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The Dark Side of Fungal Melanin; *Alternaria alternata* as Example

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Abstract: Melanins are dark, brown to black, high molecular weight pigments produced by organisms ranging from animals and plants to micro-organisms. They are formed by the oxidative polymerization of phenolic or indolic compounds. Melanins appear to have an indirect as well as a direct function in virulence. Also melanins act as body armour protecting fungi against environmental stress or unfavourable conditions like extreme temperatures, UV-, ionizing and gamma radiations, and compounds secreted by microbial antagonists. The potential protection role of melanins against radiations was investigated in this study through the assessment of the inhibitory effect of UV-radiation on the growth rate of *Alternaria alternata* as a melanized fungus and *Fusarium oxysporum* and *Penicillium digitatum* as non-melanized fungi. Spore suspensions of these fungi were exposed to different wavelengths of UV-radiation (300nm & 600nm). The results performed that the inhibitory effect of UV-radiation against the nonmelanized fungi; *F. oxysporum* and *P. digitatum* was achieving significant when compared to the melanized fungus *A. alternata* which tolerated the radiation with growth rates of 0.07mm/h, 0.06mm/h and 0.23mm/h, respectively. When putting in consideration the wide application of the classical methods for sterilization by UV-radiation, the gloomy picture of the protection role of melanin was obviously cleared.

Keywords: Melanin, UV-radiation, wavelength, *Alternaria alternata*

Introduction

Some fungal pigments are natural products associated with developmental structures, the most common being melanins, which are generally dark brown pigments formed by oxidative polymerization of phenolic compounds and are synthesized during spore formation for deposition in the cell wall. Melanins biosynthesis has been well studied in pathogenic fungi. The biosynthetic pathway of DHN melanin was first characterized in *Verticillium dahliae* by using melanin-deficient mutants. In general, melanins accumulate in fungal cell walls and have been considered to confer tolerance to environmental stresses, such as UV radiation, microbial lysis (Kawamura *et al*. 1997), melanins can also act as a scavenger of free oxygen radicals, which can be components of host defense against pathogens. Also synthetic melanin has immunosuppressive characteristics *in vitro* on mammalian cells; it is tempting to speculate that similar activities could also be displayed in plant infections. Where the pigment not only contributes to the survival of the fungal spore but is also an important virulence factor. In *Colletotrichum lagenarium*, melanin biosynthesis has been associated with the formation of appressoria, the infection structures required for host penetration, and any impairment in their formation can reduce virulence. In *Alternaria alternata*, melanin deposition is also involved in

spore development. Disruption of the *A. alternata* melanin biosynthetic gene *brm2* dramatically decreases melanin pro-duction in this fungus. The conidia produced are reduced in diameter and are more sensitive to UV light than the wild type (Kawamura *et al*. 1999). In another fungus, the maize pathogen *Cochliobolus heterotrophus*, fitness studies of albino spore mutants in a greenhouse indicated that melanin production is required for survival of this fungus (Leonard 1977). The effect of melanin biosynthesis on the virulence of fungal human pathogens has also been studied. In *Aspergillus fumigatus*, a cause of invasive aspergillosis in immunocompromised patients, spore pigment is a virulence factor. Disruption of the *alb1* gene, which encodes a putative polyketide synthase, creates a pigmentless conidial phenotype and leads to a significant reduction in fungal infection of a murine model (Calvo *et al*. 2002).

Spoilage of food products by fungi, especially those that produce mycotoxins, is a worldwide economic problem. Most of these fungi are saprophytes in a variety of environments, and their conidia are borne aerially and can be found everywhere as contaminants. For long time, irradiation sterilization of fungi in food products, laboratories and hospitals by gamma and UV irradiation have been well known and adopted (Saleh *et al*. 1988).
In this study, the potential protection role of melanins produced in fungal cell walls against radiations was investigated by assessing the inhibitory effect of UV-radiation on the growth rates of *Alternaria alternata* as a melanized fungus and *Fusarium oxysporum* and *Penicillium digitatum* as non-melanized fungi.

Materials and Methods

Pure cultures of the three fungi were grown for 5 to 7 days on 15 ml of potato dextrose agar (Difco Laboratories, Detroit, Mich.) in in 9-cm-diameter Petri dishes at room temperature. Spore suspensions of each of the three fungi were prepared by pouring 10ml sterilized distilled water in the PDA plates containing confluent mat of colony growth over the entire agar surface and agitated well. Fungal propagules in prepared suspensions were measured using haemocytometer slide to prepare approximately concentrations of 6.0×10^5 spores ml^{-1} for each fungus. Cuvette tubes filled with 1.5ml spore suspensions of each fungus were exposed to 300nm and 600nm wavelengths of UV-radiation in Ultra violet spectrophotometer. After radiation, 0.3ml of each suspension was poured in holes made by cork borer in the middle of plates containing sterilized potato dextrose agar medium. Then, plates were incubated at $25^oC \pm 2$. Growth rates (mm/hr) were assessed after 3, 6 and 9 days of incubation (Kiraly *et al*. 1974).

