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Interactive effects of activated charcoal and zinc supplementation in the casing layer on growth, yield, biological efficiency, and chemical composition of button mushroom (*Agaricus bisporus*)

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ABSTRACT

This study evaluated the individual and interactive effects of activated charcoal and zinc supplementation in the casing layer of white button mushroom (*Agaricus bisporus*). A 3 × 3 factorial completely randomized design was used, with activated charcoal at 0, 10, and 20 g experimental unit^{-1} (C1–C3) and elemental Zn at 0, 200, and 400 mg experimental unit^{-1} (Z1–Z3), with three replicates. Morphology and chemical composition were assessed in first-flush fruiting bodies, whereas productivity was assessed cumulatively over three flushes. Activated charcoal significantly affected stipe length, stipe diameter, cap diameter, and average fruiting-body weight. The zinc-formulation treatments significantly affected fruiting-body number, average weight, total yield, biological efficiency (BE), protein, and phosphorus. Significant C × Z interactions occurred for fruiting-body number, average weight, protein, phosphorus, and sodium, but not for total yield or BE. C3Z3 recorded the highest fruiting-body number and the highest numerical total yield and BE (79.46%), C_3Z_2 recorded the highest average fruiting-body weight, and C2Z2 recorded the highest protein content. Zinc concentrations in fruiting bodies and the physicochemical and microbial properties of the casing were not measured; therefore, no conclusions are drawn about zinc uptake, biofortification, or a specific casing-mediated mechanism.

1. INTRODUCTION

The white button mushroom, *Agaricus bisporus* (J.E. Lange) Imbach, is one of the most widely cultivated edible mushrooms and remains important to commercial production because of its consumer acceptance, nutritional value, and suitability for intensive cultivation. Its fruiting bodies provide protein, dietary fiber, vitamins, minerals, and other bioactive constituents [1,2]. For growers, however, performance is not defined by yield alone; fruiting-body size, morphology, and chemical composition also influence crop quality and market value.

Fruiting in *A. bisporus* depends strongly on the casing layer placed over fully colonized compost. The casing supplies water, maintains pore space and gas exchange, supports surface mycelial development, and helps establish conditions favorable for primordium formation. Casing depth and water-supplying capacity have been linked with yield and dry matter content [3], while differences in casing physicochemical properties can alter fruiting-body weight, dimensions, and production pattern [4]. Peat remains widely used because of its water-holding capacity and structural stability, although interest in alternative materials and amendments has increased because of cost, availability, and environmental concerns [5]. The casing is also biologically active; recent work has shown that its microbial community changes during crop development and may contribute to mycelial growth and fruiting [6]. In Iraq, *A. bisporus* cultivation has been investigated in university and commercial mushroom facilities, and recent local research has emphasized casing management as a practical production variable. A University of Diyala dissertation evaluated casing-associated bacteria and microelements using materials from mushroom facilities in Iraq [7]. Related Iraqi work applied Fe, Zn, Mn, and Mo to the casing layer at graded concentrations and reported concentration-dependent changes in fruiting-body composition [8]. These studies support the local relevance of improving casing management while also showing that amendment responses depend on the material, dose, and cultivation setting. Accordingly, the present experiment provides facility-specific evidence from Diyala rather than an estimate of national production performance.

Activated charcoal is a potentially useful casing amendment because of its porous structure, large surface area, and capacity to adsorb organic compounds. Long and Jacobs [9] showed that activated charcoal could restore fruiting in sterilized casing, supporting the idea that adsorption of inhibitory compounds may contribute to primordium formation. Later work found that activated charcoal improved mushroom

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production in a grain-based system, although the response was less clear with conventional compost [10]. These observations suggest that its effect is likely to depend on the amount applied and on the physical and biological environment of the casing.

Zinc may influence mushroom growth through a different set of processes. It is an essential micronutrient that participates in the structure or catalytic activity of many proteins and enzymes [11]. Fungi regulate zinc uptake, transport, and storage closely because both deficiency and excess can disturb cellular function [12]. Mineral supplementation has also been used to alter the elemental composition of cultivated mushrooms. For example, Rzymski *et al.* [13] reported increased accumulation of supplemented zinc, selenium, and copper in *A. bisporus*, although the minerals in that study were added to the cultivation substrate rather than to the casing layer.

Applying zinc together with activated charcoal may create additional interactions within the casing. Activated carbon can adsorb Zn^{2+} from aqueous solution, and adsorption depends on the properties of the carbon and the surrounding chemical conditions [14]. In a peat-based casing, such binding could alter the local distribution or availability of zinc, but the behavior observed in aqueous systems cannot be assumed to occur in the same way within a heterogeneous casing matrix.

Previous work has established three separate points: casing properties influence fruiting and production [3–6], activated carbon can affect fruiting under some casing and substrate conditions [9,10], and zinc or other microelement supplementation can modify *A. bisporus* responses when applied through the substrate or casing [7,8,13]. What remains unresolved is whether the response to casing-applied zinc changes across activated-charcoal levels when both factors are evaluated factorially under the same crop conditions.

The primary objective was therefore to test the activated charcoal × zinc (C × Z) interaction. Secondary objectives were to quantify the main effects of activated charcoal and the zinc formulation on first-flush fruiting-body morphology, three-flush productivity and biological efficiency (BE), and selected fruiting-body chemical constituents (protein, phosphorus, potassium, and sodium). Zinc concentration in the fruiting bodies was not measured; therefore, the study was not designed to assess zinc uptake or biofortification.

2. MATERIALS AND METHODS

2.1. Experimental Site and Spawn Preparation

The experiment was conducted during the 2024–2025 growing season in a controlled mushroom cultivation facility in Diyala Governorate, Iraq. Before the experimental units were introduced, the room was cleaned, chemically disinfected, thoroughly dried, and ventilated. The commercial white strain A_{15} of *A. bisporus* (J.E. Lange) Imbach was obtained from Al-Wadaq Agricultural Investment Company (Mushroom Farm) and used throughout the experiment.

