ANNALES HORTICULTURAE

VOL. XXXV (1) 2026

https://doi.org/10.24326/ah.2026.5678

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Local plant biomass and biodegradable organic waste-derived compost as a sustainable alternative to peat in casing layers for *Agaricus bisporus* cultivation

Abstract. Progressive restrictions on peat extraction in Europe create the need for sustainable alternatives for casing layers in *Agaricus bisporus* cultivation. This study evaluated compost-based peat-free humus casing as a substitute for conventional peat casing under commercial conditions. Physicochemical properties, yield performance, and mineral composition of fruit bodies from white and brown strains were analyzed across two flushes. Compared with peat, the humus casing showed lower water-holding capacity, higher pH, and higher electrical conductivity. Nevertheless, colonization dynamics and fruit body initiation proceeded similarly in both systems. In the white strain, first-flush yield under humus casing was significantly higher than under peat one, while in the brown strain yields were statistically equivalent. Mineral composition responses were strain- and flush-dependent. In the brown strain, humus increased Ca, Fe, and Se concentrations, particularly in the second flush. No consistent evidence of reduced mineral quality was observed. under humus. The second flush showed intensified positive effects, suggesting dynamic interactions between casing properties and nutrient availability. Overall, compost-derived humus casing provides yield performance comparable to peat and may enhance selected mineral traits, supporting its potential in sustainable peat-free mushroom production.

Keywords: peat-free casing, compost-based humus, cultivated mushroom, sustainable horticulture

ABBREVIATIONS AND SYMBOLS

OH – humus casing
OT – peat-based casing
BiOH – white mushroom cultivated on humus casing
BiOT – white mushroom cultivated on peat-based casing
BrOH – brown mushroom cultivated on humus casing
BrOT – brown mushroom cultivated on peat-based casing

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WHC – water holding capacity, % wt.
EC – electrical conductivity (salinity), mS·cm^{-1}
σ_p^2 – pooled variance of yield, kg²·m^{-4}
s_i^2 – variance of the *i*-th yield measurement series, kg²·m^{-4}
n_i – number of individual measurements in the *i*-th yield measurement series
P_{OH} – yield obtained with humus casing, kg·m^{-2}
P_{OT} – yield obtained with humus peat-based casing, kg·m^{-2}
σ_Δ – variance of the yield difference $\Delta = P_{OH} - P_{OT}$
σ_{OH}^2 – assumed variance of yield under humus casing, kg²·m^{-4}
σ_{OT}^2 – assumed variance of yield under peat-based casing, kg²·m^{-4}
k_α – critical quantile value at significance level α
Φ^{-1} – inverse cumulative distribution function of the standard normal distribution
Z – test statistic for the difference $P_{OH} - P_{OT}$
Φ – cumulative distribution function of the standard normal distribution
$\Delta_{rel} w_i$ – relative increase index of mineral component content, % wt.
w_i^{OH} – content of mineral component *i* in fruit body tissue of mushrooms cultivated on humus casing, mg·kg^{-1} dry mass
w_i^{OT} – content of mineral component *i* in fruit body tissue of mushrooms cultivated on peat based casing, mg·kg^{-1} dry mass
L – natural logarithm of the ratio $\frac{w_i^{OH}}{w_i^{OT}}$
s_L^2 – variance of *L*
$s_{w_i^{OH}}^2$ – variance of mineral component *i* content in fruit bodies cultivated on humus casing, mg^2·kg^{-2}
$s_{w_i^{OT}}^2$ – variance of mineral component *i* content in fruit bodies cultivated on peat-based casing, mg^2·kg^{-2}
$\Delta_r {w_i}^{G,D}$ – upper/lower confidence limit of the relative increase index of mineral component content, % wt.
$L^{G,D}$ – upper/lower confidence limit of expression *L*
$t_{\alpha,\nu_{ef}}$ – parameter from the Student's *t*-distribution for significance level α and effective degrees of freedom ν_{ef}

INTRODUCTION

Poland has for years maintained the position of an undisputed leader in mushroom production on the European market, and the domestic cultivation sector constitutes a cornerstone of the European Union's supply of this species [Walkowiak 2025]. Although this trend has been characterized by considerable growth dynamics, it is currently facing barriers arising from macroeconomic factors, such as increasing energy costs and wage pressure accompanied by a shortage of qualified specialized personnel. Under these conditions, further development of the mushroom sector is possible only through the implementation of innovation and research and development outcomes aimed at optimizing production costs [Sakson and Hreczuch 2025].

A strategic challenge, extending beyond current economic constraints, concerns the availability of raw materials, particularly peat. At present, peat constitutes the primary and indispensable component of the casing layer used in mushroom cultivation and largely determines cultivation success within the existing technological model [Szumigaj-Tarnowska and Uliński 2023].

Peat is a material with unique sorptive and biological properties, however, its resources are non-renewable on a human timescale. The formation of peat deposits meeting economic extraction criteria takes thousands of years, whereas current exploitation rates significantly exceed the natural accumulation rate of this material. This imbalance necessitates the search for alternatives in accordance with the principles of sustainable development [Żebrowska and Kociołek-Balawejder 2010].

Peatland exploitation is also associated with irreversible ecosystem degradation and substantial greenhouse gas emissions, as confirmed by environmental assessments [Goglio et al. 2025]. This aspect, combined with the prospect of future environmental charges and increasing pressure from Western European retail chains – which are progressively requiring peat-free products from their suppliers – renders continued reliance on peat as the sole casing material economically risky [Sakson 2025].

At present, the mushroom sector, as well as the broader horticultural industry, faces the necessity of gradually phasing out peat use, driven by a stringent and multi-level legal framework that severely restricts access to new deposits. These barriers arise from a set of interrelated national and European Union regulations forming part of the EU's transformation agenda toward a modern and competitive economy with minimal environmental impact, a cornerstone of the European Green Deal strategy [European Commission 2019]. In light of these conditions, it appears justified to assume that the implementation of the Green Deal objectives may, in the longer term, result in substantial limitations or even a complete ban on peat extraction, thereby placing the mushroom sector before a profound structural challenge.

Despite numerous studies conducted worldwide, the development of an alternative casing material that is not only effective but also cost-competitive with peat remains unresolved [Sakson 2025]. Identifying a functional substitute for peat in mushroom production technology is particularly challenging due to the complex role of the casing layer in cultivation. Its function extends beyond mere mechanical protection of the substrate; rather, it constitutes a key technological component indispensable for the initiation of fruiting. For this process to proceed properly, the casing material must exhibit a unique combination of high water-holding capacity and porosity, ensuring continuous gas exchange [Szumigaj-Tarnowska and Uliński 2023].

