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Antifungal activity of essential oils against *Aspergillus flavus* and *Aspergillus parasiticus* isolated from food commodities

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1. Introduction

The characterisation and identification of fungal contaminants of food are essential for the control of contamination by these microorganisms and the possible production of mycotoxins. Entirely related to this identification is the adoption of a tool capable of preventing the development of these fungi. In this context, the exploitation of the biological activity of secondary compounds present in plant essential oils herbs has been a potential way to control fungi. The aim of this study was to identify and characterise mycotoxigenic fungi in food commodities and to test antifungal activity of essential oils from plants against them.

2. Material and Methods

2.1. Fungal isolates: Thirty-six isolates of *Aspergillus* Section *Flavi,* isolated from differente products (e.g. peanuts, Pará chestnut, rice, corn and cereal bars) bought in a local market in Lavras, MG, Brazil were used in this study. Mycotoxigenic isolates of *A. flavus* and *A. parasiticus* were used as reference strains for testing antifungal activities.

2.2 Morphological characterisation: Morphological characterisation of fungal isolates was done by inoculation on different culture media and temperatures. Analysis of colony colour, presence and size of sclerotia, head seriation and conidial morphology were performed.

2.3 Mycotoxigenic profile of the isolates: The analysis of aflatoxins and cyclopiazonic acid (CPA) were made by the agar plug method in HPLC with detector fluorescence and UV, respectively.

2.4 Essential oils: The essential oils of anise (*Pimpinella anisum*), ginger (*Zingiber officinale*), mint (*Mentha piperita*), sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*) obtained of Ferquima Industry and Trade Ltda were used. To analyse the chemical constituents of essential oils High Resolution Gas Chromatography was used.

2.5 Antifungal tests:

2.5.1 Minimum inhibitory concentration (MIC): The essential oils MIC were found by a qualitative method using the solid medium diffusion procedure (Souza *et al*., 2005). To determine the MIC the procedure of diffusion test on solid medium was used. A spore suspension (10^6 spores/mL) was prepared. A drop of 120µL was spread on the culture medium (MEA) in Petri dishes. After absorption, a sterile filter paper disc (6 mm) was added to the centre of the plate, on which 10µL of different dilutions of essential oils were added.

The plates were incubated for 48-72h at 25 °C. At the end of the incubation period, the MIC was the lowest essential oil concentration showing growth inhibition zones.

2.5.2 Antifungal activity: Using the same procedure as above, 25µL of essential oils in the same concentration established as MIC were added to paper discs and plates were incubated at 25 ° C for nine days. At 5, 7 and 9 days the extent of inhibition zone of fungal growth were recorded. The spore production in these different conditions were also determined using a Neubauer chamber.

2.6 Statistical analysis of experiments: Statistical package, System of Variance for Balanced Data - Sisvar. Data were submit to ANOVA followed by Tukey's test (P=0.05).

3. Results

3.1 Morphological characterization: Based on morphological characteristics of the colour of colony and conidia ornamentation, it was possible to distinguish five distinct species, among which two stand out: *A. flavus* (29 isolates, 80.6%) with yellow-green colonies, globose and smooth to finely rough conidia, and *A. parasiticus* (4 isolados, 11.1%) with dark-green colonies and rough conidia. Single isolates of *A. tamarii*, *A. oryzae* and *A. sojae* were identified.

3.2 Mycotoxigenic profile of the isolates: All *A. parasiticus* isolates produced AFB and AFG, but no CPA was detected. For *A. flavus* 86.1% of isolates showed no detectable aflatoxin , in contrast 69.4% were able to produce CPA. The isolate of *A. tamarii* was positive for CPA production.

3.3 High Resolution Gas Chromatography of essential oils: Analysis by High Resolution Gas Chromatography of the essential oils showed that trans-anethole (51.5%), zingiberene (32.7%), menthol (55.4%), bornyl acetate (54.2%) and thymol (54.9%) respectively are the major components of essential oils of sweet fennel, ginger, mint, sage and thyme that can be related to the antifungal effect.

3.4 Antifungal activity: The MICs obtained for the essential oils of sage, ginger, sweet fennel, mint and thyme were 100%, 80%, 50%, 50% and 50% essential oil, respectively.

For the *in vitro* tests of antifungal activity the essential oil of sage was not used due to the impracticability of using pure essential oil. All other essential oils showed an antifungal effect against fungal mycelium growth and sporulation. However, no statistical significant differences were observed between days 5, 7 and 9. Thyme was the greater inhibitor and *A. parasiticus* was more resistant than *A. flavus* to essential oils.

4. Conclusion

The morphological characteristics associated with the production of aflatoxins and CPA contributed to the identification of toxigenic species of Section *Flavi.*

The essential oils, especially thyme oil, had a significant inhibitory effect on the fungi. Thus, the essential oils can be an alternative to be used as a chemical preservatives.

References

Souza, EL, Lima, EO, Freire, KRL, Sousa, CP (2005). Inhibition action of some essential oils and phytochemicals on the growth of moulds isolated from foods. Braz. Arch. Biol. Technol. 48: 245-250.