Enhanced mycelial biomass production of *Cordyceps militaris* Q5 and evaluation of selected bioactive compounds

Nguyen Nhu Ngoc*, Giang Lao Di, Vu Kim Dung, Nguyen Thi Hong Gam
Vietnam National University of Forestry

Nâng cao khả năng thu nhận sinh khối hệ sợi *Cordyceps militaris* Q5 và đánh giá hàm lượng một số hợp chất có hoạt tính sinh học

Nguyễn Như Ngọc*, Giàng Lao Di, Vũ Kim Dung, Nguyễn Thị Hồng Gấm
Trường Đại học Lâm nghiệp
**Corresponding author: ngocnn@vnuf.edu.vn*

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ABSTRACT

Submerged mycelia cultivation of Cordyceps militaris has actracted growing interest due to its short cultivation cycle, scalability, and potential for producing valuable bioactive metabolites. This study aimed to optimize submerged culture for maximizing the mycelial dry weight (MDW) of Codyceps militaris strain Q5 and evaluate the contents of selected bioactive compound. Preliminary screening identified glucose, soybean powder, $MgSO_4 \cdot 7H_2O$, agitation speed, and initial pH as important factors affecting mycelial growth with baseline levels selected at 20 g/L glucose, 10 g/L soybean powder, 0.25 g/L $MgSO_4 \cdot 7H_2O$, 200 rpm, and pH 6.5. Response surface methodology (RSM) was subsequently applied to optimize glucose concentration, initial pH, and agitation speed, while maintaining the remaining parameters constant. The optimal conditions were determined to be 19.9 g/L glucose, pH 6.6, and 195 rpm, yielding a maximum MDW of 39.8 g/L. Analysis of the mycelial extract revealed adenosine, cordycepin, polysaccharides, and polyphenols at concentrations of 2.86, 4.32, 158.23, and 43.87 mg/g, respectively. These findings demonstrate the potential of culture optimization to enhance C. militaris Q5 biomass production and provide a solid basis for process scale up and applications in functional foods and biotechnology.

Từ khóa:
Cordyceps militaris, hợp chất có hoạt tính sinh học, nuôi cấy chìm, phương pháp bề mặt đáp ứng, sinh khối hệ sợi.

TÓM TẮT

Nuôi cấy hệ sợi nấm Cordyceps militaris ngày càng được quan tâm nhờ thời gian nuôi cấy ngắn, khả năng mở rộng quy mô và tiềm năng sản xuất các hợp chất có hoạt tính sinh học. Nghiên cứu này nhằm tối ưu hóa điều kiện nuôi cấy chìm để đạt khối lượng khô hệ sợi (MDW) tối đa của chủng Q5 và xác định hàm lượng một số hợp chất có hoạt tính sinh học. Kết quả sàng lọc ban đầu xác định glucose, bột đậu tương, $MgSO_4 \cdot 7H_2O$, tốc độ lắc và pH ban đầu là các yếu tố quan trọng ảnh hưởng đến sinh trưởng hệ sợi, với các mức cơ sở được lựa chọn gồm: glucose 20 g/L, bột đậu tương 10 g/L, $MgSO_4 \cdot 7H_2O$ 0,25 g/L, tốc độ lắc 200 vòng/phút và pH 6,5. Phương pháp bề mặt đáp ứng (RSM) được sử dụng để tối ưu hóa nồng độ glucose, pH ban đầu và tốc độ lắc, trong khi cố định các thông số còn lại. Điều kiện tối ưu được xác định gồm: glucose 19,9 g/L, pH 6,6 và tốc độ lắc 195 vòng/phút, cho MDW đạt 39,8 g/L. Phân tích dịch chiết hệ sợi cho thấy hàm lượng adenosine, cordycepin, polysaccharide và polyphenol lần lượt là 2,86; 4,32; 158,23 và 43,87 mg/g. Những kết quả này khẳng định hiệu quả của việc tối ưu hóa điều kiện nuôi cấy nhằm nâng cao sinh khối chủng C. militaris Q5, đồng thời tạo cơ sở khoa học cho việc mở rộng quy mô sản xuất và ứng dụng trong lĩnh vực thực phẩm cũng như công nghệ sinh học.

