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UNVEILING THE CHEMICAL AND BIOLOGICAL INTERPLAY OF PHYLLOSTICTA SPP.: INSIGHTS INTO ENDOPHYTIC–PHYTOPATHOGENIC FUNGAL INTERACTIONS IN CITRUS.

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Phyllosticta spp. comprises several fungal species, among which *P. citricarpa* and *P. capitalensis* stand out as antagonists sharing the same citrus host. Citrus Black Spot (CBS), caused by *P. citricarpa*, is responsible for necrotic lesions, premature fruit drop, and severe economic losses in citrus production worldwide. Although some virulence factors have been described, the chemical arsenal of this pathogen remains poorly characterized. In contrast, *P. capitalensis* is a nonpathogenic endophyte frequently reported in the literature for its antagonistic activity against *P. citricarpa*, highlighting its potential role in biocontrol. However, little is known about the molecular basis of this interaction, particularly regarding the Specialized Metabolites (SMs) involved. In this study, the inhibitory potential of *P. capitalensis* against *P. citricarpa* was evaluated in dual-culture assays on potato dextrose agar (PDA). Morphological analysis revealed a significant reduction in pathogen growth, with marked hyphal alterations and an inhibition rate of 67.32%. Metabolites from mono- and co-cultures were extracted with ethyl acetate (AcOEt) and analyzed by LC-HRMS (Orbitrap). Data were processed using MZmine 4.5 for feature-based molecular networking, MetaboAnalyst 6.0 for multivariate statistics, and SIRIUS for propagation of annotation. Annotation propagation in SIRIUS revealed characteristic metabolites of *P. capitalensis*, including 2-pyrone derivatives and guignardone-type meroterpenes. In contrast, *P. citricarpa* exhibited predicted families of modified and glycosylated terpenes. Notably, these same ions contributed most strongly to the separation observed in principal component analysis (PCA), confirming their role as discriminant metabolic features between experimental groups. To validate the production of these metabolites, the endophyte was cultured in three different liquid media (Potato-Dextrose, Czapek and Yeast Broth) for periods of 10, 14 and 21 days. Screening demonstrated that Czapek medium was suitable for the biosynthesis of 2-pyrones and guignardones. To validate the biological relevance of these metabolites, the antifungal activity of the extracts of Czapeck obtained at different cultivation times against the phytopathogenic isolate CBS of *P. citricarpa* was evaluated. In the assays performed at 500 and 250 ppm, the 14-day extract showed the highest antifungal activity, with an effect comparable to the positive control (Imazalil). Overall, these results highlight the chemical potential of *P. capitalensis* as a source of bioactive specialized metabolites, provide insights into the metabolic basis of endophyte–pathogen interactions, and point to promising candidates for the development of novel antifungal agents for CBS management.

Keywords: *Phyllosticta, citrus black spot, endophytic fungi, Citrus.*