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Comunicación Breve

Ultrastructural alterations induced by the essential oil of cinnamon in *Fusarium solani* isolated from onychomycosis

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ABSTRACT

Previously, antifungal activity was documented for an essential oil of cinnamon against *Fusarium* spp., a non-dermatophytes fungus related with onychomycosis. This report showed ultrastructural alterations in spore suspensions treated with that essential oil. The most relevant finding was vacuolization of the cytoplasmic membrane, which could be associated with the lipophylic activity described for this essential oil. This activity apparently disassembles the structure of the cytoplasmic membrane, blocks membrane synthesis, inhibits the germination of spores, and inhibits cellular respiration.

Key words: essential oil, cinnamon, *Fusarium solani*, onychomycosis

RESUMEN

Alteraciones ultraestructurales inducidas por el aceite esencial de canela en *Fusarium solani* aislado de onicomicosis

Previamente fue documentada la actividad antifúngica de un aceite esencial de canela contra *Fusarium* spp., un hongo no dermatofito asociado con onicomicosis. En este informe, se muestran alteraciones ultraestructurales en una suspensión de esporas tratadas con ese aceite esencial. El hallazgo más relevante fue la vacuolización de la membrana citoplasmática, lo cual podría asociarse con la actividad lipofílica descrita para este aceite esencial, que aparentemente desensambla la estructura de membrana citoplasmática, bloquea su síntesis, inhibe la germinación de esporas y la respiración celular.

Palabras clave: aceite esencial, canela, *Fusarium solani*, onicomicosis

INTRODUCTION

Fusarium species are plant pathogen fungi throughout the world, causing significant crop losses, affecting mainly cereals. Some species of this genus have been incriminated in human infections, especially in immunodeficient hosts, due to clinical conditions such as hematologic malignancies, aplastic anemia for patients undergoing chemotherapy (1). In these hosts the fungus disseminates through the blood and causes lesions in different organs, with a high mortality rate (2). This agent is included in the list of non-dermatophytes fungi related with onychomycosis in immunocompetent persons, a trivialized infection, but one that significantly impacts quality of life and causes psychosocial problems. In Costa Rica, *Fusarium* is the most prevalent non-dermatophyte agent causing onychomycosis (3).

The *Fusarium* onychomycosis has two possible complications. First, the nail lesions can

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be a source for disseminated infections in patients with risk factors (2). Second, onychomycosis is relatively resistant to most anti-fungal agents and requires long term treatments, along with aggravating secondary effects. Further, the infection sometimes relapses (4). For this reason, alternative therapies are imperative and the essential oils of some plants have been evaluated for their antifungal properties against fungus in crops, in grain storage, and include different agents associated with human onychomycosis (5). A previous report documented the antifungal activity of a commercial essential oil of cinnamon against *F. solani*, using an *in vitro* bioassay with the disc diffusion method (6). Thus, the aim of this report is to evaluate the ultrastructural alterations caused by this essential oil against a strain of *F. solani* isolated from human onychomycosis patients.

Aliquots of 100 µl of spore suspensions (0.5 McFarland) were mixed with 100 µl of a 1/50 dilution of cinnamon oil (*Cinnamomum zeylanicus*, Carpe Deim world SA) in RPMI. As controls, similar spore suspensions were treated with 100 µl of RPMI instead of the essential oil. The mixtures (tests and controls) were then incubated for 70 minutes at room temperature, washed three times with sterile distilled water, and centrifuged three times (14000 rpm/5 minutes). The last buttons were fixed with the Karnovsky solution and processed for transmission electron microscopy using the rapid microwave methodology (7). All the tests were conducted in triplicate.

Typical electron microscopy images of thin sections of well-fixed negative control samples of cytoplasmic membrane were characterized by three distinct layers, arranged as two electron-dense layers with a middle lucent lamina (**Figure 1**). However, that pattern appeared altered in the spores treated with the essential oil of cinnamon. Alterations were evident as multiple vacuoles in the cytoplasmic membrane, which in some samples formed a chain of vacuoles (**Figure 2**), and moreover, in some cells the membrane was

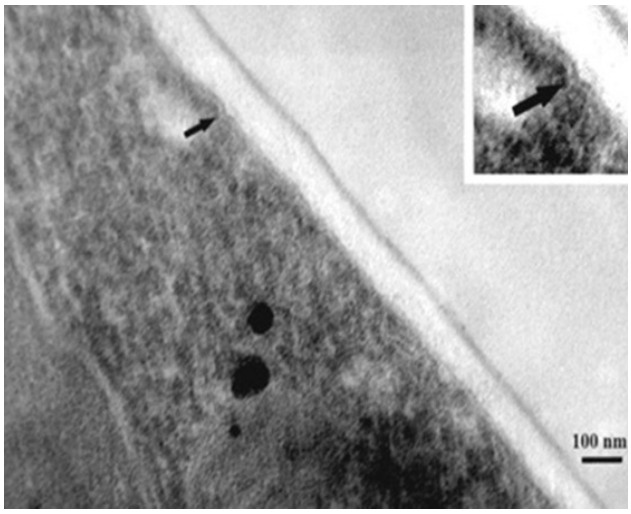

Figure 1. Transmission electron micrograph of the negative control. The arrow points to a section of the cell membrane where appreciates the typical ultrastructure. The box of the figure shows that section with more detail

