

INFLUENCE OF TEMPERATURE AND PH LEVEL ON MYCELIAL GROWTH IN LIQUID CULTURES OF *CORDYCEPS MILITARIS* MUSHROOM MYCELIUM

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Abstract

Cordyceps militaris is an entomopathogenic fungus, belongs to the Ascomycetes class and has important pharmacological principles, the most important are polysaccharides and mannitol. Given that in most cases, mushrooms are grown on different substrates of solid consistency, and most supplements containing mycelium from the *Cordyceps militaris* mushroom are obtained by drying and shredding the mycelium, which has been grown on a solid substrate consisting of brown rice flour or other cereals, so that they very often contain more starch than active ingredients. In this paper we looked at the influence of temperature and pH level on the growth of the mycelium of this mushroom in liquid culture media. The development of liquid mycelium cultures was tested at temperatures between 20-30 °C and at a pH level between 5-7. The highest amount of mycelium was obtained from samples raised at 26 °C and a pH level of 5.5.

Keywords: *Cordyceps militaris*, medicinal mushroom, pH, temperature.

1. INTRODUCTION

Medicinal mushrooms are a valuable source of biologically active principles such as polysaccharides, ergosterol, cordycepin and mannitol, which are used in traditional and modern medicine (Go et al., 2007).

The *Cordyceps militaris* mushroom has been used in folk medicine in East Asia since antiquity, being used as a food with tonic and invigorating properties (Mizuno, 1999), in subtropical and temperate regions (Nag and Wang, 2005).

The main bioactive component of medicinal interest of the *Cordyceps militaris* mushroom is represented by cordycepin (Cunningham et al., 1995), and the therapeutic and medicinal properties are represented by: anticancer activity (Shonkor et al., 2010), antioxidant activity (Wu et al., 2011), antiviral activity (Yuko et al., 2007), anti-fungal activity (Byung et al., 2009), antibacterial activity (Young et al., 2000), immunomodulatory agent for the treatment of immunological diseases (Shin et al., 2009; Rozsa et al., 2016 a).

Collecting *Cordyceps militaris* mushrooms grown in natural conditions is very expensive, and their cordycepin content is much lower than the content of artificially grown mycelial biomass (Guo et al., 1998; Rozsa et al., 2016 b).

In recent years, various methods for the production of fungal biomass have been developed and perfected, both on solid substrate (Rozsa et al., 2016 c, d, e) and on liquid surface cultures (Masuda et al., 2007) or submerged cultures (Mao et al., 2005).

The amount of cordycepin and active principles contained in the fungal biomass vary depending on the strain used (Guo et al., 1998; Rozsa et al., 2016 c), the pH of the culture media (Rozsa et al., 2017 a, b), the incubation and drying temperatures of mycelial biomass (Rozsa et al., 2017 c, d) as well as its chemical composition (Rozsa et al., 2019).

Given all these considerations, in this study were compared two very important factors in a mycelial biomass crop, namely the influence of temperature and pH of the culture medium on the amount of mycelial biomass obtained, following the values that increase production.

2. MATERIALS AND METHODS

The pure culture of *Cordyceps militaris* from which it started, was maintained in refrigeration conditions at a temperature of 4 °C, this being used as a material for inoculation.

The liquid culture medium was prepared according to the following recipe: for 1 litre of sterilized distilled water, we used 5 g of ammonium sulphate, 5 g of magnesium sulphate, 5 g of potassium phosphate, 15 g of peptone and 15 g of yeast.

The pH of the culture media was acidified with citric acid or basified with sodium hydroxide, so that 5 experimental variants were performed for the following pH values: 5, 5.5, 6, 6.5 and 7.

The media thus obtained were incubated at temperatures of 20, 22, 24, 26, 28 and 30 °C.

After incubation, the liquid cultures were centrifuged and the obtained mycelial biomass was washed with distilled water and then dried in an oven at 105 °C until a constant mass was obtained.

The mycelial biomass thus obtained was used for later chemical determinations.

3. RESULTS AND DISCUSSIONS

Following the unilateral influence of the incubation temperature on the obtained amount of mycelial biomass, it can be stated that the highest amount of mycelial biomass was obtained in the 24-28 °C temperature range. Table 1.

Table 1. The unilateral influence of the incubation temperature on the obtained amount of mycelial biomass

Incubation temperature	Mycelial biomass		Difference ±D	Signification of difference
	g/100 ml liquid culture	%		
T0 - mean of experience	25.97	100.0	0.00	Control
T1 - 20°C	20.20	77.8	-5.77	000
T2 - 22°C	22.83	87.9	-3.14	00
T3 - 24°C	27.15	104.6	1.18	**
T4 - 26°C	34.17	131.6	8.20	***
T5 - 28°C	28.87	111.2	2.90	**
T6 - 30°C	22.60	87.0	-3.37	00
	LSD (p 5 %)		0.17	
	LSD (p 1 %)		0.24	
	LSD (p 0.1%)		0.34	

Several authors mention the temperature of 24 °C as optimal for incubating and growing the mycelium of the *Cordyceps militaris* mushroom (Mao et al., 2005; Patel et al., 2013), and others (Guo et al., 1998; Masuda et al., 2007) mention temperature of 28 °C.

