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Using Copper-Based Nanomaterials To Improve Plant Development By Means Of Microbial Siderophores Generation In The Plant Rhizosphere And Consequent Effect On Iron Absorption

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Abstract: The unique physicochemical properties and interactions within the rhizosphere, copper-based nanomaterials (Cu-NMs) have been identified as efficient agents for boosting plant growth and improving nutrient absorption. This study investigates how Cu-NMs affect microbial activity in the plant rhizosphere, with a focus on how they encourage the synthesis of siderophores by helpful soil microbes. Compounds known as siderophores have a strong affinity for iron and help to solubilize and absorb this essential nutrient, which is essential for plant growth. According to our findings, when Cu-NMs are sprayed at the right quantities, they promote the development of microorganisms that produce siderophores, which raises the amount of iron available in the rhizosphere. Improvements in biomass accumulation, root growth, and chlorophyll levels were linked to the increased iron intake. This study presents a sustainable, nanotechnology-driven approach to increasing crop yield through microbiome-mediated nutrient uptake and provides fresh insights into the indirect channels through which Cu-NMs can improve plant nutrition. Flacourtia montana leaf extract was used in an environmentally benign reaction to produce copper nanoparticles. The ability of bacterial colonies to produce siderophores is demonstrated by the creation of yellow zones. By using a visual observation method, we can identify bacteria that improve the absorption of iron by plants. Evaluation of Cu- NMs-induced siderophore synthesis was made possible by the bacterial growth patterns and medium color changes seen during the study. The Debye-Scherrer equation analysis's 42.28 nm crystallite size result was consistent with the SEM results. The (111), (200), and (220) peaks in the X-ray diffraction investigation showed that the Cu_2O nanoparticles have crystalline characteristics. Our scientific studies verified that significant increases in iron availability were brought about by copper nanoparticles and their microbial siderophore enhancing capabilities in rhizosphere circumstances.

Keywords: Copper-based nanoparticles (Cu-NMS), Siderophore production, Rhizosphere, PGPR (Plant Growth- Promoting Rhizobacteria), Antimicrobial properties

I. INTRODUCTION

Plant health reaches better levels after interactions with Plant Growth-Promoting Rhizobacteria (PGPR) because these bacteria establish their presence in rhizospheres while improving plant nutrient availability [3,4,5,8,10]. Siderophores produced by these microorganisms bind soil iron to form plant-accessible compounds, which enhance plant growth and resistance by supporting metabolic processes, photosynthesis, and enzyme function [1]. The availability of iron in most soils proves challenging for standard plant growth because the necessary levels remain restricted [9]. Current developments in nanotechnology utilize copper metal oxide nanoparticles to improve both plant microbial processes and nutrient absorption potential[2,13,15].

Research has shown that copper metal oxide nanoparticles have a unique set of chemical properties which includes large surface area and catalytic activity together with antimicrobial effects [2,6,15]. Copper metal oxide nanoparticles exist in numerous fields including environmental remediation while showing applications in biomedical research and sensing technologies and agricultural settings [14,15]. Plant-based research demonstrates how copper metal oxide nanoparticles may influence rhizosphere PGPR interactions to increase microbial siderophore production which enhances plant iron absorption [1,2]. Antifungal and antibacterial characteristics of copper metal oxide nanoparticles help to create a better microbial habitat and defend plants from harmful diseases [2,6,12]. Novel sustainable agricultural solutions are produced by means of PGPR-based techniques integrating copper metal oxide nanoparticles [2,3,4,5]. When iron deficiency exists, these remedies assist in lower reliance on chemical fertilizers and concurrently increase productivity of crops and stress tolerance [1,3,4].

Nanotechnology in Agriculture

Nanotechnology innovation has brought positive changes to agriculture through advanced methods that raise farming productivity and boost nutrient efficiency as well as sustainable farming practices [2,13,15]. Researchers develop better versions of fertilizers pesticides and soil conditioners by using metal oxide nanoparticles at the nanoscale for addressing major agricultural problems [2,13,15]. Studies prove that nanoparticles prepared from copper oxide demonstrate better seed germination and stronger growth of shoots and roots compared to conventional iron-based treatments. Copper nanoparticles demonstrate outstanding antimicrobial characteristics which protect crops from major diseases like bacterial blight [2,15].

Identifying Siderophores Production Soil Microorganism

Biological tests for siderophore-producing microorganisms were started by extracting bacterial isolates through EDTA extraction [1]. We used bacterial isolates and incubated them on the specialized Chrome Azurol Sulphonate (CAS) agar medium for siderophore detection purposes. CAS agar has an iron-dye complex which displays color transformations because of siderophore release. The incubation process at room temperature between 24-48 hours revealed bacterial colonies creating yellow zones as evidence of their siderophore production capability. A visual observation method provides us with the ability to detect microorganisms that enhance plant iron absorption through this approach [1].

