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AFM Structural Analysis of *Colletotrichum gloeosporioides* Interacting with ZnO Nanoparticles

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The anthracnose is a disease caused by fungus *Colletotrichum gloeosporioides*, which produces significant economic losses in papaya (*Carica Papaya*) and avocado (*Persea Americana*) crops. Some of the symptoms are sunken spots of different colors on the leaves, necrosis on the stems, fruits, or flowers that can lead to a withering or death of plant tissue [1]. To combat this fungus is often used various types of chemical fungicides but eventually, it responds developing resistance against them [2]. Therefore it is necessary to find environmental friendly alternatives. Thus, in this work we studied the structural damage in cell of the *C. gloeosporioides* when it interacts with ZnO nanoparticles (NPs) using AFM.

The synthesis of the ZnO NPs was done as follows, 100 mL of NaOH water solution (0.4 M) was added dropwise to 100 mL of $Zn(NO_3)\cdot 6H_2O$ water solution at 0.2 M under constant stirring. This reaction was treated hydrothermally (Parr, 4649) at 100° C for 24 h. Then, the sample was centrifuged and washed with deionized water (Millipore, RiOs 8) and ethanol (99.9%) 1:1, and the precipitate was dried at 50° C. The broth microdilution method and mycelial radial growth (MRG) techniques were used to determine the minimum inhibitory concentration (MIC) and inhibition of the mycelial growth on the two strains of *C. gloeosporioides* isolated from the fruit of avocado and papaya. To evaluate the structural damage of the strains by AFM, a portion of the sample from the MRG experiment was transferred to a microscope slide. The AFM images were captured under ambient conditions (Rel. Hum.=45 %, T=25° C) using R-TESPA probe (Bruker) in semi contact mode with a spring constant of k = 40 N/m at a scanning rate of 0.7 Hz. An analysis of energy dissipation was performed with the AFM images following the ref. [3].

Figure 1a shows the XRD pattern of the ZnO NPs, indicating high crystallinity of the powder with zincite as structural phase. The crystallite size was 37 nm (calculated with the Scherrer equation). The SEM (Figure 1b) shows some prisms with pyramidal ends with average particle size around 77 ± 31 nm. From the images of AFM (Figure 2), we observed a structural deformation of both strains in contact with NPs compared with control (fungus without NPs) and it is confirmed by the height profile of the cross section of the hyphae (Figure 3a). Figure 3b shows the energy dissipation measured with the cantilever, where the dissipated energies of the strains were higher than control. The structural damage observed by AFM could be due the deformation of hyphae via swelling or vacuolar expansion as effect of the ZnO NPs which is also reflected in the energy dissipated.

In conclusion, ZnO NPs obtained by hydrothermal synthesis had an antifungal activity with MICs of 0.156 mg/mL and 0.312 mg/mL for papaya and avocado strains, respectively. The percentage of radial inhibition was 80 ± 5% for both strains with NPs concentrations of 0.312 mg/mL. AFM allowed to evaluate the structural damage of the fungus with a significant height change of the strains as a consequence of the interaction with ZnO NPs. Additionally, the analysis of dissipated energy suggests that the NPs produces an increase of viscosity in both strains with respect to control.

References:

[1] S Freeman et al., Applied and Environmental Microbiology **62** (1996), p. 1014.
[2] H Ishii and DW Hollomon, "Fungicide Resistance in plant Pathogens: Principles and a Guide to Practical Management", (Springer).
[3] JP Cleveland et al., Applied Physics Letters **72** (1998), p. 2613.

