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RESEARCH ARTICLE
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Biofertilizers Production and Climate Changes on Environmental Prospective Applications for some Nanoparticles Produced from some Microbial Isolates

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ABSTRACT

This study aimed to enhance microbial isolates' growth and nutrient availability through the use of a mixed biofertilizer and various nutritional media. Additionally, the impact of adding ZnO nanoparticles on agricultural soil fertility and the development of microbial isolates was investigated. The preservation of microbial isolates, maintenance of the ideal food-to-microorganism ratio, and vitality assurance were achieved through this procedure. The resulting liquid biological fertilizer, containing a beneficial and eco-friendly community of living microorganisms, can be safely applied to agricultural soil. The application of zinc oxide as a nano-composite to a solution containing microorganisms effectively fertilized banana plants without causing harm or pollution. Biofertilizers, which are organic and contain specific microorganisms, offer a greener alternative to chemical fertilizers, meeting plant nutritional needs while minimizing environmental pollution. Correct utilization of biofertilizers is crucial for preserving soil quality, increasing crop yields, and protecting the environment.

Keywords: Microorganisms, Eco-friendly, Chemical fertilization, Biological control, Biocidal, Fungicide

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INTRODUCTION

These days, finding safe and natural options for agricultural soil fertility and plant growth requires research and validation. This is required to prevent the use of chemical fertilizers, which have the potential to harm the ecosystem in many ways, impair soil fertility and cause other issues (Zafar et al., 2020; Kamal et al., 2024). The importance of microbes in supporting plant growth, and yield, and improving resistance to pests and diseases is highlighted in this study. These elements have a significant impact on crop productivity, which can result in shortages, which can cause harm to the environment and the economy as well as lower farmer income (Gaballa, 2017; Zafar et al., 2022). Lactic acid bacteria and Saccharomyces cerevisiae are thought to be viable substitutes for stimulating plant development in certain crops. Protein (47%), carbohydrates (33%), nucleic acid (8%), lipids (4%), and a variety of minerals (8%), including Na, Fe, Mg, K, P, S, Zn, Mn, Cu, Si, Cr, Ni, Va, and Li, are additional well-known properties of yeast (Tiwari et al., 2008; Noman & Azhar, 2023). Due to the yeast's abundance of tryptophan, an amino acid precursor that leads to indole acetic acid (IAA), prior reports have emphasized the beneficial benefits of yeast application on vegetative and fruit growth (Aslam et al., 2023).

A new approach to fighting different infections is the use of microorganisms as biocontrol agents (Elyass et al., 2021; Razzaq et al., 2021; Razzaq et al., 2023). Recent studies explore the potential applications of yeasts and lactobacillus as plant growth boosters and biocontrol agents against soil-borne fungal plant diseases (El-Tarabily and Sivasithamparam 2006; Ren et al., 2019). Using several yeasts was successful in suppressing fungal activity in sugar beet plants affected by *Rhizoctonia solani* (Kumar et al., 2016). All types of organic fertilizers are important sources of vital nutrients for all types of plants, big and tiny. Additionally, they provide a major contribution to enhancing the soil's chemical, biological, and physical characteristics. The use of liquid organic fertilizers has

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become more and more important in recent years as a clean substitute for giving These fertilizers include amino acids, and other materials, including organic acids like fulvic and humic acids. They are renowned for being reasonably priced, simple to use, low in environmental pollution, and capable of improving the properties of soil, which in turn helps different plants thrive and produce more (Alfaytouri et al., 2024). These materials are easily taken up by the roots of the plant, where they release their ions and swiftly engage in physiological functions, giving the plant the energy it needs to absorb them, especially in the early phases of growth. Achieving a balance between greater output and environmental conservation requires a return to integrated agriculture, also known as clean agriculture (Youssef & Eissa, 2014). This strategy guarantees the production of wholesome, pollution-free food. To assist plants in accessing vital nutrients, biofertilizers which are mixed into the soil—contain advantageous microbes (Napitupulu et al., 2021; Aloo et al., 2022). These microbial inoculants provide growth-promoting chemicals including hormones and regulators and assist in transforming nutrients into forms that plants can readily absorb. There has not been much research done on conservation agriculture in various agroclimatic zones or subregions (Babu et al., 2010). Numerous aspects of the use of biofertilizers in agricultural production systems are being actively studied by national research stations and agricultural institutions at both the national and international levels. Biofertilizers are solutions containing active or inactive microorganisms with the ability to fix nitrogen, dissolve phosphate, and break down cellulose (Ahmed et al., 2023). Through the application of these preparations to seeds or soil, beneficial microorganisms in the soil are increased in number and biological activity, hence improving soil fertility and stimulating plant growth. By raising the population and metabolic activity of beneficial microbes, biofertilizer microbial inoculants can be applied to soil, compost pits, or seeds (Aslam et al., 2020). This can speed up microbial activities and increase the availability of nutrients in forms that are easily digested by plants. Two factors make the use of biofertilizers essential: first, the economical and ecologically friendly nature of biofertilizers; second, the worries about soil fertility, texture, and environmental issues brought on by overuse of conventional fertilizers (Deng et al., 2021). Combining chemical and biological fertilizers is a reliable way to supply nutrients to agricultural fields (Zheng et al., 2024; Zakaria et al., 2023). A nation's ability to feed and nourish its people is a key factor in that nation's growth and development. Fertilizers are used to address the growing demand for food that results from an increasing human population. Fertilizers are materials that increase soil productivity by providing vital nutrients for the development of plants (Tiwari et al., 2008).