In response to the above limitations and constraints, the private sector has undertaken efforts to develop technologies that combine the physical properties of peat with the renewability of raw materials. An example of such a solution is the innovation developed by Afirma Sp. z o.o. (Wojnowo, Poland), which has developed, patented, and commercialized an innovative Peat-Free Humus Casing [Patent application P.452691, 2025]. This technology is based on the composting of locally sourced plant materials to obtain a material with a stable granular structure, intended to replicate the water-retention properties of peat without its associated environmental drawbacks.

Despite promising theoretical premises, the key research issue remains the verification of the suitability of the new humus casing under real industrial production conditions, where factors such as cultivation scale, irrigation systems, and the microclimate of production halls critically test material stability.

The aim of the present study is to verify the feasibility of using compost (humus) as an alternative to peat in mushroom casing, with particular emphasis on assessing its effects on yield performance and the accumulation of macro- and micronutrients.

It was hypothesized that compost-derived peat-free humus casing (OH), despite its distinct physicochemical characteristics compared to conventional peat-based casing (OT), is capable of sustaining fruit body development and yield performance of *Agaricus bisporus* at levels not inferior to those obtained with peat casing, while maintaining comparable mineral composition of mushroom fruit bodies under commercial production conditions.

The study focuses on comparing the innovative humus casing with a standard peat-based casing in terms of physicochemical properties and production efficiency, expressed by mushroom yield, fruit body quality, and chemical composition. The defined research problem arises from a technological gap between the necessity of phasing out peat and the lack of proven substitutes suitable for commercial production. The core of the problem lies in the need to identify and validate a renewable raw material (compost) that, under intensive irrigation conditions, maintains the physical parameters required for proper fruit body development, matching the performance standards of peat-based casing.

MATERIAL AND METHODS

Material

Peat-free humus casing

The production process of the peat-free humus casing layer was described in detail in the patent application [Patent Application P.452691, 2025].

Compost, constituting the main component of the humus casing layer, was produced by composting selectively collected green waste and other biodegradable plant residues originating from gardens, forests, orchards, parks, cemeteries, and marketplaces.

The feedstock included, among others, grass clippings, leaves, stems, branches, tree limbs, untreated wood, bark, and wood chips, as well as other biodegradable organic wastes classified within groups 02, 16, and 20 of the Polish Waste Catalogue, comprising agricultural, forestry, agri-food processing, and municipal biowastes.

The entire waste processing cycle, from feedstock reception to storage of the final product, lasted 84 days and was carried out in a chamber-type biocomposting facility. After shredding and homogenization, the feedstock was subjected to a composting process lasting 10–12 weeks and consisting of three consecutive stages.

Upon completion of composting, the material was screened through a 20-mm mesh sieve to remove non-composted fractions and obtain a homogeneous structure. The resulting compost was characterized by a pH of 8.0 ±0.4, total nitrogen content of 0.95 ±0.05%, phosphorus (P_2O_5) content of 0.34 ±0.02%, potassium (K_2O) content of 0.79 ±0.04%, magnesium (MgO) content of 0.45 ±0.02%, calcium (CaO) content of 3.32 ±0.16%, and an organic matter content of 25.0 ±1.25% on a dry matter basis.

The second component of the humus casing layer was sugar beet lime, a by-product of the sugar industry generated during purification of sugar beet juice using lime milk ($Ca(OH)_2$).

Sugar beet lime consists mainly of calcium carbonate ($CaCO_3$), with minor amounts of calcium oxide (CaO), organic matter, and trace quantities of plant nutrients, including nitrogen, phosphorus, potassium, and micronutrients. Depending on the degree of dewatering, sugar beet lime may occur as a moist material or as a dried granular or powdered product. In the present study, sugar beet lime was used in the form of a dry powder with a moisture content below 10% and a particle size ranging from 0.5 to 1.0 mm.

The investigated humus casing layer consisted of 95% vol. compost and 5% vol. sugar beet lime. The resulting casing material exhibited a pH ranging from 7.5 to 9.0, salinity of 2.5–4.0 $mS \cdot cm^{-1}$, total nitrogen content of 0.30–1.10%, phosphorus (P_2O_5) content of 0.10–0.50%, potassium (K_2O) content of 0.20–0.95%, and an organic matter content not lower than 15% of dry matter.

Control peat-based casing

The control casing (peat-based): a standard mixture of highmoor and lowmoor peat with a structure adapted for mechanical application, deacidified with defecation lime [Peat-based casing 2025].

Method

Preparation of the humus casing layer

The humus casing layer was prepared by mixing plant-derived compost with sugar beet lime at a volumetric ratio of 95:5. The components were mechanically blended until a homogeneous mixture was obtained. The material was subsequently screened using a star screen to obtain a particle size fraction of 10–25 mm. The larger wood fragments, compacted material, and other undesirable fractions were removed during this process. When necessary, the moisture content of the mixture was adjusted by watering to approximately 30–50%. The casing material was then conditioned in a screw structuring unit to improve its physical properties through loosening and aeration of the material and the disruption of potential aggregates. The resulting humus casing layer was used directly in the cultivation experiments.

Cultivation

Cultivation experiments were conducted in two production halls (No. 5 and No. 12) at the Grzybek mushroom farm owned by Piotr Pasoń (Opole, Poland). The study compared two types of casing materials: the tested casing (peat-free, humus-based) and the control casing (peat-based).

The experimental design comprised four variants, designated and arranged as follows:

Hall No. 12 (white mushroom):

BiOT – shelf cultivated with white A. bisporus using traditional peat casing, serving as the reference treatment.

BiOH – shelf cultivated with white *A. bisporus* using humus (peat-free) casing, serving as the experimental treatment.

Hall No. 5 (brown mushroom):

BrOT – shelf cultivated with brown *A. bisporus* using traditional peat casing, serving as the reference treatment.

BrOH – shelf cultivated with brown *A. bisporus* using humus (peat-free) casing, serving as the experimental treatment.

In both halls, standard cultivation technology was applied, including casing incubation, thermal shock induction, and the cropping phase.

An identical layer configuration was maintained across all variants to eliminate the influence of external factors: the tested or control casing was mechanically applied to Phase III compost at a thickness of approximately 4 cm.

Microclimatic parameters (air and substrate temperature, relative humidity, and CO_2 concentration) were controlled for each strain: white (K55X) and brown (LUX).

A key aspect of the study was monitoring the structural behavior of the casing materials during irrigation and assessing their water stability.

Physicochemical analyses of the casing materials were performed according to standard laboratory procedures commonly applied in soil science [Pawłowska and Wysocka 2013].

Gravimetric moisture content was determined using the oven-drying method by drying samples at 105°C to constant weight [PN-ISO 11465]. Maximum water-holding capacity was determined using the funnel method, calculated as the difference in mass between a fully water-saturated sample and the sample after cessation of gravitational drainage [Pawłowska and Wysocka 2013].

