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Optimizing Growth and Bioactive Compound Production in Split Gill Mushroom (*Schizophyllum commune*) Using Methyl Jasmonate

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Abstract: The split gill mushroom (*Schizophyllum commune*) is a valuable natural resource with high nutritional value and diverse bioactive metabolites, underscoring its potential for sustainable applications. By applying elicitors, this study highlights the quality enhancement of *S. commune* fruiting bodies, a commercially significant resource. While elicitors have been shown to stimulate beneficial bioactive compound production, research on their use in *S. commune* remains limited. This study applied methyl jasmonate (MeJA) at various concentrations (0, 4, 13, 22, 31, and 40 μM) to optimize growth, improve nutritional value, promote triterpenoid and phenolic compound synthesis, and boost antioxidant activity in *S. commune*. The results demonstrated that MeJA's effects on growth and bioactive compounds are concentration-dependent. A concentration of 22 μM was identified as the most effective, resulting in the highest growth performance, including cap diameter (2.01 cm), fresh weight (24.10 g), and biological efficiency (15.21%). Furthermore, all MeJA treatments significantly enhanced triterpenoid, phenolic compound, and antioxidant activity compared to the control. These findings present a promising approach to enhance the sustainable use of *S. commune* as a natural resource by improving its quality and bioactive properties. Additionally, this research contributes to understanding the role of MeJA in promoting the growth and production of bioactive compounds in mushrooms, offering insights for advancing mushroom-based natural resource management.

Keywords: split gill mushroom; methyl jasmonate; triterpenoid compounds; phenolic compounds; antioxidant activity

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1. Introduction

Mushrooms are an essential natural resource, widely valued for their high nutritional content and their role as a source of bioactive compounds and health-promoting nutrients. Their significant protein content and diverse nutrient profile make them an important component of global food systems. In addition to their nutritional benefits, mushrooms are recognized for their potential as sustainable sources of bioactive compounds, which offer considerable health advantages and contribute to natural resource utilization [1]. The increasing demand for mushrooms has spurred the cultivation of various species, establishing mushrooms as one of the most important agricultural resources [2]. This demand underscores their economic and ecological significance, with the global mushroom market now valued at approximately 42 billion USD annually [3]. In 2017, 2189 novel fungal species were identified, encompassing numerous substantial macrofungi, including mushrooms. The bulk of these species were classified under the phylum Ascomycota (68%, or 1481 species), followed by Basidiomycota (31%, or 684 species). To date, 1789 species of mushrooms are classified as edible, 798 as medicinal, and 561 species are recognized for their dual role as both edible and medicinal [4]. These attributes highlight the importance of mushrooms as a renewable and sustainable natural resource with broad applications in nutrition, medicine, and biotechnology.

The split gill mushroom (*Schizophyllum commune*), a globally distributed natural resource, is widely consumed in many Asian countries, including North East India, Thailand, Malaysia, Laos, and Myanmar [5,6]. It is valued both as a food source and in traditional medicine due to its rich nutritional composition, featuring high protein (24.5%) and fiber content (19.9%) along with a low-fat profile [7]. These attributes position *S. commune* as a sustainable and health-promoting resource with broad applications. Beyond its nutritional value, *S. commune* is a source of bioactive compounds such as phenolic compounds and triterpenoids. Its cell wall contains a unique polysaccharide structure of β-glucan cross-linked with chitin. The β-glucan in this mushroom, known as schizophyllan, forms a triple-helical structure and is characterized as a β-1,3-D-glucan polymer with β-1,6-D branches [8,9]. Schizophyllan is renowned for its diverse biological activities, including immune system enhancement through macrophage activation and anti-inflammatory, antioxidative, and anticancer properties, particularly in gastric, lung, and cervical carcinoma cells. It also exhibits prebiotic, antiviral, and antibacterial effects [10]. The remarkable biological properties of *S. commune* make it a valuable natural resource for multiple industries, including medicinal and pharmaceutical sectors, as well as alternative and traditional foods across Asia. These applications demonstrate the sustainable utility of *S. commune* as a renewable natural resource with the potential to address nutritional and health challenges while supporting various industrial needs [8].

Despite the popularity and economic value of *S. commune*, its production in Thailand faces several challenges that hinder its potential as a sustainable natural resource. These challenges include limited availability of natural substrate sources and an unsuitable seasonal environment for fruiting body development, resulting in low product yields, small fruiting bodies, and faster substrate degradation compared to other edible mushrooms. This leads to low biological efficiency and limits the full utilization of *S. commune* as a valuable natural resource. Therefore, there is an urgent need for the development of improved cultivation methods to enhance both the yield and quality of *S. commune*. Plant hormones, which play crucial roles in regulating various biological processes, offer a promising solution for improving mushroom production. These hormones stimulate fungal growth [11,12], induce morphogenetic changes [12,13], promote spore germination [12,14], and enhance secondary metabolism [12,15]. Methyl jasmonic acid (MeJA), a phytohormone and signaling molecule associated with plant responses to injury, is widely present in plants and has been found to influence fungal development as well. External application of MeJA induces the expression of fungal genes and enhances the activity of defense proteins, regulating enzymes that lead to the accumulation of beneficial secondary metabolites, including triterpenoids and phenolic compounds [16–20]. This study aims to explore the impact of supplementing MeJA to improve the yield, triterpenoid and phenolic contents, and antioxidant activity of *S. commune*. The findings could contribute to the management and cultivation of *S. commune*, as MeJA plays a crucial role in enhancing antioxidant activity in mushrooms by activating antioxidant enzymes, stimulating secondary metabolite production, and improving stress tolerance. Its application provides an effective strategy for increasing the nutritional and medicinal value of mushrooms, making it a valuable tool in sustainable agriculture and natural resource management.

2. Materials and Methods

2.1. Preparation of the Substrate and Cultivation of S. commune

The cultivation of *S. commune* fruiting bodies took place concurrently in Pathum Thani, Thailand, in April 2024. The substrate used for cultivation was prepared by combining 100 kg of rubberwood sawdust with 5 kg of fine bran. Lime was added in an amount sufficient to adjust the pH of the substrate to neutral. The mixture was thoroughly blended, and its moisture content was adjusted to 65–70% by adding water. For the cultivation process, 900 g of the prepared substrate was packed into mushroom-growing bags.

The substrate was sterilized in an autoclave at a temperature of 123 °C and a pressure of 15 pounds per square inch for 2 h. After sterilization, the substrate was allowed to cool to ambient temperature over a 24 h period. Once cooled, the substrate was inoculated with *S. commune* spawn. The inoculated bags were then transferred to a greenhouse with adequate ventilation, maintained at a temperature of 25 °C and a relative humidity of 85%, to promote the colonization of *S. commune* mycelium.

