EFFECTS OF NANOPARTICLES ON GROWTH ENHANCEMENT OF TOMATO PLANT (*Solanum lycopersicum*) GROWTH PROMOTING BACTERIA

By

Eunice Ojimaojo OBONUMAH (Miss)

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UNIVERSITY OF BENIN

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CERTIFICATION

We certify that this research work was carried out by **Eunice Ojimaojo OBONUMAH (Miss)** of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

Prof. H. O. Shittu _____

Project Supervisor Signature and Date

Prof. E. D. Vwioko _____

Head of Department Signature and Date

DEDICATION

This work is dedicated to God Almighty for His guidance, direction, and strength, as well as to my parents and siblings for their support throughout.

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ABSTRACT

Nanotechnology offers a promising solution to soil degradation and environmental pollution caused by traditional agrochemical practices, enhancing crop productivity and nutrient-use efficiency at lower doses. The present study investigated the effect of nanoparticles (NPs) on the growth enhancement potential of plant growth-promoting bacteria (PGPB). Bacteria from tomato rhizospheric soil were isolated and characterized for growth promotion. Magnesium oxide nanoparticles (MgONPs) and zinc oxide nanoparticles (ZnONPs) were synthesized using *Azadirachta indica* and *Moringa oleifera* leaves, respectively. The nanoparticles' effect on PGPB growth was evaluated by culturing them on potato dextrose agar (PDA) supplemented with different concentrations of NPs (100, 75, 50, and 25, and 0%). The number of colonies forming units were counted after 2 days of incubation. The results indicated that *Escherichia coli* and *Bacillus* species were the isolated PGPB. The MgONP significantly enhanced the growth of *E. coli*, with colony counts increasing from 300 cfu/ml in the control group to 1,550 cfu/ml at 100% concentration. This suggests that MgONP creates a favourable environment for bacterial proliferation, potentially enhancing beneficial traits, such as nitrogen fixation. Conversely, ZnONP exhibited a complex interaction, completely inhibiting *E. coli* growth at 100% concentration while negatively affecting *Bacillus* sp. growth as well. Despite this, *Bacillus* sp. showed increased colony counts with MgONP treatment, highlighting its potential role in promoting plant health. These findings underscore the potential of NPs, particularly MgONP, to enhance beneficial bacterial populations in agricultural settings, thereby improving crop health and yield. The research advocates for further investigation into the field trial and mechanisms of action and the environmental implications of nanoparticle use in agriculture, aiming to optimize formulations for sustainable agricultural practices.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND STUDY

Nanoparticles have emerged as a promising tool to enhance the growth and activity of Plant Growth Promoting Bacteria (PGPB), which are beneficial microorganisms that stimulate plant growth and development through various mechanism, including nutrient solubilization, phytohormones production, and biocontrol activities. Nanoparticles have the potential to offer several advantages over traditional agricultural methods, such as their large surface area to volume ratios, enhanced mass transfer capabilities, and the ability to deliver lower concentrations of nutrients or pesticides in a slow, controlled, and targeted manner to boost crop productivity when applied correctly (Hussain *et al*., 2023). While the use of nanoparticles has significantly advanced crop production, the effects of Nano-based amendments on plant-associated microorganisms remain poorly understood. Similar to conventional chemical fertilizers and pesticides, these Nano-based solutions may influence the microorganisms that support plant health. The impact of nanoparticles on these microorganisms can be either beneficial or detrimental, depending on various factors. Therefore, further research into the interactions and responses of plant-associated microorganisms to nanoparticles is essential to ensure sustainable practices in precision agriculture.

A vast majority of plant-associated microorganisms are found in the soil, near plant roots in the region called the rhizosphere, where they serve essential ecological functions, such as promoting plant growth (Bello-Akinosho *et al*., 2021). Although plants associated microorganisms can exert

both negative and positive impacts of on the host plants, the focus of this review is majorly on the impact on beneficial plant associated microorganisms. Plant beneficial bacteria residing in the rhizosphere are termed plant growth-promoting bacteria (PGPB) while microorganisms that colonize the endosphere (interior of the plant) are termed endophytes. Both endophytes and PGPR could be classified as Plant growth-promoting microorganisms (PGPMs) if they are able to improve plant growth directly and indirectly. Examples of plant microbes that could be beneficial for such relationships are Bacillus and Pseudomonas that have been identified as predominant and diverse genera of PGPR. They play an important role as biocontrol agents through the protection of plants against phytopathogens (Santoyo *et al*., 2012).

PGPR plays an important role in enhancing plant growth through a wide variety of mechanisms. The mode of action of PGPR that promotes plant growth includes (i) abiotic stress tolerance in plants; (ii) nutrient fixation for easy uptake by plant; (iii) plant growth regulators; (iv) the production of siderophores; (v) the production of volatile organic compounds; and (vi) the production of protection enzyme such as chitinase, glucanase, and ACC-deaminase for the prevention of plant diseases (Choudhary, *et al*., 2011). However, the mode of action of different PGPR varies depending on the type of host plants. PGPR are very effective at promoting plant growth and development, a select few bacterial species may inhibit growth. However, this negative impact may only occur under certain specific conditions and also by some particular traits. Thus, the selection of a particular strain is of the utmost importance in obtaining maximum benefits in terms of improved plant growth and development (Dey *et al*., 2004).

1.2 PLANT GROWTH-PROMOTING BACTERIA

Microorganisms flourish in the rhizosphere by utilizing root exudates as sources of carbon and nutrients necessary for their growth and metabolic processes (Dlamini *et al*., 2022). The rhizosphere extends from 2 to 80 mm away from the root surface, varying with the plant species. This zone may expand due to increased exudation, which can be triggered by heightened microbial activity. For example, mycorrhizal fungi enhance the ability of plant roots to access a larger soil volume through their hyphal networks, forming a mutualistic relationship that allows them to extract nutrients for the host plants beyond the immediate rhizosphere (Lanfranco *et al*., 2017). The microorganisms associated with plants in this area serve various roles, acting as symbionts, pathogens, and food sources for other microbes (Munir *et al*., 2022). Notable genera of plant growth-promoting rhizobacteria (PGPR) that contribute to increased crop yield include *Azospirillum, Bacillus, Burkholderia, Enterobacter, Flavobacterium, Pseudomonas, Rhizobium, Frankia, Clostridium, Klebsiella, Serratia,* and *Streptomyces* (Lopes *et al*., 2021). Additionally, several fungal groups, such as *Aspergillus, Fusarium, Penicillium, Piriformospora, Phoma, and Trichoderma,* are vital for enhancing agricultural productivity (Hossain *et al*., 2017). Among the various interactions between plants and rhizosphere microorganisms, biological nitrogen fixation has been the focus of extensive research (Chen *et al*., 2019; Li *et al*., 2019). Nitrogen-fixing bacteria, such as *Rhizobia*, convert unavailable atmospheric N_2 into a form accessible to plants. Free-living nitrogen-fixing rhizobacteria, including *Azotobacter, Azospirillum, Bacillus, and Klebsiella,* operate independently, while symbiotic partners like *Rhizobium, Bradyrhizobium, Mesorhizobium,* and *Sinorhizobium* collaborate with plants to exchange nitrogen for essential growth nutrients and protection (Munir *et al*., 2022). Beyond nitrogen fixation, plant growth-promoting microorganisms (PGPMs) produce organic chelating agents that enhance the

availability of nutrients such as phosphorus, manganese, iron, zinc, and copper for plants, as well as secondary metabolites and phytohormones that promote bio control, plant stimulation, and overall health within the rhizosphere (Emmanuel and Babalola, 2020).

