishes, which were then incubated at 25 °C (incubator type: BD115 EC, Binder GmbH, Germany).

Colony growth was monitored daily by measuring two perpendicular diameters. Measurements were made in mm. *P. ostreatus* colony diameter was calculated as the mean of two perpendicular measurements in each Petri dish.

2.3.2. Oyster mushroom fructification

Each substrate (500 g) was adjusted to 65% moisture content (with sterile water for control, and the four supplement solutions C1, C2, K1, and K2) and distributed into cultivation bottles (1 L), then sterilized (121 °C, 15 psi, 90 min). All bottles were then inoculated with a pure culture of oyster mushroom at 1% (w/w) of the total weight. Inoculated bottles were incubated at 25 °C in the dark until the mushroom mycelium completely invaded the bottles. During the incubation phase, ventilation was applied intermittently using an automatic electric fan system (LRG-400B, Zhengzhou Labao Instrument Equipment Co., Ltd, China), operating for approximately 5 min per ventilation cycle. This limited air exchange was intended primarily to prevent excessive heat and carbon dioxide accumulation while maintaining favorable environmental conditions for mycelial growth. After this stage, bottles were opened, and a 12 h photoperiod (1,500–2,000 lux) and an ambient temperature of 20 °C were applied to induce the fructification phase. Relative humidity was maintained at 85–90% by spraying sterile water twice daily. After six days of oyster mushroom fructification, all growing mushrooms were collected and weighed using a balance (PS 1000.R2, RADWAG, Poland). Fructification surveys were conducted for two months, and each oyster mushroom fruit body was collected after six days without calculating the flush fruiting number.

The final oyster mushroom yield was measured and recorded for each substrate and treatment by multiplying the ratio (fresh weight of fruiting bodies or total harvest/fresh weight of substrate) by 100.¹⁵ The means of the three replicates for each tested substrate are presented in Section 3.

2.4. Mineral composition of oyster mushroom fruit bodies

Fresh mushrooms were harvested, manually cleaned, and then dried at 45 °C for 48 h. The contents of dried oyster mushrooms on carbon (C) were measured in accordance with the French Association for Standardization. Phosphorus (P) content was determined colorimetrically

using the vanadomolybdate method, with absorbance measured at 420 nm. K and sodium (Na) contents were determined using an emission flame photometer, while nitrogen (N) content was determined using the Kjeldahl method.

2.5. Statistical analysis

The variances of multiple parameters (colony diameter, substrate type, fertilization, mineral content, and oyster mushroom yield) were analyzed using a generalized linear model. Multiple comparisons of means were performed using the Student–Newman–Keuls test at $\alpha = 0.05$. Principal component analysis (PCA), hierarchical agglomerative clustering (HAC), and regression analyses were used to explore relationships among yield and mushroom mineral composition. HAC was also used to classify the substrate groups. All these tests were processed using Statistical Package for Social Sciences (SPSS 20.0, IBM, United States).

3. Results

The growth curves of oyster mushroom colony diameter on raw and fertilized *T. latifolia* substrates (C1, C2, K1, and K2) exhibited broadly similar sigmoidal patterns, with approximately overlapping trends among treatments (Figure 2). Overall, mycelial development followed the classical triphasic growth pattern, consisting of an initial lag phase of approximately three days, an exponential growth phase lasting nearly three weeks, and a subsequent stationary phase.

In contrast, the mycelial growth observed on *T. latifolia* substrate fertilized with 10 g/L K (K1) showed a slightly modified kinetic profile. After a similar lag phase of three days, an acceleration phase was observed from day 5 to approximately day 18. The exponential phase lasted about 15 days, and the stationary phase was reached around day 33 post-inoculation.

Among *T. latifolia*-based treatments, supplementation with 20 g/L glucose (C2) resulted in the greatest mycelial elongation, indicating a positive effect of C enrichment on fungal growth performance under these conditions.

Considering all tested substrates, those derived from dried *A. mauritanicus* biomass supplemented with glucose (10 g/L, C1) and K (10 g/L, K1) exhibited enhanced mycelial development compared with other treatments. In particular, the combined fertilization strategies promoted higher colony extension rates and improved overall growth performance.

The *A. mauritanicus* C2 treatment also showed strong performance (Figure 3), characterized by high mycelial

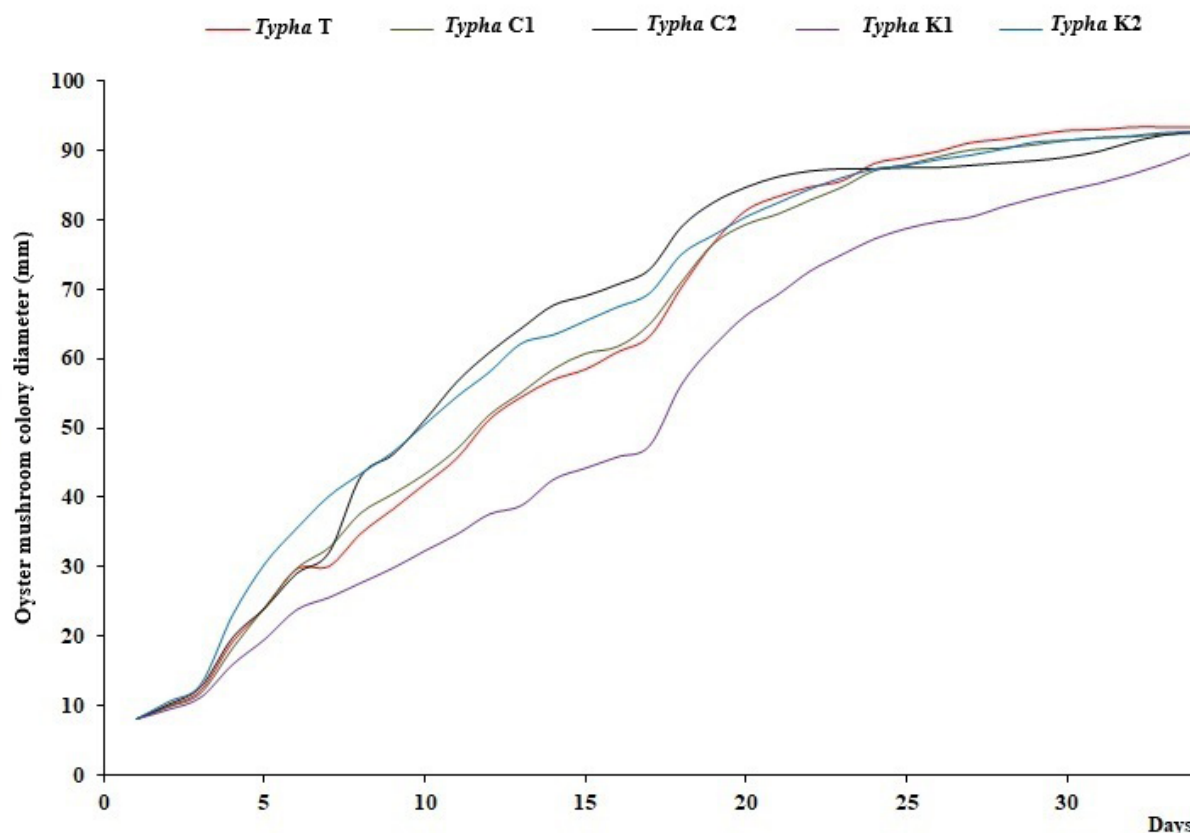


Figure 2. Oyster mushroom diameter growth colony on fertilized and brut *Typha latifolia* (T: Brut substrate; C1: Fertilization with 10 g/L glucose; C2: Fertilization with 20 g/L glucose; K1: Fertilization with 10 g/L potassium, and K2: Fertilization with 20 g/L potassium).

elongation and a shortened exponential phase relative to other substrates. This accelerated transition toward the stationary phase suggests more efficient substrate colonization under this condition.

