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THE IMPACT OF SYNTHETIC METAL OXIDE NANOMATERIALS ON XANTHOMONAS CITRI GROWTH

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ABSTRACT: Xanthomonas citri is a hardy pathogen that causes significant crop damage in citrus lemons, a valuable commercial crop. The study's goal is to determine the efficacy of synthetic metal oxide nanoparticles in removing X. citri. The fungus X. citri, which causes citrus canker, was isolated from infected citrus lemon fruits. Titanium dioxide (TiO2), copper oxide (CuO), zinc oxide (ZnO), and FeO nanoparticles were tested for their ability to prevent the growth of isolated X. citri. Unlike TiO2, FeO, and CuO, which did not suppress X. citri growth, ZnO showed a definite zone of inhibition against the bacterium. The inhibitory effect of ZnO was validated by measuring the optical density of X. citri's growth curve. Zinc oxide slowed the progression of X. citri toward the logarithmic portion of its growth curve. The findings of this study will contribute to existing information about the usage of nanomaterials in agriculture. The findings will help farmers who are struggling to eradicate X. citri, a disease that affects citrus lemons, a crop with significant economic value.

Key words: Citrus canker, Citrus lemon, Synthetic metal oxide Nanomaterials, Xanthomonas citri.

1.INTRODUCTION

According to reports, antibiotic resistance makes it difficult to control the infection of Xanthomonas citri, a Gram-negative rod-shaped bacillus. This bacteria is a major pathogen that causes canker disease in citrus plants. The disease is distinguished by the development of canker lesions on the fruits, stem, and foliage. Farmers face huge financial losses as a result of canker disease, which causes a scarcity or absence of fruit output.

Copper is an essential mineral for the nutrition and overall health of plants. Copper nanoparticles are a well-known antibacterial agent. Synthetic metal oxide nanoparticles have a significant inhibitory effect on the multiplication of dangerous microbes. Copper oxides can eliminate bacteria through contact killing. The popularity of copper oxide nanomaterials is expanding as microbes become more resistant to antibiotics. Copper can destroy methicillin-resistant Staphylococcus aureus, a superbug, with pure copper having the strongest antibacterial capabilities. Scientists are looking at the antibacterial and anticancer effects of copper oxide.

The presence of iron (Fe) nanoparticles can have either positive or negative impacts on bacterial growth and development, depending on their concentration. A research group at the National Institute of Technology in Rourkela created iron oxide nanoparticles (IONP) with diameters ranging from 10 to 20 nm. The IONPs were encased in chitosan molecules with a positive charge, producing a negative surface potential (n-IONP) akin to magnetite. The particles were found to effectively inhibit the growth of Bacillus subtilis and Escherichia coli. This may be connected to the chitosan molecule's ability

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to reverse the positive surface potential (IONP) and increase the formation of reactive oxygen species (ROS).

At the nanoscale, zinc oxide (ZnO) demonstrates significant antibacterial activity. Nanosized ZnO can enter a cell and interact with the surface and/or center of bacteria, thereby preventing several bactericidal actions.Zinc oxide nanoparticles, also known as ZnO-NP, exhibit antibacterial activity against carbapenem-resistant Acinetobacter baumannii. Zinc oxide is thought to produce reactive oxygen species. This can accelerate the process of membrane lipid peroxidation, causing the release of reducing sugars, DNA, and proteins from the membrane and, eventually, cell death. A study conducted by the Sanford School of Medicine in the United States discovered that the antibacterial activity of ZnO nanoparticles rises as particle size decreases.

Titanium dioxide (TiO2) is regarded as a safe antibacterial agent due to its several benefits, including non-toxicity, chemical stability, photocatalytic characteristics, and cost. Multiple studies have proven that this metal oxide has strong antibacterial capabilities against a variety of Gram-positive and Gram-negative pathogens. Titanium dioxide nanoparticles (TiO2 NPs) were created and demonstrated considerable improvements in their properties. Modified TiO2 with decreased graphene oxide has been shown to have antibacterial characteristics.

The current study uses metallic oxide nanoparticles as a therapeutic intervention to combat the citrus canker bacterial disease in citrus lemons. The research will lead to the identification of safe metal oxide nanoparticles with antibacterial properties suitable for agricultural crop protection. The findings could have ramifications for future study and the adoption of efforts to eliminate additional ailments.