1.2.1 Definition and importance of Plant growth-promoting bacteria

Plant growth promoting bacteria (PGPB) is a group of bacteria that can be found in the rhizosphere (Ahmad *et al*., 2008). The term "plant growth promoting bacteria" refers to bacteria that colonize the roots of plants (rhizosphere) that enhance plant growth. Rhizosphere is the soil environment where the plant root is available and is a zone of maximum microbial activity resulting in a confined nutrient pool in which essential macro- and micronutrients are extracted. The microbial population present in the rhizosphere is relatively different from that of its surroundings due to the presence of root exudates that function as a source of nutrients for microbial growth (Burdman *et al*., 2000). Weller and Thomashow (1994), prove that the narrow rhizosphere zone is rich in nutrients for microbes compared to the bulk soil; this is shown by the quantity of bacteria that are present surrounding the roots of the plants, generally 10 to 100 times higher than in the bulk soil. The microbial colonizing rhizosphere includes bacteria, fungi, acticomycetes, protozoa, and algae. However, bacteria are the most abundant microbial present in the rhizosphere (Weller and Thomashow 1995). The enhancement of plant growth by the application of these microbial populations is well known and proven (Saharan and Nerah, 2011). The term "plant growth promoting bacteria (PGPB)" for these beneficial microbes was introduced by Kloepper and Schroth (1978), paving the way for greater discoveries on PGPB. PGPR are not only associated with the root to exert beneficial effects on plant development but also have positive effects on controlling phytopathogenic microorganisms(Son *et al*., 2014). Therefore, PGPB serve as one of the active ingredients in bio fertilizer formulation. Based on

their interactions with plants, plant growth-promoting bacteria (PGPB) can be classified into two categories: symbiotic bacteria, which inhabit the interior of plants and directly exchange metabolites, and free-living rhizobacteria, which exist outside of plant cells (Grey and Smith, 2005). The mechanisms through which PGPB operate can also be divided into direct and indirect methods. Direct mechanisms include bio fertilization, root growth stimulation, rhizoremediation, and the management of plant stress. In contrast, indirect mechanisms involve biological control, where rhizobacteria promote plant growth by mitigating the effects of diseases through processes such as antibiosis, systemic resistance induction, and competition for nutrients and habitats (Egamberdieva and Lugtenberg, 2014). Symbiotic bacteria typically reside in the intercellular spaces of their host plants, though some can form mutualistic relationships that allow them to penetrate plant cells. Additionally, a few species can integrate their physiology with that of the plant, leading to the development of specialized structures. Notably, rhizobia are well-known mutualistic bacteria that form symbiotic associations with leguminous crops, fixing atmospheric nitrogen in specific root structures called nodules.

Plant growth-promoting bacteria (PGPB) play a crucial role in enhancing plant growth and productivity through various mechanisms. These beneficial microorganisms can directly promote plant development by facilitating resource acquisition or modulating plant hormone levels (Rocheli *et al*., 2015). PGPB provide plants with essential nutrients like fixed nitrogen, iron, and phosphorus that may be lacking in agricultural soils, thus reducing dependence on chemical fertilizers (Bernard, 2012). Using PGPB instead of chemical fertilizers can contribute to more sustainable agriculture practices by reducing production costs and environmental pollution associated with fertilizer use (Rocheli *et al*., 2015). Many PGPB strains have been commercialized and used as biofertilizers and biopesticides in agriculture, though their extensive

adoption is still limited compared to traditional farming methods (Bernard, 2012). PGPB can also indirectly enhance plant growth by decreasing the inhibitory effects of pathogens on plant development (Rocheli *et al*., 2015). They protect plants from disease and abiotic stresses through various mechanisms, including microbial antagonism and induced systemic resistance (Swamy, 2023). This protection helps reinforce plant defenses and triggers signal responses that activate the plant's defense system (Swamy, 2023). The success and efficiency of PGPB as inoculants for agricultural crops are influenced by factors such as root colonization efficiency, plant root exudation, and soil health (Rocheli *et al*., 2015). Further research is needed to identify optimal PGPB strains for different crops and environments, develop effective application methods, and understand interactions between PGPB and other soil microorganisms (Rocheli *et al*., 2015). PGPB also represent a promising approach to promoting sustainable agriculture by enhancing crop productivity while reducing chemical fertilizer use and environmental impacts. Continued research and development of PGPB-based approaches could significantly improve agricultural practices worldwide.

1.2.2 Mechanism of plant growth-promoting bacteria

Plant growth-promoting bacteria (PGPB) enhance plant growth through both direct and indirect mechanisms (Glick, 1995). Direct mechanisms involve bacterial traits that directly foster plant growth, such as the production of hormones like auxin, ACC deaminase, cytokinin, gibberellin, nitrogen fixation, phosphorus solubilization, and iron sequestration via siderophores. Indirect mechanisms involve traits that inhibit plant pathogens, including the production of antibiotics, cell wall-degrading enzymes, competition for resources, hydrogen cyanide, and induced systemic resistance.

Different PGPB can possess various traits, making them more suitable for specific environmental conditions such as temperature or pH levels. No single organism can utilize all mechanisms available for promoting plant growth, and various PGPB inoculants enhance growth through at least one of these methods (Saharan and Nehra, 2011).

a) Direct mechanism

Plant growth-promoting bacteria (PGPB) enhance plant growth through various mechanisms, primarily by producing phytohormones that influence physiological processes from germination to senescence (Vessey, 2003). Understanding the concentrations of these hormones is crucial for elucidating their roles in plant development.

I. **Indole-3-acetic acid (IAA):** As a natural auxin, IAA plays a significant role in plant growth, affecting cell division, elongation, differentiation, tropism, flower development, and vascular patterning (Gravel *et al*., 2007). It is synthesized from L-tryptophan by both plants and various microorganisms, including PGPR. Root tissues are particularly sensitive to IAA levels, which can stimulate root hair density and enhance nutrient and water absorption (Tanimoto, 2005). Bacteria such as *Pseudomonas* and *Rhizobium* are known for their IAA-producing capabilities, which correlate with their plant growth-stimulating effects (Patten and Glick, 2002; Gravel *et al*., 2007).

