

INVESTIGATION ON THE INITIATION OF CULTURES OF *PENICILLIUM SPP* AND THEIR RESPONSE TO THE PRESENCE IN THE CULTURE MEDIUM OF VARIOUS CONCENTRATIONS OF BORAX

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Abstract

By meeting environmental conditions favorable genus Penicillium fungi can colonize different art media with organic or inorganic, which makes no proper treatment they undergo a series of aesthetic and structural damage. To identify an effective method to eradicate fungal hyphae have grown vegetative spp Penicilium a basal medium (MB) obtained in 1000 ml distilled water and 30 g sucrose, solidified with 7 g of agar - agar. To determine their evolution under the influence of different amounts of borax I added the following experimental getting MB: M-MB control (control), devoid of borax, V1-MB supplemented with 0.05 mg / l borax, V2-MB supplemented with 0.10 mg / l borax, V3-MB supplemented with 0.15 mg / l borax and V4-MB supplemented with 0.20 mg / l borax, V5-MB supplemented with 0.25 mg / l borax.

After 15 days of insemination we found that the presence in the culture medium to a concentration of 0.25 mg / l borax inhibited the utmost growth and development of Penicilium spp micelle surface coverage of the culture medium was reduced by 79%, compared to controls, but the amount of borax added was not sufficient to eradicate the fungus attack.

Keywords: mycelium, pH, cultural medium, borax

INTRODUCTION

Besides moisture, temperature, pH and light, presence of a wide range of organic and inorganic molecules contained in a cultural cause them to be an environment for growth and development of fungi of the genus *Penicillium* for example (Andersen and colab., 2003; Li and colab., 2010; Peltola and colab., 2001; Tuthill and colab., 2001), which are in the air as fungal fragments (Baohua and colab., 2012; Gorney and colab., 2002). By installing them, fungi, produce a series of metabolites (toxic for both humans and animals) that plays a crucial role in their habitat, for example mycotoxins (Nielsen, 2003; Singh, 1977; Wouters and colab., 2000), glucans (Andersen and colab., 2003), extracellular polysaccharides (Duowes and colab., 1999; Tuomi and colab., 2000), exoenzyme (Nieminen and colab., 2002), solvents (Burge, 1990) and other substances, which came into contact with the surface 'appeal' cause at its aesthetic and structural damage. Of aesthetic damage such as: changing pigmentation, blemishes or even formation of a biofilm.

This experiment is focused on the influence of borax in the culture medium on growth and development of fungi of the genus *Penicillium*.

MATERIAL AND METHODS

Knowing that the nutritional requirements of fungi are so diverse that there is no standard environment for all species, we used a nutrient substrate represented by a basal medium (MB) that I obtained in 1000 ml distilled water, 30 g sucrose and the solidification we added 7 g of agar - agar (Claeson and colab., 2002). How to obtain basic environment (MB) was as follows: we heated glucose water agar and then I said I boiled the environment until it has melted. I left after boiling medium to cool and when it reached 25 ° C have final pH adjusted to 5.5 ± 0.2 .

In this experiment we wanted to see how the presence of a nutrient substrate different amounts of borax influence growth and development of fungi of the genus *Penicillium*. In this respect with blank - probably without borax - we added different amounts of borax MB obtaining the following: M-MB control (control), without addition of borax, V₁-MB supplemented with 0,05 mg/l borax; V₂-MB supplemented with 0,1 mg/l borax, V₃-MB supplemented with 0,15 mg/l borax, V₄-MB supplemented with 0,2 mg/l borax, V₅-MB supplemented with 0,25 mg/l borax.

After this followed the environmental sterilization by autoclaving at 121°C for 15 minutes (Wayne and colab., 2010). Averages thus obtained were cast in Petri capsules - each divided into four boxes using glass plates - sterile, for each variant of the culture dishes seeded with 20 boxes. For sowing i used biological material represented by vegetative hyphae of *Penicillium spp.* cultivated new solid culture media. Seeded containers were kept for 15 days in daylight and at a constant temperature of 27°C. To reflect the action borax (on growth and fructification of the genus *Penicillium* fungi), introduced in varying amounts in the culture medium of 3 in three days, i made eye examinations and magnification, noting they were the size and percentage of representation of the mycelium on the surface of the culture medium and the macroscopic appearance of mold grew on the substrate. At the same time i realized and measurements of pH changes of culture medium as a result of metabolic activity of the fungus.

