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**Plant Pathology**

# EFFECT OF ANTIBIOTICS ON FUNGAL COLONIZATION OF AGAR DISCS FROM SOIL INOCULA

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Effect of griseofulvin, polymyxin-β-sulphate, Benzyl penicillin and Actidion in varying concentrations was studied on colonization of fungi on agar discs from mixed inocula of soil. It was noted that as the concentrations of antibiotics increased, the number of fungal colonies and representative species decreased. In the highest concentration of each antibiotic the colonization of agar discs was restricted to only a few genera of fungi.

## INTRODUCTION

Effect of antibiotics on fungal growth has been studied by Brian (1960), Maniar *et al.* (1965) and Wright (1951); while the effect of some antibiotics on colonization of fungi on agar plates has been studied by Dwivedi (1970, 1972). The present paper aims at the study of some synthetic antibiotics on appearance of fungi on agar discs from soil inocula of a grassland.

## MATERIALS AND METHODS

Four antibiotics viz., Griseofulvin, Polymyxin-β-Sulphate, Benzyl penicillin and Actidion were chosen in different concentrations of 200, 600, 1000 and 2000 ppm in sterilized distilled water. The quantity of each antibiotic was mixed in the basal sterilized Czapek-Dox+0.05% yeast extract medium (at 40° C) in such a quantity as to maintain the desired concentration after mixing in the nutrient agar. For control, basal agar medium without any antibiotic was used. One ml of 1: 1000 dilution of a grassland soil was poured in Petri dishes and sets of four dishes for each concentration were prepared. Ten ml of the above nutrient medium supplemented with desired concentration of antibiotics were poured in Petri dishes, and latter were incubated for 6 days at 25°C. The number of colonies appearing in dishes was counted and that of representative species recorded.

## RESULTS AND DISCUSSION

As the concentration of antibiotics increased, the number of colonies and representative species decreased; 1000 ppm and 2000 ppm were highly effective to reduce the colony number and the number of representative species.

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TABLE I

*Effect of different concentrations (ppm) of Antibiotics on colonization of fungi on nutrient agar plates from mixed inocula of soil (Average of colonies of 4 replicates)*

| Name of the species | Control | | | | Concentrations in ppm | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 200 | | | | 600 | | | | 1000 | | | | 2000 | | | |
| | G | P | B | A | G | P | B | A | G | P | B | A | G | P | B | A | G | P | B | A |
| *Mucor luteus* | 1.50 | 3.00 | — | — | — | 3.00 | — | — | — | 1.50 | — | — | — | — | — | — | — | — | — | — |
| Sterile phycomycetes | — | — | 0.25 | — | — | — | 1.00 | — | — | — | 2.30 | — | — | — | — | — | — | — | — | — |
| *Cunninghamella* sp. | 0.50 | — | 0.50 | 1.00 | 0.25 | — | 0.30 | — | — | — | 0.60 | — | — | — | — | — | — | — | — | — |
| *C. bertholletiae* | — | 2.25 | — | — | — | 2.00 | — | — | — | 0.50 | — | — | — | — | — | — | — | 1.00 | — | — |
| *Gymnoascus* sp. | — | — | — | 3.80 | — | — | — | 17.00 | — | — | — | 13.33 | — | — | — | 6.66 | — | — | — | — |
| *Aspergillus nidulans* | 3.25 | 2.50 | 2.30 | 3.00 | 4.22 | 1.50 | — | 2.00 | 4.00 | — | — | — | 4.00 | 3.00 | — | — | 4.66 | 3.00 | 0.60 | — |
| *Penicillium javanicum* | 1.00 | — | 1.00 | 2.40 | 2.00 | — | — | 0.66 | — | — | — | 2.00 | — | — | — | 0.33 | — | — | — | — |
| *Aspergillus niger* | 7.00 | 8.00 | 3.30 | 1.50 | 4.50 | 2.00 | 3.00 | — | 15.00 | 1.50 | 1.00 | — | 13.00 | 2.00 | 1.00 | — | 5.00 | — | — | — |
| *A. flavus* | 4.35 | 5.50 | 1.60 | 4.50 | — | 1.50 | 0.60 | — | — | 2.00 | 1.30 | — | — | 1.00 | 1.00 | — | — | — | — | — |
| *A. terreus* | 2.25 | 1.00 | 2.30 | 3.80 | 1.66 | — | 0.30 | — | 0.33 | — | — | — | — | — | 1.00 | — | — | — | — | — |
| *A. candidus* | — | 3.25 | 2.00 | 2.10 | — | — | 3.60 | — | — | 3.50 | 1.30 | 0.66 | — | 1.00 | 2.30 | — | — | — | 1.60 | — |
| *A. luchuensis* | 5.00 | 1.50 | 2.00 | — | 6.00 | 3.00 | — | — | 5.33 | 2.00 | 0.30 | — | 6.00 | — | — | — | 4.00 | — | — | — |
| *A, sydowi* | — | 1.00 | 1.50 | 2.50 | — | — | — | 0.33 | — | 1.50 | 0.30 | — | — | — | — | — | — | — | — | — |
| *Penicillium Citrinum* | 2.50 | 1.00 | 1.50 | 2.20 | 1.33 | 1.50 | — | — | 1.00 | — | — | — | 1.00 | — | 1.00 | 4.00 | 0.33 | 1.00 | 1.60 | — |
| *P. nigricans* | — | 2.50 | 0.50 | — | — | 3.50 | — | — | — | — | — | — | — | 1.25 | — | — | — | — | — | — |
| *Pencillium* sp. | — | 2.00 | — | — | — | 4.00 | — | — | — | 3.00 | — | — | — | 2.50 | — | — | — | 1.50 | — | — |
| *P. janthinellum* | — | — | 0.50 | — | — | — | — | — | — | — | — | — | — | — | 1.00 | — | — | — | 2.60 | — |
| *P. braziliense* | — | — | 1.60 | 3.20 | — | — | 0.60 | — | — | — | 1.30 | — | — | — | 1.30 | — | — | — | — | — |
| *P. roseum* | — | — | — | 1.50 | — | — | — | 0.33 | — | — | — | 1.33 | — | — | — | — | — | — | — | — |
| *Trichoderma lignorum* | 3.25 | 1.50 | 3.20 | 2.60 | 3.66 | 2.00 | — | — | 2.33 | 2.00 | 0.60 | — | 1.00 | 3.00 | 0.30 | — | 5.00 | 1.50 | — | — |