2.EXPERIMENTAL

PlantMaterial

Diseased plant specimens were obtained from the villages of Mittamedapalli and Vellatur in the Kadapa district. Examination of afflicted citrus fruits revealed the presence of the bacterium X. citri, which causes citrus canker disease. Individual organisms were kept alive during the studies using HiMedia's nutritional agar (NA). The antibacterial properties of metal oxide nanomaterials, specifically copper oxide (CuO) nanoparticles, FeO, ZnO, and TiO2, were examined using agar diffusion to determine their efficiency against various infections. Copper nitrate and copper chloride were used in the manufacture of copper oxide. ZnO nanoparticles were synthesized using sodium chloride and zinc nitrate hexahydrate. The ferrous chloride precipitation procedure involved the use of isobutanol and ammonium hydroxide to produce iron oxide nanoparticles. The antibacterial effectiveness of produced metal and metal oxide nanoparticles against X. citri was evaluated.

IsolationofPlantPathogens

Infected plant material was collected in the fields after identifying sickness symptoms. Twigs and leaves from infected citrus fruits were collected to extract X. citri. To eradicate surface bacteria, the affected plant portions were first cleaned twice with distilled water before being treated with a 0.1% mercuric chloride solution. To

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isolate bacteria, modest amounts of infected plant material were equally spread over Nutrient Agar. The NA medium plates were incubated at 39°C. Following a 24-hour interval, the plates were examined for signs of bacterial multiplication, and the bacterial cultures were then subcultured. Microscopic examination revealed that the subcultured bacteria were indeed pure cultures. The isolated pure culture of bacteria was used for further investigation.

MicroscopicObservationofBacteria

To study bacterial growth under a light microscope, the Gram staining technique was used, with crystal violet as the primary stain and safranin as the counter stain. The germs were studied with objective lenses at 45× and 100× magnifications.

BiochemicalTests

A variety of biochemical studies were carried out, including the methyl red and Voges-Proskauer tests, the oxidase test, the nitrate reduction test, the KOH test, the H2O2 test, the starch hydrolysis test, the Simmons citrate test, and the gelatin liquefaction test.

Scanning Electron Microscopic (SEM) Observation of Bacteria

The aseptic bacteria colony was taken from the inoculation chamber, evenly dispersed on a glass slide, and then dried in ambient air before being examined under a scanning electron microscope.

Inhibitory Effect of Synthetic Metal Oxide Nanomaterials

The study used the X. citri growth curve optical density measurement method and the agar diffusion method to investigate the inhibitory effects of metal oxide nanoparticles on bacterial growth.

Preparation of Nanomaterials for Testing Antimicrobial

Characteristics Test tubes containing varying levels of nanomaterials, especially 25 mg, 50 mg, 75 mg, and 100 mg, were used to produce various amounts of TiO2, CuO, ZnO, and FeO. The samples were freeze-dried in 1000 µl distilled water and labeled accordingly. The antimicrobial test was carried out using recently prepared test solutions.

Bacterial Growth Inhibition by Agar Diffusion Method

The antibacterial properties of metals and metal oxides were investigated utilizing the Agar Diffusion Method. A newly created bacterial culture in nutrient-rich broth with a 100 microliter inoculum was cultured for 18-24 hours. The culture was then equally distributed on 100 mm Petri plates coated with Nutrient Agar (NA). A cup borer was used to perforate agar material, resulting in 6 mm diameter wells. Using a micropipette, exact amounts of ten and twenty microliters of each nanomaterial—TiO2, CuO, ZnO, and FeO—were put into the wells. The nanomaterials were synthesized in various quantities, including 25 micrograms per milliliter, 50 micrograms per milliliter, 75 micrograms per milliliter, and 100 micrograms per milliliter. After sealing the plates, they were incubated at 37°C for 24 hours. Following that, the growth inhibition zones were identified and quantified in millimeters. The experiment was repeated three times in sterile conditions, and the average diameter of the inhibitory zone was calculated.

BacterialGrowthInhibitionbyOpticalDensityMeasurements

To test the suppression of bacterial proliferation, 100 µl of freshly cultivated bacterial cultures (18-24 hours) were

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added to 10 ml of nutrient broth. TiO2, CuO, ZnO, and FeO nanomaterials were mixed in 500 µl at a concentration of 100 µg/mL. The combination was then placed in an incubator set to 37°C for 24 hours. The changes in bacterial growth were measured by quantifying the optical density at a wavelength of 600.

3.RESULTSANDDISCUSSION

IsolationofPlantPathogens

Citrus canker disease was found on both the leaves and fruits of the plants in the field. Fruits and leaves that showed symptoms of infection were collected to isolate the pathogen (Figure 1).

