Effect of microalgae extract Fortification and Timing of Application in the Casing Layer on Yield of *Agaricus bisporus*

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Abstract

This study aimed to evaluate the effect of fortifying the casing layer with the cynobacterium *Spirulina platensis* and the microalga *Chlorella vulgaris*, as well as different application timings, on enhancing the yield and quality characteristics of the cultivated white mushroom Agaricus bisporus. The trial was carried out at the station belonging to Department of Horticulture – College of Agriculture / University of Diyala in a complete randomized design (CRD) as factorial experiment in three factors,, including three addition timings (at casing, after 8 days from casing, and at the beginning of primordia formation), and three concentrations for each of Spirulina and Chlorella (0, 2000, 4000 mg), with three replicates. The results showed significant differences in most studied traits according to algal type, its concentration, and the timing of its addition. The timing of addition after 8 days from casing achieved the highest mean for total yield and number of fruiting bodies compared with the other timings. Spirulina treatments, particularly at 4000 mg, were superior in increasing total yield and number of fruiting bodies, whereas Chlorella treatments contributed to a greater extent to improving quality traits, particularly the mean fruiting body weight and cap diameter. The three-way interaction between the timing of addition after 8 days from casing and Spirulina at 4000 mg with the control treatment recorded the highest total yield.

Keywords: white button mushroom, Agaricus bisporus, casing layer, marine algae, Spirulina, Chlorella.

Introduction

The genus Agaricus is classified within the macrofungi and encompasses a number of species of nutritional and therapeutic importance, most notably Agaricus bisporus and Agaricus subrufescens. According to modern classifications, this genus comprises six subgenera and 24 sections, in addition to more than 500 species scientifically described to date [1, 2]. A. bisporus, commonly known as the button mushroom or white mushroom, is the most widely cultivated and distributed mushroom species globally, particularly in Europe, North America, and China [3]. China holds the top rank worldwide in the production of this mushroom, followed by the United States of America and a number of European and Asian countries with varying proportions, reflecting the significant economic importance of this species within the global food production system [4].

The importance of *A. bisporus* is highlighted by its status as a relatively complete food, containing high proportions of high-quality proteins, in addition to most of the essential amino acids that the human body cannot synthesize, include lysine, methionine, leucine, and threonine. This makes it an important dietary alternative, especially in vegetarian diets. It is also characterized by low fat and calorie content, and contains dietary fiber, vitamins, and essential mineral elements, placing it among what are known as functional foods with a positive impact on human health [5, 6].

In addition to its nutritional value, A. bisporus has gained a growing medical and health importance due to its presence of bioactive components including the polyphenols, beta-glucans, ergothioneine, and ergosterol. Recent research demonstrates their positive effects on immune system, the antioxidant activity, heart disease risk reduction and preventing some types of cancer, as well as their anti-inflammatory and antimicrobial properties. This has also driven interest in mushrooms not only as a food, but also as dietary factors for promoting overall health [7, 8].

Mushroom *Agaricus bisporus* is cultivated through a series of well-defined technical stages which have direct influence on the productivity and efficiency performance. The casing layer (Cl) is often regarded as one of the most critical steps in the cultivation cycle, since it significantly promotes the switch from vegetative growth of mycelium to fruiting stage. The casing layer also plays a role in controlling moisture, allowing gas exchange and the supply of a stimulatory microbial environment for fruiting body formation; thus, its physical, chemical and biological properties are one of the determining factors for production quantity and quality [9, 10].

The casing layer is typically made of relatively inert substances such as peat moss and agricultural soil supplemented with calcium carbonate; its main function is to serve as the proper medium in terms of porosity and water retention abilities, but not a direct food source for the mushroom. Nutrient exhaustion of the growing medium during multiple flushes usually is associated with reduced yield and gradual loss in quality characteristics of fruit bodies thus leading research to investigate possible enhancement of nutritional value of the casing soil by adding various substances in order to enhance its productivity and functional efficiency [11]. In this regard, the Biostimulant trend has captured attention as a sustainable alternative to traditional supplements with low negative effects on human health and environmental safety without decreasing the overall efficiency of microbial activity without causing negative impacts on the ecosystem. Indeed, several studies have shown that amending the casing layer with organic materials or natural extracts leads to higher numbers and mean weight of fruiting bodies and better protein and mineral content, which translates into a better biological efficiency and total mushroom yield [4].

