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ANTIMICROBIAL ACTIVITY OF PIGMENT FROM *FUSARIUM SOLANI*

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Abstract : *Fusarium solani* (Mart.) Sacc. produces a red diffusible quinone pigment. The pigment is shown to be anti-mycotic, effective in low concentrations,against human pathogenic fungi, namely *Candida albicans* and a *Fusarium* isolate obtained from an infected toe nail, the MIC of the pigment extract on growth of the two molds being 0.03 μg% and 0.02 μg% respectively.

Key words: *Candida*, Onychomycosis, Pathogens.

INTRODUCTION

Pigments have been widely used in industry as food additives (1), and in dyeing of textiles, leather, paper and other items.Pigments produced as secondary metabolites by fungi, have been found importance not only for their use in dyeing (2), but also for their antimicrobial effect (3).In fact, a number of pigments produced by *Fusarium* spp. are quinone derivatives (3, 6) and quinones are reported to have antibiotic properties(3-8).

The culture under study, *Fusarium solani* (Mart) Sacc. produces a diffusible orange-red pigment. Studies on the antibiotic effect on two human pathogenic molds are given below.

MATERIALS AND METHODS

Organisms : The pigment-producing culture *Fusarium solani* (Mart) Sacc. was isolated in the laboratory as a chance contaminant on ferulic acid solution (Nazareth and Mavinkurve,1987).

The test cultures, (a) *Candida albicans* was obtained from the microbiology department of the Goa Medical College, Goa, and (b) fungal culture was isolated from an infected human toe nail, on potato dextrose agar (PDA). The culture was identified based on its cultural and morphological characteristics.

Growth of the culture : The fungal isolate *Fusarium solani* (Mart) Sacc. was grown for two days, at room temperature (30°C), at 150-200 rpm, in minimal medium containing per litre distilled water: 0.1g $Na_2HPO_4.2H_2O$, 0.7g KH_2PO_4, 0.2g $MgSO_4.7H_2O$, 0.5g $(NH_4)_2SO_4$, 0.1g $CaCl_2.2H_2O$, 0.05g NaCl, 0.01g $FeSO_4.7H_2O$; 1ml of trace mineral solution containing (%) : 1.1g $ZnSO_4$, 0.5g $MnSO_4$, 0.2g $Na_2(MoO_4)$, 5mg $CoSO_4$, 5mg boric acid, 0.7mg $CuSO_4$ supplemented with 0.05% malt extract, 0.5% glucose. The medium was designated as MMG.

Extraction and purification of pigment: The culture medium after 2d growth of fungus, was filtered, and the filtrate shaken with chloroform, for extraction of the pigment .

The pigment extract in chloroform was concentrated to a minimal volume and run on thin layer chromatography in chloroform.

The concentrated extract was purified on a silica gel column, eluted with increasing concentration of ether (0-10%) in chloroform.

Absorption scan of the pigment : Small volumes of the original extract and separated components of the pigment were gently heated to remove the chloroform and immediately dissolved in water. The aqueous solution was scanned

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between 700-400, and max noted.

MIC Test : Candida albicans was grown in Wickerham's broth containing the pigment or its isolated components as indicated in the results.The growth was monitored at 650 for 3d.

Fungal isolate was grown in potato dextrose broth containing the pigments or its isolated components, as above, incubated for 3d. Growth was estimated in terms of dry weight of the filtered mycelia.

RESULTS

Fusarium solani produced a wine-red diffusible pigment designated R in medium MMG. The pigment was separated into two components on TLC (Fig. 1), one less polar and orange in colour (O); the other more polar fraction was purple (P). UV scan (Fig. 2), showed the pigment R had a max of 500nm, while max of the components O and P were at 499 and 497nm respectively.

The fungal culture isolated from the infected human toe nail was identified as *Fusarium* based on its cultural and morphological characters.

The growth of *Candida albicans* and the *Fusarium* isolate decreased with increasing concentrations of the pigment as shown in Fig. 3 and 4 respectively.

MIC of crude extract (R) on *C.albicans* was 0.03 mg%; for components O and P it was 0.02 mg% and 0.03 mg% respectively.(Fig 3).

MIC on the *Fusarium* isolate by R, O and P was 0.020 mg% for each.(Fig4).

