

## In vitro antifungal activity of Lippia sidoides essential oil on Aspergillus niger by different methods

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Lippia sidoides, family Verbenaceae, is a plant that occurs mainly in regions of semiarid climate, such as northeast region in Brazil, popularly known as "alecrim pimenta" (1). Its popular use as antimicrobial in conventional treatments has been affirmed through studies, proving the antibacterial and antifungal action of its essential oil (EO) when applied in the food industry and in edible coatings of fruits (2). Considering that fundal contaminants are the main cause of deterioration in bakery products, essential oils have been evaluated as a potential natural alternative in the preservation of this kind of product, replacing the use of synthetic preservatives. The aim of this study was to verify the antifungal activity of L. sidoides EO on Aspergillus niger species, by the agar dilution method and by exposure to volatile EO compounds. The L. sidoides EO used was obtained by steam distillation and provided by Agropaulo company (Fortaleza-CE), chemical characterization was performed by gas chromatography coupled to a mass spectrometer (GC / MS) and the quantification of its constituents by flame ionization detector (GC / FID). An isolated of the fungal species A. niger was obtained from bread samples and its identity confirmed by molecular techniques. Antifungal activity was evaluated in both methods using doses of 0 (control), 200, 300, 400, 500 and 600 ppm. The amount of EO was calculated based on the volume of agar or atmospheric air in petri dishes (90 mm x 15 mm), according to each method. In the agar dilution method, the EO was previously homogenized by the Tween 80 emulsifier and added to the still melting BDA (Potato Dextrose Agar) medium. The volatile exposure method was performed by depositing the EO on filter paper discs, fixed the Petri dish lid. In both methods, 5 µl of a solution (10<sup>6</sup> spores / mL) was deposited on a filter paper circle positioned in the center of each plate and incubated at 25 ° C for 14 days. At the end of the incubation period, the MIC (Minimum Inhibitory Concentration) was determined. The EO characterization provided the identification of 27 compounds, having as major compounds: thymol (59.4%), cymene (12.6%), trans-caryophyllene (12.2%), gamma-terpinene (3.62%). alpha-terpinene (1.59%), mircene (1.55%) alphathujene (1.18%) and copaene (1%). The MIC observed by the agar dilution method was between 400 and 500 ppm. In the method of exposure to volatiles, the MIC was between 300 and 400 ppm, and it can be stated that the antifungal activity of L. sidoides EO was more effective by exposure to volatiles. Therefore, results obtained in this study suggests that the inhibition of fungal growth in breads through exposure to volatile from L. sidoides EO may be a technique to be considered to control fungal growth. However, further studies are required to optimize and verify the safety and acceptability of bread consumers.

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