

THE INFLUENCE OF TEMPERATURE AND pH ON *CORDYCEPS MILITARIS* MUSHROOM MYCELIUM GROWTH

INFLUENȚA TEMPERATURII ȘI A pH-ULUI ASUPRA CREȘTERII MICELIULUI CIUPERCII *CORDYCEPS MILITARIS*

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Abstract. *Cordyceps* is from the Greek *kordyle* meaning “club” and *ceps* for “head”. *Sinensis* means “from China”. *Militaris* is related to the growth pattern that looks like a regiment of toy soldiers. *Cordyceps* is the fruiting body of fungi parasitizing other fungi, such as the deer truffle (*Elaphomyces* spp.), or insects, such as caterpillars of moths, ants, and beetles. It grows inside the caterpillars and other insects to produce hyphae. When they die, the fungus produces a fruiting body that sporulates into the wind to infect another generation. For the successful cultivation of any mushroom on a small scale or commercial scale, one of the most important requirements is the mycelium of that species or variety. The spawn is a pure culture of the mycelium grown on a special medium. The growing temperature and substrate pH are specific for each species and variety.

Key words: mushrooms, *Cordyceps militaris*, spawn, temperature, pH

Rezumat. *Cordyceps* provine din grecescul *kordyle* care înseamnă "club" și *ceps* care înseamnă "cap". *Sinensis* înseamnă "din China". *Militaris* este legat de modul de creștere, care arată ca un regiment de soldați de jucărie. *Cordyceps* este un organ de fructificare al unei ciuperci, care parazitează alte ciuperci, cum sunt Truftele de cerb (*Elaphomyces* spp.) sau insectele, cum sunt omizii de molii, furnicile și gândacii. Ea crește în interiorul omizi și în insecte pentru a produce hife. Când mor, ciuperca produce un corp fructifer, care sporulează în vânt pentru a crea o altă generație. Pentru cultivarea cu succes a oricărui ciuperci la scară mică sau la scară comercială, una dintre cele mai importante cerințe este miceliul acestei specii sau varietății. Miceliul este o cultură pură pe un mediu special. Temperatura de creștere și pH-ul substratului sunt specifice fiecărei specii și tulpini.

Cuvinte cheie: ciuperci, *Cordyceps militaris*, miceliu, temperatură, pH

INTRODUCTION

Cordyceps sinensis is the most well-known, but the genus can claim several other medicinal stars. It parasitizes the larvae of a bat moth. It is estimated that over 680 varieties of *Cordyceps* have been discovered and named, and more than thirty are common in alpine terrain.

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In ancient times, its use was restricted to the Emperor's Palace, and baked in duck as a tonic similar to Ginseng. It sold at one time for four times its weight in silver. It has a spicy cinnamon fragrance that lends itself to soups and broths.

Cultivation

Although the use of wild fungi for medicinal purposes is appealing, the fermentation of mycelium on wheat and other grains produces a safer and more consistent product. Various studies indicate similar antioxidant properties between wild and cultivated strains. Another advantage of the fermented product is that it is 100 percent vegetarian. Various substrates have been used including wheat, rice, sorghum, and even organic purple corn.

Isaka *et al.* (2005) isolated a new *C. sinensis* anamorph with potential to improve cultivated lines.

Traditional Uses

Adder's tongue is mild, slightly acrid, and used in traditional Chinese medicine as a lung and kidney tonic. It increases production of red blood cells, increases sperm production, and strengthens qi.

It combines well with Garden Burnet (*Sanquisorba officinalis*) root for menorrhagia and irregular menstrual cycles. This is probably due to two compounds isolated from the mycelium that possess estrogenic activity (Kawagishi *et al.* 2004).

Chinese Caterpillar mushroom (*C. sinensis*) has been used as an aphrodisiac for nearly two thousand years, with the first written record in the Classic Herbal of the Divine Plow-man from 200 AD.

Cordyceps strengthen both the mind and body at a very basic level, replenishing yin jing and restoring the deep energy depleted by excessive stress.

In traditional Chinese medicine, the mushroom is used for fatigue, wheezing, shallow breathing, and loss of stamina associated with kidney and lung yang deficiency.

Because it tonifies both yin and yang, it can be used safely in nearly any endocrine condition to help relieve fatigue and calm the nervous system. To be more exact, Cordyceps is both yin-nourishing and yang-invigorating.

Medicinal Use

Chemical Constituents

■ *C. sinensis*: various sterols, polysaccharides, galactomannans, cordycepic acid, protein, adenine, adenosine, uridine, uracil, cordycepin, mannitol, eighteen amino acids, ergosterol, vitamin B12, trace elements, and saturated and unsaturated fatty acids.