Extracts of marine algae attracted much more scientific attention in the agricultural sector, because it is natural protein, amino acid, vitamin and mineral sources present in algal extract as well as containing active growth promoting substances and effective antioxidants. Recent research has shown that microalgae are potentially sustainable biostimulants giving rise to increase plant growth and yield in various crops, exhibiting new opportunities for enhancing modern farming systems due to their non-toxic, biodegradable and environment-friendly nature [12].

Among these algae, Chlorella and Spirulina stand out, as recent studies have shown they possess a high capacity to improve the accumulation of active compounds within the tissues of some plants with ex vivo medicinal uses, in addition to their role in improving the physiological and chemical characteristics of cultivation substrates [13].

The work of Oztekin and Kurt [14] reported the influence marine alga extract *Ecklonia maxima* on mycelial growth of

Pleurotus ostreatus. The two scientists have employed different concentrations of the algal extract in PDA growth medium. The results demonstrated that mycelium of treatments supplemented with 2% *E. maxima* extract had the highest growth rate compared to control (without any supplement) and among all the treatments. The growth speed was also decreased, but accentuated at higher level such as 3 and 4%, indicating that moderate concentration is recommended for accelerating mycelial development and enhancement of mushroom yield which ultimately led to increase the biological efficiency of cultivated mushroom. This enhancement of growth was attributed to the presence of biostimulatory substances in algal extracts such as auxins and gibberellins and some mineral elements like iron, magnesium and zinc, besides amino acid and sugars enhancing the biological activity of the mushroom. Moreover, Sezen and Turunçoğlu [15] emphasized that incorporating microalgae such as *Cladophora glomerata* (green algae) and *Spirulina platensis* (blue-green algae) into the casing layer of the white mushroom (*Agaricus bisporus*), had a beneficial impact on mushroom growth and yield.

The addition of Spirulina platensis led to an increase in total yield by up to 15%, whereas Cladophora glomerata achieved an increase of about 7% compared with the standard treatment. The results indicated that higher concentrations of algal extracts improved the formation of fruiting bodies and improved the structure of mycelial growth and its density, and increased the nutritional content of the mushroom, especially (protein and heavy minerals), which makes Spirulina platensis the most effective in enhancing productivity and quality.

The possibility of using extracts of these algae as an alternative to conventional supplements in the casing layer opens new horizons for producing cultivated mushrooms with standard organic specifications, which contributes to filling the food gap with a high-value product and using innovative and environmentally friendly agricultural technologies. Based on the above, this study was conducted to achieve the following objectives:

1- Evaluation of the effect of fortifying the casing layer (Casing) with Spirulina and Chlorella algae in improving productive traits and biological efficiency of the white cultivated mushroom.

2- Study of the effect of the timing of adding marine algae to the casing layer at different growth stages on productive traits and the nutritional value of the produced mushroom.

3- Determination of the effect of the interaction between the type of marine algae and the time of addition in improving the nutritional content of the mushroom and identifying the best treatment that achieves the highest quality and productivity.

Materials and Methods

Stages of edible mushroom production

Preparation of the production hall

The experiment was conducted at the research station to the Department of Horticulture, College of Agriculture, University of Diyala. A production hall with dimensions of 4 × 7 m was selected. The hall was disinfected with commercial formalin solution (37%) at a concentration of 4% by spraying the floors and walls, then it was tightly closed for three consecutive days to ensure elimination of microbial contaminants. Then, a comprehensive ventilation process was carried out to remove residues of the disinfectant substance.

