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INFLUENCE OF MAGNESIUM (Mg) SOURCE ON THE *Cordyceps militaris* (L.) MUSHROOM MYCELIUM GROWTH

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Abstract
Magnesium (Mg) is an element with a role in oxidation processes. The optimal concentration of magnesium for the development of fungi is associated with an optimal concentration of phosphorus (P), which through its essential constituents, is an important element for the existence of biological systems in nature through nucleic acids, phospholipids, phosphoglycerides, phytin and phosphates. Magnesium has a role in the activity of certain enzymes and in respiration, being a component of protein substances with a special importance for microorganisms being included in reducing and phosphorylating enzymes and for protein synthesis. In this study, magnesium sulphate (MgSO4), which is an accessible source of both magnesium and sulphur, as well as magnesium carbonate (MgCO3) were used as sources of magnesium, both being used in different concentrations in the growth medium. The culture medium or substrate influences the growth of the fungal mycelium, through the mineral, nutritive and stimulating substances in their composition. Using experimental culture media, an isolated tissue culture was performed from 2 strains of Cordyceps militaris (L.) fungus. The culture was performed in Petri dishes, incubated at a temperature of 24 ° C, aiming to increase the mycelium for 15 days. The highest increase was recorded by strain CI 32 with the addition of magnesium sulphate.

Keywords: Cordyceps militaris (L.), magnesium, mushroom, mycelium.

1. INTRODUCTION

Mushrooms are a complete food with a high nutritional value, the chemical composition of mushrooms differs with the species, the stage of ontogenetic development, with different parts of the carposome (hat, leg) and the nutrient substrate on which they grow (Hoa and Wang, 2015; Rózsa, 2017a).

In general, mushrooms contain: 82-92% water, 0.5-1.5% mineral salts (potassium, calcium, magnesium, phosphorus, silicon), 1-3% sugars (mannite, glucose, trehalose, glycogen and cellulose), 2-4% nitrogenous substances (proteins), very low amounts of fats (1% lecithin), tannins, organic acids (malic, citric, tartaric) and vitamins (A in the form of carotene, B_1, B_2 and D) (Kalyoncu et al., 2010; Rózsa et al., 2016a; Rózsa et al., 2017b).

It is recommended that mushrooms be eaten at a young age and as soon as possible after harvest, as they contain easily altered substances. Mushrooms can be preserved fresh or preserved (dried, pickled or marinated) (Zhu et al., 1998; Rózsa et al., 2016b; Rózsa et al., 2017f).

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Ascomycetes are characterized by a well-developed vegetative apparatus, most fungi in this class have a filamentous stem, the mycelium being composed of multicellular hyphae often branched, isolated or anastomosed (Kalyoncu et al., 2010; Rózsa et al., 2016c; Rózsa et al., 2017c).
Their multiplication is asexual and is achieved by different spores (conidia, pycnospores).
Filamentous thallus is the most widespread vegetative apparatus of fungi. It is represented by simple or branched multicellular filaments, each compartment representing a cell. Both the siphonoplast and the filamentous thallus are also known as mycelium, and the filaments that make it up are called mycelial hyphae. The filamentous thallus is found in fungi of the class *Ascomycetes*, *Basidiomycetes* and the group *Deuteromycetes* (Patel and Ingalhalli, 2013; Rózsa et al., 2016d).
A culture medium can be defined as a sterile nutrient support, which allows the development and study of a microorganism outside the natural ecological niche. In order to allow the development of microorganisms, any culture medium must meet certain conditions: to contain nutrients necessary for metabolism, to have a certain pH reaction within which the fungus can grow, to correspond to the physiological characteristics of fungi, taking into account their type, breathing and be sterile.
For mother cultures and pure cultures in the laboratory, a liquid or solid culture medium containing all the minerals necessary for the growth of the mycelium is needed (Rózsa et al., 2016e).
The passage of the mycelium in the laboratory, from one culture medium to another, is important for keeping it in a viable state for as long as possible. Ironing is done every three months but can be performed even earlier. But if this is delayed a lot and the mycelium has nothing to feed on, it can perish. This is all the more important when we keep new, valuable stems that can no longer be reconstituted (Patel and Ingalhalli, 2013; Rózsa et al., 2016f).
The mineral elements with a role in the growth of the mycelium both on the laboratory culture medium and on the granular medium, i.e., the one based on cereals, but also on compost or lignocellulosic substrate are very important (Rózsa et al., 2019; Rathore et al., 2019).
Magnesium (Mg) is an element with a role in oxidation processes. The optimal concentration of magnesium for the development of fungi is associated with an optimal concentration of phosphorus (P), which through its essential constituents, is an important element for the existence of biological systems in nature through nucleic acids, phospholipids, phosphoglycerides, phytin and phosphates. Magnesium has a role in the activity of certain enzymes and in respiration, being a component of protein substances with a special importance for microorganisms being included in reducing and phosphorylating enzymes and for protein synthesis (Wang and Shiao, 2000; Rózsa et al., 2016g).
Through its essential constituents, phosphorus (P) is an important element for the existence of biological systems in nature, through nucleic acids, phospholipids, phosphoglycerides, phytin and phosphates (Xiao and Zhong, 2007; Rózsa et al., 2016h). Phosphorus is found in organic waste in the form of organic compounds. Soluble phosphates are important nutrients for all biological systems (Rózsa et al., 2017d). The mineralization of organic compounds takes place under the action of enzymes such as: phytases, phosphatases, nucleases, phospholipases, glycerophosphatases, etc. secreted by various microorganisms. The mineralization process is activated by an alkaline pH and a temperature above 20 °C. By mineralizing the organic compounds with phosphorus, soluble orthophosphates are born, which can be used as nutrients by biological systems, plants, microorganisms, or insoluble phosphate is formed with calcium, magnesium, iron and aluminum. Immobilized forms of phosphorus in the form of insoluble phosphates, constitute a reserve of nutrients and can be enhanced by their solubilization, calcium intake having an essential role in this case (Yang et al., 1994; Rózsa et al., 2017e).