II. **Ethylene**: This gaseous hormone regulates several physiological processes, including seed dormancy, root and shoot differentiation, adventitious root formation, and fruit ripening (Babalola, 2010b). Ethylene production can be significantly increased under stress conditions, promoting root development. However, excessive ethylene can inhibit root growth and result in abnormal plant development. Ethylene is synthesized from

methionine, with ACC serving as a precursor. Some PGPB possess the enzyme ACC deaminase, which breaks down ACC, lowering ethylene levels and providing nitrogen and carbon sources for the bacteria (Dobbelaere *et al*., 2003). This action helps mitigate the inhibitory effects of high ethylene concentrations (Shaharoona *et al*., 2006).

III. **Gibberellins (GA):** A group of hormones that regulate various growth processes, including seed germination, stem elongation, and flower induction (Boemke and Tudzynski, 2009). The first gibberellin discovered was GA3, isolated from the fungus *Gibberella fujikuroi*. PGPR such as *Azospirillum* can produce GA1 and GA3, influencing plant growth through their metabolic pathways (Gutierrez-Manero *et al*., 2001).

IV. **Cytokinins**: These hormones are involved in numerous physiological processes, including the regulation of root and shoot growth, branching, and leaf senescence (Ortiz-Castro *et al*., 2009). Cytokinin signaling is critical for mediating plant responses to PGPB, such as *B. megaterium*. The presence of cytokinins in both bacterial biomass and culture media can promote plant growth, but excessive levels may inhibit root development and disrupt normal growth patterns.

V. **Nitrogen Fixation:** Nitrogen is a crucial nutrient for the growth of all living organisms, including plants and bacteria. Although nitrogen makes up about 78% of the Earth's atmosphere, it exists primarily as gaseous N_2, which is not easily usable by most organisms. To be assimilated by plants, nitrogen must first be converted to ammonia, a process that requires significant energy due to the strong triple bond in N_2 (Baas *et al*., 2014). This energy can come from fossil fuels, biological nitrogen fixation, or other nitrogen input mechanisms (Zhang *et al*., 2015). Various nitrogen-fixing bacteria have been identified, particularly those that form symbiotic relationships with legumes, such as

Rhizobium, *Sinorhizobium*, and *Mesorhizobium* (Babalola, 2010; Pérez-Montaño *et al*., 2014; Turan *et al*., 2016). These bacteria colonize plant root cells, leading to the formation of root nodules, where they exist as wall-less bacteroids and fix atmospheric nitrogen using the enzyme nitrogenase, producing ammonia. In return, the plant supplies the bacteria with organic acids from photosynthesis, which are essential for the bacteria's growth. While some free-living bacteria can also fix nitrogen, they do so less efficiently and are not commonly used commercially. A challenge in nitrogen fixation is a side reaction where nitrogenase reduces protons (H+) to hydrogen gas (H2), resulting in energy loss and reducing the overall efficiency of nitrogen fixation by about 30%. However, certain rhizobia strains possess an enzyme called hydrogenase, which can recover H2 from the atmosphere and convert it back into H+, thereby conserving energy for further nitrogen fixation (Adams *et al*., 1981). This ability helps improve the efficiency of the nitrogen-fixing process.

VI. **Phosphate Solubilization:** Phosphate solubilizing bacteria (PSB) are crucial for making phosphorus more accessible to plants without harming the environment (Alori *et al*., 2017). They convert insoluble organic and inorganic phosphates into forms that plants can readily absorb. The effectiveness of these bacteria is influenced by environmental, plant, soil conditions, and the specific bacterial strains present (Gupta *et al*., 2015). Powerful phosphate solubilizers are primarily found in genera such as *Bacillus*, *Rhizobium*, and *Pseudomonas*, as well as non-symbiotic nitrogen fixers like *Azotobacter* and *Azospirillum* (Banerjee *et al*., 2005; Saharan and Nehra, 2011). Their main mechanism for solubilizing inorganic phosphate involves producing mineral-dissolving compounds, including hydroxyl ions, organic acids, protons, siderophores, and carbon

dioxide (CO2) (Rodríguez and Fraga, 1999). Organic acids, such as gluconic and keto gluconic acids, lower the pH of the surrounding environment, facilitating the release of phosphate ions by chelating cations (Khosro, 2012). The production of protons and bicarbonate, alongside gas exchanges (O2/CO2), contributes to this acidification process (Sharma *et al*., 2017). Consequently, there is an inverse relationship between the pH of the rhizosphere and phosphorus availability. A significant amount of phosphorus in soil exists in organic forms, with up to 30-50% potentially being organic phosphorus, primarily from inositol hex phosphate (phytate). Phytate is generally not accessible to plants due to the low production of the phytase enzyme by plant roots. However, many plant growth-promoting bacteria (PGPB) can solubilize phytate and other organic phosphorus compounds, such as phosphomonoesters, phosphodiesters, and nucleic acids, which must be broken down into lower molecular weight forms for plant assimilation (Rodríguez and Fraga, 1999; Peix *et al*., 2001). The process of phosphorus mineralization refers to the solubilization of organic phosphorus and the degradation of its molecular structure triggered by insufficient phosphate availability in the soil.

VII. **Siderophores:** Siderophores are small peptide molecules with functional groups that bind to ferric ions, acting as iron chelators and carriers (Goswami *et al*., 2016). These molecules are essential for microbes, as they help transport iron, which is crucial for various biological processes. Many siderophores have been identified in different microbial species and can be species-specific (Sandy and Butler, 2009). Siderophore-producing plant growth-promoting bacteria (PGPB) can inhibit the growth of pathogens by reducing the availability of iron in the environment (Shen *et al*., 2013). By secreting these high-affinity siderophores, PGPB effectively bind to most of the available Fe3+ in

the rhizosphere, taking up the iron for themselves or for the host plant. This competition for iron prevents fungal and bacterial pathogens from accessing the necessary iron for their growth, thereby limiting their ability to proliferate and act as pathogens. The biocontrol effectiveness of this mechanism relies on the significantly higher affinity of PGPB siderophores for iron compared to those produced by fungi (Kloepper *et al*., 1980). Various studies have demonstrated the iron-supplying capabilities of siderophores from different bacterial species. For instance, siderophores from *Chryseobacterium spp*. have been shown to effectively supply iron to tomato plants when delivered to their roots (Radzki *et al*., 2013). Additionally, certain *Pseudomonas* strains have been linked to increased germination and plant growth due to their siderophore production (Sharma and Johri, 2003). Overall, siderophores play a critical role in promoting plant health by facilitating iron uptake and enhancing resistance to pathogens.

