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BIOLOGICAL EFFICACY OF MWCNTS-ZNO NANOCOMPOSITES AGAINST RALSTONIA SOLANACEARUM AND FUSARIUM SOLANI: A NANOBIOTECHNOLOGICAL APPROACH FOR PLANT DISEASE MANAGEMENT

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Abstract

In the current study, it is reported that multi-walled carbon nanotube-zinc oxide (MWCNTs-ZnO) nanocomposites were synthesized, their structure characterized, and demonstrated to be useful in antimicrobial applications. We prepared the MWCNTs, which have been cleaned and carboxyl-modified (easy water dispersion), and, later, ZnO nanoparticles were coated on the nanotubes with sol-gel technique. UVVis spectroscopy, FTIR, XRD and SEM were used to prove structural integrity and good functionalization. Antimicrobial activity of the nanocomposites was floated against two agricultural important pathogens viz. Ralstonia solanacearum (Gram-negative bacterium) and Fusarium solani (fungal pathogen), using disc diffusion technique of microbial assay. Findings showed that the antimicrobial activity of MWCNTs was dose-dependent thus having a synergistic effect with ZnO. The composite's bioactivity is likely due to ROS generation, membrane disruption, and enhanced cellular uptake. This study supports the potential application of MWCNTs-ZnO nanocomposites in sustainable agriculture

INTRODUCTION

Nanotechnology has been acknowledged as an important area in materials science and agriculture. Nanomaterials' special physicochemical characteristics, characterized by a large surface area relative to volume and the influence of quantum phenomena, enable better interaction with biological systems. Carbon nanotubes (CNTs), and more specifically multi-walled carbon nanotubes (MWCNTs), are standing out among these materials because of their remarkable electrical conductivity, mechanical strength, and surface functionalization ability. An example of this is that it is a routine engineering of functionalized carbon nanotubes to interact in a direct manner with the cell membrane of microbes.

Due to their low cytotoxicity and the ability to produce the reactive oxygen species (ROS), zinc oxide (ZnO) nanoparticles have been of particular interest to the scholars. Combination of ZnO with multi-walled carbon nanotubes (MWCNTs) shows a synergetic bioactivity increase that has been explained to be due to the oxidative-stress propagation and physical perturbation of the microbial cells.

The main causes of threat to potato production are Ralstonia solanacearum; the causative agent of bacterial wilt, and Fusarium solani which causes Fusarium wilt. These organisms survive in the soil and are recalcitrant to traditional therapy and thus pose serious problems in managing diseases. The study in question thus examines the synthesis, characterization and bioefficacy of MWCNTs-ZnO nano composites against these two pathogens with evaluation of their use in agriculture as alternates to traditional agrochemicals in use.

2. Materials and Methods

2.1 Materials

2.2 Functionalization of MWCNTs

Carboxyl functionalization was used to improve MWCNTs' dispersibility and capacity to interact with metal oxides and biological systems. 150 mL of an acid mixture made up of concentrated HNO_3 and H_2SO_4 in a 1:3 ratio was used to disperse one gram of pristine MWCNTs. After that, the suspension was refluxed for six hours at 120°C while being continuously stirred by magnetic means. Impurities were eliminated during this procedure, and carboxylic acid (-COOH) groups were added to the MWCNTs' surface. Following the reaction, the mixture was left to cool to room temperature before being repeatedly rinsed with deionized water until the filtrate's pH reached a neutral level. A 0.22 µm PTFE membrane was used to filter the resulting black residue, which was then oven-dried at 60°C and stored in a desiccator until further use.

2.3 Synthesis of ZnO Nanoparticles

ZnO nanoparticles were synthesized using a sol-gel technique. At room temperature, 5 grams of zinc acetate dihydrate were dissolved in 100 milliliters of absolute ethanol while being constantly stirred. The precipitation process was started by adding a 0.1 M sodium hydroxide (NaOH) solution dropwise to the mixture to raise the pH to 10. To allow for additional aging, the reaction mixture was left undisturbed overnight after being stirred for three hours. After centrifuging the resulting white precipitate for 10 minutes at 8000 rpm, it was successively cleaned with ethanol and deionized water to get rid of any unreacted species. Finally, it was dried at 80°C. Pure ZnO was formed by calcining the dried product one last time for two hours at 400°C nanoparticles.

2.4 Fabrication of MWCNTs-ZnO Nanocomposite

For 60 minutes, carboxylated MWCNTs (0.5 g) were ultrasonically treated to disperse them in 50 mL of ethanol. A different 50 mL ethanol solution was used to disperse 1 g of ZnO nanoparticles in parallel. To guarantee consistent interaction, the two suspensions were then mixed together and constantly swirled for three hours at 70°C. After the solvent evaporated, the resultant solid was calcined for three hours at 120°C. After drying, the material was ground into a fine powder and kept for later use as the MWCNTs-ZnO nanocomposite.