1. INTRODUCTION

Cordyceps militaris is a medicinal mushroom of considerable scientific interest due to its diverse bioactive metabolites and pharmacological activities. Both fruiting bodies and mycelia contain key compounds, including cordycepin, adenosine, polysaccharides, and phenolics, with antioxidant, anti-inflammatory, and immunomodulatory effects [1, 2]. Cordycepin (3'-deoxyadenosine) and adenosine are key chemical markers of therapeutic efficacy, while polysaccharides and polyphenols contribute to immunomodulatory and radical-scavenging activities. [3].

Growing evidence indicates that mycelial biomass from submerged fermentation is an efficient alternative to fruiting bodies, with cordycepin levels comparable to or exceeding those in natural fruiting bodies under optimized conditions [2]. This underscores the potential of mycelial cultivation as a reliable, scalable, and standardized platform for the production of high-value fungal metabolites.

Conventional cultivation of *C. militaris* fruiting bodies is limited by long production cycles and environmental sensitivity, whereas submerged mycelial cultivation enables rapid biomass production, precise process control, and industrial scalability [4]. Therefore, optimizing biomass recovery and metabolite accumulation in the Vietnamese *C. militaris* strain Q5 is essential for the sustainable development of the functional food and pharmaceutical industries.

2. RESEARCH METHODS

2.1. Materials

C. militaris strain Q5, provided by College of Forestry Biotechnology. The strain was previously authenticated by the provider using ITS sequencing. For stock maintenance, the fungus was cultured on Potato Dextrose Agar (PDA; 200 g/L potato infusion, 20 g/L glucose, and 15 g/L agar), stored in the dark at 4°C, and transferred weekly to fresh PDA medium using mycelial plugs (5 × 5 mm) for subsequent studies.

2.2. Methods

2.2.1. Effects of Nutritional and Environmental Factors on Mycelial Biomass Production

The effects of nutritional and environmental factors on the mycelial growth of *C. militaris* strain Q5 were evaluated using a one-factor-at-a-time (OFAT) approach in submerged culture. The basal medium (potato extract 200 g/L, dextrose 20 g/L, pH 6.0) was modified to assess different nutritional sources: carbon sources (glucose, sucrose, maltose, and soluble starch) at 15 g/L; nitrogen sources (yeast extract, peptone, NH_4NO_3, soybean extract, and dried silkworm pupae powder) at 5 g/L; and mineral salts ($MgSO_4 \cdot 7H_2O$, KCl, $ZnSO_4$, $CaCl_2 \cdot 2H_2O$, and Na_2SO_4) at 0.25 g/L. Initial pH (4.5–7.5) and agitation speed (100–250 rpm) were also investigated. All media were sterilized at 121°C for 30 min and dispensed into 500 mL Erlenmeyer flasks (200 mL per flask). Each flask was inoculated with 5% (v/v) of a 5-day-old seed culture and incubated at 22°C for 10 days in darkness. Mycelial biomass was harvested using a 200-mesh nylon filter, washed three times with distilled water, and weighed to determine fresh biomass. Dry biomass was subsequently determined after oven-drying at 60°C to constant weight (SD ≤ 0.5 mg).

2.2.2. Statistical Optimization of Culture Conditions for Enhanced Mycelial Biomass

Mycelial biomass optimization of *C. militaris* Q5 was performed using a Box–Behnken design (BBD) with Design-Expert 7.1.5 (State-Ease, Inc., USA). Based on preliminary results, three variables were selected: glucose concentration (5–25 g/L), agitation speed (100–250 rpm), and initial pH (5.5–7.5), comprising 17 experimental runs with five center points. After fermentation, mycelia were filtered through a 200-mesh nylon filter, washed three times with distilled water, and dried at 60°C to constant weight (SD ≤ 0.5 mg). All experiments were conducted in triplicate, and results were expressed as mean values.

2.2.3. Quantification of bioactive compound content

Adenosine and cordycepin: Freeze-dried mycelia (1.0 g) were extracted with 30 mL of 15% (v/v) methanol by ultrasound-assisted extraction (UAE) at 55°C for two 30-min cycles. The extract was centrifuged (10,000 rpm, 10 min, 4°C) and filtered through a 0.45 µm nylon membrane. HPLC analysis was performed using a Shimadzu-2030C 3D plus system equipped with a C18 column (250 × 4.6 mm, 5 µm) at 30°C. The mobile phase comprised water (A) and methanol (B), with a gradient of 3% B (0–5 min), 25% B (5–25 min), and 3% B (25–30 min). The flow rate, injection volume, and detection wavelength were 0.5 mL/min, 10 µL, and 260 nm, respectively. Adenosine and cordycepin (Sigma-Aldrich) served as external standards ($R^2 > 0.999$)[5].