b) Indirect Mechanism

Indirect mechanisms improve plant health by suppressing pathogens and enhancing the plant's defense systems. On average, various plant diseases reduce plant yields by around 10%/year in more developed countries and by about 20%/year in less developed countries of the world. In an efort to decrease the widespread use of chemicals as a means of preventing phytopathogen damage to plants, scientists have been developing the use of certain environmentally friendly PGPB as biocontrol agents (Glick and Bashan 1997; Lucy *et al*. 2004) with many of these organisms already available commercially. This mechanism can be seen in the following:

I. **Antibiotics:** The main way plant growth-promoting bacteria (PGPB) help protect plants from harmful pathogens is by producing antibiotics (Couillerot *et al*., 2009; Haas and Keel, 2003; Raaijmakers and Mazzola, 2012). However, an antibiotic effective against

one pathogen might not work against another, and the effectiveness of PGPB can change depending on field conditions and how they are grown and applied (Glick, 2015c). The role of antibiotics in disease control by PGPB comes from two types of studies (de Jesus Sousa and Olivares, 2016). First, bacteria that do not produce antibiotics have been shown to lose their ability to protect plants from certain pathogens (Heimpel and Mills, 2017). Second, specific antibiotics isolated from PGPB have been found to inhibit the same pathogens that the bacteria themselves can control (Glick, 2015c). Many antibiotics are produced by bacteria like *Bacillus* and *Pseudomonas*, which create various substances that are antifungal, antibacterial, and more. For example, *Bacillus* produces antibiotics such as bacilysin and surfactin, while *Pseudomonas* produces compounds like phenazine-1-carboxylic acid and rhamnolipids (Goswami *et al*., 2016). Researchers have identified gene clusters in *Bacillus subtilis* that coordinate the production of these antibiotics through specific enzymes (Chang *et al*., 2007).

II. **Cell wall degrading enzymes:** Many plants respond to infections from fungal pathogens by activating the production of enzymes that degrade fungal cell walls. These enzymes include chitinase, which breaks down chitin—a key component of the cell walls of many fungi (Husson *et al*., 2017)—and β-1,3-glucanase, which targets cell wall carbohydrates (Vaddepalli *et al*., 2017). Additionally, proteases degrade proteins in the fungal cell wall, while lipases break down certain lipids associated with it. Together, these enzymes can help lyse fungal cells (Friedrich *et al*., 2012; Gortari and Hours, 2008). Some biocontrol plant growth-promoting bacteria (PGPB) also produce similar enzymes (Chernin *et al*., 1995). Laboratory experiments have demonstrated that genetically modifying PGPB strains to include genes for these enzymes enhances their effectiveness as bio control

agents (Koby *et al*., 1994). For example, chitinase genes can be overexpressed, and strains can be co-transformed with an acetamidase gene, which improves chitinase activity, as shown in studies using a specific promoter (Kowsari *et al*., 2016; Limon *et al*., 1999). Enzymes like chitinases, peroxidases, and β-1,3-glucanases belong to a group called pathogenesis-related (PR) proteins, and their activation can induce systemic resistance (ISR) in plants (Yedidia *et al*., 1999). *Bacillus sp*. JS has been shown to enhance the expression of PR-2 and PR-3 genes, which encode for β-1,3-glucanase and chitinase, respectively (Kim *et al*., 2015).

III. **Hydrogen Cyanide:** Several biocontrol plant growth-promoting bacteria (PGPB) are capable of producing hydrogen cyanide (HCN). If HCN were the sole mechanism for biocontrol, its low concentration would generally be ineffective against most fungal pathogens. However, many HCN-producing PGPB also produce antibiotics or enzymes that degrade cell walls (Ramette *et al*., 2006). Additionally, the low levels of HCN can enhance the effectiveness of antifungal agents, helping to prevent the development of resistance in fungi. This suggests that HCN works synergistically with other biocontrol strategies employed by the same bacteria. HCN exerts its toxicity by inhibiting cytochrome C oxidase and other crucial metalloenzymes (Nandi *et al*., 2017). Many bacterial genera, including *Rhizobium, Pseudomonas, Alcaligenes, Bacillus,* and *Aeromonas*, have been identified as HCN producers (Ahmad *et al*., 2008; Das *et al*., 2017; Zachow *et al*., 2017). For example, HCN has been linked to the suppression of tomato root knot disease caused by *Meloidogyne javanica* (Siddiqui *et al*., 2006) and the control of the pest *Odontotermes obesus* in India (Kumar *et al*., 2015).

IV. **Induced Systemic Resistance (ISR):** Induced systemic resistance (ISR) is a defense mechanism activated by non-pathogenic microbes, including certain plant growth-promoting bacteria (PGPB), to help plants resist pathogens (Van Loon *et al*., 1998). Studies have shown that ISR can protect plants from damage caused by various pathogens, such as when *Pseudomonas fluorescens* strain WCS417r was used against *Fusarium oxysporum* (Halfeld-Vieira *et al*., 2006; Van Peer and Schippers, 1992). When plants are pre-treated with PGPB, they become primed to respond more effectively to future attacks. ISR offers broad protection against different pathogens, activating dormant defense mechanisms in plants through coordinated signaling pathways regulated by plant hormones (Pieterse *et al*., 2012, 2014; Walters *et al*., 2013). Some PGPB can produce salicylic acid (SA), which triggers another defense mechanism known as systemic acquired resistance (SAR), typically induced by pathogens (Chen *et al*., 1999). While SA can activate protective mechanisms, its role in PGPB-induced resistance is rare compared to ISR (Zhang *et al*., 2002). SAR involves the activation of pathogenesis-related (PR) genes, with PR-1 being a well-known biomarker for this response (van Loon *et al*., 2006). The regulation of both ISR and SAR is heavily influenced by the protein NPR1, which is crucial for transcriptional regulation and is synthesized in the cytoplasm. NPR1 plays a significant role in the ISR activated by *Pseudomonas fluorescens* and other PGPB (Pieterse *et al*., 1998; Abo-Elyousr *et al*., 2009; Lucas *et al*., 2014; Weller *et al*., 2012; Yi *et al*., 2013). It helps establish SAR by activating PR genes in response to SA accumulation. Although both ISR and SAR are effective defense strategies, they operate through different signaling pathways, leading to slight variations in their effectiveness (Ton *et al*., 2002).