X. citripure culture

After developing bacteria in isolation on NA, colonies with a full edge and a yellow hue were chosen for subculture on fresh NA-containing plates. Individual colonies were evaluated on the plates, and to ensure the preservation of a homogeneous culture, a single colony was chosen and placed in fresh NA media and broth.

MicroscopicObservationofBacteria

A sample of X. citri was taken from a subcultured plate and placed on a clean glass slide for examination under a microscope. Then, Gram staining was done. Figure 2 shows the observation of individual bacterium with a pink tint and a rod-like shape. The absence of additional bacterial development verified the presence of a pure X. citri culture, as evidenced by the appearance of pink-colored rod-shaped germs.

Biochemical Tests for X. citri

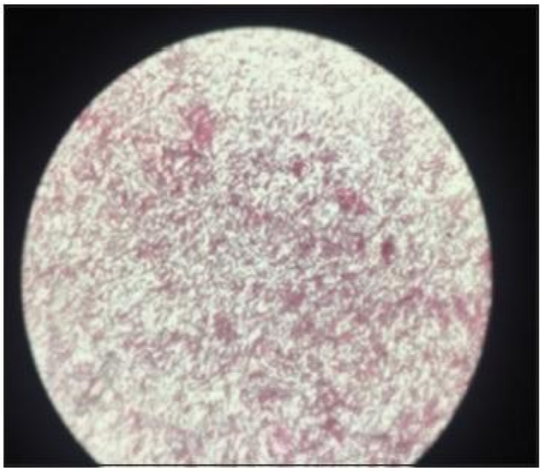
Biochemical assays were carried out on pure X. citri cultures. The gelatin liquefaction, oxidase, and nitrate reduction tests were all negative, however the KOH, H2O2, starch hydrolysis, Simmons citrate, MR-VP, and other tests provided positive results (Table 1).



Figure 1: Citrus canker disease on lemon fruit.

Entry	Biochemical test	Result
1	KOH	+
2	H^2O^2 test	+
3	Starch hydrolysis test	+
4	Simmons citrate test	+
5	MR-VP test	+
6	Gelatin liquefaction test	+
7	Oxidase test	-
8	Nitrate reduction test	-

Table 1: Biochemical tests for Xanthomonas citri



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Figure 2: Pink-colored rod-shaped bacteria – Xanthomonascitri under (100×).

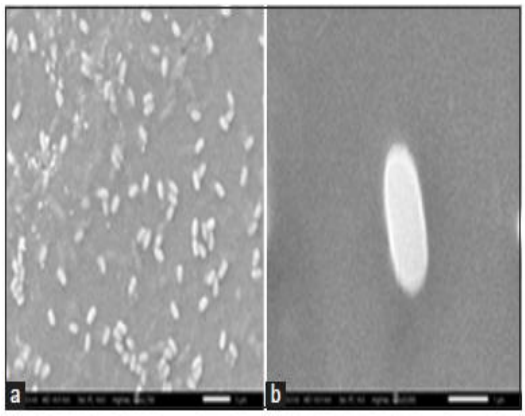


Figure 3: (a) Xanthomonas citri under SEM. (b) Xanthomonascitri single bacillus.

SEM observation of bacteria

Prior to scanning electron microscopy (SEM) investigation, the X. citri colony was carefully removed from the inoculation chamber, spread out on a glass slide, and air dried. Figure 3 depicts the bacteria's cylindrical shape, measuring 1 µm in diameter and 2 µm in length.

Inhibitory Effect of Synthetic Metal Oxide Nanomaterials

The researchers investigated how nanoparticles of TiO2, CuO, ZnO, and FeO impeded the development of X. citri when grown in isolation. The wells were filled with ten and twenty microliters of each nanomaterial (TiO2, CuO, ZnO, and FeO) using a micropipette. The nanomaterial concentrations were 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL respectively. After sealing the plates, they were placed in an incubator set to 37°C for 24 hours. Afterward, the millimeter-sized growth inhibition zones were visible. Under aseptic circumstances, the experiment was performed three times to establish the average inhibitory zone diameter (Table 2). Figure 4b shows a clear zone of growth suppression, demonstrating that only ZnO had significant antibacterial activity against X. citri, as opposed to the control (Figure 4a). These data show that ZnO efficiently suppresses the growth of both Gram-positive and Gram-negative bacteria. The study demonstrated that ZnO had high antibacterial capabilities. These findings are astounding when one considers Xanthomonas' tenacity. The investigated nanomaterials TiO2, CuO, and FeO did not limit Xanthomonas bacterial growth (Figure 4c–e). To restrict the spread of X. citri, we will examine the use of ZnO nanoparticles.

