horticulturae

MDPI

Article

A Comparative Study of Calcium Sulfate Alternatives in Compost Production for White Button Mushroom (*Agaricus bisporus*)

András Misz [1,2], Amanda Sándorné Szőke [1], Judit Bajzát [1], Dániel Kökény [1], Marianna Visnyei [1], László Kredics [2], Henrietta Allaga [2], Attila Szűcs [2], Sándor Kocsubé [2], Csaba Csutorás [1,3,*] and Csaba Vágvölgyi [2,*]

1 Új Champignons Ltd., Bartók B. Str. 162, H-1224 Budapest, Hungary; andras.misz@gmail.com (A.M.); amanda.szoke14@gmail.com (A.S.S.); bajud98@gmail.com (J.B.); kokenydani5@gmail.com (D.K.); laborujchamp2@gmail.com (M.V.)
2 Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Közép Fasor 52, H-6726 Szeged, Hungary; kredics@bio.u-szeged.hu (L.K.); henrietta.allaga@gmail.com (H.A.); a.szunyi@windowslive.com (A.S.); shigsanyi@gmail.com (S.K.)
3 Institute of Chemistry and Physics, Eszterházy Károly Catholic University, Eszterházy Sqr. 1, H-3300 Eger, Hungary
* Correspondence: csutoras.csaba@uni-eszterhazy.hu (C.C.); csaba@bio.u-szeged.hu (C.V.)

Abstract: This study explores various potential substitutes for gypsum in the production of compost for white button mushrooms (*Agaricus bisporus*). During compost preparation, calcium sulfate ($CaSO_4$) was replaced with calcium carbonate ($CaCO_3$), ammonium sulfate ($(NH_4)_2SO_4$), and monocalcium phosphate ($Ca(H_2PO_4)_2$). Complete replacement of gypsum with calcium carbonate led to a significant pH increase during the second phase of composting, adversely affecting mushroom mycelium growth. Compost parameters were observed to be similar in scenarios where calcium sulfate was supplemented with calcium carbonate in 8:2 and 6:4 ratios, both with and without the presence of ammonium sulfate, and in 3:1 and 1:1 mixtures of calcium sulfate and monocalcium phosphate, when compared to traditional gypsum-based processes. All experimental compost mixtures yielded comparable mushroom crops in cultivation trials. Notably, the 8:2 mixture of calcium sulfate and calcium carbonate demonstrated superior performance in cultivation trials relative to the 6:4 mixture. However, supplementing these mixtures with ammonium sulfate resulted in similar crop yields. Monocalcium phosphate also emerged as a promising partial gypsum substitute, showing comparable crop production in both 3:1 and 1:1 ratios to the technological optimum. The exploration of alternative calcium sources like calcium carbonate and monocalcium phosphate reflects the adaptability of the industry in response to resource availability challenges. The potential use of byproducts like ammonium sulfate from the composting process itself offers a cost-effective and environmentally friendly approach to compost formulation, underscoring its worldwide relevance.

Keywords: mushroom compost production; compost additives; white button mushroom; compost base materials; gypsum; mushroom yield

Citation: Misz, A.; Szőke, A.S.; Bajzát, J.; Kökény, D.; Visnyei, M.; Kredics, L.; Allaga, H.; Szűcs, A.; Kocsubé, S.; Csutorás, C.; et al. A Comparative Study of Calcium Sulfate Alternatives in Compost Production for White Button Mushroom (*Agaricus bisporus*). *Horticulturae* **2024**, *10*, 378. https://doi.org/10.3390/horticulturae10040378

Academic Editor: Chenyang Huang

Received: 9 March 2024
Revised: 28 March 2024
Accepted: 31 March 2024
Published: 9 April 2024

1. Introduction

The cultivated mushroom *Agaricus bisporus* is a predominant choice in Europe's mushroom consumption. The cornerstone of industrial-scale mushroom production is mushroom compost, a selective substrate crafted through controlled chemical and microbiological processes. The changing availability of resources due to geopolitical issues, coupled with environmental sustainability concerns, necessitates the exploration of alternative compost ingredients that could maintain or enhance mushroom yield while mitigating environmental impact. Essential components of mushroom compost include horse manure, wheat straw, chicken manure, gypsum as an additive, and substantial water [1,2]. Depending on

regional practices, the base mixture might predominantly consist of horse manure with added straw, possibly straw with a bit of horse manure, or even straw without horse manure. This base is then blended with a specific quantity of chicken manure and gypsum.

The composting process initiates with a chemical analysis of the base materials [3]. Adjustments in the amounts of various base materials are made based on different management systems to achieve optimal values for several parameters, primarily chemical compounds, including nitrogen, ammonium, dry matter, organic matter, ash content, pH, and the carbon/nitrogen ratio [4]. Monitoring the cellulose, hemicellulose, and lignin content is sometimes practiced, though these are typically not primary factors in determining the basic mix recipe; they are more often used for guiding the technology and monitoring the process [5].