1.2.3 Role of Plant growth-promoting bacteria in food scarcity

Plant growth-promoting bacteria play a crucial role in addressing food scarcity through multiple mechanisms that enhance crop productivity and plant health [Ahmad *et al*., 2016; Bashan *et al*., 2017]. These beneficial microorganisms increase crop yields and productivity by producing plant growth regulators, enhancing nutrient acquisition, and improving stress tolerance in plants [Bashan *et al*., 2017; Chen *et al*., 2020]. They also contribute to improved water use efficiency through ACC deaminase production and drought tolerance enhancement [Ahmad *et al*., 2016]. Additionally, PGPB facilitate nutrient cycling by solubilizing phosphates and fixing atmospheric nitrogen, making these essential nutrients more accessible to plants [Khan *et al*., 2020]. Their interaction with soil microorganisms improves overall soil health, while their ability to enhance plant stress tolerance allows crops to thrive in challenging environments [Ahmad *et al*., 2016]. Furthermore, PGPB exhibit synergistic effects when combined with other beneficial organisms like mycorrhizal fungi or beneficial nematodes [Lugtenberg *et al*., 2016]. By addressing key factors such as increased yields, improved resource utilization, enhanced soil quality, and stress resilience, PGPB help mitigate food scarcity challenges in agriculture [Chen *et al*., 2020; Khan *et al*., 2020]. As research continues to advance our understanding of PGPB-plant interactions, their potential role in sustainable agriculture and global food security becomes increasingly significant [Glick *et al*., 2007], offering a promising approach to address the complex issue of food scarcity.

1.3 NANOPARTICLES AND THEIR BENEFITS

Nanotechnology has been described as the understanding and control of matter in the range of 1 to 100 nm (Rajput *et al*., 2018). Particle dimensions within this range are considered nanoparticles (NPs) (Taghavi *et al*., 2013). Nanoparticles are distinguished based on their core material (organic or inorganic). Inorganic NPs are further divided into metal (Al, Bi, Co, Cu, Au,

Fe, In, Mo, Ni, Si, Ag, Sn, Ti, W, Zn), metal oxide (Al2O3, CeO2, CuO, Cu2O, In2O3, La2O3, MgO, NiO, SiO2, TiO2, SnO2, ZnO, ZrO2), of which Ag, ZnO, TiO2, FeO, and CuO are often utilized and their harmful effects on the activity, diversity, and abundance of flora and fauna are closely observed (Rajput *et al*., 2018). The impact of direct exposure of plants to nanoparticles should not be ignored as they may pose both negative and/or positive effects on soil health as well as crop growth and quality. The factors that influence the effects of nanoparticles include the type and size of the nanoparticle, plant species, nanoparticle concentration, and length of time that the soil/crop was exposed to the nanoparticles (Duan and Li, 2013). In a study done by An *et al*. (2008) silver nanoparticles boosted ascorbate and chlorophyll in the leaves of asparagus (*Asparagus officinalis* L.). These findings provide examples of the beneficial effects of nanoparticles. In a different study, silica nanoparticles applied to maize seedlings increased seed germination, root and shoot length, photosynthesis, and dry weight (Suriyaprabha *et al*., 2012)**.**

1.3.1 Plant-mediated synthesis of nanoparticles

Plant-mediated biosynthesis of nanoparticle is considered a widely acceptable technology for rapid production of metallic nanoparticles for successfully meeting the excessive need and current market demand and resulting in a reduction in the employment or generation of hazardous substances to public health. Similar to microbes which have been used as a "bio-factory" in the synthesis of metallic nanoparticles, plants are also the natural "chemical factories" which are economical and require minimal maintenance (Nyoman Rupiasih *et al*. 2013). Plants have several cellular structures and physiological processes to combat the toxicity of metals and maintain homeostasis. They also possess dynamic solutions to detoxify metals and hence scientists have now turned into phytoremediation (Abboud *et al*. 2013). The modus operandi of detoxification includes immobilization, exclusion, chelation, and compartmentalization of the

metal's ions, and the expression of more general stress response mechanisms, such as ethylene and stress proteins (Sánchez *et al*. 2011). The ability to tolerate inimical concentrations of toxic metals is found in the plant kingdom from ages. Their ability to accumulate high concentrations of metals was observed for both essential nutrients, such as copper (Cu), iron (Fe), zinc (Zn), and selenium, as well as non-essential metals, such as cadmium (Cd), mercury (Hg), lead (Pb), aluminum (Al), and arsenic (As) (Sahayaraj *et al*. 2012). In plants or plant derived materials, a wide range of metabolites with redox potentials is determined, which are playing a principal role as a reducing agent in the biogenic synthesis of nanoparticles. In comparison to the microbial synthesis of nanoparticles, highly stable nanoparticles are synthesized by plant or plant extracts with the higher rate of production. Consequently, the advantages of plant-mediated preparation of metal nanoparticles lead researchers to in search of further exploration of the bio-reduction mechanism of metal ions by plants and the possible mechanism of formation of metal nanoparticle in and by the plants (Ahmad and Sharma 2012). In recent years, biosynthesis of metal nanoparticles, especially silver and gold nanoparticles, using plant extracts as Nano-factories becomes an important subject of researches in the field of bio-nanotechnology (Iravani 2011). Based on all aforementioned information, a schematic diagram of a proposed mechanism for plant-mediated fabrication of metal nanoparticles is illustrated in Fig. 2. Generally, the bio-reduction mechanism of metal nanoparticle in plants and plant extracts includes three main phases (Makarov *et al*. 2014). The activation phase in which the reduction of metal ions and nucleation of the reduced metal atoms occur. The growth phase, referring to the spontaneous coalescence of the small adjacent nanoparticles into particles of a larger size, accompanied by an increase in the thermodynamic stability of nanoparticles, or a process referred to as Ostwald ripening and the termination phase in which the final shape of the nanoparticles formed.

1.3.2 Advantages of plant-mediated synthesis of nanoparticles

Due to their easy availability, green preparation of nanoparticles using plant extracts turns out to be an important research subject in the field of bio-nanotechnology in this era. Principally, the biogenic synthesis employs plant extracts in aqueous form in the fabrication of noble nanoparticles for the reason that the availability of reducing agent is higher in the extract than the whole plant (Huang *et al*. 2007). Besides, plant-mediated synthesis of nanoparticles is simpler and easier to be conducted without requiring any specific operating conditions as compared to typical physical and chemical methods. The synthesized products of the process including waste products are resulted from natural plant extracts, and hence this technique is also more environmental green. Nevertheless, both strong and weak chemical reducing agents and capping agents such as sodium citrate, sodium borohydride, and alcohols, which are mostly toxic, flammable, and cannot be degraded easily, are required in the physical and chemical methods (Lalitha *et al*. 2013). Through this bio-based protocol of nanoparticles synthesis, higher reproducibility of the process and higher stability of the synthesized nanoparticles can be attained. Therefore, this green-based fabrication of nanoparticles is suitable for large scale production with more effective cost investment, eco-friendly, and safe for human therapeutic use. Apart from the aspects of reproducibility and stability, the rate of bio-reduction of metal ions using biological agents is showed to be much faster and also at ambient temperature and pressure conditions (Pasupuleti *et al*. 2013). On the contrary, previous studies reported that the bio-reduction potential of the plant extracts is comparatively higher than the microbial culture (Khalil *et al*. 2014). Moreover, the waste products resulted from the microbial-based method is likely to be more harmful to the environment depending on the type of microbes involved in the synthesis (Moghaddam 2010). Hence, plant-mediated synthesis brings less or almost zero

contamination and so reducing the impact on the environment. With all the aforementioned advantages and outstanding features over other methods, the biosynthetic method employing plant extracts has now turned as a simple, effective and viable technique as well as a good alternative to conventional chemical and physical nanoparticle preparation methods, and even microbial methods (Huang *et al*. 2007).