The pH was measured potentiometrically using a combined glass electrode, with measurements performed in a water suspension to determine active acidity and in a 1M KCl solution to determine exchangeable acidity [Pawłowska and Wysocka 2013].

Electrical conductivity (EC) was determined conductometrically to assess the total concentration of soluble salts [Pawłowska and Wysocka 2013].

Yield assessment

Fruit bodies were harvested selectively at commercial maturity. Each experimental shelf constituted an independent measurement unit. The yield collected from each shelf was weighed after every harvest to the nearest 0.01 kg, allowing precise determination of productivity per 1 m^2 of cultivation area for each type of casing material.

Quality and chemical composition analysis

Sampling

To determine the nutritional value of the final product, fruit body samples were collected from each experimental variant. Mushrooms for laboratory analysis were randomly selected from different locations across the cultivation surface to ensure sample representativeness.

Dry matter, water content

Dry matter content of fresh mushrooms was determined gravimetrically by drying the sample at 105°C to constant mass. Samples were homogenized immediately prior to analysis and weighed into pre-dried and pre-weighed weighing dishes. After drying, samples were cooled to room temperature in a desiccator and weighed. Constant mass was defined as a difference between successive weighings, not exceeding 0.5% of the lower value or 2 mg. Each sample was analyzed in three independent replicates. Results were reported as % dry matter (mean of three determinations), and water content was calculated by difference to 100%.

Mineral content

The contents of Ca, Fe, K, Mg, P and Se were determined by ICP-OES using a PlasmaQuant 9100 spectrometer (Analytik Jena, Germany) at MCBR UO (International Research and Development Center of the University of Opole). Samples were subjected to microwave-assisted digestion with HNO_3 (65%) and H_2O_2 (30%) until a clear solution was obtained; the digests were then diluted with deionized water to the final measurement volume. Quantification was performed using calibration curves prepared from element standard solutions and with a reagent blank included. Calibration ranges were as follows: 0.1–8.0 $mg \cdot L^{-1}$ (Ca, Mg, K), 0.01–0.8 $mg \cdot L^{-1}$ (Fe, Se) and 1.0–80.0 $mg \cdot L^{-1}$ (P). The following analytical wavelengths (nm) were used: Ca 317.933; Fe 259.940; K 766.491; Mg 285.213; P 178.287; Se 196.028. Each sample was analyzed in three independent replicates; results were corrected for the blank. Concentrations were expressed as mg of element per kg of dry matter ($mg \cdot kg^{-1}$ d.m.).

RESULTS AND DISCUSSION

Origin and technological determinants of the investigated casing materials

The fundamental difference between peat and compost arises from the distinct dynamics of their formation and the environmental conditions under which they develop. Peat consists of organic deposits accumulated in waterlogged environments, where excess moisture and oxygen deficiency lead to slowed humification of plant residues. This long-term process creates a specific environment in which organic matter is preserved for millennia [Skreczko and Trepka 2016]. Owing to the rate of deposit accumulation (approximately 1 mm per year), peat is regarded as a non-renewable resource on an economic timescale. In contrast to geological formation processes, composting represents a controlled biotechnological process. It occurs under aerobic conditions and involves microbial succession, including thermophilic fungi that intensively degrade lignocellulosic material. When properly conducted, the process enables the transformation of plant biomass into a stable humified product within a matter of weeks, thereby conferring the status of a fully renewable raw material [Pudełko 2015].

The production process of casing material, regardless of the raw material base, aims to obtain a medium with specific quality parameters; however, the technological pathways leading to this objective differ substantially. In the case of standard peat-based casing, production relies on an extractive model combined with physicochemical conditioning of the mineral resource [Żebrowska and Kociołek-Balawejder 2010]. The process begins with mechanical extraction and fractionation of highmoor and lowmoor peat, which are subsequently blended in proportions ensuring a balance between water absorption capacity and air-filled porosity [Szumigaj-Tarnowska and Uliński 2023].

A fundamental technological challenge in this model is the natural acidity of the raw peat material, typically ranging from pH 3.5 to 5.0 [Żebrowska and Kociołek-Balawejder 2010]. This necessitates an energy consuming neutralization process using defecation lime or chalk. Moreover, due to the potential presence of pathogens in natural deposits, the finished peat mixture often requires preventive chemical disinfection prior to application in cultivation [Sakson 2023].

A fundamentally different strategy is represented by the technology for producing peat-free humus casing [Patent application P.452691, 2025], which constitutes a bioprocess engineering approach. The starting material is renewable plant biomass subjected to controlled aerobic stabilization. A key distinguishing feature of this method is the utilization of the natural activity of thermophilic microorganisms.

During the intensive phase of the process, the temperature within the compost pile exceeds 60–65°C, leading to autopasteurization of the raw material and effective elimination of pathogens and weed seeds without the use of chemical agents [Pudełko 2015]. Importantly, this process is accompanied by natural pH stabilization to levels above 7.5, thereby eliminating the need for liming, which is characteristic of peat-based casing. Nevertheless, a controlled addition of defecation lime is applied to maintain pH stability across successive production batches, to facilitate the formation of the desired granular structure of the humus casing (OH), and to regulate its bulk density depending on the type of compost used.

The compost itself may vary in origin (e.g., organic), depending on producer requirements, market expectations, and applicable regulations. The final stage of production involves precise mechanical fractionation, imparting the material with the desired granular structure. The resulting product is biologically stable and obtained through biosynthesis rather than through mere processing of a mineral resource [Patent application P.452691, 2025].

The choice of casing technology therefore determines not only agronomic practices but also the strategic position of the producer in the context of climate and market changes. Peat extraction is associated with the degradation of natural carbon sinks and CO_2 emissions, which, according to life cycle assessment analyses (LCA), constitute the principal environmental burden of mushroom production [Goglio et al. 2025, Skreczko and Trepka 2016]. Furthermore, in contrast to the extractive model, humus-based technology aligns with the principles of the Circular Economy. The use of locally sourced biomass enables the reduction of carbon dioxide emissions and the return of organic matter to the soil after the cultivation cycle, thereby eliminating the issue of waste generation [Pudełko 2015].

The Polish mushroom sector, partially dependent on imported peat, is exposed to currency and logistical risks [Kayzer 2017]. The implementation of technology based on locally sourced agricultural raw materials (e.g., straw and green waste) reduces producers' dependence on raw material geopolitics and contributes to the stabilization of production costs [Sakson and Hreczuch 2025]. While peat provides excellent water retention capacity [Żebrowska and Kociołek-Balawejder 2010], humus casing offers enhanced phytosanitary safety due to the thermal autopasteurization process [Patent application P.452691, 2025]. The broad spectrum of the ecological and functional benefits outlined above positions humus casing as an innovative foundation for the development of sustainable mushroom production.