Bacterial Growth Inhibition by Optical Density Measurements

To test the effect of nanomaterials CuO and ZnO on bacterial growth, 100 µl of freshly cultivated cultures were combined with 500 µl of each material in a nutrient broth solution containing 10 µg/mL. Following that, the solution was incubated in an incubator set to 37°C for 24 hours. As a control, 100 microliters of freshly cultivated bacterial cultures were mixed into a solution containing 10 milliliters of nutrient-rich broth. Starting with 600, OD measurements were taken every 2 hours for a total of 24 hours. Xanthomonas growth was slowed in ZnO-containing cultures compared to controls and CuO-mixed cultures. Only cells treated with CuO or left alone throughout the logarithmic and stationary phases revealed evidence of Xanthomonas growth. Zinc oxide (ZnO) inhibited Xanthomonas cell development, preventing the bacteria from reaching the logarithmic phase of their growth curve (Figure 5). These results indicate that ZnO inhibits X. citri cell proliferation. Xanthomonas cultures

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treated with CuO went through a logarithmic growth phase that lasted between 2 and 10 hours, followed by a stationary phase that lasted 10 to 24 hours. In contrast, there was no apparent growth in the Xanthomonas culture treated with ZnO for 2–24 hours. After 2-10 hours of logarithmic growth, the Xanthomonas bacteria reached a stationary phase lasting 10-24 hours.

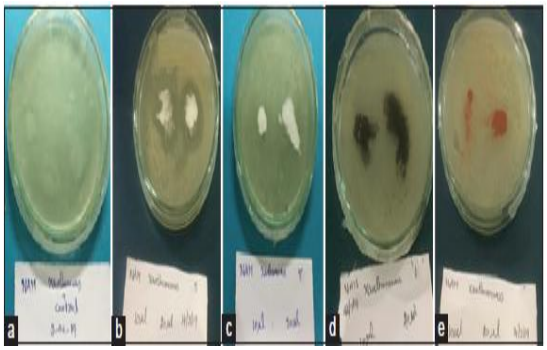


Figure 4: (a) Control: Xanthomonas growth on nutrient agar. (b) Effect of ZnO (100 µg/mL) on Xanthomonas growth (zone of growth inhibition 2.5 and 3 cm). (c) Effect of titanium dioxide (100 µg/mL) on Xanthomonas growth (no zone of growth inhibition). (d) Effect of CuO (100 µg/mL) on Xanthomonas growth (no zone of growth inhibition). (e) Effect of FeO (100 µg/mL) on Xanthomonas growth (no zone of growth inhibition)

Nanomaterials	Conc. µg/mL	Zone of growth inhibition	Conc. µg/mL	Zone of growth inhibition
TiO_2	25	0	50	0
FeO	25	0	50	0
ZnO	25	2±0.57	50	5±1.52
CuO	25	0	50	0
TiO_2	75	0	100	0
FeO	75	0	100	0
ZnO	75	20±2	100	30±3
CuO	75	0	100	0

Table 2: Effect of nanomaterials on Xanthomonas

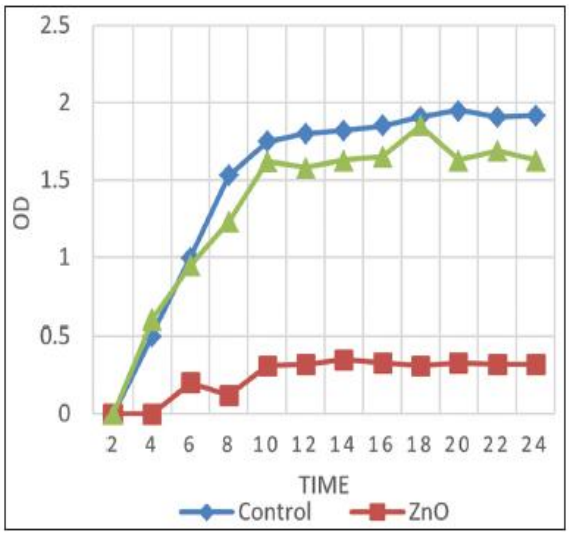


Figure 5: Xanthomonas growth curve.

4.CONCLUSION

The experimental control is Xanthomonas growth on nutrient agar. (b) Zinc oxide (ZnO) at 100 µg/mL inhibited Xanthomonas growth, resulting in 2.5-3 cm zones. (c) Despite being exposed to 100 µg/mL of titanium dioxide, Xanthomonas continued to develop without inhibition. (d) CuO at 100 µg/mL did not reduce Xanthomonas growth. FeO at 100 µg/mL had no effect on the proliferation of Xanthomonas bacteria.

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