In Vitro Culture Conditions of *Colletotrichum gloeosporioides* for Spore Production:

(a) Effect of medium composition and light on spore production

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Introduction

***Colletotrichum gloeosporioides*, the imperfect stage of the ascomycete *Glomerella singulata*, is responsible for anthracnose in a wide variety of plant species. *Hypericum perforatum*, which is responsible for the synthesis of hypericin, a metabolite with important pharmaceutical applications, is one of the species sensitive to *C. gloeosporioides*.**

Since *H. perforatum* tolerance to *C. gloeosporioides* is dependent on plant variety, we intend to select tolerant varieties *in vitro* for metabolite production without fungicide application. *C. gloeosporioides* spores are needed to inoculate and induce a selective pressure on plants, but *in vitro* sporulation is particularly difficult to achieve. The establishment of *in vitro* conditions for sporulation was the main goal of the trials here presented.

Two variables were tested for *C. gloeosporioides* spore production: medium composition and light. The effect of medium composition was tested by growing the fungus in PDA (Potato Dextrose Agar) and MMN (modified Melin & Norkrans). We also tested the influence of transfering the fungus from PDA to MMN and vice-versa. Growth and spore formation was recorded for a period of three months. Light influence was tested by submitting the fungus to light and dark conditions, for both media. Growth and spore formation under these conditions was recorded for a period of two months.

Materials and Methods

1 - Inoculation of the different cultures in PDA and MMN medium.

2 - Growth in the different conditions as shown:

3 – The growth in radius was measured weekly.

4 – Plates with spores were counted.

5 - The percentage of plates with spores and average growth in radius were calculated.

Culture	PDA-PDA	PDA-MMN	MMN-PDA	MMN-MMN
Day 1 to 10	37ºC 4ºC Dark Dark Light Dark	37ºC 4ºC Dark Dark Light Dark	37ºC 4ºC Dark Dark Light Dark	37ºC 4ºC Dark Dark Light Dark
Day 10 to 20	Dark Dark 37ºC 4ºC	Dark Dark 37ºC 4ºC	Dark Dark 37ºC 4ºC	Dark Dark 37ºC 4ºC
After 20th day	Dark Dark Dark	Dark Dark Dark	Dark Dark Dark	Dark Dark Dark

Results

Figure 1 - Influence of medium composition on growth

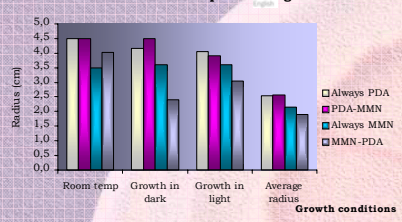


Figure 2 - Influence of medium composition and changing medium on spore production

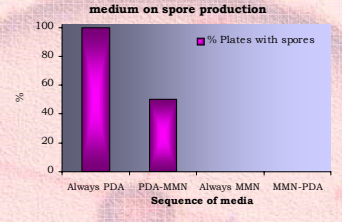


Figure 3 - Influence of medium composition and light on growth

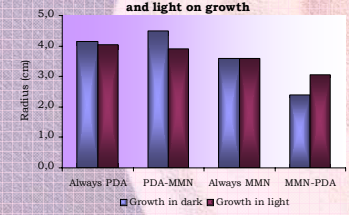
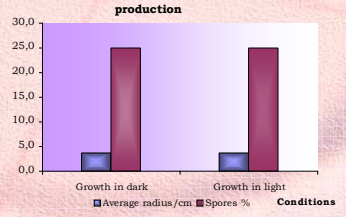
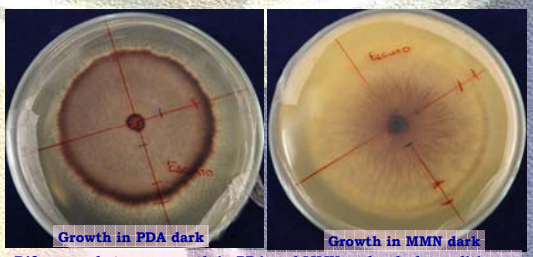
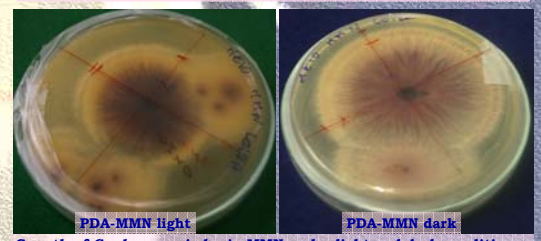


Figure 4 - Influence of light on growth and spore production





Diferences between growth in PDA and MMN under dark conditions



Growth of *C. gloeosporiodes* in MMN under light and dark conditions after growth in PDA medium with spore production.

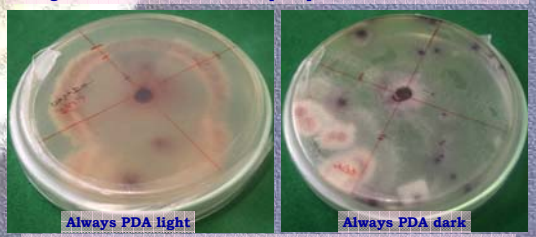
Conclusion

1 - Preliminary results showed PDA to be a better medium for spore production of *C. gloeosporioides* (Fig. 2).

2 - The presence or absence of light did not influence spore production of *C. gloeosporioides* (Fig. 3 & 4).

3 - Media composition and light influence growth and the fungus mycelia colour.

Influence of the medium on colour (growth in dark conditions)



Diferences between growth in PDA under light and dark conditions with spore production.

4 - The best growth was obtained when transferring from PDA medium to PDA and from PDA to MMN (Fig. 1).

5 – From the results obtained we can hypothesize that the medium where the fungus was growing before transference is more important for growth pattern than the actual medium.

6 – After two months of growth under the described media and light conditions spore production occurred in both PDA or MMN medium but just when the fungus came from PDA.

Complementary trials on spore production and *in vitro* plant inoculation are being developed.

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