RESULTS AND DISCUSSION

Making a comparative analysis of pH of the culture and its coverage of mycelia of *Penicillium spp.* is noted that even if these species are recognized as being very tolerant to an acidic pH (Wheeler and colab., 1991), where our experiment, the media with the highest acidity - pH 4,1 - have the lowest yield of biomass. This experimental variants manifested in

the culture medium to which we added 0,20 mg/l borax (V₄) and 0,25 mg/l borax (V₅). However we can say that organic acid excretion was not abundant, and therefore not influenced aggressive nutritional substrate pH, probably due to the decrease in biomass yield of *Penicillium spp.*

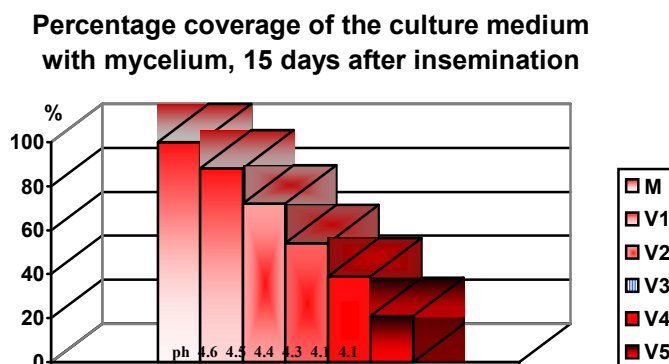


Fig.1. Coverage area of the culture medium, 15 days after insemination, the mycelium of *Penicillium spp.* nutrients on different substrates, namely: M-MB version control arm (control), devoid of borax, V₁-MB supplemented with 0,05 mg/l borax, V₂-MB supplemented with 0,15 mg/l borax, V₃-MB supplemented with 0,15 mg/l borax, V₄-MB supplemented with 0,2 mg/l borax, V₅-MB supplemented with 0,25 mg/l borax.

It is accepted that pH is one of the most important environmental parameters which directly influence the germination of spores (Li and colab., 2010). If this research blank (no added sodium borate) was initially a pH value of $5,5 \pm 0,2$, and after 15 days of experiment, a pH of 4,6 and a coverage with mycelium of 100% (Fig. 1), which is consistent with previously published studies which show that the optimum pH for germination of spores of *Penicillium* is around 5 (Li and colab., 2010). Decreasing pH in the nutrient substrate has not, as expected, directly and to maintain its coverage of the mycelium, so a pH of 4,5 the culture medium supplemented with 0,05 mg/l borax (V₁) this parameter was below control by 12% to 28% less on a culture medium supplemented with 0,10 mg/l borax (V₂) at pH 4,4, in while the culture medium supplemented with 0,15 mg/l borax (V₃) at pH 4,3 and mycelium of *Penicillium spp.* did not cover more than 72% of its surface. It is noted that the culture media supplemented with 0,20 mg/l borax (V₄) and 0,25 mg/l borax (V₅), pH is maintained at a value of 4,1, but coverage of the environment culture is declining compared with control, with 61% in the first case and 89% in the second case (Fig. 1).

Based on the results obtained in this experiment we can say that the biomass yield decreased in *Penicillium spp.* even at pH obtin nutrient substrate is due to the presence in the culture medium of various

concentrations of borax, which according to the results previously published (Tuthill and colab., 2001) is very effective in combating molds produced by filamentous fungi at a pH below 5, and the more effective the closer the value of this parameter is approaching 2. In this case, analysis of population dynamics of *Penicillium spp.* showed that with increasing concentration of borax in the culture medium decreases both its pH and mycelial biomass, due to the effects of boron, which inhibits mitochondrial functions of the fungus and lead to death it (Wayne and colab., 2010).

Based on readings taken every three days during the 15 days the experiment lasted current, operation at light labor protection rules and in accordance with the methods described in "Materials and Methods", i noted that dynamic diameter Average hyphae of *Penicillium spp.* is significantly influenced by amount of borax present in the culture medium

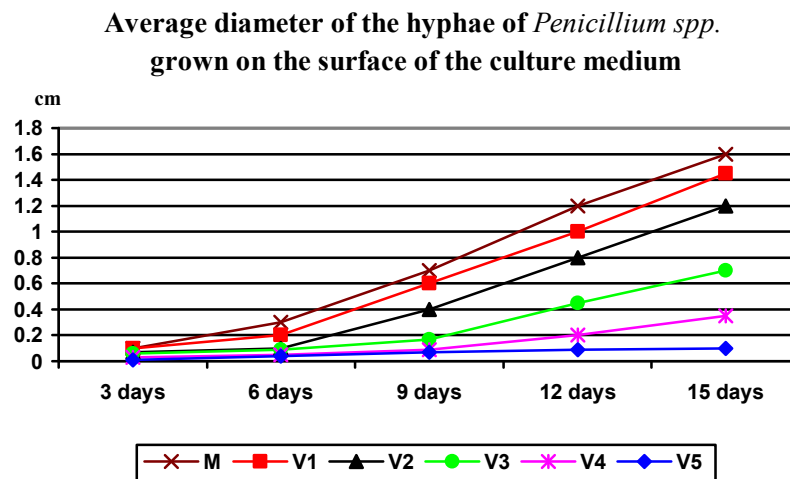


Fig.2. Average diameter of the hyphae of *Penicillium spp.* at certain times of their development and nutritional grown on different substrates, namely: M-MB version control arm (control), devoid of borax, V₁-MB supplemented with 0,05 mg/l borax, V₂-MB supplemented with 0,15 mg/l borax, V₃-MB supplemented with 0,15 mg/l borax, V₄-MB supplemented with 0,2 mg/l borax, V₅-MB supplemented with 0,25 mg/l borax.