■ *C. militaris*: cordycepin, ergosterol, beta sitosterol, adenosine, adenine, and D-mannitol.

C. militaris contains cordycepin that shows reverse transcriptase inhibition (Penman *et al.*, 1970). According to Christopher Hobbs, in his inspiring book Medicinal Mushrooms, cordycepin (3'-deoxyadenosine) was dropped as a clinical isolate for cancer due to its toxic side effects.

Hui Mei Yu *et al.* (2006) compared the oxidative damage protection of *C. militaris* and *C. sinensis*. The content of adenosine and cordycepin is higher in the former and both show antioxidant protection.

Kim *et al.* (2006) found *C. militaris* more hypoglycaemic in activity than in *C. sinensis*. Rukachaisirikul *et al.* (2004) identified cordycepin, pyridine-2,6-dicarboxylic acid, and cepharosporides C, E, and F in this species.

Work by Lin *et al.* (2007) on sub fertile boars found both the quality and quantity of sperm increased by supplementation with *C. militaris* powder.

Water extracts of *C. militaris* have been found to induce apoptosis and growth inhibition of U937 leukaemia cells. The regulation of several major growth gene products such as Bcl-2 family expression and caspase protease activity suggests therapeutic potential for human leukaemia (Park *et al.*, 2005).

The fungi induced IL-18 and acted on IFN- γ production (Kim *et al.* 2008).

Extracts of *C. militaris* have been found to exhibit anti-angiogenic properties and repress growth of B16-F10 melanoma cells in mice compared to Controls (Yoo *et al.*, 2004).

Pokhrel *et al.* (2006) found a hot-water extract of *C. militaris* inhibited cancer cell proliferation by inducing cell apoptosis through the activation of caspase-3, and that the extract may have potential in human leukemia.

A polysaccharide isolated from culture, CPS-1 has been shown to possess significant anti-inflammatory activity and suppressed the humoral immunity in mice, but no significant effect on cellular and non-specific immunity has been found (Yu *et al.*, 2004).

MATERIAL AND METHOD

For the experiments with the *Cordyceps militaris* mushroom mycelium was used a semisolid agar medium using the following formula/recipes:

1. PDA – potatoes dextrose agar, composed by 200g sliced potatoes, 20g agar, 20g dextrose, 1000ml distillate water, sterilized at 121 °C for 1 hour (Rózsa *et al.*, 2016a, b).

2. CEA – compost agar, composed by 50g dry *Agaricus* compost extract, 20g agar, 1000ml distillate water, sterilized at 121°C for 1 hour in the first day, repeated after 24 hours and 48 hours (Rózsa *et al.*, 2016b, c).

3. MEA – malt extract agar, composed by 50g malt extract, 20g agar and 1000ml distillate water, sterilized at 121°C for 1 hour (Rózsa *et al.*, 2016c, d).

The tested temperature ranges in this study were: 22, 23, 24, 25, 26, 27, 28, 29, 30, 31°C. The pH ranges used in the experience were: 4*, 4.5*, 5* (* - adjusted with citric acid C₆H₈O₇), 5.5, 6**, 6.5**, 7**, 7.5** and 8** (** - adjusted with sodium hydroxide 1n NaOH).

The results were recorded during 10 days of mycelium run and was expressed in mm/day.

RESULTS AND DISCUSSION

The influence of temperature on mycelial growth, is presented in figure 1.

Following the results presented in figure 1, we can see that the maximum mycelial growth was recorded at 26 °C on PDA recipe with 9.8 mm / 10 days, being followed by MEA recipe at 27 °C with 9.7 mm of growth on 10 days.

