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THE EFFECT OF MELANIN ON THE TOLERANCE OF FUNGAL CONIDIA TO ULTRAVIOLET AND VISIBLE LIGHT EXPOSURE

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Abstract: Melanin is a group of dark, brown to black compounds composed of high molecular weight pigments produced by many organisms, ranging from animals and plants to micro-organisms. In fungi; melanin provides a protective functionagainst unfavorable conditions such as extreme temperatures, UV, ionizing and gamma radiations, and compounds secreted by microbial antagonists. The potential protective role of melanin against radiation was investigated in this study through the assessment of the inhibitory effect of different wavelengths of radiation on the growth rate of *Alternariaalternata* as a melanized fungus, and *Fusariumoxysporum* and *Penicilliumdigitatum* as non-melanized fungi. Spore suspensions of these fungi were exposed to UV radiation at wave length 300nm and visible light at wave length 600nm. The study confirmed the protective role of fungal melanin against UV-radiation. The result suggests the use of UV-radiation in hospitals and laboratories will be infective against melanized fungal conidia. The inhibitory effect of UV-radiation (300nm) against the non-melanized fungi (*F. oxysporum* and *P.digitatum*) after 216 hrs of incubation was significant when compared to the melanized fungus (*A.alternata*), with growth rates of 19.3, 33.2 and 53.7%, respectively, of normal mycelial growth. Regarding the wide application of the classical methods for sterilization by UV-radiation, the gloomy picture of the protective role of melanin was obviously cleared.

Keywords: Melanin, radiation, wavelength, *Alternariaalternata, Fusariumoxysporum*, *Penicilliumdigitatum.*

Introduction

Melanin is an amorphous polymer that is produced by one of two synthetic pathways. Fungi may synthesize melanin from endogenous substrate via a 1,8-dihydroxynaphthalene (DHN) intermediate. Alternatively, some fungi produce melanin from L-3,4-dihydroxyphenylalanine (L-dopa). The detailed chemical structure of melanin is not known. However, microscopic studies show that it has an overall granular structure. In fungi, melanin granules are localized to the cell wall where they are likely cross-linked to polysaccharides. Recent studies suggest

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the fungal melanin may be synthesized in internal vesicles akin to mammalian melanosomes and transported to the cell wall (Eisenman and Casadevall, 2012). The biosynthetic pathway of melanin was first characterized in *Verticillium dahlia* (Kleb.) using melanin-deficient mutants (Kawamura, et al., 1997).

Melanin appears to have an indirect as well as a direct function in virulence. In general, melaninaccumulates in fungal cell walls and has been considered to confer tolerance to environmental stressessuch as radiation and microbial lysis (Kawamura, et al., 1997). Melanin can also act as a scavenger of free oxygen radicals, which can be a component of plant defense against pathogens. Radioresistance of some fungal species which have been isolated from within and around the Chernobyl plant has been linked to the presence of melanin, which has been shown to have emerging properties of acting as an energy transporter for metabolism and has been implicated in enhancing hyphal growth and directed growth of sensitized hyphae towards sources of radiation (Dighton, et al., 2008).

In addition, synthetic melanin has immunosuppressive characteristics *invitro* on mammalian cells. It is tempting to speculate that melanin could play a similar role in plant infections by plant fungal pathogens, as a virulence factor. In *Colletotrichumlagenarium* (Passerini) melanin biosynthesis has been associated with the formation of appressoria. These infection structures are required for host penetration andany impairment in their formation can reduce virulence. In *A. alternata*, melanin deposition is also involved inconidial development. Disruption of the *A. alternate* melaninbiosynthetic gene *brm2* dramatically decreases melanin production in this fungus. The conidia produced are reduced indiameter and are more sensitive to UV light than the wild type (Kawamura, et al., 1999). In another fungus, the maize pathogen *Cochliobolusheterostrophus* (Drechsler), fitness studies of albino spore mutants in a green house suggested that melanin production is required for the survivalof the conidia of this fungus (Leonard, 1977).

The effect of melanin biosynthesis on the virulence of plant fungal pathogens has also been studied. In *Aspergillusfumigatus*, which can cause invasive as per gillosis in immune compromised patients, conidial pigmentation is a virulence factor.Sterilization of food products, laboratories and hospitals by gamma and UV-radiation widely used to control fungi and bacteria (Saleh, 1988).

In this study, the protective role of melanin produced in fungal cell walls against different wavelengths of radiation was investigated by assessing the inhibitory effect of these

radiations on the growth rates of *A.alternata*as a melanized fungus, and *Fusariumoxysporum* (Schlecht.) and *Penicilliumdigitatum* (Pers.) as non-melanized fungi.

