

**IMPACT OF MELATONIN ON CuO NPs INDUCED STRESS
TOLERANCE AND PLANT GROWTH-PROMOTING
ACTIVITY OF RHIZOBACTERIA**

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APRIL, 2026

Impact of Melatonin on CuO NPs Induced Stress Tolerance and Plant Growth-Promoting Activity of Rhizobacteria

by

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Submitted

In fulfillment of the requirements for the degree of

Doctor of Philosophy

to the



Indian Institute of Technology Delhi
Hauz Khas, New Delhi-110016, India

April, 2026

CERTIFICATE

This is to certify that the thesis entitled "**Impact of melatonin on CuO NPs induced stress tolerance and plant growth-promoting activity of rhizobacteria**", being submitted by **Mr. Gyan Datta Tripathi**, to the **Indian Institute of Technology, Delhi**, for the award of **Doctor of Philosophy**, is a record of bonafide research work carried out by him. He has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis. To the best of my knowledge, the results contained in this thesis have not been submitted in part or in full to any other university or institute for the award of any degree or diploma.

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ACKNOWLEDGEMENTS

I sincerely express the Almighty God my heartfelt thanks for giving me the vitality and divine intervention to complete this work.

*First and foremost, I want to express my gratitude to my supervisor, **Prof. Kavya Dashora**, for her motivational talks, compassion, enthusiastic guidance, instruction, and mentoring. Throughout my PhD, she had an immense stake in my overall welfare, much like my family, and has been an excellent advisor outside of the academic realm.*

*I am grateful to all the faculty members of the Centre for Rural Development and Technology, IIT Delhi, for their help and encouragement while I finished this thesis, I want to express my gratitude to my SRC committee members, **Prof. Stayawati Sharma** (Chairperson, CRDT), **Prof. Anushree Malik** (Internal Expert, CRDT) and **Prof. Arun Kumar** (External Expert, Department of Civil Engg. IIT Delhi) for bringing me the outstanding support they offered, always prepared with insightful suggestions, constructive feedback, and sincere, valuable advice to enhance the quality of my research endeavors. I extend my heartfelt thanks to the laboratory personnel and administrative staff at **CRDT, IIT Delhi**, for their assistance and support concerning research and academic services, and resources.*

*I would like to profoundly appreciate my colleagues in the lab and seniors, **Dr. Mansi Mishra, Dr. Vinayak Fasake, Dr. Ayushi Srivastava, Dr. Ajay Yadav, Dr. Zoya Javed, Meghana Gattupalli, Mohona Munshi, Onkar Sarma, Shantanu Kumar, Mr Sagar and Mr. Chetan Kumar** for assisting me across this journey, constantly motivating me to keep a positive attitude and accomplish my objectives.*

*My deepest gratitude to my good friends **Mr. Kuldeep Singh, Mr. Adarsh Singh, Dr. Himanshu Arora, Dr. Gaurav** and my well-wisher **Wg Cdr. Rohit Srivastava and Dr. Anupam Singh** for their unwavering support and tenacious presence during my thesis journey*

*Above all, I cannot overstate the tremendous influence and contribution of my beloved parents, **Mr. Madan Gopal Tiwari and Mrs. Dhandhari**. Their frequent epistolary contact and unwavering encouragement got me through the hardest of times. Without their boundless love and motivation, this accomplishment would have remained an unrealized*

*dream, as this thesis attests. My sibling, **Mr. Anand Datta Tripathi**, deserves special recognition for their intense support, encouragement, and prayers, which helped me feel emotionally connected even though we were miles apart. I am thankful to have you all on this road with me since you have all given me so much in so many ways. Numerous more people have offered me support and assistance in different ways. I want to express my gratitude to each one of them and ask for their pardon for not naming them here.*

Gyan Datta Tripathi

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 the 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 μ 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.

