

**TITLE:** PHOTODYNAMIC EFFECT OF HYPERICIN AGAINST BIOFILM FORMATION BY *FUSARIUM OXYSPORUM*

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**ABSTRACT:**

In the last decades there was a significant increase of *F. oxysporum* as an opportunistic pathogen, mainly on patients using medical devices. Hypericin-based photodynamic Therapy (PDT+Hyp) has been shown to be an effective therapy against cancer cells and for microorganisms inactivation. In this context, the aim of study was to evaluate the *in vitro* antifungal potential of PDT+Hyp in the biofilms formation by *F. oxysporum*. For this, conidia from *F. oxysporum* (ATCC 48112) were collected in sterile saline solution (0.85%) after 7 days on potato dextrose agar (PDA). The inoculum was adjusted in RPMI 1640 ( $1 \times 10^6$  conidias/mL), and incubated into 96-well plates without shaking at 25° C for 2 h. Subsequently, the wells were washed with sterile saline, and added 25 µM (2x minimal inhibitory concentration, 2xMIC), 12.5 µM (MIC) and 6.25 µM (0.5xMIC) of hypericin in wells in triplicate. The plate was again incubated in the dark for 2 h at 25° C, then the suspensions were irradiated by an LED (3.0 mW/cm<sup>2</sup>). Two controls of biofilm in formation were included: one with saline solution irradiated (CC) and other with saline solution non-irradiated (C). After irradiation, the plate was incubated in the dark for 24 h. Then, each well was scraped twice with saline solution and serial dilutions (1:10) were performed. Each concentration were plated on PDA and incubated at 25° C for up to 48 h. For to evaluate the metabolic activity *in situ* mitochondrial's biofilm formation assay by XTT (tetrazolium salt 2,3-(2-methoxy-4-nitro-5-sulphophenyl)-5-([phenylamino] carbonyl) 2H tetrazolium hydroxide). The results showed that PDT+Hyp was efficient anti-biofilm activity in all concentrations tested. The concentration 6.25 µM had reduction, however in 12.5 and 25 µM the assay showed greater reduction than 3 logs ( $p < 0.05$ ), and were showed an percentage of cell viability only of 35% ( $p < 0.05$ ), compared to controls (CC) and (C). Between in the controls there not statistical difference. These results point to the PDT+Hyp success in the prevention of biofilm formation, suggesting a promising application of this therapy on hospital medical devices.

**Keywords:** biofilm, *Fusarium oxysporum*, hypericin, photodynamic therapy.

**Development Agency:** National Council of Scientific and Technological Development (CNPQ) State University of Maringá.