Chemical fertilizers are made in factories using either liquid or solid raw chemicals that are carefully formulated to fulfil the nutritional requirements of plants. In addition to other nutrients, these fertilizers usually include nitrogen, phosphorus, and potassium (NPK) (Mandal et al., 2024).

MATERIALS & METHODS

To conduct studies to ascertain the existence of specified elements, samples of soil and banana leaves were collected from a designated place. In this study, banana trees and their leaves were treated with a microbial solution, commonly referred to as biological fertilizer. The objective was to watch the live fertilization process and evaluate the biological fertilizer's effects on the plants and soil.

Characterizations of ZnO Nanoparticle

The chemically produced ZnO nanoparticles were characterized using the Shimadzu UV-1800 UV–vis Spectroscopy. Measuring the UV-visible light absorption between 200 and 600 nm allowed researchers to calculate the optical absorption of biogenic ZnO nanoparticles. To further characterize the nanoparticles, X-ray diffraction (XRD) was carried out using a step size of 0.0260◦ in the 2θ range of 1◦ to 80◦. Fourier Transform Infrared (FTIR) spectroscopy (Thermo Scientific Nicolet iS10 FT-IR), scanning electron microscopy (SEM) (FEI Quanta 250 TE scan vega3), and transmission electron microscopy (TEM) images (JEOL JEM-1230) were used for surface characterization and chemical analysis. Using an FTIR spectrometer (model Nicolet iS-10 Thermo Scientific, USA), scans were performed at 4 cm-1 resolution and the FTIR spectra were obtained at room temperature throughout the range of 4000–400 cm-1. Energy Dispersive X-ray (EDX) analysis was used to examine the elemental compositions of the various sections to guarantee the homogeneity of nanomaterial distribution over the sensor's surfaces. Using the JEOL JSM-6390LV (TE scan vega3model), scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM-EDX) pictures were captured.

Preparation of ZnO and Microbial Solution

ZnO nanoparticles weighing one gram were refined using a dispersion of ten liters of deionized water, 30 minutes of ultrasonication, and a mixture of several microbial species.

The Effects of ZnO Nano Priming on the Soil Treatment

The following technique is used to remediate soil using ZnO microbial solutions. For each crop, 4g of ZnO suspended in 4L of water with microorganisms is used.

Estimation of Biochemical Components

Thirty-day-old plants were subjected to measurements of the following biochemical components: cations, nematodes, bacterial and fungal analyses of a 250g soil sample at 25°C, both before and after treatment.

Statistical Examination

Every test was run in three duplicates. The data were analyzed using one-way variant analysis and presented as mean and efficiency % before and after treatment.

RESULTS and DISCUSSION

Optical Studies

Through the use of UV–vis spectrophotometric measurement, the synthesis of ZnO nanoparticles from zinc acetate was confirmed. The produced ZnO nanoparticles showed absorption peaks around 396nm, which can be related to their surface resonance plasmonic feature, according to an analysis of the UV–vis spectra (Fig. 1).