1.3.3 Types of nanoparticles

a) Metallic nanoparticles

Metallic nanoparticles are popular for their excellent properties and are widely used in various applications, especially in biomedicine for drug delivery, gene delivery, bio sensing, imaging, and as antimicrobial agents (Hossain, Khan, & El-Denglawey, 2021). Common metallic nanoparticles include gold, silver, copper, and iron. Metal oxides, which are often more stable, can have different electrical properties and are used in many fields, including sensors, batteries, and energy devices. Key oxide nanoparticles include zinc oxide, iron oxides, aluminum oxide, and silica (Manzoor, Ashraf, Tayyaba, & Hossain, 2021).

b) Semiconductor nanoparticles

Semiconductor nanoparticles have special properties because of their tiny size. They're neither good conductors nor good insulators. Their ability to control light and electricity makes them useful for many things like lights, computers, lasers, and medical tools (Hossain *et al*., 2021). These nanoparticles come in different types based on what elements they're made of. Some common ones are silicon, zinc sulfide, and gallium nitride (Hossain *et al*., 2021). They can be made in different shapes too, like spheres with a core inside. This allows scientists to create materials with just the right properties for specific jobs (Dahman,

2017; Nayak *et al*., 2017). The unique properties of these tiny particles make them very useful in lots of industries and technologies. Scientists are still learning how to make and use them effectively (Hossain *et al*., 2021)

c) Carbon - based nanoparticles

Carbon- based nanoparticles predominantly; carbon-based nanoparticles are split into carbon nanotubes (CNTs) and fullerenes. The use of these nanoparticles tends to focus on structural reinforcement as they are 100 times stronger than steel. CNTs can be classified into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). CNTs are unique as they are thermally conductive along the length and non-conductive across the tube. So far, CNTs have been used to build transistors, aircraft, sensors and biosensors, drug delivery vehicles, batteries and other energy storage, they are also used to reinforce concrete and treat water (khan *et al.*, 2019).

d) Ceramic nanoparticles

Ceramic nanoparticles are inorganic solids at the nanoscale, primarily composed of oxides, carbides, phosphates, or carbonates of metals and nonmetals [Diao, Gall, & Dunn, 2003]. Their porous nature provides protection against physical degradation. Discovered in the early 1980s via the sol-gel process, these nanoparticles have gained popularity due to their superior mechanical properties and high-temperature resistance. They can be broadly categorized into oxide-based and monoxide-based types, with examples including silica, alumina, titania, zirconia, and silicon carbide. These materials exhibit unique properties due to their size and molecular structure, often demonstrating exceptional strength and durability compared to larger-scale ceramic materials. Recent advancements in nanotechnology have led to the development of novel structures with remarkable

mechanical properties, showcasing the versatility and potential of ceramic nanoparticles in various applications.

e) Lipid-based nanoparticles

Lipid-based nanoparticles represent a clinically advanced class of systems specialized as nanocarriers. The most vital reason why these particles garnered wide attention is their application in cancer treatment and drug delivery. Unique chemical structure makes them versatile with the capability of transporting hydrophobic, hydrophilic, and amphiphilic molecules. Moreover, they exhibit high biocompatibility and almost no toxicity. Significant research is still ongoing to engineer their functionality with help of novel as well as modified composition. Based on the architecture, lipid-based nanoparticles are classified into several types such as solid lipid nanoparticles (SLN), nanostructured lipid carriers, lipoproteins, liposomes, lipid-core micelles, hybrid lipid-nanoparticle complexes (HLNC), lipoplexes, lipid Nano emulsions, etc. (Khalid and Saadbin, 2022)

f) Polymeric nanoparticles

Polymeric nanoparticles refer to colloidal structures comprising of active compounds entrapped within or structure, polymeric nanoparticles can be further classified into either Nano capsules surface-adsorbed onto polymeric core. They are promising drug carriers because of their biocompatibility, controlled releasing profile, and non-toxic and non-immunogenic properties. Based on the morphological or Nano spheres. The distinguishing factors between these two types are: bioactive compounds are chemically integrated within polymer matrix in Nano spheres, while they are entrapped within the polymeric shell in Nano capsules. (Khalid and Saadbin, 2022).

1.3.4 Nanoparticles in agriculture

The application of nanotechnology in agriculture could have multiple benefits. In addition to field applications of agrochemicals, this technique can deliver pesticides encapsulated in nanomaterial for controlled release, stability of bio-pesticides, gradual release of nanomaterial-assisted fertilizers, bio-fertilizers, and micronutrients for efficient usage. Recently, this technology has also been used to transport genetic material for crop development. The use of nano-sensor technology to detect in the health sector, as well as in cross-border export and import. Nanoparticles, particularly porous hollow silica-based, clay-polyester, plastic starch coated or cemented nanoparticles, are also important for soil conservation, and silver nanoparticles as antifungal and antibacterial agents play a role in agricultural crop protection, where these particles also regulate proper nutrition to plants, according to the study. Furthermore, the use of this technology may be envisaged in improving the quality of agricultural products based on pesticide use, disease detection, Nano fertilizer, chemical, and bio-pesticide application, residual quantity detection in agro-products, the content of nucleic acids using Nano sensors, and soil structure maintenance by producing natural Nano-clays, among other things. As a result, it is possible to conclude that appropriate agricultural sector innovations in India will be a challenging phenomenon in the twenty-first century, with the potential to revolutionize agricultural and agricultural food production while also assisting in the proper sustainability of farm products.

1.4 JUSTIFICATION OF THE STUDY

This research aims to investigate the effects of nanoparticles on the growth enhancement of plant growth-promoting bacteria (PGPB). PGPB are known for their role in improving plant health and stress resilience, while nanoparticles have shown promise in boosting microbial activity and plant growth. By examining the interaction between nanoparticles and PGPB, this study aims to

uncover new insights into how nanoparticles may enhance the effectiveness of PGPB in promoting plant growth. The findings could contribute to developing more efficient and sustainable agricultural practices, leading to improved crop yields and better plant health.

