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Ecological Enhancement of *Agaricus bisporus* L. Mushrooms' Vitamin Content Using Carbon Nanotubes, Magnetic Iron Nanoparticles, and Biostimulants

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ABSTRACT

The edible mushroom *Agaricus bisporus* L. plays a crucial ecological role in nutrient cycling and organic matter decomposition, alongside its increasing importance in the food and nutrition industry. This study explored ecological interventions to enhance the mushroom's vitamin content by enriching its cultivation substrate with nanomaterials and biostimulatory agents. The experiment was conducted within the mushroom production project at Al-Qadisiyah Governorate, Iraq. The compost-based medium was amended with magnetic iron nanoparticles (N-FeO), carbon nanotube (CNT) suspensions, EM biofertilizer, and Atonik growth stimulant. Their ecological impact on the enrichment of fat-soluble (A, D, E) and water-soluble (B2, B3, B5, B6) vitamins in mushrooms was assessed. The study employed a Completely Randomized Design (CRD) with three replicates. Results revealed that the synergistic application of these eco-friendly treatments significantly enhanced the vitamin profiles of *A. bisporus*. The highest concentrations of vitamins B2 and B5 (5.16 and 17.70 mg kg^{-1}, respectively) and vitamin A (6.87 IU ml^{-1}) were recorded under the combined quadruple treatment. Additionally, the triple treatment (N-FeO + EM + Atonik) notably increased levels of vitamins B2 (4.47 mg kg^{-1}), B6 (25.66 mg kg^{-1}), D (34.76 mg kg^{-1}), and vitamin A (6.87 IU ml^{-1}). Dual treatments (EM + Atonik) also significantly improved vitamin B2 (4.54 mg kg^{-1}) and vitamin E (3.30 mg kg^{-1}) contents. These findings demonstrate that integrating nanomaterials and biostimulants can serve as an ecological strategy to improve the nutritional quality of

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mushrooms while promoting sustainable agricultural practices.

Keywords: *Agaricus bisporus* L.; Ecological Cultivation; EM Biofertilizer; Iron Nanoparticles; Carbon Nanotubes; Sustainable Agriculture; Water-Soluble Vitamins; Fat-Soluble Vitamins

1. Introduction

The production of the cultivated mushroom, *Agaricus bisporus* L., is of agricultural interest due to its nutritional value. According to the FAO, edible mushroom production rose from 30 to 2 million tons worldwide in 2010 to 48 million tons in 2017. As a leading producer, China increased its mushroom production from 22.6 million tons in 2010 to 38.4 million tons in 2017, representing 75% of global production. Of the 16,000 known species of mushrooms, approximately 7,000 have varying degrees of edibility. Over 3,000 edible mushroom species and 700 species are considered safe medicinal mushrooms [1–4].The global food system is increasingly threatened by challenges such as climate change, water scarcity, and land degradation, highlighting the urgent need to explore alternative, sustainable. These challenges require seeking non-traditional food sources for a healthy and sustainable diet [5]. In this context, mushrooms are considered a healthy food source that has not yet been fully exploited. The classification of edible mushrooms as a healthy and sustainable food source for humans depends primarily on their species, chemical composition, and growing conditions [6]. Thirty-five species of mushrooms are grown commercially. Edible mushrooms are gaining increasing importance for their health benefits, playing a pivotal role in supporting the immune system, combating cardiovascular disease, and preventing the risk of cancer [7–9]. Mushrooms, whether consumed as whole foods or extracts, offer a valuable combination of essential nutrients, including proteins, dietary fiber, vitamins, minerals, and beneficial fatty acids, making them a promising component of a balanced diet. Furthermore, mushrooms contain numerous active compounds, such as polysaccharides and phenols, which possess important bioactive properties, providing essential nutrients for boosting the immune system and preventing life-threatening diseases [10].

Vitamins are classified according to their solubility in liquids into fat-soluble vitamins, including A, D, E, and K, and water-soluble vitamins, including B-complex and C [11]. Vitamins also participate in normal metabolic processes and regulate cell function and metabolism. They are essential for growth and development and are chemicals the body requires to maintain good health [12]. Growth and productivity can be improved by enriching the culture medium of *A. bisporus* to increase the productive qualities and nutritional value of the mushroom's bioactive compounds. [13] demonstrated through their studies that nanotechnology improves agricultural production's quality and nutritional properties and increases the efficiency of nutrient and fertilizer use. It may meet growing market needs and enhance food security at the local and global levels, as it is a promising technology characterized by its high ability to target and highly efficient nutrient delivery intelligently.