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In this study, magnesium sulphate ($MgSO_4$), which is an accessible source of both magnesium and sulphur, as well as magnesium carbonate ($MgCO_3$) were used as sources of magnesium, both being used in different concentrations in the growth medium.

2. MATERIALS AND METHODS

Magnesium sulphate ($MgSO_4$) is a salt of sulfuric acid with magnesium, having a molar mass of 120.37 g / mol and a density of 2.66 g / cm^3, being a water-soluble substance (269 g / L (0 °C)) MgSO4 · 7H2O: 710 g / L (20 °C), used as a chemical fertilizer in agriculture, serving as a source of magnesium for plants (S1 experimental variant).
Magnesium carbonate ($MgCO_3$) is magnesium salt with carbonic acid, having a molar mass of 84.31 g / mol and a density of 2.96 g / cm^3 (20 °C), being a poorly soluble substance in water (0.106 g / L). Magnesium carbonate, which is chemically neutral, is converted by heat release hydration into basic magnesium carbonate and determines the hardness of the water (S2 experimental variant).
The biological material used in the experiment was represented by 2 similar strains of the *Cordyceps militaris* (L.) fungus, CI 32 and CI 44, from pure cultures from the Ciupercaria SRL company, from Aghireșu-Fabrici, Cluj County (T1 and T2 experimental variants).
The culture medium used was PDA (potato-dextrose-agar), being the most used medium in mycology, being favorable for the growth of most fungi. The medium was prepared from 200g potatoes, 20g dextrose, 20g agar and 1000ml distilled water, to which were added different concentrations of magnesium salts (1g, 3g and 5g → C1, C2 and C3 experimental variants).
To prepare the medium, the potatoes were washed and cleaned, and then cut into cubes of about 12 mm. Weighed 200 g of peeled potatoes, rinsed in water and boiled in a glass bowl for one hour, until the potatoes were softened and crushed. Strain as much pulp as possible through the gauze, then we add the agar and boil until it has dissolved. It was set on fire; dextrose was added and mixed until dissolved. Make up to 1 liter with distilled water, then was sterilize at 121 °C for 15 minutes. The culture medium was poured into sterile Petri dishes under a sheet flow hood. During casting, the culture medium was stirred continuously so that the distributed medium was uniform.
The mycelium increase was recorded daily and the obtained results are presented in millimeters over 15 days of growth (Rózsa et al., 2019).
The combination of experimental factors resulted in 12 experimental variants. The results obtained were interpreted statistically, using the Statistica 10 program.

3. RESULTS AND DISCUSSIONS

In table 1 are presented the results on the unilateral influence of strain (T) on the growth of the C. militaris L. mushroom mycelium.

Table 1. The unilateral influence of strain (T) on the growth of the C. militaris L. mushroom mycelium

Experimental variant	Mycelial growth(mm)/15 days		Difference (mm) ±D	Signification of difference
	Obtained values	%		
T1	41.23	90.8	-4.19	-
T2	49.60	109.2	4.18	-
Average	45.41	100.0	0.00	Avg.
	LSD (p 5%)		7.16	
	LSD (p 1%)		35.86	
	LSD (0.1%)		358.63	

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Following the results presented in above table, it can be seen that none of the studied strains show statistically assured results, regarding the source of magnesium used. However, the analyzed T2 strain, registered for the 15 days an increase of 49.60 mm, the experience average being 45.41 mm for 15 days.
In table 2 are presented the results on the unilateral influence of magnesium source (S) on the growth of the C. militaris L. mushroom mycelium during the experience.