Grain spawn was prepared from wheat grains. The grains were boiled, drained to remove excess water, supplemented with calcium sulfate ($CaSO_4$) and calcium carbonate ($CaCO_3$), and dispensed at 250 g into 1-L glass flasks. The flasks were autoclaved at 121°C and 15 psi for 60 minutes, cooled, and inoculated with actively growing pieces of pure A_{15} mycelial culture. They were incubated at 25°C ± 2°C until the grains were fully colonized and were shaken periodically to promote uniform mycelial distribution [15].

2.2. Compost Preparation and Inoculation

Compost for *A. bisporus* production was prepared from approximately 900 kg wheat straw, 520 kg poultry manure, 70 kg agricultural gypsum, and 9 kg urea, providing approximately 1.5 t of initial solid ingredients. Composting was carried out with adaptation from the procedure described by Hassan *et al.* [16]. The ingredients were moistened and mixed thoroughly. Phase I composting continued for approximately 14 days, with turning every 3 days and water added as needed, and the material subsequently underwent Phase II pasteurization and conditioning. Temperature was raised to approximately 60°C for 6 hours and then reduced to 47°C –49°C; conditioning continued until ammonia was no longer detectable, after which the compost was cooled to approximately 25°C before inoculation [16,17]. Compost pH, total N, and C:N ratio were not determined in the present experiment.

The compost was packed into polyethylene bags measuring 60 × 40 × 20 cm, with 18 kg of wet compost per bag. Compost moisture at inoculation was 58%, determined from a representative sample of the complete compost batch before bag filling; therefore, the corresponding initial dry compost mass used for biological-efficiency calculation was 7.56 kg per experimental unit. Each bag represented one independent experimental unit. The compost was inoculated with A15 spawn at 2% (w/w), mixed thoroughly to distribute the spawn, and incubated at 25°C ± 2°C for approximately 3 weeks or until complete mycelial colonization.

2.3. Experimental Design and Treatment

The experiment was arranged as a 3 × 3 factorial in a completely randomized design, with three activated-charcoal levels (C) and three zinc-supplementation levels (Z). The nine treatment combinations were replicated three times, giving 27 biological experimental units. Each compost bag was the experimental unit. The 27 units were assigned completely at random to positions within the production room before treatment evaluation. After placement, the bags remained in their assigned positions throughout the experiment and were not relocated or rotated. Temperature, relative humidity, and ventilation were controlled automatically. Complete randomization was used to distribute any residual within-room spatial variation among treatments and reduce systematic positional bias.

Activated charcoal was incorporated into the casing material at 0, 10, and 20 g experimental unit^{-1} and designated C1, C2, C3, respectively. Each unit received approximately 4 kg of wet casing material; thus, the charcoal treatments corresponded approximately to 0, 2.5, and 5.0 g kg^{-1} wet casing. The zero control plus two evenly spaced non-zero additions provided a simple graded range for evaluating dose-related and factorial responses. Previous work showed that *A. bisporus* responses to activated carbon in casing can depend on inclusion rate, although the earlier concentration basis differed [10]. The assigned charcoal amount was mixed thoroughly with the casing material before casing application. Casing moisture was not measured, so rates are not expressed on a dry-casing basis. The activated-charcoal manufacturer/source, particle-size distribution, activation method, surface area, pH, and ash content were not retained in the experimental records; this limitation is acknowledged explicitly.

Zinc was supplied as DISPER Zn Sinergy 19 (EDEN Modern Agriculture S.L., Spain), a commercial formulation containing 19% water-soluble Zn, of which 15.1% was lignosulfonate-complexed and 3.9% ethylenediaminetetraacetic acid (EDTA)-chelated. Treatments supplied 0, 200, and 400 mg of elemental Zn experimental unit^{-1} and were designated Z1, Z2, and Z3, respectively. Based on approximately 4 kg wet casing per unit, these rates corresponded approximately to 0, 50, and 100 mg of elemental Zn kg^{-1} wet casing. The zero control and two evenly spaced non-zero rates were selected to provide a graded treatment range and test whether the response to the zinc formulation varied with activated-charcoal level. Iraqi casing-layer research has likewise used graded microelement concentrations, including Zn [7,8], although the

formulations and concentration bases were not directly equivalent. Three days after application of the casing layer, the zinc treatments were applied once as an irrigation to the casing. For Z2, 1.05 g of DISPER Zn Sinergy 19 was dissolved in 500 ml of irrigation water per experimental unit and distributed uniformly over the casing surface, supplying approximately 200 mg of elemental Zn experimental unit^{-1}. For Z3, 2.10 g of the product was dissolved in 500 ml of irrigation water and applied in the same manner, supplying approximately 400 mg of elemental Zn experimental unit^{-1}. Control units (Z1) received the same 500-ml volume of irrigation water without the product. Thus, the application volume was standardized at 500 ml per experimental unit across all zinc treatments. Because increasing DISPER Zn Sinergy 19 also increased its lignosulfonate and EDTA components, the experiment does not independently separate elemental-Zn effects from possible formulation-component effects.

2.4. Casing and Environmental Management

After complete colonization of the compost, each experimental unit was covered with approximately 4 kg of a ready-to-use commercial casing material supplied by Al-Wadaq Agricultural Investment Company (Mushroom Farm), applied to a depth of approximately 4 cm. During casing colonization, air temperature was maintained at 24°C–25°C and relative humidity above 90% [18]. Casing pH, electrical conductivity, moisture content, bulk density, water-holding capacity, porosity, microbial activity, and nutrient or zinc availability were not measured. The experiment therefore quantified mushroom-level agronomic and compositional responses rather than physicochemical or microbial mechanisms within the casing.