Physicochemical parameters of the investigated casing materials

The differences in the origin of the two materials were reflected in the results of laboratory analyses. The findings confirmed that the humus casing creates a growth environment with characteristics distinct from those of the standard peat-based casing.

Table 1. Comparison of physicochemical parameters of casing materials used in studies

Shelf	Flush no.	Moisture (% wt.)	pH (in water)	pH (in KCl soln.)	Salinity (EC) ($mS \cdot c^{-1}$)	WHC (% wt.)
BiO**H**	I	49.79	8.15	7.67	3.74	171.03
	II	50.75	8.20	7.51	3.14	152.83
	average	**50.27**	**8.18**	**7.59**	**3.44**	**161.93**
BiO**T**	I	76.60	7.67	7.29	1.95	249.38
	II	76.58	7.80	7.34	1.09	263.47
	average	**76.59**	**7.74**	**7.32**	**1.52**	**256.43**
BrO**H**	I	52.04	8.21	7.47	3.65	154.15
	II	45.93	8.26	7.49	2.97	173.47
	average	**48.99**	**8.24**	**7.48**	**3.31**	**163.81**
BrO**T**	I	73.70	7.78	7.31	2.20	261.56
	II	71.07	7.89	7.37	1.23	284.10
	average	**72.39**	**7.84**	**7.34**	**1.72**	**272.83**

A comparison of the key parameters (Table 1) indicates pronounced differences in the hydrodynamic properties of the tested substrates. The analysis of the collected data reveals fundamental differences in the characteristics of the two compared casing materials.

The most pronounced discrepancies concern water management properties, where the humus casing (BiOH, BrOH shelves) exhibited substantially lower maximum water-holding capacity (on average approximately 162–164%) and lower moisture content (approximately 50%) compared to the peat-based casing, which retained water at levels exceeding 250%.

The humus casing was naturally more alkaline (pH > 8.1) than the limed peat casing (pH ~7.7–7.8). Another parameter potentially influencing yield quality is salinity (EC); the humus variants showed electrical conductivity values of 3.3–3.4 mS·cm^{-1}, which were higher than those recorded for the peat casing.

Course of the experimental cultivation cycles

Dynamics of casing colonization and fruit body initiation

The previously described differences in the origin and physicochemical parameters of the investigated materials were reflected in the course of the mushroom production cycle. However, observations indicated that despite structural differences between the casing materials, the key developmental stages proceeded within comparable time frames, although clear differences in mycelial morphology were noted.

During the casing colonization phase, proper vegetative growth was observed in all variants. The humus casing (BiOH, BrOH), characterized by a dark color and granular structure, was colonized by the mycelium at a rate comparable to that of the peat-based control (BiOT, BrOT), reaching the stage of full surface mycelial coverage (“mycelial blanket”) by the end of the first week after casing application. The process of fruit body initiation (generative phase) occurred at a similar time in both the experimental and control variants. This suggests that the relatively higher pH and specific microbiological profile of the humus casing did not exert an inhibitory effect on the signals initiating primordia formation.
A key agronomic observation was the high structural stability of the humus material; despite intensive irrigation during the shock phase, the casing maintained its porosity.

Crop health status

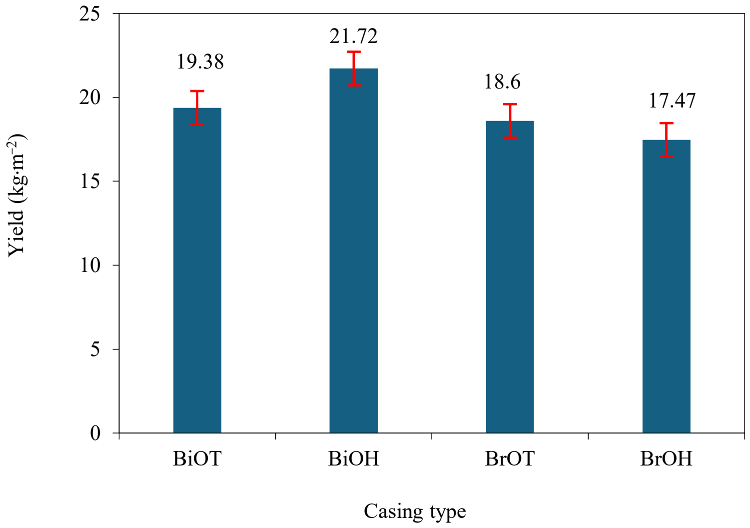
A notable event during the experiment was the occurrence of green mold outbreaks in the cultivation hall with white mushrooms during the second flush. It should be emphasized, however, that the infection affected both the experimental variant (BiOH) and the control variant (BiOT) to a comparable extent. The fact that the pathogen developed with similar intensity on both types of casing materials allows for an important conclusion, the source of infection was not the casing material itself but an external factor. The humus casing did not exhibit either increased susceptibility or enhanced resistance to colonization by competing mold fungi compared to the standard peat-based casing.

Yield performance

Figure 1 presents a comparative analysis of yield results for white (Bi) and brown (Br) mushrooms obtained using two types of casing materials: peat-based (OT) and experimental humus-based (OH).

In the case of white mushrooms, the yield in the first flush reached 19.38 kg·m^{-2} on peat casing (BiOT) and 21.72 kg·m^{-2} on humus casing (BiOH), corresponding to a nominal increase of 2.34 kg·m^{-2} when the humus casing was applied. For brown mushrooms, the yield obtained on peat casing (BrOT) was 18.60 kg·m^{-2}, whereas on humus casing (BrOH) it reached 17.47 kg·m^{-2}, representing a difference of -1.13 kg·m^{-2}.

Figure 1. First flush yield by casing type and mushroom strain



A statistical comparison of the obtained yield results requires consideration of the natural variability inherent in the cultivation process, particularly given the screening character of the comparative experiment, in which a single observation was performed for each variant.

Such an experimental design precludes the direct determination of empirical measures of dispersion (variance and standard deviation), which constitute the basis of classical comparative analysis.

At the same time, the observed differences in yield between humus and peat casing required evaluation with reference to the range of random variability inherent in the cultivation process, in order to determine whether the effects exceeded stochastic fluctuations and could therefore be considered statistically significant.

To establish a formal framework for this assessment, a probabilistic model was adopted assuming a normal distribution of yield variability around the expected value.

Dispersion parameters were estimated on the basis of historical yield data obtained from peat casing during earlier studies conducted at the MEXEO enterprise within the BIOMEX research project [Raport INHORT 2021].

Table 2 presents the results of yield assessments obtained from five independent measurement series based on cultivation experiments conducted using standard peat casing under conditions comparable in scale to those of the present study (i.e., 50–100 m^2 of experimental cultivation area).