Table 2. The unilateral influence of magnesium source (S) on the growth of the C. militaris L. mushroom mycelium

Experimental variant	Mycelial growth(mm)/15 days		Difference (mm) ±D	Signification of difference
	Obtained values	%		
S1	57.59	126.8	12.18	**
S2	33.23	73.2	-12.16	00
Average	45.41	100.0	0.00	Avg.
	LSD (p 5%)		3.85	
	LSD (p 1%)		8.89	
	LSD (0.1%)		28.28	

Following the results presented in above table, it can be seen that the S1 magnesium source ($MgSO_4$), registered 12.18 mm difference from the experience average, the result being significantly positive, compared to the experience average considered as control. For the S2 magnesium source ($MgCO_3$) registered a significantly negative difference.
Table 3 presents the result on the unilateral influence of magnesium source concentration (C) on the growth of the *C. militaris* L. mushroom mycelium.

Table 3. The unilateral influence of magnesium source concentration (C) on the growth of the C. militaris L. mushroom mycelium

Experimental variant	Mycelial growth(mm)/15 days		Difference (mm) ±D	Signification of difference
	Obtained values	%		
C1	46.21	101.8	0.80	-
C2	50.16	110.4	4.74	**
C3	39.87	87.8	-5.54	00
Average	45.41	100.0	0.00	Avg.
	LSD (p 5%)		2.66	
	LSD (p 1%)		3.87	
	LSD (0.1%)		5.81	

Analyzing the concentration of the used magnesium source, the results obtained highlighted the C2 concentration, being the most optimal for our experience.
For this experimental variant, we recorder 50.16 mm mycelial growth per 15 days of experience, the result being significantly positive, compared to the experience average considered as control 45.41 mm per 15 days.
For C1 experimental variant, the obtained results, 46.21 mm mycelial growth per 15 days, were not statistically assured. C3 experimental variant, registered 39.87 mm mycelial growth per 15 days, the result being significantly negative, compared to the experience average considered as control.

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During the course of the experiment was followed the combined influence of factors magnesium source (S) and its concentration (C) used on the growth of the mycelium of the *C. militaris* L. mushroom, the data obtained are presented in table 4.

Table 4. The combined influence of factors magnesium source (S) and its concentration (C) used on the growth of the mycelium of the C. militaris L. mushroom

Experimental variant	Mycelial growth(mm)/15 days		Difference (mm) ±D	Signification of difference
	Obtained values	%		
S1 C1	58.91	127.5	12.70	**
S2 C1	33.51	72.5	-12.68	00
Average	46.21	100.0	0.00	Avg.
S1 C2	63.21	126.0	13.06	**
S2 C2	37.10	74.0	-13.02	00
Average	50.16	100.0	0.00	Avg
S1 C3	50.67	50.67	10.79	**
S2 C3	29.08	29.08	-10.77	00
Average	39.87	100.0	0.00	Avg.
	LSD (p 5%)		4.70	
	LSD (p 1%)		8.67	
	LSD (0.1%)		21.35	

The combined influence of factors magnesium source (S) and its concentration (C) used on the growth of the mycelium of the C. militaris L. mushroom, they highlighted S1 magnesium source ($MgSO_4$), this registering significantly positive differences, regardless of the concentration to which it was applied (C1, C2 or C3). In this case, mycelial growth recorded values between 50.67 and 63.21 mm per 15 days of experiment. The data obtained by us are comparable to those found in the scientific literature.

In table 5 are presented the values obtained for the combined influence of factors, magnesium source (S) and the used mycelium strain (T) on the growth of the of the *C. militaris* L. mushroom mycelium.