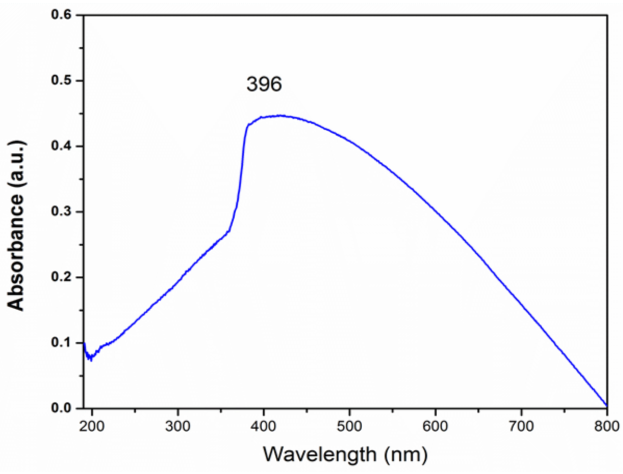


Fig. 1: UV Vis absorption spectrum.

Structural Studies

The synthetic ZnO nanoparticles' structural features are shown in the X-ray diffraction (XRD) pattern shown in Fig. 2. The findings suggest that the crystalline structure of these nanoparticles is hexagonal. Their crystalline nature is further confirmed by the presence of particular planes in the XRD pattern, such as (100), (002), (101), (102), (1100, (103), (220), (112), (201), (004), and (202). Interestingly, no peaks representing contaminants were found, indicating that the ZnO nanoparticles that were created are only one phase in nature. Aldalbahi et al.'s 2020 discoveries led to the determination of the lattice parameter, which was 0.323420nm. The Scherer equation was used to determine the ZnO nanoparticles' crystallite size. The full-width half-maximum (FWHM) of the most noticeable peak, which corresponds to the (101) plane, was utilized in this calculation. D is the crystallite dimension, k is the Scherer constant, λ is the X-ray wavelength, β is the FWHM of the (101) plane, and θ is the Bragg diffraction angle. The equation is as follows: D = kλ βcosθ nm. It was found that the produced ZnO nanoparticles had an average crystallographic size of between 20 and 30 nm. In addition, 4.44690 Å was found to be the lattice parameter.

FT-IR Studies

The interaction of the freshly produced ZnO nanoparticles' active metabolites was examined using FT-IR analysis. The 400–4000cm^{-1} range was the location of the peaks in the FT-IR spectra (Fig. 3). Notably, lesser peaks at 2948cm^{-1} showed C–H vibration, while a significant peak at 3359 cm - 1 suggested O – H vibration. Furthermore, stretching vibrations with a -C≡C- corresponded to a peak at 2185cm^{-1}. C=O and C–O were shown to be functional groups at 1732 cm-1 and 1652cm^{-1} (stretching), respectively. Furthermore, C–C stretching in aromatic compounds was linked to the peak at 1444cm^{-1}. There were Zn–O stretching modes found in the absorption peaks between 1120 and 400cm^{-1}.

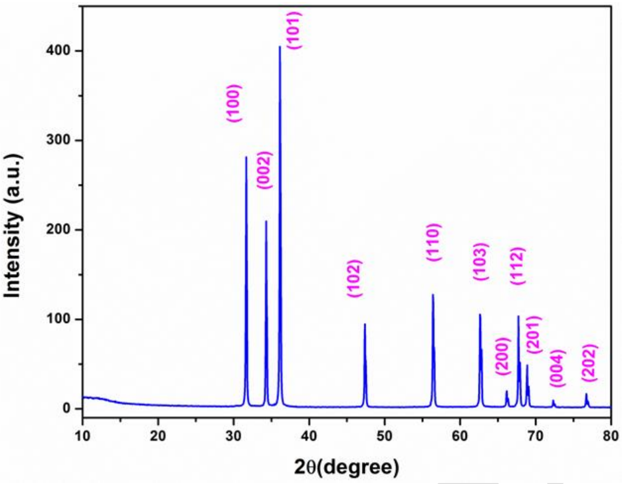


Fig. 2: XRD pattern of ZnO nanoparticles.