Carbon nanotubes are a promising and sustainable technology for increasing both quantitative and qualitative production in the agricultural sector, given their impact on regulating the growth of living tissue, their ability to penetrate cell walls, and their rapid transport and assimilation. Carbon nanotubes have received significant attention in agricultural applications compared to other nanomaterials. Given their ability to act as innovative nutrient delivery platforms with slow release and as bio-growth stimulants, carbon nanotubes are considered a novel nutrient for enhancing the growth environment of fungi and improving the quantity and quality of production of *A. bisporus* [14].

Nanomagnetic iron oxide is an innovative and important nanomaterial with unique magnetic and nanoscale properties that are interesting for agriculture. It is a promising solution for sustainable agriculture, improving agricultural production in efficient and environmentally friendly ways. It improves the structure of the culture medium and increases its ability to retain water and essential nutrients for growth and production. Nanoscale magnetic iron oxide can be an enzyme activator to enhance growth parameters and precisely control biochemical reactions in living tissue [15]. Biostimulants have positive effects, as they promote the growth and development of living tissue, increase enzyme activity, and enhance nitrogen uptake [16, 17]. Using biostimulants under unfavorable conditions leads to tolerance to abiotic stress in tissues and metabolic sites[18]. With the growing awareness of safe food, research into the potential of mushroom production using effective microorganisms (Ems) as growth promoters and preventative protection against disease is of great importance. Effective organisms are an example of a complementary compound that can be used in mushroom production. EMs are defined as beneficial strains of effective organisms that provide natural benefits when applied as fertilizer to the ecosystem of a food fungus culture medium. They are an additive to increase microbial diversity in the substrate[19]. In addition, the use of fungal organisms improves soil properties[20]. Therefore, this study aimed to determine the effect of adding nanomagnetic iron, carbon nanotubes, the Atonik biostimulant, and the EM bioenhancer as a single application and in binary, ternary, and quaternary combinations on the content of biologically active compounds and water-soluble and fat-soluble vitamins in *A. bisporus* mushrooms.

2. Materials and Methods

Due to its diverse protein and nutritional content, the cultivated mushroom *A. bisporus* is of increasing economic and nutritional importance. The experiment was conducted at the Mushroom Production Project in Diwaniyah Governorate—Iraq (N3541283.97 E501886.60) from December 15, 2023, to June 2, 2024. The experiment was carried out in a controlled indoor facility measuring 2.6 × 8 meters. The cultivation environment was sterilized using 4% formalin (prepared from a 37% commercial stock solution). After sterilization, the facility was ventilated for three days to ensure the complete removal of residual formaldehyde. The compost culture medium was prepared in two stages through an open-air fermentation process. The first stage involved preparing the culture medium by moistening wheat straw for 6 days. Then, two batches applied 50% of the poultry manure to the wheat straw. The first application time was made after checking moisture content, and the second seven days later. During this period, moistening and mixing were carried out to maintain aerobic fermentation. In the second stage, the medium produced from the first stage was pasteurized at 60 °C for 12 hours. The temperature was then gradually reduced for six days, with the first and second days being 50–60 °C, the third and fourth days 45–48 °C, and the fifth and sixth days 25 °C. The most important indicators confirming the completion of this stage are measuring ammonia and nitrogen levels, as well as moisture content[21].

Representative random samples were taken for laboratory testing after the medium was prepared for the fungal inoculum. The experimental bags were filled with 18 kg of compost medium. 175 g of the inoculum was applied to each bag. After creating the appropriate conditions of temperature, humidity, oxygen, and light inside the farm hall, and after the mycelium had spread throughout parts of the growing medium, a 5 cm thick cover soil was added. The soil, which was composed of 70% peat moss, 20% sand, and 10% calcium carbonate, had been prepared in advance within the mushroom production project. The fungal inoculum was prepared using the method instructed by[22]. Wheat grains were boiled in water, and 2% calcium sulfate and 8% calcium carbonate were applied based on the dry weight of the grain. These grains were mixed well, distributed into glass bottles, sealed with cotton, and autoclaved for one hour at 121°C and 15 psi. The bottles were then left to cool down. Then, they were inoculated with pieces of the parent culture of *A. bisporus*, a white strain of Dutch origin, under sterile conditions[9, 23].

