Mo-CBP3: A seed-derived protein from Moringa oleifera as a potential biocontrol agent for melon postharvest rot

LAÍS OLIVEIRA LEITE[1], ARIANE FERNANDES FERREIRA SILVA[1], CAMILA RIBEIRO DOS SANTOS GOMES[1], DANIELE DE OLIVEIRA BEZERRA DE SOUSA[1], RAQUEL GUIMARÃES BENEVIDES[1]

[1.] UNIVERSIDADE FEDERAL DO CEARÁ, FORTALEZA / CE, BRASIL

Fungi of the genus *Fusarium* are among the most important phytopathogenic fungi found worldwide, and their spores act as infectious propagules that initiate infection. In agriculture, *Fusarium* species play a significant role as they can cause devastating crop diseases. In some cases, they are also indirectly responsible for allergic reactions and food poisoning among consumers, since cereals are the primary substrate available during the growing season and fungal contamination is accompanied by the production of mycotoxins, which are subsequently ingested by consumers. Melon (*Cucumis melo* L.) is Brazil's third-most-exported fruit, with an annual production of 862,387 tons. Reports from Brazilian melon exporters indicate that 15% of exported melons show symptoms of postharvest diseases upon arrival at their destination and are consequently discarded; one of the main postharvest diseases is rot caused by *Fusarium* sp. One of the main problems resulting from the misuse of conventional fungicides is resistance to their mechanisms of action, which is considered one of the most serious threats to food security. The discovery of compounds with antifungal potential are essential for the sustained control of the main diseases affecting crops. In this context, plant-based proteins emerge as potential alternatives for combating fungal diseases. Given this context, the present work aimed to test the antifungal potential of *Mo*-CBP$_3$, a protein purified from moringa (*Moringa oleifera*) seeds and to explore its possible mechanisms of action against the filamentous fungus *Fusarium jinanense*, a strain that causes rot in melon crops grown in northeastern Brazil. The fungus was cultured on plates containing Potato Dextrose Agar (PDA) medium and incubated at 30 °C for 5 days. Fungal suspension was prepared in sterile 0.9% saline solution and standardized to 10^6 conidia/mL in a Neubauer chamber. The inoculum was uniformly distributed in each of the 24 wells of the plates. The protein was diluted in PDA medium with concentrations ranging from 1.0–0.01562 mg/mL. Growth control contained only culture medium and inoculum. Minimum inhibitory concentration (MIC) was determined qualitatively. The effect of *Mo*-CBP$_3$ on fungal membrane integrity and the induction of reactive oxygen species production were evaluated using propidium iodide and DCFH-DA, respectively. The comparison of the non-treated control with treated fungal sample was performed using fluorescence microscopy. The leakage of intracellular proteins and sugars in samples treated with MIC concentration was quantified. Aliquots of the supernatant were taken daily over 4 days. Soluble proteins and sugars were quantified using Bradford and Dubois methods, respectively. The MIC of *Mo*-CBP$_3$ was determined to be 0.25 mg/mL. The sugar content was undetectable in the growth control and in the sample treated with *Mo*-CBP$_3$. Fluorescence microscopy results demonstrated that the protein was able to damage the fungal cell membrane and induce the formation of reactive oxygen species, which consequently delayed mycelial growth. This demonstrated the protein's fungistatic potential. Although the sugar content was undetectable, there was a progressive increase in the detection of soluble proteins in

the supernatant from the second day of treatment, confirming severe membrane damage. These results demonstrate that *Mo*-CBP_3 can be a promising candidate for the development of new biofungicides for the control of melon rot.

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