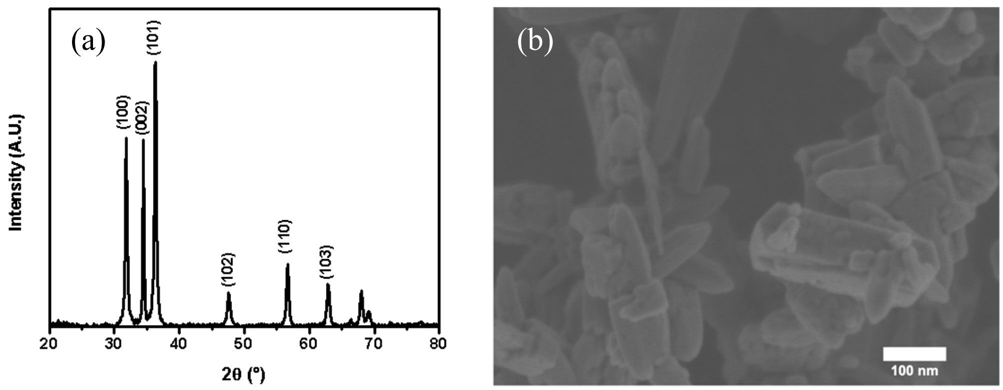

Figure 1. (a) XRD pattern and (b) SEM image of ZnO NPs.

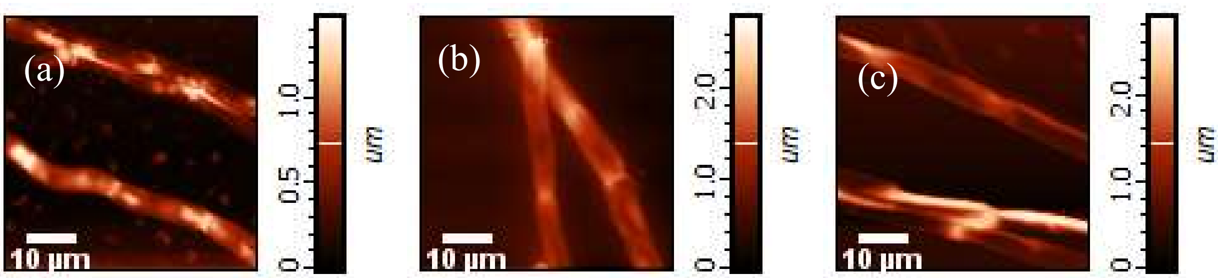

Figure 2. AFM images of (a) control, (b) avocado and (c) papaya strains with NPs.

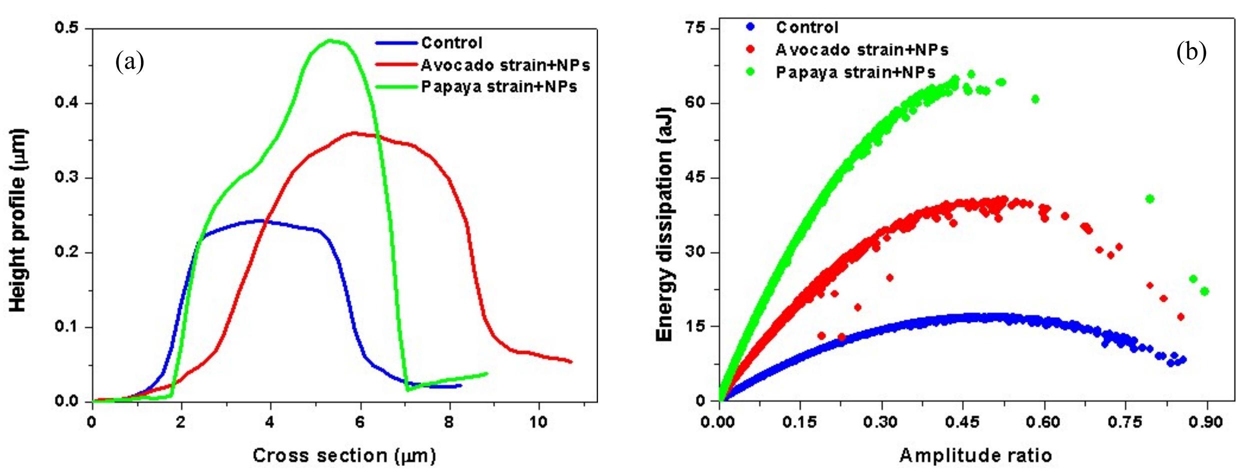

Figure 3. (a) Height profile and (b) dissipated energy of the hyphae.

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