2.1. Study Treatments

The experiment included the application of:

- Magnetic iron nanoparticle suspension (N-FeO), as magnetic nanoparticles, possess distinct properties compared to other materials due to their large surface area.
- Carbon nanotubes (CNTs) possess distinct structural characteristics. They have a cylindrical nanostructure with a length-to-diameter ratio much higher than any other material[24].
- Liquid biofertilizer (EM), which consists of 10 genera belonging to 5 different families, including photosynthetic bacteria such as *Rhodopseudomonas palustrus* and *Rhodobacter sphaeroides*; lactic acid bacteria such as *Lactobacillus plantarum*, *L. casei*, and *Streptococcus lactis*; yeasts such as *Saccharomyces cerevisiae* and *Candida utilis*; actinomycetes such as *Streptomyces* and *Saccharomyces* spp.; and fermenting fungi. However, lactic acid bacteria, yeasts, and

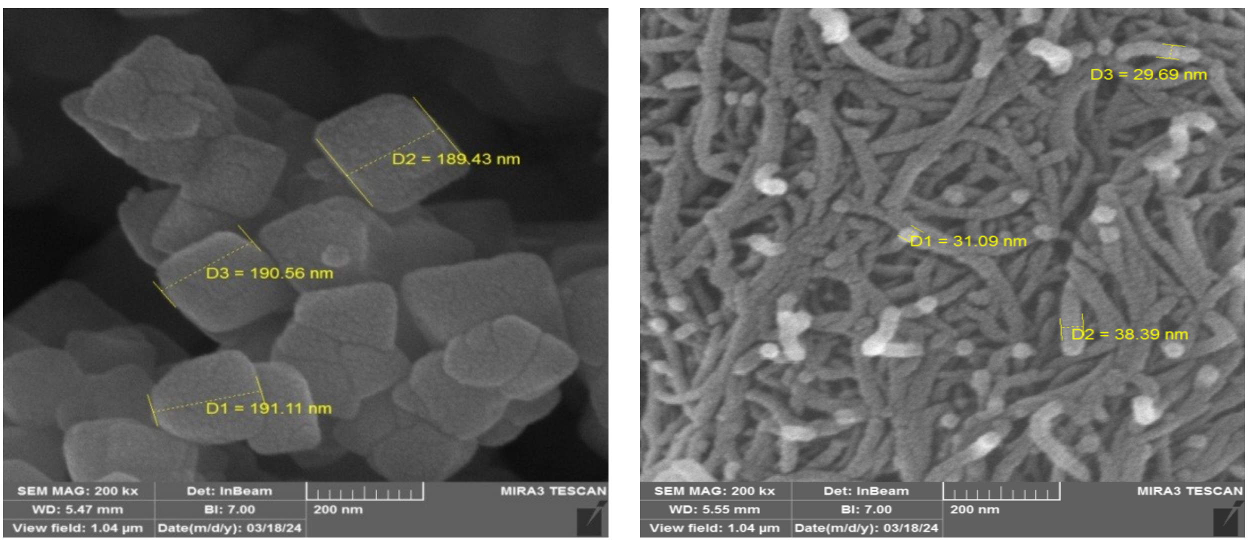
photosynthetic bacteria are the essential components of EM biofertilizer. All these organisms can coexist and interact compatible [25].

- The Atonik biostimulant is an aromatic nitro compound [26]. It comprises three nitrophenol compounds: sodium 5-nitrogualacolate 0.1%, sodium para-nitrophenolate 0.3%, and sodium ortho-nitrophenolate 0.2% (**Table 1**).

Table 1. Chemical characteristics of the compost medium before planting.