In many systems, the straw component is pre-soaked and left to settle for a few days, whether aerated or not, to initiate the microbiological breakdown of the straw. The basic mixture is then processed in specialized mixing lines, where the correct proportions of base materials are homogenized and moistened to a certain level [2]. The next stage, Phase I composting, occurs on aerated floors or, more commonly, in aerated "bunkers"—special concrete structures equipped with high-pressure floors to ensure an aerobic composting process [6]. Temperature control is vital in these units, and depending on the system, various aeration regimes are applied to provide adequate oxygen for different mesophilic, thermophilic, and chemical stages. Numerous bacterial and fungal species contribute to this process; many are unidentified, with only a few known for their role in producing high-quality compost. At the end of Phase I composting, the compost's chemical and microbiological characteristics are optimized, but it is not yet ready for the inoculation of mushroom mycelium [1,2]. To prepare for this, Phase I compost undergoes peak heating in a closed system. The most crucial stages of peak heating include a pasteurization process at 58–60 °C for eight hours to eliminate pests and diseases, followed by a conditioning period of 2–2.5 days at 48 °C to proliferate beneficial thermophilic microbes [7]. These microbes can serve as a nutrition source for button mushrooms and help to reduce the ammonia level to below 10 ppm since 10 ppm or higher levels of it are toxic for mushroom mycelium. The compost is then cooled to 25 °C, making it suitable for inoculation with mushroom mycelium.

In the above-described composting process, calcium sulfate (gypsum) plays a significant role [8]. It helps to lower the compost's pH, affecting the $NH_4^+ = NH_3 + H^+$ dissociation equilibrium. While the ammonium ion can nourish mushrooms, gaseous ammonia above a certain level is toxic to the mycelium. Ammonia is crucial during Phase I composting for softening the straw and is necessary to eliminate harmful microorganisms during the Phase II pasteurization process. Gypsum also benefits the texture of the compost, preventing it from becoming too dense and aids in maintaining the required aerobic conditions and proper structure during spawn-run and mushroom cultivation [7,8].

Historically, composters in many European countries have sourced their gypsum from coal-fired thermal power plants, which remove sulfur (specifically sulfur dioxide) from their flue gas by introducing calcium carbonate. Prior to the Ukrainian war, the climate goals aimed at shutting down such coal-fired thermal power plants; for instance, the planned closure of the Visonta Power Plant in Hungary, a major gypsum supplier for compost-producing companies in the whole region, was slated for 2025. Although the dramatic changes in the availability of energy carriers might temporarily delay the shutdown of these plants, it is almost certain that in the foreseeable future, the drive to transition to energy production with a lower carbon footprint will pick up again. This shift is likely to lead to a regional shortage of gypsum supply compared to the current levels.

The potential of a gypsum-free composting process of wheat straw for mushroom production was evaluated by Mouthier et al. [9]. The results revealed a faster, gypsum-free alternative for Phase I; however, according to the recommendation of the authors, gypsum is still needed at the end of Phase I according to Phase II condition requirements.

The objective of this study was to identify alternative additives that could partially or fully replace gypsum in mushroom compost. We experimented with calcium carbonate and monocalcium phosphate added to gypsum in various ratios. We hypothesize that calcium carbonate could buffer the pH of mushroom compost during cultivation, as button mushrooms produce oxalic acid as a metabolic product [10]. Keeping the compost pH 6–7 is crucial to mitigate the risk of competitive microorganisms like *Trichoderma* species causing green mold disease in mushroom cultivation [11].

2. Materials and Methods

2.1. Analysis of Basic Compost Parameters

For compost production, the samples were analyzed using basic laboratory approaches and Foss NIR spectrometry (NIRS DS2500, Hilleroed, Denmark). For the determination of total nitrogen and ammonium ion content, the nitrogen compounds in the compost samples (2.5 g) were transformed into ammonium sulfate through digestion with concentrated sulfuric acid (12.5 mL). Then, 33% (*w*/*w*) sodium hydroxide (60 mL) was added, followed by distillation and absorption in a boric acid solution (1% (*w*/*w*), 50 mL). The resultant ammonium borate was titrated using a 1 M sulfuric acid solution. For the determination of pH, the measurement of hydrogen ions was conducted using an ion-selective electrode (ADVA AD8000 multimeter, with pH electrode AD1131B, Szeged, Hungary). One hundred mL of water was added to 100 g of compost, the mixture was stirred for 15 min, and the pH of the aqueous solution was measured. Dry matter and moisture content were determined through mass measurement with a ME204 precision balance (Mettler Toledo, Budapest, Hungary) after drying to a constant mass at 105 °C for 24 h in a heat chamber (HŐKER HK-45/1100C, Miskolc, Hungary). For the determination of organic matter, wet compost samples (17 g) were dried at 105 °C for 24 h and then incinerated in a burning chamber (HŐKER, HK-45/1100C, Miskolc, Hungary) at 600 °C for 6 h. Ash content was measured with a ME204 precision balance. Organic matter was calculated from ash content: $100 \times (17 - \text{ash content})/17$ (%). EC/N ratio, cellulose, hemicellulose, and lignin fractions were measured using the Foss NIRs device (NIRS DS2500, Hilleroed, Denmark) on fresh, moist samples with a calibration line provided by Masterlab (Boxmeer, The Netherlands).

Composting intensity evaluation was based on color, softness, and temperature build-up. In case of good composting intensity, the color of the straw-based basic materials changes from yellow to dark brown in 12–14 days, the hard straw particles soften to a compressible status, and the temperature build-up achieves a minimum of 1.5 °C/h. The wax layer of the compost modifies from a shiny one to a matte status, or almost completely disappears. Temperature was measured with a CLAAS 147861.0 agricultural thermometer (CLAAS E-systems GmbH, Dissen, Germany). Mycelium growth was evaluated by color, visible mycelium mass, and smell. Good mycelium growth was recorded when, after the spawn run period, the color of the compost mass changed from dark brown to reddish-brown, when the mycelium mass formed a continuous net of mycelium in the whole mass, and when the smell was medium to strong mushroom smell. Poor mycelium growth was indicated by black straw particles found in the compost mass, with areas ungrown by mushroom mycelium and a neutral smell. The absence of mycelium growth was indicated by the black color of the compost with no mycelium growth and, unattached mushroom mycelium from the spawn to the compost particles and an unpleasant smell like rotten or mold smell.

