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”Effect of CuO, ZnO, and TiO₂ Nanoparticles on Withanolide Production in *Withania somnifera*”

The vegetative phase (60 days) of *Withania somnifera* was assessed for the effects of CuO, ZnO, and TiO₂ nanoparticles on withanolide biosynthesis through HPLC quantification. Results indicated that nanoparticle exposure caused significant, dose-dependent alterations in withanolide content. Withaferin A accumulated the most in ZnO at 25 ppm (~7.5 mg/g DW), showing that it has a lot of potential to boost secondary metabolism. TiO₂ at 8 ppm was the best at increasing Withanolide A levels (about 2.8 mg/g DW), and moderate amounts of CuO (75 ppm) also increased withanolide levels compared to the control. Conversely, elevated concentrations of CuO and ZnO (100 ppm) inhibited withanolide synthesis. These results show that the type and amount of nanoparticles have different effects on the production of secondary metabolites. ZnO and TiO₂ were the best treatments for increasing withanolide yield during vegetative growth. This suggests that they could be useful in nanotechnology-based ways to improve the productivity of medicinal plants.

KEYWORDS: *Withania somnifera*; CuO, ZnO, TiO₂ nanoparticles; Withaferin A; Withanolide A; HPLC; Vegetative phase

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