	Qualitative Traits	Value	Measurement Units	Reference
1	C	11.63		
2	N	0.45		
3	C/N	18.25	%	
4	P	0.33		
5	K	0.325		
6	Ca	0.391		
7	Mg	10.08		
8	Fe	28.00		[27]
9	Cu	7.03		
10	Mn	29.12		
11	Zn	28.91	mg^{-1} kg	
12	Mo	0.006		
13	Co	4.52		
14	B	1.03		
15	PH	7.4		
16	Ec	411	1-µSm	

The experimental treatments used were composed of individual treatments for magnetic nano-iron (N-FeO), carbon nanotubes (CNTs), the liquid biostimulant (EM), the growth stimulant (ATO), and binary, ternary, and quaternary combinations, **Table 2**. The experiment used a Completely Randomized Design (CRD) with three replicates. An ultrasonic device and deionized water at 42°C were used. The nanomaterials and biostimulants were applied to the bags by spraying after the coating stage. The application of nanomaterials and stimulants to the bags was repeated according to the treatments after each fruiting body harvest (**Figure 1**).



(**a**) (**b**)

Figure 1. scanning electron microscope SEM picture. (**a**) Magnetic iron oxide nanoparticles; (**b**) Carbon nanotubes

Table 2. Experimental treatments and their applied concentrations.

Treatment	Treatment Concentration
0	Control
N-FEO	magnetic iron oxide Nanoparticles (50 mg l^{-1})
CNT	*Carbon* nanotubes (50 mg l^{-1})
EM	EM Effective Microorganisms (100 ml l^{-1})
ATO	Atonik (0.5 ml l^{-1})
N-Feo+CNT	magnetic iron oxide Nanoparticles (50 mg l^{-1}) + *Carbon* nanotubes (50 mg l^{-1})
N-Feo+EM	magnetic iron oxide Nanoparticles (50 mg l^{-1}) + EM (100 ml l^{-1})
N-Feo+ATO	magnetic iron oxide Nanoparticles (50 mg l^{-1}) + ATO (0.5 ml l^{-1})
CNT+EM	*Carbon* nanotubes (50 mg l^{-1}) + EM (100 ml l^{-1})
CNT+ATO	*Carbon* nanotubes (50 mg l^{-1}) + ATO (0.5 ml l^{-1})
EM+ATO	EM (100 ml l^{-1}) + ATO (0.5 ml l^{-1})
N-Feo+ CNT+EM	magnetic iron oxide Nanoparticles (50 mg l^{-1}) +*Carbon* nanotubes (50 mg l^{-1}) + EM (100 ml l^{-1})
N-Feo+ CNT+ATO	magnetic iron oxide Nanoparticles (50 mg l^{-1}) + *Carbon* nanotubes (50 mg l^{-1}) + ATO (0.5 ml l^{-1})
N-Feo+ EM+ATO	magnetic iron oxide Nanoparticles (50 mg l^{-1}) + EM (100 ml l^{-1}) + ATO (0.5 ml l^{-1})
CNT+ EM+ATO	*Carbon* nanotubes (50 mg l^{-1}) + EM (100 ml l^{-1}) + ATO (0.5 ml l^{-1})
N-Feo+CNT+EM+ATO	magnetic iron oxide Nanoparticles (50 mg l^{-1}) +*Carbon* nanotubes (50 mg l^{-1}) + EM (100 ml l^{-1}) +ATO (0.5 ml l^{-1})

2.2. Fat-Soluble Vitamins Measurements

The sample was extracted by taking 1 g of mushroom powder mixed with 3 ml of ethyl acetate, and the sample was placed in a shaking incubator for 15 minutes at 50 ± 2 °C[27]. The sample was centrifuged at 4000 rpm for 15 minutes. The sample was then filtered using 0.45 µm membrane filters. The sample was then analyzed for HPLC[28]. Measurements were performed using a SYKAM-HPLC device under analytical conditions according to **Table 3**.

2.3. Water-Soluble Vitamins Measurements

The sample was extracted using the following method[29]. The sample was then examined by an HPLC device according to the analytical program[28]. The measurements were performed using a SYKAM HPLC device (Germany) under the analysis conditions as per **Table 3**.

Table 3. Conditions required for the detection of water-soluble and fat-soluble vitamins in *A. bisporus* fungi samples using an HPLC device.