| | | | | | | | | | | | | | | | | | | | | |
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| *Acrophialophora fusispora* | 2.00 | 0.50 | 2.40 | 1.50 | — | — | 3.00 | 0.66 | — | — | 2.60 | — | — | — | 1.00 | — | — | — | 0.60 | — |
| *Cladosporium herbarum* | 1.50 | 1.75 | 1.60 | 1.20 | — | — | 2.00 | 0.33 | — | — | — | — | — | — | 1.60 | — | — | — | .. | — |
| *Helminthosporium anomalum* | — | 0.50 | — | 2.50 | — | — | — | — | — | — | — | 0.33 | — | — | — | — | — | — | — | — |
| *Curvularia lunata* | 2.00 | 1.00 | 1.00 | — | — | 1.75 | 1.30 | — | — | 0.50 | — | — | — | — | — | — | — | — | — | — |
| *Fusarium* sp. | 2.00 | — | — | — | 1.33 | — | — | — | 1.00 | — | — | — | — | — | — | — | — | — | — | — |
| *F. Poae* | — | 2.00 | — | — | — | 1.50 | — | — | — | 2.50 | — | — | — | 0.50 | — | — | — | — | — | — |
| *F. chlamydosporum* | — | — | 1.50 | — | — | — | 1.60 | — | — | — | 2.30 | — | — | — | 0.30 | — | — | — | 1.30 | — |
| *Humicola sp.* | — | 0.50 | 0.50 | — | — | 2.00 | 0.30 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| *Harmodenrum cladosporoides* | — | — | 0.50 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1.00 | — |
| *Scopulariopsis* sp. | — | — | — | 2.60 | — | — | — | 1.00 | — | — | — | 1.66 | — | — | — | 2.00 | — | — | — | — |
| Sterile white mycelium | 3.00 | 4.00 | 3.60 | 2.00 | — | 1.50 | 2.30 | — | — | 3.00 | 1.00 | — | — | — | 3.00 | — | — | — | 2.60 | — |
| Sterile dark mycelium | — | 1.50 | 2.50 | 1.50 | — | 3.00 | 2.30 | — | — | 2.50 | 1.30 | — | — | — | 1.00 | — | — | — | 1.00 | — |
| Total average No. of colonies | 41.10 | 46.75 | 37.65 | 45.40 | 24.95 | 32.75 | 22.20 | 22.31 | 28.99 | 24.40 | 16.20 | 19.31 | 25.00 | 14.25 | 15.80 | 12.99 | 18.99 | 8.00 | 13.90 | — |

*C*, Control; *G*, Griseofulrin; *P*, Polymyxin-β-Sulphate; *B*, Benzyl pencillin; *A*, Actidion.

Griseofulvin at the latter concentration reduced the colony number to 18.9 representing only five species. Polymyxin-β-Sulphate showed similar result. At 200 ppm level the number of colonies and representative species was 33.7 and 15 respectively, whereas at 2000 ppm the colony number and representative species decreased to 8 and 5 species respectively. At the highest concentration of Griseofulvin, only *A. niger, A. luchuensis, A. nidulans, P. citrinum* and *T. lignorum* showed the high tolerant capacity; while at the same concentration of Polymyxin-β-Sulphate the representative species were restricted to only a few fungi like *Cunninghamella bertholletiae, A. nidulans, P. citrinum, Penicillium* sp. and *T. lignorum,*

Almost similar result appeared with Benzyl penicillin. At 200 ppm the average colony number was 22.2 representing 14 species, while at the highest concentration the number of colony declined to 13.9 representing 9 species. In case of Actidion also the average number of colonies and representative species were higher at 200 ppm which declined at higher concentrations. *A. nidulans, A. candidus, P. citrinum, P. janthinellum, F. chlamydosporum, Acrophialophora fusispora, Hormodendrum cladosporoides* and sterile white and black mycelia could appear at the highest concentration (2000 ppm) of Benzyl penicillin. In case of Actidion, only *P. javanicum, P. citrinum, Gymnoascus* sp. and *Scopulariopsis* sp. were able to grow at 1000 ppm while at the highest concentration of 2000 ppm not a single species could grow. Thus this antibiotic is most toxic at this concentration against the soil fungi. The tolerant capacity of such fungi which could appear on agar discs supplemented with the highest concentration of antibiotics might be due to production of antibiotic substances of their own to be able to overcome the toxic effect of antibiotics supplemented in agar discs. It is known that *A. nidulans* produces 'nidulin' and nor-nidulin', *P. citrinum* 'citrinin', *T. lignorum* 'gliotoxin', *A. candidus* 'Candidulin' and *F. chlamydosporum* 'Chlamydosporin'. These substances might have reduced the effect of supplemented synthetic antibiotics to allow the growth of these fungi on agar plates. These findings support the biological control of soil-borne fungal pathogens by antifungal antibiotics.

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