2.2. Industrial Scale Composting Experiments

Experimental mixes were prepared in a clean mixing line, segregated from conventional materials. To ensure distinct separation, experimental mixes were composted on aerated floors. The Phase II and III processes in the peak-heating and spawn-running tunnels were demarcated using pulling net loops. The different mixing ratios used in the experiment are summarized in Table 1.

Table 1. Salt mixtures used in the experiments.

Treatment	Ratio in %			Ammonium Sulfate Solution (kg per 1 kg Calcium Carbonate)
	Calcium Sulfate	Calcium Carbonate	Monocalcium Phosphate	
Basic technology	100	-	-	-
1	-	100	-	-
2	80	20	-	-
3	60	40	-	-
4	80	20	-	0.7
5	60	40	-	0.85
6	75	-	25	-
7	50	-	50	-

During the experiments, we homogenized the wheat straw, horse manure, and chicken manure mixture in a proportion of 100:20:82 on a mixing line specifically designed for this purpose. After homogenization, the appropriate amounts of the experimental substances were added to the base mixture units, and then the entire mixture was homogenized using clean mechanical equipment (Hoving Holland, Stadskanaal, The Netherlands). The composting experiments and mycelium runs of the compost were conducted in 4 replicates for all 7 experimental mixtures, along with complete laboratory analyses (pH, moisture content, organic matter, total nitrogen, ammonium content, C/N ratio, cellulose, hemicellulose, and lignin fractions) of the intermediate and final products.

2.3. Mushroom Cultivation Experiments in Bags

The experiments were performed in 2023 in a mushroom growing room of the Új Champignons Ltd. in Kerecsend, Northern Hungary. Phase III compost, ready for growth, was filled into plastic bags, standardized by weight, and placed in the same growing houses as their control (containing only gypsum additive) to minimize differences in yield or quality due to varying climates. The casing soil was sourced from the same supplier (Alsópáhok, Hungary) and batch for both the control and experimental materials. Cultivation was carried out in 3 replicates. Mushroom growing details are given in Supplementary File S1, including all detailed parameters of compost temperature, air temperature in the growing house, carbon-dioxide concentration, and relative humidity in the air of the growing house, as well as the watering regime by days at different stages of mushroom growing. Harvesting was performed through normal hand picking: mushrooms were taken with 2–2.5 cm of stems between a cap size of 5–5.5 cm. The weight of mushrooms was measured using a digital scale (ME 204, Mettler Toledo, Budapest, Hungary).

First class, healthy mushrooms were characterized by a white color and firmness of both cap and stem, with caps remaining closed. In contrast, second-class mushrooms displayed a light gray or yellow coloration, softer caps and stems, and caps that were opened.

2.4. Statistical Analysis

The experimental data were statistically evaluated using the Meta.Numerics library (https://github.com/dcwuser/metanumerics, accessed on 31 January 2024). For the evaluation of the results, standard deviation (SD) values were calculated, and the Student's *t*-test was performed to assess significance levels of differences. The experimental mixes were benchmarked against a 'technological optimum range'. This range is derived from extensive cultivation experiences accumulated over decades. For statistical comparison, an artificial series consisting of the lowest, highest, and average values of the technological optimum range was created, and their mean and standard deviation values were determined.

3. Results

3.1. Full Replacement of Calcium Sulfate with Calcium Carbonate

In the first phase of composting, temperatures aligned with technological specifications, and no differences were observed in the structure of the mixture, composting intensity, or breakdown of the straw fraction's wax layer. At the end of Phase I, a slight, non-significant rise in pH was noted, with total nitrogen, ammonium ion, and organic matter ratio remaining within the technological optimum range parameters (Table 2).

Table 2. Composting parameters (organic matter, C/N ratio, total N, NH_4^+, cellulose, hemicellulose, and lignin as percentage of dry matter) of different experiments. Ash content (%) can be calculated from organic matter as 100%—organic matter (%). Values are means ± SD based on 4 replicates.