Material and Methods

Pure cultures of *A. alternata*, *F. oxysporum* and *P. digitatum* were grown for 7 days on 15 ml of potato dextrose agar (Difco Laboratories, Detroit, Michigan) in 9cm diameter Petri dishes at room temperature. Spore suspensions of each fungi was prepared by pouring 10ml sterilized distilled water in the PDA plates containing pure cultures of the fungi, agitated with a glass rod and spore suspension was transferred to a 50ml falcon tube and vortexed many times to release the spores from the mycelia of the suspension. Spore concentrations of suspensions were measured using haemocytometer slide to prepare approximately concentrations of 6.0×10^5 spores $ml^{-1}$for each fungus. Quartz cuvette (Heinz Herenz, Hamburg, Germany) containing 1.5ml of the spores suspension of each fungus were then exposed to UV at 300nm and visible light at 600nm wavelengthsfor themaximum relative intensity (90%) in a UV/Vis spectrophotometer-1800 (Shimadzu, Japan) for two minutes. Three replicates of each fungus were not exposed to radiation as untreated control treatments. After exposure, 0.3ml of each suspension was poured into holes made by a cork borer in the middle of the PDA plates. Then the plates were incubated at $25^oC \pm 2$. Growth rates (%) were assessed relative tothe Untreated Controls of each fungusafter 72, 144 and 216 hours of incubation (Kiraly, et al., 1974). This experiment was replicated three times. The experimental design adopted was a completely randomized design. ANOVA was conducted using SPSS version 13.0. Duncan`s multiple range test (DMRT) was used to compare treatment means.

Results and Discussion

The highest *A. alternata* growth rates of 53.7% and 77.2%were recorded after 216 hrs of incubation when the fungus was exposed to the high energy and low energy of radiation (300 and 600nm), respectively. However, growth rates of *F. oxysporum and P. digitatum* for the same treatments were 19.3%, 33.2%, and 16.632%, 25.2%, respectively (Fig. 1, 2).

The statistical analysis revealed that the use of high and low energy of radiation significantly ($P \leq 0.05$) suppressed the growth of *F. oxysporum and P. digitatum*. While the suppression effect of radiation against *A. alternate* was almost imperceptible (Table1).

The study confirmed the protective role of fungal melanin against UV-radiation. The result suggests the use of UV-radiation in hospitals and laboratories will be infective against

melanized fungal conidia. The inhibitory affect of UV-radiation against the non-melanized fungi such as *F*. oxysporum and *P. digitatum* was significant when compared to the melanized fungus, *A. alternata,* which grew relatively well after UV-radiation exposure. It appears that melanin protected fungal conidia from UV damage, which enhanced the survivability of the melanized fungus. This ability of melanin to enhance the survivability over extreme conditions led Tseng et. al. (Tseng, et al., 2011) conducted an experiment aiming to transfer the gene responsible of melanin production to entompathogenic fungal races that lack these gene.

This finding agreeswith the reports of Kawamura *et. al*. (1999) and Thomma *et al.,*(2003)who suggested that melanin acts as a "body armour", protecting fungi against environmental stress andunfavorable conditions such as extreme temperatures, UV-radiation and compounds secreted by microbial antagonists. Also our finding agrees with Eisenman and Casadevall (Eisenman and Casadevall, 2012) who stated that melanin is a unique pigment with myriad functions that is found in all biological kingdoms. It is multifunctional, providing defense against environmental stresses such as ultraviolet (UV) light, oxidizing agents and ionizing radiation. Melanin contributes to the ability of fungi to survive in harsh environments. In addition, it plays a role in fungal pathogenesis.

Moreover this finding correspond and agrees precisely withthe data collected from the area contaminated by the Chernobyl nuclear power plant accident which proved that certain melanized fungi survive chronic irradiation from multiple radionuclides (Tugay, et al., 2006). The lowest growth rates of *F. oxysporum* and *P.digitatum* obtained in this study indicated that the high energy treatment of UV (300 nm) was only a fungistaticfor non-melanized fungi even high energy treatments of UV-radiation would be ineffective in protecting food from melanized fungi such as *Alternaria* spp.

Conclusion

Our findings revealed that the use of high and low energy of radiation significantly suppressed the growth of *F. oxysporum and P. digitatum*. While the suppression effect of radiation against *A. alternate* was almost unnoticeable. The result proposes the use of UV-radiation inlaboratories will be infective against melanized fungal conidia. The inhibitory affect of UV-radiation against the non-melanized fungi such as *F*. oxysporum and *P. digitatum* was significant when compared to the melanized fungus such as *A. alternata,* which grew relatively well after UV-radiation exposure. Regarding the wide application of

the classical methods for sterilization by UV-radiation, the gloomy picture of the protective role of melanin was obviously cleared.