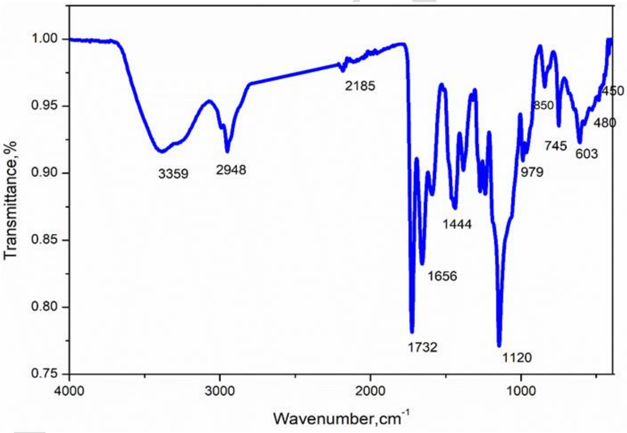
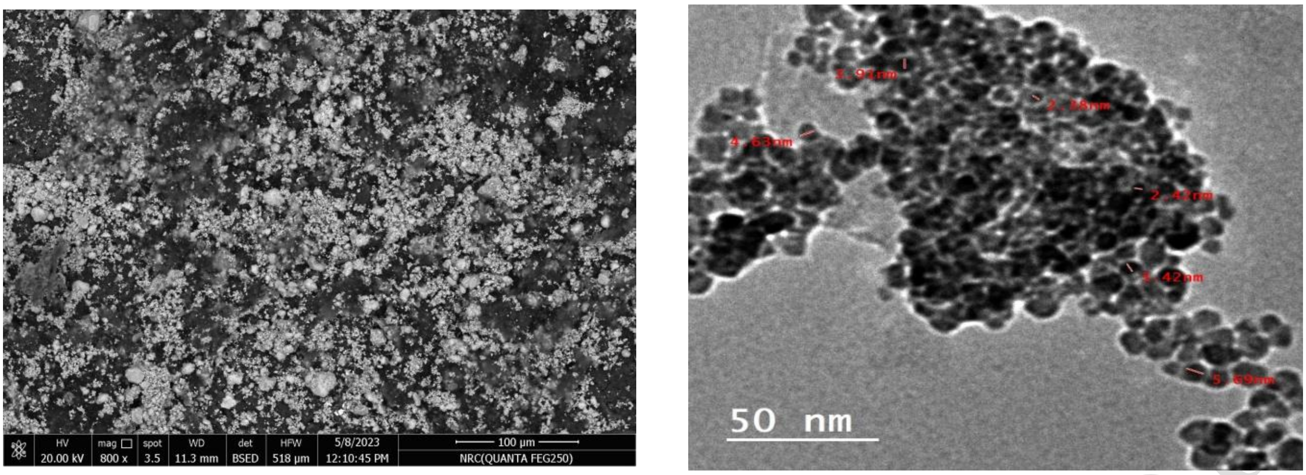


Fig. 3: FT-IR spectrum of ZnO nanoparticles.

Surface Morphological Studies

The structure of the generated ZnO nanoparticles was examined by surface morphology examination using SEM and TEM methods, as shown in Fig. 4. The agglomerated particles visible in the image indicated that the green ZnO nanoparticles were spherical in shape. The ZnO nanoparticles had an average particle size between 3 and 10 nm. Furthermore, Fig. 4(II) shows the particle size distribution histogram of the ZnO nanoparticles that were manufactured.

It investigated how the ZnO-NPs and microbial mixture affected the treatment of plants. There was a clear relationship between the addition of ZnO-NPs and the microbial solution and the pace of plant development. The plants responded favourably to the addition of bacteria and ZnO-NPs. Table 1 unequivocally shows that the application of ZnO-NPs and microbial solution greatly increased soil efficiency. Following the



SEM image (I) TEM image (II)

Fig. 4: (A) SEM and (B) TEM images of synthesized ZnO nanoparticles.

Table 1: Estimation of soft and large elements in soil (mg/sq.m) Before and after treatment (Banana plant)

Sample	B	Zn	Mg	Cu	Fc	K	P	N
Before treatment	-	106	21	3	213	53	48	32
After treatment	85	165	39	7.0	295	93	75	46
Efficiency %	-	35.7	46.1	57.1	27.7	43.0	36.0	30.4

treatment, there was an increase in the efficiency values of Zn, Mg, Cu, Fe, K, P, and N by 35.7, 46.1, 57.1, 27.7, 34.0, 6.0, and 30.4%, respectively.

Table 2 shows a significant improvement in soil efficiency after ZnO/microbial solution treatment. Significant increases were observed in the efficiency percentages for Zn, Mg, Cu, Fe, K, P, and N, which came to 33.3, 42.7, 43.8, 50, 28.3, 36.7, and 53.6%, respectively.