Compost Sample	Phase of Composting	pH	Moisture Content (%)	Total N (%)	NH_4^+ (%)	Organic Matter (%)	C/N Ratio	Hemicellulose (%)	Cellulose (%)	Lignin (%)
Technological optimum ($CaSO_4$)	End of Phase I	7.8–8.3	73–75	1.8–2.2	0.25–0.5	76–79	19–21	14–18	32–42	8–12
	End of Phase II	7.4–7.8	66–70	2.0–2.4	<0.1	73–76	16–17	10–15	26–33	11–14
	End of Phase III	6.2–6.7	61–65	2.2–2.6	0.05–0.15	70–73	14–15	8–12	25–29	9–11
Full replacement of $CaSO_4$ with $CaCO_3$	End of Phase I	8.63 ± 0.27	73.02 ± 0.10	2.08 ± 0.08	0.27 ± 0.02	78.18 ± 1.15	18.84 ± 1.11	15.72 ± 0.04	42.02 ± 0.41	8.45 ± 0.15
	End of Phase II	8.54 ± 0.06 *	67.20 ± 0.46	2.16 ± 0.04	0.01 ± 0.00	78.13 ± 0.25 *	18.27 ± 0.35 *	12.01 ± 0.07	36.65 ± 1.03 *	12.53 ± 0.33
	End of Phase III	-	-	-	-	-	-	-	-	-
Mixture of $CaSO_4$ and $CaCO_3$ in ratio of 8:2	End of Phase I	8.36 ± 0.02	73.22 ± 0.18	1.74 ± 0.05	0.22 ± 0.01	73.79 ± 0.38 *	21.45 ± 0.50	17.25 ± 0.31	39.50 ± 1.04	6.62 ± 0.50 *
	End of Phase II	7.57 ± 0.03	68.65 ± 0.24	2.27 ± 0.05	0.05 ± 0.00	72.30 ± 0.69	16.06 ± 0.27	13.52 ± 0.63	29.09 ± 0.43	11.62 ± 0.36
	End of Phase III	6.47 ± 0.03	62.95 ± 0.49	2.14 ± 0.09	0.10 ± 0.01	69.83 ± 1.06	16.32 ± 0.84 *	8.27 ± 0.17	26.96 ± 0.43	9.55 ± 0.25
Mixture of $CaSO_4$ and $CaCO_3$ in ratio of 6:4	End of Phase I	8.31 ± 0.02	73.51 ± 0.26 *	1.90 ± 0.03	0.22 ± 0.01	76.05 ± 0.98	20.39 ± 0.43	16.94 ± 0.72	38.54 ± 1.17	8.43 ± 0.74
	End of Phase II	7.97 ± 0.03 *	68.19 ± 0.67	1.99 ± 0.02	0.00 ± 0.00	73.60 ± 0.92	18.51 ± 0.42	13.42 ± 0.07	32.03 ± 0.88	12.12 ± 0.39
	End of Phase III	6.43 ± 0.06	67.10 ± 0.40	2.23 ± 0.05	0.07 ± 0.01	69.29 ± 1.61	15.62 ± 0.64	9.35 ± 0.23	25.73 ± 1.07	10.10 ± 0.21
Mixture of $CaSO_4$ and $CaCO_3$ in ratio of 8:2, with $(NH_4)_2SO_4$ solution	End of Phase I	8.19 ± 0.06	73.72 ± 0.15	2.03 ± 0.09	0.24 ± 0.01	77.59 ± 1.19	19.45 ± 0.91	17.90 ± 0.83	37.19 ± 0.91	8.57 ± 0.65
	End of Phase II	7.92 ± 0.04 *	68.76 ± 0.06	2.23 ± 0.04	0.01 ± 0.00	74.70 ± 0.28	16.79 ± 0.35	14.51 ± 0.36	30.33 ± 0.35	10.97 ± 0.38
	End of Phase III	6.67 ± 0.07	66.75 ± 0.77 *	2.45 ± 0.05	0.07 ± 0.00	72.82 ± 0.50	14.88 ± 0.22	10.13 ± 0.78	26.75 ± 0.58	10.05 ± 0.26
Mixture of $CaSO_4$ and $CaCO_3$ in ratio of 6:4, with $(NH_4)_2SO_4$ solution	End of Phase I	8.23 ± 0.07	73.04 ± 0.37	2.03 ± 0.04	0.27 ± 0.01	78.24 ± 0.28	19.53 ± 0.39	17.09 ± 0.51	34.19 ± 0.98	11.20 ± 0.37
	End of Phase II	7.73 ± 0.04	68.40 ± 0.31	2.48 ± 0.04 *	0.00 ± 0.00	74.39 ± 0.16	15.03 ± 0.24 *	12.70 ± 0.42	29.26 ± 0.62	12.58 ± 0.40
	End of Phase III	6.52 ± 0.02	63.76 ± 0.24	2.39 ± 0.07	0.06 ± 0.01	73.91 ± 0.34 *	15.45 ± 0.46	10.08 ± 0.40	28.02 ± 0.38	9.28 ± 0.36
Mixture of $CaSO_4$ and $Ca(H_2PO_4)_2$ in ratio of 75:25	End of Phase I	8.28 ± 0.01	74.85 ± 0.26	1.81 ± 0.06	0.28 ± 0.03	78.71 ± 0.32	22.04 ± 0.66 *	19.32 ± 0.96 *	41.97 ± 0.68	5.60 ± 0.17 *
	End of Phase II	7.82 ± 0.08	70.81 ± 0.24 *	2.33 ± 0.06	0.01 ± 0.00	75.31 ± 0.07	16.42 ± 0.22	14.78 ± 0.39	28.63 ± 0.16	12.03 ± 0.38
	End of Phase III	6.49 ± 0.02	64.31 ± 0.43	2.44 ± 0.03	0.08 ± 0.00	72.59 ± 1.00	14.92 ± 0.16	9.18 ± 0.95	27.08 ± 0.28	7.58 ± 0.67 *
Mixture of $CaSO_4$ and $Ca(H_2PO_4)_2$ in ratio of 50:50	End of Phase I	8.09 ± 0.06	75.06 ± 0.31	1.95 ± 0.04	0.28 ± 0.01	77.76 ± 0.42	20.17 ± 0.48	19.08 ± 0.74 *	39.19 ± 0.93	8.03 ± 0.55
	End of Phase II	7.98 ± 0.06 *	70.78 ± 0.22 *	2.28 ± 0.02	0.01 ± 0.01	74.19 ± 0.33	16.26 ± 0.14	14.61 ± 0.30	29.33 ± 0.41	12.80 ± 0.39
	End of Phase III	6.49 ± 0.06	66.22 ± 0.32 *	2.27 ± 0.07	0.03 ± 0.01 *	72.52 ± 1.16	15.98 ± 0.32 *	9.83 ± 0.27	26.19 ± 0.80	8.10 ± 0.35 *

*: significantly different from the technological optimum (Student's *t*-test, $p < 0.05$).