Table 2. The yield performance on peat-based casing (archival data) [Raport INHORT 2021]

Series 1 (kg·m^{-2})	Series 2 (kg·m^{-2})	Series 3 (kg·m^{-2})	Series 4 (kg·m^{-2})	Series 5 (kg·m^{-2})
13.44	18.89	18.62	14.33	16.57
13.76	15.42	17.58	12.39	17.52
13.96	16.66	–	–	–
15.13	17.98	–	–	–

To establish a basis for the statistical evaluation of variability in the obtained yield results, it was assumed that the most appropriate estimator of variance in this case would be the pooled variance of historical yield data (Table 2), calculated according to Equation (1):

$$\sigma_{\mathrm{p}}^2 = \frac{\sum_{\mathrm{i}=1}^{5}(n_i - 1)s_i^2}{\sum_{\mathrm{i}=1}^{5}(n_i - 1)} \tag{1}$$

The comparison of the obtained yield results, and thus the preliminary evaluation of the humus casing material with respect to yield performance, was formulated as a test of a conservative null hypothesis, constructed in accordance with the commonly applied principles of formal statistical inference [Riley and Hobson 2006, Czermiński and Iwasiewicz 1989].

The null hypothesis postulated that the yield obtained with humus casing is at most equal to the yield achieved with peat casing (2):

$$H_0\colon P_{OH} \le P_{OT} \tag{2}$$

against the alternative hypothesis defined by the strict inequality (3):

$$H_1\colon P_{OH} > P_{OT} \tag{3}$$

indicating the existence of statistical grounds to assume that yield under humus casing may exceed that obtained under peat casing.

Since yield variance was estimated independently on the basis of archival data and subsequently applied to future single observations, the inference was based on the principles of predictive testing for the estimation of confidence intervals for future observations derived from the variability characteristics of historical data [Hahn 1977, Geisser 1993].

In the applied inference procedure, the test statistic *Z* defined by Equation (4):

$$Z = \frac{P_{OH} - P_{OT}}{\sigma_\Delta} \tag{4}$$

was adopted as the verification measure for hypotheses (2) and (3). The statistic σ_Δ representing the variance of the difference $\Delta = P_{OH} - P_{OT}$, was estimated in accordance with the principle of variance propaga-tion, where the sources of variability are P_{OH} and P_{OT} (5):

$$\sigma_\Delta = \sqrt{\sigma_{OH}^2 + \sigma_{OT}^2} = \sqrt{2 \cdot \sigma_p^2} = \sigma_p \cdot \sqrt{2} \tag{5}$$

The calculated values of σ_p^2, σ_p, σ_Δ are respectively 0.98, 0.99 and 1.40.
As the test criterion, the critical quantile value *k* (6):

$$k_\alpha = \Phi^{-1}(1 - \alpha) \tag{6}$$

corresponding to the adopted significance level $\alpha = 0.05$, was applied, where Φ^{-1} denotes the inverse cumulative distribution function of the standard normal distribution.

The decision rule for rejecting the null hypothesis H_0, constructed in accordance with the previously described framework, reduces – consistent with the principles of statistical hypothesis testing – to satisfying inequality (7):

$$Z \geq k \tag{7}$$

The values of *k* were calculated from (8) for $\Phi(1-0.05)$

$$\Phi(k) = \frac{1}{2}\left[1 + \mathrm{erf}\left(\frac{k}{\sqrt{2}}\right)\right] \tag{8}$$

Where the symbol erf(*x*) denotes an error function given by Equation (9):

$$\mathrm{erf}(x) = \frac{2}{\sqrt{\pi}}\int_0^x e^{-t^2}\mathrm{d}t \tag{9}$$

For the adopted significance level ($\alpha = 0.05$) the critical quantile value $k = \Phi^{-1}(0.95)$ calculated according to Equation (8), was equal to 1.64.

The value of the test statistics *Z* using Equation (4) amounted:

$$Z = \frac{2.34\ kg{\cdot}m^{-2}}{1.40\ kg{\cdot}m^{-2}} = 1.67 \tag{10}$$

which satisfies the test criterion (7) indicating rejection of the null hypothesis H_0.

The test result indicates that the observed difference in yield between BiOH and BiOT, equal to 2.34 kg·m^{-2} in favor of BiOH, exceeds the typical process variability (standard deviation of the difference ≈ 1.40 kg) and is statistically significant in a one-sided test at the level α = 0.05 (*Z* = 1.67; *p* = 0.047). This result therefore provides statistical grounds for concluding that yield performance in the BiOH variant was superior to that of BiOT under the defined experimental conditions.

The yield of brown mushrooms obtained using the experimental casing (BrOH) was 17.47 kg·m^{-2}, which is 1.13 kg·m^{-2} lower than the yield recorded for the reference peat-based casing (BrOT).

To assess the statistical significance of the difference in favor of BrOT, an inference procedure analogous to that described above was conducted, adopting the forms of hypotheses H_0 and H_1 as specified in Equations (2) and (3).

The value of Z calculated from Equation (4) amounted:

$$Z = \frac{1.13\ kg{\cdot}m^{-2}}{1.40\ kg{\cdot}m^{-2}} = 0.81 \tag{11}$$

The obtained value *Z* of the test statistic was clearly lower than the critical quantile value for the test, i.e. $k_{0.05} = 1.64$ indicating that there are no grounds for rejecting the null hypothesis H_0.

The result of the above one-sided test therefore indicates the absence of a statistically significant difference between the yield values of BrOH and BrOT.

It can thus be concluded that the yield of brown mushrooms obtained from the humus casing, although numerically lower than that recorded for the peat-based casing, falls within the range of natural process variability, and there are no grounds to consider it statistically significantly lower than P_{OT}. Due to the occurrence of green mold infection originating from the substrate after the first flush, yield analysis was not continued for the second flush in the conducted experiment.

Mineral content in mushroom fruit bodies

The results of the analysis of mineral content in white and brown mushroom fruit bodies cultivated on the experimental humus-based casing and on the reference peat-based casing are presented in Tables 3 and 4.

The reported concentration values, expressed as means of three independent measurements, are accompanied by confidence intervals constructed using the Student's *t*-distribution.