Table 3 makes clear that the application of ZnO/microbial solution changed the pH, EC, and Sp of the soil and leaves. The soil and leaves saw a pH shift from 8.95 and 8.16 to 8.38 and 8.0, correspondingly. Furthermore, following ZnO NP treatment, EC levels rose from 3.21 and 1.14 to 4.12 and 1.93, respectively. Moreover, Sp values for soil and leaves increased from 8.0 and 9.3 to 14 and 11.8, respectively.

Table 4 unequivocally shows that after the ZnO/microbial solution treatment, the levels of SO4, HCO3, and CO3 in the soil and leaves were changed. While the concentration of SO4 in leaves increased from 4.16 to 9.6, it increased from 8.9 to 14.3 in the soil. Furthermore, following ZnO NP treatment, the HCO3 levels went up a little bit—from 0.65 to 0.68 in the soil and from 0.43 to 0.72 in the leaves. Conversely, the soil's CO3 levels dropped from 3.5 to 2.96 in both the soil and the leaves.

It is clear from the results in Table 5 that after the ZnO/microbial solution treatment, the cation levels in the soil and leaves changed. In particular, the soil and leaf potassium (K) values dropped from 3.2 and 2.0 to 2.1 and 1.4, respectively. Following ZnO NP therapy, sodium (Na) levels similarly dropped, going from 11.2 and 2.0 to 9.4 and 1.8, respectively. Furthermore, the soil and leaf magnesium (Mg) values dropped from 7.2 and 3.6 to 4.3 and 2.1, while the calcium (Ca) values dropped from 21 and 7.3 to 18 and 5.6, respectively (as shown in Fig. 5).

Table 2: Estimate the soft and large elements in the leaf as mg/kg dry plant before treatment and after treatment (Banana plant)

Sample	B	Zn	Mg	Cu	K	P	N
Before treatment	86	83	11.0	1.8	58	43	19
After treatment	129	145	19.6	3.6	81	68	41
Efficiency %	33.3	42.7	43.8	50	28.3	36.7	53.6

Table 3: Results of chemical analysis of soil paste extract before and after treatment (before and after sample application banana plant)

Sample	PH		Ec		Sp	
	Before	After	Before	After	Before	After
Soil	8.95	8.38	3.21	4.12	8.0	14
Leave	8.16	8.0	1.14	1.93	9.3	11.8

Table 4: Anions analysis (mg/L) before and after treatment (Banana plan

Sample	SO4		HCO3		CO3	
	Before	After	Before	After	Before	After
Soil	8.9	14.3	0.65	0.68	3.5	2.96
Leave	4.16	9.6	0.43	0.72	----	-----

Table 5: Cation analysis (mg/L) before and after treatment (Banana plant)

Sample	K		Na		Mg		Ca	
	Before	After	Before	After	Before	After	Before	After
Soil	3.2	2.1	11.2	9.4	7.2	4.3	21	18
Leave	2.0	1.4	2.3	1.8	3.6	2.1	7.3	5.6

The effect of ZnO and microbiological solution on the growth of strawberry plants (250g at 25ºC) is clearly shown in Table 6. The effects were investigated both before and after the solution was added. After the soil was treated with ZnO and a microbiological solution, the results demonstrated a considerable improvement in the soil's ability to regulate egg and larva populations. The quantity of eggs dropped from 170 to 53, yielding a remarkable 68.8% efficiency rate. In a similar vein, there were 36 larvae instead of 260, indicating an 86.1% efficiency rate.

Table 6: Table of Nematode, bacterial and fungal analysis of a soil sample of 250g at a temperature of 25°C before and after treatment (Strawberry plant)

Samples		Soil sample 250 grams		Efficiency percentage	
		Egg	larva	Egg	Larva
Before treatment	Nematuda Root Nodes	170	260	68.8	86.1
After treatment	Nematuda Root Nodes	53	36		

Fig. 5: Images of banana plant after treatment with ZnO nanoparticles – microbial solution in the beginning and after complete growth.

These results demonstrate the beneficial benefits of ZnO/microbial solution on the general well-being and yield of strawberry plants.

The effect of ZnO/microbiological solution on cucumber plants (250g at 25°C) was investigated both before and after application, based on the data shown in Table 7. After being treated with ZnO/microbial solution, the soil's ability to decrease the populations of eggs and larvae changed. The number of eggs dropped from 115 to 25, yielding a 78.2% efficiency rate. In a similar vein, the number of larvae dropped from 230 to 36, exhibiting an 84.3% efficiency rate. Furthermore, Table 8 shows that the efficiency rates for the bacteria and fungi Fusarium, Verticillium, and Rhizoctonia were, respectively, 62, 63.8, and 65.8% following treatment.