Name	Water-Soluble Vitamins	Fat-Soluble Vitamins
Carrier Phase	Acetonitrile, formic acid	Methanol, acetonitrile
Separation Column	C18-ODS (25 cm × 4.6 mm)	C18-ODS (250 mm × 4.6 mm, 5 µm)
Detector	215 - UV, 210 nm	UV–VIS, 264 nm
Flow Rate	1.0 ml/min	1.0 ml/min

Statistical Analysis

The Gnestat statistical analysis computing program was used to analyze variance (ANOVA). Means of experimental parameters were compared using Duncan's multiple range test[30].

3. Results and Discussion

The impact of magnetic iron nanosuspensions, carbon nanotubes, and biostimulants on the water-soluble vitamin B2, B3, B5, and B6 content of A. bisporus fungi.

3.1. Mushroom's Vitamin B2 Content

The individual treatments and their combinations had an apparent positive effect on the mushroom's vitamin B2 content compared to the control, **Table 4**. The quadruple combination treatment, CNT+N-FeO+EM+ATO, resulted in the highest vitamin B2 content, 5.16 mg kg^{-1}, compared to the control, 2.09 mg kg^{-1}, outperforming the binary and ternary treatments. While the binary combinations resulted in significant increases, the binary combination, EM+ATO, had the highest significant value compared to its counterparts, reaching 4.54 mg kg^{-1} compared to the control. The ternary combinations also achieved significant differences in mushroom vitamin B2 content, reaching a maximum of 4.47 mg kg^{-1} in the treatments of N-FeO+EM+ATO.

3.2. Mushroom's Vitamin B3 Content

The single treatments and their binary, ternary, and tetramer combinations achieved significant differences in mushroom vitamin B3 content, 24.00 mg kg^{-1}, compared to the control. No significant differences were recorded between them (**Table 4**).

3.3. Mushroom's Vitamin B5 Content

The quadruple treatment, CNT + N-FeO + EM + ATO, resulted in a significant increase in mushroom vitamin B5 content, 17.70 mg kg^{-1}, compared to the control, 15.45 mg kg^{-1}. It is higher than the 25% ternary and 50% binary treatments. The 50% binary combinations treatments and all individual treatments resulted in non-significant differences compared to the control (**Table 4**).

3.4. Mushroom's Vitamin B6 Content

The triple treatment, N-FeO + EM + ATO, resulted in 25.66 mg kg^{-1} of B6 vitamin, which is the highest significant value for mushroom vitamin B6 content compared to the control 18.41 mg kg^{-1} with nonsignificantly from all ternary and binary combinations treatments. However, it outperformed most individual treatments, except for the Atonik biostimulant treatment, which had a value of 23.30 mg kg^{-1}.

Table 4. The Effect of nanomagnetic iron suspensions, carbon nanotubes, and biostimulants on the water-soluble vitamin B2, B3, B5, and B6 content of *A. bisporus* in the fruiting body.

Traits / Treatment		Water-Soluble Vitamins (mg kg^{-1})							
		B2		B3		B5		B6	
1	C	2.09	I	24.00	b	15.45	f	18.41	d
2	N-FeO	2.57	k	33.64	a	15.75	ef	22.78	bc
3	CNT	2.47	k	33.70	a	15.62	f	21.92	C
4	EM	2.82	j	33.17	a	15.70	ef	22.66	bc
5	ATO	2.84	j	34.55	a	15.94	ef	23.30	abc
6	N-FeO+CNT	3.12	i	33.92	a	16.28	def	23.02	abc
7	N-FeO+EM	3.59	g	35.54	a	16.55	bcdef	24.06	abc
8	N-FeO+ATO	3.39	h	34.39	a	16.51	cdef	23.78	abc
9	CNT+EM	3.74	f	35.89	a	16.83	abcde	24.04	abc
10	CNT+ATO	4.08	e	35.82	a	17.37	abcd	24.61	abc
11	EM+ATO	4.54	d	36.27	a	17.47	abc	24.98	ab
12	N-FeO+CNT+EM	4.65	cd	36.71	a	17.57	abc	24.94	ab
13	N-FeO+CNT+ATO	4.17	e	36.40	a	17.68	ab	24.86	ab
14	N-FeO+EM+ATO	4.92	b	36.80	a	16.27	def	25.66	a
15	CNT+EM+ATO	4.74	c	36.65	a	16.84	abcde	24.48	abc
16	N-FeO+CNT+EM+ATO	5.16	a	37.35	a	17.70	a	25.21	ab