I reported to blank (no added borax), presence in the culture medium of a quantity of 0,05 mg/l borax (V₁) and 0,10 mg/l borax (V₂) influenced in the slightest as the average diameters of *Penicillium spp.* hyphae so three days after insemination in the first case this makes no difference, while in the latter case the value of this parameter was less than 0,03 cm. The following readings mean micelle diameters of *Penicillium spp.* were constantly under the control situation, with larger differences, respectively 0,2 cm in six days, nine days and 0,3 cm by 0,4 cm of the twelfth day before the end of the experiment to which we added to version 0,10 mg/l borax

(V₂) while the sample to which we introduced in the culture medium 0,05 mg/l borax (V₁) the differences are minor, throughout the growing season, topping them with 0,1 cm below the standard in the sixth and ninth day, 0,2 cm in the twelfth day and 0,15 cm after fifteen days (Fig.2). Diameter hyphae of *Penicillium spp.* on culture media with the addition of 0,15 mg/l borax (V₃) had significantly higher differences relative to witness M, so three days after insemination this parameter amounted to 0,04 cm below the standard, by 0,21 cm in the sixth day and 0,53 cm in the ninth day of culture, after which the differences widened reaching the twelfth day 0,75 cm and 0,9 cm at the end of the experiment (Fig.2).

Significantly greater differences were obtained from mycelium grown on culture media supplemented with 0,20 mg/l borax (V₄) and 0,25 mg/l borax (V₅), where three days after insemination the average diameter of hyphae of *Penicillium spp.* were below the control by 0,07 cm in the first case and 0,09 cm in the second case, after six days of culture this parameter was decreased by 0,25 cm, 0,26 cm and nine days after starting the experiment the difference was 0,61 cm and 0,63 cm. This difference will increase from the twelfth day of culture when the average diameter of hyphae grown on culture medium supplemented with 0,20 mg/l borax (V₄) is 1,0 cm below the witness (M) and 1,11 cm from the culture medium supplemented with 0,25 mg/l borax (V₅), while the end of the experiment that this parameter is below the benchmark by 1.25 cm in the first case and 1,5 cm in the second case (Fig.2).

The results show that the best development of variants present experiment had a population of *Penicillium spp.* grown on culture medium without addition of borax (M), also a quantity of 0,05 mg/l borax (V₁) and 0,10 mg/l borax (V₂) no major influence fungal growth and development of this kind. At a quantity of 0,15 mg/l borax (V₃) added into the culture medium began to notice an antagonist effect between the presence of this substance and the growth and development of *Penicillium spp.* Increasing the concentration of borax to 0,20 mg/l borax (V₄) and 0,25 mg/l borax (V₅) significantly inhibits the growth and development of mycelium of *Penicillium spp.*, pointing out however that the eradication of attack requires a larger amount of borax.

Confirm these results previously published studies that the presence of a boron concentration of 0,25% is effective in the control of blue mold caused by *Penicillium spp.*, but a significant influence on population dynamics of this kind has to apply a fungal concentrations 0,5% of boron [18]. Based on the results we conclude that a pH between 3 and 6 obtim, within the optimal enzyme activity are fungi *Penicillium spp.* (Baohua and colab., 2012), the presence of borax in the culture medium inhibited the activity. Knowing that *Penicillium spp* at a pH between 4,6 to 2,8 and a

resistance to copper salts of iron, nickel, cobalt and chromium in relatively high concentrations (Singh, 1977) treatment with boron can be an alternative to eradication of the fungus *Penicillium* genus, according to some studies even organically (Wayne and colab., 2010).