As the mycelium advanced through the casing and approached fruiting, air temperature was gradually lowered to 16°C–18°C and ventilation was increased to encourage primordium formation while avoiding direct airflow over the casing surface. Relative humidity was maintained at 85%–90% during primordium formation and fruiting. Irrigation was applied as a very fine mist, using equal and uniform water applications for all experimental units.

Fruiting bodies were harvested manually while fresh and at commercial maturity, before rupture of the partial veil beneath the cap. Fruiting-body number, average fruiting-body weight, total fresh yield, and BE were determined cumulatively over the first three flushes. Morphological measurements and samples for chemical analysis were obtained from the first flush.

2.5. Yield and Morphological Traits

2.5.1. Number of fruiting bodies

The cumulative number of fruiting bodies harvested from each experimental unit during the first three flushes was recorded and expressed as fruiting bodies experimental unit^{-1}.

2.5.2. Average fruiting-body weight

Average fruiting-body weight was calculated for each experimental unit by dividing cumulative fresh yield from the first three flushes by the corresponding cumulative fruiting-body number.

Average fruiting-body weight (g fruiting body^{-1}) = total fresh yield (g) / total number of fruiting bodies

2.5.3. Total yield

Total yield was the cumulative fresh weight of fruiting bodies harvested from each experimental unit during the first three flushes and was expressed as g experimental unit^{-1}.

2.5.4. Biological efficiency

Biological efficiency was calculated as cumulative fresh fruiting-body yield over the first three flushes relative to the initial dry weight of compost [19]. Compost moisture at inoculation was 58%, measured from a representative sample of the complete compost batch before bag filling. Because each experimental unit contained 18 kg wet compost, dry matter represented 42% of this mass and the initial dry compost weight was 7.56 kg (7560 g) per unit. All BE values were therefore recalculated from the cumulative yield of each experimental unit using the common dry-mass denominator.

BE (%) = [cumulative fresh fruiting-body yield (g) / 7560 g initial dry compost] × 100

2.5.5. Morphological traits

Five representative, freshly harvested fruiting bodies were selected randomly from the first flush of each experimental unit. Stipe length was measured from the base of the stipe to its point of attachment to the cap. Stipe diameter was measured at the midpoint of the stipe, cap diameter across the widest part of the cap, and cap thickness at the center (central crown region) of the cap. Measurements were made with a digital caliper with a resolution of 0.01 mm. The mean of the five fruiting bodies constituted the experimental-unit observation for each morphological trait.

2.6. Chemical Analysis of Fruiting Bodies

2.6.1. Sample preparation

Representative fruiting-body samples were collected from the first flush of each experimental unit. Samples were dried in a forced-air oven at 60°C to constant weight, ground, passed through a 0.5-mm sieve, and stored in airtight containers at 4°C until analysis [20].

2.6.2. Crude protein content

Total nitrogen was determined by the micro-Kjeldahl method. Crude protein was calculated using a nitrogen-to-protein conversion factor of 4.38 [21].

Crude protein (%) = total nitrogen (%) × 4.38

2.6.3. Preparation of digests for mineral analysis

A 0.2-g subsample of dried, ground fruiting-body material was transferred to a 250-ml digestion flask and subjected to tri-acid wet digestion using 5.0 ml of HNO_3 (65%–70%), 1.0 ml of H_2SO_4 (95%–98%), and 0.5 ml of $HClO_4$ (60%–72%), corresponding to a 10:2:1 (v/v/v) ratio, in a procedure adapted from Allen [22]. Digestion was carried out at 200°C–250°C. Rather than using a fixed digestion time, heating was continued until a clear, colorless digest was obtained. After cooling, the digest was quantitatively transferred and brought to 100 ml with distilled water. A reagent blank was processed in parallel through the digestion and measurement steps. All mineral determinations were performed in three analytical replicates for each experimental unit. The resulting digest was used for phosphorus, potassium, and sodium determinations. Zinc concentration in the casing or fruiting bodies was not determined; therefore, the study cannot be used to quantify zinc uptake or biofortification.

Aliquots of the resulting mineral digest were subsequently diluted according to the element being determined. Concentrations were expressed as mg/kg dry weight after correction for the applicable

dilution factor, the 100-ml digest volume and the original dry sample mass (0.2 g).

2.6.4. Phosphorus content

Phosphorus was measured by the ascorbic acid–molybdenum blue colorimetric method, following Olsen and Sommers [23]. The mineral digest was diluted twofold. A 5.0-mL aliquot of the diluted digest was transferred to a 50-mL volumetric flask, and 1–2 drops of 0.25% p-nitrophenol indicator were added. The medium was adjusted with 4 N NaOH as needed, followed by 5 N H2SO4 until the yellow indicator color disappeared (approximately pH 5). Freshly prepared reagent B (4.0 ml) was added, and the mixture was made up to 50 ml with distilled water, mixed, and allowed to develop the characteristic blue color. Absorbance was determined at 882 nm with a Model 721 spectrophotometer (China), using a reagent blank.

Reagent A was prepared by dissolving 12 g of ammonium molybdate in 250 ml of distilled water and 0.2908 g of potassium antimonyl tartrate in 100 ml of distilled water, combining the two solutions with 1 L of 5 N H2SO4, and making up to 2 L with distilled water. Reagent B was prepared by dissolving 1.056 g of ascorbic acid in 200 ml of reagent A and used within 24 h. The indicator solution contained 0.25 g of p-nitrophenol per 100 ml, and 4 N NaOH was prepared using 16 g per 100 ml.

Phosphorus working standards of 0, 2, 4, 6, 8 and 10 mg/l were subjected to the same color-development procedure as the samples. The calibration concentrations referred to the working standards before color development. With a twofold preliminary dilution, phosphorus was calculated as P (mg/kg DW) = Cworking × 1,000, where Cworking is the blank-corrected concentration in the diluted digest expressed by the calibration curve (mg/l). Equivalently, using the concentration in the final 50-mL colored solution, P (mg/kg DW) = Cfinal × 10,000. The two expressions are alternatives and are not applied together.