Induction of Siderophore Production in Soil Microorganism by Copper Nanoparticles

The conditions for measuring CuO NP effects on siderophore production consisted of bacteria samples with an optical density matching OD 600 nm = 0.1 (containing approximately 10^8 CFU/mL)[1].

We poured the bacterial cultures into Siderophore Induction Medium (SIM) containing CuO NPs before running time-based incubation [2,15]. The bacterial responses to CuO NPs were investigated because these nanoparticles might initiate defense processes or support siderophore enzyme activity [6,10]. Scientific research shows that microbes produce more effectively with lower doses of metal nanoparticles [2,15]. The bacterial growth patterns and medium color changes observed during the research allowed assessment of CuO NPs induced siderophore production [1,2].

Evaluation of the Production of Siderophores between Natural and Cu-treated Nanoparticle Soil Microorganism

The Chrome Azurol S (CAS) method served as the basis for measuring siderophore production between untreated bacterial strains and those exposed to CuO NP [1]. This method shows siderophore activity through a transformation of blue CAS solution to orange/yellow color, and the observed intensification of color indicates growing siderophore concentration levels [1,6]. After CuO NP treatment, bacterial cultures produced dramatically elevated levels of siderophores in comparison to untreated control samples which shows nanoparticles enhance the microorganisms' iron retrieval ability [6,15]. The research demonstrates that CuO NPs represent a sustainable agricultural tool capable of improving plant nutrient acquisition, specifically in environments with iron deficiency [2,15].

II. Results and Discussion

Synthesis and Characterization of Cu nano-particle

The Production of copper nanoparticles occurred through an environmentally friendly reaction using *Flacourtia montana* leaf extract to reduce and stabilize natural components [2]. The leaves underwent preparation before cleaning and subsequent drying until they became powdered. We filtered an extract while boiling the powdered substance in distilled water at 80°C for 30 minutes. 1 mL of Benedict's solution was mixed with an equal volume *of F. montana* leaf extract and heated at 100°C for 10 minutes to initiate the reaction as shown in the fig.1. During the synthesis reaction, the copper reduction into CuO nanoparticles resulted in the simultaneous development of dark reddish-brown color together with green color transformation. We performed the analysis resulting in a fine nanoparticle powder that was isolated through centrifugation at 10,000 rpm and then cleaned repeatedly with ethanol and distilled water. The result of 42.28 nm crystallite size from Debye-Scherrer equation analysis aligned with SEM findings [15]as shown in the fig.2 **.**The synthesis purity of nanoparticles was evaluated through Energy- Dispersive X-ray Spectroscopy (EDX) to show clear results during its elemental analysis by detecting a Cu peak [2,15]. Plants constitute an effective synthesis method that produces high-quality Cu_2O nanoparticles at low prices for environmental and biomedical applications [2].Different analytical techniques served to study the synthesized Cu-NPs. SEM analysis revealed spherical nanoparticles distributed well across the surface through which we conducted the study [2,13,15]. The X-ray Diffraction analysis revealed crystalline properties for Cu_2O nanoparticles through the examination of (111), (200), and (220) peaks which corresponded to the JCPDS file No. 05-0667 [2,15]as shown in fig.3. Results obtained from the Debye-Scherrer equation analysis verified the SEM results with a crystallite size of 42.28 nm [15]. Chemical identification through Energy-Dispersive X-ray Spectroscopy (EDX) proved that the nanoparticle production method was pure since it displayed a distinct peak containing the element copper in its analysis [2,15]. The synthesis procedure employing plants produces effective, high-quality Cu_2O nanoparticles which offer both sustainability and economical production properties for biomedical and environmental purposes [2].

Table.1 Identifying Siderophores Production Soil Microorganism

Morphological and cultural characteristics					
Bacteria Isolates	**Colony on nutrient agar**	**Shap e**	**Arrangem ent**	**Grams reaction**	**Endospo re**
BI-1	r regular, undulate creamish dull, with Round with entire margin	Rod	gl e and in chains	Gram (+)	Present
BI-2	r regular, undulate creamish dull, with Round with entire margin	Rod	gl e and in chains	Gram (-)	Absent
BI-3	rregular, undulate creamish dull, with Round with entire margin	Rod	gl e and in chains	Gram (+)	Present
BI-4	rregular, undulate creamish dull, with Round with entire margin	Rod	gl e and in chains	Gram (-)	Absent
BI-5	rregular, undulate creamish dull, with Round with entire margin	Rod	gl e and in chains	Gram (+)	Present

