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## **BIOCHEMICAL ANALYSIS OF WALL TELIOSPORES OF *Thecaphora frezzii*, A PATHOGENIC FUNGUS OF PEANUT CROPS**

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Peanut cultivation in Argentina is significantly threatened by the fungus *Thecaphora frezzii*, of the Basidiomycota family, and it is the causal agent of peanut smut. Despite its detrimental impact on crop yield, effective control measures are still being investigated. A deeper understanding of the structure and composition of *T. frezzii* teliospores is crucial for developing more efficient fungicidal treatments. Our previous studies revealed that these resistant spores consist of an endospore, with chitin as the main component of the wall. This endospore is enveloped by a multi-layered coating with distinct structural conformations. The thick, ornamented outermost layer serves as the primary protective barrier for the germinal cell. The aim of this study was to analyze the composition of this outer layer, which would contribute to explore alternative control methods. High-temperature alkaline hydrolysis was employed, leading to the disintegration and solubilization of the coating in NaOH. After precipitation and purification, the resulting material was then subjected to infrared spectroscopy (FTIR), UV/Visible spectrometry, high-performance liquid chromatography with a PDA detector (HPLC/PDA), and mass spectrometry detector (HPLC/MS). A commercial eumelanin standard served as a control. FTIR analysis revealed that the spectra of pigments isolated from the spores closely resembled those of the commercial eumelanin, with significant peaks observed between 3500-3100 cm<sup>-1</sup> and 1680-1550 cm<sup>-1</sup>, corresponding to N-H, C-N, C=C, O-H, and CO<sub>2</sub>H vibration frequencies related to primary and secondary amines and amides. UV/VIS spectrometry indicated a characteristic spectrum with strong absorption between 220-250 nm in both the isolated pigments and the commercial eumelanin. HPLC/PDA chromatograms showed a prominent peak in the commercial standard, which was also present in the spore-derived pigments along with additional compounds extracted during hydrolysis. Although HPLC/MS analysis did not detect the melanin polymer peak, it did identify peaks corresponding to tyrosine and 5,6-dihydroxyindole, two intermediates in the eumelanin biosynthetic pathway. These findings strongly suggest that the primary component of the outermost layer of *T. frezzii* spores is eumelanin. This study is the first to report that *T. frezzii* produces melanized resistant structures, which could play a vital role in protecting the fungus against

adverse environmental conditions.

Palabras clave: peanut smut- melanin- teliospores