सार

वर्तमान अध्ययन ने मिट्टी में मौजूद राइजोबैक्टीरियल (पीजीपीआर) प्रजातियों को बढ़ावा देने वाले पौधों के विकास पर कॉपर ऑक्साइड नैनो कणों प्रेरित विषाक्तता की मेलाटोनिन-मध्यस्थ शमन रणनीतियों के दायरे पर प्रकाश डाला। कॉपर ऑक्साइड नैनो कणों का उपयोग कई कृषि रसायनों जैसे कीटनाशकों, कवकनाशी और उर्वरकों में किया जाता है। इसके अतिरिक्त, ये कई इलेक्ट्रिकल, फार्मा और सौर उद्योगों का हिस्सा हैं। मजबूत रोगाणुरोधी गुणों के कारण, यह विषाक्त हो सकता है यदि वे मिट्टी में प्रत्यक्ष या अप्रत्यक्ष तरीकों से प्रवेश करने के बाद मिट्टी के माइक्रोबियल समुदायों के साथ संपर्क में आते हैं। मेलाटोनिन एंटीऑक्सीडेंट अणुओं में से एक है जिसमें तनाव शमन क्षमता की एक श्रृंखला है। इसमें आरओएस केलेशन क्षमता होती है, जो एक अणु है जो सेलुलर क्षति, लिपिड पेरोक्सीडेशन और माइक्रोबियल कोशिकाओं के ऑक्सीडेटिव तनाव के लिए जिम्मेदार है। इसमें, अलग-थलग पीजीपीआर प्रजातियों से संस्कृति पर निर्भर अध्ययन और तनाव की स्थिति में पौधों के साथ उनकी संपर्क के साथ-साथ गैर-लक्षित जीवाणु समुदाय के लिए सूक्ष्म जगत अध्ययन कॉपर ऑक्साइड नैनो कणों की विषाक्तता पर मेलाटोनिन के प्रभाव का निरीक्षण करने के लिए किया गया था। इसके अतिरिक्त, फाइटोकेमिकल गुणों के कारण मेलाटोनिन का उपयोग सुरक्षित कॉपर ऑक्साइड नैनो कणों के विकास के लिए भी किया गया है।

गेंदे की मिट्टी के राइजोस्फीयर मिट्टी से पीजीपीआर प्रजातियों का अलगाव संस्कृति के लिए एक प्रतिनिधि बैसिलस प्रजातियों को प्राप्त करने के लिए किया गया था, जो इन विट्रो अध्ययनों में निर्भर करता है। अलगाव पूर्व-प्रसंस्कृत मिट्टी के क्रमिक कमजोर पड़ने के बाद शुद्ध संस्कृति द्वारा किया गया था। प्रणालीगत जैव रासायनिक लक्षण वर्णन और आणविक लक्षण वर्णन किया गया था जो बैसिलस प्रजातियों की पुष्टि करता है। उनके कृषि महत्व की पुष्टि करने के लिए पीजीपीआर लक्षणों की जांच की गई है। अंत में, उच्च IAA उत्पादक प्रजातियों को आगे के अध्ययन के लिए चुना गया।

कॉपर ऑक्साइड नैनो-कणों विषाक्तता की पुष्टि बैसिलस मेगाटेरियम के परस्पर प्रभाव को अध्ययन करके की गई। इन नैनो-कणों को न केवल संपर्क मोड विषाक्त तंत्र के माध्यम से सेलुलर लिफाफे को नुकसान पहुंचाकर आकृति को बदल दिया गया था, बल्कि सांद्र और आकार जैसे कारकों के आधार पर आईएए उत्पादन को 39-81% की सीमा में कम करने में भी सक्षम थे, जो पौधों के विकास

के लिए जिम्मेदार प्रमुख पौधों के विकास हार्मोनों में से एक है। मेलाटोनिन के अनुप्रयोग को पृथक पीजीपीआर बी मेगाटेरियम पर कॉपर ऑक्साइड नैनो कणों की मध्यस्थता वाले तनाव को कम करने में उपयोगी पाया गया है। मेलाटोनिन का अनुप्रयोग जीवाणु द्वारा इन विट्रो IAA उत्पादन को वापस करने में भी सक्षम था, जो शुरू में CuO NPs प्रेरित तनाव के कारण कम हो गया था।

प्लांट में नैनो-कणों के साथ बढ़ी हुई मिट्टी में पौधों की वृद्धि पर नैनो-कणों के प्रभाव का निरीक्षण करने के लिए अध्ययन किए गए थे। मिट्टी में किसी भी प्रकार के माइक्रोबियल प्रभुत्व को हटाने के लिए मिट्टी को निष्फल किया गया था। मेलाटोनिन (50 माइक्रोमोलर) और बैक्टीरिया के अनुप्रयोग ने CuO नैनो-कणों की विषाक्तता को कम कर दिया, जिसे रूट मॉर्फोलॉजी, न्यूनतम प्रोपोडियम आयोडाइड अवशोषण और अन्य पौधों के शारीरिक मापदंडों जैसे एंटीऑक्सीडेंट एंजाइम और पोषक तत्व अवशोषण द्वारा सहसंबद्ध किया जा सकता है। इसके साथ ही, सूक्ष्म जगत के अध्ययनों ने सुझाव दिया कि मेलाटोनिन के अनुप्रयोग ने CuO नैनो-कणों नुकीली मिट्टी की उच्च सांद्रता पर माइक्रोबियल भार में वृद्धि की।