Total polysaccharide: Defatted mycelial powder (90% ethanol) was extracted twice with hot water (1:20, w/v, 80°C, 2 h). The concentrated extract was precipitated with 95% ethanol (1:3, v/v) at 4°C for 24 h, followed by deproteinization using the Sevag method [6]. Total polysaccharide content was determined by the phenol-sulfuric acid method. Briefly, 1,000 µL of sample was reacted with 5% phenol and 98% H_2SO_4 at 95–100°C for 5 min, and absorbance was measured at 490 nm using a D-glucose calibration curve ($R^2 > 0.999$)[7]. Results were expressed as mg glucose equivalents per gram of dry weight (mg/g DW).

Total phenolic content (TPC): TPC was determined according to TCVN 9745-1:2013[8]. Mycelial powder was extracted with 96% ethanol for 72 h (three cycles). The Folin–Ciocalteu method was used, with absorbance measured at 765 nm. TPC was calculated using a gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW).

2.2.4. Statistical Analysis

All experiments were conducted in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel (2019/365). Differences among means were evaluated by ANOVA followed by Student's t-test, with significance set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effects of nutritional sources on mycelial growth of *C. militaris* Q5

Various carbon and nitrogen sources were evaluated by replacing the corresponding components in the basal medium. After 10 days of incubation, mycelial fresh and dry biomass were determined, as shown in Figure 1.

As illustrated in Figure 1, glucose supported the highest biomass accumulation (249.1 g/L wet; 22.8 g/L dry), followed by sucrose (236.7 g/L wet; 21.4 g/L dry). In contrast, maltose and starch yielded significantly lower wet and dry biomass (118.9 g/L wet; 15.4 dry and 106.7 g/L wet; 12.2 g/L dry, respectively). The superiority of glucose is attributed to its role as a readily fermentable monosaccharide that enters glycolysis directly, providing efficient energy for rapid growth. Conversely, the structural complexity of starch and maltose likely imposes a higher enzymatic demand. Regarding nitrogen- rich source, organic sources consistently outperformed inorganic counterparts. The results indicated that yeast extract and soybean meal yielded the maximum fresh and dry biomass (298.3 g/L wet; 29.1 g/L dry and 296.2 g/L wet; 28.9 g/L dry, respectively), whereas NH_4NO_3 produced the minimum (231.4 g/L wet; 20.7 g/L dry). This indicates that for *C. militaris* Q5, nitrogen-rich organic sources have a better effect on mycelial growth than their inorganic counterparts, possibly due to the essential vitamins and growth factors contained therein. These results align with Rohit Rawat et al and Park et al [9, 10] who identified glucose and yeast extract as optimal substrates for *C. militaris*. The observed growth reduction with starch and inorganic nitrogen is also consistent with Hye-Jin Park et al and Trang et al [10, 11] reinforcing the strain's preference for easily assimilable nutrients.