On wheat straw substrate (Figure 4), potassium supplementation at 10 g/L (K1) led to greater improvement in mycelial growth than the other groups (control, C1, C2, and K2) as well as the control. This indicates a beneficial effect of K enrichment on the utilization of lignocellulosic substrates by *P. ostreatus*.

Similarly, coffee waste substrates (Figure 5) supplemented with K (K1 and K2) showed greater mycelial growth enhancement than the control. However, glucose supplementation induced irregular growth dynamics, characterized by multiple inflection points and temporary reductions in growth rate, suggesting possible metabolic instability or substrate imbalance.

Oyster mushroom mycelial growth exhibited a

sigmoidal pattern across all tested substrates (Figure 6). Compared to wheat straw (control), substrates were grouped into two categories: (i) lower-performing substrates, including *T. latifolia* and coffee waste, and (ii) a higher-performing group mainly represented by *A. mauritanicus*, which showed growth dynamics comparable to wheat straw but with variations in the duration of the exponential phase (approximately 12 days). Overall, *A. mauritanicus* (C2) achieved the highest mycelial growth, followed by wheat straw (K1), then *A. mauritanicus* (K1), and finally wheat straw (C1).

Statistical analysis revealed a significant effect of substrate type and incubation time on mycelial growth ($p < 0.0001$) (Table 1). Furthermore, fertilization significantly affected oyster mushroom production, with differential effects depending on substrate type (Table 2, Figure 7).

Oyster mushroom yield data showed that fertilization with K and glucose increased the performance of *T. latifolia* and *A. mauritanicus* substrates (Table 2). K addition

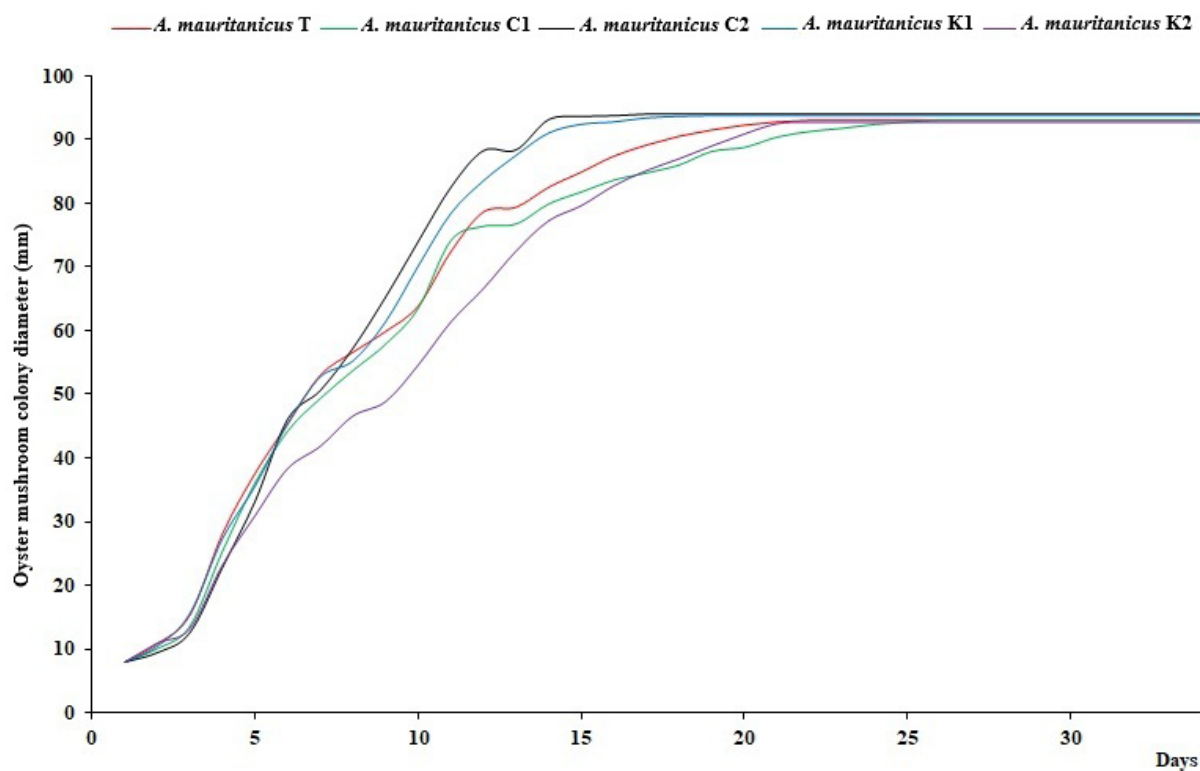


Figure 3. Oyster mushroom diameter growth colony on fertilized and brut *Ampelodesmos mauritanicus* (T: Brut substrate; C1: Fertilization with 10 g/L glucose; C2: Fertilization with 20 g/L glucose; K1: Fertilization with 10 g/L potassium; and K2: Fertilization with 20 g/L potassium).

