Moringa seed-derived protein Mo-CBP4: A sustainable alternative for pitaya anthracnose management

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Fungi of the genus *Colletotrichum* are widely distributed in tropical and subtropical regions. Epidemics caused by fungi of this genus are generally initiated from conidia that disperse and reach the surface of plants, germinating and penetrating the host tissue under favorable conditions. Considered one of the top 10 pathogenic fungi of plants, *Colletotrichum* sp. is the causal agent of anthracnose in more than 3.000 plant species, leading to a considerable reduction in the productivity of economically important crops. Anthracnose infection commonly occurs in fields during the flowering and fruiting stages. The most visible symptoms are sunken, black or dark brown lesions containing masses of conidia on the surface of infected fruits. Pitaya is included among the crops affected by anthracnose caused by species of the genus *Colletotrichum*. Anthracnose is considered a very damaging disease that affects both the plant and the fruit. The use of chemical substances have been suggested for controlling the disease, but regulatory pressure regarding agrochemicals and their effects on human health and the environment has become a growing concern. Natural products, such as plant-derived proteins, have shown promising potential for the development of new fungicides. Given this context, the present work aimed to test the antifungal potential of *Mo*-CBP$_4$, a protein purified from moringa (*Moringa oleifera*) seeds and to explore its possible mechanisms of action against the filamentous fungus *C. tropicale*, a strain that causes anthracnose in pitaya crops. 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$_4$ 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 content 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 *Mo*-CBP$_4$ protein showed a MIC of 0.25 mg/mL, demonstrating fungistatic potential based on cell membrane damage and the induction of reactive oxygen species, as evidenced by fluorescence microscopy. This mechanism was responsible for delaying the mycelial growth of the fungus. Although the sugar content remained

undetectable in the control and treated groups, a progressive increase in soluble proteins in the supernatant was observed from the second day of treatment. This confirms the occurrence of severe membrane lesions. *Mo*-CBP_4 demonstrates itself to be a sustainable biotechnological alternative to conventional chemical fungicides, possessing promising potential for the development of new biofungicides intended for the management of anthracnose in pitaya crops.

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