Essential oil from *Lippia gracilis* Schauer: a potential antifungal agent against *Fusarium* biofilm

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Fusarium are ubiquitous soil inhabiting fungi, including phytopathogenic and non-phytopathogenic species. Moreover, some superficial infections in human and animals, such as keratitis and onychomycosis, can be caused by *Fusarium* species. Furthermore, some studies have reported the biofilm formation from *Fusarium*. Biofilms are composed by microbial cells that are irreversibly associated with a surface and enclosed in a matrix of polymeric material. The search for potential phytochemicals as anti-biofilm agents has become an active area of research. Thus, the present work aims to evaluate *in vitro* the antifungal and antibiofilm activity of the essential oil (EO) from *Lippia gracilis* Schauer against *Fusarium oxysporum* URM6704 and *Fusarium solani* URM6749. *L. gracilis* EO was evaluated at the concentrations ranging from 1 to 0.0078 % (v/v). The antifungal activity of the EO was determined by inhibition of the mycelial growth (measured for 10 days) in Petri dishes containing potato dextrose agar (PDA) with different concentrations of the EO. Moreover, the fungal vegetative growth was evaluated in presence of the EO using 96-wells plate. The wells containing 2.0×10^5 spores/mL in potato dextrose broth (PDB) were incubated with different concentrations of the EO at 25 °C and the fungus germination was evaluated by absorbance mensuration at 620 nm, at intervals of 24 h, up to 72 h after initiation of incubation. Biofilms were developed in 96-well plates with and without the presence of EO for 72 h. The biofilms were characterized by total biomass (crystal violet staining) and metabolically active cells by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay. Additionally, images in Scanning Electronic Microscopy (SEM) were obtained. The results showed that in both species, the EO completely inhibited the mycelial growth at concentrations of 1 to 0.125 %. Furthermore, the EO showed inhibitory effect on the vegetative growth of *Fusarium* species at concentrations ragging from 1 to 0.031%. Regarding biofilm formation, in general, the EO reduced significantly the biomass formation and cell viability of the fungal biofilms. Moreover, SEM analysis of *F. oxysporum* biofilm treated with EO showed morphological changes in the hyphal structure. In summary, the essential oil from *L. gracilis* have potential to be an effective alternative antifungal to prevent biofilm formation of *Fusarium* species.

Keywords: *Fusarium*, biofilm, essential oil, antifungal

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