2.5 Characterization of Nanocomposites

UV-Visible Spectroscopy: A UV-Visible spectrophotometer was used to record the optical characteristics of the nanocomposite in the 200–800 nm range. With a maximum optical density (OD) of 3.875 and a noticeable absorbance peak at 390 nm, the composite and ultraviolet light interacted effectively.

FTIR Spectroscopy: To find functional groups, FTIR spectra (400–4000 cm^{-1}) were examined to find functional groups. Relevant functional moieties were confirmed by peaks at 1022, 1095 (C=O), 1648 (C=C), 2855 and 2950 (C-H), 3410 (O-H), and 495, 570, and 750 cm^{-1} (ZnO bending).

XRD: XRD was used to examine the nanocomposite's crystalline structure. ZnO's phase purity was confirmed by matching its standard crystalline planes with sharp peaks. The (002) plane of MWCNTs was linked to a broad diffraction peak at about $2\theta \approx 23°$, suggesting the presence of graphitic carbon.

SEM: SEM was used to analyze the MWCNTs-ZnO composite's surface morphology. The micrographs showed that ZnO nanoparticles were evenly spaced across the carbon nanotubes' surface. With an average diameter of roughly 18.5 nm, the MWCNTs maintained their distinctive tubular shape. Attached to the nanotube structure, the ZnO particles showed up as granular deposits.

2.6 Pathogen Culture and Microscopy

Fusarium solani: For seven days, the fungal strain was cultivated on potato dextrose agar (PDA) and incubated at 28°C. Under a 40× magnification microscope, hyaline, septate hyphae and banana-shaped conidia with a length of roughly 10–20 µm were visible.

Ralstonia solanacearum: For twenty-four hours, the bacterial strain was cultivated in Luria-Bertani (LB) broth at 28°C. A McFarland standard of 0.5 was then applied to the culture. Gram staining and light microscopy at 40× magnification showed rod-shaped, Gram-negative cells that were 1.5–3.0 µm long and 0.5–1.0 µm wide.

2.7 Disc Diffusion Method Antibacterial Activity

The disc diffusion method was used to assess the MWCNTs-ZnO nanocomposite's antibacterial activity. 10 µL aliquots of MWCNTs-ZnO solutions at concentrations of 75, 105, 135, 165, 195, and 225 µg/mL were loaded onto sterile filter paper discs (6 mm in diameter). Ralstonia solanacearum-inoculated LB agar plates were used to hold the prepared discs. For 48 hours, the plates were incubated at 30°C. Zones of inhibition were measured in millimeters following the incubation period; the diameters varied based on the concentration, ranging from 14 mm to 23 mm. As a positive control, gentamicin (30 µg/disc) created a 14 mm inhibition zone. There was no discernible antibacterial activity on blank discs without test material.

2.8 Activity Against Fungi

Using the disc diffusion method on potato dextrose agar (PDA), the antifungal activity of the MWCNTs-ZnO nanocomposite against Fusarium solani was evaluated. Each PDA plate had a 6 mm fungal plug of F. solani positioned in the middle. Around the fungal plug, sterile filter paper discs impregnated with 10 µL of MWCNTs-ZnO suspensions at concentrations of 130, 230, 330, 430, 530, and 630 µg/mL were placed equally apart. For five days, the plates were incubated at 28°C. The observed zones of inhibition, which ranged in diameter from 10 mm to 17 mm, were found to increase with increasing concentrations of the nanocomposite. The positive control, miconazole (30 µg/disc), created an 18 mm zone of inhibition.