The cellulose, hemicellulose, and lignin fractions were consistent with the technological optimum. However, at the heat treatment's end, pH values spiked to 8.54, exceeding the technological optimum (7.4–7.8). Moisture content was lower than in the case of the technological optimum, and the ammonium ion content remained below the inhibitory threshold of mycelial growth. Other chemical parameters fell within the expected phase range. Mycelial growth was completely inhibited in the compost by the end of the spawn run, indicated by the significantly higher pH of Phase II compost. The compost color remained black, no mushroom mycelium growth was noticed from the surfaces of the inoculated

mushroom spawn into the compost particles, and the mushroom smell of the compost was entirely missing. Thus, the raw material was excluded from the cultivation experiment.

3.2. Mixture of Calcium Sulfate and Calcium Carbonate in Ratios of 8:2 and 6:4

In the first composting phase, no significant differences were noted in temperatures, composting intensity, or straw fraction breakdown. At the end of Phase I, the 8:2 mixture had a higher pH than the 6:4 mixture (Table 2), both within the technological optimum range. Moisture content, ammonium ion, hemicellulose, and cellulose content showed no significant variance between the mixtures. The 8:2 mixture had significantly lower total nitrogen, higher ash content, and a greater carbon–nitrogen ratio. Lignin values varied, ranging from 6.1 to 7.4 for the 8:2 mixture and 7.2 to 9.1 for the 6:4 mixture. During heat treatment, the 8:2 mixture's pH dropped significantly by 0.8, indicative of favorable microbiological and chemical processes, whereas the 6:4 mixture showed a lesser decrease of 0.45. No significant differences were observed in other chemical parameters (Table 2). The 6:4 mixture exhibited a smaller decrease in cellulose content during Phase II compared to the 8:2 mixture. By the end of Phase III, differences in chemical parameters, including pH, partially equalized, likely due to oxalic acid production through the mycelium. Mycelial growth was more pronounced in the 8:2 mixture, with minimal non-spawn-run parts in the 6:4 mixture at a low rate of 2–3%.

In cultivation experiments, the 8:2 mixture's first flush started 20–24 h later than the control but yielded uniform, healthy mushrooms. The 8:2 mixture significantly outperformed the control in yield, as shown in Table 3.

Table 3. Results of bag cultivation experiments. Values are means ± SD based on 3 replicates.

Treatment	Mushroom Yield (g/18 kg Compost)		
	1st Flush	2nd Flush	Total
Control: 100% $CaSO_4$	5077 ± 187	4412 ± 139	9489 ± 180
8:2 mixture of $CaSO_4$ and $CaCO_3$	6256 ± 220 *	3961 ± 141 *	10,217 ± 120 *
6:4 mixture of $CaSO_4$ and $CaCO_3$	4699 ± 164	4597 ± 148	9296 ± 99 *
8:2 mixture of $CaSO_4$ and $CaCO_3$, with $(NH_4)_2SO_4$	5907 ± 180 *	4448 ± 114	10,355 ± 130 *
6:4 mixture of $CaSO_4$ and $CaCO_3$, with $(NH_4)_2SO_4$	2651 ± 92 *	4467 ± 118	7118 ± 90 *
75:25 mixture of $CaSO_4$ and $Ca(H_2PO_4)_2$	5377 ± 140	5214 ± 161 *	10,591 ± 125 *
50:50 mixture of $CaSO_4$ and $Ca(H_2PO_4)_2$	4970 ± 116	5522 ± 132 *	10,492 ± 106 *

*: significantly different from the control (Student's *t*-test, $p < 0.05$).

The crop flush of the 6:4 calcium sulfate to calcium carbonate mixture commenced on schedule, yet the mushrooms exhibited variability in size and shape, with much larger, slightly distorted fruiting bodies (Figure 1).

(**A**) (**B**) (**C**)

Figure 1. Mushroom fruiting bodies from the cultivation experiments in bags. (**A**): healthy fruiting bodies in control bags with 100% calcium sulfate addition to compost; (**B**,**C**): distorted fruiting bodies in bags with 6:4 calcium sulfate to calcium carbonate mixture added to the compost.

Notably, instances of hollow-stemmed mushrooms were observed. Comparatively, the 8:2 mixture demonstrated a more consistent and favorable outcome. In the first flush, the yield from the 8:2 mixture surpassed the control by 23%, while the 6:4 mixture produced 8% fewer mushrooms. During the second flush, the 8:2 mixture continued to outperform, yielding 10% more mushrooms than the control, and the 6:4 mixture showed a modest increase of 4% over the control (Table 3).

The total yield results are also shown (Table 3). The 8:2 mixture exhibited an 8% higher combined yield compared to the control, affirming its efficacy. Conversely, the 6:4 mixture's combined yield was 2% lower than that of the control. Beyond the quantitative aspects, qualitative observations also favored the 8:2 mixture. The quality of mushrooms cultivated using this mixture was observed to be higher compared to those grown in the 6:4 mixture, indicating that the 8:2 ratio not only enhances yield but also improves mushroom quality.