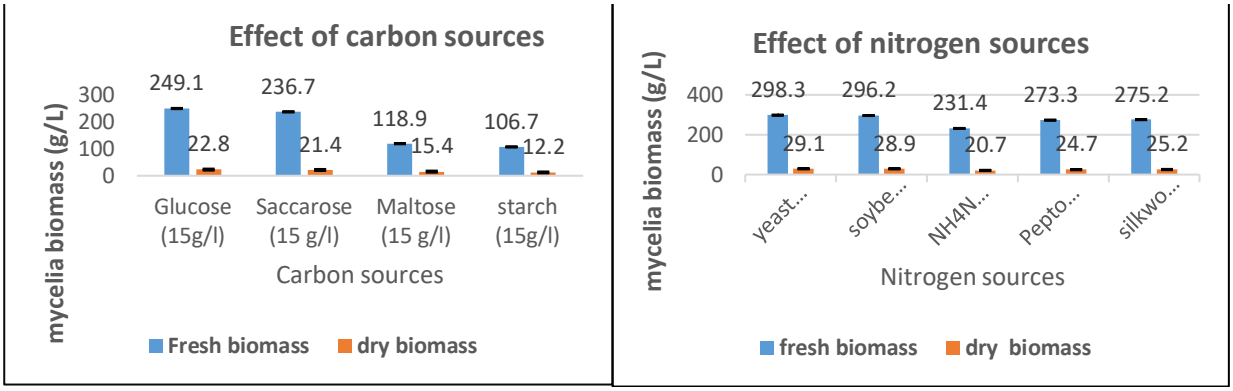


Figure 1. Effect of Carbon and nitrogen sources on mycelia biomass production

3.2. Effect of glucose and soybean meal concentrations on mycelial growth of *C. militaris* Q5

The effects of glucose and soybean meal concentrations on mycelial biomass and pellet morphology were investigated after 10 days of cultivation. The results are presented in Table 1.

Table 1. Effect of glucose and soybean concentrations on mycelia biomass production

No.	Glucose (g/L)	Fresh biomass (g/L)	Dry biomass (g/L)	Pellet morphology
1	5	154.13 ± 2.25	16.73 ± 1.55	Few pellets, small in size, and sparsely distributed
2	10	199.61 ± 1.91	19.67 ± 1.37	Small, sparse pellets with a loose structure
3	15	251.47 ± 2.70	22.70 ± 1.40	Moderate-sized pellets, densely and evenly distributed
4	20	273.53 ± 2.28	25.53 ± 1.96	Uniform pellets with high density and even distribution
5	25	275.7 ± 2.17	25.47 ± 1.32	Dense, large pellets with pronounced aggregation
No.	**Soybean meal (g/L)**	**Fresh biomass (g/L)**	**Dry biomass (g/L)**	**Peellet morphology**
6	2.5	286.80 ± 1.26	26.57 ± 0.93	Moderate Pellets number with high and uniformly distributed density
7	5	294.57 ± 2.66	28.47 ± 1.56	Moderate Pellets number with high and uniformly distributed density
8	7.5	335.10 ± 3.40	33.30 ± 1.31	Low Pellets number, a sparse but uniformly distributed density
9	10	338.53 ± 2.87	35.00 ± 1.31	uniform Pellets number, a high and uniformly distributed density
10	12.5	340.90 ± 3.64	34.77 ± 0.85	moderate Pellets number, a high and uniformly distributed density

Note: Data are expressed as mean ± standard deviation (SD) of three biological replicates (n = 3)

Glucose and soybean meal significantly modulated the mycelial growth and pellet morphology of *C. militaris* Q5. Biomass accumulation gradually increased as the glucose concentration increased from 5 g/L and peaked at 20 g/L (yielding 273.53 g/L wet; 25.53 g/L dry biomass) and showed no substantial increase at 25 g/L (25.47 g/L), suggesting that excessive carbon levels may induce osmotic stress. This concentration-dependent response aligns with Trang et al [11], who noted that supra-optimal glucose levels reduce metabolic efficiency in *Cordyceps* species.

Biomass production reached its maximum at soybean meal concentrations of 10–12.5 g/L, yielding approximately 338.53 g/L wet; 35 g/L dry biomass. However, no significant difference was observed between these two concentrations. Therefore, a soybean meal

concentration of 10 g/L was considered optimal for mycelial biomass accumulation, indicating that this concentration was sufficient to satisfy the nitrogen-rich nutrient requirement for the mycelial growth of *C. militaris* Q5. These results support the findings of Rohit Rawat et al and Li et al [9, 12] which reported that organic nitrogen sources at concentrations ranging from 1 to 10 g/L are generally suitable for the growth of *C. militaris*, emphasizing the strain's requirement for readily assimilable organic substrates.

Notably, a morphological transition from small, loose structures to dense, uniform pellets was observed at optimal nutrient concentrations. This shift is closely associated with enhanced nutrient uptake and balanced metabolic activity regulated by the optimized C/N ratio.

3.3. Effects of mineral sources on mycelial growth of *C. militaris* Q5

The effects of mineral sources, supplemented individually into the culture medium at a concentration of 0.25 g/L, on the growth of *C. militaris* Q5 are summarized in Table 2.