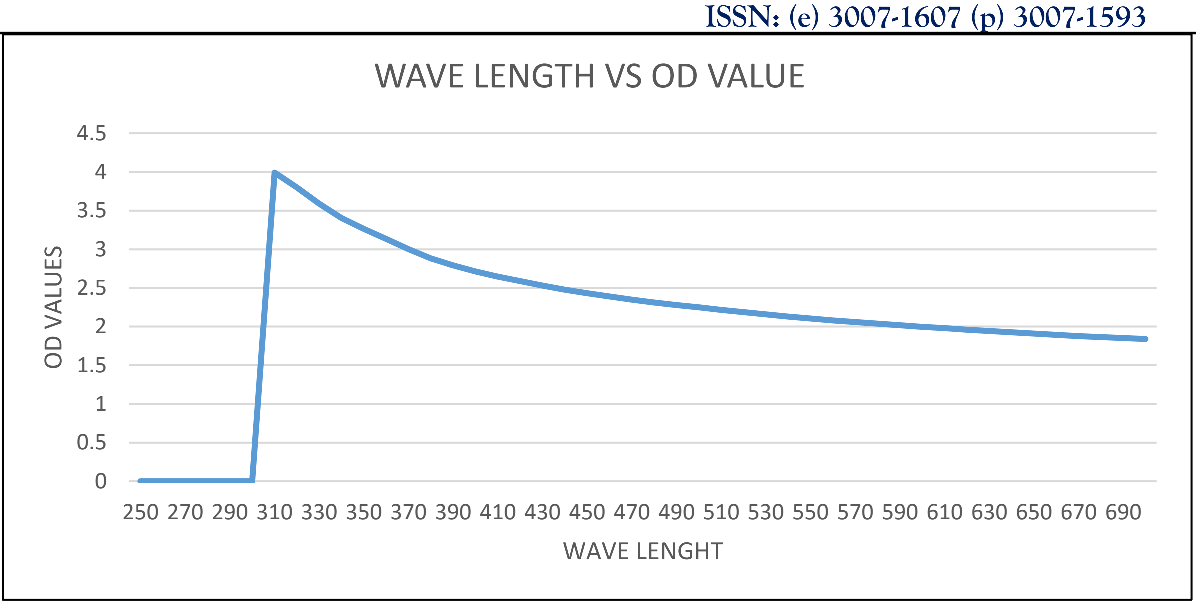
2.9 GC-MS Pathogen Metabolite Profiling

The chemical profiles of Fusarium solani and Ralstonia solanacearum were compared before and after treatment with MWCNTs-ZnO nanocomposites using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. After treatment, both microbial cultures' supernatants were gathered and extracted for GC-MS examinationThis paper addressed the metabolic fingerprint of two research animals, Danio rerio and Caenorhabditis elegans under exposure to MWCNTs-ZnO nanomaterials. Both species experienced significant variations, those being their fatty acid derivatives and volatile organic compounds; these findings showed that nanomaterial exposure resulted in metabolic stress and interfered with normal biochemical processes. The reproducibility was provided by performing all the experiments three times, and the values were provided in the way of means and the standard deviations (mean give SD). Regression models have also been advanced in order to analyze concentration-related patterns that are evident in the experimental antimicrobial activities recorded.

3. Results

3.1 Spectroscopy in the UV

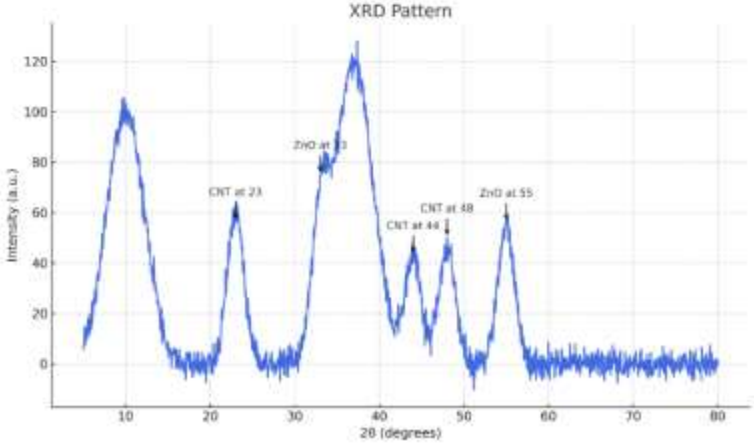
The optical characterization of fabricated MWCNTs-ZnO nanocomposites was done with the help of UV-visible spectroscopy. At 390 nm there was a prominent peak of absorbance that signified the band-gap transition of ZnO. This high peak intensity indicates the fact that there is a great optical interaction between the composite and the incoming ultraviolet light since the maximum absorbance 3.875 was recorded at this wavelength. Such an absorption nature is found as a train of the semiconducting property of ZnO, as well as the optical sensitivity of the multi-walled carbon nanotubes. The spectroscopic data therefore confirms the fact that the nanocomposite material has a potential application in the area of photo catalytic systems, ultraviolet sensing and the use in optoelectronic devices.



The ultraviolet absorption spectrum of multi-walled carbon nanotubes (MWCNTs) is characterized by the resonance of the wavelength and optical density (OD) values within the visible range.