1.5 AIM AND OBJECTIVES

This study was aimed at investigating the effect of nanoparticle on growth enhancement of plant growth promoting bacteria. The objectives of the study were to:

a) Isolate and characterize bacteria from tomato rhizosphere using morphological and biochemical tests.
b) Characterize the bacteria isolates for plant growth-promoting abilities.
c) Synthesize and characterize nanoparticles.
d) Determine the effect of biologically synthesized nanoparticles on bacterial growth.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS USED

2.1.1 Source of rhizospheric soil

The rhizospheric soil sample was collected from the root zone of a healthy tomato plant located at 14 Ogboghodor Street, Benin Technical Road, Benin City, Edo State.

2.1.2 Source of chemicals used

The chemicals and reagents used during the project include synthetic nutrient agar and broth, antifungal drug (ketoconazole), ethanol, crystal violet, iodine, methylated spirit, distilled water, hydrogen peroxide, saffranine, citrate medium, kovac reagent, tryptophan broth, peptone water, picric acid, sodium hypochloride, sodium bicarbonate and salvowski reagent used to test for plant growth promoting bacteria were purchased from Solution biotechnology laboratory and Pyrex Nigeria Limited in Benin City, Edo State, Nigeria.

2.1.3 Source of plant materials

Fresh leaves of Moringa (*Moringa oleifera*) and Neem (*Azadirachta indica*) were used for the synthesis of Zinc nitrate and Magnesium nitrate respectively. The plant materials were gotten from Market Road, Ekosodin, Benin City, Nigeria.

2.1.4 Equipment Used

Research materials used for this experiment include equipment like Microscope, pressure cooker, spectrophotometer, spirit lamp, inoculum loop, weigh balance, foil paper, glass rods, petri dish,

insulation tape, masking tape, beakers, conical flasks, test tubes, micro pipette and pipette tips, U. V light, inoculation cabinet, incubator, fridge, bowls and measuring cylinders

2.2 EXPERIMENTAL METHODOLOGY

2.2.1 Preparation of nutrient agar

The nutrient agar medium was prepared according to the manufacturer's instruction by dissolving 2.31g of powdered nutrient agar in 100ml of distilled water into a clean conical flask of 250ml. an antifungal drug (ketoconazole) was calculated and the appropriate amount was measured and added to the solution. Foil paper was used to cover the conical flask which acted as a cork to avoid contamination from the environment, after which the conical flask containing the solution was then carefully placed in a pressure cooker containing water and it was sterilized for 15 minutes. After which it was then allowed to cool to about 27 C and then poured into 4 plates (20 ml) each and allowed to gel.

2.2.2 Isolation of rhizospheric bacteria

The bacteria were isolated from the rhizospheric soil of tomato by taking 1g of each soil sample and then serial dilution was carried out. Diluent from 10^{-2} and 10^{-3} were inoculated and incubated for 24 hours.

2.2.3 Sub-culturing of rhizospheric bacteria

After 24 hours the growth of different colonies of bacteria was observed. A single and distinct colony was picked up using a sterile loop and streaked on a freshly prepared nutrient agar medium. This culture was then incubated for 24 hours.

2.2.4 Cultural and biochemical identification of rhizospheric bacteria

Cultural characteristics were observed on the nutrient agar plates. The cultural characteristics include form, shape, margin, elevation, pigmentation and optical density. Pigmentation was done by visibly observing the plate. Series of biochemical test were carried out to further identify the bacteria isolates, this includes:

a) **Catalase test**: This test helps to differentiate bacteria that can produce an enzyme called catalase. A small number of bacteria was collected using a sterile loop and placed on a clean glass slide. Two drops of hydrogen peroxide were added to the glass slide containing the bacteria and was observed for immediate and active bubbling for a positive test.

b) **Citrate test**: most bacteria use citrate as a source of carbon, this test was carried based on their ability to use the citrate or not. Simmon Citrate agar was prepared following the manufacturer's instruction in a test tube. Then a sterile loop was used to inoculate the bacteria into the medium and incubate it for 48 hours. It was observed for a colour change; a bright blue color indicates a positive result.

c) **Indole Test**: this test was carried out to test for indole production by the bacteria. A sterile wire loop was used to inoculate a number of bacteria in 2 ml of tryptophan broth and incubated for 48 hours. After which drops of Kovac reagent was added to the medium to check for indole-positive and indole negative bacteria.

d) **Gram staining**: the gram staining technique was used to differentiate between the gram-positive and gram-negative bacteria. A drop of sterile water was dropped on a clean glass slide, and an amount of bacteria isolate was mixed in it and was stirred to scatter the colony. The smear was fixed by passing it through the fire from the spirit lamp, it was

then flooded with crystal violet for 1 minute, then washed off with distilled water. It ws flooded with iodine also for 1 minute and washed off with distilled water, one drop of ethanol was then added for 5 – 10 seconds and immediately washed off , then finally the smear was flooded with saffranine for 45 seconds, this acted as a counterstain. Then it was left to air dry, after which was viewed under the microscope Gram-positive bacteria appeared dark purple, while Gram-negative bacteria appeared pink.

2.2.5 Plant growth promoting tests

a) **Indole Acetic acid (IAA) Production**: IAA is a plant growth hormone involved in promoting root growth and development. Not all Bacteria can produce IAA . Indole acetic acid (IAA) production is a property of rhizosphere bacteria that stimulate and facilitate plant growth. Bacterial cultures were grown on different Nutrient broth media for 4 days. Fully grown cultures were Centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution). Development of pink colour indicated IAA production.

b) **Production of Hydrogen Cyanide (HCN):** Hydrogen Cyanide is a volatile compound produced by several species of plant growth promoting bacteria. While HCN is known for its toxicity, its production by certain bacteria is associated with beneficial effects on plants particularly in disease resistance and growth enhancement. All the isolates were screened for the production of hydrogen cyanide. Nutrient agar was amended with 4.4 g glycine/l and bacteria were streaked on modified agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed in the top of the plate. Plates were

sealed with insulating tapes and incubated at room temperature for 4 days. Development of orange to red colour indicated HCN production.

c) **Ammonia production test**: Bacterial isolates were tested for the production of ammonia in peptone water following the manufacturer's instruction. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 4 days at room temperature. Nessler's reagent (0.5 ml) was added in each tube. Development of yellow to brown colour was a positive test for ammonia production.