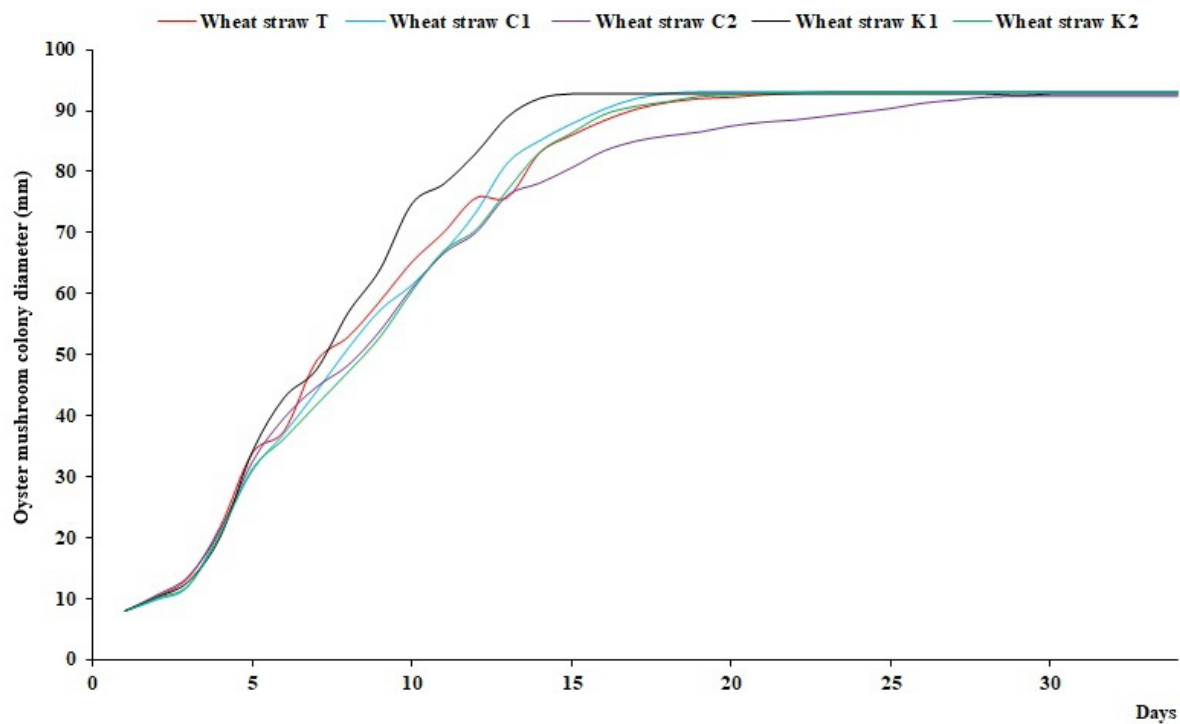


Figure 4. Oyster mushroom diameter-growth colony on fertilized and brut wheat straw (T: Brut substrate; C1: Fertilization with 10 g/L glucose; C2: Fertilization with 20 g/L glucose; K1: Fertilization with 10 g/L potassium, and K2: Fertilization with 20 g/L potassium).

Table 1. One-way analysis of variance of yield, mycelial growth, and mineral elements

Dependent variable	Source	DDL	Type III SS	Mean square	F	p-value
Yield (%)	Substrate	17	4,273.72	51.40	1,399.52	< 0.0001
	Minerals	13	234,487	3,048,332	–	< 0.0001
Growth (mm)	Substrate	19	287,227.72	15,117.25	275.57	< 0.0001
	Time	35	1,403,198.59	40,091.39	730.82	< 0.0001
N (%)	Substrate	19	306.02	16.11	719.36	< 0.0001
P (%)	Substrate	19	0.76	0.04	3.30	0.0007
K (%)	Substrate	19	9.07	0.48	21.62	< 0.0001
Na (%)	Substrate	19	1.90	0.10	6.36	< 0.0001
C (%)	Substrate	19	5,026.04	264.53	11,969.60	< 0.0001
Mn (%)	Substrate	19	44.24	2.33	105.40	< 0.0001
Cu (ppm)	Substrate	19	321.61	16.93	766.26	< 0.0001
Zn (ppm)	Substrate	19	803.70	42.30	1,914.90	< 0.0001
Cd (ppm)	Substrate	19	0.76	0.04	1.80	0.0582
Ni (ppm)	Substrate	19	3.18	0.17	7.58	< 0.0001
Fe (ppm)	Substrate	19	229.59	12.08	547.02	< 0.0001
Pb (ppm)	Substrate	19	0	0	–	–
Co (ppm)	Substrate	19	9.08	0.48	21.64	< 0.0001
Cr (ppm)	Substrate	19	0	0	–	–
Ca (ppm)	Substrate	19	4,264.41	224.44	10,160.40	< 0.0001

Abbreviations: DDL: Degree of freedom; SS: Sum of squares.

yielded the highest number of oyster mushrooms, followed by glucose addition on *T. latifolia* and *A. mauritanicus* substrates, respectively. It was demonstrated that K2-treated *T. latifolia* increased oyster mushroom yield by approximately 8.6-fold compared to unfertilized *T. latifolia* (Table 2). Tested fertilizers and concentrations produced different outcomes depending on the substrate's basic composition. Our results generally show a 1.5- to 8.0-fold increase in oyster mushroom yield following fertilization, except in coffee waste substrates, where glucose additions at 10 g/L and 20 g/L reduced production. This result was confirmed by statistical analysis, which showed that variation in oyster mushroom yield according to substrate type (Table 2) was highly significant ($p < 0.0001$).

Table 2. Oyster mushroom yield (%) in different tested substrates

Substrate	Oyster mushroom yield (%)
PT	6.75 ± 0.5
PC1	9.65 ± 0.3

(Cont'd...)

Table 2. (Continued)

Substrate	Oyster mushroom yield (%)
PC2	5.69 ± 0.4
PK1	10.91 ± 0.6
PK2	12.15 ± 0.5
TyT	3.46 ± 0.5
TyC1	9.96 ± 0.5
TyC2	4.20 ± 0.13
TyK1	19.28 ± 0.3
TyK2	29.91 ± 0.5
DT	6.72 ± 0.4
DC1	22.10 ± 0.3
DC2	29.14 ± 0.5
DK1	22.88 ± 0.6
DK2	3.60 ± 0.2
CT	7.61 ± 0.2
CC1	0.20 ± 0.2
CC2	0.20 ± 0.3

(Cont'd...)