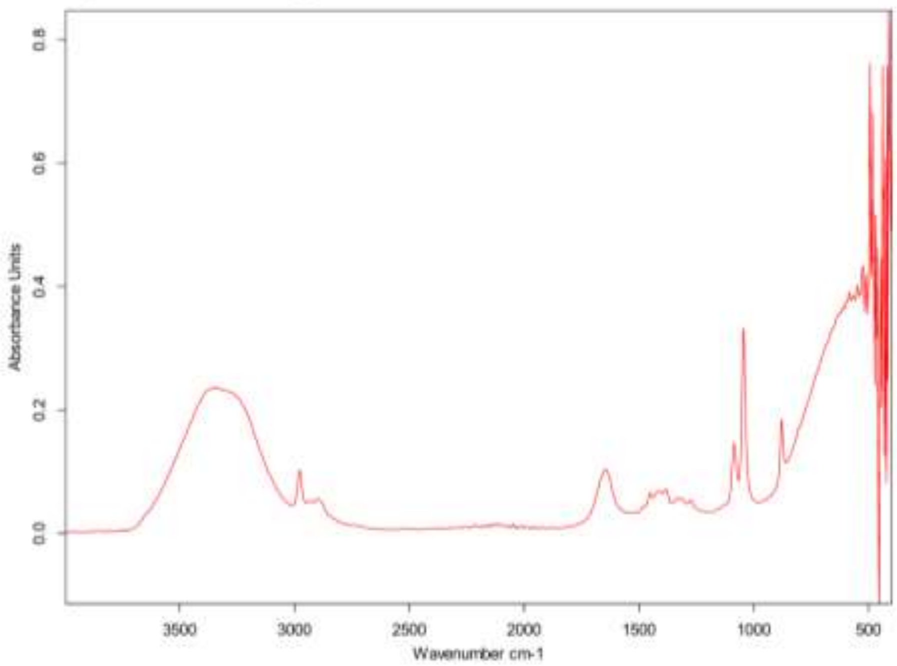
3.2 Analysis of X-ray Diffraction (XRD)

The X-ray diffraction (XRD) analysis of the MWCNTs-ZnO nanocomposite showed that the Cu-Ka radiation was used on the range of 2- theta that ranged between 10 o and 80 o. The trend was a different set of diffraction peaks due to ZnO and MWCNTs. The presence of a broad diffraction peak which is centred at 2- 0 23 0]/span doubly peaked, and it is assigned to the disorder graphitic structure of the MWCNTs which is centred at (002). The peaks of diffraction of ZnO are sharp and well-resolved, which signify that ZnO nanoparticles developed crystalline domains. These peaks indicate indeed that ZnO was introduced in MWCNT matrix composition as in the composite fabrication process the structural integrity of the nanotubes was observed through the preservation of the MWCNTs-specific diffraction peak.X-ray diffraction (XRD) was performed using the Shimadzu XRD-6000 model with Cu-K alpha radiation. The observed characteristic peaks indicate the presence of both MWCNTs and ZnO features in the composite.



3.3 Fourier Transform Infrared Spectroscopy (FTIR)

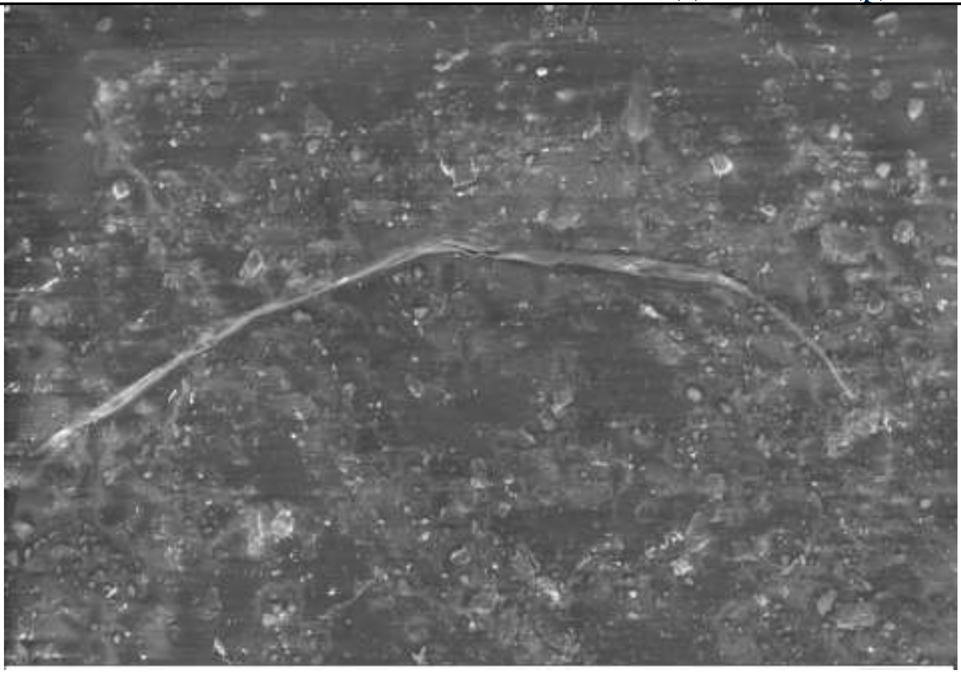
Surface chemistry and functional groups of the MWCNTs-ZnO nanocomposite were characterized employing Fourier Transform Infrared (FTIR) spectroscopy. The 400–4000 cm^{-1} range was used to record the spectrum. Different chemical bonds were represented by the distinct absorption bands that were seen. Carbonyl (C=O) group vibrations were identified as the cause of the peaks at 1022 and 1095 cm^{-1}, whereas C=C stretching—a sign of alkene structures—was identified as the cause of the peak at 1648 cm^{-1}. At 1348 and 1452 cm^{-1}, bending vibrations of C–H were detected. C–H stretching in alkyl groups was represented by additional peaks at 2855 and 2950 cm^{-1}. The presence of hydroxyl groups was suggested by a broad absorption band at about 3410 cm^{-1} that showed O–H stretching vibrations. Zn–O bending vibrations were identified as the cause of peaks at lower frequencies, 750, 570, and 495 cm^{-1}. These The successful functionalization of MWCNTs with ZnO nanoparticles and the preservation of crucial organic functional groups required for ensuing sbiological interactions were validated by these spectral features.