The experimental design adopted was complete randomized design. Statistical analysis was accomplished in SPSS 13.0 version, and the Duncan`s multiple range test (DMRT) was adopted to compare means. Least significant difference values at $P \leq 0.05$ were used to separate treatment means when ANOVA indicated a significant *F* value.

Results and Discussion

As shown in Figs.1, 2 and 3, the highest growth rates of 0.433, 0.370 and 0.380 were obtained from the untreated control of *A. alternata*, *F. oxysporum* and *P. digitatum*, respectively. The least growth rate of 0.231mm/hr of *A. alternata* was recorded, after 216 hours of incubation, when the fungus exposed to the highest UV dose 600nm. While the least growth rates of 0.071mm/hr and 0.063mm/hr of *F. oxysporum* and *P. digitatum*; respectively; were recorded for the same dose and time.

The results showed that there were highly significant differences ($P \leq 0.05$) among treatments in growth rates 216 hours after commencement of the experiment. Exposure to 600nm UV-

wavelength significantly suppressed the growth of *F. oxysporum* and *P. digitatum in vitro* in comparison with *A. alternata* and the untreated control of these fungi (Table.1).

These results revealed that the inhibitory effect of UV-radiation against the non-melanized fungi; *F. oxysporum* and *P. digitatum* was achieving significant when compared to the melanized fungus *A. alternata* which survived the higher radiation exposure. Also these findings proved that melanins are protecting from UV damage and appear to enhance the survivability of melanized fungi, which agreed with the reports by Kawamura *et al*. (1999) and Thomma (2003) who indicated that melanins act as "body armour" protecting fungi against environmental stress or unfavourable conditions like extreme temperatures, UV-radiation and compounds secreted by microbial antagonists.

This experiment highlighted that even high doses of UV-radiation would be insufficient for efficacious sterilization and food pasteurization if a melanized fungus such as *Alternaria* spp. is present.