2.6.5. Potassium and sodium contents

Potassium and sodium were determined by flame photometry using a Jenway PFP7 instrument according to Al-Tamimi [24]. For potassium determination, the mineral digest was diluted tenfold before measurement (DF = 10), and calibration standards of 0, 2, 4, 6, 8 and 10 mg/l were used. Sodium was calibrated with standards of 0, 10, 20, 30 and 40 mg/l. A reagent blank was included in the analytical sequence. Potassium concentration on a dry-weight basis was calculated as K (mg/kg DW) = Ck × 10 × 0.100/0.0002 = Ck × 5,000, where Ck is the blank-corrected concentration (mg/l) in the diluted measurement solution.

2.7. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) for a 3 × 3 factorial experiment arranged in a completely randomized design. The model included the main effects of activated charcoal (C) and zinc supplementation (Z) and their C × Z interaction. The experiment comprised nine treatment combinations with three replicates per combination; each bag was an independent experimental unit. For morphological traits, the mean of the five fruiting bodies sampled from the first flush of each bag constituted the experimental-unit observation. The ANOVA degrees of freedom were 2 for C, 2 for Z, 4 for C × Z, 18 for residual error, and 26 total.

When the factorial ANOVA indicated a significant effect at $p \leq 0.05$, means for that effect were separated using Duncan's multiple range test (DMRT) at the same probability level [25]. Statistical analyses were

Table 1. Effects of activated charcoal (C), zinc (Z), and their interaction on morphological traits of first-flush *A. bisporus* fruiting bodies.

Activated charcoal (C)	Zinc (Z)	Stipe length (cm)	Stipe diameter (cm)	Cap diameter (cm)	Cap thickness (cm)
Interaction (C × Z)					
C1	Z1	2.71	1.72	4.48	2.46
C1	Z2	2.85	1.98	4.78	2.81
C1	Z3	2.61	2.36	4.61	3.22
C2	Z1	3.00	2.56	5.96	3.05
C2	Z2	3.00	2.20	5.48	2.83
C2	Z3	2.73	2.16	5.10	3.02
C3	Z1	3.14	1.65	4.53	2.65
C3	Z2	3.94	2.03	6.26	2.76
C3	Z3	3.93	1.42	4.98	2.44
Main effect of activated charcoal (C)					
C1		2.72 b	2.02 ab	4.63 b	2.83
C2		2.91 b	2.30 a	5.51 a	2.97
C3		3.67 a	1.70 b	5.25 a	2.62
Main effect of zinc (Z)					
	Z1	2.95	1.97	4.99	2.72
	Z2	3.26	2.07	5.51	2.80
	Z3	3.09	1.98	4.90	2.89
Pooled SEM (interaction means)		0.36	0.25	0.35	0.24
Pooled SEM (main-effect means)		0.21	0.14	0.20	0.14

Means: interaction $n = 3$; main effects $n = 9$. Letters are shown only for significant factorial ANOVA effects ($p \leq 0.05$). Within a column and section, means sharing a letter do not differ by DMRT at $p \leq 0.05$; unlettered sections were nonsignificant. SEM values are pooled from residual MS.

Table 2. Effects of activated charcoal (C), zinc (Z), and their interaction on yield and productivity traits accumulated over the first three flushes.

Activated charcoal (C)	Zinc (Z)	Number of fruiting bodies (bag^{-1})	Average fruiting-body weight (g fruiting body^{-1})	Total yield (g bag^{-1})	Biological efficiency (%)
Interaction (C × Z)					
C1	Z1	161.67 bcd	27.75 b	4478.1	59.23
C1	Z2	177.33 bc	26.59 bc	4690.1	62.04
C1	Z3	191.67 bc	26.55 bc	5064.5	66.99
C2	Z1	159.00 cd	29.24 b	4608.4	60.96
C2	Z2	207.00 b	26.53 bc	5480.7	72.50
C2	Z3	172.00 bcd	28.09 b	4824.4	63.81
C3	Z1	170.67 bcd	27.30 bc	4640.8	61.39
C3	Z2	131.00 d	37.20 a	4836.0	63.97
C3	Z3	248.33 a	24.19 c	6006.8	79.46
Main effect of activated charcoal (C)					
C1		176.89	26.96 b	4744.2	62.75
C2		179.33	27.96 ab	4971.1	65.76
C3		183.33	29.56 a	5161.2	68.27
Main effect of zinc (Z)					
	Z1	163.78 b	28.10 b	4575.7 b	60.53 b
	Z2	171.78 b	30.11 a	5002.3 ab	66.17 ab
	Z3	204.00 a	26.28 c	5298.6 a	70.09 a
Pooled SEM (interaction means)		13.87	0.96	292.9	3.87
Pooled SEM (main-effect means)		8.01	0.55	169.1	2.24

Means: interaction $n = 3$; main effects $n = 9$. Productivity traits cover the first three flushes; BE uses 7.56 kg initial dry compost per unit. Letters are shown only for significant factorial ANOVA effects ($p \leq 0.05$); unlettered sections were nonsignificant. SEM values are pooled from residual MS.

Table 3. Effects of activated charcoal (C), zinc (Z), and their interaction on first-flush fruiting-body chemical composition.