The FTIR spectrum of MWCNTs-ZnO provides detailed information regarding the molecular structureand the functional groups present in the composite material. Captured at the wave numberin the range of 400 to 4000 cm -1, the spectrum exhibited different peaks, each representing various functional groups.

3.4 Scanning Electron Microscopy (SEM)

The MWCNTs-ZnO nanocomposite surface morphology was investigated by Scanning Electron Microscopy (SEM). With an average diameter of about 18.5 nm, the images revealed that the tubular structure of the MWCNTs had been retained. The ZnO nanoparticles were found to be well-distributed granular particles on the nanotubes' surface. To provide more interaction surface area and enhance the composite's biological and physicochemical performance, this homogeneous deposition was believed to be crucial. The accomplishment of composite formation and structure integrity of the MWCNTs was confirmed by maintaining the morphology of the nanotubes after ZnO coating.



The SEM micrograph of the MWCNTs-ZnO composite showed the surface morphology of the composite at a magnification of 1000 µm.

3.5 Microscopic Examination of Pathogens

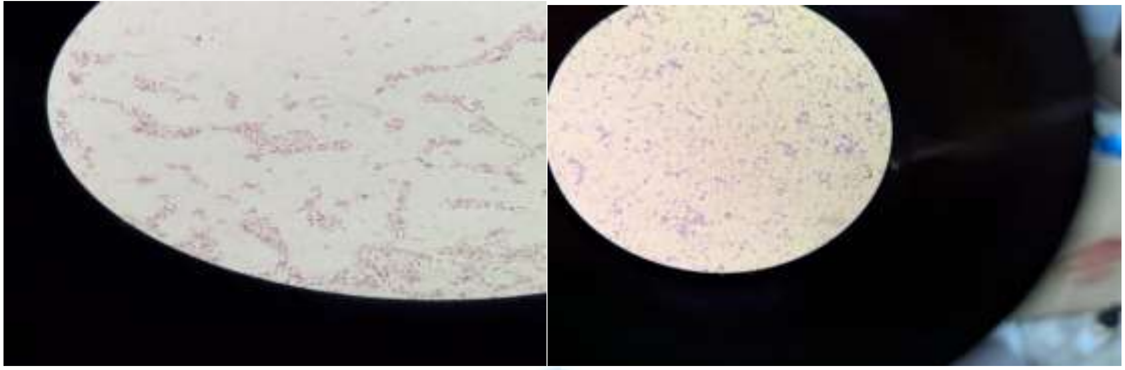
Fusarium solani

A compound light microscope set to 40× magnification was used to examine Fusarium solani under a microscope. Hyaline, septate hyphae with long, banana-shaped conidia were seen in the fungal structures. The conidia were seen alone or in tiny clusters, and they ranged in length from 10 to 20 µm. The overall morphology and the existence of septa within the conidia were found to be in line with accepted descriptions of F. solani. These findings were thought to be adequate to verify the fungal species' identity.

The morphology of Fusarium solani is illustrated in two discrete formats. The foremost representation is a macroscopic observation of the culture, manifesting as a powdery white colony, a distinctive phenotype of the species. The complementary image, attained with a compound microscope at 40X magnification, delineates the organism's microscopic architecture.