3.3. Mixture of Calcium Sulfate and Calcium Carbonate in Ratios of 8:2 and 6:4, Combined with Ammonium Sulfate Solution

In this part of the experiment, 0.7 and 0.85 L of 20% (*w*/*w*) ammonium sulfate solution was added per kilogram of calcium carbonate in the 8:2 and 6:4 mixtures, respectively. Throughout Phase I of composting, no notable differences were observed in composting temperatures, composting intensity, or the breakdown of the straw fraction's wax layer. By the end of Phase I, chemical parameters were largely consistent with the control, except for a slight increase in ash content and a non-significant decrease in lignin content in the 8:2 mixture (Table 2).

At the conclusion of Phase II, the 8:2 mixture exhibited a minor but significant increase in pH in relation to the technological optimum. This difference was not noted in the 6:4 mixture. Other chemical parameters remained comparable to the technological optimum. By the end of Phase III, no significant differences were observed in most of the chemical characteristics of either mixture (Table 2). The compost structure and mycelial growth were consistent with technological expectations, rendering the composts suitable for cultivation experiments.

The crop yields of this experimental setup are summarized in Table 3. In the first flush, the 8:2 mixture produced 16% more mushrooms, while the 6:4 mixture yielded 48% less compared to the control. In the second flush, both the 8:2 and 6:4 mixtures showed a marginal 1% increase in yield over the control. The overall yield was 9% higher for the 8:2 mixture but 25% lower for the 6:4 mixture compared to the control. Mushroom quality was similar to that of the control in both cases.

When ammonium sulfate was present, a significant increase in crop yield was observed for the 8:2 mixture, and a significant decrease in yield for the 6:4 mixture compared to the control and to results without ammonium sulfate (Table 3). This suggests that while ammonium sulfate can positively influence yield in certain mixtures, its impact varies depending on the specific calcium-salt ratio used.

3.4. Mixture of Calcium Sulfate and Monocalcium Phosphate in Ratios of 75:25 and 50:50

Throughout the composting process, the temperatures, intensity of composting, and the breakdown of the straw fraction's wax layer showed no differences from the technological optimum for both mixtures. At the end of the first phase, the 50:50 mixture exhibited a significantly lower pH value, a significantly higher total nitrogen content, and a significantly increased lignin content compared to the 75:25 mixture (Table 2). During the second phase, both mixtures resulted in a slight pH decrease, with values slightly but significantly exceeding the technological limit in the 50:50 mixture. The ash content in the 50:50 mixture was higher than in the 75:25 mixture. Ammonium ion levels were detectable in both mixtures but remained below the germination inhibitory threshold. By Phase III, chemical parameters were almost identical, except for ash content, where the difference between the two mixtures persisted. At the end of the mycelium run, the amount

of mycelium and compost structure was deemed adequate, qualifying the experimental samples for cultivation experiments.

In the first flush, the 75:25 mixture produced 6% more mushrooms, while the 50:50 mixture yielded 2% less compared to the control (Table 3). In the second flush, the 75:25 mixture showed an 18% increase, and the 50:50 mixture showed a 25% increase in mushroom yield compared to the control. The combined yield was significantly higher in both cases compared to the control, with a 12% increase for the 75:25 mixture and an 11% increase for the 50:50 mixture (Table 3). Mushroom quality in both cases was found to be comparable to the control.

4. Discussion

Various attempts are known to meet the calcium requirements of different cultivated edible mushrooms. Interestingly, most such research did not aim to alter the technological bases but focused on enhancing the quality and nutritional value of the cultivated mushrooms [12]. Experiments were conducted with species including *Pleurotus eryngii*, *Flammulina velutipes*, *Lentinula edodes*, and *Hypsizygus marmoreus*, utilizing a range of calcium sources for calcium accumulation [13–15]. Since the calcium sources were primarily used as additional supplements, a wide array of calcium sources was examined, such as calcium chloride, calcium amino acid chelates, calcium lactate, calcium nitrate, and calcium carbonate, as well as complex additives like agricultural lime, starfish powder, eggshells, and oyster shells, which predominantly contain calcium carbonate [16,17]. Although the main objective was the calcium fortification of mushrooms, in some cases, these supplements also led to increased yields, e.g., Fan et al. [18] demonstrated that the addition of calcium carbonate and shellac resulted in denser mycelia of *F. velutipes* and improved the quality and yield of fruiting bodies.

The current study explores the use of calcium carbonate, ammonium sulfate, and monocalcium phosphate as viable partial substitutes for gypsum in compost production for *A. bisporus* cultivation. This research highlights the complex relationship between compost chemistry and optimal mushroom growth conditions, emphasizing the potential for resource optimization in mushroom farming [5].

Our findings indicate that the complete replacement of gypsum with calcium carbonate resulted in an unfavorable pH increase. The rise in pH during Phase II composting can be attributed to calcium bicarbonate formation from the reaction of calcium carbonate with carbon dioxide, a byproduct of bacterial and fungal metabolism. Calcium bicarbonate, unlike water-insoluble calcium carbonate, is water-soluble and can raise pH levels. In Phase I, continuous aeration mitigates carbon dioxide accumulation, keeping pH closer to the optimal value. However, in Phase II, without aeration, significant calcium bicarbonate formation elevates pH beyond the optimal range.