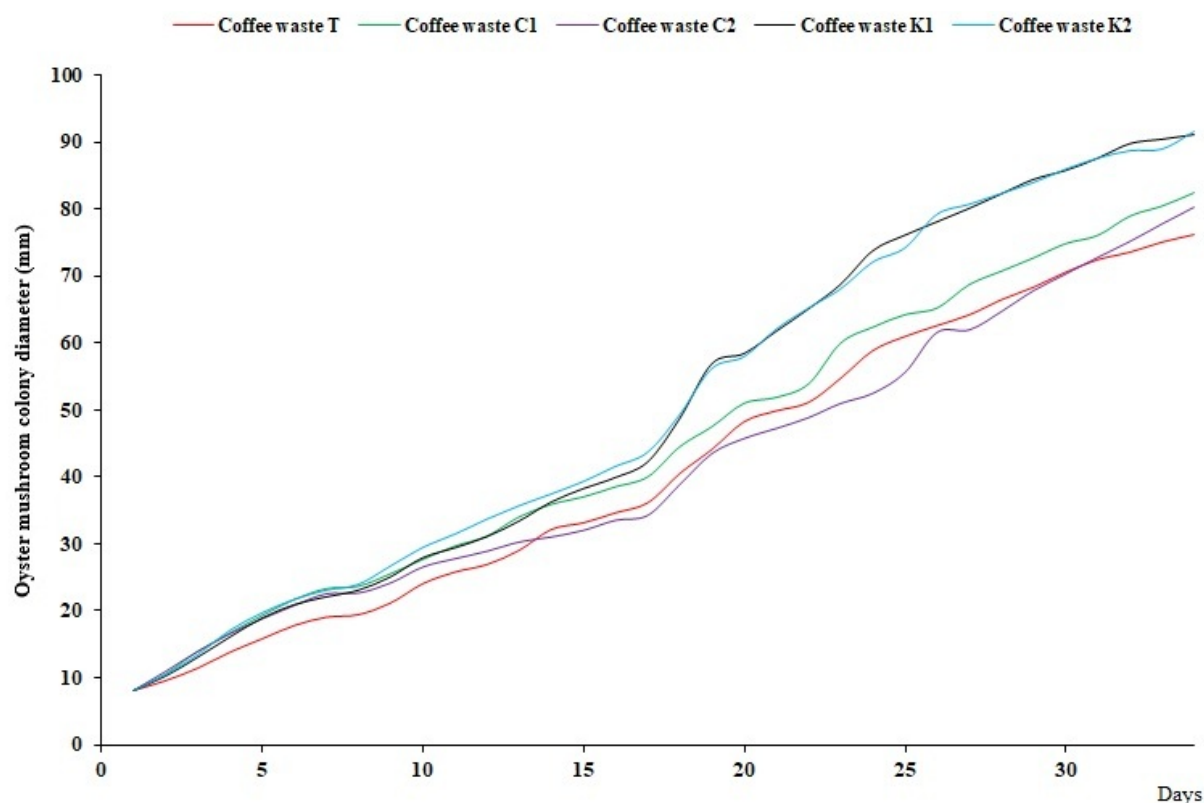


Figure 5. Oyster mushroom diameter growth colony on fertilized and brut coffee waste (T: Brut substrate; C1: Fertilization with 10 g/L glucose; C2: Fertilization with 20 g/L glucose; K1: Fertilization with 10 g/L potassium, and K2: Fertilization with 20 g/L potassium).

Table 2. (Continued)

Substrate	Oyster mushroom yield (%)
CK1	16.90 ± 0.4
CK2	19.28 ± 0.3

Note: Results are presented as mean ± standard deviation. Abbreviations: C: Coffee waste; C1: Fertilization with 10 g/L glucose; C2: Fertilization with 20 g/L glucose; D: *Ampelodesmos mauritanicus*; K1: Fertilization with 10 g/L potassium; K2: Fertilization with 20 g/L potassium; P: Wheat straw; T: Brut substrate; Ty: *Typha latifolia*.

According to analyzed minerals (Table 3), the hierarchical tree using average distance (between classes) of class combinations resized (Figure 8) segregated four groups of substrates: the first group included DC1, DK1, DC2 and TK2; the second group was represented by PC1, PK1, PK2, PC2, DT and DK2; the third one included TT, TC2, TC1, PT and TK1 and the last group comprised CK1, CK2, CC1, CC2 and CT2. Based on the oyster mushroom yield results, the first group showed the highest yield. It is important to note that this group includes substrates based on *T. latifolia* and *A. mauritanicus*, both fertilized with glucose and K, respectively. The second and third groups,

which contained substrates with straw, showed results similar to each other in oyster mushroom production. The lowest substrates for oyster mushroom production were those made with coffee (control and fertilized ones with glucose and K).

Multiple linear regression analysis revealed a significant relationship between substrate mineral composition and yield variation (Table 4). The regression model showed a strong correlation between observed and predicted yield values ($R = 0.805$) and explained 64.8% of the total yield variability, with an adjusted R^2 of 0.538. The overall model was statistically significant ($F = 5.912$, $p < 0.001$), indicating that the selected mineral variables significantly contributed to explaining yield differences among experimental conditions (Table 4).

Among the tested predictors, several mineral components showed significant effects on yield. N exhibited a positive and significant contribution ($\beta = 3.567$, $p = 0.005$), suggesting that increased nitrogen availability was associated with enhanced yield. C also showed a significant positive effect ($\beta = 0.978$, $p = 0.002$), indicating the importance of organic C availability in supporting

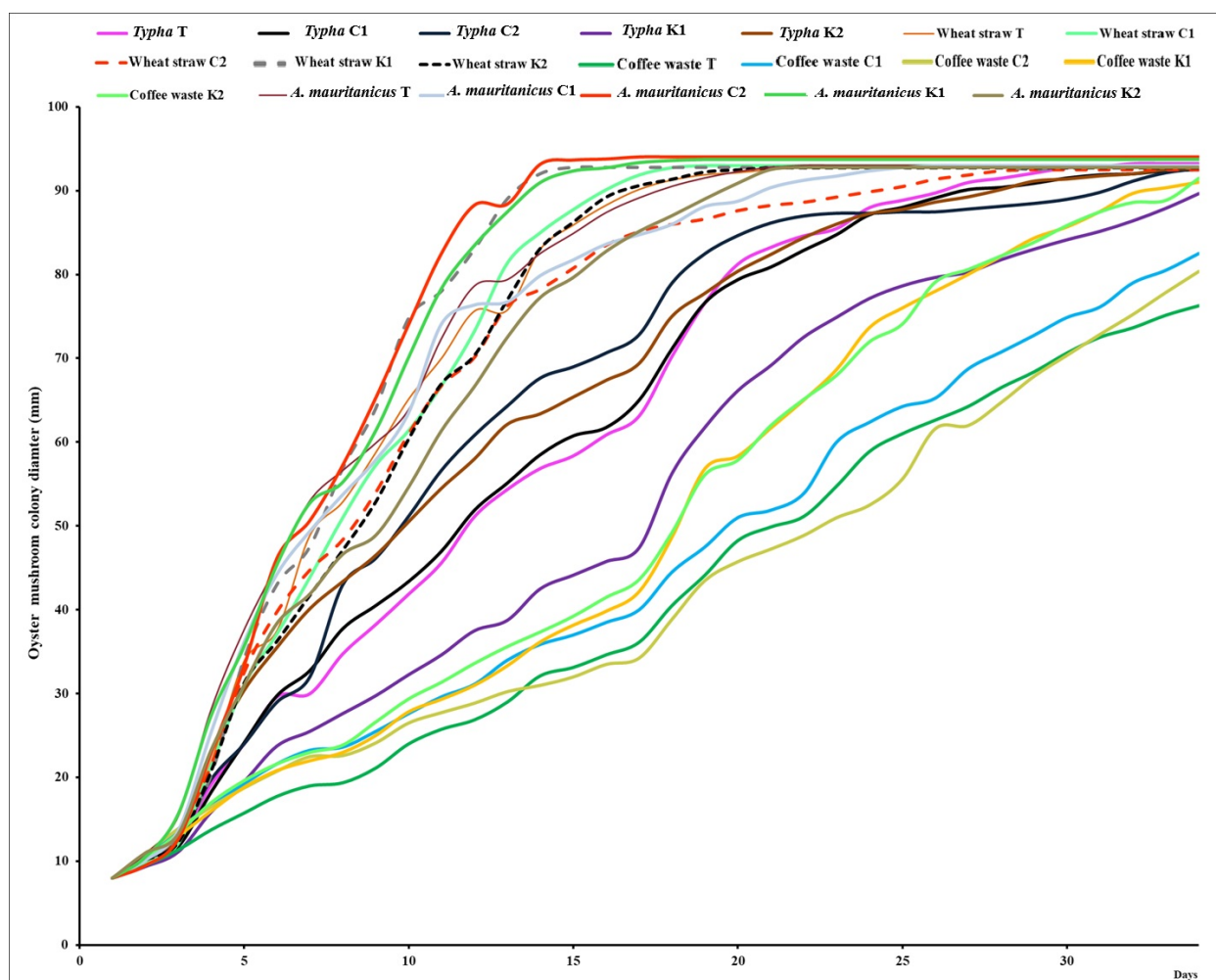


Figure 6. Oyster mushroom diameter growth colony on studied substrates (T: Brut substrate; C1: Fertilization with 10 g/L glucose; C2: Fertilization with 20 g/L glucose; K1: Fertilization with 10 g/L potassium, and K2: Fertilization with 20 g/L potassium).