अधिक गहराई से समझने के लिए, मेलाटोनिन डोपड नैनो-कणों को संश्लेषित किया गया है और पाया गया है कि कॉपर के साथ मेलाटोनिन का मिश्रण अकेले संश्लेषित कॉपर ऑक्साइड नैनोकण की तुलना में अधिक प्रभावी था। कम सांद्रता पर इसका बेसिलस प्रजातियों पर कम विषाक्त प्रभाव पड़ता है। हालांकि, कणों में इन विट्रो स्थितियों के तहत रोगाणुरोधी और एंटीफंगल दोनों गतिविधियां होती हैं। जबकि, सूक्ष्म जगत अध्ययन के दौरान यह देखा गया था कि मेलाटोनिन डोपड कॉपर ऑक्साइड नैनोकण माइक्रोबियल समुदाय पर कोई विषाक्त प्रभाव नहीं दिखाता है।

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LIST OF ABBREVIATIONS

AFM	Atomic Force Microscopy
APX	Ascorbate Peroxidase
Blast	Basic Local Alignment Search Tool
CAS MEDIUM	Chrome Azurol S
CAT	Catalase
Chl a	Chlorophyll a
Chl b	Chlorophyll b
CHN	Carbon, Hydrogen, and Nitrogen
CLSI	Clinical and Laboratory Standards Institute
CLUSTAL W	Clustering Method for Weighted Multiple Sequence Alignment
CMC AGAR PLATE	Carboxymethyl Cellulose Agar.
Cu	Copper
Cu ₂ O	Cuprous Oxide
CuO	Copper oxide
DCFH-DA	2',7'- Dichlorodihydrofluorescein Diacetate
DI WATER	Deionized Water
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
FTIR	Fourier Transform Infrared Spectroscopy
H ₂ O ₂	Hydrogen Peroxide
HCL	Hydrochloric Acid
HClO ₄	Perchloric Acid
HCN	Hydrogen Cyanide
IAA	Indole-3-acetic acid
ICPMS	Inductively Coupled Plasma Mass Spectrometry
KI	Potassium Iodide
LB	Luria-Bertani
MBC	Minimal Bactericidal Concentration
MDA	Malondialdehyde
MEGA 6	Molecular Evolutionary Genetics Analysis version 6

Mel	Melatonin
MHA	Muller Hilton Agar
MIC	Minimum Inhibitory Concentration
NA	Nutrient Agar
Na ₂ CO ₃	Sodium Carbonate
NaOH	Sodium Hydroxide
NB	Nutrient Broth
NCBI	National Center for Biotechnology Information
NPs	Nanoparticles
NTA	Nanoparticle Tracking Analysis
O.D.	Optical Density
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PGP	Plant Growth-Promotion
PGPR	Plant Growth Promoting Rhizobacteria
pH	Potential of Hydrogen
PI	Propidium Iodide
POD	Peroxidase
ROS	Reactive Oxygen Species
rRNA	Ribosomal RNA
RT	Room Temperature
SEM	Scanning Electron Microscope
SOD	Superoxide Dismutase
SRL	Sisco Research Laboratories
TEM	Transmission Electron Microscopy
TTC	2,3,5-triphenyltetrazolium chloride
UV/VIS	Ultraviolet-Visible Spectroscopy
XRD	X-ray Diffraction

LIST OF SYMBOLS AND UNITS

°C	Degree Celsius
µg/ ml	Micrograms Per Milliliter
ml	Milliliter
Hrs.	Hours
RPM	Revolutions Per Minute
g/L	Gram Per Liter
%	Percentage
g/ml	Gram Per Milliliter
mg/ml	Milligram Per Milliliters
mg	Milligram
µM	Micromolar
µL	Microliter
mM	Millimolar
cm ⁻¹	Inverse Centimeters
mg/gm	Milligrams Per Gram
mg/kg	Milligrams Per Kilogram
w/v	Weight Per Volume
mM ⁻¹ cm ⁻¹	Per Millimeter Per Centimeter
nmol g ⁻¹	Nanomoles Per Gram
CFU ml ⁻¹	Colony Forming Units per milliliter
v/v	Volume by Volume
mg L ⁻¹	Milligrams Per Liter