Activated charcoal (C)	Zinc (Z)	Protein (%)	Phosphorus (mg kg^{-1} DW)	Potassium (mg kg^{-1} DW)	Sodium (mg kg^{-1} DW)
Interaction (C × Z)					
C1	Z1	18.85 c	4327.0 bcd	25707	4230.8 abc
C1	Z2	21.90 b	5098.3 ab	28851	3643.2 c
C1	Z3	21.43 b	5635.2 a	27230	4209.4 abc
C2	Z1	18.73 c	3805.2 d	29393	4700.9 ab
C2	Z2	24.73 a	5429.7 a	28563	5000.0 a
C2	Z3	22.40 b	4916.3 abc	26504	3782.1 bc
C3	Z1	18.92 c	5279.5 a	28236	3803.4 bc
C3	Z2	21.29 b	4058.7 cd	26304	4145.3 abc
C3	Z3	22.77 b	5792.7 a	27276	4326.9 abc
Main effect of activated charcoal (C)					
C1		20.72	5020.2	27262	4027.8
C2		21.95	4717.1	28153	4494.3
C3		21.00	5043.7	27272	4091.9
Main effect of zinc (Z)					
	Z1	18.83 b	4470.6 b	27778	4245.0
	Z2	22.64 a	4862.2 b	27906	4262.8
	Z3	22.20 a	5448.1 a	27003	4106.1
Pooled SEM (interaction means)		0.62	286.9	1259.6	305.7
Pooled SEM (main-effect means)		0.36	165.6	727.2	176.5

Means: interaction $n = 3$; main effects $n = 9$. Mineral data are on a dry-weight basis. Letters are shown only for significant factorial ANOVA effects ($p \leq 0.05$); unlettered sections were nonsignificant. SEM values are pooled from residual MS.

Table 4. Factorial ANOVA summary for morphological, productivity, and chemical traits of *A. bisporus*.

Trait	Activated charcoal (C) F (P)	Zinc (Z) F (P)	C × Z F (P)	Residual MS	CV (%)
Stipe length	5.82 (0.0112)	0.57 (0.5763)	0.68 (0.6164)	0.39296	20.22
Stipe diameter	4.58 (0.0246)	0.15 (0.8642)	1.99 (0.1394)	0.18024	21.15
Cap diameter	5.01 (0.0187)	2.61 (0.1013)	2.75 (0.0606)	0.37466	11.93
Cap thickness	1.57 (0.2344)	0.38 (0.6906)	1.37 (0.2838)	0.17923	15.10
Fruiting-body number	0.17 (0.8492)	7.07 (0.0054)	7.91 (0.0007)	577.296	13.36
Average fruiting-body weight	5.66 (0.0124)	12.02 (0.0005)	20.44 (<0.0001)	2.74810	5.89
Total yield	1.52 (0.2447)	4.62 (0.0241)	2.59 (0.0718)	257429.6	10.23
Biological efficiency	1.52 (0.2447)	4.62 (0.0241)	2.59 (0.0718)	45.04170	10.23
Protein	3.27 (0.0616)	33.93 (<0.0001)	3.41 (0.0306)	1.15025	5.05
Phosphorus	1.21 (0.3215)	8.82 (0.0021)	7.22 (0.0012)	246950.7	10.09
Potassium	0.50 (0.6176)	0.45 (0.6436)	1.54 (0.2317)	4759714.7	7.92
Sodium	2.05 (0.1574)	0.24 (0.7920)	3.01 (0.0459)	280410.7	12.59

Factorial ANOVA degrees of freedom: C = 2, Z = 2, C × Z = 4, residual error = 18, total = 26. *P* values are shown in parentheses. Biological efficiency was recalculated from cumulative fresh yield using 7.56 kg initial dry compost per experimental unit; because this is a constant linear transformation of yield, yield and BE have identical F and P values. CV = coefficient of variation.

performed using SAS software (SAS Institute Inc., Cary, NC, USA). Replicate-level data were rechecked during revision, and the factorial ANOVA was recalculated for all measured traits. Table 4 reports F values and P values for C, Z, and C × Z together with residual mean square and coefficient of variation (CV). Pooled SEM values derived from the residual mean square are reported in Tables 1–3.

3. RESULTS

3.1. Morphological Traits of Fruiting Bodies

First-flush morphology showed a selective response to activated charcoal (Table 1). Activated charcoal significantly affected stipe length ($p = 0.0112$), stipe diameter ($p = 0.0246$), and cap diameter ($p = 0.0187$), whereas cap thickness was unaffected. C3 produced the longest stipes, while C2 and C3 had larger cap diameters than C1; stipe diameter was greatest at C2 and lowest at C3. Zinc had no significant main effect on any morphological trait.

No C × Z interaction reached $p \leq 0.05$ for the four morphological traits (stipe length $p = 0.6164$, stipe diameter $p = 0.1394$, cap diameter $p = 0.0606$, and cap thickness $p = 0.2838$). Although C3Z2 had the largest numerical cap diameter and C3Z2 and C3Z3 showed the greatest numerical stipe lengths, these combination-level differences are not interpreted as significant factorial interactions.

3.2. Yield and Productivity Traits

Yield and productivity responses accumulated over the first three flushes differed among traits (Table 2). Activated charcoal significantly affected average fruiting-body weight ($p = 0.0124$) but not fruiting-body number, total yield, or biological efficiency. Zinc significantly affected fruiting-body number ($p = 0.0054$), average fruiting-body weight ($p = 0.0005$), total yield ($p = 0.0241$), and biological efficiency ($p = 0.0241$). Because BE was calculated from yield using the same 7.56-kg dry-compost denominator for every unit, its factorial F and *p* values are identical to those for total yield.

Across zinc levels, Z3 produced the highest fruiting-body number, total yield, and biological efficiency, whereas average fruiting-body weight peaked at Z2 and declined at Z3. Relative to Z1, Z3 increased both total yield and biological efficiency by 15.8%, reflecting their direct proportional relationship. The higher zinc-formulation rate therefore favored cumulative productivity and fruiting-body number rather than maximum individual mass.

C × Z interactions were significant for fruiting-body number ($p = 0.0007$) and average fruiting-body weight ($p < 0.0001$), but not for total yield or biological efficiency ($p = 0.0718$ for both). C3Z3 produced the highest fruiting-body number and the highest numerical total yield and BE (6006.8 g bag^{-1} and 79.46%, respectively), whereas C3Z2 produced the highest average fruiting-body weight (37.20 g) but the lowest fruiting-body number. Thus, the strongest interaction evidence concerned the allocation between fruiting-body number and individual mass, not cumulative yield or BE.