yield formation. In contrast, K and copper (Cu) showed significant negative relationships with yield (K: $\beta = -28.630$, $p = 0.001$; Cu: $\beta = -10.820$, $p < 0.001$), suggesting that excessive concentrations of these elements may reduce productivity. Calcium (Ca) showed a significant positive association with yield ($\beta = 1.938$, $p = 0.001$), highlighting its potential to improve substrate quality and biological performance (Table 4).

Other elements, including iron (Fe), P, Na, manganese (Mn), zinc (Zn), cadmium (Cd), nickel (Ni), and cobalt (Co), did not significantly explain yield variation individually ($p > 0.05$), although some showed tendencies toward positive or negative effects (Table 4). The high variance inflation factor (VIF) values observed for several predictors, particularly Cu, Ca, Mn, and Co, indicated strong multicollinearity among mineral variables.

Therefore, the individual contribution of several elements should be interpreted cautiously. Despite this limitation, residual analysis confirmed the regression model's reliability, with standardized residuals ranging from -2.110 to 1.727 , a low maximum Cook's distance (0.071), and acceptable prediction errors (Table 4).

The effects of substrate type, treatment, and their interaction were further evaluated using two-way analysis of variance (Table 5). All investigated variables (N, K, C, and Ca) were significantly influenced by substrate, treatment, and the substrate $\times$ treatment interaction ($p < 0.001$), demonstrating that both the initial substrate characteristics and applied treatments strongly affected mineral composition. N content was highly affected by substrate ($F = 2,686.56$), treatment ($F = 89.50$), and their interaction ($F = 437.51$), indicating a strong dependence of

Table 3. Quantities of elements in the oyster mushroom in each tested substrate

S	N ^a	P ^a	K ^a	Na ^a	C ^a	Mn ^a	Cu ^b	Zn ^b	Cd ^b	Ni ^b	Fe ^b	Pb ^b	Co ^b	Cr ^b	Ca ^b
PT	3.64 ± 0.01	0.19 ± 0.006	1.01 ± 0.03	0.22 ± 0.06	52.50 ± 1.60	0.60 ± 0.01	12.63 ± 0.80	64.76 ± 0.70	0.70 ± 0.01	3.70 ± 0.60	73.40 ± 0.60	BDL	2.80 ± 0.06	BDL	98.90 ± 1.90
PC1	5.63 ± 0.20	0.23 ± 0.006	1.50 ± 0.01	0.26 ± 0.01	68.00 ± 0.50	0.50 ± 0.01	13.02 ± 0.20	66.30 ± 0.01	0.60 ± 0.01	3.00 ± 0.01	74.00 ± 0.50	BDL	2.50 ± 0.01	BDL	99.00 ± 0.01
PC2	4.02 ± 0.26	0.32 ± 0.05	1.60 ± 0.01	0.25 ± 0.01	65.50 ± 0.50	0.54 ± 0.01	13.60 ± 0.10	66.80 ± 0.01	0.60 ± 0.01	3.00 ± 0.01	74.20 ± 0.10	BDL	2.50 ± 0.01	BDL	99.20 ± 0.01
PK1	6.03 ± 0.40	0.30 ± 0.06	1.20 ± 0.13	0.26 ± 0.06	70.20 ± 0.70	0.61 ± 0.06	14.56 ± 0.60	65.20 ± 0.06	0.60 ± 0.06	3.00 ± 0.16	75.10 ± 0.30	BDL	2.50 ± 0.06	BDL	99.60 ± 0.50
PK2	7.50 ± 0.33	0.30 ± 0.1	1.30 ± 0.10	0.26 ± 0.10	69.02 ± 0.10	0.64 ± 0.10	14.65 ± 0.10	62.30 ± 0.50	0.60 ± 0.10	3.00 ± 0.10	75.30 ± 0.50	BDL	2.50 ± 0.10	BDL	99.80 ± 0.60
Ty T	3.10 ± 0.33	0.19 ± 0.01	1.50 ± 0.06	0.35 ± 0.10	51.20 ± 0.40	0.80 ± 0.10	11.20 ± 0.13	71.20 ± 0.50	0.50 ± 0.10	3.00 ± 0.10	77.20 ± 0.50	BDL	2.50 ± 0.10	BDL	100.00 ± 0.80
Ty C1	5.48 ± 0.10	0.18 ± 0.02	1.60 ± 0.06	0.36 ± 0.05	52.30 ± 0.60	0.84 ± 0.03	10.98 ± 0.50	72.10 ± 0.80	0.50 ± 0.05	3.00 ± 0.10	77.90 ± 0.60	BDL	2.50 ± 0.07	BDL	101.20 ± 1.05
Ty C2	5.98 ± 0.70	0.68 ± 0.01	2.10 ± 0.05	0.86 ± 0.06	52.80 ± 0.60	1.34 ± 0.05	11.48 ± 0.90	72.60 ± 0.80	1.00 ± 0.01	3.50 ± 0.30	78.40 ± 0.60	BDL	3.00 ± 0.06	BDL	101.70 ± 1.20
Ty K1	6.20 ± 0.80	0.19 ± 0.01	1.60 ± 0.40	0.39 ± 0.04	58.60 ± 1.50	0.68 ± 0.05	11.03 ± 1.50	73.30 ± 2.45	0.50 ± 0.02	3.00 ± 0.20	77.50 ± 2.50	BDL	2.50 ± 0.60	BDL	102.00 ± 1.50
Ty K2	6.32 ± 0.70	0.20 ± 0.01	1.70 ± 0.50	0.40 ± 0.05	71.30 ± 1.50	0.91 ± 0.10	11.40 ± 1.80	73.60 ± 2.56	0.50 ± 0.02	3.00 ± 0.10	77.50 ± 2.30	BDL	2.50 ± 0.60	BDL	101.60 ± 1.50
DT	3.56 ± 0.70	0.21 ± 0.01	1.80 ± 0.08	0.14 ± 0.06	66.30 ± 1.40	0.70 ± 0.07	12.03 ± 2.30	70.20 ± 2.50	0.50 ± 0.02	3.00 ± 0.20	77.00 ± 2.40	BDL	2.50 ± 0.50	BDL	108.00 ± 1.50
DC1	5.62 ± 0.50	0.19 ± 0.01	1.84 ± 0.1	0.16 ± 0.06	75.20 ± 1.20	0.68 ± 0.02	13.02 ± 2.40	71.30 ± 2.40	0.50 ± 0.01	3.00 ± 0.50	77.00 ± 3.10	BDL	2.50 ± 0.40	BDL	110.00 ± 1.50
DC2	6.15 ± 0.60	0.12 ± 0.01	1.90 ± 0.01	0.19 ± 0.01	74.30 ± 0.10	0.78 ± 0.01	13.50 ± 0.50	71.50 ± 0.70	0.50 ± 0.01	3.00 ± 0.05	77.00 ± 0.80	BDL	2.50 ± 0.10	BDL	111.20 ± 2.00
DK1	5.62 ± 0.80	0.16 ± 0.06	1.70 ± 0.06	0.15 ± 0.1	75.01 ± 0.90	0.65 ± 0.10	13.60 ± 0.20	71.20 ± 0.50	0.50 ± 0.10	3.00 ± 0.05	77.00 ± 0.60	BDL	2.50 ± 0.09	BDL	111.00 ± 0.90
DK2	5.80 ± 0.70	0.15 ± 0.01	1.80 ± 0.01	0.18 ± 0.01	75.80 ± 1.50	0.70 ± 0.01	14.00 ± 0.60	71.60 ± 1.30	0.50 ± 0.05	3.00 ± 0.05	77.00 ± 1.40	BDL	2.50 ± 0.50	BDL	111.60 ± 1.80
CT	5.44 ± 0.80	0.24 ± 0.01	0.82 ± 0.10	0.05 ± 0.01	54.00 ± 1.80	2.76 ± 0.80	17.76 ± 2.60	73.70 ± 2.30	0.60 ± 0.05	3.73 ± 0.90	79.60 ± 2.10	BDL	1.70 ± 0.50	BDL	115.80 ± 2.30
CC1	0.35 ± 0.03	0.25 ± 0.01	0.90 ± 0.02	0.06 ± 0.01	55.00 ± 1.60	2.60 ± 0.70	17.80 ± 3.50	74.06 ± 2.50	0.60 ± 0.02	3.20 ± 0.30	79.00 ± 2.60	BDL	1.70 ± 0.70	BDL	120.00 ± 2.00
CC2	0.37 ± 0.03	0.26 ± 0.01	0.92 ± 0.03	0.02 ± 0.05	55.20 ± 2.30	2.65 ± 0.80	17.56 ± 2.40	74.50 ± 2.80	0.60 ± 0.03	3.20 ± 0.80	80.00 ± 2.50	BDL	1.70 ± 0.60	BDL	121.00 ± 1.70