2.2. Analysis of the Physical Properties and Nutrient Content of a Mushroom Cultivation Substrate

The physical characteristics and nutrient composition of the substrate were assessed prior to the cultivation of *S. commune*. To prepare the substrate for analysis, it was oven-dried at 60 °C for 48 h and then passed through a 2 mm sieve to evaluate the specific physical properties of the substrate. The pH and electrical conductivity of each substrate sample were measured in water at a 1:10 weight-to-volume (*w*/*v*) ratio. A PC950 pH meter (Apera Instrument, Columbus, OH, USA) was used to determine pH, while electrical conductivity measurements were conducted using a Eutech CON 2700 conductivity meter (Thermo Fisher Scientific, Waltham, MA, USA). The organic carbon (OC) content was determined with a CHNS/O Analyzer (628 series) from Leco Corporation, St. Joseph, MI, USA. Organic matter (OM) was estimated by multiplying the organic carbon value by 1.724, and the carbon-to-nitrogen (C/N) ratio was calculated by dividing the organic carbon content by the nitrogen content.

The nutrient composition, including total nitrogen, phosphorus, and potassium, was analyzed following the method outlined by Thepsilvisut et al. [21]. Total nitrogen in the mushroom cultivation substrates was measured using a CHNS/O Analyzer, an elemental analyzer. Phosphorus content was determined using a UV Spectrophotometer (UV-1280, Shimadzu, Japan) at a wavelength of 420 nm, while potassium levels were evaluated with flame atomic absorption spectroscopy with a Thermo Scientific iCE™ 3400 (Waltham, MA, USA).

2.3. Determination of Growth and Yield of S. commune Using MeJA

In the cultivation process of *S. commune*, six MeJA treatments were injected, which included 0 (control), 4, 13, 22, 31, and 40 μM. For each treatment, 120 mL of MeJA was injected into each bag, divided into four applications, with each application using 30 mL per bag. When the fruiting body opening stage was reached, the prepared MeJA solution was injected using a syringe. The solution was injected above the slits (each bag had three slits for fruiting body opening, as shown in Figure 1), with 10 mL per slit. The solution was administered in the morning (08:00–09:00) for 4 consecutive days, once daily, on alternate days, and then they were left for 10 days. Following the final application, bags were left undisturbed for 10 days until the first generation of mushrooms was ready for harvest.

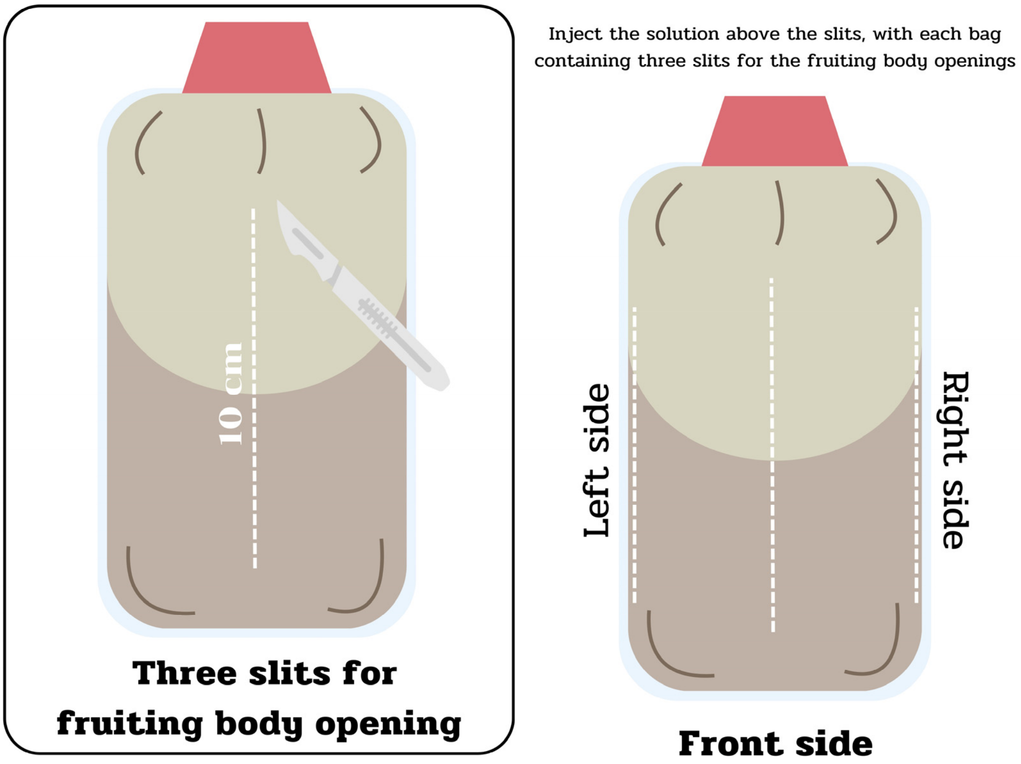

Figure 1. Characteristics of the fruiting body cultivation of the *S. commune* by cutting the bag into 3 compartments, 10 cm each.

After the harvest period, the initial batch of mushroom yield was collected in a single harvest. The harvested mushrooms were evaluated based on the following parameters: fruiting body density, cap diameter (in cm), fresh weight (in grams), and dry weight (in grams). To measure the cap diameter, 10 fruiting bodies were randomly selected, and their diameters were determined using a vernier caliper. The average value of these measurements was recorded. The biological efficiency (%BE) was calculated using the following formula:

$$\text{Biological efficiency } (\%\text{BE}) = \frac{\text{Fresh fruiting body weight } (\text{g})}{\text{Dry weight of substrate } (\text{g})} \times 100 \tag{1}$$

Biological efficiency refers to the ability of mushrooms to extract nutrients from the substrate to produce the biomass of the fruiting bodies. This efficiency is expressed as the ratio of the fresh fruiting body weight to the dry weight of the substrate, presented as a percentage [22].

2.4. Analysis of Mineral Content and Proximate Composition of the S. commune Fruiting Bodies

2.4.1. Sample Preparation

The fresh fruiting bodies of *S. commune* were dried in an oven at 60 °C for 48 h. Once dried, the samples were ground into a fine powder using a commercial blender to facilitate the analysis of their nutritional composition. The analysis included determining the moisture content, crude protein, crude fat, crude fiber, ash, total carbohydrates, and energy content. The results will be reported as percentages based on dry weight, with energy values expressed in kilocalories (kcal). All assessments were carried out following the methods specified by AOAC [23].

