

## ABSTRACT

The present study highlighted the scope of melatonin-mediated mitigation strategies of CuO nanoparticles (NPs) induced toxicity on the plant growth-promoting rhizobacterial (PGPR) species present in the soil. CuO NPs are used in multiple agrochemicals such as pesticides, fungicides, and fertilizers. Additionally, these are part of multiple electrical, pharma, and solar industries. Due to their strong antimicrobial properties, these nanoparticles might be toxic if they interact with soil microbial communities after entering into soil in either direct or indirect ways. Melatonin is one of the antioxidant molecules with a range of stress mitigation potential. It has the ROS chelation potential, which is a molecule responsible for cellular damage, lipid peroxidation, and oxidative stress of microbial cells. Herein, a culture-dependent study from the isolated PGPR species and their interaction with plants under stress conditions, as well as microcosm studies for nontargeted bacterial communities, was performed to observe the effect of melatonin on the toxicity of CuO NPs. Additionally, due to phytochemical properties, melatonin has been utilized for the development of safe CuO NPs also.

The isolation of PGPR species from the rhizosphere soil of marigold was performed to get a representative *Bacillus* species for the culture-dependent *in vitro* studies. The isolation was performed by serial dilution of pre-processed soil followed by pure culture. The systemic biochemical characterization and molecular characterization was performed which confirms the *Bacillus* species. The PGPR traits have been checked to confirm their agricultural significance. Finally, higher IAA producer species were selected for further studies.

The CuO NPs toxicity was confirmed by applying CuO NPs against the *Bacillus megaterium*. These NPs not only altered the morphology by damaging the cellular envelope through contact mode toxic mechanisms but were also able to reduce the IAA production in the range of 39-81% depending on factors like conc. and size, which is one of the key plant growth hormones responsible for the growth of the plants. The application of melatonin has been found to be useful in the mitigation of CuO NPs-mediated stress on the isolated PGPR *B. megaterium*. The application of the melatonin was also able to revert the *in vitro* IAA production of the bacterium, which was initially reduced due to the CuO NPs-induced stress.

*In planta* studies were conducted to observe the impact of NPs on the plant growth in the soil spiked with NPs. The soil was sterilized to remove any type of microbial dominance in the soil. The application of melatonin (50  $\mu$ M) and bacteria reduced the toxicity of CuO NPs, which

can be correlated by the root morphology, PI staining, and other plant physiological parameters like antioxidant enzymes and nutrient absorption. Along with that, the microcosm studies suggested that application of melatonin increased the microbial load at higher concentrations of CuO NPs-spiked soil.

To understand deeper, melatonin-doped NPs have been synthesized, and, it was found that the mixing of melatonin with CuO was more effective than the synthesized CuO NPs alone. At lower concentrations it has a less toxic effect on the *Bacillus* species. However, the particles have both antimicrobial and antifungal activities under *in vitro* conditions. While, during microcosm studies, it was observed that melatonin-doped CuO NPs show no toxic effect on the microbial communities.