(Cont'd...)

Table 3. (Continued)

S	N ^a	P ^a	K ^a	Na ^a	C ^a	Mn ^a	Cu ^b	Zn ^b	Cd ^b	Ni ^b	Fe ^b	Pb ^b	Co ^b	Cr ^b	Ca ^b
CK1	0.39 ± 0.01	0.25 ± 0.01	0.95 ± 0.02	0.12 ± 0.05	54.30 ± 1.60	2.48 ± 0.50	17.02 ± 2.60	75.20 ± 3.00	0.60 ± 0.03	3.20 ± 0.60	80.00 ± 1.80	BDL	1.70 ± 0.70	BDL	122.00 ± 2.50
CK2	0.31 ± 0.01	0.25 ± 0.01	0.90 ± 0.03	0.13 ± 0.05	51.60 ± 1.80	2.86 ± 0.60	17.03 ± 2.50	75.09 ± 1.80	0.60 ± 0.40	3.20 ± 0.40	80.00 ± 1.50	BDL	1.70 ± 0.20	BDL	122.50 ± 1.80

Notes: ^a(%); ^b(ppm). Abbreviations: BDL: Below detection limits; C: Coffee waste; C1: Fertilization with 10 g/L glucose; C2: Fertilization with 20 g/L glucose; D: *Ampelodesmos mauritanicus*; K1: Fertilization with 10 g/L potassium; K2: Fertilization with 20 g/L potassium; P: Wheat straw; S: Substrate; T: Brut substrate; Ty: *Typha latifolia*.

nitrogen dynamics on substrate composition and treatment conditions. Similarly, C content showed extremely significant effects of substrate ($F = 50,484.44$), treatment ($F = 8,990.23$), and interaction effects ($F = 3,334.04$), confirming that C availability was strongly modified by the experimental factors (Table 5).

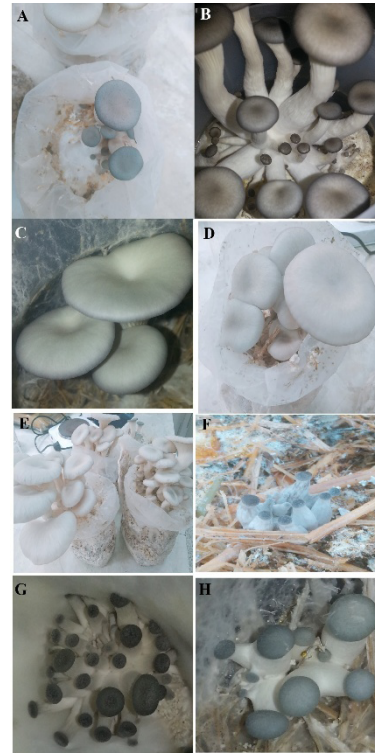


Figure 7. *Pleurotus ostreatus* fructification aspects in some studied substrates. (A) Oyster mushroom fructification in CK2 substrate. (B) Oyster mushroom fructification in CK1 substrate. (C) Oyster mushroom fructification in DK1 substrate. (D) Oyster mushroom fructification in DC2 substrate. (E) Oyster mushroom fructification in TyK2 substrate. (F) Oyster mushroom fructification in TyK1 substrate. (G) Oyster mushroom fructification in PK1 substrate. (H) Oyster mushroom fructification in PK2 substrate.

Abbreviations: C: Coffee waste; C1: Fertilization with 10 g/L glucose; C2: Fertilization with 20 g/L glucose; D: *Ampelodesmos mauritanicus*; K1: Fertilization with 10 g/L potassium; K2: Fertilization with 20 g/L potassium; P: Wheat straw; T: Brut substrate; Ty: *Typha latifolia*.

Concentration of K was also significantly affected by substrate and treatment ($p < 0.001$), although the interaction effect was comparatively weaker ($F = 2.30$, $p = 0.024$). Ca exhibited the strongest response among mineral elements, with highly significant effects of substrate ($F = 62,540.77$), treatment ($F = 907.44$), and their interaction ($F = 149.58$), suggesting that Ca accumulation was highly dependent on the combination of substrate characteristics and treatment application (Table 5).