Table 4 shows the effects of magnetic iron oxide (N-FeO) nanosuspensions, carbon nanotubes (CNTs), and biocatalysts (EM and ATO) on the content of water-soluble vitamins (B2, B3, B5, and B6) in the fruiting bodies of Agaricus bisporus. The combined treatment containing all components (N-FeO + CNT + EM + ATO) recorded the highest concentration of all vitamins, with B2 (5.16 mg/kg), B3 (37.35 mg/kg), B5 (17.70 mg/kg), and B6 (25.21 mg/kg), indicating that this combination has a strong synergistic effect in enhancing the nutritional value of mushrooms. It was also observed that most of the combined treatments showed a gradual improvement in vitamin levels compared to the stan-

dard treatment (C), reflecting the effectiveness of using nano- and bio-technologies in improving the nutritional content of edible mushrooms.

Impact of magnetic iron nanosuspensions, carbon nanotubes, and biocatalysts to A. bisporus mushrooms on the fat-soluble vitamin content A, D, and E.

3.5. Mushroom's Vitamin A Content

Significant differences were found between the single treatments and the binary, ternary, and quadruple combinations treatments in the fat-soluble vitamin content of mushrooms, **Table 5**. The quadruple combination treatments CNT + N-FeO + EM + ATO resulted in a significant increase in vitamin A content 6.87 ml iu compared to the control 2.71 ml iu, outperforming most binary and ternary combinations except for N-FeO + EM + ATO that had 6.57 ml iu. No single treatment resulted in a significant increase in vitamin A, except for the ATO and EM treatments that made values of 3.98 and 3.66 ml iu, respectively.

3.6. Mushroom's Vitamin D Content

The highest mushroom vitamin D content was achieved in the tetrameric combination treatment of CNT + N-FeO + EM + ATO, 35.08 mg kg^{-1}, compared to the control, 31.04 mg kg^{-1} (**Table 5**). This value is higher than most binary and ternary combination treatments, except for the ternary treatment of ATO + N-FeO + EM, which had 34.76 mg kg^{-1}. The individual treatments significantly outperformed the control, except for the EM treatment, which had a value of 31.73 mg kg^{-1}.

Table 5. Magnetic iron nanosuspensions, carbon nanotubes, and biostimulants impact the fat-soluble vitamin A, D, and E content of *A. bisporus* mushrooms.

Treatment	Traits	Fat-Soluble Vitamins					
		A (iu)		D (mg kg^{-1})		E (mg kg^{-1})	
1	C	2.71	j	31.04	f	0.98	h
2	N-FeO	2.84	j	32.36	e	1.35	gh
3	CNT	3.25	ij	32.35	de	1.25	gh
4	EM	3.66	hi	31.73	ef	1.63	fg
5	ATO	3.98	gh	32.44	de	2.01	ef
6	N-FeO+CNT	4.24	fgh	33.55	c	2.30	de
7	N-FeO+EM	4.57	fg	33.46	c	2.20	def
8	N-FeO+ATO	4.68	f	32.54	de	2.38	de
9	CNT+EM	4.78	ef	33.73	c	2.69	bcd
10	CNT+ATO	5.47	cd	33.24	cd	2.59	cde
11	EM+ATO	5.91	bcd	34.01	bc	3.30	ab
12	N-FeO+CNT+EM	5.93	bcd	33.95	bc	3.08	abc
13	N-FeO+CNT+ATO	5.37	de	33.58	de	3.26	ab
14	N-FeO+EM+ATO	6.57	ab	34.76	ab	3.36	a
15	CNT+EM+ATO	6.12	bc	34.15	bc	3.64	a
16	N-FeO+CNT+EM+ATO	6.87	a	35.08	a	3.62	a

3.7. Mushroom's Vitamin E Content

The quadruple and triple combinations treatments resulted in the highest mushroom vitamin E content values, followed by the binary treatments of ATO + EM, which had 3.30 mg kg_{-1}. The highest values were found in the triple CNT + EM + ATO treatments, which had 3.64 mg kg_{-1}, outperforming all other combinations and single treatments.