Partial substitutions with calcium carbonate and monocalcium phosphate did not compromise compost quality or mushroom yield. This is especially notable in the 8:2 ratio of calcium sulfate to calcium carbonate, which not only maintained a conducive growth environment but also slightly enhanced mushroom yield compared to traditional gypsum-based compost. Earlier, calcium carbonate has been successfully used as an additive in the substrates of oyster and shiitake mushrooms, enhancing their growth and yield [17,19]. These observations underline the potential of calcium carbonate as a partial substitute for gypsum, consistent with studies highlighting the role of calcium in supporting mushroom growth and development [18,20].

The addition of ammonium sulfate in mixtures with higher calcium carbonate content proved to be an effective strategy to mitigate pH elevation, an essential consideration given the sensitivity of *A. bisporus* to compost pH levels. Ammonium sulfate, a byproduct continuously generated in compost plants during the ammonia removal process from exhaust gases of Phase I composting [21], appears to be a valuable addition for maintaining optimal composting conditions. Although the introduction of ammonium sulfate and monocalcium phosphate led to minor fluctuations in some parameters of the intermediate

products, our research indicates that these additives, in various mixing ratios, are viable for cultivation experiments. The composting and chemical parameters of each experimental additive largely remained within the optimal values. The beneficial effect of ammonium sulfate aligns with its known role in composting processes, acting as a pH stabilizer and providing nitrogen, crucial for mycelial growth [5,22].

Furthermore, this study emphasizes the feasibility of using monocalcium phosphate as an alternative calcium source in compost formulations. Both 3:1 and 1:1 mixtures of calcium sulfate to monocalcium phosphate showed promising results, with mushroom yields comparable to the control. The slightly better performance of the 50:50 mixture in the second flush and its overall higher combined yield suggests that this ratio might be more effective for long-term cultivation. This finding is significant, given the dual benefit of monocalcium phosphate as both a calcium and phosphorus source, crucial for energy transfer and mycelium growth.

The comparative analysis of different calcium sources and their ratios contributes to a deeper understanding of their impact on compost quality and mushroom cultivation. For example, the detrimental effects of high calcium levels observed in other studies [18,23] were not evident in our controlled partial substitution scenarios, suggesting the negative impact of calcium on mycelial growth can be mitigated through careful management of calcium sources and concentrations.

5. Conclusions

The results of this study indicate that mixtures of calcium sulfate with calcium carbonate or monocalcium phosphate can effectively replace 100% calcium sulfate in compost production, with the modified mixtures not only yielding comparable but, in some cases, superior mushroom crops.

Our findings not only validate the critical role of gypsum in mushroom compost but also highlight the potential of alternative calcium sources to enhance compost formulations. The strategic use of calcium carbonate, ammonium sulfate, and monocalcium phosphate within specific ratios presents a viable approach to optimize mushroom cultivation practices worldwide. It also opens avenues for the utilization of byproducts like ammonium sulfate in compost production. The insights gained offer valuable guidance for the mushroom cultivation industry on a global scale, particularly in adapting to changing resource availabilities and environmental considerations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10040378/s1.

Author Contributions: Conceptualization, A.M., C.C. and C.V.; methodology, A.M., J.B. and H.A.; software, A.S. and S.K.; validation, A.S., L.K. and C.C.; formal analysis, A.M., C.C. and C.V.; investigation, A.M., A.S.S., J.B., D.K., H.A. and M.V.; resources, A.M., C.C. and C.V.; data curation, A.M., C.C. and A.S.; writing—original draft preparation, A.M. and C.C.; writing—review and editing, A.M., C.C., C.V. and L.K.; visualization, H.A. and S.K.; supervision, C.C. and C.V.; project administration, C.C., C.V. and L.K.; funding acquisition, C.C., C.V. and L.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by grants 2020-1.1.2-PIACI-KFI-2020-00111 and 2023-1.1.1-PIACI_FÓKUSZ-2024-00044 from the National Research, Development and Innovation Office, Hungary. Additional backing came from the Doctoral Student Scholarship Program of the Co-operative Doctoral Program of the Ministry of Innovation and Technology, funded by the National Research, Development and Innovation Fund (grant No. KDP-2021-C1764158 to A.M.).

Data Availability Statement: All relevant data are presented within the manuscript.

Conflicts of Interest: Authors A.M., A.S.S., J.B., D.K., M.V., and C.C. were employed by the company Új Champignons Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships.

