# RESEARCH CONCERNING THE RESPONSE OF *CLADOSPORIUM HERBARUM* CULTURES GROWN ON CELLULOSE SUPPORT, NAMELY PAPER, LINEN AND CARDBOARD, THE ACTION $NH_4^+$ CONCENTRATION OF 0,2% AND 0,5%

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***Abstract***

*The biodegradation of the culturally and economically marked goods is causing huge damage, this type of process is launched when several circumstances, as the following, come to meet: optimum medium environmental conditions, the source of food, and the presence of the pathogen agent. One of the microorganisms, often isolated, on the surface of the works of art is the chroma-togenous fungus, Cladosporium herbarum, species we have inseminated, in the present experiment, upon three cellulose based strata as arranged: paper, flax fabric, cardboard. We watched its evolution for 14 days, after which we pulverized a lair of $NH_4^+$ 0,2%, and 0,5%, and, after four days to make new observations on the growth and mycelium development, as well as the nutritive sub layer Ph to a certain extent. The insemination was performed with already grown mycelium on solid media, in sterile environments, on cellulose material deposited in Petri boxes and leveled the ph to neutral.*

*In the long run it has been noticed that in all cases the fungus metabolic activity led to acidifying the cultural layer, both before applying the treatment and after having applied the $NH_4^+$ solution in a 0,2%, and 0,5%, dilution concentration, which proved insufficient to combat and eradicate the fungus attack. The most vulnerable cellulose sub layer exposed to biodegradation, determined by the Cladsporium herbarum species, was the paper.*

**Keywords:** mycelium, paper, flax fabric, cardboard, Ph

## INTRODUCTION

Genus *Cladosporium* fungi can be isolated from natural environment almost anywhere in the world (Schubert and colab., 2005a; Yano and colab., 2003) they are part of common micromycetes. Genus *Cladosporium* is saprophyte but contains pathogenic species (Masclaux and colab., 1995; Rippon, 1988), the most common species *Cladosporium herbarum remember, C. sphaerospermum, C. cladosporioides* and *C. elatum* (Deacon, 1999; Qiu-Xia and colab., 2008).

The activity of various living organisms is due to various degradation processes taking place at the cultural heritage movable or immovable, of which the assets are micromycetes. Biodegradation mechanisms are different depending on the nature of the substrate - organic or inorganic. Action of fungus on cellulose media is common cellulolytic fungi which are capable of external digestion via enzymes degrade cellulose.

When the mycelium of *Cladosporium*, during growth, the cell wall are synthesized a series of enzymes (Williamson and colab., 2000), complex

polysaccharides and various secondary metabolites, including phenolic complexes that allow degradation of cellulose (Sadena and colab., 1988). Genus *Cladosporium* external enzymes synthesize a CDH (Celulo dehydrogenase) first discovered in *Sporotrichum pulverulentum, Polyporus versicolor* and (Westermark and colab., 1974; Westermark and colab., 1975) and was subsequently found in many other *Ascomycetes* and imperfect fungi (Bao nd colab., 1993; Coudary and colab., 1982; Dekker, 1988; Henriksson and colab., 2000). Importance of CDH is linked celulolitică activity, but also participates in iron uptake, oxidative phosphorylation and kill competing organisms (Ander and colab., 1996; Henriksson and colab., 2000) also, this enzyme has many applications in the pulp and paper (Henriksson and colab., 2000; Qiu-Xia and colab., 2008).

Cellulose degradation by the fungus *Cladosporium* is closely related to the presence of CDH enzyme whose production is dependent on pH and temperature. The largest amount of CDH occurs at a pH of 4,5 and a temperature of 28 °C (Fang and colab., 1999; Henriksson and colab., 2000; Sadena and colab., 1985; Westermark and colab, 1975; Wood and Wood, 1992). Knowing all this, in this experiment, we sought to examine how the evolving culture of *Cladosporium herbarum* supports three types of cellulose, ie paper, linen and cardboard, and pH changes in the culture medium before and after be treated with a solution of $NH_4^+$ 0,2% and 0,5%.