Ralstonia solanacearum

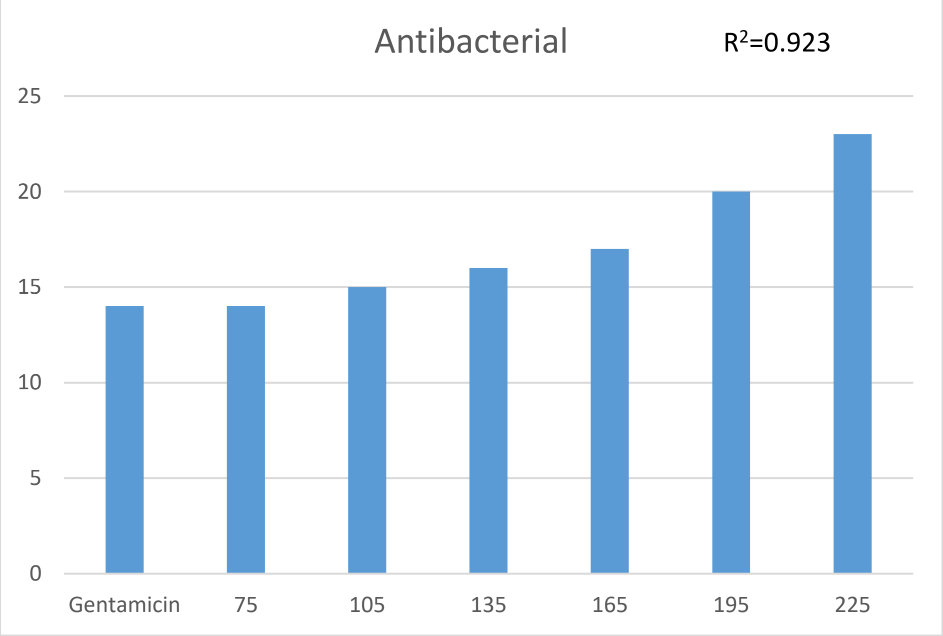
Morphological characterization of Fusarium solani is presented in two complementary formats. The initial representation is a macroscopic observation of the colony, manifesting as a granular, white, and powdery mycelial growth, consistent with the species' recognized phenotype. The accompanying microphotograph, acquired with a compound microscope at 40× magnification, elucidates the hyphal architecture and reproductive structures of the organism.



Two sequential microscopic examinations of Ralstonia solanacearum were performed using a compound microscope set to 40X magnification. The initial micrograph presents the canonical morphology of the bacterium, depicting small, straight, rod-shaped cells that exhibit the characteristic morphology of Gram-negative organisms. The second image offers a clearer view of the bacterial cells compared to the first, and they maintain the same rod-shaped, motile form, measuring approximately 0.5-1.0 micrometers in width and 1.5-3.0 micrometers in length.

3.6 Antibacterial Activity Results

Using the disc diffusion method, the antibacterial activity of the MWCNTs-ZnO nanocomposite against Ralstonia solanacearum was evaluated at different concentrations (75, 105, 135, 165, 195, and 225 µg/mL). Following a 48-hour incubation period, zones of inhibition were measured. With inhibition zone diameters of 14 mm, 15 mm, 16 mm, 17 mm, 20 mm, and 23 mm, respectively, a concentration-dependent increase in antibacterial activity was noted. The negative control (blank disc) showed no inhibition, whereas the positive control, gentamicin (30 µg/disc), created a 14 mm zone of inhibition. To assess the correlation between nanocomposite concentration and antibacterial activity, statistical analysis was performed. With an R2 value of 0.923, a strong positive correlation was found. This evidence justified the prospective use of MWCNTs-ZnO as an effective bactericidal agent by demonstrating significant and direct influence of enhancing nanocomposite concentration on inhibition of bacterial growth.



The outcomes of the disc diffusion technique, employed to evaluate the antibacterial properties of MWCNTs-ZnO composites, were depicted.