The effectiveness of Nematuda Spiral, Najar Nematuda, and Nematuda Root Nodes on Tomato plant Egg and Larva is greatly increased by the ZnO-microbial solution; the corresponding percentages are 93.3, 90.3, and 84.5% for Egg and 90.7, 77.7, and 90.7% for Larva. Furthermore, 77.7% is the efficiency percentage for fungus and bacteria (Table 9).

Enhancing the effectiveness of Najar Nematuda and Nematuda Root Nodes on Egg and Larva in the Peanut plant is largely dependent on the ZnO-microbial solution. The corresponding efficiency rates for Larva are 85.0 and 86.8%, while for Egg they are 72.8 and 72.5%. Furthermore, Table 10 shows that the efficiency percentage for fungus and bacteria was 69.2.

Nematuda root nodes on eggs and larvae in orange trees are significantly more efficient when treated with the ZnO-microbial solution (Table 11), increasing the efficiency to 90.8% for Egg and 86.9% for Larvae. Furthermore, 68.9% is reported as the efficiency percentage for fungus and bacteria.

Table 7: Nematode, bacterial and fungal analysis of a soil sample of 250g at a temperature of 25°C before and after treatment (cucumber plant)

Sample		Soil sample		Efficiency percentage	
		Egg	larva	Egg	Larva
Before treatment	Nematuda Root Nodes	115	230	78.2	84.3
After treatment	Nematuda Root Nodes	25	36		

Table 8: The efficiency rates for the bacteria and fungi Fusarium, Verticillium, and Rhizoctonia

Sample	Soil sample	Efficiency percentage		
	Bacteria and fungi	Fu.	Ver.	Rhiz.
Before treatment	- *Verticillium* 29%	62.0	63.8	65.3
	- *Fusarium* 36%			
	- *Rhizoctonia* 26 %			
After treatment	-*Fusarium* 13%			
	- *Verticillium* 11%			
	- *Rhizoctonia* 9 %.			

Table 9: Nematode, bacterial and fungal analysis of a soil sample of 250g at a temperature of 25°C before and after treatment (Tomato plant)

Sample		Soil sample		Efficiency percentage	
		Egg	larva	Egg	larva
Before treatment	Nematuda Spiral	209	65	93.3	90.7
After treatment	Nematuda Spiral	14	6		
Before treatment	Najar Nematuda	156	36	90.3	77.7
After treatment	Najar Nematuda	15	8		
Before treatment	Nematuda Root Nodes	136	206	84.5	90.7
After treatment	Nematuda Root Nodes	21	19		
Before treatment	Bacteria and fungi	*Rhizoctonia* 36%		77.7	
After treatment	Bacteria and fungi	*Rhizoctonia* 8%			

Table 10: Table of Nematode, bacterial and fungal analysis of a soil sample of 250g at a temperature of 25°C before and after treatment (Peanuts plant)

Sample		Soil sample		Efficiency percentage	
		Egg	larva	Egg	larva
Before treatment	Najar Nematuda	140	145	72.8	86.8
After treatment	Najar Nematuda	38	19		
Before treatment	Nematuda Root Nodes	200	100	72.5	85.0
After treatment	Nematuda Root Nodes	55	15		
Before treatment	Bacteria and fungi	*Fusarium* 26%		69.2	
After treatment	Bacteria and fungi	*Fusarium* 8%			

Table 11: The Nematode, bacterial and fungal analysis of a soil sample of 250g at a temperature of 25°C before and after treatment (Orange trees)

Samples		Soil sample		Efficiency percentage	
		Egg	larva	Egg	larva
Before treatment	Nematuda Root Nodes	230	115	90.8	86.9
After treatment	Nematuda Root Nodes	21	15		
Before treatment	Bacteria and fungi	*Verticillium* 29%		68.9	
After treatment	Bacteria and fungi	*Verticillium* 9%			

Effect of Biofertilizers on Soil Health previous studies have shown that the use of biofertilizers positively influences soil health and nutrient availability (Tyagi and Kumar, 2021). The application of bioinoculants as soil or seed treatments increases plant nutrient availability, leading to improved agricultural output and growth (El-Tarabily, 2004). In our study, the use of a mixed biofertilizer resulted in a significant increase in the effectiveness and percentage of biofertilizer usage in treating root rots, diseases, and nematode resistance. Nematode, bacterial, and fungal analyses revealed gains of 62, 65, and 68%, respectively, in treated soil samples. These findings highlight the importance of utilizing biofertilizers to preserve soil quality and enhance crop productivity.