**MATERIALS AND METHODS**

In this experiment we used for insemination me celia fungus *Cladosporium herbarum* cultivated by us, under sterile conditions in Petri capsules on solid agar medium.

*Cladosporium herbarum* is part of celulolitice fungi, for which we used as a nutrient substrate paper, linen and board, obtaining three different experiments as follows:

$V_1$ - under the nutritive substrate paper;

$V_2$ - under the nutritive substrate linen;

$V_3$- under the nutritive substrate cardboard.

For a more accurate as the results for each of the three experimental variants ($V_1$, $V_2$, $V_3$) i made every 30 samples. The support material was sterilized by dry heat with the iron at a temperature of 200-300 °C, then using sterile tweezers i inserted in Petri capsules also sterile.

PH meter with pH found support material - paper, linen and cardboard - which was brought to neutral (pH 7). To this end i distilled water diluted to 0,1% HCl and 0,1% NaOH, spraying, as appropriate, the support base or acid material. As wetted material was inoculated with mycelium of *Cladosporium herbarum*, using sterile tweezers. The operation

took place within the laminar flow hood and sterile air was performed quickly to avoid penetration of other microorganisms from the outside.

Such containers were sown kept for 14 days in daylight and at a constant temperature of 28 °C. After 14 days of insemination observations were made (to the naked eye and magnifying glass) on mycelium size, color and its appearance, and nutritional substrate pH. The operation was followed by treatment of a solution of $NH_4^+$ 0,2% and 0,5%, and after four days, during which cultures were kept in the same microclimate conditions, we repeated readings.

**RESULTS AND DISCUSSION**

Comparing the average size of the mycelium is noted that 14 days after insemination of media with vegetative hyphae of *Cladosporium herbarum* growing mycelium with the nutrient substrate is represented in the board ($V_3$) where its average diameter was 1,0 cm, 0,2 cm higher than the average diameter of mycelium grown on linen ($V_2$) and 0,1 cm above the average diameter of mycelium grown on paper ($V_1$) (Fig.1).

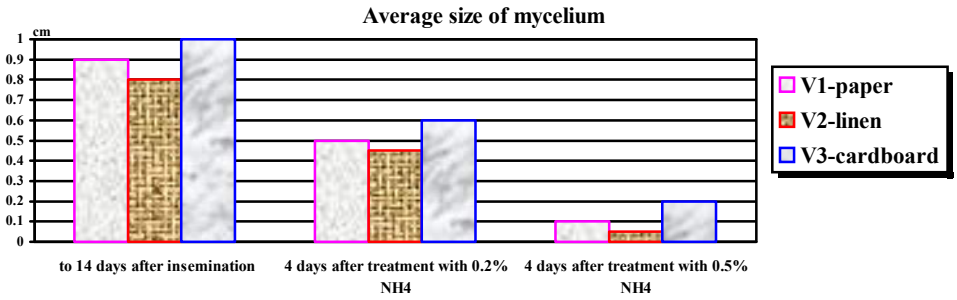


Fig.1. Evolution of the average micelle size of *Cladosporium herbarum* - 14 days after insemination and 4 days after treatment with $NH_4^+$ 0,2% and 0,5% - expressed in absolute terms, grown on different substrates nutrients, namely: version $V_1$ represented the paper substrate nutrients on nutritive substrate, variant $V_2$ is the linen represented and represented the nutrient substrate $V_3$ variant of cardboard.