2.4.2. Mineral Analysis

The mineral content of the powdered dried fruiting body of *S. commune* was analyzed, including total nitrogen, phosphorus, potassium, calcium, magnesium, and zinc. Total nitrogen in the mushroom cultivation substrates was measured using a CHNS/O Analyzer, an elemental analyzer. The total phosphorus content was measured calorimetrically at 820 nm using a UV Spectrophotometer with the vanadomolybdate method [23]. The analysis of potassium, magnesium, calcium, and zinc was conducted using flame atomic absorption spectroscopy with a Thermo Scientific iCE™ 3400.

2.4.3. Moisture Content

Approximately 1.0 g of the dried mushroom powder sample, as described in Section 2.4.1., was placed into the crucible for drying. After that, it was dried in an oven at 120 °C for 3 h [1], and then transferred into a desiccator. The dish containing the dried sample was then reweighed, and the heating and cooling process was repeated until a constant weight was obtained. The moisture content was calculated using the following equation:

$$\text{Moisture } (\%) = \frac{\text{weight lost (g)}}{\text{weight of sample}} \times 100 \tag{2}$$

2.4.4. Crude Protein Content

The Kjeldahl method was used to calculate the crude protein. Ground samples of the *S. commune* weighing 0.5 g were digested in a Kjeldahl flask with 98% sulfuric acid, followed by steam distillation. The titration of the resulting distillate was carried out using 0.1 N sulfuric acid, and the protein percentage was then determined using the provided formula.

$$\text{Crude protein content } (\%) = \frac{(\text{A} - \text{B}) \times \text{N} \times 1.4007 \times 5.71}{\text{weight of sample}} \tag{3}$$

where

A = the amount of sulfuric acid used to titrate a sample;
B = the amount of sulfuric acid used to titrate a blank;
N = normality (0.1) of sulfuric acid.

2.4.5. Crude Fat Content

The crude fat content was determined using an automatic extraction system (AnkomXT 15 extractor). A 1.0 g sample of finely ground mushroom was placed in the extractor, and petroleum ether (boiling point of 35–65 °C) was added. The extraction process lasted for 1–2 h, after which the ether was evaporated until the flask was dry. The fat content was calculated by measuring the difference in weight of the flask before and after the evaporation of the ether. The fat percentage was then determined using the following formula:

$$\text{Crude fat content } (\%) = \frac{\text{W1} - \text{W2}}{\text{weight of sample}} \times 100 \tag{4}$$

where

W1 = sample weight with filter bag XT4;
W2 = sample weight with filter bag after extraction.

2.4.6. Crude Fiber Content

The crude fiber content was determined using the ANKOM Delta system. The procedure involved boiling a precise amount of air-dried powdered sample with sulfuric acid, followed by rinsing with water to remove the acidity. The residue was then boiled with potassium hydroxide (KOH) and rinsed with water to remove the alkalinity. The insoluble residue was dried at 120 °C, weighed, and then burned at 550 °C until only ash remained. The crude fiber content was calculated using a specific formula:

$$\text{Crude fiber content } (\%) = \frac{(\text{W2} - (\text{W1} \times \text{C1}))}{\text{weight of sample}} \times 100 \tag{5}$$

where

W1 = weight of the filter bag;
W2 = total weight of the fiber and filter bag;
C1 = weight after burning divided by the weight of the empty filter bag before extraction.

2.4.7. Ash Content

The ash content was determined by placing approximately 1 g of mushroom sample in a crucible and heating it at 550 °C for 4 h in a muffle furnace (CARBOLITE, ELF models, Hope Valley, UK). After the ashing process, the crucible was allowed to cool in a desiccator. The weight of the crucible before and after ashing was compared to calculate the ash content using the following formula:

$$\text{Ash content } (\%) = \frac{\text{W1} - \text{W2}}{\text{weight of sample}} \times 100 \tag{6}$$

where

W1 = weight of the sample after burning;
W2 = weight of the crucible.

2.4.8. Total Carbohydrate

The content of the available carbohydrates was calculated using the following formula:

$$\text{Total carbohydrates } (\%) = 100 - (\%\text{moisture} + \%\text{protein} + \%\text{fat} + \%\text{ash} + \%\text{fiber}) \tag{7}$$

2.4.9. Energy Values

The energy values of the mushroom samples were calculated using the following formula:

$$\text{Energy (kcal)} = (\text{Protein (g)} \times 4) + (\text{Fat (g)} \times 9) + (\text{Carbohydrates (g)} \times 4) \tag{8}$$

2.5. Measurement of Bioactive Compounds and Antioxidant Activity

2.5.1. Preparation of *S. commune* Extract

The ethanol extraction process was conducted following the method outlined by Chutimanukul et al. [24]. The *S. commune* fruiting bodies were first dried in an oven at 60 °C for 48 h. After drying, the fruiting bodies were ground into a fine powder and stored in sealed containers until further analysis. To perform the extraction, 5 g of powdered mushroom was mixed with 50 mL of 95% ethanol (w/v) and homogenized at room temperature. The mixture was then filtered using Whatman® Grade 1 qualitative filter paper. The maceration and extraction process was repeated every 3 days over a total period of 9 days. Following the extraction, the ethanol-containing extract was concentrated and dried under a vacuum using a Rotavapor® R-300 (BUCHI, Flawil, Switzerland).

2.5.2. Determination of the Total Triterpenoid Content Analyses of *S. commune* Extracts

The total triterpenoid content in *S. commune* extracts was measured using a modified method based on the procedure by Chutimanukul et al. [24]. A 300 µL sample of the extract was placed in a centrifuge tube, to which 50 µL of a vanillin–acetic acid solution (5 mg/mL) and 800 µL of 70% perchloric acid were added. The mixture was incubated for 15 min at 60 °C in a water bath and then cooled in an ice bath. Following this, 5 mL of acetic acid was added, and the mixture was allowed to sit undisturbed for 15 min at room temperature. The absorbance at 548 nm was measured using a Multiskan GO microplate reader (Thermo Scientific, Waltham, MA, USA), with the blank solution used as a reference. The triterpenoid content was determined by converting the absorbance values into mg of Ursolic acid equivalents (mg Urs/g DW).

2.5.3. Determination of the Total Phenolic Content of *S. commune* Extracts

The total phenolic content of *S. commune* extracts was determined using a modified Folin–Ciocalteu method [25], based on adjustments by Rahimah et al. [26]. The ethanol extract stock solution was prepared by dissolving the extract in absolute ethanol. For the assay, 20 µL of the extract was combined with 100 µL of Folin–Ciocalteu's reagent (diluted 1:10) and 80 µL of 7.5% sodium carbonate. The mixture was incubated for 30 min, after which the absorbance was measured at 765 nm using a microplate reader. The total phenolic content was quantified by converting the absorbance readings into mg of Gallic acid equivalents (mg GAE) per g of DW (mg GAE/g DW).

