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CHEMICAL ACTIVITY OF THE LIQUID CORDICEPS MILITARIS CULTURE FROM RADIX TARAXACI CUM HERBA MEDIUM

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Abstract
Even though many consider it a weed without any use, dandelion (Taraxacum officinale) is a valuable plant, used both as food and as medicine. However, more and more research show that dandelion root is one of the most powerful weapons in the fight against cancer. Dandelion is known to have diuretic properties, cleanses the liver, helps treat allergies and is a good ally in the fight against high cholesterol. Dandelion root is a good source of potassium, vitamin A, iron, calcium, folic acid, magnesium and vitamin C. In this study we used Radix Taraxaci cum herba (RTH) as a medium for the cultivation of Cordyceps militaris mushushroom liquid mycelium culture to investigate the amount of cordicepine. In the classical liquid culture after 21 days of fermentation without stirring, 23.33 ppm cordicepine was determined, and in the liquid culture with RTH, 29.72 ppm cordicepine was determined.

Keywords: cordycepine, Cordyceps militaris, medicinal mushroom, Taraxacum officinale.

1. INTRODUCTION

Amongst mushrooms with medicinal properties, *Cordyceps militaris* mushroom is well known in traditional Chinese medicine, with a very long history, being an entomopathogenic fungus belonging to the Ascomycetes class (Zhu, 2013).

The pharmacological principles of this mushroom and its active compounds, such as polysaccharides, adenosine and mannitol, have been studied by many researchers (Smiderle, 2013).

The polysaccharides contained in the fungus and the mycelium of the *Cordyceps militaris* mushroom have antitumor, immunomodulatory, hypoglycaemic, anti-inflammatory and antioxidant effects (Li, 2003). Adenosine has a cardioprotective effect, and mannitol has antiviral and diuretic properties (Yu, 2004; Rozsa et al., 2016 a).

In *Cordyceps militaris* mushrooms, cordycepin is the main bioactive compound, with pharmacological and biological properties, showing anticancer, antiviral and immune system stimulating activities (Cunningham et al., 1995; Ahn et al., 2000; Zhou et al., 2002).

In the current practice of producing mycelium and mycelial biomass, the cereal grain substrate is commonly used, which usually lasts longer than liquid cultures (Rozsa et al., 2016 b).

Also, the growth of the fungal mycelium is influenced by the pH of the culture media, temperature, as well as by the nitrogen content of these media (Rozsa et al., 2016 c, d, e).

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In the case of nutritional supplements obtained from solid culture media, the active principles contained are in a smaller amount than those obtained from liquid media, as they contain a high amount of starch from cereal seeds from mycelial biomass culture media. (Rozsa et al., 2017 a, b).
The content in active principles of mycelial biomass is directly influenced by the method of drying and processing (Rozsa et al., 2017 c), the active principles being in direct correlation with the soluble dry matter content (Rozsa et al., 2017 d), and the vitamin content is influenced by the chemical composition of the culture medium (Rozsa et al., 2019).
Some researchers have tried to enrich the content of active ingredients by using herbal additives in culture media, thus Yu-Wei and Chiang in 2008, performed experiments on the antitumor activity of the fermentation broth of *Cordyceps militaris* grown in *Radix astragali* medium.
Dandelion (*Taraxacum officinale*) is considered one of the most widespread species of Asteraceae, in sunny places, in meadows and pastures, orchards, roadsides, uncultivated places, from the plains to the subalpine area and has been used since ancient times in traditional Chinese medicine. From this medicinal plant are used the leaves (Taraxaci folium), the aerial part with roots (Radix Taraxaci cum Herba - RTH) and the roots (Taraxaci radix).
Dandelion leaves contain flavonoids, chicory acid, bitter substances (lactucopicrin). The root contains triterpene alcohols (taraxasterol), sterols, vitamins, mineral salts and inulin ($C_6H_{10}O_5$), which is a reserve substance and is found in the amount of 30-40% of the plant mass (Muntean et al., 2016).

2. MATERIALS AND METHODS

The mycelium of the *Cordyceps militaris* mushroom was inoculated on PDA (potato-dextrose-agar) medium in Petri dishes, then incubated for 10 days at 26 °C. The biological material thus obtained was used to inoculate liquid media with 1 cm^2 of culture.
For one litre of liquid culture medium were used: 1 litre of sterilized distilled water, peptone (as an additional source of nitrogen) 15g, magnesium sulphate 5g, ammonium sulphate 5g, potassium phosphate 5g and yeast extract 15g.
The culture was established and incubated in 750 ml bottles containing 100 ml of culture medium.
The aerial part of the dandelion with roots was harvested before flowering, with flower buds, in spring. The leafy roots were shaken to remove the soil, they were washed. Drying was performed at 45 °C, the drying yield being 5:1.
The first experimental variant was represented by the culture medium described above, and for the second experimental variant 15 grams of powder from dandelion leaf and root (RTH) were added to one litre of medium.
The obtained data were processed using the Statistica 10 program by Stat Soft Inc.