Table 5 showed that the use of nanosuspensions of magnetic iron oxide (N-FeO), carbon nanotubes (CNT), and biocatalysts (EM and ATO), individually or in combination, had a significant effect in increasing the content of fat-soluble vitamins (A, D, and E) in Agaricus bisporus. The combined treatment of N-FeO, CNT, EM, and ATO (Treatment 16) achieved the highest concentrations of all vitamins, with vitamin A (6.87 IU), vitamin D (35.08 mg/kg), and vitamin E (3.64 mg/kg), indicating a synergistic effect between these compounds in enhancing the nutritional value of the mushroom. It was also noted that the gradual improvement was evident as the combination of treatments increased, emphasizing the importance of combining these bio- and nano-treatments to obtain the highest nutritional benefits.The increase in the mushroom content of water-soluble vitamins B2, B5, and fat-soluble vitamins A and D with the

N-FeO+CNT+EM treatment is likely due to the combined synergistic impact of carbon nanotubes on improving the growth of mycelium dispersed in the mulch soil and culture medium. This effect can be attributed to the high penetration capacity of carbon nanotubes into living tissues, making them an innovative tool for intelligent targeting and precise delivery of nutrients. They may influence biochemical pathways involved in carbon metabolism, though their role as a direct carbon source remains uncertain. This improves the nutritional quality of fruiting bodies and their vitamin content by enhancing metabolic processes and antioxidant activity, representing a promising approach for sustainable agriculture. Vitamin D stimulates the synthesis of calcium transport proteins in the small intestine, enhancing calcium absorption as a nutrient and thus reducing the risk of osteoporosis in adults and rickets in children.

Iron is a transition element essential for all living cells. It participates in numerous physiological processes, including respiration, redox reactions, and enzymatic activity. It is also an essential component of many proteins (hemoglobin, myoglobin, cytochromes, and enzymes) involved in vital metabolic functions such as oxygen transport, oxidative energy production, mitochondrial respiration, inactivation of harmful reactive oxygen species (ROS), and DNA synthesis and repair. Iron oxide nanoparticles, which are smaller than regular iron oxide particles, form more complexes that increase iron availability [31–33]. Mushrooms are a valuable source of vitamins. The most abundant vitamins in mushrooms include niacin and riboflavin. Other important vitamins include alpha-tocopherol, ascorbic acid, vitamin B1, and vitamin B3 [34, 35]. Magnetic iron nanoparticles possess unique surface characteristics, making them a more effective and innovative delivery vehicle. It enhances the culture medium's physicochemical, electrical, and optical properties, such as controlling the gradual release of nutrients and reducing their loss through leaching, fixation, or evaporation from the growth medium [36]. This positively impacts the content of biologically active substances, including water-soluble and fat-soluble vitamins. A small amount of nanoparticles meets growth requirements, reducing environmental impacts. It intelligently targets the sites of biosynthesis and production of biologically active substances in mushroom tissues, unlike traditional iron sources and the problems associated with iron absorption and release into mushroom tissues [37].

The application of EM compost, containing strains of practical and beneficial organisms such as *Rhodopseudomonas palustrus* and *Rhodobacter sphaeroides*, lactic acid bacteria such as *Lactobacillus plantarum, L. casei*, and *Streptococcus lactis*, yeasts such as *Saccharomyces cerevisiae* and *Candida utilis*, and actinomycetes such as *Streptomyces* and *Saccharomyces* spp., has been shown to enhance the growth of these beneficial organisms under natural conditions. These organisms occupy the same niche and interact with each other, and these interactions influence fungal morphology, growth patterns, and biochemical processes [38]. Bacteria and fungi present in EM compost can form symbiotic relationships, often altering the feeding process of the symbiotic organisms. These interactions can also significantly contribute to biogeochemical cycles for mineralizing nutrients such as nitrogen, phosphorus, potassium, calcium, trace elements, and biotechnological processes. They are important in agriculture, environmental protection, food production, and medicine.