2.5.4. DPPH Radical Scavenging Activity

The antioxidant activity of *S. commune* extracts was evaluated using their ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, following a method adapted from Soares et al. [27]. Extracts of *S. commune* were dissolved in absolute ethanol to prepare concentrations of 1, 2, 3, 4, and 5 mg/mL. A 100 µL aliquot of each sample was mixed with 100 µL of a 6×10^{-5} M DPPH solution in a 96-well microplate. The mixture was incubated in the dark at room temperature for 30 min to prevent light interference. The absorbance was measured at 520 nm using a microplate reader. The percentage of DPPH inhibition was calculated using the following formula:

$$\text{Inhibition}(\%) = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100 \quad (9)$$

where

A_{control} is the absorbance of the DPPH solution;
A_{sample} is the absorbance of the solution containing the sample.

The antioxidant activity from the DPPH radical scavenging assay was expressed as the IC_{50} value, which is the concentration of the extract required to scavenge 50% of the DPPH radicals. Butylated hydroxytoluene (BHT) was used as the positive control, while the negative control consisted of samples without mushroom extract. The IC_{50} values were reported in milligrams per milliliter (mg/mL).

2.6. Statistical Analysis

The experiment was conducted using a completely randomized design (CRD) with five replicates for each treatment. Each replicate consisted of 10 mycelium bags, and all experiments were performed simultaneously. The experimental data were analyzed using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to compare means. Statistical significance was determined when the *p*-value was less than 0.05. All statistical analyses were performed using IBM SPSS Statistics 21.

3. Results and Discussion

3.1. Physical Properties and Nutrient Content of *S. commune* Substrate Cultivation Were Studied

In the experiment, the *S. commune* was grown on a substrate with a pH value of 5.94, which displayed specific physical and chemical characteristics. The pH of the substrate plays a crucial role before spawning, influencing the substrate's ionic state and the structure, appearance, and biological functions of fungal cells. This, in turn, affects the absorption of nutrients and their synthesis [24,28]. According to the report by Imtiaj et al. [29], the optimal pH range for mushroom cultivation is between 5 and 9, which is favorable for mycelium proliferation. The substrate's electrical conductivity (EC) was found to be 1.39 dS m^{-1}, with the optimal range for mycelium growth and the development of mushrooms being 0.87 to 1.98 dS m^{-1} [30]. Additionally, the organic carbon content and organic matter content of the substrate were 25.09% and 43.25%, respectively (Table 1). The carbon sources derived from sawdust, such as cellulose and hemicellulose, are complex carbohydrates that need microbial activity to degrade into simpler compounds. Subsequently, the mushroom mycelium uses organic carbon and organic matter to generate fresh microbial cells in the fermentation process, which in turn releases vital nutrients to support mushroom growth [24].

Table 1. Chemical properties and nutrients of *S. commune* substrate used in this study.

Components of the Substrate	Contents
pH	6.94 ± 0.75
EC (dS m^{-1})	1.39 ± 1.54
Organic carbon (%)	25.09 ± 1.99
Organic matter (%)	43.25 ± 0.87
Total nitrogen (%)	0.60 ± 2.54
Total phosphorus (%)	0.02 ± 1.73
Total potassium (%)	0.01 ± 1.94
C/N ratio	41.72 ± 1.85

The data are presented as mean ± standard deviation (SD) (n = 5).

The examination of the substrate's nutrients indicated that nitrogen is a crucial element for the efficient growth of *S. commune*, with a concentration of 0.60%. Before spawning, the substrate contained 0.02% total phosphorus and 0.01% total potassium. Although present in smaller amounts, phosphorus and potassium contribute to the development of mushroom mycelium and support normal physiological processes [1]. This ratio is critical as it influences nutrient utilization from the substrate and directly affects the growth and development of the mushroom mycelium. Therefore, it is vital to establish a balanced nitrogen and carbon ratio to maintain an optimal C/N ratio between 35 and 55 for successful mushroom cultivation [31].

3.2. Study on Growth and Yield of *S. commune*

Mushrooms provide numerous nutritional benefits that make them a valuable component of natural resources, offering a sustainable source of essential nutrients, bioactive compounds, and health-promoting properties. As natural resources, mushrooms contribute significantly to human health, agricultural sustainability, and the broader ecosystem. The development of *S. commune* showed that the growth substrate plays a crucial role in supplying necessary nutrients for mushroom growth and development. Adding MeJA to the substrate improves the ability of the mushroom to break down complex organic compounds by releasing enzymes, enabling the mycelia to absorb smaller nutrients. This process effectively stimulates the growth and productivity of *S. commune*. The study found that the application of MeJA at concentrations of 22 and 31 μM resulted in an increased density of *S. commune* fruiting bodies around the slits, leading to a high density (+++) of mushrooms in these slits. Furthermore, the application of MeJA at concentrations of 3 and 40 μM resulted in a medium density (++) of mushrooms around the slits, while the control treatment showed a low density (+) of mushrooms around the slits (Figure 2). In addition, the use of MeJA at a 22 μM concentration led to an increased diameter of the *S. commune* cap compared to the control treatment. Upon measuring the cap diameter, it was determined that the average cap diameter with MeJA treatment at 22 μM was 2.01 cm. In contrast, the control treatment resulted in an average cap diameter of 1.65 cm (Table 2). This difference may be attributed to methyl jasmonate (MeJA), a natural plant growth regulator that plays an important role in various physiological processes, including the development of *S. commune* mycelium, which ultimately forms the fruiting bodies. Mushroom mycelium can facilitate the transport of phytohormones like MeJA through phytohormone receptors. According to Hérivaux et al. [32], studies on fungal phytohormone receptors have identified fungal histidine kinases that exhibit similarities to plant hormone receptors. These fungal receptors respond to MeJA by inducing changes in growth patterns. Phytohormones are known to significantly influence mushroom growth, and recent research highlights the role of MeJA in regulating biosynthetic and metabolic processes [33]. MeJA's mechanism of action in mushroom development involves a cascade of biochemical and molecular events that modulate physiological processes such as growth, development, secondary metabolite synthesis, and stress responses.