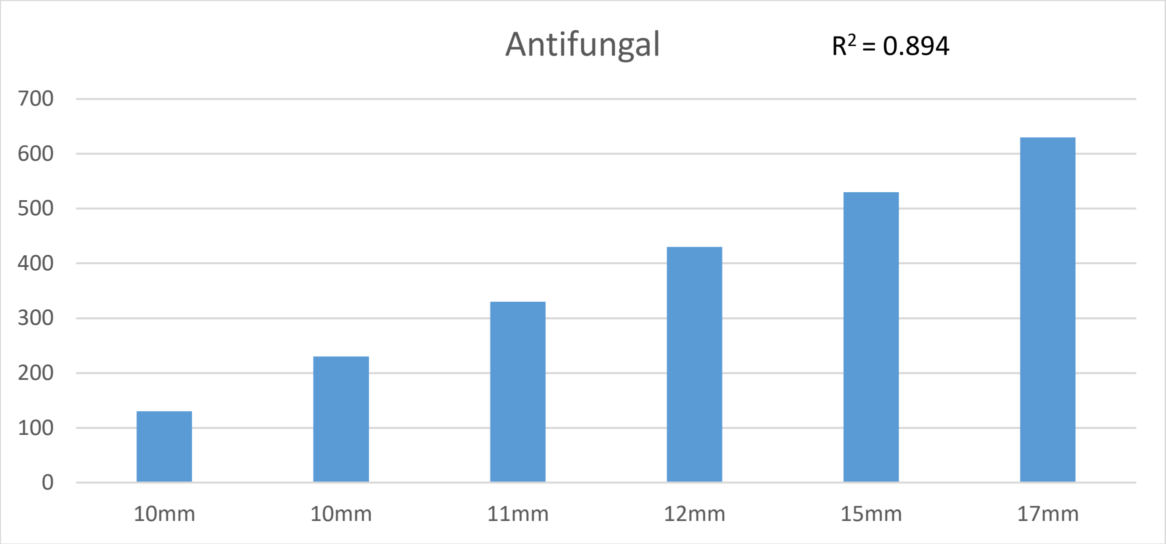
The statistical correlation, with an R-squared of 0.923, indicates a high positive correlation between the antibacterial efficacy of the MWCNTs-ZnO and its concentration. This implies that higher concentrations of MWCNTs-ZnO promote its inhibitory effect on bacterial growth, which could validate its application as an antibacterial drug. (Concentrations on X-axis, Zones on Y-axis)

3.7 Antifungal Activity Results

By the disc diffusion technique, the antifungal potential of the MWCNTs-ZnO nanocomposite against Fusarium solani was examined at 130, 230, 330, 430, 530, and 630 µg/mL concentrations. The zone of inhibition was 10 mm, 10 mm, 11 mm, 12 mm, 15 mm, and 17 mm with each concentration. The standard antifungal medication miconazole (30 µg/disc) formed an 18 mm inhibition zone.

The correlation between nanocomposite concentration and antifungal activity was determined by linear regression analysis. An R2 of 0.894 demonstrated the evidence of a strong positive correlation. This proved the efficiency of the material as a potential antifungal agent by evidence that increased concentrations of the MWCNTs-ZnO composite resulted in greater inhibition of fungal growth.

The disc diffusion method results, which were used to evaluate the antifungal properties of MWCNTs-ZnO composites, were illustrated.



The R-squared value of 0.894 from the statistical analysis suggests a strong positive relationship between the concentration of MWCNTs-ZnO and its antifungal effectiveness. (Concentrations on Y-axis, Zones on X-axis)

3.8 GC-MS Profiling of Treated vs. Untreated Pathogens

Ralstonia solanacearum GC-MS Profile

To determine the chemical structure of Ralstonia solanacearum supernatant fluids prior to and following treatment with MWCNTs-ZnO nanocomposites, the Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted. To untreated cultures, bioactive metabolites such as methyl glyoxal, acetic acid, and hydroxyacetone and various lactones were observed. A different metabolic profile of characteristics resulted following treatment. New compounds, like dimethoxy derivatives and cyclopentasiloxane, were identified, indicating that the bacterium features significant disruptions in the anthropogenic biochemical pathway. The changes in metabolite expression indicate that exposure to MWCNTs-ZnO induces disruption in the usual metabolism of R. solanacearum either due to the cell membrane interaction or induction of oxidative stress. The occurrence of siloxane-based metabolites can be a sign that stress responses or regulation of enzyme activity is related to exposure to nanocomposite.

The results of the Gas Chromatography-Mass Spectrometry (GC-MS) analysis of Ralstonia solanacearum were presented, highlighting the primary microconstituents identified in the bacterial samples.

Sr. No.	Microconstituent	Molecular Formula	Molar Mass (g/mol)	Retention Time (Min)	Peak Area (%)
1.	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	370.77 g/mol	6.051 min	64.23
2.	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444.92 g/mol	7.569 min	1.54
3.	2,3-Dimethoxy-2	$CH_3C(OCH_3)_2COCH_3$	146.19 g/mol	7.645 min	19.42
4.	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	667.39	11.474	1.05

Fusarium solani GC-MS Profile

The metabolic profile of Fusarium solani was analyzed by the analysis of Gas Chromatography-Mass Spectrometry (GC-MS) analysis before and after exposure to MWCNTs-ZnO nanocomposites was done. Major metabolites identified in the untreated fungal samples included alpha-linolenic acid (21.08% peak area), palmitic acid, di(2-ethylhexyl) phthalate

(DEHP) and gamma-sitosterol. These were mainly connected with the lipid metabolism and structural integrity of the membrane.