3. RESULTS AND DISCUSSIONS

Following the unilateral influence of the addition of RTH on the production of mycelial biomass, it can be seen that the data obtained are statistically assured, registering a significant positive difference compared to the average of 3.62 g / 100 ml liquid culture medium. Table 1.
Comparing the results obtained by us with those found in the literature, we can say that they are found in similar limits, Patel and Ingalhalli 2013 reporting 37.25 g of mycelial biomass per 100 ml of liquid culture.
Following the unilateral influence of RTH addition on the soluble dry matter content of the obtained mycelial biomass, it can be seen that the obtained data are statistically assured, registering a

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significant positive difference compared to the average of experience by 0.24 g soluble dry matter per 100 ml liquid culture medium (Table 2).

Table 1. The unilateral influence of the addition of RTH on the production of mycelial biomass

Recipe	*Mycelial biomass*		Difference ±D	Signification of difference
	g/100 ml liquid culture	%		
-	38.33	100.0	0.00	Control
1 – Without RTH addition	34.72	90.6	-3.62	0
2 – With RTH addition	41.95	109.4	3.62	*
LSD (p 5 %)			3.33	
LSD (p 1 %)			7.70	
LSD (p 0.1%)			24.50	

Table 2. Unilateral influence of RTH addition on the soluble dry matter content of the mycelial biomass

Recipe	*Soluble dry matter*		Difference ±D	Signification of difference
	g/100 ml liquid culture	%		
-	2.57	100.0	0.00	Control
1 – Without RTH addition	2.33	90.5	-0.24	0
2 – With RTH addition	2.82	109.5	0.24	*
LSD (p 5 %)			0.21	
LSD (p 1 %)			0.49	
LSD (p 0.1%)			1.55	

The results obtained are similar to those presented in the literature for the recipe without the addition of RTH, 2.25 g soluble dry matter / 100 ml liquid culture were determined by Fengyao et al. (2011).

For the recipe with added RTH no data were found in the literature, but they show significant positive differences for all repetitions performed compared to the recipe without added RTH.

Given that most of the bioactive components in the *Cordyceps militaris* mushroom, are present in the form of complex polysaccharides in various forms, the correlation between the soluble dry matter and the total sugar content is intended to highlight these components.

Following the correlation coefficient between the amount of soluble dry matter and the sugar content we obtained r = 0.95537 (Figure 1).

Comparing this value with the correlation coefficient by the probability of 5%, respectively 1%. r = 0.95> 0.67 and 0.80 based on these comparing, it can be stated that between the amount of soluble dry matter and the sugar content of the *Cordyceps militaris* mushroom, the correlation coefficient is significantly positive, indicating the close dependence of the amounts of soluble dry substance, mycelial biomass and sugar content.

A higher amount of soluble dry matter obtained from mycelial biomass will record higher values of sugars (figure 1).

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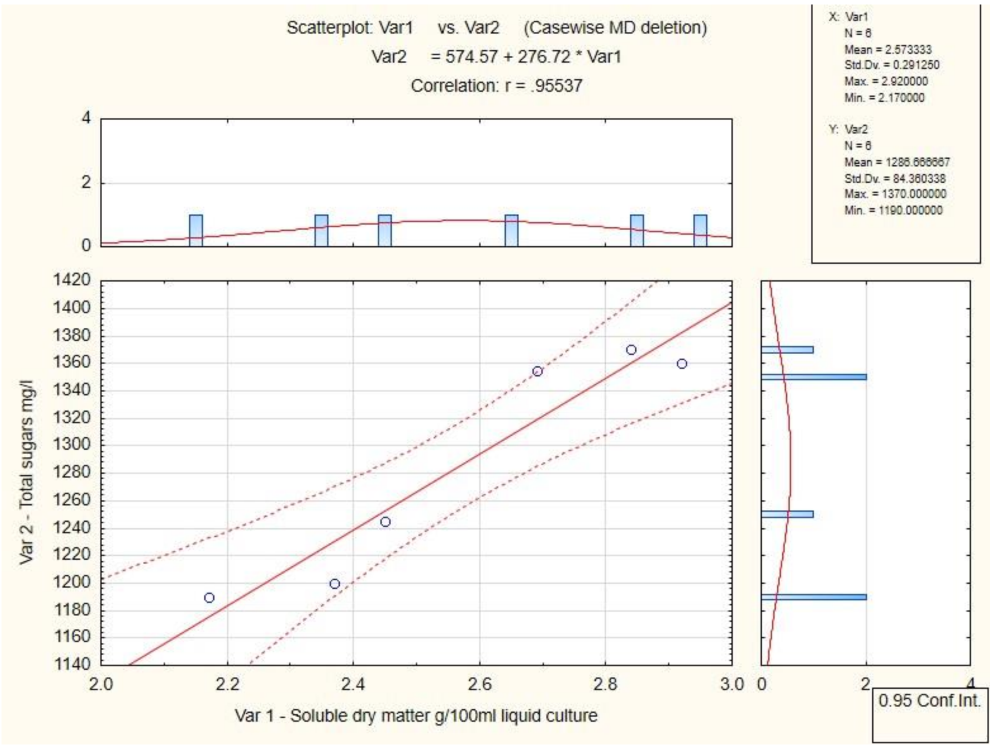