Preparation of organic compost (Compost)

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Two thousand kilograms of wheat straw were mixed with 1200 kg of poultry residues, 30 kg of calcium sulfate and 10 kg of calcium ammonium nitrite were mixed. Then, the compound was moistened and spread out as a heap 3 m wide and 1.7 m high. Fermentation was carried out for 2 weeks with rotation every three days until moisture reached 60–70% and temperature was within the range of 60–70˚C. After that, transfer of the heap took place for steam pasteurization by air circulation (400 m^3/ton/hour) until ammonia concentration and temperature were cooled to 25°C. The medium showed values of moisture content at 69%, pH=7.7, ash at 2.7%, nitrogen at a level of 2% and N:C ratio as low as1:17.8

Pasteurization and preparation of the growth medium:

In mid-January 2025, the pile was transferred to the pasteurization room, where steam was pumped and an air current was provided at a rate of 400 m^3/ton/hour. On the second day, the temperature was raised to 60°C for 6 hours, then it was gradually reduced until the sixth day to reach 47°C. After confirming the disappearance of the ammonia odor, the temperature was lowered to 25°C, and the medium became ready for inoculation.

Inoculation and incubation:

On 19/1/2025, the medium was inoculated with Agaricus bisporus spawn at a rate of 1–2% of the compost weight. The trays were transferred to dark incubation conditions for 14–18 days, while maintaining a temperature of 25±2°C and a relative humidity of not less than 70% to ensure the spread of the mycelium throughout the medium.

Casing stage:

When hyphal growth above the organic medium was completed, a casing layer of peat moss with a thickness of 4 cm was added. Relative humidity was raised to ≥70% to maintain layer moisture, and the temperature was maintained at 25±2°C.

Primordia (Pin-head) formation stage:

With the beginning of the appearance of tissue aggregates of hyphae under the casing layer, the hall temperature was reduced to 16°C, and humidity was increased to approximately 90%, while avoiding direct air currents. Water was sprayed using fine sprayers several times daily.

Cropping stage:

Relative humidity was reduced to <80%, CO_2 concentration was maintained between 1100–1300 ppm, and temperature was maintained at 17–18°C. After 5 days from the appearance of pinheads, the first three flushes (economic flushes) were harvested and the required measurements were conducted.

Experimental design and number of treatments:

The experiment was carried out according to a Completely Randomized Design (CRD) as a factorial experiment with three factors. The first factor (D) was the timing of addition (at casing, after 8 days from casing, and at the beginning of pinhead appearance). The second factor (C) was fortification with Chlorella algae at three levels (0, 2000, and 4000) mg per experimental unit (40 ×30 cm) . The third factor (S) was fortification with Spirulina algae at three levels (0, 2000, and 4000) mg per experimental unit. Each treatment was replicated three times, so that the number of experimental units became 81 experimental units. Data were taken from each experimental unit (40 ×30 cm) and analyzed according to the statistical program SAS (2003), and arithmetic means were compared using Duncan's multiple range test at a probability level of 0.05 [16].

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The powders were distributed on the casing layer uniformly at each timing according to the specified concentration for each treatment.

Studied traits

Total yield (g):

It was estimated by summing the yield produced for each experimental unit from three harvests (economic flushes), and it was expressed on the basis of g per experimental unit.

Number of fruiting bodies (fruiting body):

It was estimated by calculating the total number of fruits produced for each experimental unit from three harvests (economic flushes).

Fruiting body weight (g):

It was estimated using the following equation:

$$\text{Fruiting body weight} = \frac{\text{total weight of fruiting bodies produced from an experimental unit}}{\text{number of fruiting bodies produced from an experimental unit}}$$

Stipe length of the fruiting body (cm):

The stipe length of each fruiting body was estimated using a graduated ruler.

Cap diameter of the fruiting body (cm):

The cap diameter of each fruiting body was estimated using a graduated ruler.

Results and Discussion

Total yield

The results in Table 1 show the presence of significant differences in total yield. The second timing was superior by giving the highest total yield of 1344.06 g, whereas the third timing recorded the lowest total yield of 1105.04 g. Regarding the effect of Spirulina algae treatments, the 4000 mg treatment recorded the highest total yield of 1287.77 g, whereas the 2000 mg treatment recorded the lowest total yield of 1163.66 g. The control treatment and Chlorella algae at a concentration of 4000 mg also showed significant superiority by giving the highest total yield of 1262.69 and 1233.88 g, respectively.

As for the two-way interactions, the interaction between the second timing and Spirulina algae at a concentration of 4000 mg was superior with the highest total yield of 1559.40 g, whereas the interaction between the third timing and the control gave the lowest total yield of 978.99 g. The two-way interaction between the second timing of addition and the control treatment also recorded the highest total yield of 1396.95 g.