Table 4. Multiple regression analysis explaining yield variation and the model diagnostics component

Component	Variable/statistic	Estimate	p-value	VIF
Model performance	Number of observations (n)	60.000		
	R	0.805		
	R ²	0.648		
	Adjusted R ²	0.538		
	F-value (df = 14,45)	5.912	< 0.001	
	Standard error of estimate	6.079		
Regression coefficients	Durbin–Watson	0.814		
	N	3.567	0.005	12.295
	C	0.978	0.002	12.231
	K	−28.630	0.001	18.234
	Cu	−10.820	< 0.001	54.021
	Ca	1.938	0.001	36.427
	Fe	−4.509	0.058	33.441
	P	−7.932	0.566	6.339
	Na	11.732	0.417	14.094
	Mn	18.030	0.089	131.721
	Zn	−0.137	0.885	19.341
	Cd	35.610	0.270	45.025
	Ni	−17.936	0.127	14.663
	Co	−7.159	0.735	118.792
Residual diagnostics	Mean residual	0.000		
	Residual standard deviation	5.309		
	Standardized residual range	−2.110 to 1.727		
	Maximum Cook's distance	0.071		
	Maximum Mahalanobis distance	28.334		

Notes: Multiple linear regression was performed with yield as the dependent variable and mineral composition parameters (N, P, K, Na, C, Mn, Cu, Zn, Cd, Ni, Fe, Co, and Ca) as predictors; The model was significant ($F = 5.912$, $p < 0.001$) and explained 64.8% of yield variability (adjusted $R^2 = 0.538$); VIF values were examined to assess multicollinearity among predictors; Residual diagnostics confirmed the adequacy of the regression model. Abbreviation: VIF: Variance inflation factor.

The PCA was performed to identify relationships among mineral variables, substrate characteristics, and yield (Table 6). The Kaiser–Meyer–Olkin index ($KMO = 0.694$) indicated an acceptable sampling adequacy for PCA. Four principal components were retained, explaining 88.72% of the total variance. The first component (principal component 1) accounted for 29.28% of the variance and showed high positive loadings for Zn, Fe, substrate, Ca, Mn, Cu, K, Na, Co, Cd, Ni, C, and N, indicating that this component mainly represented overall mineral enrichment and substrate fertility gradients.

The second component (principal component 2) explained 25.07% of the variance and was mainly associated with Zn, Fe, substrate, and Ca, suggesting a differentiation

related to specific nutrient accumulation patterns. The third component (principal component 3) explained 20.08% of the variance and was strongly associated with Cd, P, and Ni, reflecting a separate mineral contamination or availability gradient. The fourth component (principal component 4), accounting for 14.29% of the variance, was characterized primarily by C and yield loadings, indicating a close relationship among organic C, N availability, and productivity (Table 6). Overall, the PCA confirmed that yield variation was primarily associated with substrate fertility parameters, particularly C- and N-related factors, while mineral balance and elemental interactions contributed to the overall differentiation among treatments (Table 6).

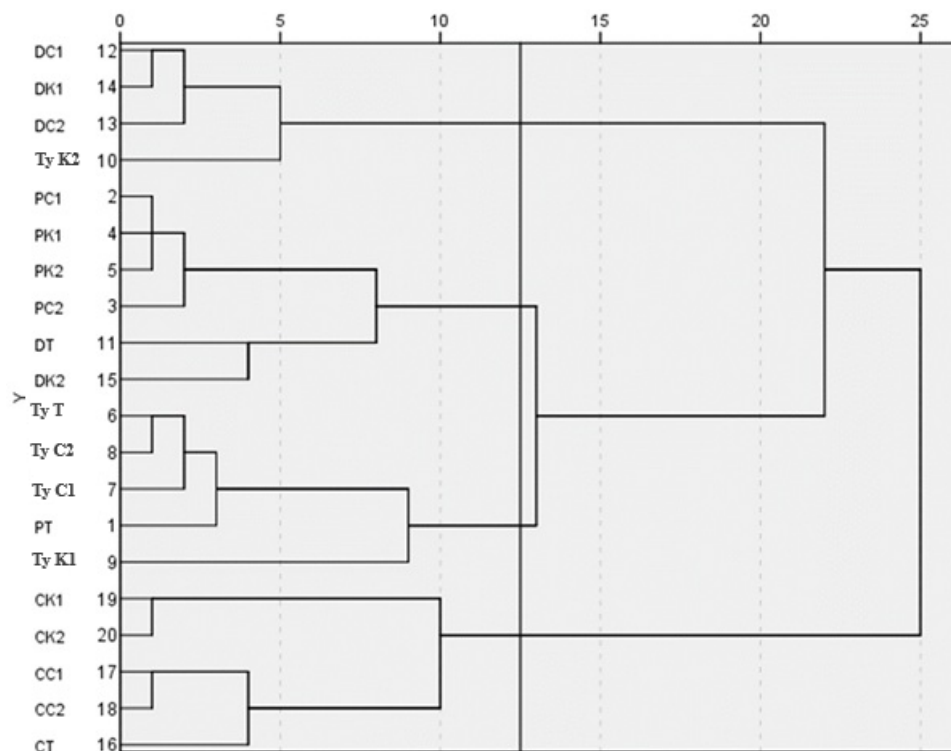


Figure 8. Hierarchical tree using the average distance of substrate type, resized

Abbreviations: C: Coffee waste; C1: Fertilization with 10 g/L glucose; C2: Fertilization with 20 g/L glucose; D: *Ampelodesmos mauritanicus*; K1: Fertilization with 10 g/L potassium; K2: Fertilization with 20 g/L potassium; P: Wheat straw; T: Brut substrate; Ty: *Typha latifolia*.

Table 5. Two-way analysis of variance of substrate, treatment, and their interaction on measured variables

Variable	Source	df	F-value	p-value
N	Substrate	3	2,686.56	<0.001
N	Treatment	4	89.50	<0.001
N	Substrate × treatment	12	437.51	<0.001
K	Substrate	3	115.54	<0.001
K	Treatment	4	9.13	<0.001
K	Substrate × treatment	12	2.30	0.024
C	Substrate	3	50,484.44	<0.001
C	Treatment	4	8,990.23	<0.001
C	Substrate × treatment	12	3,334.04	<0.001
Ca	Substrate	3	62,540.77	<0.001
Ca	Treatment	4	907.44	<0.001
Ca	Substrate × treatment	12	149.58	<0.001

Notes: The experiment consisted of four substrates × five treatments with three replicates per combination ($n = 60$); A general linear model was used, including substrate, treatment, and substrate × treatment interaction.

Although K showed a positive trend in some treatments, the multiple regression model showed a negative coefficient for K ($\beta = -28.63$, $p = 0.001$). This apparent contradiction may result from multicollinearity among the mineral variables, as indicated by a high VIF value of K (18.23). K effects should therefore be interpreted in relation to the combined nutrient profile rather than as an isolated predictor. In fact, K is an essential nutrient for fungal growth; the high K_2O supplementation levels applied in this study (10 and 20 g/L) were associated with a reduction in the measured parameter. This negative effect may be related to an increase in substrate ionic strength or osmotic stress resulting from K_2O addition, rather than solely reflecting a nutritional effect of K.