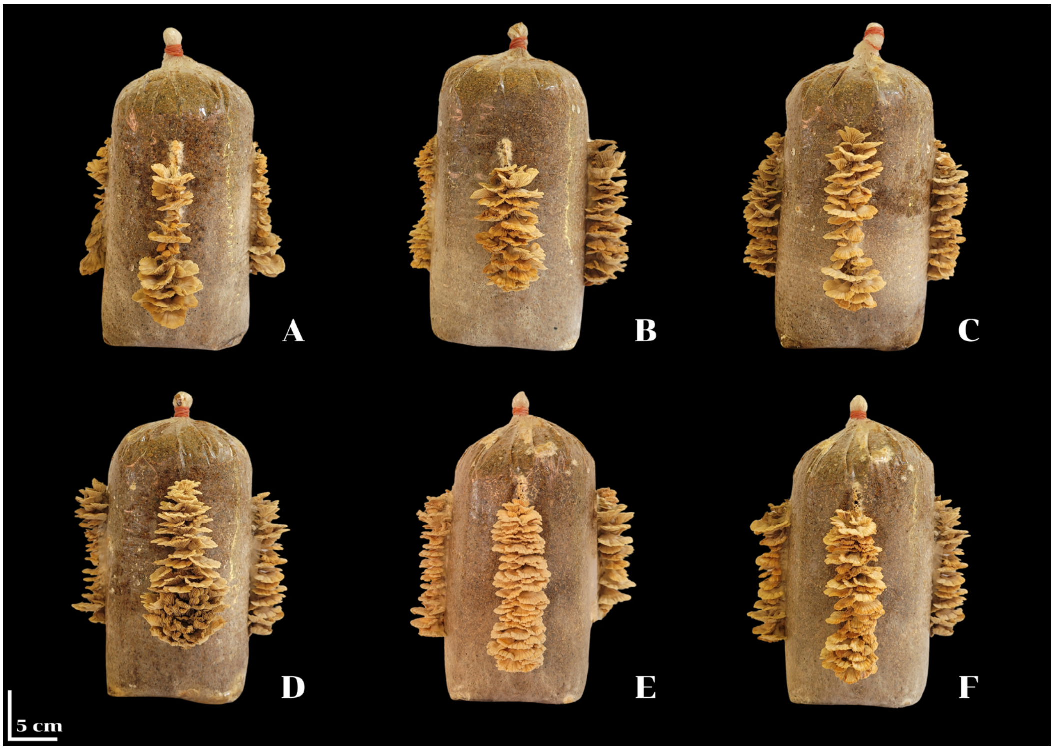

Figure 2. The densities of the fruiting bodies of *S. commune:* (**A**) 0 μM (control), (**B**) 4 μM, (**C**) 13 μM, (**D**) 22 μM, (**E**) 31 μM, and (**F**) 40 μM. The fruiting body density is categorized; + is low density, ++ is medium density, and +++ is high density.

Table 2. The growth and yield of the *S. commune* were evaluated under the influence of various concentrations of MeJA.

Concentration of MeJA (μM)	Cap Diameter (cm)	Fresh Mushroom Weight (g)	Dry Mushroom Weight (g)	Biological Efficiency (%)
0 (control)	1.65 ± 0.23 ^{b}	15.30 ± 2.71 ^{bc}	5.63 ± 0.75	6.59 ± 1.73 ^{d}
4	1.46 ± 0.07 ^{b}	16.62 ± 2.68 ^{bc}	5.78 ± 0.80	10.05 ± 1.01 ^{bc}
13	1.53 ± 0.12 ^{b}	14.95 ± 3.48 ^{bc}	5.66 ± 0.78	11.25 ± 1.20 ^{b}
22	2.01 ± 0.20 ^{a}	24.10 ± 4.99 ^{a}	7.44 ± 1.49	15.21 ± 1.52 ^{a}
31	1.76 ± 0.24 ^{ab}	19.88 ± 3.10 ^{ab}	7.02 ± 1.92	12.04 ± 1.47 ^{b}
40	1.58 ± 0.09 ^{b}	13.81 ± 0.88 ^{c}	5.67 ± 0.57	8.12 ± 0.67 ^{cd}
F-test	*	**	ns	**
C.V.%	11.88	22.19	13.07	12.88

The data are presented as mean ± standard deviation (SD) (n = 5). Distinct letters within the same column indicate significant differences between treatments, as determined by Duncan's multiple range test (DMRT) at $p < 0.05$. Asterisks (** and *) indicate significant differences at $p < 0.01$ and $p < 0.05$, respectively, while "ns" denotes no significant differences.

The results of the application of MeJA on *S. commune* yield indicate that adding MeJA to the substrate increased the yield compared to the control treatment. When MeJA was added to the substrate, it enhanced the yield performance of the *S. commune* fruiting body. For mushroom production, the application of 22 μM MeJA resulted in the highest fresh weight, with the *S. commune* reaching 18.79 g. The dry weight, which represents the organic mass of the mushrooms after complete dehydration, is also correlated with the fresh weight. MeJA concentrations ranging from 4 to 40 μM produced a dry weight between 5.66 and 7.44 g. Additionally, the biological efficiency of the *S. commune* varied significantly with different concentrations of MeJA. The highest biological efficiency of 15.21% was observed at a 22 μM concentration, whereas the control treatment showed the lowest biological efficiency at 6.59% (Table 2). Biological efficiency serves as an important indicator of substrate conversion effectiveness in mushroom cultivation. It is calculated as the ratio of the fresh weight of harvested mushrooms to the dry weight of the cultivation substrate. A higher BE value signifies more efficient utilization of the substrate for mushroom growth and development [34]. In this study, it was found that using MeJA resulted in a higher biological efficiency for *S. commune* compared to the control treatment. However, the biological efficiency in the result may be slightly lower than that in general production. Nonetheless, these findings indicate that MeJA effectively stimulates growth, thereby enhancing both the yield and biological efficiency of *S. commune*. Additionally, MeJA plays a role in regulating vital activities such as cell division and meristem formation, which influence the growth of the *S. commune*. The experimental findings are in line with the notion that the presence of MeJA could be another significant element influencing growth and productivity. The study provides evidence that MeJA has a crucial impact on the structural changes in fungi by stimulating the formation of tissues and the division of cells. This stimulation of mycelial growth leads to the development of fruiting bodies [1]. Thus, the application of MeJA and its effect on stimulating phytohormone production within the mushroom mycelium is a key factor in regulating various developmental processes in mushrooms, contributing to the sustainable cultivation and optimization of *S. commune* as a renewable natural resource.

