INFLUENCE OF DIFFERENT TYPES OF GRANULATED SUBSTRATE ON *CORDYCEPS MILITARIS* MUSHROOM MYCELIUM GROWTH

INFLUENŢA DIFERITELOR TIPURI DE SUBSTRAT GRANULAT ASUPRA CREŞTERII MICELIULUI CIUPERCII *CORDYCEPS MILITARIS*

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Abstract. *Mushroom mycelium is a biological preparate that is obtained under sterile laboratory conditions and which, in an optimal microclimate, can reproduce the mushrooms from which it originates. Mushroom mycelium is used to inoculate the prepared substrates. This inoculum consists of a supportive material completely colonized by fungal mycelium. The type of support material varies depending on the cultivated mushroom species, although rye is the choice of most mycelium producers. In our experience, we have been looking at how to grow Cordyceps militaris mushroom mycelium on various substrates of cereal grains. The growth was followed for 10 days under laboratory conditions, and the most intense increase was recorded on millet grains with an average increase of 1.65 mm/day and the worst growth was recorded in the case of rye with an increase average of 1.05 mm/day.*
Key words: Cordyceps, mycelium, protein addition

Rezumat. *Miceliul de ciuperci este un preparat biologic ce se obţine în condiţii sterile de laborator şi care, plasat într-un microclimat optim, poate reproduce ciuperca din care provine. Miceliul de ciuperci este utilizat pentru a inocula substraturile preparate. Acest inocul este format dintr-un material de suport complet colonizat de miceliul ciupercii. Tipul de material de suport, variază în funcţie de speciile de ciuperci cultivate, cu toate că boabele de secară sunt alegerea majorităţii producătorilor de miceliu. În experienţa derulată am urmărit modul de creştere a miceliului ciupercii Cordyceps militaris pe diferite substraturi formate din boabe de cereale. Creşterea s-a urmărit timp de 10 zile în condiţii de laborator, iar creşterea cea mai intensă s-a înregistrat pe boabele de mei cu o creştere medie de 1,65 mm/zi, iar creşterea cea mai slabă s-a înregistrat în cazul boabelor de secară cu o creştere medie de 1,05 mm/zi.*
Cuvinte cheie: Cordyceps, miceliu, adaos proteic

INTRODUCTION

Cordyceps militaris (L.) Link is an entomopathogenic fungus, belonging to the Ascomycetes class, is one of the most important medicinal mushrooms which has been used popularly as a folk tonic food and a crude drug in East Asia (Ying *et*

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al., 1987). This medicinal mushroom, contains many kinds of active components, such as polysaccharides, cordycepin, mannitol and ergosterol, and due to its several physiological activities, it is currently used in medicine (Mizuno, 1999; Song *et al.,* 1998; Nag and Wang 2005). In the nature, is widely distributed, from sub-tropical to temperate regions around the world (Mains, 1958).

Until now, more than 375 species of Cordyceps were discovered all around the world, of which about 135 species have been originated in China (Jiang, 2004).

Cordyceps militaris (L.) Link is the type of Cordyceps species, which naturally parasitizes larvae or pupae of lepidopteran insects, and has a worldwide distribution (Kryukov *et al.,* 2011; Shrestha *et al.,* 2012).

All Cordyceps mushrooms are endoparasitoid species, mainly on insects and other arthropods, just a few species are parasitic other fungi (Shrestha *et al.* 2004).

Brownbridge *et al*. (1993) have studied common entomopathogenic fungi in forest soils and Hajek et al. (2000), have been isolated entomopathogenic fungi from the interface between leaf litter and the organic layer of soil.

In natural condition, Cordyceps militaris mycelium colonizes the body of an insect, the spores of the fungus germinate and produce a germ tube that penetrates the cuticle, allowing the mycelia to grow inside the host body (Tanada and Kaya 1993, Inglis *et al.,* 2001).

In last year's Cordyceps militaris is cultivated in liquid or in solid media (Das *et al.* 2010). In solid media different supplemented grain types and seeds are used (Chen *et al.* 2011, Shrestha *et al.* 2012, Wen *et al.* 2014, Yi *et al*. 2014, Rozsa *et al*. 2016 a, b, c, d).

Mushroom mycelium is used to inoculate prepared substrates. This inoculum consists of a support material completely colonized by the mushroom mycelium. The type of support material varies depending on the cultivated mushroom species, although rye beans are the choice of most mycelium producers (Rozsa *et al.,* 2016 a, b).