Table 2. Effects of mineral sources on mycelia biomass production

Mineral sources (0,25g/l)	Fresh biomass (g/L)	Dry biomass (g/L)	Pellet mophology
$MgSO_4.7H_2O$	369.60 ± 2.45	37.43 ± 1.65	Small, porous pellets with high density and uniform distribution (Fig 2.a)
$CaCl_2$	316.43 ± 2.91	34.50 ± 1.90	Medium-sized, porous pellets with high density and uniform distribution (Fig 2.b)
$ZnSO_4$	332.37 ± 1.97	31.47 ± 1.75	Small, porous pellets with medium density and uniform distribution (Fig 2.c)
Na_2SO_4	293.33 ± 2.45	26.33 ± 1.60	Medium-sized pellets with moderate density and uniform distribution (Fig2.d)
KCl	285.40 ± 1.70	25.73 ± 1.25	Large pellets with moderate density and uniform distribution (Fig2.e)

Note: Data are expressed as mean ± standard deviation (SD) of three biological replicates (n = 3)

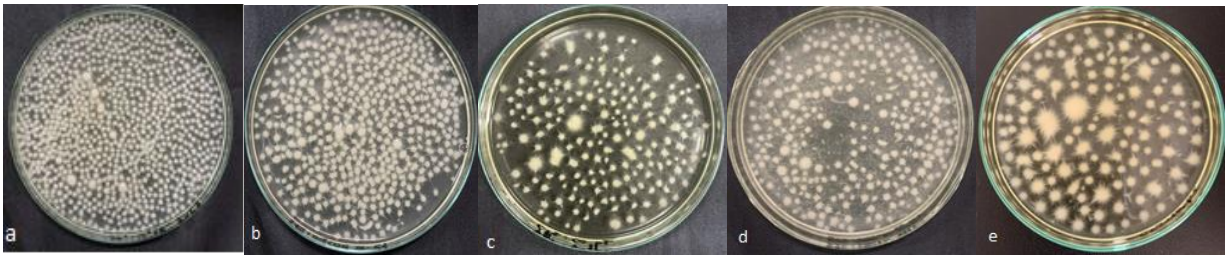


Figure 2. *C. militaris* Q5 Pellet morphology under different mineral sources
(a) $MgSO_4.7H_2O$, (b) $CaCl_2$, (c) $ZnSO_4$, (d) Na_2SO_4 ,(e) KCl

As shown in Table 2, $MgSO_4 \cdot 7H_2O$ supported the highest mycelial biomass (369.60 g/L fresh and 37.43 g/L dry weight), significantly exceeding other mineral sources ($p < 0.05$). This effect may be attributed to the role of Mg^{2+} as an essential enzymatic cofactor in fungal metabolism and ATP-dependent reactions. $CaCl_2$ and $ZnSO_4$ also promoted relatively high biomass, whereas KCl resulted in the lowest yield (285.4 g/L fresh and 25.73 g/L dry weight). Mineral composition also affected pellet morphology, with $MgSO_4$ favoring small, dense, porous pellets that may enhance nutrient and oxygen diffusion compared with larger or irregular aggregates (Fig. 2). Comparison with Tiwari et al[13] highlights the importance of mineral supplementation in maintaining hyphal cell wall integrity and promoting mycelial branching in *C. militaris*. However, unlike the present study, they identified potassium sulfate as the optimal mineral source, while sodium chloride resulted in the lowest mycelial growth and biomass yield.

3.4. Effect of agitation speed on mycelial growth of *C. militaris* Q5

Agitation speed significantly affected biomass yield and pellet morphology of *C. militaris* Q5 ($p < 0.05$; Table 3). Dry biomass increased from 24.5 g/L at 100 rpm to a

maximum of 39.0 g/L at 200 rpm, before declining to 33.1 g/L at 250 rpm. Thus, 200 rpm provided an optimal balance between oxygen transfer and mechanical shear stress. Morphologically, low agitation (100 rpm) produced large, compact pellets, potentially limiting nutrient and oxygen diffusion, whereas intermediate speeds (150–200 rpm) favored smaller, loosely structured aggregates with greater surface-area-to-volume ratios. At 250 rpm, increased shear stress may have induced hyphal fragmentation, reducing biomass accumulation (Fig. 3). These results differ from Yang et al[14], who identified 150 rpm as optimal for mycelial growth and cordycepin production in *C. militaris* CGMCC 2909. Overall, 200 rpm was optimal for maximizing Q5 biomass in shake-flask cultures.