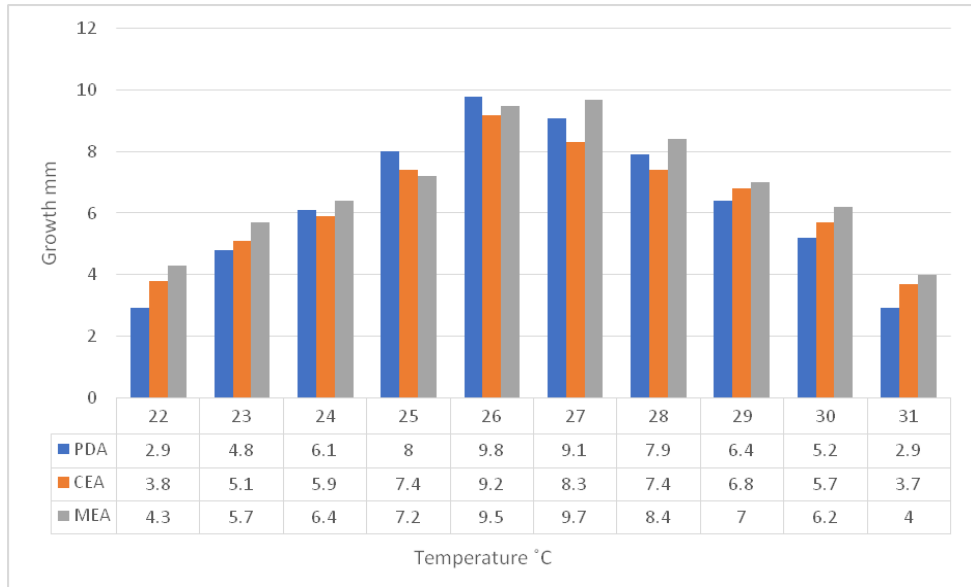


Fig. 1 The influence of temperature and recipe on *Cordyceps militaris* mycelium growth.

Taking into account the unilateral influence of recipe used in experience on the *C. militaris* mycelial growth (fig.1), we recorded a difference of 1.85 mm/day being very significant positive, to PDA taken as controls which registered value 9.8 mm/ 10 days.

Taking into account the unilateral influence of temperature used in experience on the *C. militaris* mycelial growth, we recorded a difference of 2.21 mm/day at 26 °C being very significant positive, to 24 °C taken as controls (fig. 1) which registered 6.1 mm/ 10 days.

The influence of combined factors, temperature on the growth recipe of mycelium, at 26°C, were recorded the highest values, on PDA recipe, followed by 27 °C and 26 °C on MEA recipe. On the last place was located 22 and 31 °C, both temperatures for PDA recipe.

The influence of pH on mycelial growth, is presented in figure 2.

Following the results presented in Fig. 2, we can see that the maximum mycelial growth was recorded at 5.5 pH value on CEA recipe with 9.9 mm / 10 days, being followed by MEA recipe at the same pH value with 8.9 mm of growth on 10 days.

Taking into account the unilateral influence of recipe used in experience on the *C. militaris* mycelial growth (fig. 2), we recorded a difference of 1.35

mm/day being very significant positive, to PDA taken as controls which registered value 9.2 mm/ 10 days.

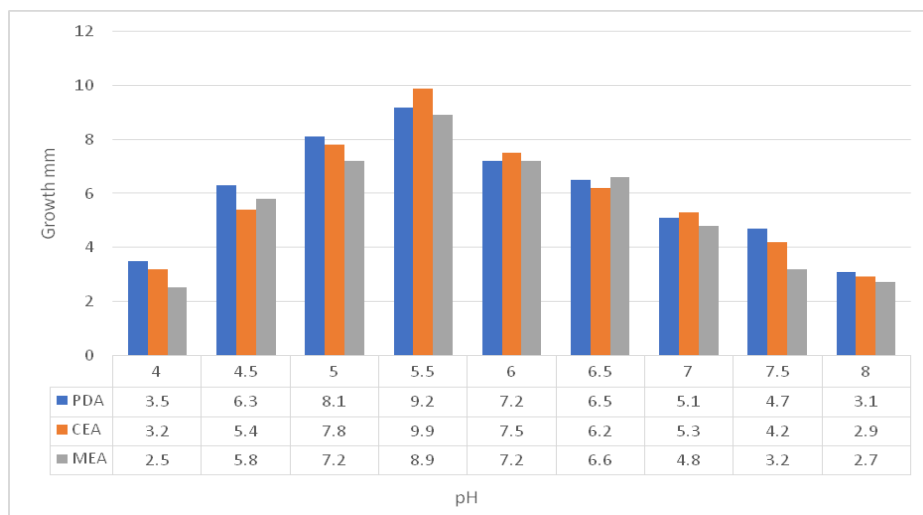


Fig. 2 The influence of pH and recipe on *Cordyceps militaris* mycelium growth.

Taking into account the unilateral influence of temperature used in experience on the *C. militaris* mycelial growth, we recorded a difference of 1.27 mm/day at 5.5 pH value, being very significant positive, to pH value 5 taken as controls (fig. 2) which registered 8.1 mm/ 10 days.

The influence of combined factors, pH on the growth recipe of mycelium, at 5.5 pH value, were recorded the highest values, on CEA recipe, followed by 5 pH value with 8.1 mm / 10 days on PDA recipe and 7.5 mm of growth / 10 days at 6 pH value. On the last place was located pH 4 with 2.5 mm growth on MEA recipe and pH 8 with 3.1 mm of growth on PDA recipe.

CONCLUSIONS

Mycelium run days varied significantly due to different recipes, pH and temperature values.

The optimal temperature range for *Cordyceps militaris* studied strain is 26-28 °C, on PDA and MEA recipe, at 5.6 pH value.

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