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Antifungal Evaluation of Zn and Zn-Cu Oxychloride Nanoparticles.

L. A. Hermida-Montero[1*], I.C. Ruiz-Leyva[1], F. Paraguay-Delgado[1], D. Lardizábal-Gutiérrez[1], L.N. Muñoz-Castellanos[2], C.V. Villalba-Bejarano[2]

[1.] Centro de Investigación en Materiales Avanzados S.C. (CIMAV), Laboratorio Nacional de Nanotecnología, Chihuahua, Chih. México.
[2.] Universidad Autónoma de Chihuahua (UACH), Facultad de Ciencias Químicas, Circuito Universitario S/N, Campus UACH II, Chihuahua, Chih. México.
* Corresponding author: luis.hermida@cimav.edu.mx

Fungal phytopathogens is one of the main problems in agriculture that greatly reduces crop yields and causes economic losses worldwide [1]. While there are many commercially available fungicides, many of them have a low efficiency due to the constant progress of antifungal resistance. Therefore, nanoparticles (NPs) are currently being studied as efficient alternative fungicides. Simonkolleite is a Zn oxychloride has been studied in photocatalysis, as anticorrosive, among others, but their antifungal activity has not been widely studied. Alves *et al*. showed that the antifungal activity of simonkolleite thin films was higher than ZnO thin films on *Candida albicans* [2] which shows it's potential as an antifungal nanomaterial. On the other hand, zincian paratacamite is a Zn-Cu based oxychloride, which has not been studied as an antifungal material. In this study simonkolleite (OxZn) and zincian paratacamite (OxZnCu) NPs were synthesized by hydrothermal method. Their antifungal activity was evaluated against three types of phytopathogenic fungi from the *Fusarium* genus (*F. fujikuroi*, *F. oxysporum* and *F. solani*) at three different concentrations (1000, 1500 and 2000 mg/L). Fluodioxonil and CuOH, which are common agricultural fungicides, were also evaluated at the same conditions to compare.

Oxychloride nanoparticles (OxZn and OxZnCu) were synthesized by a hydrothermal method. Briefly, for OxZn NPs, 3.2 g of NaOH and 6.8 g of $ZnCl_2$ were mixed with 100 mL of distilled water at 60 rpm. The mixture was heated at 75 °C for 2 h. Then the reaction was transferred to a Teflon vial and heat treated at 100 °C for 24 h. For OxZnCu NPs, 3.6 g of NaOH, 6 g of $CuCl_2$ and 2.7 g of $ZnCl_2$ were mixed with 100 mL of distilled water, they were mixed at 60 rpm and 75 °C for 2 h as well and heat treated at 100 °C for 24 h. Both synthesis products were filtered and washed several times with distilled water. TEM micrographs were obtained with a JEOL JEM 2200FS+CS TEM. Antifungal activity of OxZn and OxZnCu NPs, as well as fluodioxonil and CuOH (commonly used fungicides in agriculture), were evaluated against three phytopathogenic species of *Fusarium* fungi (*F. fujikuroi*, *F. oxysporum* and *F. solani*). For this, NPs were suspended in 10 mL of potato dextrose agar (PDA) at three different concentrations (1000, 1500 and 2000 mg/L). After the PDA solidified, 5 µL of a spore suspension (1 x 10^6 CFU/mL) was inoculated at the center of the petri dish for each *Fusarium* specie. A control for each species of *Fusarium* was also prepared; the procedure was the same with the difference being a lack of NPs or fungicides. After preparation, the samples were incubated at 29 °C for 7 days. Each treatment was done by triplicate.

To confirm the obtained phases on the synthesized nanomaterials, X-ray diffraction (XRD) was obtained. OxZn XRD data was indexed with a mixture of Zincite (ZnO) with the JCPDS card 00-036-1451 and simonkolleite ($Zn_5(OH)_8Cl_2 \cdot H_2O$) with the JCPDS card 00-007-0155. On the other hand, OxZnCu sample was indexed with a mixture of the previously mentioned ZnO phase and zincian