DISCUSSION

Microbial secondary metabolites displaying antimicrobial effects are well known (Miller, 1961; Paper and Rehm, 1986). Prominent among these are penicillin and griseofulvin produced by *Penicillium* spp., and streptomycin from *Streptomyces*.

Fusarium spp. are well documented for their production of antimetabolites, in particular, mycotoxins(Mall and Chauhan, 1990). However, Fusaria are also known for their beneficial effects, namely, destruction of the nematode which causes root rot of *Phaseolus vulgaris* (Vadhera *et al.*, 1995), production of gibberellins and the synthesis of cyclosporin.(9).

Fusarium solani produces a red coloured pigment R consisting of two components: orange (O) and purple (P) in colour.The antimicrobicity of the individual components was therefore checked against *Candida albicans* and the *Fusarium* isolate from an infected toe nail. Both *C.albicans* and *Fusarium* spp. have been reported as causative agents of onychomycosis or fungal infection of nails by non-dermatophytes (Singh, 1990) Both O and P contributed to antimicrobicity against *Candida albicans*, the P component having greater effect. However in the case of the *Fusarium* isolate, R, O and P fractions showed equal effect in inhibiting the growth of the culture. Thus while growth of *C. albicans* was inhibited at as low a concentration as 0.003mg% of P, the *Fusarium* isolate was unaffected at this concentration, requiring an MIC of 0.020 mg%.

Antifungal property of quinones has been reported (Brewer eta., 1997; 7,8), including the action of 1,4-napthoquinones on C.albicans (7). The pigment produced by *Fusarium solani* (Mart) Sacc.shows an inhibitory effect on the test cultures, in very low concentrations, which makes it a highly potential candidate for use in chemotherapeutic topical applications.However, its use for treatment of Candidiasis would need to be restricted to dermatophytic and superficial infections. Use in oral thrush or in genital tract candidal infections would require an in-depth study of levels of toxicity.Its application for other fungal dermatophysis as that caused by *Fusarium* may be of interest, subject to test for possible toxic effects .

Fusarium spp. have been shown to be resistant to standard MIC of

imidazoles (Weinberg, 1989) used for topical treatment of dermatophytic infections.The search for newer potential drugs is therefore of extreme importance in the face of increasing incidence of drug-resistant microorganisms.

The cure of nail infections by dermatophytes is much higher than non- dermatophytic onychomycosis.Antimycotics have not proved ideal because of adverse side-effects, and narrow range of activity(Singh, 1990). Little attention has been paid to the problem of onychomycosis in India, where its incidence is fairly high.(Singh, 1990) This certainly calls for greater efforts to find suitable remedies.

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LEGENDS TO FIGURES

Fig 1A. TLC of pigment extract R.

1B. Scan of pigment extract R, isolated components O and P.

Fig 2.Growth of *Candida albicans* in presence of pigment R and its *components O and P.*

Fig 3. Growth of Fusarium isolate in presence of pigment R and its *components O and P.*