Table 3. Effects of agitation speed on mycelia biomass production

Agitation speed (rpm)	Fresh biomass (g/L)	Dry biomass (g/L)	Mycelial morphology
100	248.47 ± 2.73	24.50 ±2.05	Formation of large, compact pellets with smooth surfaces; uneven distribution (Fig3.a)
150	398.47 ±1.99	30.50 ±2.02	Looser pellets with filamentous hyphae; improved dispersion (Fig3.b)
200	413.33 ± 2.86	39.0 ± 1.72	Well-dispersed mycelia; moderate (Fig3.c)
250	418.27 ±2.36	33.1 ± 1.91	Highly dispersed but possibly fragmented mycelia due to shear stress (Fig3.d)

Note: Data are expressed as mean ± standard deviation (SD) of three biological replicates (n = 3)

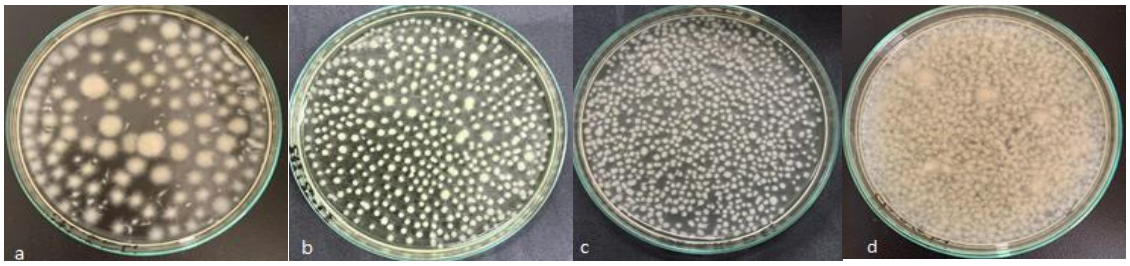


Figure 3. *C. militaris* Q5 Pellet morphology under different agitation speed
(a): 100 rpm; (b): 150 rpm; (c): 200 rpm; (d): 250 rpm

3.5. Effect of initial pH value on mycelial growth *C. militaris* Q5

The effects of different initial pH values on the mycelial growth of *C. militaris* Q5 are presented in Table 4.

Table 4. Effect of initial pH on mycelial growth and morphology of *C. militaris* Q5

Initial pH	Wet biomass (g/L)	Dry biomass (g/L)	Mycelial morphology
4.5	218.47 ± 2.73	20.5 ± 2.05	Small, compact pellets; low density; uneven distribution
5.5	328.47 ± 1.99	27.50 ± 2.02	Medium-sized, loose pellets; high density; uniform distribution
6.5	423.33 ± 2.86	38.9 ± 1.83	Medium-sized, loose pellets with rough surfaces and abundant hyphae; high density; uniform distribution
7.5	410.27 ± 2.36	36.7 ± 1.91	Large pellets; low density; uneven distribution

Note: Data are expressed as mean ± standard deviation (SD) of three biological replicates (n = 3)

Table 4 shows that initial pH significantly affected biomass accumulation and pellet morphology in *C. militaris* Q5 ($p < 0.05$). Dry biomass increased from 20.5 g/L at pH 4.5 to a maximum of 38.9 g/L at pH 6.5, before declining to 36.7 g/L at pH 7.5, indicating that slightly acidic conditions (pH 6.5) favored mycelial growth. Morphologically, pH 4.5 resulted in small, dense, and irregular pellets, potentially restricting oxygen and nutrient diffusion. In contrast, pH 5.5–6.5 promoted moderately sized, loosely structured, well-

dispersed pellets with abundant surface hyphae, potentially facilitating mass transfer. At pH 7.5, large, loose, and unevenly distributed pellets were observed, which may have impaired nutrient uptake and contributed to reduced biomass accumulation. These findings are consistent with those of Trang et al. and Li et al.[11, 12]. who reported optimal *C. militaris* growth and cordycepin production at pH 5.5–6.5. Overall, this pH range appears favorable for nutrient uptake and cellular metabolism, supporting enhanced mycelial growth and favorable pellet morphology.