Table 3. Mineral content (mg·kg⁻¹ dry weight) in mushroom fruit bodies (first flush)

Shelf	Se (mg·kg⁻¹ d.w.)	Fe (mg·kg⁻¹ d.w.)	Ca (mg·kg⁻¹ d.w.)	Mg (mg·kg⁻¹ d.w.)	P (mg·kg⁻¹ d.w.)	K (mg·kg⁻¹ d.w.)	Moisture content (%)
BiOH	2.82 ±0.70	49.20 ±1.40	59.0 ±1.5	1550 ±20	13100 ±200	18900 ±300	90.6 ±0.3
BiOT	3.65 ±0.67	50.30 ±1.50	241 ±10	1670 ±20	13300 ±400	24500 ±900	91.5 ±0.5
BrOH	2.96 ±0.75	66.90 ±2.40	390 ±14	1780 ±30	13500 ±300	20200 ±500	90.7 ±0.2
BrOT	3.13 ±0.26	50.00 ±2.30	278 ±30	1600 ±10	12300 ±400	22600 ±600	91.8 ±0.2

Table 4. Mineral content (mg·kg⁻¹ dry weight) in mushroom fruit bodies (second flush)

Shelf	Se (mg·kg⁻¹ d.w.)	Fe (mg·kg⁻¹ d.w.)	Ca (mg·kg⁻¹ d.w.)	Mg (mg·kg⁻¹ d.w.)	P (mg·kg⁻¹ d.w.)	K (mg·kg⁻¹ d.w.)	Moisture content (%)
BiOH	3.72 ±0.27	68.10 ±2.60	173 ±2	2360 ±20	12900 ±100	20100 ±1000	91.6 ±0.5
BiOT	2.04 ±0.08	68.50 ±1.50	238 ±7	2330 ±40	12800 ±600	29200 ±1500	91.8 ±0.4
BrOH	3.09 ±0.34	97.50 ±1.60	338 ±10	2200 ±50	13400 ±200	18200 ±100	91.6 ±0.3
BrOT	2.39 ±0.20	63.50 ±0.60	178 ±2	2280 ±30	12900 ±800	26900 ±900	92.9 ±0.5

The results of mineral content determinations in white and brown mushroom fruit bodies obtained in the first flush from the experimental humus casing (BiOH, BrOH) and the reference peat-based casing (BiOT, BrOT) indicate that selenium concentrations ranged from 2.82 to 3.65 mg·kg⁻¹ dry weight (d.w.), with no clear differences observed between casing types or mushroom strains.

Iron content was higher in brown mushrooms (66.9 mg·kg⁻¹ d.w., BrOH) than in white mushrooms (approximately 49–50 mg·kg⁻¹ d.w.).

Calcium levels showed marked variation among the variants – particularly high in BrOH (390 mg·kg⁻¹ d.w.) and lowest in BiOH (59 mg·kg⁻¹ d.w.).

Magnesium and phosphorus concentrations remained at comparable levels across variants; for phosphorus, values ranged from 13.000 to 13.500 mg·kg⁻¹ d.w.

The highest absolute concentrations among the analyzed elements were recorded for potassium (18.900–24.500 mg·kg⁻¹ d.w.), with the maximum value observed in the BiOT variant.

In the second flush, the range of variability differed partially. Selenium content was higher in the humus casing variants (3.09–3.72 mg·kg⁻¹ d.w.) than in the peat-based variants (2.04–2.39 mg·kg⁻¹ d.w.).

Iron reached higher values in brown mushrooms (up to 97.5 mg·kg^{-1} d.w. in BrOH) compared with white mushrooms (approximately 68 mg·kg^{-1} d.w.).

Calcium again exhibited pronounced differences between strains and casing variants, with the highest value recorded in BrOH (338 mg·kg^{-1} d.w.).

Magnesium and phosphorus remained at relatively stable levels, ranging from 2200 to 2360 mg·kg^{-1} d.w. (Mg) and from 12.800 to 13.400 mg·kg^{-1} d.w. (P), respectively.

As in the first flush, the highest absolute concentrations were observed for potassium (18.200–29.200 mg·kg^{-1} d.w.), with the maximum value recorded in the BiOT variant.

In both flushes, potassium was the quantitatively dominant element. Phosphorus and magnesium showed relatively stable concentrations across variants, whereas calcium and iron displayed more pronounced variation between strains and casing types.

In all cases, water content was similar and exhibited limited variability between variants, remaining within the range of 90.6–92.9% (w/w) in the fruit bodies.

The differences observed between the first and second flush indicate inter-flush variability, particularly evident for selenium, iron, and potassium.

To quantitatively assess the capacity of the experimental humus casing material to enhance the accumulation of key mineral components relative to peat-based casing, an index of relative increase in mineral content was defined for the purposes of this evaluation, as expressed in Equation (12):

$$\Delta_{rel} w_i = \frac{w_i^{OH} - w_i^{OT}}{w_i^{OT}} \cdot 100\% \tag{12}$$

representing a measure of the percentage increase (positive or negative) in the accumulation capacity of mineral components.

The estimation of the variability interval of the index defined by Equation (12), due to the mathematical properties of its functional form, required a separate analytical approach.

It should be noted that for statistics defined as a ratio of random variables, classical linear approximation may lead to substantial amplification of the uncertainty associated with the denominator.

Under conditions of small sample size and moderate variability, this may result in overestimation of the variance – and consequently the standard deviation – sometimes by several orders of magnitude relative to the mean, as well as in asymmetric confidence intervals.

To stabilize the estimation of uncertainty associated with the relative change in microelement content, an approach based on the logarithm of the ratio of means (log response ratio, lnRR) was applied. This method is widely used for estimating effect sizes for multiplicative quantities in biological meta-analyses [Hedges and Gurevitch 1999, Lajeunesse 2011].

The application of logarithmic (log-ratio) transformation is recommended for ratio-type variables, as it limits the amplification of denominator uncertainty and provides more reliable confidence interval coverage under small sample conditions.

Following the adopted methodology, Equation (12) was transformed into an algebraically equivalent ratio form Equation (13):

$$\frac{w_i^{OH}}{w_i^{OT}} = \Delta_r w_i + 1 \tag{13}$$

As a consequence of the adopted analytical approach, the variability of the index defined by Equation (12) (was estimated by evaluating the variance propagation of the logarithmic expression Equation (14):

$$L = \ln \frac{w_i^{OH}}{w_i^{OT}} \tag{14}$$

based on the well-established rule of variance propagation described in Equation (15):

$$s_L^2 = \left(\frac{\partial L}{\partial w_i^{OH}}\right)^2 s_{w_i^{OH}}^2 + \left(\frac{\partial L}{\partial w_i^{OT}}\right)^2 s_{w_i^{OT}}^2 \tag{15}$$

Thus

$$s_L^2 = \frac{s_{c_i^{OH}}^2}{\left(w_i^{OH}\right)^2} + \frac{s_{c_i^{OT}}^2}{\left(w_i^{OT}\right)^2} \tag{16}$$

The confidence interval limits of $\Delta_r c_i$ were calculated from Equation (17):

$$\Delta_r w_i^{G,D} = e^{L^{G,D}} - 1 \tag{17}$$

where:

$$L^{G,D} = L \pm t_{\alpha,\nu_{ef}} \cdot \frac{s_L}{\sqrt{n}} \tag{18}$$

The value of the parameter $t_{\alpha,\nu_{ef}} = 2.871$ from the Student's t-distribution, appearing in Equation (18) was determined by interpolation assuming a significance level of $\alpha = 0.05$ and an effective number of degrees of freedom $\nu_{ef} = 3.69$ calculated using the Welch-Satterthwaite equation [JCGM (GUM) 2008], taking into account the differential expressions appearing in Equation (15).