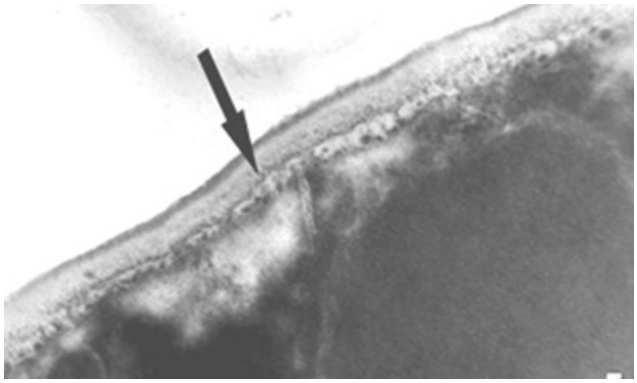

Figure 2. Transmission electron micrograph of samples treated with cinnamon. The cytoplasmic membrane shows damage, characterized by a vacuolization process such as chain vacuoles (arrow)

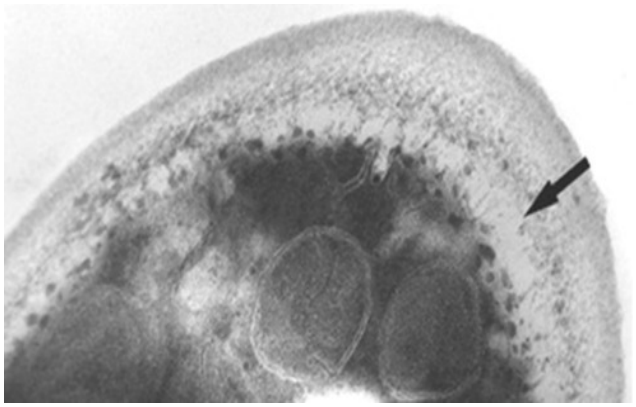

Figure 3. Transmission electron micrograph of samples treated with cinnamon. In some cells the damage of the cytoplasmic membrane is such that virtually destroy it, and there are only remnants between the cytoplasm and the cell wall (arrow)

lost (**Figure 3**). However, the rest of the cellular structures, including mitochondrial membranes, do not show major alterations.

The antimicrobial activity of the essential oils is not fully explained. It is thought to be related with its lipophylic properties (8), which apparently disassembles the structure of the cytoplasmic membrane, blocking membrane synthesis, which inhibits the germination of spores, proliferation, and cellular respiration (5). Previously, we reported that the essential oil of cinnamon inhibits the spore germination and growth of *F. solani* (6). Nowadays, the electron microscopy evidence presented in this report (**Figures 2** and **3**), involves that effect with a vacuolization of the cytoplasmic membrane of *F. solani.* The correlation between both findings, the growth inhibition and the alteration of the cytoplasmic membrane, argues that cinnamon oil could be an alternative therapy against onychomycosis-related with *F. solani*, and may likely treat others species of this fungi.

The potential medical use of the essential oil of cinnamon against *Fusarium* must be based on the investigation of the chemical components of this essential oil to evaluate their functional groups and their possible synergistic interactions; as a new field in the research of drugs derived from plants.

REFERENCES

1. **Nucci M, Anaissie E.** *Fusarium* infections in immunocompromised patients. Clin Microbiol Rev 2007; 20: 695-704.
2. **Bourgeois GP, Cafardi JA, Sellheyer K, Andea A.** Disseminated *Fusarium* originating from toil nail paronychia a neutropenic patient. Cutis 2010; 85: 191-4.
3. **Salas-Campos I, Gross-Martínez NT.** Agentes etiológicos de onicomicosis diagnosticadas en el laboratorio de micología médica de la Universidad de Costa Rica. Acta Méd Costarricense 2012; 54: 114-8.
4. **Carrillo-Muñoz AJ, Tur-Tur C, Hernández-Molina JM, Santos P, Cárdenas D, Guisiano G.** Antifúngicos disponibles para el tratamiento de las micosis ungueales. Rev Iberoam Mycol 2010; 27: 49-56.
5. **De Bona da Silva C, Guterres SS, Wisheimer V, Schapoval E.** Antifungal activity of the lemongrass oil and citral against *Candida* spp. Brazilian J Infect Dis 2008; 12: 63-6.
6. **Camacho-Umaña E, Salas-Campos I, Hernández-Chavarría F.** Onicomicosis: Medicina natural y *Fusarium*. Rev Col Microbiólogos y Químicos Clínicos de Costa Rica 2010; 16: 17-20.
7. **Hernández-Chavarría F & Vargas-Montero M.** Rapid contrasting of ultrathin sections using microwave irradiation with heat dissipation. J Microbiol 2001; 203: 227-230.
8. **Park MJ, Gwak KS, Yang I, Kim KW, Jeung EB, Chang JW, et al.** Effect of citral, eugenol and α-terpeneol on the ultrastructural changes of *Trichophyton mentagrophytes*. Fitoterapia2009; 80: 290-6.