Table 1. Mean growth rates of *A. alternata*, *F. oxysporum* and *P. digitatum* exposed to UV-radiation and visible light

Fungus name	UV and visible light treatment (nm)	Growth rates after different times as percentage of an untreated control 72 hr.	144 hr.	216 hr.
A. alternata	300	54.068 c	48.315 c	53.715 b
	600	92.190 a	84.788 a	77.235 a
F. oxysporum	300	0.00 d	20.630 e	19.275 e
	600	75.665 b	69.376 b	33.213 c
P. digitatum	300	75.665 b	69.376 b	16.632 e
	600	54.170 c	39.958 d	25.200 d
F		618.76	423.06	79.57
p- value		2.17	5.93	5.03
LSD (0.05)		8.19	6.36	6.26

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

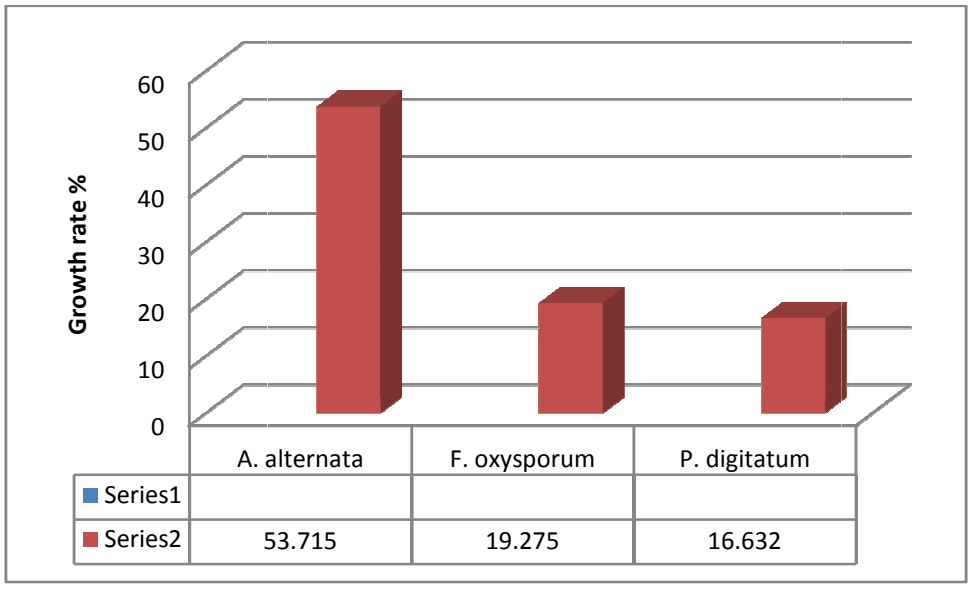

Fig. 1. Average growth rates of the three fungi exposed to the high energy treatment of UV-radiation (300 nm) after 216 hrs.

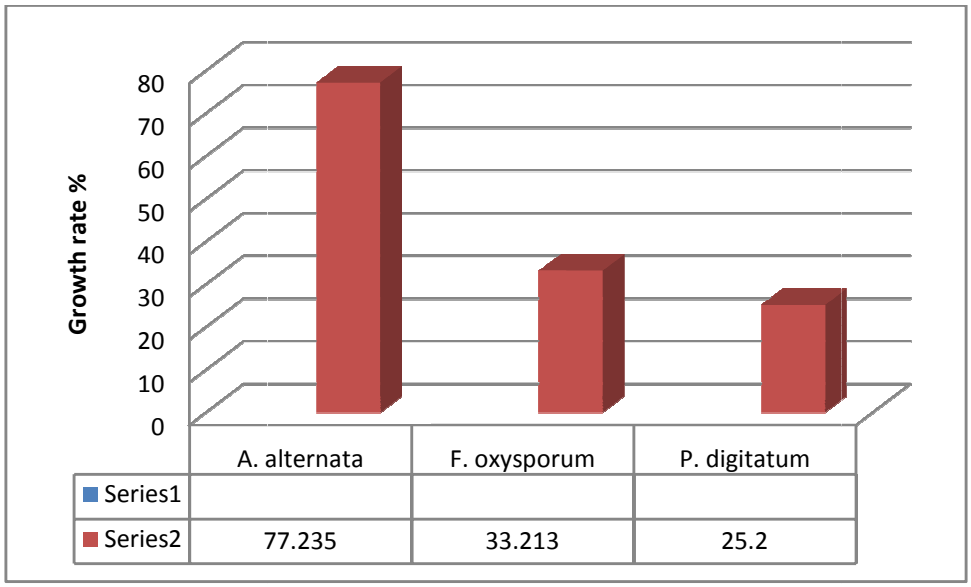

Fig. 2. Mean growth rates of the three fungi exposed to the low energy treatment of visible light radiation (600 nm) after 216 hrs.

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