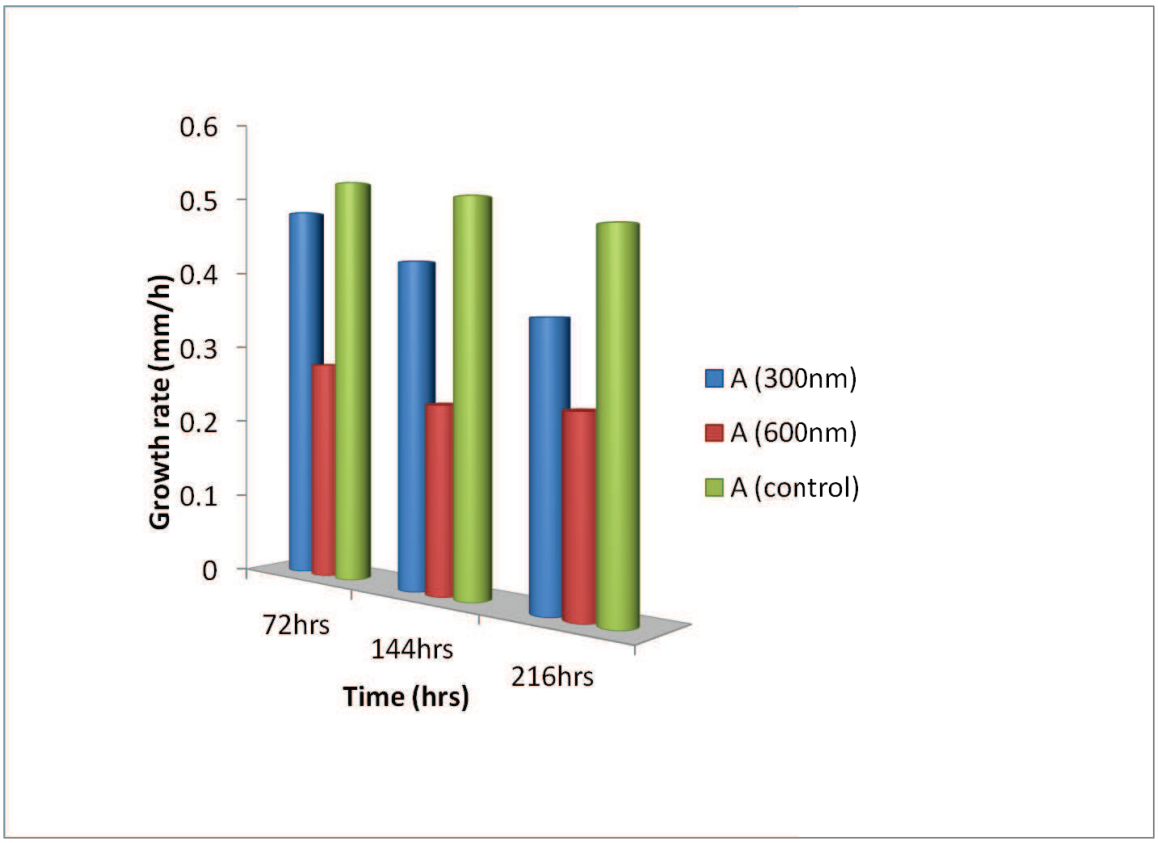

Figure1. Average growth rate of *A. alternata* exposed to different UV-radiation wavelengths

A: *Alternaria alternata*, **F:** *Fusarium oxysporum*, **P:** *Penicillium digitatum*, **300nm:** the UV-radiation wavelength 300nm, **600nm:** the UV-radiation wavelength 300nm

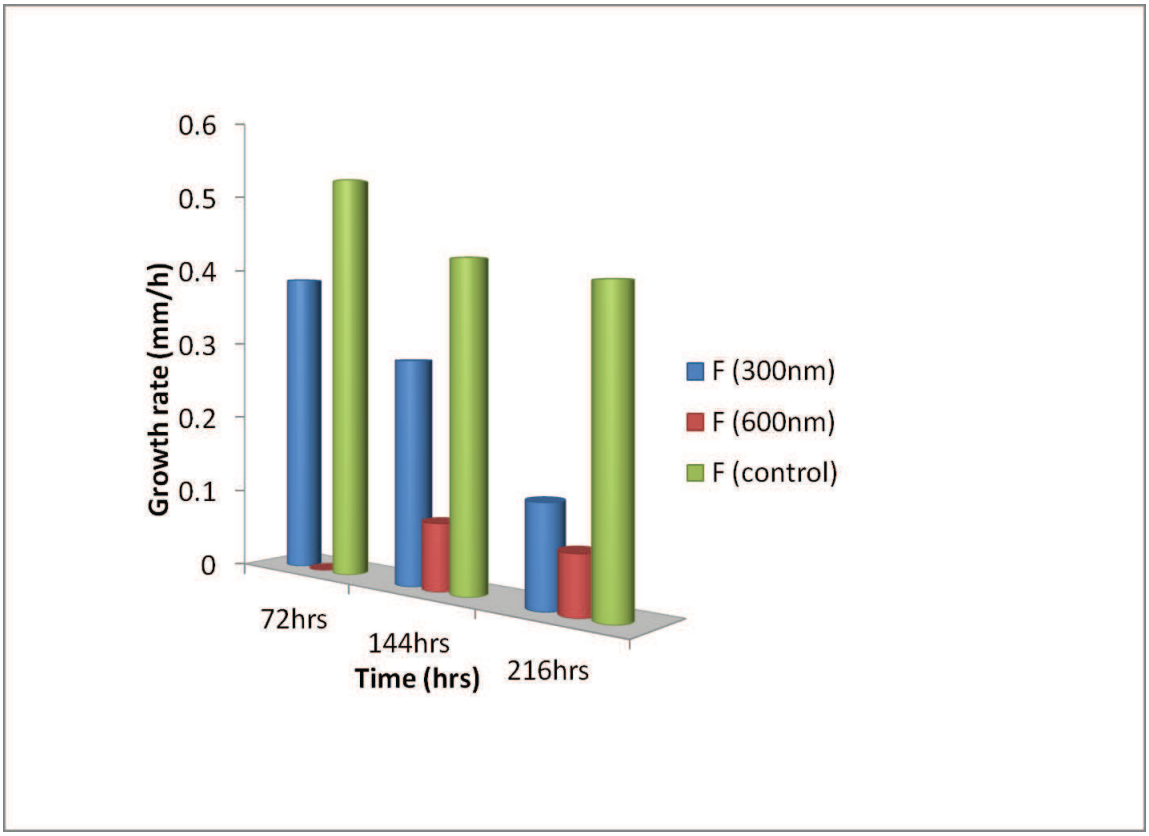

Figure2. Average growth rate of *F. oxysporum* exposed to different UV-radiation wavelengths

A: *Alternaria alternata*, **F:** *Fusarium oxysporum*, **P:** *Penicillium digitatum*, **300nm:** the UV-radiation wavelength 300nm, **600nm:** the UV-radiation wavelength 300nm