With respect to the interaction between the marine algae, significant differences among interactions were observed. The interaction between Spirulina algae and the control treatment recorded the highest total yield of 1561.12 g, whereas the yield decreased to its lowest level in the control treatment (861.66 g).

Regarding the three-way interaction, the interaction of the second timing with Spirulina algae at 4000 mg with the control recorded the highest total yield of 1869.14 g, whereas the interaction between the second timing with no addition of Spirulina and Chlorella algae recorded the lowest yield of 793.78 g.

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Table 1. Effect of supplementing the casing layer with spirulina and chlorella and the timing of their addition on the total yield (g) of *Agaricus bisporus*.

Addition timing	Spirulina (mg L⁻1)	Chlorella 0	Chlorella 2000 mg	Chlorella 4000 mg	timing × spirulina
Addition at casing	0	901.82 hi	1526.13 b	1664.43 b	1364.13 ab
	2000 mg	1319.18 cd	929.97 ghi	1123.09	1124.08
	4000 mg	1254.64 de	955.09	868.47 i	1026.06 de
Addition 8 days after casing	0	793.78 i	1227.29 de	1471.49 bc	1164.19
	2000 mg	1527.94 b	1246.50 de	1151.36	1308.60 bc
	4000 mg	1869.14 a	1244.72 de	1564.35 b	1559.40 a
Addition at the start of pin	0	889.38 hi	917.90 hi	1129.70	978.99 e
	2000 mg	1248.69 de	983.51	942.68 ghi	1058.29
	4000 mg	1559.60 b	1084.56	1189.39 de	1277.85

Addition timing × chlorella				
Addition at casing	1158.54 ab	1137.06 ab	1218.66 ab	1171.42 B
Addition 8 days after casing	1396.95 a	1239.50 ab	1395.73 a	1344.06 A
Addition at the start of pin	1232.56 ab	995.32 b	1087.26 b	1105.04 C

Spirulina × chlorella				
0	861.66 d	1223.78 bc	1421.87 ab	1169.10 B
2000 mg	1365.27 ab	1053.32 cd	1072.38 cd	1163.66 B
4000 mg	1561.12 a	1094.79 c	1207.40 bc	1287.77 A

Overall means of chlorella		
Chlorella 0	Chlorella 2000 mg	Chlorella 4000 mg
1262.69 A	1123.96 B	1233.88 A

Number of fruiting bodies

The results in Table 2 show significant differences in the number of fruiting bodies. The second timing was superior by giving the highest number, 84.074 fruiting bodies, whereas the first timing recorded the lowest number, 63.11 fruiting bodies. Regarding the effect of Spirulina algae treatments, the 2000 mg treatment recorded the highest number, 81.186 fruiting bodies, whereas the control treatment recorded the lowest number, 69.48 fruiting bodies. It appears that the control treatment for Chlorella was superior by giving the highest number of fruiting bodies, 79.96, whereas the Chlorella 4000 mg treatment gave the lowest number of fruiting bodies, 69.85.

As for the two-way interactions, the interaction between the second timing and Spirulina algae at 2000 mg gave the highest number of fruiting bodies, 93.33, whereas the interaction between the first timing and Spirulina algae at 4000 mg gave the lowest number, 53.33. The interaction between the second timing of addition and the control treatment was also superior by giving the highest number of fruiting bodies, 92.44, whereas the first timing with Chlorella algae at 2000 mg gave the lowest number, 59.89. Regarding the interaction between the marine algae, the two interactions between Spirulina algae at 2000 mg and the control recorded the highest number of fruiting bodies, 95.66, whereas the control treatment gave the lowest number of fruiting bodies, 59.78.

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For the three-way interaction effect, the interaction of the second timing with Spirulina algae at 2000 mg with the control recorded the highest number of fruiting bodies, 108.0, whereas the interaction between the first timing with marine algae at 4000 mg recorded the lowest number, 46.667 fruiting bodies.

Table 2. Effect of supplementing the casing layer with spirulina and chlorella and the timing of their addition on number of fruiting bodies of *Agaricus bisporus*.