In 1932, Dr. James Sinden patented a new process of mycelium production, using cereal grains, as a support material for mycelium. Since then, rye has been the most commonly used cereal, although both millet and wheat have been used. The new approach of Sinden, has set a new standard for inoculation and is the basis for most modern inoculum productions. The distinct advantage of inoculation on cereal grains is the increase in the number of inoculation places. Each coarse grain thus becomes a point through which the mycelium can spread. Thus, one liter of the inoculum on rye grains contains about 25,000 grains, representing a vast improvement of the inoculum, compared to the coarse materials that have been used before (Rozsa *et al.,* 2016 a, b, c, d).

MATERIAL AND METHOD

To produce mycelium on cereal grains, these are moistened at 48-52% humidity, then sterilized in the autoclave or in a pressure cooker for 1 hour at 121 °C, in the containers to be inoculated (fig. 1).

After the cereal jars were autoclaved, they were placed in the sterile room and allowed to cool.

After removing the hot jars from the pressure cooker or autoclave, they were stirred to break up the grains caught on top and evenly distribute the wet and dry cores, according to the method described by (Rozsa *et al.*, 2016 a, b, c, d). Shaking prevents the beans from sticking and hardening on the bottom of the jar.

Fig. 1 Preparation of jars with cereals for sterilization (original).

The biological material used to inoculate cereal grains was a pure mycelium culture on agar substrate (fig. 2).

Fig. 2 Pure culture of *Cordyceps militaris* mycelium (original).

Bottle inoculation (fig. 3), was carried out at the flame of a gas bulb according to the method described by (Rozsa *et al*., 2016 a, b, c, d).

Fig. 3 Bottle inoculation (original)

In this experience we used as a protein additive dried and ground silkworm (*Bombyx mori*) chrysalis, in concentration of 10 grams per litre of cereals.

RESULTS AND DISCUSSIONS

The evolution of mycelium growth on the substrates used in this experiment with protein addition is shown in figure 4. The evolution of mycelium growth on the substrates used in this experiment without protein addition is shown in figure 5.

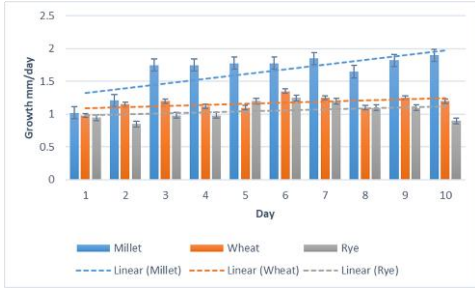

Fig. 4 The evolution of mycelium growth substrates with protein addition.

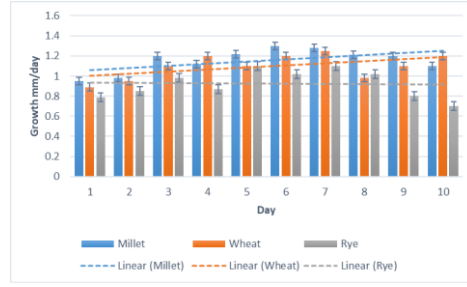

Fig. 5 The evolution of mycelium growth substrates without protein addition.

Following the unilateral influence of the substrate on mycelium growth, considering the substrate with rye, considering that most of the industrial producers of mycelium use as rye substrate, the millet substrate recorded very significant differences of growth 0.43 mm compared to the control considered, being followed by the wheat substrate with distinctly significant differences 0.15 mm.

Following the unilateral influence of protein addition on mycelium growth, there was a significant difference of 0.11 mm on substrates with additional protein addition and a significant negative difference on those without additional protein addition.

Following the combined influence of the factor's additional protein addition and substrate, at the substrate with millet and additional protein addition, there was a significant difference of 0.24 mm compared to the considered control.

The combined influence of substrate factors and additional protein addition once again revealed thousands based on or without additional protein addition, which in the case of additional protein addition recorded very significant differences, 0.60 mm compared to the average of the experience, and in the absence of additional protein addition, positive distinct significantly differences 0.26 mm from the control of experience.

The results obtained are comparable to those found in the literature, thus Wen *et al.* 2014 mentions an average increase of 0.3 mm / day, but without mentioning whether or not additional protein was used.

According to Yi et al. 2014 mentions an increase of 0.25 mm / day, with the addition of peptone.

CONCLUSIONS

1. The fastest growth of the mycelium of the *Cordyceps militaris* mushroom was recorded on millet grains, followed by wheat.

2. Adding additional protein powder represented by cocoons of silkworm (*Bombyx mori*) has positively influenced the growth and development of the mycelium fungus Cordyceps Militaris.

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