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paratacamite ($(Cu,Zn)_2Cl(OH)_3$) with the JCPDS card 00-050-1558. Fig. 1a shows the particle shape and size of commercially acquired CuOH, there are long shaped groups of particles with sizes that varies from approximatively 50 nm up to 600 nm and some are agglomerated. OxZn NPs (Fig.1b) have irregular shaped and their size ranges from approximatively 20 to 250 nm. Finally, OxZnCu NPs are shown in Fig. 1c, these are the smallest NPs among all samples, they have a spherical shape with a range of size of 20 to 50 nm. The red circle signaled in Fig. 1c shows the ZnO phase that was also found on XRD analysis (this was confirmed by EDS analysis). OxZn and OxZnCu NPs showed a higher antifungal activity than organic fungicide fluodioxonil (Fig 2) on all *Fusarium* species. However, CuOH showed a higher antifungal activity on *F. fujikuroi* at 1500 and 2000 mg/L, and on *F. oxysporum* at 1500 mg/mL. *F. solani* is the specie that resisted the most on all antifungal evaluation, nonetheless, OxZn NPs had the highest antifungal activity. Overall, OxZn NPs had the highest antifungal activity on *F. oxysporum* and *F. solani* at the highest concentration (2000 mg/L) and while OxZnCu NPs showed some antifungal activity and had the lowest particle size, it was not as high as OxZn NPs on all cases. On the other hand, CuOH had a higher activity with *F. fujikuroiI* and *F. oxysporum* at 1500 and 2000 mg/L in some cases and the highest on *F. fujikuroi* showing that antifungal activity can depend on the species that is being treated and type of NPs that is being used.

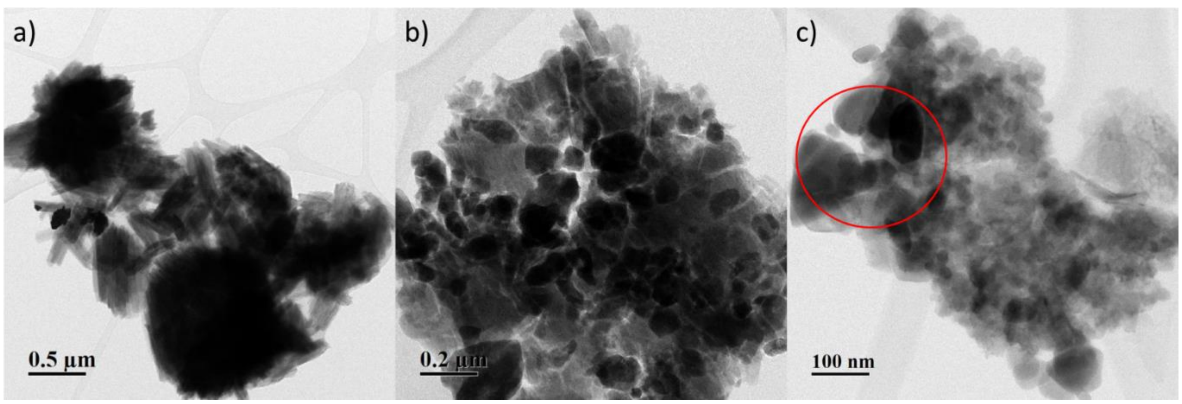

Figure 1. TEM micrographs of CuOH (a), OxZn (b) and OxZnCu (c).

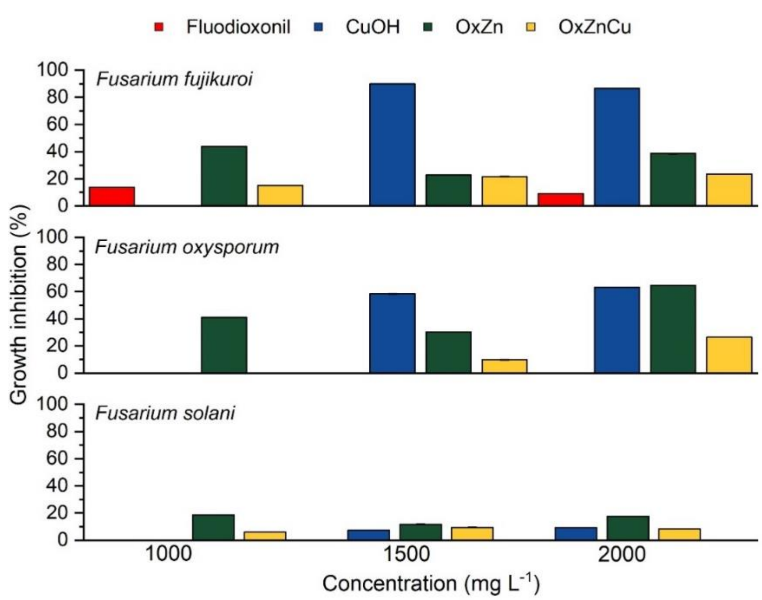

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Figure 2. Antifungal activity of OxZn, OxZnCu and commercial fungicides agains *F. fujikuroi*, *F. oxysporum* and *F. solani*.

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