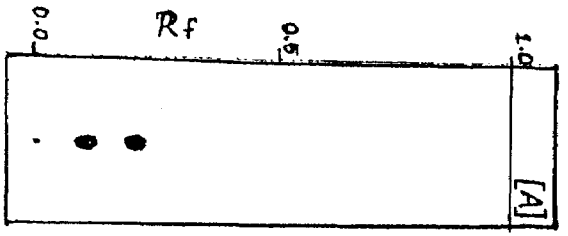

0.0
Rf
0.5
1.0
[A]

Fig. 1 A

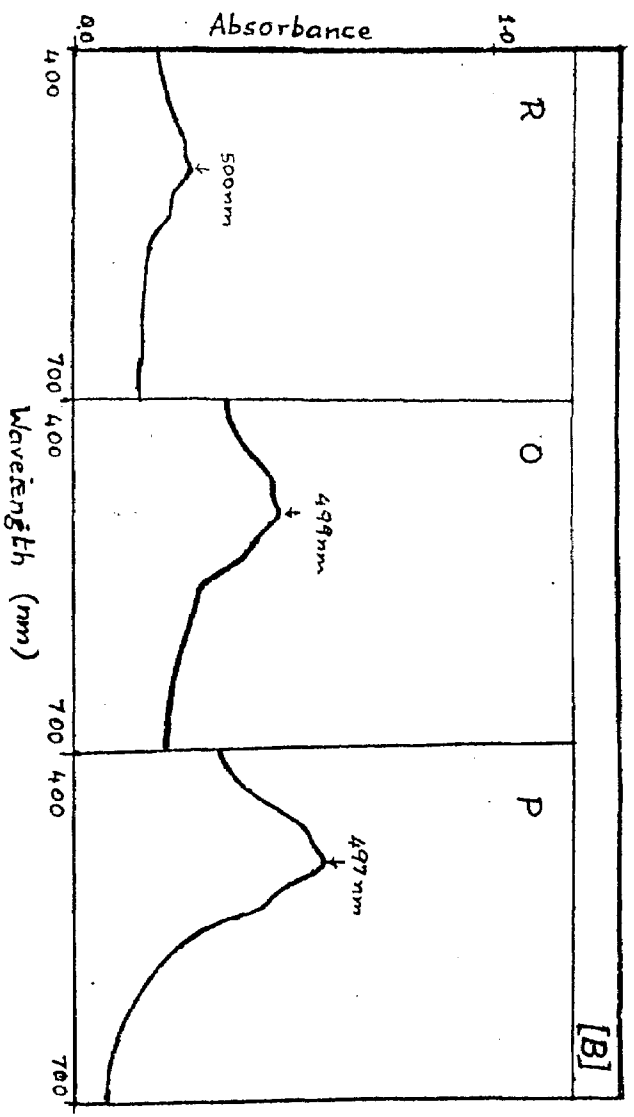

0.0
Absorbance
1.0
400
700
Wavelength (nm)
R
500nm
O
499nm
P
497nm
[B]

Fig. 1 B

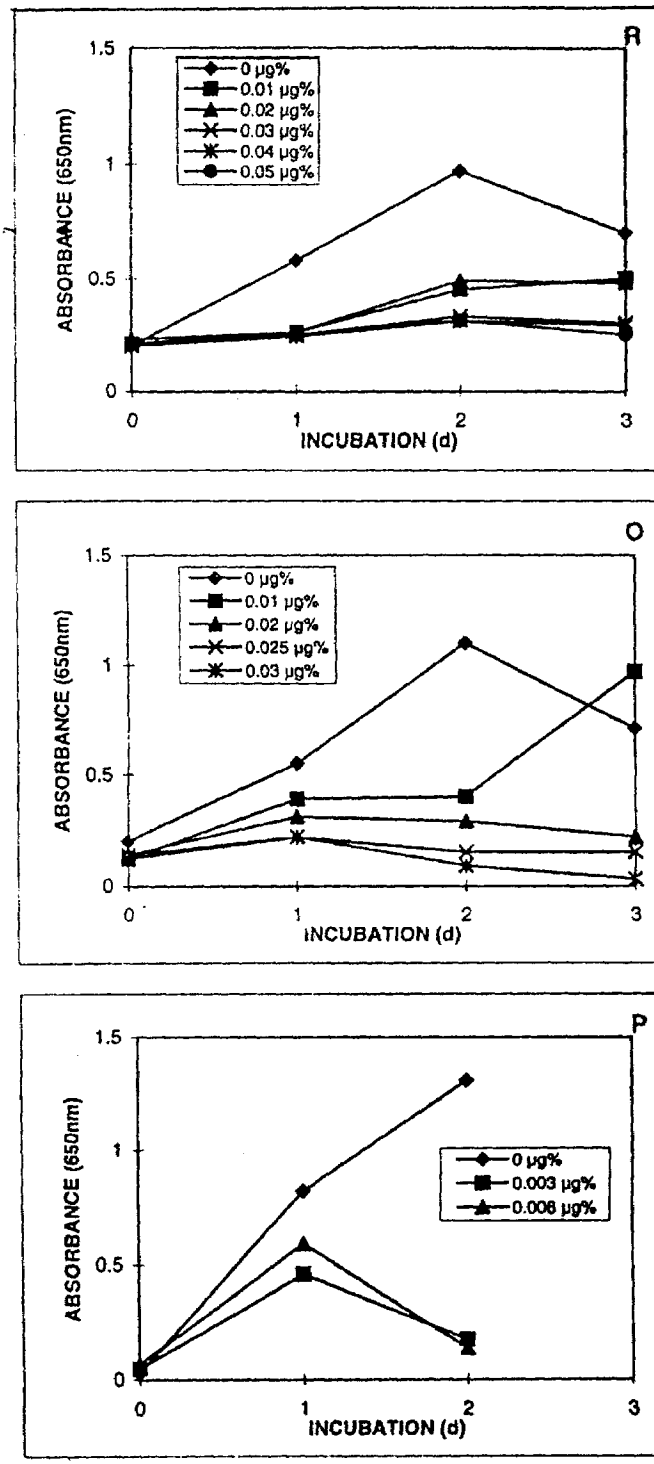
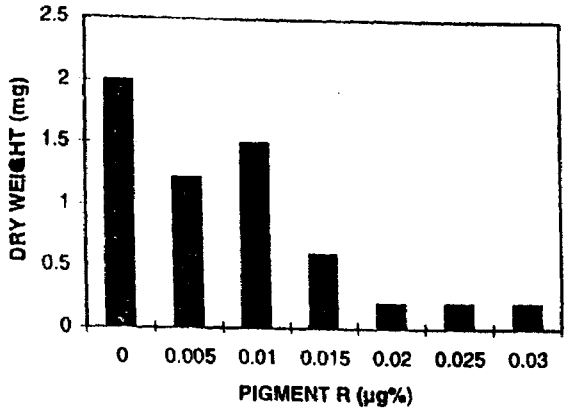