Figure 1. The correlation between the amount of soluble dry matter and the total sugars content of Cordyceps militaris liquid biomass culture

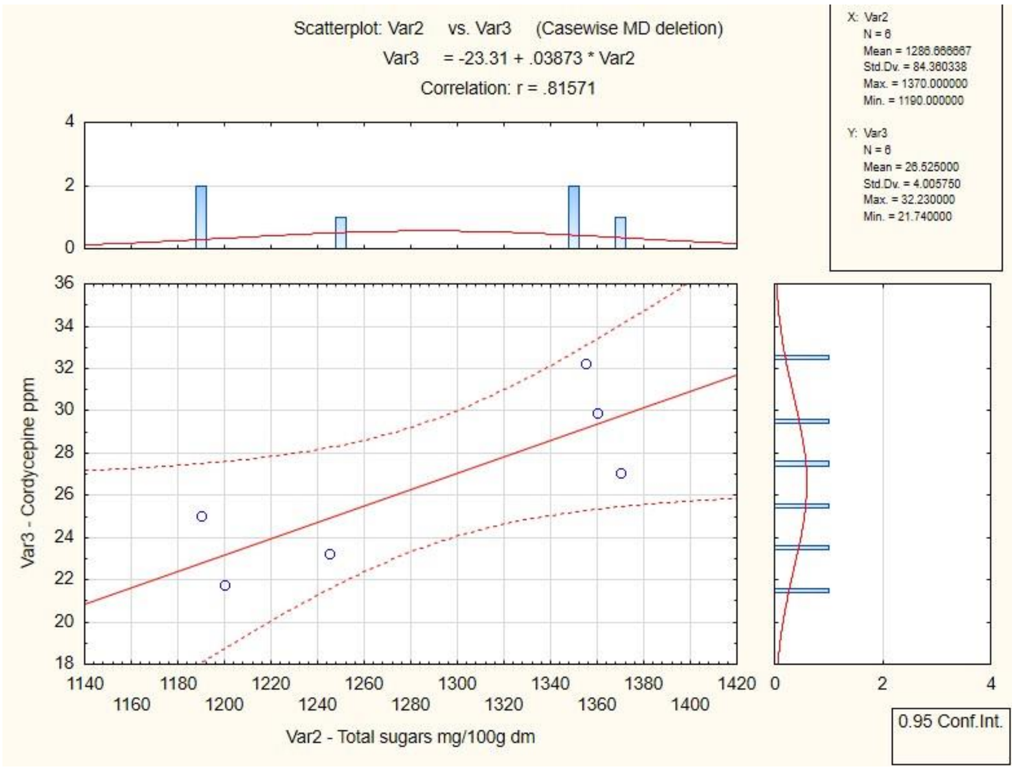

Figure 2. The correlation between the amount of total sugars and the cordycepine content of Cordyceps militaris liquid biomass culture

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The addition of RTH influences the amount of dry matter, sugars and also the amount of cordycepin in the mycelial biomass of the *Cordyceps militaris* mushroom (figure 2).
It is observed that the results are real, experimental and faithfully follow the correct regression.
The relationship between the two characters estimated by simple linear regression is illustrated by the following equation of degree I: y = -23.31 + 0.03873 * soluble dry matter and the value of the coefficient of determination $R^2 = 0.81571$.
Based on these comparisons, it can be stated that the correlation coefficient is significant between the amount of dry mycelial biomass, the addition of RTH and the cordycepin content.

4. CONCLUSIONS

The addition of RTH influences significantly positively the obtained amount of mycelial biomass, in our experience we obtained with 3.62 g / 100 ml of culture medium more mycelial biomass, compared to the simple culture medium.
The addition of RTH also influences the dry matter content of mycelial biomass.
In the classical liquid culture after 21 days of fermentation without stirring, was determined 23.33 ppm cordycepin, and in the liquid culture with RTH, 29.72 ppm cordycepin, which is in direct correlation with the polysaccharide content and dry matter contained in mycelial biomass.

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