3.6. Optimization of culture conditions for mycelial biomass production of *C. militaris* Q5.

Based on preliminary results, glucose concentration (X_1), agitation speed (X_2), and initial pH (X_3) were selected as optimization variables. Their combined effects on *C. militaris* Q5 biomass were assessed using a second-order experimental design. The 17 experimental runs and corresponding dry biomass yields are presented in Table 5.

Table 5. Experimental matrix for mycelial biomass production

Test	X_1 (g/L)	X_2 (rpm)	X_3	Dry biomass (g/L)	Test	X_1 (g/L)	X_2 (rpm)	X_3	Dry biomass (g/L)
1	15	150	6.5	17.9	10	20	250	5.5	11.2
2	25	150	6.5	20.4	11	20	150	7.5	20.5
3	15	250	6.5	15.2	12	20	250	7.5	17.3
4	25	250	6.5	12.2	13	20	200	6.5	38.9
5	15	200	5.5	21.9	14	20	200	6.5	39.2
6	25	200	5.5	19.6	15	20	200	6.5	39.1
7	15	200	7.5	25.2	16	20	200	6.5	37.9
8	25	200	7.5	23.7	17	20	200	6.5	38.7
9	20	150	5.5	18.8					

As shown in Table 5, dry mycelial biomass ranged from 11.2 to 39.2 g/L, reflecting considerable variation in response to the selected factors. ANOVA (Table 6) confirmed that the quadratic model was highly significant ($F = 514.15$, $p < 0.0001$), with a non-significant lack of fit ($p = 0.3003$), indicating an adequate fit to the experimental data. All three linear factors (X_1, X_2, and X_3), together with the interaction terms X_1X_2 and X_2X_3, significantly affected biomass production ($p < 0.05$). The model showed excellent predictive performance, as indicated by the adjusted R^2 (0.9966), predicted R^2 (0.9854), low coefficient of variation (CV = 2.4%), and high adequate precision (60.8052). The relationship between the independent variables and dry biomass yield (Y, g/L) was described by the following second-order polynomial equation: $Y = 38.76 – 0.5375*X_1 – 2.71*X_2 + 1.9*X_3 - 1.38*X_1*X_2 + 0.2*X_1*X_3 + 1.1*X_2*X_3 - 8.34X_1^2 - 13.99X_2^2 - 7.92*X_3^2$.

Numerical optimization using the desirability function (100 search runs) predicted optimal conditions of 19.89 g/L glucose, 195.41 rpm agitation, and an initial pH of 6.61. Under these conditions, the predicted dry mycelial biomass yield was 39.00 g/L (Figure 4).

Table 6. Analysis of variance (ANOVA) for the fitted model

Parameter	Variance	Standard F	Significant level p	Parameter	Variance	Standard F	Significant level p
Model	1622.54	514.15	< 0.0001	$X_2.X_3$	4.84	13.80	0.0075
Glucose concentration (X_1)	2.31	6.59	0.0371	X_1^2	293.04	835.73	< 0.0001
Shaking speed (X_2)	58.86	167.87	< 0.0001	X_2^2	824.38	2351.05	< 0.0001
pH value (X_3)	28.88	82.36	< 0.0001	X_3^2	257.32	733.85	< 0.0001
$X_1.X_2$	7.56	21.57	0.0024	Lack of fit	1.38	1.72	0.3003
$X_1.X_3$	0.16	0.4563	0.5210				