Addition timing	Spirulina $(mg\ L^{-1})$	Chlorella 0	Chlorella 2000 mg	Chlorella 4000 mg	timing × spirulina
Addition at casing	0	54.333 klm	76.667 efgh	79.333 defg	70.111 cde
	2000 mg	86.320	51.010 mn	60.340	65.890 de
	4000 mg	61.333 ijkl	52.000 lmn	46.667 n	53.333 f
Addition 8 days after casing	0	62.667 ijk	84.000	87.333 bcd	78.000 bcd
	2000 mg	108.000 a	89.667 bc	82.333 cdef	93.333 a
	4000 mg	106.667 a	70.000 ghi	66.000 ij	80.889 bc
Addition at the start of pin	0	62.333 ijk	56.000	62.667 ijk	60.333 ef
	2000 mg	92.667 b	84.333	76.000 fgh	84.333 ab
	4000 mg	85.333	75.653 fgh	68.013 hi	76.333 bcd

Addition timing × chlorella				
Addition at	67.329 bc	59.892 c	62.113 c	63.111 C
Addition 8	92.444 a	81.222 ab	78.556 ab	84.074 A
Addition at the	80.111 ab	71.996 bc	68.893 bc	73.667 B

Spirulina × chlorella				
0	59.778 d	72.222 bcd	76.444 bc	69.481 B
2000 mg	95.662 a	75.003 bc	72.89 bcd	81.186 A
4000 mg	84.444 ab	65.884 cd	60.227 d	70.185 B

Overall means of chlorella		
Chlorella 0	Chlorella 2000 mg	Chlorella 4000 mg
79.961 A	71.037 B	69.854 B

Fruiting body weight (g fruiting body^{-1})

The results in Table 3 show significant differences in fruit weight. The first timing was superior by giving the highest weight of 18.5848 g, whereas the third timing recorded the lowest weight of 15.1522 g. Regarding the effect of Spirulina algae treatments, the 4000 mg treatment recorded the highest weight of 18.5063 g, whereas the 2000 mg treatment recorded the lowest weight of 14.6393 g. It also appears that the 4000 mg Chlorella treatment was superior by giving the highest weight of 17.8537 g, whereas the control gave the lowest fruit weight of 15.8726 g.

With respect to the two-way interactions, the interaction between the second timing of addition and Spirulina algae at a concentration of 4000 mg gave a fruit weight of 19.6233 g, whereas the interaction between the third timing of addition and the 2000 mg level gave the lowest fruit weight of 12.5100 g. Significant differences were present among the two-way interactions. The first timing of addition with Chlorella algae at concentrations of 4000 and 2000 gave the highest fruit weights of 19.414 and 18.859 g, respectively, whereas the third timing of addition recorded the lowest fruit weight of 14.132 g.

The two-way interactions between the marine algae gave significant differences, where the 4000 mg concentration recorded the highest fruit weight of 19.913 g, whereas the interaction at a concentration of 2000 mg gave the lowest fruit weight of 14.327 g.

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Regarding the effect of the three-way interaction, the second timing and the 4000 mg concentration of the marine algae recorded the highest fruit weight of 23.5667 g, whereas the third timing of addition with marine algae at a concentration of 2000 mg gave the lowest fruit weight of 11.6567 g.

Table 3. Effect of enriching the casing layer with spirulina and chlorella and addition timing on mean fruit body weight (g) of Agaricus bisporus