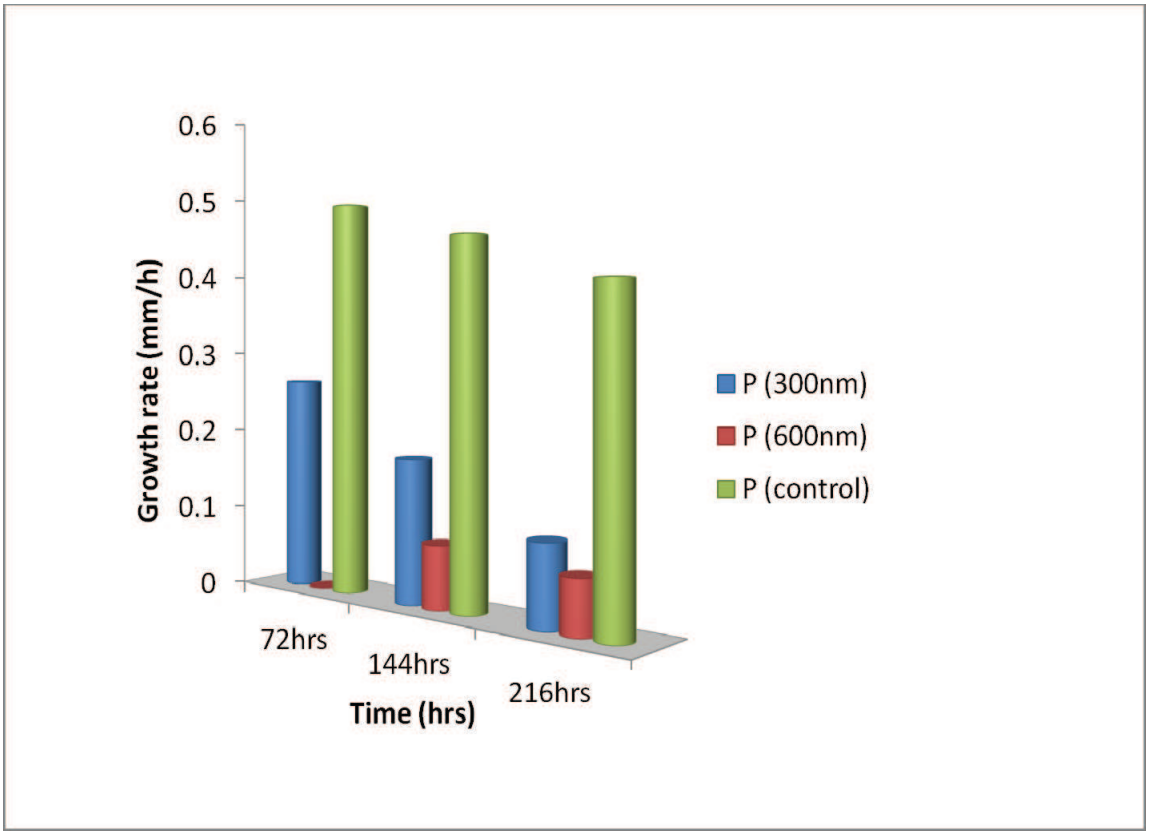

Figure3. Average growth rate of *P. digitatum* exposed to different UV-radiation wavelengths

A: *Alternaria alternata*, **F:** *Fusarium oxysporum*, **P:** *Penicillium digitatum*, **300nm:** the UV-radiation wavelength 300nm, **600nm:** the UV-radiation wavelength 300nm

Table1. Average growth rates of *A. alternata*, *F. oxysporum* and *P. digitatum* exposed to different UV-radiation wavelengths

Treatments	Time after culturing (hrs)		
	72h	**144h**	**216h**
A (300nm)	0.481^{d}	0.406^{e}	0.334^{d}
A (600nm)	0.277^{b}	0.231^{c}	0.231^{c}
A (control)	0.513^{e}	0.479^{g}	0.433^{f}
F (300nm)	0.387^{c}	0.280^{d}	0.123^{ab}
F (600nm)	0.00^{a}	0.083^{a}	0.071^{a}
F (control)	0.514^{e}	0.403^{e}	0.370^{e}
P (300nm)	0.263^{b}	0.174^{b}	0.096^{a}
P (600nm)	0.00^{a}	0.076^{a}	0.063^{a}
P (control)	0.486^{de}	0.438^{f}	0.380^{e}

* Means followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

A: *Alternaria alternata*, **F:** *Fusarium oxysporum*, **P:** *Penicillium digitatum*, **300nm:** the UV-radiation wavelength 300nm, **600nm:** the UV-radiation wavelength 300nm

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