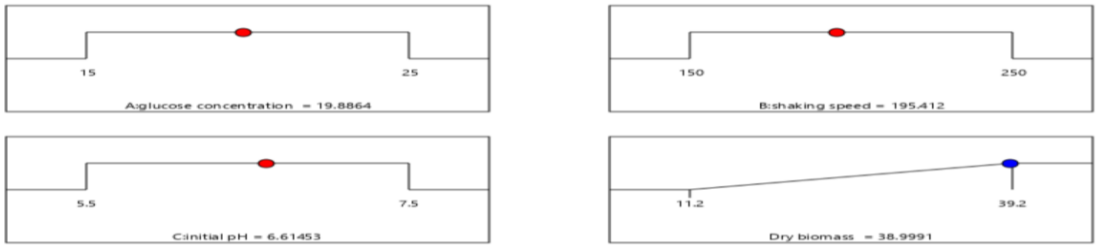


Figure 4. Dry mycelial biomass under optimal culture conditions

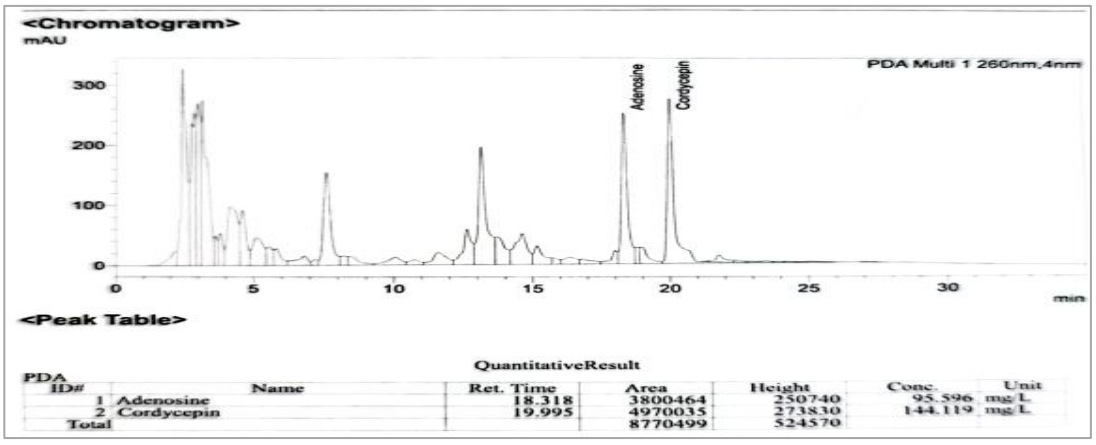
To validate the predictive performance of the model, a verification experiment was conducted in triplicate under the predicted optimal conditions (19.9 g/L glucose, 195 rpm, and initial pH 6.6). The experimentally measured dry biomass yield was 39.8 ± 0.15 g/L, closely matching the predicted value of 39.00 g/L and falling within the 95% confidence interval. The prediction error of 2.05% further supported the model's predictive accuracy and its suitability for optimizing *C. militaris* mycelial biomass production.

3.7. Content of Major Bioactive Compounds in mycelia biomass of *C. militaris* Q5

As shown in Table 7 and Figure 5, *C. militaris* Q5 mycelia contained considerable levels of bioactive compounds. Adenosine and cordycepin contents reached 2.86 and 4.32 mg/g, respectively. While the adenosine level fell within the range of 0.8–3.77 mg/g reported by Jedrejko et al [15], cordycepin exceeded the upper value, suggesting that the optimized culture conditions may have favored its accumulation. Total polysaccharide content reached 158.23 mg/g, comparable to the 144.02 mg/g reported by Mohd Azrie et al[16]. Total polyphenol content was also higher than the 1.6 mg/g reported by Mayuree et al.[17] These compounds have been associated with immunomodulatory and antioxidant activities [10] Overall, the findings highlight the potential of optimized *C. militaris* Q5 mycelial biomass as a source of bioactive compounds for further development as a functional ingredient or pharmacological raw material.

Table 7. Content of bioactive compounds in the mycelial biomass of *C. militaris* Q5

Bioactive compound	Content (mg/g DW)	Analysis method
Adenosine	2.86 ± 1.83	HPLC
Cordycepin	4.32 ±1.05	HPLC
Total Polyphenols	43.87 ± 1.16	UV-Vis
Total Polysaccharides	158.23 ± 1.85	UV-Vis



(Dilution factor (DF) of 3)

Figure 5. HPLC chromatogram of the dried mycelial biomass of *C. militaris* Q5

4. CONCLUSION

This study established optimal culture conditions for maximizing *C. militaris* Q5 mycelial biomass, yielding 39.8 g/L dry weight. The optimized medium contained 19.9 g/L glucose, 10 g/L soybean meal, and 0.25 g/L $MgSO_4$, with agitation at 195 rpm and an initial pH of 6.6. The harvested biomass contained adenosine (2.86 mg/g), cordycepin (4.32 mg/g), polysaccharides (158.23 mg/g), and polyphenols (43.87 mg/g). These findings highlight the potential of *C. militaris* strain Q5 as a promising source of bioactive-rich mycelial biomass for further processing and industrial applications.

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