3.3. Chemical and Mineral Composition

First-flush chemical composition responded selectively to the treatments (Table 3). Activated charcoal had no significant main effect on protein, phosphorus, potassium, or sodium. The zinc formulation significantly affected protein ($p < 0.0001$) and phosphorus ($p = 0.0021$), but not potassium or sodium.

Protein was highest at Z2 and remained statistically similar at Z3, whereas Z1 had the lowest mean. Phosphorus increased to its highest mean at Z3, approximately 21.9% above Z1. The absence of significant activated-charcoal main effects indicates that compositional responses were driven more by the zinc-formulation factor and specific treatment combinations than by a consistent charcoal response.

Significant C × Z interactions occurred for protein ($p = 0.0306$), phosphorus ($p = 0.0012$), and sodium ($p = 0.0459$). C2Z2 produced the highest protein content (24.73%), whereas C3Z3 produced the highest phosphorus concentration (5792.7 mg kg^{-1} DW). Potassium showed no significant main or interaction effect. Sodium responded only to the interaction, indicating a combination-specific pattern rather than a consistent main-factor response.

4. DISCUSSION

The factorial reanalysis shows that treatment interactions were trait-specific rather than universal. Significant C × Z interactions were detected for fruiting-body number, average fruiting-body weight, protein, phosphorus, and sodium, whereas total yield, biological efficiency, and the measured morphological traits did not show

significant interactions at $p \leq 0.05$. This more precisely addresses the study gap: the response to the two casing amendments depended on their combination for selected crop components and compositional traits, but not for every outcome. Mechanistic interpretation must remain cautious because casing pH, electrical conductivity, water-holding capacity, microbial activity, nutrient status, and zinc availability were not measured, and the commercial zinc source also supplied lignosulfonate and EDTA.

4.1. Morphological Traits of Fruiting Bodies

The morphological pattern was associated primarily with activated charcoal rather than with zinc or a factorial interaction. This broadly agrees with evidence that casing characteristics can influence fruiting-body dimensions [4,5], but the non-linear response prevents a simple conclusion that increasing charcoal consistently improves morphology. Activated carbon has a porous adsorptive structure and has affected fruiting in previous casing experiments [9,10]; however, water-holding capacity, porosity, pH, electrical conductivity, and adsorption were not measured here. The observed morphological differences should therefore be treated as amendment-associated responses rather than evidence of change in a specific casing property.

Previous studies have linked C8 volatile compounds and casing-associated microorganisms with primordium formation in *A. bisporus*, while a dedicated review has summarized critical factors involved in primordia development [26–29]. Those findings provide biological context for why casing amendments can influence fruiting, but they do not establish the mechanism operating here because microbial activity, community structure, and volatile compounds were not quantified in this experiment.

Zinc is required for numerous fungal proteins and enzymes and is subject to regulated uptake, transport, and storage [11,12]. The absence of significant zinc main effects on morphology indicates no consistent morphological response across the tested formulation rates. Likewise, because none of the morphological C × Z interactions reached $p \leq 0.05$, the numerical combination differences in cap or stipe dimensions should not be interpreted as evidence that activated charcoal modified zinc availability.

Where significant C × Z interactions did occur in this experiment, they are best interpreted as statistical evidence that the response to one treatment factor depended on the level of the other, not as proof that activated charcoal altered zinc availability. Direct testing of an adsorption or availability mechanism would require treatment-level measurements of available zinc in the casing and zinc concentration in harvested fruiting bodies.

4.2. Yield and Productivity Traits

The yield results distinguish effects on fruiting-body number and individual mass from effects on cumulative crop productivity. Activated charcoal affected average fruiting-body weight but not fruiting-body number, total yield, or BE as a main factor. Zinc significantly affected all four productivity traits. The significant C × Z interactions for fruiting-body number and average weight, combined with nonsignificant interactions for total yield and BE, show that treatment combinations altered how productivity was partitioned among fruiting bodies more clearly than they altered cumulative crop mass.

The zinc formulation treatment produced the broadest productivity response, with Z3 increasing fruiting-body number, total yield, and BE relative to Z1, while Z2 maximized individual mass. This is directionally consistent with Iraqi work showing concentration-dependent responses to casing-applied microelements, including zinc [7,8], although the formulations, concentration bases, response variables, and cultivation conditions differ. The present data therefore support a graded treatment response but not a universal monotonic effect across all productivity components.

The contrasting number-versus-mass response is biologically compatible with competition among developing fruiting bodies for water and assimilates: a larger number of sinks may limit mass attained per fruiting body, whereas fewer sinks may permit greater individual mass. This interpretation is consistent with the significant interaction patterns for number and individual weight, but it was not tested directly because primordium survival, water flux, and assimilate partitioning were not measured.

Activated carbon can adsorb compounds associated with inhibition of fruiting [9,26], whereas zinc has established metabolic roles in fungi [11,12]. These properties provide physiological context, but they do not establish the mechanism responsible for the observed treatment responses. In particular, the nonsignificant C × Z interactions for total yield and BE should not be described as evidence of an interaction mechanism affecting cumulative productivity. Measurements of casing chemistry, water relations, microbial activity, volatile compounds, and zinc availability would be required for such inference.

Rzymski *et al.* [13] demonstrated that *A. bisporus* can accumulate supplemented trace elements when those elements are measured directly in harvested fruiting bodies. The present experiment used a different application site and did not determine tissue zinc. Consequently, the productivity response observed here cannot be interpreted as evidence of zinc uptake or accumulation, and differences from substrate-supplementation studies may also reflect strain, zinc formulation, compost composition, and crop management.

