doi:10.1017/S1431927622005566
Microsc. Microanal. 28 (Suppl 1), 2022

Antifungal Activity Changes of Zinc Oxide and Zinc Peroxide Nanoparticles

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Phytopathogenic fungi can cause significant economic losses on agricultural crops each year [1]. While there are many commercially available fungicides, there is evidence of the development of antifungal resistance among pathogens, this is why nanoparticles (NPs) are being studied more nowadays as alternative antifungal agents [2], [3]. Zinc oxides (ZnO and ZnO_2) are materials that are widely used in different industries due to their properties, such as their antimicrobial activity [4]. In this study, size and shape of commercially available ZnO NPs (ZnOc), synthesized plate-like ZnO NPs (ZnOp) and ZnO_2 NPs (ZnO_2) were analyzed by TEM and SAED. Their antifungal effect against phytopathogenic fungi was evaluated for three different *Fusarium* species by poisoned medium method.

The ZnOp and ZnO_2 NPs were synthesized by hydrothermal and colloidal method, respectively. And ZnOc was purchased from Sigma-Aldrich. ZnOp were synthesized by dissolving 1.09 g of zinc acetate and 0.84 g of NaOH, both in 15 ml of distilled water. Afterwards, 15 ml of ethanol was added by slow dripping while stirring. The resultant suspensions were transferred to a Teflon vial and treated at 180ºC for 18 h. In the case of ZnO_2, 1.316 g of zinc acetate that was dissolved in 120 mL of ethanol. The solution was stirred and heated at boiling point in a reflux system. After that, a solution of 1.1 g of $NaBH_4$ in 40 mL of ethanol was added and then 5 mL of H_2O_2 (30%) was added. Both types of NPs were washed with distilled water and dried at 80 °C for 8 h. Sample micrographs and SAED patterns were obtained on a Hitachi HT7700 TEM. Antifungal activity was evaluated on *F. fujikuroi*, *F. oxysporum* and *F. solani*. For this purpose, NPs were suspended at different concentrations in 10 mL of potato dextrose agar (PDA) (1000, 1500 and 2000 mg/L). Aside from the previously mentioned NPs (ZnOc, ZnOp and ZnO_2), fluodioxonil, a commonly used fungicide, was evaluated at the same concentrations. After the PDA medium solidified, 5 µL of a spore suspension of the fungi (1 x 10^6 CFU/mL) were inoculated at the center of the petri dish and were incubated at 29 °C for 7 days. A control was also tested the same with the absence of NPs or fungicides. Each test was done by triplicate.

Fig. 1 shows TEM micrographs and SAED patterns of ZnOc, ZnOp and ZnO_2 NPs. Fig. 1a shows ZnOc with diameter size between f 20 nm and 250 nm, The ED patterns, show they are monocrystals. For ZnOp (Fig. 1b) we can observe thin plate like ZnO NPs, with an approximate size of 200 to 400 nm and thickness of 60 nm, SAED patters show that these particles are crystalline. Finally, for ZnO_2 (Fig. 1c) there are small, agglomerated NPs with a size below 10 nm, the diffuse ring SAED patter shown, itis due to the polycrystalline nature of the agglomerates. These NPs showed a higher antifungal activity when compared to fluodioxonil (Fig. 2) on all *Fusarium* species, being *F. fujikuroi* (Fig. 2a) the most inhibited on all concentrations and *F. solani* (Fig.2c) the most resistant. ZnOc NPs showed the most antifungal activity of all Zn based oxides on *F. fujikuroi*. However, ZnOp NPs were most efficient against *F. solani* at 1000 and 1500 mg/L. In the case of ZnO_2, the NPs were most efficient on *F.*

oxysporum at 1500 and 2000 mg/L. These results show that ZnO and ZnO_2 NPs have a better effect than a commonly used organic fungicide. It's also evident that even though size was different among the different samples, they each inhibited a specific *Fusarium* specie more than the rest. This means that phase and synthesis method have an influence on the antifungal activity of ZnO and ZnO_2 NPs.

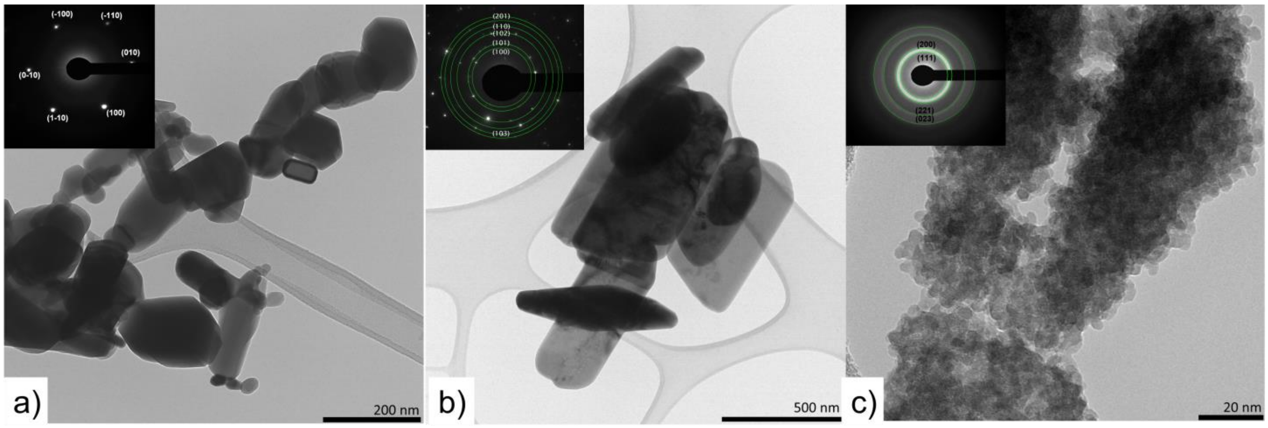

Figure 1. TEM micrographs and SAED patterns of (a) ZnOc, (b) ZnOp and (c) ZnO_2.

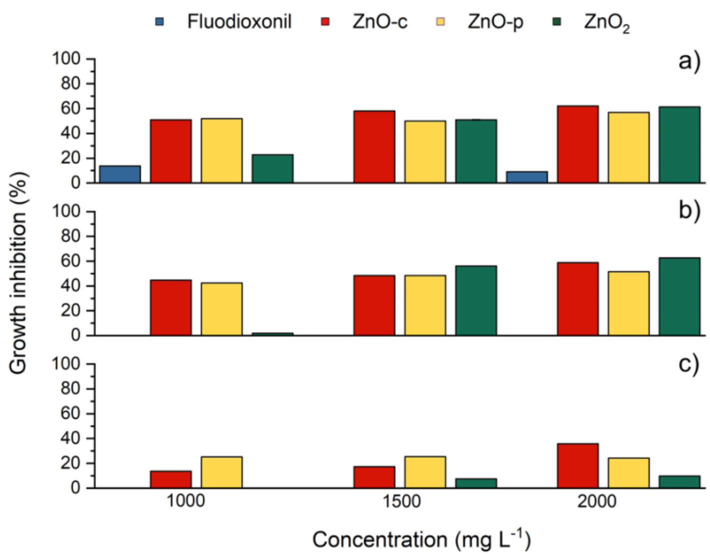

Figure 2. Antifungal effect of ZnOc, ZnOp and ZnO2 nanoparticles on (a) *F. fujikuroi*, (b) *F. oxysporum* y (c) *F. solani*.

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