Addition timing	Spirulina (mg L⁻ ¹)	Chlorella 0	Chlorella 2000 mg	Chlorella 4000 mg	timing × spirulina
Addition at casing	0	16.6233 fgh	19.9100 bc	20.9833 b	19.1722 ab
	2000 mg	15.3500 hi	18.2400 de	18.5833 cd	17.3911 bc
	4000 mg	20.4700 b	18.4267 de	18.6767 cd	19.1911 ab
Addition 8 days after casing	0	12.6933 klm	14.6200 ij	16.9367 efg	14.7500 de
	2000 mg	14.1667 ijk	13.8900 ijk	13.993 ijk	14.0167 ef
	4000 mg	17.5200 defg	17.7833 defg	23.5667 a	19.6233 a
Addition at the start of pin formation	0	14.2933 ij	16.3967 gh	18.0367 def	16.2422 cd
	2000 mg	13.4633 jkl	11.6567 m	12.4100 lm	12.5100 f
	4000 mg	18.2733 de	14.3433 ij	17.4967 defg	16.7044 c
		Mean		Addition timing × chlorella	
Addition at casing		17.481 abc	18.859 a	19.414 a	18.5848 A
Addition 8 days after casing		14.793 d	15.431 cd	18.166 ab	16.1300 B
Addition at the start of pin formation		15.343 cd	14.132 d	15.981 bcd	15.1522 C
		Mean		Spirulina × chlorella	
0		14.537 d	16.976 bc	18.652 ab	16.7215 B
2000 mg		14.327 d	14.596 d	14.996 cd	14.6393 C
4000 mg		18.754 ab	16.851 bc	19.913 a	18.5063 A
Overall means of chlorella					
		Chlorella 0	Chlorella 2000 mg	Chlorella 4000 mg	
		15.8726 B	16.1407 B	17.8537 A	

Stipe length of the fruiting body

The results in Table 4 show significant differences in stipe length. The first and third timings gave the highest stipe length, 27.8222 and 26.8148 mm, respectively, whereas the second timing recorded the lowest stipe

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length of 24.6926 mm. Regarding the effect of Spirulina algae treatments, the 2000 mg treatment was superior with the highest stipe length of 28.8889 mm, whereas the control treatment recorded the lowest stipe length of 24.3111 mm. It appears that the 4000 mg Chlorella treatment and the control were superior by giving the highest stipe lengths of 27.0667 and 27.0037 mm, respectively, whereas the Chlorella 2000 mg treatment gave the lowest stipe length of 25.2593 mm.

As for the two-way interactions, the interaction between the third timing and Spirulina algae at 2000 mg gave the highest stipe length of 31.978 mm, whereas the control at the second timing gave the lowest stipe length of 22.889 mm. Significant differences were found in the interaction between the third timing of addition and the control, which gave the highest stipe length of 30.589 mm, whereas the third timing with Chlorella algae at 2000 mg gave the lowest stipe length of 24.178 mm.

With regard to the interaction between the marine algae, significant differences among interactions were observed. The interaction between Spirulina at 2000 mg and Chlorella at 4000 mg gave the highest stipe length of 30.900 mm, whereas the 4000 mg treatment with the control gave the lowest value of 22.778 mm.

Regarding the effect of the three-way interaction, the interaction of the third timing with Spirulina at 2000 mg and Chlorella at 4000 mg recorded the highest stipe length of 36.700 mm, whereas the interaction between the third timing with Chlorella at 2000 mg and the control recorded the lowest stipe length of 18.700 mm.

Table 4. Effect of enriching the casing layer with spirulina and chlorella and addition timing on stipe length (mm) of Agaricus bisporus

Addition timing	Spirulina (mg L⁻ ¹)	Chlorella 0	Chlorella 2000 mg	Chlorella 4000 mg	timing × spirulina
Addition at casing	0	23.267 ijk	29.100 cde	21.167 kl	24.511 c
	2000 mg	27.533 defg	30.767 bcd	28.800 def	29.033 ab
	4000 mg	24.067 ghijk	23.233 ijk	33.400 ab	26.900 bc
Addition 8 days after casing	0	22.367 ijk	24.400 ghijk	21.900 jkl	22.889 c
	2000 mg	26.300 efghi	23.467 hijk	27.200 defgh	25.656 bc
	4000 mg	27.733 defg	23.833 ghijk	25.033 fghijk	25.533 bc
Addition at the start of pin formation	0	32.633 bc	18.700 l	25.267 efghij	25.533 bc
	2000 mg	28.633 def	30.600 bcd	36.700 a	31.978 A
	4000 mg	30.500 bcd	23.233 ijk	24.133 ghijk	25.956 bc
Addition timing × chlorella					Mean
Addition at casing		24.956 bc	27.700 abc	27.789 abc	26.8148 A