In the case of the strain used by us, the highest amount of mycelial biomass was obtained at the temperature of 26 °C. This temperature was mentioned by Shonkor et al., 2010.

Following the unilateral influence of pH on the obtained amount of mycelial biomass, it can be stated that at the 5.5 pH level, the highest amount of mycelial biomass was obtained, the obtained results being statistically ensured by very distinctly significant positive differences. (Table 2).

Table 2. Unilateral influence of pH on the obtained amount of mycelial biomass

Broth pH value	Mycelial biomass		Difference ±D	Signification of difference
	g/100 ml liquid culture	%		
P0 – mean of experience	25.97	100.0	0.00	Control
P1- 5	28.67	110.4	2.70	**
P2 – 5.5	28.37	109.2	2.40	***
P3 – 6	26.40	101.7	0.43	**
P4 – 6.5	24.79	95.4	-1.18	00
P5 - 7	21.63	83.3	-4.35	000
LSD (p 5 %)			0.16	
LSD (p 1 %)			0.22	
LSD (p 0.1%)			0.28	

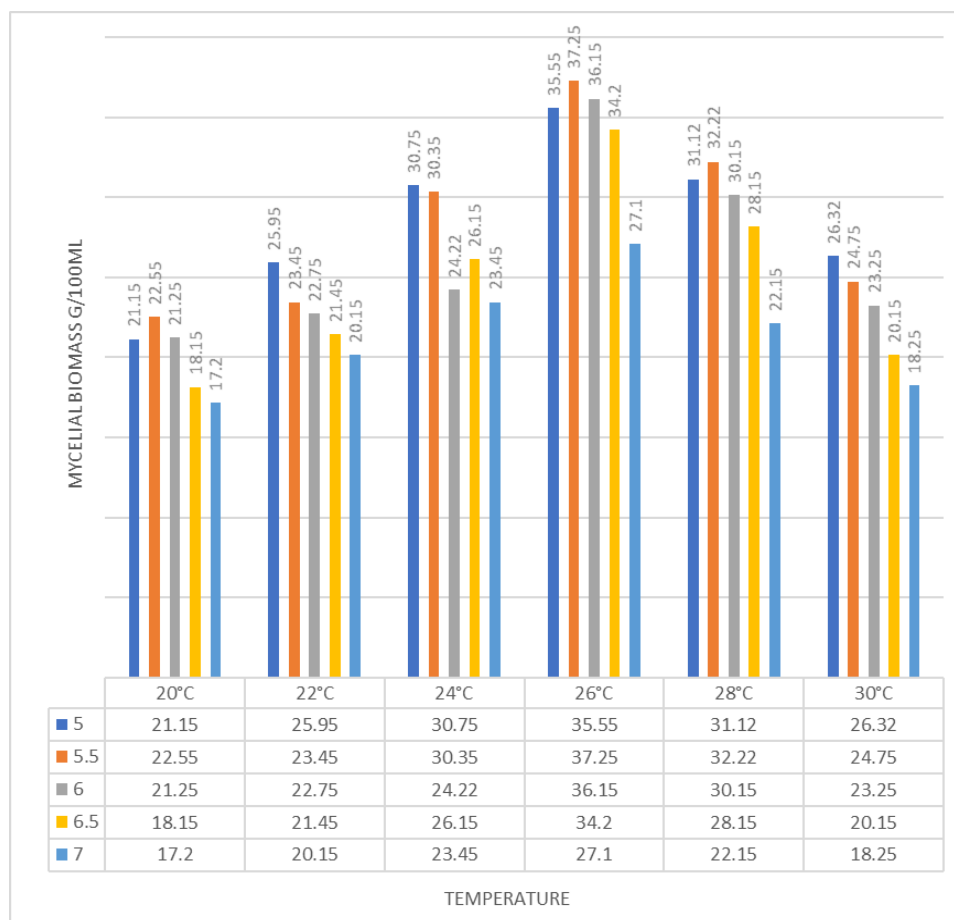


Figure 1. Combined influence of experimental factors, temperature and pH, on *Cordyceps militaris* mushroom mycelial biomass production

As in the case of temperature, the pH level of the culture medium influences the amount of obtained mycelial biomass, so the values we determined are found in the intervals mentioned by other authors (Masuda et al., 2007; Patel et al., 2013).

Following the combined influence of experimental factors, temperature and pH (Figure 1), in the case of the *Cordyceps militaris* strain used by us in this study, the highest amount of mycelial biomass was obtained at a temperature of 26 °C and 5.5 pH value, in this case the cell growth rate was the highest.

Previous studies by Xiao et al., 2004, Kwon et al., 2009, Cheng et al., 2011, also argued that the culture medium at a pH of 5.5 produces a higher amount of mycelium.

4. CONCLUSIONS

In liquid culture media, the acidic pH is optimal for mycelial biomass, because in the acidic environment the metabolites necessary for the growth and development of the mycelium of Ascomycete fungi are produced faster.

For the used strain of *Cordyceps militaris* mushrooms, the optimal temperature for the best mycelial biomass production was 26 °C.

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