Table 5. The combined influence of factors magnesium source (S) and the used mycelium strain (T) on the growth of the of the C. militaris L. mushroom mycelium

Experimental variant	Mycelial growth(mm)/15 days		Difference (mm) ±D	Signification of difference
	Obtained values	%		
S1 T1	53.00	128.6	11.74	*
S2 T1	29.46	71.4	-11.76	0
Average	41.23	100.0	0.00	Avg.
S1 T2	62.19	125.4	12.57	**
S2 T2	37.01	74.6	-12.59	00
Average	49.60	100.0	0.00	Avg
	LSD (p 5%)		5.44	
	LSD (p 1%)		12.57	
	LSD (0.1%)		39.99	

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Following the data in the table above, for the S1 T2 experimental variant, we recorded 62.19 mm per 15 days mycelial growth, the result being significantly positive, compared to the experience average considered as control. For the S1 T1 experimental variant, we recorded 53.00 mm per 15 days mycelial growth, in this case the result being positive, compared to the experience average considered as control. For S2 T1 and S2 T2 experimental variants, we recorded negative and significantly negative differences, compared to the experience average considered as control.
Table 6 presents the obtained values for the combined influence of factors magnesium concentration (C) and the used mycelium strain (T) on the growth of the of the *C. militaris* L. mushroom mycelium in our experience.

Table 6. The combined influence of factors magnesium concentration (C) and the used mycelium strain (T) on the growth of the of the C. militaris L. mushroom mycelium

Experimental variant	Mycelial growth(mm)/15 days		Difference (mm) ±D	Signification of difference
	Obtained values	%		
C1 T1	44.58	108.1	3.35	-
C2 T1	43.79	106.2	2.56	-
C3 T1	35.32	85.7	-5.91	00
Average	41.23	100.0	0.00	Avg.
C1 T2	47.84	96.4	-1.76	-
C2 T2	56.52	114.0	6.92	**
C3 T2	44.43	89.6	5.16	0
Average	49.60	100.0	0.00	Avg.
	LSD (p 5%)		3.77	
	LSD (p 1%)		5.48	
	LSD (0.1%)		8.22	

The combined influence of factors magnesium concentration (C) and the used mycelium strain (T) on the growth of the of the *C. militaris* L. mushroom mycelium, highlight the C2 T2 experimental combination, with 56.52 mm growth per 15 days of experiment, the obtained result being distinctly significantly positive, compared to the experience average considered as control.
The C3 T1 and C3 T2 experimental combinations, register distinctly significantly negatively and negatively differences, compared to the experience average considered as control. For this combination of factors, we have also experimental combinations that did not record statistically assured data, like C1 T1, C2 T1 and C1 T2.
Also, in this case the obtained data highlight the C2 concentration of magnesium supply.
Among the culture medium recipes found in the scientific literature, we can find also these concentrations of magnesium used by us during the experiment.
Taking into account that we have organized a three-factor experiment, the combination of the three factors was followed: magnesium source (S), mycelium strain (T) and magnesium concentration (C) on the growth of the of the C. militaris L. mushroom mycelium, the data obtained are presented in table 7.

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Table 7. The combined influence of factors: magnesium source (S), mycelium strain (T) and magnesium concentration (C) on the growth of the of the C. militaris L. mushroom mycelium

Experimental variant	Mycelial growth(mm)/15 days		Difference (mm) ±D	Signification of difference
	Obtained values	%		
S1 T1 C1	56.19	126.0	11.59	*
S2 T1 C1	32.98	74.0	-11.61	0
Average	44.58	100.0	0.00	Avg.
S1 T1 C2	57.72	131.8	13.93	**
S2 T1 C2	29.87	68.2	-13.92	00
Average	43.79	100.0	0.00	Avg.
S1 T1 C3	45.11	127.7	9.78	*
S2 T1 C3	25.52	72.3	-9.80	0
Average	35.32	100.0	0.00	Avg.
S1 T2 C1	61.63	128.8	13.80	**
S2 T2 C1	34.04	71.2	-13.78	00
Average	47.84	100.0	0.00	Avg.
S1 T2 C2	68.71	121.6	12.19	*
S2 T2 C2	44.34	78.4	-12.17	0
Average	56.52	100.0	0.00	Avg.
S1 T2 C3	56.22	126.5	11.79	*
S2 T2 C3	32.65	73.5	-11.77	0
Average	44.43	100.0	0.00	Avg.
	LSD (p 5%)		6.65	
	LSD (p 1%)		12.26	
	LSD (0.1%)		30.19	

Following the data in the table above, regarding the combined influence of factors, in this case, too, they highlight magnesium sulphate (S1) as the best source of magnesium in the *C. militaris* L. mushrooms culture medium, the recorded values being statistically assured, regardless of the concentration used or the mushroom strain. For magnesium carbonate (S2) we registered significantly negatively and negatively differences, compared to the experience average considered as control.

4. CONCLUSIONS

Based on the experimental results obtained on the complex influence of the magnesium source used in the culture medium of *Cordyceps militaris* L., the following can be concluded:

• the unilateral influence of the magnesium source used highlights magnesium sulphate, with statistically assured differences;

• the unilateral influence of the magnesium concentration used in the substrate recipe highlights the results obtained for the concentration of 3 grams.

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