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Addition 8 days after casing	25.467 bc	23.900 c	24.711 bc	24.6926 B
Addition at the start of pin formation	30.589 a	24.178 c	28.700 ab	27.8222 A
Spirulina × chlorella Mean				
0	26.089 bcd	24.067 cd	22.778 d	24.3111 C
2000 mg	27.489 abc	28.278 ab	30.900 a	28.8889 A
4000 mg	27.433 abc	23.433 cd	27.522 abc	26.1269 B
Overall means of chlorella				
	Chlorella 0	Chlorella 2000 mg	Chlorella 4000 mg	
	27.0037 A	25.2593 B	27.0667 A	

Cap diameter of the fruiting body

The results in Table 6 show significant differences in the mean cap diameter. The third timing was superior by giving the highest cap diameter of 48.2944 mm, followed by the first timing with a diameter of 46.9222 mm, whereas the second timing recorded the lowest cap diameter of 45.7952 mm. Regarding the effect of Spirulina algae treatments, the 4000 mg treatment recorded the highest mean diameter of 48.6419 mm, whereas the control gave the lowest cap diameter of 45.3407 mm. It appears that the Chlorella treatment at a concentration of 4000 mg was superior by giving the highest mean cap diameter of 48.7233 mm compared with the other treatments.

With respect to the two-way interactions between the timing of addition and Spirulina algae, the third timing of addition with the control was superior by giving the largest diameter of 51.856 mm, whereas the first timing with the control gave the lowest cap diameter of 42.811 mm. Regarding the interaction between the marine algae, no significant differences were observed among interactions.

Regarding the effect of the three-way interaction, the interaction of the first timing with the marine algae at a concentration of 4000 mg recorded the highest mean cap diameter of 57.867 mm, whereas the interaction between the second timing with the marine algae at a concentration of 2000 mg recorded the lowest mean cap diameter of 35.757 mm.

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Table 5. Effect of enriching the casing layer with spirulina and chlorella and addition timing on cap diameter (mm) of *Agaricus bisporus*

Addition timing	Spirulina (mg L⁻ ¹)	Chlorella 0	Chlorella 2000 mg	Chlorella 4000 mg	timing × spirulina
Addition at casing	0	37.067 ij	48.433 defg	45.633 fgh	43.711 a
	2000 mg	48.067 defg	51.433 bcde	43.967 gh	47.822 a
	4000 mg	43.300 gh	46.533 efg	57.867 a	49.233 a
Addition 8 days after casing	0	41.100 hi	47.300 efg	45.733 fgh	44.711 a
	2000 mg	46.500 efg	35.757 j	52.400 bcd	44.886 a
	4000 mg	45.533 fgh	54.200 abc	43.633 gh	47.789 a
Addition at the start of pin formation	0	55.733 ab	36.633 ij	50.433 cdef	47.600 a
	2000 mg	44.967 gh	46.440 efg	53.733 abc	48.380 a
	4000 mg	54.867 abc	46.733 efg	45.110 gh	48.903 a
Addition timing × chlorella					Mean
Addition at casing		42.811 d	48.800 abc	49.156 ab	46.9222 AB
Addition 8 days after casing		44.378 bcd	45.752 bcd	47.256 abcd	45.7952 B
Addition at the start of pin formation		51.856 a	43.269 cd	49.759 ab	48.2944 A
Spirulina × chlorella					Mean
0		44.633 a	44.122 a	47.267 a	45.3407 C
2000 mg		46.511 a	44.543 a	50.033 a	47.0293 B
4000 mg		47.900 a	49.156 a	48.870 a	48.6419 A
Overall means of chlorella					
		Chlorella 0	Chlorella 2000 mg	Chlorella 4000 mg	
		46.3481 B	45.9404 B	48.7233 A	

Discussion

The results of the total yield (Table 1) indicate that the second addition timing (after 8 days from casing) achieved the highest mean total yield compared with the other timings, which indicates that the period immediately after casing represents a physiologically sensitive period in which the mycelium is in the phase of transition toward fruiting, and the casing layer is more capable of responding to biostimulants. The treatment interactions also showed the superiority of the Spirulina treatment at a concentration of 4000 mg L⁻ ¹ at the second timing, achieving the highest total yield, whereas some Chlorella treatments recorded high values but were lower than this treatment, which confirms that algal type, its concentration, and the timing of its addition all integrate in forming the pattern of productive response.