4.3. Chemical and Mineral Composition

The compositional response was selective rather than uniform. Activated charcoal showed no significant main effect on any of the four measured chemical constituents, whereas the zinc formulation significantly affected protein and phosphorus. Significant C × Z interactions for protein, phosphorus, and sodium indicate combination-dependent responses for specific constituents, but the unchanged potassium response shows that supplementation did not produce a generalized mineral increase.

Zinc has structural and catalytic roles in many fungal proteins and enzymes [11,12], providing physiological context for the significant association between zinc-formulation level and protein concentration. However, the commercial product also supplied lignosulfonate and EDTA, and tissue zinc was not determined. The protein response therefore cannot be attributed specifically to increased elemental-zinc uptake. The significant C × Z interaction in protein similarly establishes a treatment-combination response, not a demonstrated zinc-uptake mechanism.

Although zinc enrichment of *A. bisporus* is achievable under some cultivation conditions [13], demonstrating biofortification requires direct measurement of zinc in edible tissue. Because that measurement was not included here, the present results should not be used to infer zinc accumulation, enhanced dietary zinc value, or biofortification.

The phosphorus response indicates an association between zinc treatment and phosphorus concentration in the harvested tissue,

but it does not demonstrate a zinc-phosphorus synergy. Such a mechanism would require measurements of available phosphorus, zinc availability, relevant enzymatic activity, and tissue zinc. The present data therefore support only a treatment-associated change in phosphorus concentration.

The relative stability of potassium and the interaction-specific response of sodium further emphasize that mineral responses were element-specific. Because ionic conditions and electrical conductivity of the casing were not characterized, the basis of the sodium interaction remains unresolved and should be regarded as a compositional response rather than as evidence of improved mineral nutrition.

4.4. Overall Implications and Study Limitations

The practical implication is that no amendment combination can be described as universally optimal across all measured traits. The findings should be generalized cautiously. The experiment used one A15 strain, one cultivation facility, one 2024–2025 crop cycle, and three biological replicates per treatment. Morphology and chemical composition were sampled only from the first flush, whereas yield and BE covered the first three flushes. Although the experimental units were completely randomized and environmental control was automated, positions remained fixed and within-room gradients were not quantified. Casing mass was recorded only approximately on a wet basis, detailed casing physicochemical properties were not measured, activated-charcoal specifications were incomplete, and the commercial zinc source also supplied lignosulfonate and EDTA. Casing pH, electrical conductivity, water-holding capacity, porosity, microbial activity, volatile compounds, nutrient availability, and tissue zinc were not measured.

Future studies should validate these responses across independent crop cycles, compost batches, facilities, and *A. bisporus* strains. They should determine casing moisture and dry mass precisely, characterize activated charcoal, quantify compost and casing physicochemical properties before and after amendment, assess microbial and nutrient dynamics, and directly determine zinc in the casing and fruiting bodies. These measurements would allow treatment effects to be separated from formulation effects and would clarify whether adsorption, nutrient availability, or zinc uptake contributes to the observed crop responses.

5. CONCLUSION

Activated charcoal and zinc supplementation in the casing layer altered *A. bisporus* performance, but the statistical response depended on the trait. Activated charcoal significantly affected selected morphological traits and average fruiting-body weight, whereas the zinc formulation significantly affected fruiting-body number, average weight, total yield, biological efficiency, protein, and phosphorus. C × Z interactions were significant for fruiting-body number, average weight, protein, phosphorus, and sodium, but not for total yield, BE, or morphology. C3Z3 had the highest fruiting-body number and the highest numerical total yield and BE (79.46%), while C3Z2 favored individual fruiting-body mass and C2Z2 protein content. These results support trait-specific treatment selection rather than a universal amendment rate. Because tissue zinc and casing physicochemical or microbial mechanisms were not measured, no conclusion about zinc uptake or biofortification is warranted, and commercial recommendations require validation across additional crop cycles, facilities, and strains.

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7. CONFLICTS OF INTEREST

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9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship.

11. DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

AI-assisted technology was used only to support language editing and manuscript formatting. The authors reviewed and verified the scientific content, data, statistical analyses, references, and interpretation and take full responsibility for the final manuscript. No research data or images were generated or altered using AI.

REFERENCES

1. Royse DJ, Baars JJP, Tan Q. Current overview of mushroom production in the world. In: Zied DC, Pardo-Giménez A (eds.)., Edible and medicinal mushrooms: technology and applications, : John Wiley & Sons, Chichester, UK, p 5, 2017; doi: https://doi.org/10.1002/9781119149446.ch2
2. Dissemond MH, Franken CE, Sari M. *Agaricus bisporus* grown on sustainable peat casing alternatives: a systematic review on quality characteristics. Foods 2025;14(19):3348; doi: https://doi.org/10.3390/foods14193348
3. Kalberer PP. Influence of the depth of the casing layer on the water extraction from casing soil and substrate by the sporophores, on the yield and on the dry matter content of the fruit bodies of the first three flushes of the cultivated mushroom, *Agaricus bisporus*. Sci Hortic 1985;27(1-2):33–43; doi: https://doi.org/10.1016/0304-4238(85)90052-4
4. Pardo A, Pardo JE, De Juan JA, Zied DC. Modelling the effect of the physical and chemical characteristics of the materials used as casing layers on the production parameters of *Agaricus bisporus*. Arch Microbiol 2010;192(12):1023–30; doi: https://doi.org/10.1007/s00203-010-0631-3
5. Young G, Grogan H, Walsh L, Noble R, Tracy S, Schmidt O. Peat alternative casing materials for the cultivation of *Agaricus bisporus* mushrooms: a systematic review. Cleaner Circular Bioeconomy 2024;9:100100; doi: https://doi.org/10.1016/j.clcb.2024.100100
6. Li H, Xi Z, Zhong H, Yan X, Mo T, Chen Q, *et al.* Integrative analysis reveals casing layer dynamics during *Agaricus bisporus* cultivation and the growth-promoting effect of *Pseudomonas putida* AT130. Food Biosci 2026;78:108429; doi: https://doi.org/10.1016/j.fbio.2026.108429