These results are due, perhaps, that though the three culture media are capable of cellulose in the composition of cards in the waste paper and old textiles, coatings thus obtained are joined using adhesives, which offer a wider range fungus of nutrients. Values obtained by us conform to previous research (Henriksson and colab., 2000), supporting the idea that *Cladosporium* thrive on cellulosic support, but the fabric mycelium growth is accelerated compared to the paper, and so it is more intense coloring on paper. At 4 days after treatment with $NH_4^+$ 0,2% and 0,5%, it is noted that the paper ($V_1$) and cardboard ($V_3$), the witness reported, there have been - in absolute terms - loss mycelium in the average size of 0,4 cm in the first case

and 0,8 cm in the second case. The linen ($V_2$) hyphae mycelia are found only in the spaces between fibers and fabric, this parameter has declined significantly less that the 0,35 cm and 0,75 cm (Fig.1).

The paper ($V_1$) and cardboard ($V_3$) mycelium is located entirely on the surface of the support that comes as a powder (Fig.2.I. şi 2.III.), while the linen ($V_2$) hyphae mycelia are restricted in the spaces between the fabric fibers (Fig.2.II.).

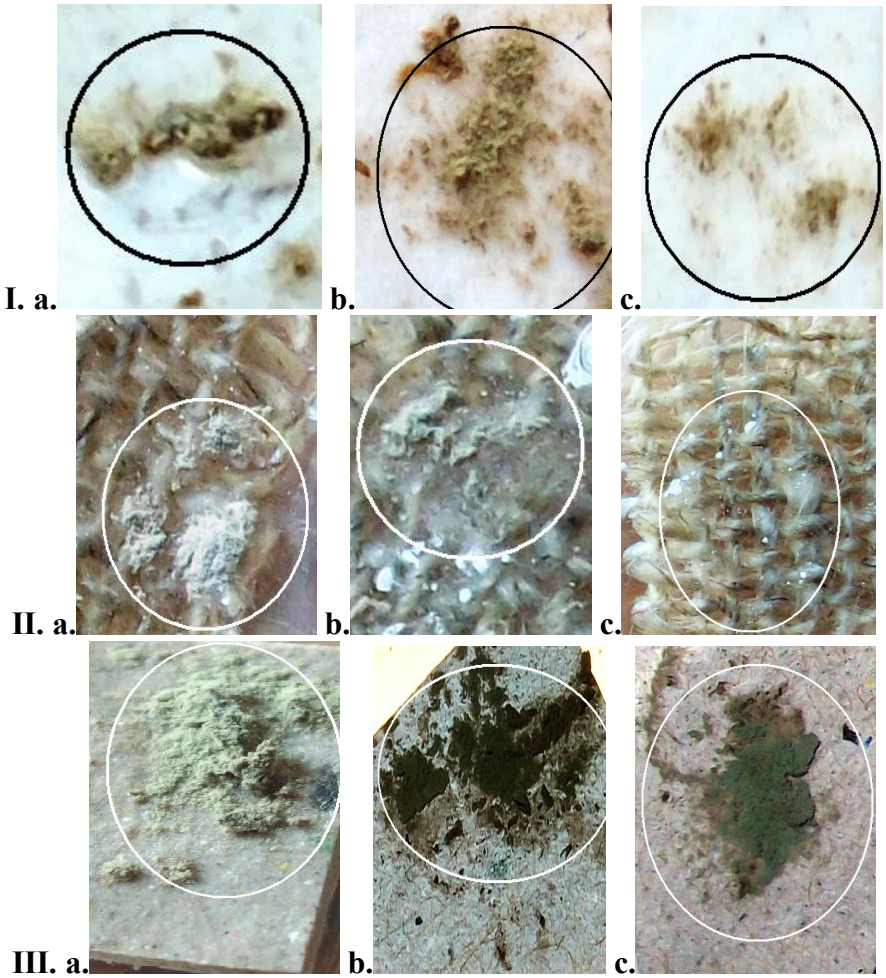


Fig.2. Images *Cladosporium herbarum* mycelium grown on different substrates nutritional insemination 14 days and 4 days after treatment with $NH_4^+$ 0,1%, respectively: I-mycelium of *C.herbarum* inoculated on paper, where: to spawn *C.herbarum* to 14 days after insemi-nation, b-mycelium of *C.herbarum* at 4 days after treatment with $NH_4^+$ 0,2%, c-mycelium of *C.herbarum* at 4 days after treatment with $NH_4^+$ 0,5% II-mycelium of *C.herbarum* inoculated on linen, where: mycelium of *C.herbarum* to 14 days after insemination, b-mycelium of *C.herbarum* at 4 days after treatment with $NH_4^+$ 0,2 %, c-mycelium of *C. herbarum* at 4 days after treatment with $NH_4^+$ 0,5%, III-mycelium of *C.herbarum* inocu-lated on board, where: mycelium of *C.herbarum* to 14 days after insemination; b-mycelium of *C.herbarum* at 4 days after treatment with $NH_4^+$ 0,2%, c-mycelium of *C.herbarum* at 4 days after treatment with $NH_4^+$ 0,5%.