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The clear improvement in total yield and its components and a number of morphological traits under the effect of adding marine algae (Spirulina and Chlorella) is attributed to their role as biostimulants that contribute to activating the micro-environment of the casing layer biologically and chemically, which is reflected positively on the efficiency of the transition of growth from the vegetative phase to the phase of primordia formation. The casing layer is considered a governing factor in the initiation of fruiting, due to its role in regulating moisture, aeration, and gas balance, in addition to providing a stimulatory microbial environment. Some marine algae also contain active compounds and plant growth regulators or similar compounds, such as auxins, gibberellins, and cytokinins, which may enhance the physiological response associated with the initiation of fruiting when they are available in the cultivation medium or in the environment surrounding the mycelium [15].

The results of the table of the number of fruiting bodies (Table 2) show that the increase in total yield in a number of treatments was mainly associated with an increase in fruiting density, as the second addition timing was superior in total fruit number compared with the first addition timing. Spirulina treatments at the second timing also achieved the highest number of fruiting bodies within the studied interactions. This is attributed to the intensity of competition for nutritional and water resources within the production unit when fruiting density increases, which leads to a decrease in the mean weight of a single fruiting body [17]. Mustaf et al. [18] also confirmed this phenomenon clearly, as they showed that the treatments that gave the highest number of fruiting bodies recorded the lowest fruit weight due to the intensity of crowding within the unit area.

The effect of crowding also appears in the morphological traits of the stipe, as correlation treatments indicated the presence of a moderate positive and significant relationship between fruit number and each of stipe length. This may be due to the response of stipes to increased fruiting body density and increased CO_2 concentration in the micro-environment surrounding them, through elongation or increased diameter as morphological adaptations under less aerated conditions. These findings are consistent with what was reported by Wang and Zhao [17] and in line with what had mentioned from Mustaf et al. [18], who found that induction treatments resulting in a higher number of mushrooms also caused greater stipe lengths because of crowding and higher CO_2 concentration in the micro-environmental condition around the fruiting bodies

As to cap diameter, it was positively and significantly correlated with total yield, suggesting that enlarged cap diameter results in increased weight of the overall yield and its quality traits. This result is in line with the study of Radmann et al. [19] who reported that an increase in nutrition and organic matter content could enhance cap tissue thickness and raise the market Mustafa et al. [18] also showed that quality. treatments in which the number of fruiting bodies decreased were superior in cap diameter due to reduced crowding, improved aeration, and availability of nutrients for each fruiting body.

The results of the current study agree with those of Sezen and Turunçoğlu [15] regarding the possibility of increasing the productivity of *A. bisporus* using marine algal extract; however, the difference in the magnitude and pattern of response between the two studies can be explained by differences in the method and timing of addition and the targeted physiological stage. Their study relied on adding the extract by injection inside the

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compost during the vegetative growth stage and before casing, whereas the current study focused on addition timings after casing. Based on the above, it is clear that the effect of Spirulina was mostly directed toward enhancing yield through increasing the number of fruiting bodies, whereas the effect of Chlorella and some interaction treatments was directed to a greater extent toward improving quality traits, especially those related to mean fruit weight and cap thickness. Accordingly, the role of algal type and the timing of its addition is not limited to controlling the magnitude of quantitative increase in yield, but extends to directing the pattern of productive response either toward increasing fruit number or toward improving their quality characteristics, within the known physiological framework of *A. bisporus* production.

Conclusion

Supplementing casing layer with marine algae enhanced yield and quality of Agaricus bisporus. Maximum total yield and number of fruiting bodies was obtained from the addition 8 days after casing. Spirulina, especially at 4000 mg, was found superior for total yield and number of fruiting bodies increase, while Chlorella was relatively beneficial to some quality traits (mean weight of fruiting body and cap diameter)." The highest yield was obtained when Spirulina was added at 4000 mg after 8 days from casing along with the control.

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Conflict of interest

The authors declare no conflict of interest.

Contribution of authors

All authors contributed to the study conception and design. Material preparation, experiment implementation, and data collection were performed by the authors. Statistical analysis and interpretation were carried out by the authors. The manuscript was written and revised by the authors, and all authors read and approved the final version.

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