The calculated values of the relative increase indices for mineral content in the fruit bodies of the investigated white and brown mushroom strains, for two harvests (flushes) and depending on casing type (OH vs. OT), are presented graphically in Figures 2 (first flush) and 3 (second flush).

Figure 2. Relative increase indices of mineral concentrations in fruit bodies cultivated on experimental (humus) casing *vs*. peat-based casing (first flush)

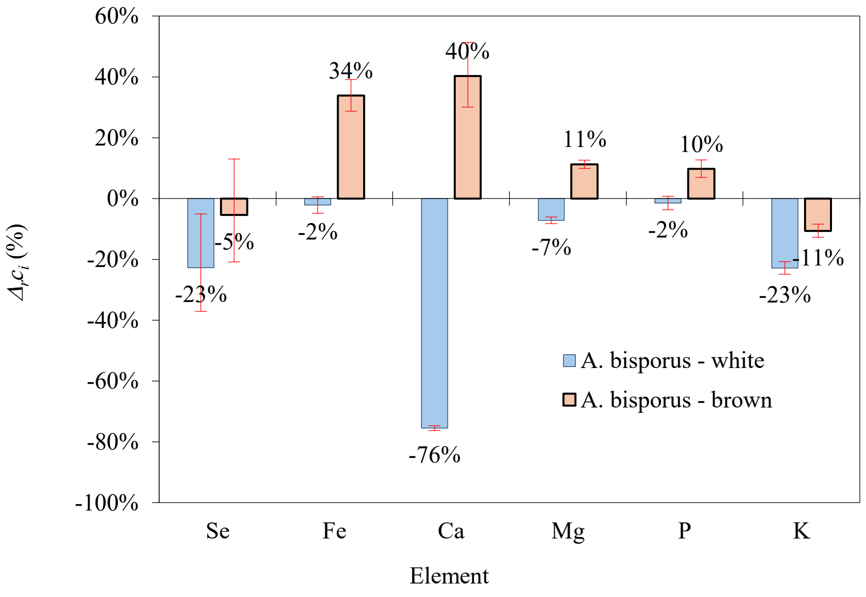
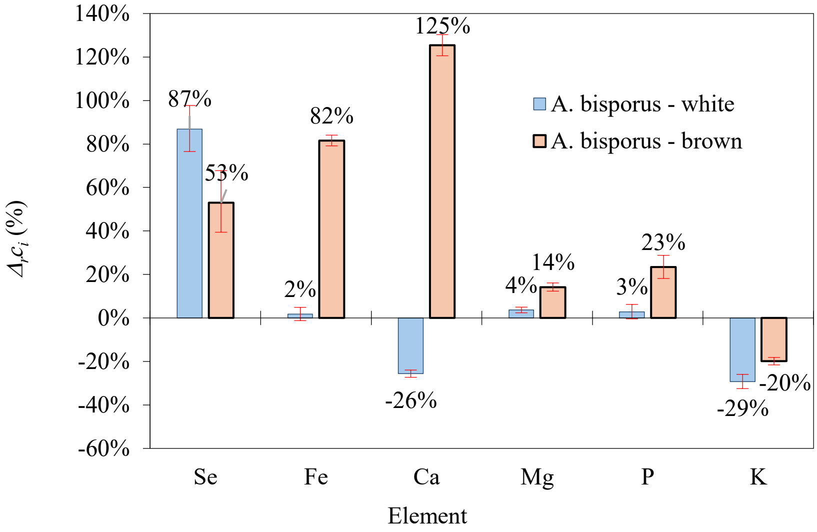


Figure 3. Relative increase indices of mineral concentrations in fruit bodies cultivated on experimental (humus) casing *vs*. peat-based casing (second flush)



In the first flush, the application of humus casing (OH) generally led to a reduction in the concentrations of most analyzed elements relative to peat-based casing (OT).

The most pronounced effect concerned calcium (Ca), for which a strong relative decrease (−76%) was observed, with a very narrow confidence interval (−76% to −75%), clearly excluding random variation. The magnitude of the change in Ca content between the white and brown strains may suggest a substantial modification in the mechanisms of Ca uptake or distribution in the compared cases.

A significant reduction in concentration was also observed for selenium (Se: −23%), potassium (K: −23%), and magnesium (Mg: −7%). In each of these cases, the confidence intervals did not include zero, indicating an effect exceeding random variability.

For iron (Fe: −2%) and phosphorus (P: −2%), the confidence intervals included zero, indicating no statistically distinguishable change relative to peat casing.

In summary, the first flush in the white strain was characterized by a predominance of reduction effects, particularly pronounced for Ca and K in fruit bodies cultivated on humus casing compared to peat-based casing.

In contrast to the white strain, the brown strain in the first flush exhibited a predominance of positive effects. This pattern could potentially serve as an indicative marker of mushrooms cultivated under organic conditions using humus casing; however, this issue requires further analysis of absolute elemental concentrations depending on casing type, based on a broader experimental dataset.

A clear increase in concentration was observed for Ca (+40%), Fe (+34%), Mg (+11%), and P (+10%). In all cases, the confidence intervals were entirely positive and did not include zero, indicating a systematic and statistically significant increase in the concentrations of these elements under humus casing conditions.

For Se (−5%, CI: −21% to 13%), the confidence interval included zero, indicating the absence of a conclusive effect. Potassium showed a moderate but statistically significant decrease (−11%).

Particularly noteworthy was the opposite response of calcium between the two strains, which may suggest strain-specific differences in the regulation of ion homeostasis.

In the second flush, a marked shift in the response profile was observed. For the white strain, a pronounced increase in selenium concentration was recorded (+87%). Moderate but statistically significant increases were observed for Mg (+4%) and P (+3%), although in the case of P the effect was borderline. For Fe (+2%), the confidence interval of the index included zero, indicating statistically indistinguishable accumulation properties of the white strain under peat and humus casing conditions.

At the same time, a clear decrease in Ca (−26%) and K (−29%) persisted, suggesting a sustained modification of macronutrient balance under humus casing conditions.

The brown strain in the second flush exhibited the strongest positive effects observed in the entire study. Calcium concentration increased by +125%, Fe by +82%, Se by +53%, Mg by +14%, and P by +23%.

All these effects were unequivocally positive and clearly exceeded the range of random variability, demonstrating high statistical significance. Potassium remained the only element with a consistently negative change (−20%).