4. Discussion

All fungal species require several nutrients for growth. C, N, P, K, and magnesium are macronutrients required by fungi for their structural and energy uses.¹⁵ Among these macronutrients, K stimulates the development of *Pleurotus* sp.^{16,17} Typically, the deficiency of several mushroom substrates is ameliorated using appropriate

Table 6. Principal component analysis showing factor loadings, explained variance, and contribution of substrate characteristics, mineral elements, and yield-related variables

Variables	Extraction	Components			
		Principal component 1	Principal component 2	Principal component 3	Principal component 4
Zn	0.621	0.972			
Fe	0.750	0.945			
Substrate	0.832	0.870			
Ca	0.878	0.756			
Mn	0.919	0.685			
Cu	0.922		-0.912		
K	0.975		0.770		
Na	0.970		0.749		
Co	0.954		0.721		
Cd	0.961			0.972	
P	0.717			0.889	
Ni	0.946			0.765	
C	0.946				0.885
Yield	0.952				0.699
N	0.963				0.555
% variance		29.275	25.073	20.079	14.290
Kaiser-Meyer-Olkin index			0.694		

natural combinations or fertilizers.¹⁸ Use of mineral supplementation in the cultivation substrates is recommended for mushroom production and particularly for the oyster mushroom.^{19,20} Likewise, the significant effects of glucose on oyster mushroom growth were specifically demonstrated.²¹ The current study showed the best effectiveness of the substrate formed by *T. latifolia* fertilized with 20 g/L glucose (C2) in promoting the growth of oyster mushroom mycelium. This substrate, along with the amount of fertilizer, could yield significant increases in oyster mushroom spawn production. However, it is important to note that K supplementation may have exerted both nutritional and ionic effects on *Pleurotus* growth. As salinity and electrical conductivity were not assessed in this study, the specific contribution of each mechanism remains to be elucidated. Likewise, other studies have shown that adding a nitrogen source increases fungal biomass and productivity.^{22–24} Indeed, nitrogen, vitamins, and mineral elements can accelerate mycelium growth and increase the mushroom yield when added to cultivation substrates.

Effects of several percentages of chemical fertilizers (N, diphosphorus pentoxide, and K₂O) on the growth, yield, and biological efficiency of *P. ostreatus* grown on rice

straw have largely been assessed.²⁵ These studies confirmed the effectiveness of adding 0.3% K₂O to improve oyster mushroom production. Similarly, the influence of K on the growth rate and nutritional value of *P. florida* has been debated.²⁶ According to previous studies, all tested concentrations (between 10 and 100%) of this fertilizer increased *P. florida* biomass and nutritional quality, but when added at 60%, the potassium yielded the highest biomass with the most nutritive content.

Styer²⁷ reported that adding Ca and K to mushroom-growing manures has a favorable effect. In addition, it is well recognized that K and glucose are essential for the growth of microorganisms.²⁸ However, the addition of any component or mineral should depend on the substrate's properties and must meet the fungi's requirements. In fact, several additives can become toxic when the substrate already contains high levels of the elements. The growth of *P. ostreatus* mycelium decreased with excessive doses.²⁹

Therefore, this study suggests that the biomass of the *T. latifolia* plant species harvested from wetlands can be used as a renewable substrate resource for oyster mushroom cultivation and to reduce environmental plant invasion. Elbagory *et al.*⁷ recommended the use of aquatic plants for cultivating oyster mushrooms and other edible

species. Another study recommended using whole cattail weed (*Typha domingensis* Pers.) as a new substrate for the cultivation of *Pleurotus* HK 37 and *Pleurotus sapidus*.³⁰ Richness of cattail on carbohydrates (80%) could explain its ability to support mushroom growing.³¹ Numerous reports on oyster mushroom cultivation have been conducted on unexploited plants, such as *Phragmites* sp. and cattails.^{32,33} It was demonstrated that the yield and quality of oyster mushrooms depend on the chemical and nutritional properties of the substrates used for their cultivation.³⁴

Several lignocellulosic by-products, including *Triplochiton scleroxylon* sawdust, rice straw, banana leaves, maize stover, corn husk, rice husk, fresh sawdust, and elephant grass, were evaluated as substrates for oyster mushroom cultivation. Mushroom yields ranged from 13.0 to 183.1 g in all tested substrates except for elephant grass, which was found to be unsuitable for mushroom cultivation by producing no fruiting bodies (0.0 g yield).³⁵ In comparison, our results of oyster mushroom yield on *T. latifolia* and *A. mauritanicus* show the greatest performance of these plant species to increase oyster mushroom cultivation. To the best of our knowledge, this is the first report studying *A. mauritanicus* as a substrate for mushroom cultivation.

The addition of glucose caused irregularity in the growth of the oyster mushroom colony diameter in a coffee-based substrate. Similarly, pasteurized coffee used as a culture substrate for oyster mushrooms showed high levels of *Neurospora* contamination resulting from the elevated internal humidity of the pasteurized substrate.³⁶ An interesting interaction effect between bacteria and fungi, enabling efficient breakdown of lignocellulose in the substrate, has been confirmed for several edible mushroom species.³⁷ This is because the role of microbes in mushroom production systems implies the function of enzymes for an efficient degradation of the raw material.

The broader relevance of the present results is also consistent with recent research on the circular valorization of agricultural and organic residues. In particular, agricultural waste-derived bioactive products have been proposed as a promising route to improve crop productivity and sustainability, while low-energy pretreatment of organic waste using a vortex layer reactor has been shown to enhance C conversion and process efficiency in biotechnological waste-processing systems.³⁸

5. Conclusion

The evaluation of the effect of the fertilization of studied substrates on oyster mushroom growth demonstrated that *A. mauritanicus* supplemented with 20 g/L of glucose and the *T. latifolia* fertilized by 10 g/L of K increased

mycelial growth. The addition of 10 g/L of glucose and K also promoted oyster mushroom production on *A. mauritanicus* and *T. latifolia*, respectively. Furthermore, K added to coffee waste improved mushroom yield compared to the control. This result highlights the potential to improve the quality of these natural substrates when used as growing media for oyster mushroom cultivation. The use of wetland and forestry plant species, along with agro-industrial by-products, represents a sustainable and promising alternative for oyster mushroom cultivation due to their low cost and availability. Furthermore, this approach could promote ecological interests by reducing the spread of invasive macrophytes in the environment.

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Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Awatef Slama, Abdelhamid Khaldi

Formal analysis: Salima Bahri, Awatef Slama

Investigation: Awatef Slama

Writing—original draft: Awatef Slama

Writing—review & editing: Awatef Slama, Faten Mezni, Marwa Khammassi, Abdelhamid Khaldi

Availability of data

Data are available from the corresponding author upon reasonable request.

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