The exposure to MWCNTs-ZnO revealed severe modifications of the metabolic profile. Among the newly found were Furanmethanol, levoglucosenone, 6-fluoroindole, and octasiloxane. Conversely, some of the lipid-associated metabolites that used to be abundant were totally missing or their abundance reduced drastically. These alterations were seen as the indicators of the metabolic shifts induced by stress that could either have been initiated by the impairment of fungal biosynthesis routes.The metabolite composition changes that were observed offered compelling proof that the MWCNTs-ZnO nanocomposite interacted with F. solani at the molecular level, causing stress reactions that resulted in decreased cellular viability and metabolic disturbance.

The key compounds detected in the GC-MS analysis of Fusarium solani exposed to multi-walled carbon nanotubes (MWCNTs) have been summarized.

Sr. No.	Microconstituent	Molecular Formula	Molar Mass (g/mol)	Retention Time (Min)	Peak Area (%)
1.	Furanmethanol	$C_5H_6O_2$	98.1g	6.267	0.19
2.	Levoglucosenone	$C_6H_6O_3$	126.14	6.267	0.19
3.	Formic acid	CH_2O_2	46.025	15.863	0.5
4.	6-Fluoroindole, TMS	C_8H_6FN	135.14	12.5	0.72
5.	Tris(tert-butyldimethylsilyloxy)	$C_18H_45ASO_3Si_3$	468.7	20.29	0.72
6.	7-Hydroxy-7,8,9,10-tetramethyl-7	$C18H_{20}O$	252.3	13.5	0.52
8.	1-(2-Thienyl)-1-propanone	C_7H_8OS	140.20 g/mol	6.583	1.51
9.	6-Amino-2,4-dioxo-1,2,3,4-tetrah.	$C_4H_7N_3O_2$	152.15 g/mol	7.220	0.50
10	Octasiloxane	$C_{18}H_{54}O_7Si_8$	607.31 g/mol	7.654	26.13

4. Discussion

It was indeed observed in this study that the MWCNTs-ZnO nanocomposites were highly antimicrobial in their effect on phytopathogenic bacteria and fungus. Several synergistic mechanisms were proposed, based on which the augmented effect occurred. Whereas the ZnO nanoparticles produce reactive oxygen species (ROS) that produce oxidative stress to the microbial cell, the large surface-area-to-volume ratio of MWCNTs enhances the point of intersection between nanoparticles and microorganisms. In addition, the entire architecture was presumed to support the penetration of the cells through increasing their permeability using microbial structures as its membranes.

The use of novel methods in many of the previous researches revealed that ZnO nanoparticles destroy microbial cell walls by inducing ROS and liberation of Zn2+ ions and therefore, disrupt membrane integrity. At the same time, MWCNTs perform the role of nanodagger, which can penetrate cell membranes and augment the absorption of nanoparticles. Both the mechanisms were found to have enhanced efficacy when combined.

The dose dependence nature of the antimicrobial activity of the nanocomposite was confirmed by the increased diameter of inhibition zones with the increment in concentration. The scanning electron microscope (SEM) allowed the authors to see that ZnO was well dispersed on the MWCNTs that increased microbial adhesion and eventual cell lysis. The enhanced dispersion in the composites was ascribed to the carboxylated MWCNTs level that enables nanoparticle to interact with biological systems.

All these results show that nano-composites of MWCNTs-ZnO can be used as substitute to the traditional use of agrochemicals, thus limiting the usage of chemical pesticides and offer a more sustainable and environmentally friendlier method of containing crop-associated diseases.

5. Conclusion

MWCNTs-ZnO nanocomposites were shown to have potent antimicrobial effects against fungus Fusarium solani and bacterium Ralstonia solanacearum that are the major phytopathogens affecting potato crop.

The outcomes showed that the effect was dose dependent: the greater concentration resulted in better inhibition. There were signs that mechanical disruption provided by MWCNTs combined with the oxidative stress produced by ZnO nanoparticles led to the destruction of the membrane and an accompaniment of the metabolic impairment in the pathogens.

Succesful synthesis, modification and nanocomposit morphology were also confirmed using characterization techniques, UV-Vis, FTIR, XRD and SEM analysis. The GC-MS analysis also proved the hypothesis to be true by showing the changes in the expression of biomolecules of importance and highlighting the biochemical stress generated by nanomaterials.

MWCNTs-ZnO nanocomposites are therefore a biologically benign alternative to the use of conventional agrochemicals which can potentially promote the health of crops and reduce environmental toxicosity and the use of pesticides. In vivo field efficacy trials, long-term environmental consequences, especially on soil microbial assemblages, and the development of cost-effective and easily scalable nanocomposite-based agrotechnologies whose application is applicable in various agricultural settings should be of future research focus.In conclusion, the research paved the way for the application of nanotechnology-based approaches to plant disease control and promoted the creation of environmentally conscious and potent next-generation antimicrobial platforms.

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