From a general perspective, several recurrent patterns can be identified. Potassium content showed a stable decrease in both strains and in both flushes, representing the most consistent trend across the entire dataset. In contrast, calcium content exhibited a strong strain-dependent response, characterized by a sustained decrease in the white strain and a marked increase in the brown strain.

The second flush was characterized by an intensification of positive effects, particularly in the brown strain, which may indicate the influence of temporal factors, changes in nutrient availability within the substrate, or cumulative effects.

Cases in which the confidence intervals included zero (Fe and P in the first flush for the white strain; Se in the first flush for the brown strain; Fe in the second flush for the white strain; and borderline P in the second flush for the white strain) should be interpreted as effects indistinguishable from random variability.

Overall, the application of humus casing resulted in clear, strain-dependent modifications of mineral accumulation patterns.

The obtained results indicate that the use of humus casing (OH) as an alternative to conventional peat-based casing (OT) does not lead to deterioration of raw material quality with respect to the analyzed mineral composition. The observed changes were selective in nature and dependent on both mushroom strain and flush (harvest), suggesting a complex interaction between casing type and mycelial physiology.

In particular, a distinctly favourable effect of OH application was observed in the brown strain. In both flushes, an increase in the concentration of most analyzed elements was recorded, with the effect markedly intensified in the second flush. This was especially evident for calcium, iron, and selenium, for which the relative increases were high and clearly exceeded the range of random variability. Such a strong and repeatable response suggests that humus casing may enhance the bioavailability of selected mineral components or modify their uptake and translocation mechanisms within the fruit body.

In the case of the white strain, the response was more heterogeneous. In the first flush, reduction effects predominated, particularly for calcium and potassium; however, in the second flush a clear improvement was observed, including a pronounced increase in selenium concentration and moderate but positive changes for magnesium and phosphorus.

It is noteworthy that the second flush was characterized by a general intensification of positive effects, which may indicate dynamic changes in nutrient availability over the course of the production cycle.

It is possible that the physicochemical properties of the humus casing – such as higher biological activity or a different ion-buffering capacity – reveal their full potential during the later stages of fruiting.

It is also important to note that some of the observed differences were indistinguishable from random variability, further confirming the absence of a systematic negative effect associated with the use of OH. This indicates that, within the analyzed scope, no unequivocal evidence of reduced mineral quality of the fruit bodies under humus casing conditions was identified.

From an overall perspective, humus casing exhibits properties comparable to peat-based casing and, in the case of the brown strain, distinctly favourable effects.

The obtained results suggest that OH not only does not represent an inferior solution, but under certain genotypic and technological configurations may offer additional benefits associated with enhanced accumulation of selected mineral components.

From both practical and environmental standpoints, this aspect acquires particular significance. As noted in the introduction, peat is a non-renewable resource on a technological timescale, and its extraction exerts pressure on peatland ecosystems. In this context, the development of alternative casing materials, such as compost-based humus casing, aligns with the concept of sustainable intensification of horticultural production.

The results of the present study provide a rationale for continued research and development aimed at optimizing the composition and technological parameters of OH.

It may be anticipated that further investigations – encompassing a greater number of production cycles, detailed analysis of physicochemical casing properties, and assessment of interactions with microclimatic parameters – will enable a more comprehensive understanding of the mechanisms underlying the observed differences. In particular, further exploration of calcium homeostasis and potential ionic antagonisms, which may differentiate strain-specific responses, appears especially warranted.

In summary, humus casing emerges as a material with tangible application potential, whose properties are not inferior to those of traditional peat-based casing and may, under certain conditions, surpass them. The obtained results constitute a solid basis for further research and technological refinement toward practical implementation and market introduction.

SUMMARY

The conducted study aimed to verify the feasibility of applying compost-based humus casing as an alternative to conventional peat-based casing in A. bisporus cultivation technology.

Both production aspects, expressed in terms of yield performance, and raw material quality, assessed on the basis of mineral composition of fruit bodies from two mushroom strains (white and brown) across two successive flushes, were analyzed.

The obtained results indicate that the application of humus casing leads to yield performance at least equivalent to that achieved with peat-based casing.

In the case of the white strain, a statistically significant increase in yield was observed in the first flush, whereas for the brown strain, differences in yield remained within the range of natural process variability, providing grounds to consider humus casing equivalent in terms of productivity.

Analysis of mineral composition demonstrated that the effect of casing type on mineral accumulation is selective and dependent on both mushroom strain and flush.

Particularly favourable effects of humus casing application were observed in the brown strain, for which clear increases in the concentrations of most analyzed elements – including calcium, iron, and selenium – were recorded in both flushes, especially in the second flush.

In the white strain, the response was more heterogeneous; however, even in this case, no unequivocal evidence of reduced mineral quality of the fruit bodies under humus casing conditions was identified.

The results of the second flush, characterized by a general intensification of positive effects, suggest that the properties of humus casing may become more fully expressed in the later stages of the production cycle. This phenomenon may be associated with dynamic changes in mineral availability and the biological activity of the cultivation substrate.

These observations support the rationale for further investigations encompassing a greater number of production cycles, as well as detailed analyses of the physiological and environmental mechanisms determining mushroom responses to alternative casing materials.

Overall, the obtained results confirm that compost-based humus casing constitutes a material with properties superior or comparable to those of traditional peat-based casing and, under certain conditions – particularly in the cultivation of brown mushrooms – may exhibit distinctly advantageous characteristics.

In the context of the finite availability of peat, increasing regulatory pressure, and rising environmental requirements, the findings of the present study provide an important premises for the implementation of peat-free technologies in mushroom production.

ACKNOWLEDGMENTS

The authors wish to express their sincere gratitude to Mr. Piotr Pasoń, owner of the Grzybek – mushroom farm (Opole, Poland), for enabling the experiment to be conducted under commercial production conditions and for his technical assistance. We also extend our thanks to the MCBR UO (International Research and Development Center of the University of Opole, Poland) for performing the chemical and laboratory analyses.

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Funding: This research was financed by the internal funds of Mexeo, 9 Energetyków St., 47-225 Kędzierzyn-Koźle, Poland and Afirma Sp. z o.o., Wojnowo, 62-095 Murowana Goślina, Poland.

Otrzymano/Received: 9.03.2026
Zaakceptowano/Accepted: 3.06.2026
Opublikowano/Published: 7.07.2026

Citation: Sakson N., Chruściel A., Pinkowski F., Wałek-Nawratek K., Frankowski P., Guziałowska-Tic J., Hreczuch W., 2026. Local plant biomass and biodegradable organic waste-derived compost as a sustainable alternative to peat in casing layers for *Agaricus bisporus* cultivation. Ann. Hortic. 35(1), 47–64. https://doi.org/10.24326/ah.2026.5678