3.3. Mineral Content and Proximate Composition of the Fruiting Body of S. commune

3.3.1. Analysis of Mineral Content of the *S. commune*

The mineral content in the powdered dried fruiting body of *S. commune* treated with varying concentrations of MeJA was analyzed. Specifically, the study focuses on total nitrogen, phosphorus, and potassium, measured as percentages, to assess the influence of MeJA on the mineral content of the *S. commune*. Treatments were applied at MeJA concentrations of 0, 4, 13, 22, 31, and 40 µM. The results reveal that total nitrogen content ranged between 3.43% and 4.47%, with the highest value observed at 4 µM MeJA. Total phosphorus was recorded between 0.06% and 0.12%, while total potassium ranged from 0.98% to 1.24%. Furthermore, the treatment with MeJA led to *S. commune* containing calcium, magnesium, and zinc in the ranges of 0.08 to 0.09%, 0.06 to 0.08%, and 0.010 to 0.014%, respectively (Figure 3 and Table S1). The experimental results are consistent with the findings of Singh et al. [7]. Their study reported that the mineral content of *S. commune* collected from the wild consisted of nitrogen, phosphorus, and potassium at levels of 3.25%, 0.79%, and 1.12%, respectively. However, the mineral content in mushrooms largely depends on the composition of the growth substrate. Mushrooms absorb minerals through their mycelium from the substrate, so altering external factors like MeJA application may not impact this process if the substrate's mineral content remains unchanged [35]. Also, MeJA is known for modulating secondary metabolite production and stress response rather than directly affecting the absorption or assimilation of minerals. If MeJA is applied at a stage when mineral uptake has already stabilized or completed, it may have no observable impact on mineral content.

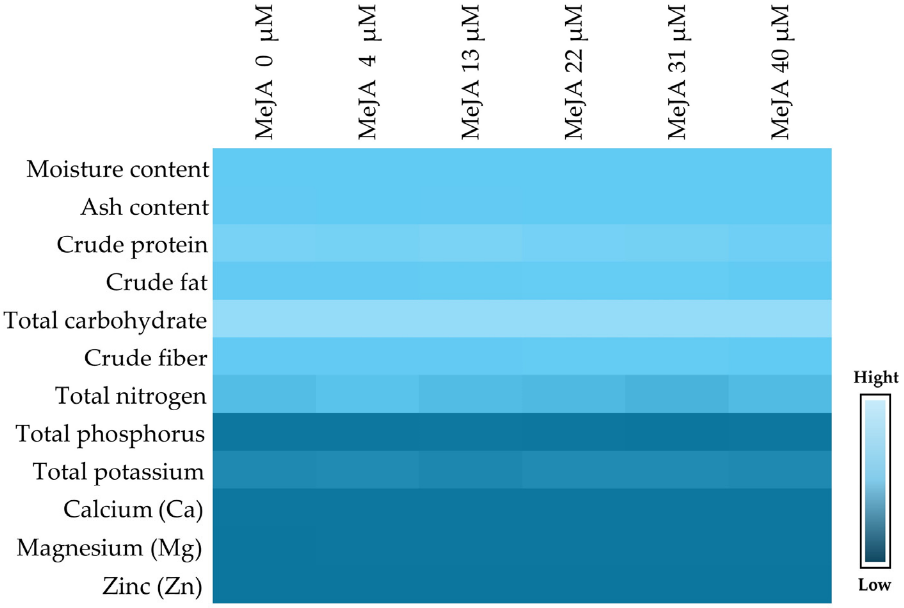

Figure 3. This heat map illustrates the proximate composition and mineral content of *S. commune* after the exogenous application of MeJA at varying concentrations. The color gradient denotes the relative values, with light shades representing higher concentrations of the respective components.

3.3.2. Analysis of Proximate Composition of the *S. commune*

The *S. commune* studied showed moisture contents ranging from 5.28% to 6.06% after MeJA application, with no statistically significant differences. This suggests that the application of MeJA, a growth regulator known to influence cell proliferation and differentiation, does not have any impact on the moisture content of *S. commune*. The ash content of *S. commune* was not significantly different, with the MeJA application showing ash contents ranging from 6.36% to 7.02% at concentrations of 4 to 40 µM. From the above experimental results, it is concluded that ash content is primarily a measure of the total mineral content in a sample after the organic material has been burned off. As mineral content in mushrooms is largely determined by the nutrients in the growth substrate, external application of MeJA is unlikely to impact it unless it alters substrate properties or nutrient uptake [35]. In summary, ash content is a reflection of the inherent mineral composition of the substrate and the mushroom's ability to assimilate these minerals. Since MeJA does not alter the substrate's mineral content or the mycelium's absorption process, it has little to no impact on the ash content of mushrooms [36]. In addition, the use of MeJA also affected the crude protein content of *S. commune*. The application of MeJA at a concentration of 13 µM resulted in the highest crude protein content of *S. commune*, equal to 25.54%, whereas the control treatment yielded a protein content of 23.76%, according to the report of Singh et al. [7] it was found that *S. commune* had a crude protein content of 24.51%, which was similar to what was analyzed. In the same way, *S. commune* crude fat levels, following exposure to MeJA concentrations from 4 to 31 µM, exhibited fat percentages ranging from 7.37% to 7.92%. Meanwhile, the application of 40 µM of MeJA resulted in the fat content being reduced to 5.97% (Figure 3 and Table S2). The production of proteins and fats in *S. commune* was suppressed due to MeJA's significant involvement in the breakdown of organic material by microorganisms. As previously reported by Chutimanukul et al. [1], the decomposition process breaks down organic matter into smaller molecules that cells use as energy and nitrogen sources for synthesizing proteins and fats. The study highlighted that phytohormones act as signaling molecules, transmitting information about environmental factors to the cell genome, thereby activating pathways involved in protein and fat synthesis [37]. Therefore, the application of higher concentrations of MeJA appears to interfere with cellular signaling pathways, potentially disrupting the normal regulatory mechanisms involved in protein and fat synthesis. This inhibition may alter key molecular processes, including gene expression and enzyme activity, which are critical for maintaining the balance of metabolic systems. As a result, the synthesis of proteins and lipids may be negatively affected, leading to changes in the biochemical composition and overall metabolic response of the organism. These findings suggest that while MeJA can serve as an elicitor, excessive concentrations may trigger stress responses that hinder its beneficial effects on metabolic pathways.

Interestingly, carbohydrates were the most abundant macronutrient resulting from MeJA applications at 4 to 40 µM concentrations, resulting in *S. commune* having the highest levels with applications between 47.11 and 56.03%. The *S. commune* carbohydrate content aligns with its protein and ash content. During the inoculated mushroom process, the degradation of carbohydrates is caused by the crude protein and ash content. This research revealed that the application of MeJA causes a decrease in the amount of protein and ash in *S. commune*, which results in an increased accumulation of carbohydrates. However, the experimental results showed that the application of MeJA did not affect the increase in the crude fiber and energy contents of *S. commune*, which ranged from 6.35 to 7.51% and 344.75 to 365.53 kcal, respectively (Figure 3 and Table S2). The energy content of *S. commune* is determined by its energy-yielding components, including carbohydrates, fats, and proteins. These macronutrients serve as the primary contributors to the mushroom's overall energy value. When combined, these elements determine the overall nutritional energy value of the mushrooms. The findings of this study are unique because this is the first time that the effects of MeJA on the nutritional composition of *S. commune* have been investigated, to the best of our knowledge. The results challenge the existing literature, offering new insights into how MeJA influences the nutritional profile of this mushroom species.