Monitoring the survival rate of the hyphae of *Cladosporium herbarum* four days after application, spraying, treatment with $NH_4^+$ 0,2% and 0,5%, compared witness (without treatment), it is noted that these values decreased substantially in all three experiments. Application of $NH_4^+$ 0,2%, both for hyphae grown on linen ($V_2$) and paper ($V_1$) resulted in a 50% decrease of this parameter, while on board ($V_3$) was decreased with 42,8% (Fig.3), compared to values recorded in control samples, samples in which no treatments were applied. $NH_4^+$ concentration increased to 0,5% caused this parameter decreased to 8% for mycelium of *Cladosporium herbarum* on linen ($V_2$) and 17% in the paper ($V_1$), while on board ($V_3$) to the highest value for this experiment, namely 23% (Fig.3). With decreasing size of mycelium decreased horizontal and amount of vegetative hyphae, it decreased in height, so it may be noted that the paper ($V_1$) and board ($V_3$) the place where fungal colony was destroyed (in presence of $NH_4^+$), material support remained stained (Fig.2.I.c şi 2.III.c). The linen ($V_2$) due to the position that he held the mycelium of *Cladosporium herbarum* can not immediately see the obvious spots caused by the presence of fungus, due, perhaps, darker color of the fabric than paper and cardboard (Fig.2.II.c).

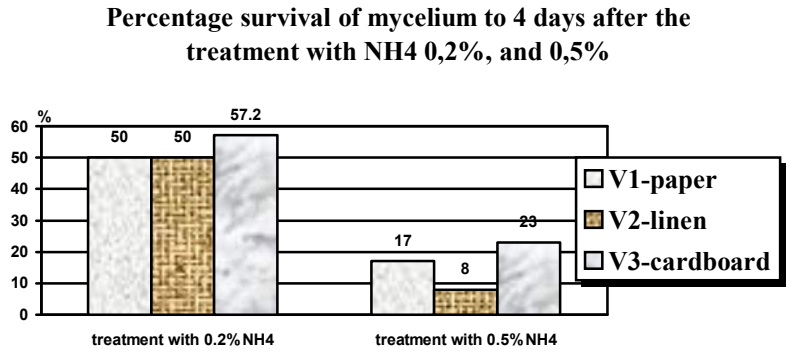


Fig.3. Evolution of the average micelle size, expressed as a percentage, of *Cladosporium herbarum* grown on different substrates, nutrients, namely: version $V_1$ represented the paper substrate nutrient, nutrient substrate represented the variant $V_2$ is the $V_3$ version linen and nutrient substrate is represented by cardboard, four days after spraying a treatment with $NH_4^+$ concentration of 0,2% and 0,5%.

The results of this experiment are similar to those published in the literature [15], which presents that the mycelium of *Cladosporium herbarum* is growing weaker in the presence of $NH_4^+$, but in all three cases investigated by us, the concentration of $NH_4^+$ (0,2%, and 0,5%) was insufficient to eradicate the fungus attack, the worst effect on hyphae of *Cladosporium herbarum* was recorded on paper ($V_3$), due possibly chemical composition of the substrate (Fig.3).