Nitrogen-fixing bacterial species also play an important role in releasing nitrogen from organic matter in the culture medium, resulting from the symbiotic relationship between beneficial organisms and fungi. They also play a significant role in vitamin synthesis and production processes by controlling the supply and release of nitrogen available for absorption [39–41]. In addition to the effective fermenting of fungi, they also participate in symbiotic relationships with fungi, serving as a key tool for decomposing organic matter in the culture medium and transforming nutrients. Beneficial organisms have specific tactics for absorbing substrates and increasing the content of biologically active compounds, including vitamins, in the fungal body [42, 43].

The Atonik growth stimulant, applied to the culture medium, directly improves nutrient absorption and the activity of living organisms. Although it is not a fertilizer, its unique composition, as an aromatic nitrogenous compound, can penetrate tissues, reach sites of metabolism, and synthesize biologically active substances, including vitamins. It also stimulates the biochemical processes of nitrogen and phosphorus, which produce many compounds, such as proteins, nucleic acids, purines, pyrimidines, and polysaccharides. Nitrogen is an essential component of many fungi's cell walls, consisting of a β(1-4)-linked unit of N-acetylglucosamine. The growth promoter affects the nitrogen-fixing bacteria present in the EM biofertilizer added to the growth medium and improves the sustainability of nutrient supply by enhancing the biomass in the culture medium, releasing nutrients, increasing their absorption, and producing more beneficial substances such as vitamins [17, 44, 45].

The increase in the content of vitamins B2, B5, A, and D in mushrooms with the N-FeO+EM+ATO treatment is attributed to the role of the growth promoters and magnetic iron nanoparticles applied to the culture medium and mycelium. It stimulates biochemical reactions and rapidly delivers nutrients and substances needed by the fungus for growth and production by acting as an innovative tool for penetrating the living tissues of the fungus and the active organisms in symbiosis with the fungus applied through EM fertilizer, intelligently targeting sites of metabolism and synthesis of beneficial substances. It promotes sustainable mushroom growth and production, increases nutritional value, and makes *A. bisporus* a safe and healthy food rich in biologically active compounds, including vitamins. It also protects the environment and reduces fungal pathogens.

The increase in vitamin E content in mushrooms with the EM+ATO treatment is attributed to the role of the active organisms applied to the growing medium through the EM biofertilizer, which is symbiotic with the fungus. The EM biofertilizer increased the active and beneficial biomass activity, thereby increasing the fertility of the growing medium, providing nutrients, and enabling the active organisms to decompose organic matter and release nutrients through mineralization processes.

The application of the growth promoter, ATO, stimulated various biochemical reactions in fungal tissues and active organisms, particularly nitrogen fixation, phosphorus release, and solubilization, leading to increased growth factors. The bioactive compounds produced by the active and beneficial microorganisms in the liquid biofertilizer (EM) may enhance the overall health and vitality of *A. bisporus*. These compounds can influence growth rates and may lead to increased production of bioactive compounds, including vitamins, in the fungal body [46, 47].

Global climate change threatens food security, necessitating urgent measures to enhance agricultural productivity and expand it to less suitable areas for agriculture. This challenge is crucial to achieving Sustainable Development Goal 2 (Zero Hunger), and effective symbiotic and fungal growth-promoting microorganisms are emerging as a promising solution to mitigate the impact of extreme climatic conditions and productivity on *A. bisporus* [48–54].

4. Conclusions

The individual application of the study treatments and the combined synergistic effect of magnetic iron nanoparticles, carbon nanotubes, liquid biofertilizer, and the Atonik growth promoter resulted in significant increases in the levels of water-soluble vitamins B2, B3, B5, and B6, and fat-soluble vitamins A, D, and E. Using the quadrilateral combination treatment resulted in a significant increase in the mushroom content of vitamins B2, B5, and A. The triple combination treatments of N-FeO+EM+ATO also significantly increased the mushroom content of vitamins B2, B6, D, and A. The single application of EM significantly increased the mushroom content of vitamins A and D, while the single ATO treatment significantly increased the mushroom content of vitamin A.

Author Contributions

Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review, editing, visualization and supervision M.A.; Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review, editing, visualization and supervision H.A.J.; Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review, editing, visualization and supervision R.A.C. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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