R
1.5
1
0.5
0
ABSORBANCE (650nm)
0 µg%
0.01 µg%
0.02 µg%
0.03 µg%
0.04 µg%
0.05 µg%
0
1
2
3
INCUBATION (d)
O
1.5
1
0.5
0
ABSORBANCE (650nm)
0 µg%
0.01 µg%
0.02 µg%
0.025 µg%
0.03 µg%
0
1
2
3
INCUBATION (d)
P
1.5
1
0.5
0
ABSORBANCE (650nm)
0 µg%
0.003 µg%
0.006 µg%
0
1
2
3
INCUBATION (d)

Fig. 2


DRY WEIGHT (mg)
2.5
2
1.5
1
0.5
0
0
0.005
0.01
0.015
0.02
0.025
0.03
PIGMENT R (µg%)

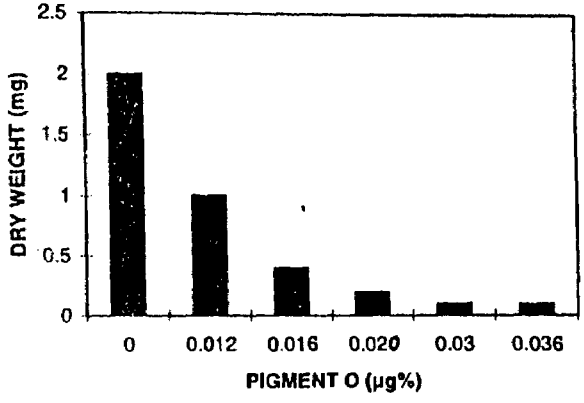
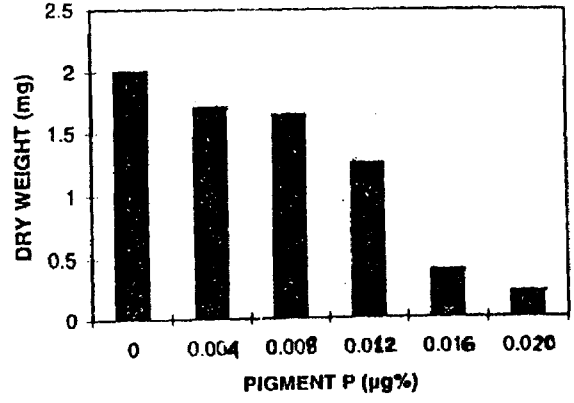

DRY WEIGHT (mg)
2.5
2
1.5
1
0.5
0
0
0.012
0.016
0.020
0.03
0.036
PIGMENT O (µg%)

Fig. 3


DRY WEIGHT (mg)
2.5
2
1.5
1
0.5
0
0
0.004
0.008
0.012
0.016
0.020
PIGMENT P (µg%)