References

1. Arya, A.; Rusevska, K. (Eds.) *Biology, Cultivation and Applications of Mushrooms*; Springer Verlag: Singapore, 2022; ISBN 9789811662560.
2. Singhal, S.; Rasane, P.; Kaur, S.; Garba, U.; Singh, J.; Raj, N.; Gupta, N. Mushroom cultivation, processing and value-added products: A patent based review. *Recent Pat. Food Nutr. Agric.* **2019**, *10*, 3–19. [CrossRef]
3. Sánchez, C. Modern aspects of mushroom culture technology. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 756–762. [CrossRef]
4. Noble, R.; Gaze, R.H. Preparation of mushroom (*Agaricus bisporus*) composts in controlled environments: Factors influencing compost bulk density and productivity. *Int. Biodeterior. Biodegr.* **1996**, *37*, 93–100. [CrossRef]
5. Kariaga, M.G.; Nyongesa, H.W.; Keya, N.C.O.; Tsingalia, H.M. Compost physico-chemical factors that impact on yield in button mushrooms, *Agaricus bisporus* (Lge) and *Agaricus bitorquis* (Quel) Saccardo. *J. Agric. Sci.* **2012**, *3*, 49–54. [CrossRef]
6. Lyons, G.A.; Sharma, H.S.S.; Kilpatrick, M.; Cheung, L.; Moore, S. Monitoring of changes in substrate characteristics during mushroom compost production. *J. Agric. Food Chem.* **2006**, *54*, 4658–4667. [CrossRef]
7. Straatsma, G.; Gerrits, J.P.G.; Thissen, J.T.N.M.; Amsing, J.G.M.; Loeffen, H.; Van Griensven, L.J.L.D. Adjustment of the composting process for mushroom cultivation based on initial substrate composition. *Biores. Technol.* **2000**, *72*, 67–74. [CrossRef]
8. Gerrits, J.P.G. The significance of gypsum applied to mushroom compost, in particular in relation to the ammonia content. *Neth. J. Agric. Sci.* **1977**, *25*, 288–302. [CrossRef]
9. Mouthier, T.M.B.; Kilic, B.; Vervoort, P.; Gruppen, H.; Kabel, M.A. Potential of a gypsum-free composting process of wheat straw for mushroom production. *PLoS ONE* **2017**, *12*, e0185901. [CrossRef] [PubMed]
10. Gąsecka, M.; Magdziak, Z.; Siwulski, M.; Mleczek, M. Profile of phenolic and organic acids, antioxidant properties and ergosterol content in cultivated and wild growing species of *Agaricus*. *Eur. Food Res. Technol.* **2018**, *244*, 259–268. [CrossRef]
11. Kredics, L.; Hatvani, L.; Allaga, H.; Büchner, R.; Cai, F.; Vágvölgyi, C.; Druzhinina, I.S.; Naeimi, S. Trichoderma green mould disease of cultivated mushrooms. In *Advances in Trichoderma Biology for Agricultural Applications*; Fungal Biology Series; Amaresan, N., Sankaranarayanan, A., Dwivedi, M.K., Druzhinina, I.S., Eds.; Springer: Cham, Switzerland, 2022; pp. 559–606. [CrossRef]
12. Tang, Z.-X.; Shi, L.-E.; Jiang, Z.-B.; Bai, X.-L.; Ying, R.-F. Calcium enrichment in edible mushrooms: A review. *J. Fungi* **2023**, *9*, 338. [CrossRef]
13. Yang, H.; Zhang, M.; Song, C.; Liu, J.; Xu, Z.; Shang, X. Effects of Fe^{2+}, Zn^{2+} and Ca^{2+} on mycelium growth and its biological enrichment in mycelia of three edible mushrooms. *Acta Edulis Fungi* **2017**, *24*, 27–33.
14. Zhang, T.; Qian, L.; Wang, W.; Li, C.; Li, F. Study on calcium enrichment in mycelia of *Hypsizygus marmoreus*. *N. Hortic.* **2022**, *4*, 104–109.
15. Zhang, J.; Xin, Z. Effect of exogenous calcium on *Lentinula edodes* mycelium growth. *N. Hortic.* **2016**, *18*, 143–145.
16. Choi, U.K.; Lee, O.; Kim, Y. Effect of calcinated oyster shell powder on growth, yield, spawn run, and primordial formation of king oyster mushroom (*Pleurotus eryngii*). *Molecules* **2011**, *16*, 2313–2322. [CrossRef] [PubMed]
17. Royse, D.J.; Sanchez-Vazquez, J.E. Influence of precipitated calcium carbonate ($CaCO_3$) on shiitake (*Lentinula edodes*) yield and mushroom size. *Biores. Technol.* **2003**, *90*, 225–228. [CrossRef]
18. Fan, L.; Jin, Q.; Feng, W.; Song, T.; Shen, Y.; Cai, W. Effects of different calcium sources and amounts on the growth and yield of *Flammulina velutipes*. *Edible Med. Mushrooms* **2018**, *26*, 91–93.
19. Ghareeb, B.A. Impact different level of calcium carbonate ($CaCO_3$) on growth and yield of oyster mushroom (*Pleurotus* spp.). *Int. J. Eng. Technol.* **2019**, *11*, 785–792. [CrossRef]
20. Feng, G.; Shi, H.; Zheng, J.; Shi, Y. Research process of metal ion on the growth and development of edible fungi. *Food Res. Dev.* **2021**, *42*, 193–198.
21. Melse, R.W.; Ogink, N.W.M. Air scrubbing techniques for ammonia and odor reduction at livestock operations: Review of on-farm research in the Netherlands. *Trans. Am. Soc. Agric. Eng.* **2005**, *48*, 2303–2313. [CrossRef]
22. Zied, D.C.; Pardo-Giménez, A.; Minhoni, M.A.; Villas, R.L.; Álvarez-Ortí, M.; Pardo-González, J.E. Characterization, feasibility and optimization of *Agaricus subrufescens* growth based on chemical elements on casing layer. *Saudi J. Biol. Sci.* **2012**, *19*, 343–347. [CrossRef]
23. Chen, Y.; Xiao, S.; Chen, Z.; Ma, L.; Liu, Q. Effect of calcium salt on the growth of Lentinan mycelium in course of shaking flask fermentation. *Food Mach.* **2013**, *29*, 58–61.