Fig.1-5 Represents Synthesis and Characterization of Nanoparticle

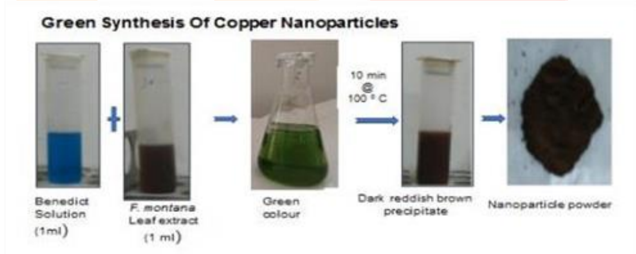
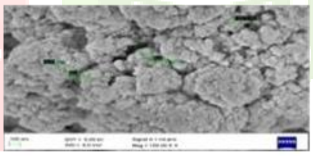
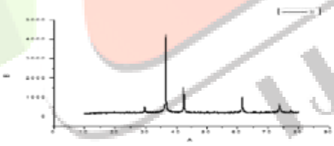


Fig Green synthesis of copper nanoparticle from plant extract





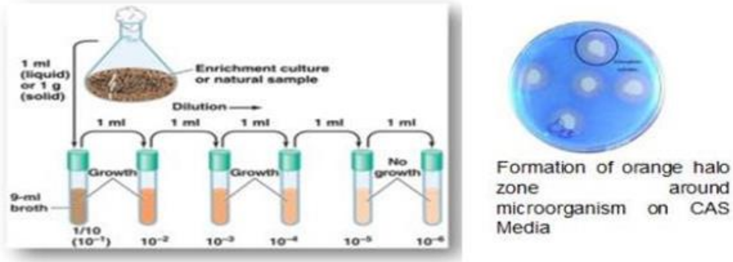


Fig: Serial Dilution of Soilsample and plating dilution on CAS Agar Plate

1. Soil samples from different sites of the same field were mixed to make uniform composite soil.
2. Incubate the flask on a shaker at RT for 6-7 hours.
3. 1ml from the incubated medium and perform serial dilutions up to 10-4, 10-5, and 10-6.
4. Diluted solution inoculated on Chrome Azurol sulphonate (CAS) agar
5. Incubate the plates at 37°C for 48 hours.
6. Observed for zones of yellow coloration around the bacterial colonies
7. Colonies were used for identification and further studies.

$$PSU = \frac{(A_r - A_s) \times 100}{A_r}$$

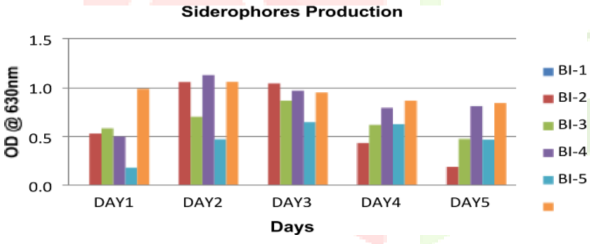
Quantitative Estimation of the Siderophores

The determination of bacterial siderophore amounts used LB broth to grow microbes, while maintaining an uninoculated broth solution for the control [1]. After undergoing incubation at 28°C for 48 hours, the cultures underwent centrifugation at 10,000 rpm for 10 minutes until cell-free supernatant was collected [1]. We measured the absorbance at 620 nm using Chrome Azurol S (CAS) reagent after combining the supernatant with this solution to determine siderophore production levels [1]. The calculation of percent siderophore units (PSU) relied on the following mathematical expression to represent the amount of produced siderophores by bacterial strains (Payne 1993) [1] -

where A_r is used to represent the reference absorbance from CAS solution containing uninoculated broth and A_s stands for absorbance from the sample solution made from cell-free supernatant [1]. A spectrophotometric analysis checked siderophore production at 630 nm wavelength throughout five consecutive days at 24-hour intervals [1]. During the initial range of days 2–3, siderophore concentration showed upward movement but it started to decrease after that period [1].

The impact of Nanoparticle on siderophores production

To see the impact of copperoxide nanoparticles on siderophore production, we added 10^8 CFU/mL (approx. OD 600 nm = 0.1) bacterial cultures into LB broth medium that contained nanoparticles [1,2]. The experiment used plan broth without bacteria as its basic reference point [1]. The cultured bacteria underwent 28-hour incubation before 10,000 rpm centrifugation needed for the separation of supernatant from cells [1]. The supernatant mixture of CAS reagent showed siderophore production levels in PSU values [1]. The PSU values came from standard Payne (1993) calculation methods, so we could assess siderophore production changes from nanoparticles [1].