Nanotechnology in Agriculture Nanotechnology has emerged as a promising field in sustainable agriculture. Nanoparticles, such as ZnO nanoparticles, have unique physicochemical characteristics that can significantly impact soil quality and plant growth (Tiwari et al., 2008; Al Jabri et al., 2022). Our study focused on using ZnO nanoparticles as a nano-biofertilizer in agricultural settings. The application of ZnO nanoparticles improved seed germination, enriched crop quality, and provided balanced nutrients to the plants. These nanoparticles have the potential to increase plant growth and production, ultimately leading to higher agricultural yields (Kumar et al., 2016; Sheteiwy et al., 2021; Shahid et al. 2023).

Environmental Benefits of Biofertilizers Chemical fertilizers have been widely used in agriculture, but their overuse has led to detrimental effects on the environment and soil quality (García-Fraile et al., 2015; Chaudhary et al., 2022). In contrast, biofertilizers offer an eco-friendly alternative that can meet plant nutritional needs without increasing chemical fertilizer usage or polluting the environment. The use of biofertilizers contributes to the improvement of soil's chemical, biological, and physical characteristics, enhancing overall soil health (Yang and Zhang, 2023).

Microorganisms, including lactic acid bacteria and Saccharomyces cerevisiae, have a significant impact on promoting plant growth and enhancing resistance against pests and diseases. These microorganisms have been recognized as promising alternatives for stimulating plant growth in different crop varieties (Gaballa, 2017).

Conclusion

To properly handle issues with soil fertility, it is essential to raise awareness of and use of biofertilizers. Adding microorganisms by inoculation can improve the water status of plants, which is especially advantageous for crops grown in arid soils. Plant nutrient needs can be met while maintaining soil health and environmental well-being by the regular application of chemical and biological fertilizers. ZnO nanoparticle synthesis, as evidenced by XRD, IR, SEM, and TEM pictures, has demonstrated encouraging outcomes in crop protection, soil productivity enhancement, and environmental safety when combined with microorganisms in plant soil water. It is therefore strongly advised for usage in the future. Researchers have some future recommendations based on the present study.

Biofertilizers Production

Customization and Optimization: Develop customized biofertilizers tailored to specific soil types, crops, and environmental conditions through the optimization of microbial consortia.

Advanced Fermentation Techniques: Utilize advanced fermentation techniques, such as solid-state fermentation and submerged fermentation, to enhance the production efficiency of biofertilizers Biotechnological Approaches: Employ biotechnological approaches, including genetic engineering and synthetic biology, to enhance the nutrient-fixing capabilities and stress tolerance of biofertilizer microbes Waste Recycling: Explore the use of organic waste materials, such as agricultural residues and food waste, as substrates for biofertilizer production to promote circular economy principles.

Field Trials and Validation: Conduct extensive field trials and validation studies to evaluate the efficacy, environmental impact, and economic feasibility of biofertilizers under diverse agroecosystems.

Climate Change and Environmental Applications of Nanoparticles from Microbial Isolates:

Nanoparticle Synthesis: Investigate novel methods for synthesizing nanoparticles from microbial isolates with enhanced efficiency, scalability, and sustainability.

Functionalization and Stabilization: Develop techniques for functionalizing and stabilizing nanoparticles to improve their dispersibility, bioavailability, and environmental persistence.

Targeted Delivery Systems: Design targeted delivery systems for nanoparticles to enhance their uptake by plants, mitigate environmental risks, and minimize off-target effects.

Biochar Nanocomposites: Explore the incorporation of nanoparticles into biochar matrices to create multifunctional nanocomposites with synergistic effects on soil fertility, carbon sequestration, and crop productivity.

Risk Assessment and Regulation: Address knowledge gaps regarding the potential ecotoxicological effects and long-term environmental fate of nanoparticle-based products through comprehensive risk assessment studies and the development of regulatory frameworks.

Authors Contribution

Aiad Abdelkareim Akhreim and Mahmoud F. Gaballa write original draft; Gomaa Sulaiman data curation; Idress Hamad Attitalla; reviewing, editing and funding.

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