3.4. Measurement of Bioactive Compounds and Antioxidant Activity of *S. commune*

Mushrooms, as natural resources, provide a wealth of bioactive compounds that contribute significantly to human health and the environment. These compounds are often responsible for the medicinal, nutritional, and ecological benefits of mushrooms. The presence of bioactive compounds in mushrooms enhances their value as renewable natural resources, supporting both sustainable agriculture and therapeutic applications.

3.4.1. Total Triterpenoid Content

The total triterpenoid content in *S. commune* was analyzed to examine the effect of MeJA on triterpenoid biosynthesis. Different MeJA concentrations were tested, revealing a significant increase in total triterpenoid content with MeJA treatment. The highest triterpenoid levels, ranging from 66.40 to 78.27 mg Urs/g DW, were observed at MeJA concentrations between 22 and 40 µM. In contrast, the control treatments showed a triterpenoid content of 47.51 mg Urs/g DW (Figure 4 and Table S2). These findings highlight a significant difference in triterpenoid levels between treated and control groups, indicating that MeJA effectively stimulates triterpenoid biosynthesis. Previous studies have demonstrated the physiological impacts of MeJA as a plant hormone on *S. commune*. Hypotheses suggest potential trade-offs in secondary metabolite synthesis, as supported by research from Xu et al. [38], which showed a 53.6% increase in triterpenoid production in *Inonotus obliquus* upon MeJA treatment. Similarly, Ren et al. [12] reported that MeJA significantly enhanced ganoderic acid production in *Ganoderma lucidum*. This aligns with findings from Meng et al. [39], which demonstrate that MeJA, as a naturally occurring plant growth regulator and inductor of gene expression, is involved in the biosynthesis of secondary metabolites. Additionally, the presence of phytohormone receptors in fungi, such as fungal histidine kinases, enables the effective stimulation of secondary metabolite biosynthesis through phytohormones like MeJA for mushroom cultivation [40]. These results provide valuable insights into the role of MeJA as a phytohormone in enhancing the production of bioactive compounds, such as triterpenoids, in *S. commune*.

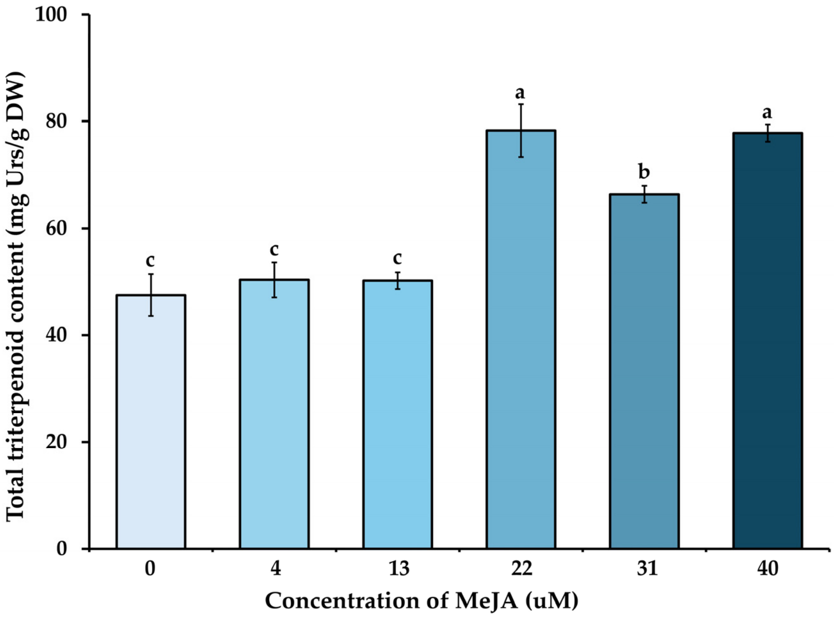

Figure 4. The total triterpenoid content in *S. commune* was assessed following the exogenous application of MeJA at varying concentrations. The findings, presented as mean values with standard deviations ($n = 5$), revealed statistically significant differences among treatments. These differences are denoted by distinct letters displayed above the bars, determined using Duncan's multiple range test at a significance level of ($p < 0.05$).

This finding is significant for the sustainable utilization of *S. commune* as a natural resource, as it highlights the potential to optimize the production of valuable bioactive metabolites through controlled cultivation practices. MeJA is a well-known plant hormone that is involved in plant stress responses and secondary metabolite production. When applied to mushroom cultures, MeJA can significantly increase the production of triterpenoids. In addition, MeJA is one of the most studied plant hormones in mushroom cultivation due to its ability to increase the production of triterpenoids and other secondary metabolites. MeJA is known to regulate gene expression and stimulate defense-related enzymes in fungi, leading to enhanced secondary metabolite production [41]. In mushrooms such as *G. lucidum* (reishi) and *Lentinula edodes* (shiitake), the application of MeJA has been shown to boost triterpenoid content significantly [42].

3.4.2. Total Phenolic Content

To examine the impact of MeJA on the biosynthesis of phenolic compounds in *S. commune*, the total phenolic content was analyzed following treatment with varying concentrations of MeJA. The results demonstrated a notable increase in total phenolic content after the MeJA application. Specifically, MeJA concentrations ranging from 4 to 40 µM enhanced phenolic compound synthesis, resulting in total phenolic contents ranging from 15.86 to 20.04 mg GAE/g DW, compared to 13.81 mg GAE/g DW in the control group (Table 3). These findings indicate that MeJA serves as an effective elicitor for enhancing the production of bioactive compounds in *S. commune*. As a plant hormone and signaling molecule, MeJA primarily facilitates plant defense responses to various biotic and abiotic stressors. When applied to fungi, including mushrooms, MeJA acts as an elicitor by activating pathways that promote growth and increase the synthesis of bioactive compounds such as phenolics and triterpenoids. This aligns with the findings of Lin et al. [43] that demonstrate the application of MeJA to enhance the production of bioactive components in the fruit body of *Cordyceps militaris*. Results showed that the effect of MeJA could increase the content of polyphenol. The phenylpropanoid pathway in fungi is typically responsible for producing secondary metabolites as a defense mechanism against environmental stimuli [44–46]. Signal molecules activate this pathway, triggering cellular defense mechanisms at the plasma membrane, which then initiate the transcription of defense-related genes [46,47]. This process leads to the accumulation of secondary metabolites through oxidative catabolism during fungal interactions or elicitor treatments [16,46]. Furthermore, Dai et al. [20] found that MeJA supplementation significantly increased secondary metabolite production, such as ergosterol, in *Hericium erinaceus*. This increase was associated with elevated enzyme activities and related defense proteins. MeJA application also stimulated the production of other secondary metabolites, such as alkaloids and phenolic acids, and regulated the release of volatile signaling compounds [48].