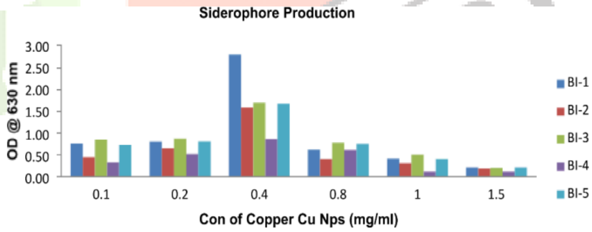


Fig.5. Siderphore production *Fig.6. Siderphore production*

The production of copper nanoparticles through *F. montana* leaf extract followed an economical and environmentally friendly synthesis method [2]. Multiple analytical tests confirmed the size of nanoparticles 42.28 nm [2,15]. Scientific tests applied CuO NPs after their synthesis successfully generated the maximum bacterial growth inhibition results using siderophores [2]. The maximal growth rate appeared at the 0.8 mg/mL concentration of CuO NP, but further elevations in the concentration caused bacterial growth to diminish [2,15]. Future research can investigate the total effects of using CuO NPs in plant systems to determine both their agricultural opportunities and their associated hazards [2,15].

III. Conclusion

A 1:1 mixture of crude Opuntia extract combined with polylactic acid successfully produced a biopolymer-based metal coating that increased iron plate corrosion resistance through weight loss reduction by 2-3%. The capabilities of siderophores and nanoparticles for improving fertilizers and antimicrobial functions form a sustainable agricultural framework using biopolymer-based metallic coatings as protective measures. Plants that easily absorb siderophore-metal complexes improve their iron uptake. Therefore, farmers can replace chemical pesticides and synthetic fertilizers with better agricultural outputs. Our scientific investigations confirmed that copper nanomaterials together with their microbial siderophore enhancement properties in rhizospheric conditions resulted in notable improvements in iron availability. CuO NPs derived from the vegetable leaf extract acted as a highly effective method for increasing siderophore levels from bacteria and increased their ability to mobilize iron better than unmodified bacterial cultures.

The methodology section outline the plan and method that how the study is conducted. This includes Universe of the

. Beta is the measure of systematic risk and has alinear relationship with return (Horn, 1993). High risk is associated with high return (Basu, 1977, Reiganum, 1981 and Gibbons, 1982). Fama and MacBeth (1973) suggested the existence of a significant linear positive relation between realized return and systematic risk as measured by β. But on the other side some empirical results showed that high risk is not associated with high return (Michailidis et al. 2006, Hanif, 2009). Mollah and Jamil (2003) suggested thatrisk-return relationship is notlinear perhaps due to high volatility.

3.4Statistical tools and econometric models

This section elaborates the proper statistical/econometric/financial models which are being used to forward the study from data towards inferences. The detail of methodology is given as follows.

3.4.1 Descriptive Statistics

Descriptive Statics has been used to find the maximum, minimum, standard deviation, mean and normally distribution of the data of all the variables of the study. Normal distribution of data shows the sensitivity of the variables towards the periodic changes and speculation. When the data is not normally distributed it means that the data is sensitive towards periodic changes and speculations which create the chances of arbitrage and the investors have the chance to earn above the normal profit. But the assumption of the APT is that there should not be arbitrage in the market and the investors can earn only normal profit. Jarque bera test is used to test the normality of data.

3.4.2 Fama-Mcbeth two pass regression

After the test statistics the methodology is following the next step in order to test the asset pricing models. When testing asset pricing models related to risk premium on asset to their betas, the primary question of interest is whether the beta risk of particular factor is priced. Fama and McBeth(1973)develop a two pass methodology in which the beta of each asset with respect to a factor is estimated in a first pass time series regression and estimated betas are then used in second pass cross sectional regression to estimate the risk premium of the factor. According to Blum (1968) testing two-parameter models immediately presents an unavoidable errors-in-the variables problem.It is important to note that portfolios (rather than individual assets) are used for the reason of making the analysis statistically feasible.Fama McBeth regression is used to attenuate the problem of errors-in-variables (EIV) for two parameter models (Campbell, Lo and MacKinlay, 1997).If the errors are in the β (beta)of individual security are not perfectly positively correlated, the β of portfolios can be much more precise estimates of the true β (Blum, 1968).

The study follow Fama and McBeth two pass regressionto test these asset pricing models.The Durbin Watson is used to check serial correlation and measures the linear association between adjacent residuals from a regression model. If there is no serial correlation, the DW statistic will be around 2. The DW statistic will fall if there is positive serial correlation (in worst case, it will be near zero). If there is a negative correlation, thestatistic will lie somewhere between 2 and 4. Usually the limit for non-serial correlation is considered to be DW is from 1.8 to 2.2. A very strong positive serial correlation is considered at DW lower than 1.5 (Richardson and smith, 1993).

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