Examining the mean values of pH in the culture medium in the three experimental variants we found that: both 14 days after insemination

mycelium of *Cladosporium herbarum* and 4 days after treatment with $NH_4^+$ 0,2%, respectively 0,5%, pH experimental substrates was acid, acidification of which is the result of metabolic activity of the fungus. Lowest values of pH were recorded on paper ($V_1$), where 14 of culture was recorded at pH 4,9, by applying $NH_4^+$ 0,2%, mean pH in four days after treatment were 5,7 and 6,0 versions where $NH_4^+$ concentration was 0,5% (Fig.4).

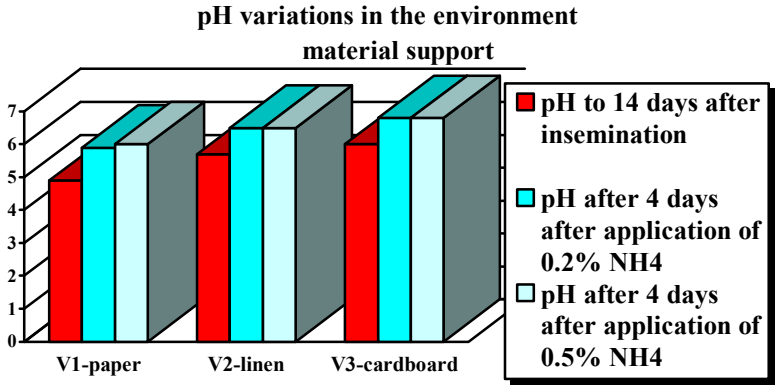


Fig.4. Evolution of mean values of pH represented by the support material: paper - version $V_1$, linen - $V_2$ version and cardboard - $V_3$ version, at 14 days after insemination mycelium of *Cladosporium herbarum* on these media and 4 days after application treatment with $NH_4^+$ 0,2% and 0,5%.

Regarding the aforementioned parameter values on linen ($V_2$) and cardboard ($V_3$), 14 days after insemination mycelium, they were respectively 5,9 and 6,0 4 days after treatment with $NH_4^+$ 0,2% pH recorded in the two growing media was 6,5 and 6,8 in the experimental variants that were applied at a concentration of 0,5% $NH_4^+$(Fig.4). Elevated pH in all three experiments, is due to $NH_4^+$ application solution. After Coudary and collaborators (1982) a pH of 4,5 promotes maximum activity of cellulose degradation by fungi *Cladosporium*, this process is directly correlated with the amount of secreted CDH based on these considerations the results obtained from our research, we justify to conclude that the support material was exposed biodegradation paper, where pH 4,9 was found which was the nearest 4,5, similar results obținutute by us and in an experiment previously (Vidican and colab., 2012).

**CONCLUSIONS**

1. After 14 days of vegetative hyphae of *Cladosporium herbarum* insemination on cellulose culture media average size of the micelles have undergone small differences, the highest increase was recorded on paper ($V_3$) where he had an average diameter of 1,0 cm, compared with the 0,8 cm recorded on linen ($V_2$) and 0,9 cm on paper ($V_1$).

2. Four days after spraying treatment with $NH_4^+$ 0,2% average micelle diameter decreased in all experimental variants, with 50% linen ($V_2$) and paper ($V_1$) and 42.8% on cardboard ($V_3$).
3. A concentration of 0,5% $NH_4^+$ has led to four days after application, a decrease in the average micelle size of *Cladosporium herbarum* with 92% linen ($V_2$), and 83% paper ($V_1$) and 77% on cardboard ($V_3$), these results show that concentrations of $NH_4^+$ year used in this experiment is not sufficient for eradication of *Cladosporium herbarum* attack.
4. The pH of the nutrient substrate so after 14 days of culture and 4 days after treatment with $NH_4^+$ 0,2% and 0,5%, was below 7, the acid environment being recorded on paper ($V_1$) or 4,9, which is most exposed to support biodegradation.

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