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Copper oxide nanoparticles and their effects on *Trichoderma asperellum* in plant growth and disease control

Authors

Enrique García-Mireles^{1*}, Claudia A. Ramírez-Valdespino², Marlyn Morales-García², Guillermo Herrera-Pérez², Joan Salas-Leiva², Erasmo Orrantia-Borunda², Yordan J. Romero-Contreras³, Socorro Tarango-Rivero⁴, Susana González-Chávez⁵

¹Bioinformatics Program, The University of Texas at El Paso, El Paso, TX

²Centro de Investigación en Materiales Avanzados (CIMAV), Chihuahua, Chihuahua, Mexico

³Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México (UNAM),

Cuernavaca, Morelos, Mexico

⁴Campo Experimental Delicias, INIFAP, Chihuahua, Mexico

⁵Laboratorio PABIOM, Facultad de Medicina y Ciencias Biomédicas, Universidad Autónoma de Chihuahua, Chihuahua, Chihuahua, Mexico

*Work conducted at Centro de Investigación en Materiales Avanzados (CIMAV), Chihuahua, Mexico; currently at The University of Texas at El Paso, El Paso, TX.

Abstract

Modern agriculture faces the challenge of increasing food production while minimizing environmental impact caused by excessive use of fertilizers and pesticides. One promising approach involves the use of beneficial fungi such as *Trichoderma* species, which not only suppress phytopathogenic fungi but also promote plant growth. Simultaneously, nanotechnology has emerged as a valuable tool in agriculture, with nanoparticles such as zinc oxide (ZnO), titanium dioxide (TiO₂), and copper oxide (CuO) being explored as fertilizers and antifungal agents. However, their effects on beneficial microorganisms like *Trichoderma spp.* remain insufficiently understood. In this study, a strain of *Trichoderma asperellum* was evaluated in the presence of CuO nanoparticles (CuO-NPs). The fungus exhibited normal growth up to 200 parts per million (ppm), demonstrating no signs of stress or morphological alterations, although spore formation occurred more slowly. These results were supported by growth rate assessments performed on media containing embedded CuO nanoparticles at different concentrations.

The antagonistic capacity of *T. asperellum* against *Fusarium* species, *Rhizoctonia solani*, and *Alternaria alternata* was also analyzed. The presence of CuO-NPs did not affect negatively *Trichoderma* species activity and, in some cases—such as interactions with *F. oxysporum*—phytopathogen control efficacy was enhanced. Gene expression analysis of *hyd2*, *epl1*, and *ech42* revealed that CuO-NPs influenced transcriptional activity, as determined by RT-PCR and qRT-PCR followed by ANOVA, suggesting a subtle molecular effect.

Finally, alfalfa plants (*Medicago sativa*) were used to assess the combined impact of *T. asperellum* and CuO-NPs. The nanoparticles promoted increased plant height, leaf number, and root development, while co-inoculation with *Trichoderma* species further improved seed germination and overall plant performance. These findings indicate that CuO-NPs are compatible with *T. asperellum* and may enhance both its phytopathogen control and growth-promoting effects, offering potential for environmentally friendly alternatives to conventional agrochemicals.