MeJA is involved in regulating gene expression in fungi by activating specific signaling pathways. MeJA binds to receptors in the cell membrane or cytoplasm, leading to the activation of jasmonic acid (JA) responsive transcription factors. These transcription factors then initiate the expression of genes that regulate the production of secondary metabolites and other growth-related factors [49]. One of the most significant effects of MeJA on mushroom growth is its role in the induction of secondary metabolites. In many mushrooms, including medicinal species like *G. lucidum* and *L. edodes*, MeJA stimulates the synthesis of bioactive compounds such as triterpenoids, phenolics, and polysaccharides (e.g., β-glucans). These metabolites play crucial roles in the medicinal properties of mushrooms, including antioxidant, anticancer, and anti-inflammatory activities. MeJA enhances the production of these compounds by activating biosynthetic enzymes involved in their pathways [42].

Table 3. The total phenolic content and antioxidant activity of *S. commune* were evaluated across various concentrations of MeJA treatment.

Concentration of MeJA (µM)	Total Phenolic Content (mg GAE/g DW)	Antioxidant Activity (IC_{50}, mg/mL)
0	13.81 ± 1.15^{d}	3.50 ± 0.58^{b}
4	15.86 ± 1.33^{c}	2.93 ± 0.07^{a}
13	16.09 ± 0.59^{c}	2.89 ± 0.12^{a}
22	17.18 ± 0.53^{bc}	2.67 ± 0.03^{a}
31	18.56 ± 0.50^{ab}	2.70 ± 0.09^{a}
40	20.04 ± 1.20^{a}	2.70 ± 0.20^{a}
F-test	**	*
C.V.%	5.35	5.83

The data are presented as mean ± standard deviation (SD) ($n = 5$). Distinct letters within the same column indicate significant differences between treatments, as determined by Duncan's multiple range test (DMRT) at $p < 0.05$. Asterisks (** and *) indicate significant differences at $p < 0.01$ and $p < 0.05$, respectively.

3.4.3. Antioxidant Activity

The DPPH radical scavenging ability of *S. commune* extracts was assessed, and the results were compared to the standardized antioxidant butylated hydroxytoluene (BHT), which exhibited an IC_{50} value of 13.33 ± 0.89 µg/mL. The assays, conducted in methanol, presented the findings as IC_{50} values, which indicate the concentration of antioxidants required to reduce the initial DPPH concentration by 50%. Generally, lower IC_{50} values correspond to stronger antioxidant properties. The results revealed that the DPPH radical scavenging ability of *S. commune* extracts treated with MeJA was significantly higher compared to the control treatment. MeJA treatments at concentrations ranging from 4 to 40 µM exhibited the strongest antioxidant activity, with IC_{50} values ranging from 2.70 to 2.93 mg/mL. In contrast, the control treatment demonstrated a higher IC_{50} value of 3.50 mg/mL, indicating lower antioxidant activity (Table 3). The improved antioxidant activity in MeJA-treated extracts is likely due to the stimulatory effects of MeJA on phenolic compound synthesis in *S. commune*. The control treatment displayed reduced antioxidant activity, which could be attributed to limited phenolic compound production. This aligns with findings by Yuan et al. [46], which reported that total polyphenols from *Sanghuangporus vaninii* cultured with MeJA supplementation exhibited strong free radical scavenging capacities. Similarly, Zeng et al. [50] found that MeJA treatment enhanced the ability of *Flammulina velutipes* to scavenge reactive oxygen species. Furthermore, phenolic compounds are well documented for their direct relationship with antioxidant activity and other biological functions [51–53]. The data from this study support the hypothesis that the application of exogenous MeJA stimulates the production of secondary metabolites, such as phenolic compounds, thereby enhancing the antioxidant activity of *S. commune*.

The mechanism of action of MeJA on antioxidant activity in mushrooms and other organisms involves activating signaling pathways that stimulate the production of antioxidant enzymes and secondary metabolites with antioxidant properties. This process enhances the organism's ability to neutralize reactive oxygen species (ROS) and protect against oxidative damage. MeJA functions as an elicitor, triggering antioxidant defense mechanisms in mushrooms by upregulating genes responsible for the synthesis of key antioxidant enzymes. These include Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPX), which collectively play a vital role in mitigating oxidative stress. They achieve this by scavenging free radicals and maintaining the cellular redox balance [54]. Additionally, MeJA stimulates the biosynthesis of secondary metabolites, such as phenolic compounds, flavonoids, and triterpenoids, all of which exhibit potent antioxidant properties [42].

4. Conclusions

This study revealed the effects of elicitors on the production of bioactive compounds in *S. commune*, highlighting the potential of MeJA application as a sustainable strategy to enhance natural resources. The application of MeJA at a concentration of 22 µM significantly improved the growth, yield, and nutritional value of *S. commune*, including crude protein, crude fat, and total carbohydrates, compared to the control treatment. Furthermore, higher concentrations of MeJA, particularly 40 µM, stimulated the production of triterpenoids and phenolic compounds, resulting in enhanced antioxidant activity. MeJA acts as an elicitor that mimics environmental stress, thereby activating metabolic pathways that lead to the production of secondary metabolites, including phenolic compounds, triterpenoids, and other antioxidants. These findings underscore the feasibility of incorporating MeJA into mushroom cultivation systems as an eco-friendly strategy to enhance productivity and the synthesis of bioactive compounds. Such advancements promote the efficient utilization of mushrooms as natural resources for food, pharmaceuticals, and nutraceuticals. Furthermore, this study lays a foundation for future research into the growth mechanisms and metabolic synthesis processes of *S. commune* under elicitor applications, contributing to the sustainable management and optimization of biological resources.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/resources14010003/s1, Table S1: The mineral content of *S. commune* from the exogenous application of MeJA at various concentrations; Table S2: Proximate composition of *S. commune* from the exogenous application of MeJA at various concentrations; Table S3. Total triterpenoid content of *S. commune* from the exogenous application of MeJA at various concentrations.

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