7. Mustaf KI. Effect of adding some bacterial strains and microelements to casing layer on production, quality, and storage characteristics of the edible mushroom *Agaricus bisporus* [doctoral dissertation]. College of Agriculture, University of Diyala, Diyala, Iraq, 2023.
8. Hammad HS, Hassan AA, Mustaf KI. Impact of the microelements supplemented in the casing layer on the chemical constituents of the edible mushroom *Agaricus bisporus*. Eur Scholar J 2023;4(10):80–5.
9. Long PE, Jacobs L. Aseptic fruiting of the cultivated mushroom, *Agaricus bisporus*. Trans Br Mycol Soc 1974;63(1):99–107; doi: https://doi.org/10.1016/S0007-1536(74)80140-3
10. Bechara MA, Heinemann PH, Walker PN, Demirci A, Romaine CP. Evaluating the addition of activated carbon to heat-treated mushroom casing for grain-based and compost-based substrates. Bioresour Technol 2009;100(19):4441–6; doi: https://doi.org/10.1016/j.biortech.2008.12.064
11. Eide DJ. Transcription factors and transporters in zinc homeostasis: lessons learned from fungi. Crit Rev Biochem Mol Biol 2020;55(1):88–110; doi: https://doi.org/10.1080/10409238.2020.1742092
12. Soares LW, Bailão AM, Soares CM de A, Bailão MGS. Zinc at the host-fungus interface: how to uptake the metal?. J Fungi 2020;6(4):305; doi: https://doi.org/10.3390/jof6040305
13. Rzymski P, Mleczek M, Niedzielski P, Siwulski M, Gąsecka M. Cultivation of *Agaricus bisporus* enriched with selenium, zinc and copper. J Sci Food Agric 2017;97(3):923–8; doi: https://doi.org/10.1002/jsfa.7816
14. Leyva Ramos R. Adsorption of zinc (II) from an aqueous solution onto activated carbon. J Hazard Mater 2002;90(1):27–38; doi: https://doi.org/10.1016/S0304-3894(01)00333-8
15. Moreaux K. Spawn production. In: Zied DC, Pardo-Giménez A (eds.). Edible and medicinal mushrooms: technology and applications. John Wiley & Sons, Chichester, UK, p 89, 2017; doi: https://doi.org/10.1002/9781119149446.ch5
16. Hassan AA, Natheer AM, Mahmoud AR. Improvement of agronomic characters and productivity of *Agaricus bisporus* Lange (Imbach) using some organic sources. Iraqi J Agriculture 2002;7(3):104–12.
17. Buth J. Compost as a food base for *Agaricus bisporus*. In: Zied DC, Pardo-Giménez A (eds). Edible and medicinal mushrooms: technology and applications. John Wiley & Sons, Chichester, UK, p 129, 2017; doi: https://doi.org/10.1002/9781119149446.ch6
18. Pardo-Giménez A, Pardo González JE, Zied DC. Casing materials and techniques in *Agaricus bisporus* cultivation. In: Zied DC, Pardo-Giménez A (eds.). Edible and medicinal mushrooms: technology and applications. John Wiley & Sons, Chichester, UK, p 149, 2017; doi: https://doi.org/10.1002/9781119149446.ch7
19. Sánchez JE, Royse DJ. Adapting substrate formulas used for shiitake for production of brown *Agaricus bisporus*. Bioresour Technol 2001;77(1):65–9; doi: https://doi.org/10.1016/S0960-8524(00)00130-9
20. Dündar A, Acay H, Yildiz A. Yield performances and nutritional contents of three oyster mushroom species cultivated on wheat stalk. Afr J Biotechnol 2008;7(19):3497–501; doi: https://doi.org/10.5897/AJB08.594
21. Bąkowski J, Kosson R, Horbowicz M. Nutritional values of different strains of mushrooms (*Agaricus bisporus*). Acta Agrobot 1986;39(1):111–21; doi: https://doi.org/10.5586/aa.1986.010
22. Allen SE, editor. Chemical analysis of ecological materials. 2nd ed. Blackwell Scientific Publications, Oxford, UK, 1989.
23. Olsen SR, Sommers LE. Phosphorus. In: Page AL, Miller RH, Keeney DR (eds.). Methods of soil analysis. Part 2: chemical and microbiological properties. 2nd ed. American Society of Agronomy and Soil Science Society of America, Madison, WI, p 403, 1982; doi: https://doi.org/10.2134/agronmonogr9.2.2ed.c24
24. Al-Tamimi RAK. Chemical analysis of soil, water and plant: principles and applications [in Arabic]. University of Anbar, Anbar, Iraq, 2016.
25. Duncan DB. Multiple range and multiple F tests. Biometrics 1955;11(1):1–42; doi: https://doi.org/10.2307/3001478
26. Noble R, Dobrovin-Pennington A, Hobbs PJ, Pederby J, Rodger A. Volatile C8 compounds and pseudomonads influence primordium formation of *Agaricus bisporus*. Mycologia 2009;101(5):583–91; doi: https://doi.org/10.3852/07-194
27. Carrasco J, Preston GM. Growing edible mushrooms: a conversation between bacteria and fungi. Environ Microbiol 2020;22(3):858–72; doi: https://doi.org/10.1111/1462-2920.14765
28. Wang YH, Yang XY, Wan LZ, Ren HX, Qu L, Guo HD, *et al.* Influence of the casing layer on the specific volatile compounds and microorganisms by *Agaricus bisporus*. Front Microbiol 2023;14:1154903; doi: https://doi.org/10.3389/fmicb.2023.1154903
29. Baars JJP, Scholtmeijer K, Sonnenberg ASM, Van Peer A. Critical factors involved in primordia building in *Agaricus bisporus*: a review. Molecules 2020;25(13):2984; doi: https://doi.org/10.3390/molecules25132984

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