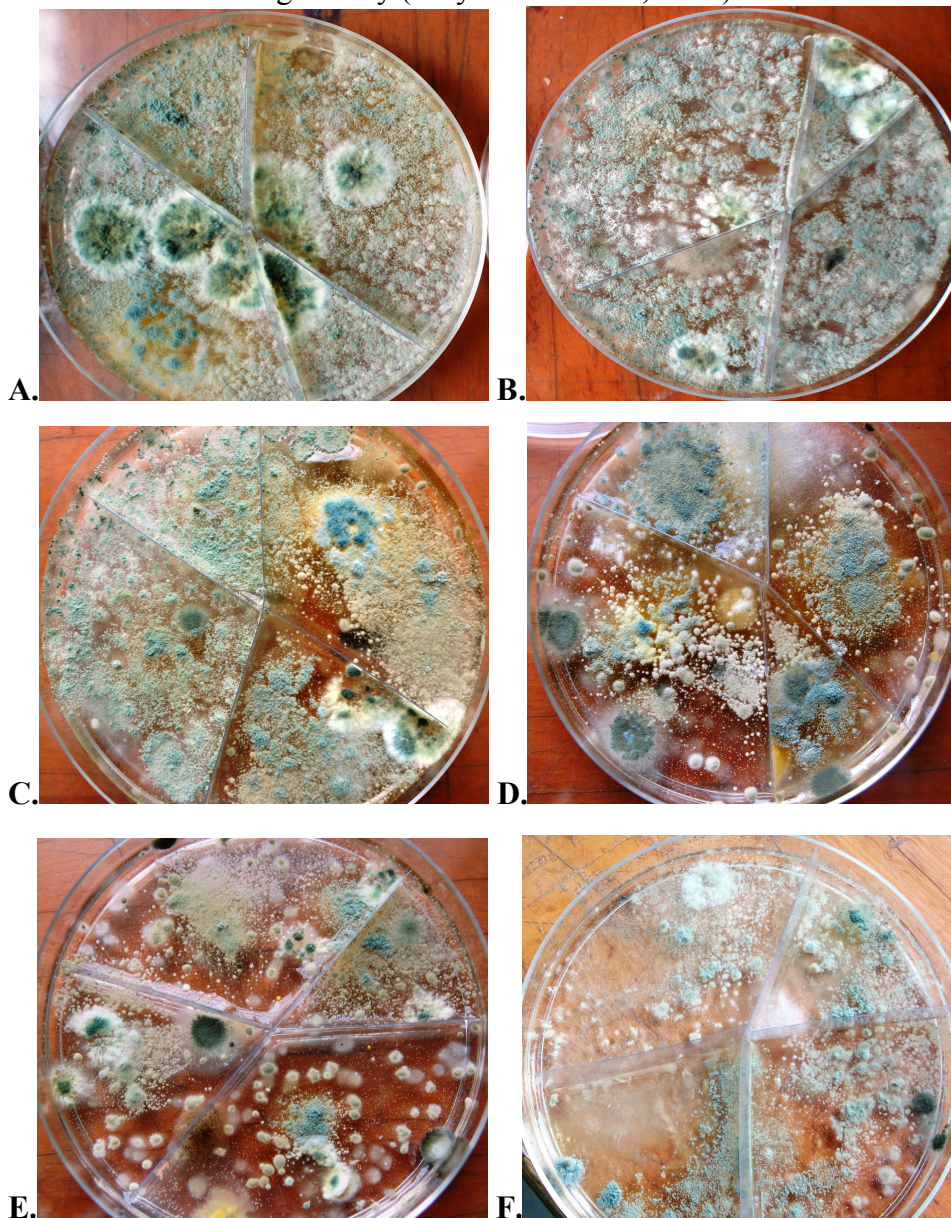


Fig.. Imagini cu miceliu de *Penicillium* spp. la 15 zile de la inseminarea pe substrat nutritiv, unde: A-miceliu de *Penicillium* spp. pe mediu control, fără adaos de borax; B-miceliu de *Penicillium* spp. pe mediu cu adaos de 0,05 mg/l borax; C-miceliu de *Penicillium* spp. pe mediu cu adaos de 0,1 mg/l borax; D-miceliu de *Penicillium* spp. pe mediu cu adaos de 0,15 mg/l borax; E-miceliu de *Penicillium* spp. pe mediu cu adaos de 0,2 mg/l borax; F-miceliu de *Penicillium* spp. pe mediu cu adaos de 0,25 mg/l borax.

In terms of macroscopic appearance of the mycelium of *Penicillium spp.* notes that regardless of the culture that developed it is presented either as a highly branched network consisting of multicellular hyphae, fluffy, white-blue or green-blue, or resembling an "muscles" thick, green, blue or green form of concentric zones, velvety, with lighter edges than the center (Fig. 3).

CONCLUSION

1. The inhibitory effect of borax can be seen in mostly the culture media supplemented with 0,25 mg/l borax, where after 15 days of initiation of the experiment there is a decrease of 79% coverage of the substrate mycelium, reported the witness.
2. After 15 days of culture is noted that the genus *Penicillium* fungi mycelium developed with the largest diameter, 1,6 cm, the culture medium without addition of borax, while the lowest values of this parameter, only 0,1 cm were recorded on nutritional substrates supplemented with 0,25 mg/l borax.
3. The maximum amount of 0,25 mg/l borax used in this experiment was not sufficient for total eradication of the fungus *Penicillium* genus.

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