2.2.6 Preparation of plant extract

The leaves of *Moringa oliefera* (moringa) and *Azadrichta indica* (neem) were collected and 40 grams was weighed using a weighing balance. The leaves were thoroughly washed with tap water (3 times) to remove any form of dirt and was washed with 1% sodium hypochloride solution for surface sterilization. The leaves were then rinsed thoroughly with distilled water, and they were shredded into tiny bits and placed in a beaker containing 100 ml of distilled water for the neem leaf and 200 ml of distilled water for the moringa leaf, the beakers were labeled and covered with foil paper then placed in a pot of water to boil for 10 minutes. After boiling it as allowed to cool and then filtered using a Whatman filter paper the extract was also labeled and covered with foil paper.

2.2.6 Preparation of precursor solution

Precursor solution of Magnesium nitrate and Zinc nitrate were prepared according to instructions. Magnesium nitrate salt (100ml) was taken from the stock solution and diluted was dissolved in 10mls of distilled water to get 0.1M Mg(NO3)2, and Zinc nitrate salt (50ml) was

also taken from the stock solution and diluted with 10 ml of distilled water to get 0.2M Zn(NO3)2 precursor solution.

2.2.7 Synthesis of nanoparticles

According to protocol used, two nanoparticles namely (magnesium nitrate and Zinc nitrate) were synthesized using two plant extract and two precursor solutions. Magnesium Nitrate nanoparticles were synthesized by adding 30mls of $Mg(NO_3)_2$ precursor to 10mls of *Azadrichta indica* (Neem plant) in the ratio 3:1 extract to make 100% MgO.NPs. Next the Zinc Nitrate was prepared by adding 50mls of $Zn(NO_3)_2$ was added to 10mls of *Moringa oleifera* plant extract in the ration 5:1 to make 100% ZnO.NPs.

2.2.8 Characterization of nanoparticles using UV-Vis spectrophotometer

Two Nanoparticles namely Magnesium Nitrate and Zinc nitrate were characterized after 24 hours of synthesis using an ultraviolet-visible (UV-Vis) spectrophotometer. The nanoparticles were characterized one after the other, each nanoparticle was 100% concentrated and was placed in a cuvette. Sterile water served as the blank and readings were gotten, which determined the wavelength and absorbance value of each nanoparticle.

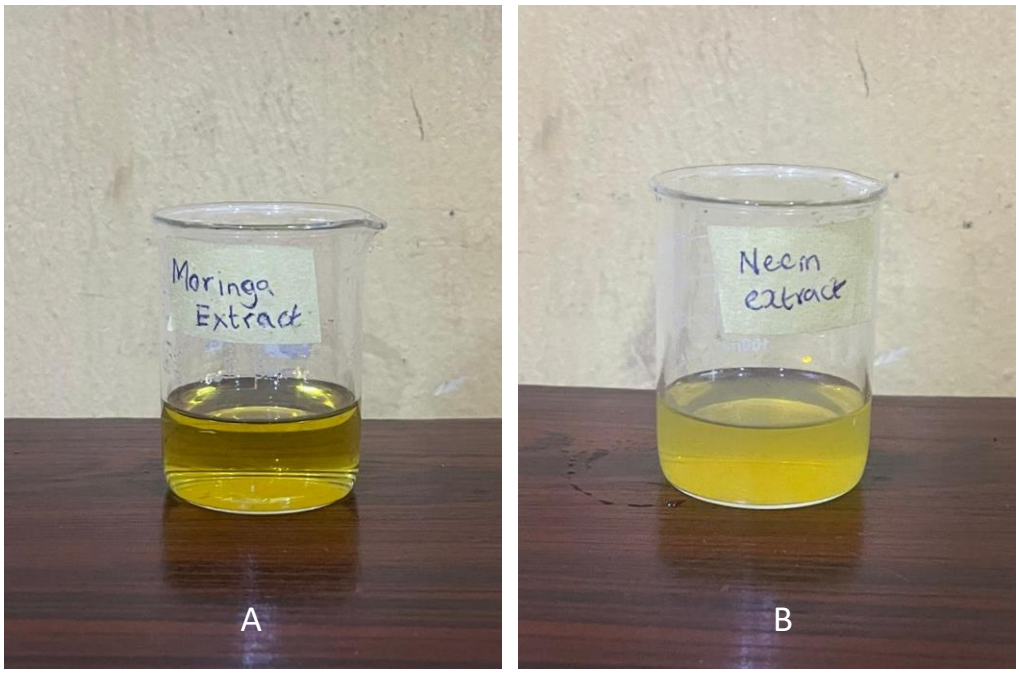

Plate 2.1: Plant extract prepared from the leaves of Moringa and Neem.

Legend

A: Moringa extract

B: Neem extract

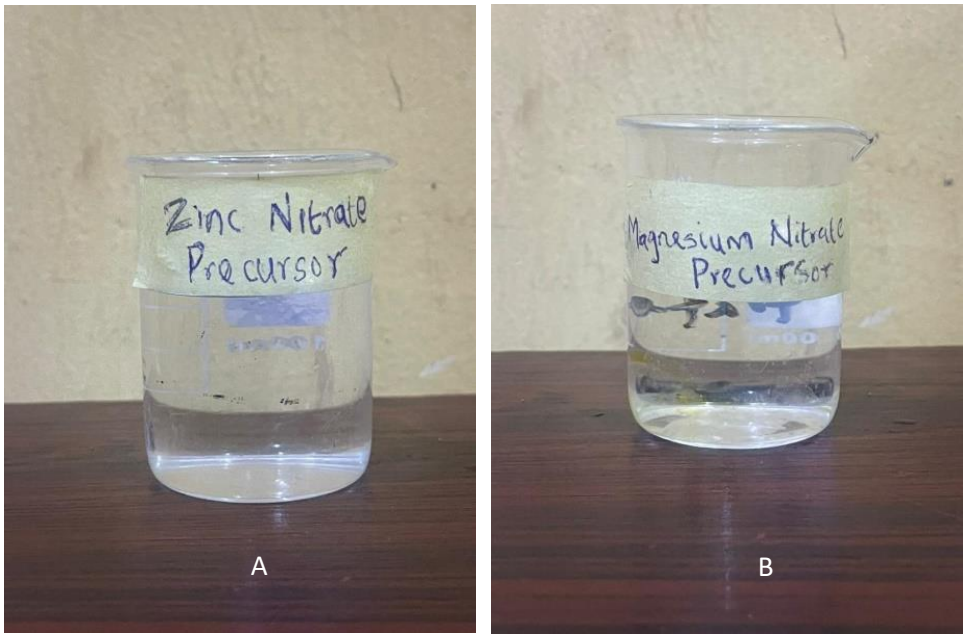

Plate 2.2: Prepared Zinc nitrate and magnesium nitrate precursor.

Legend

A: Zinc Nitrate precursor

B: Magnesium Nitrate precursor

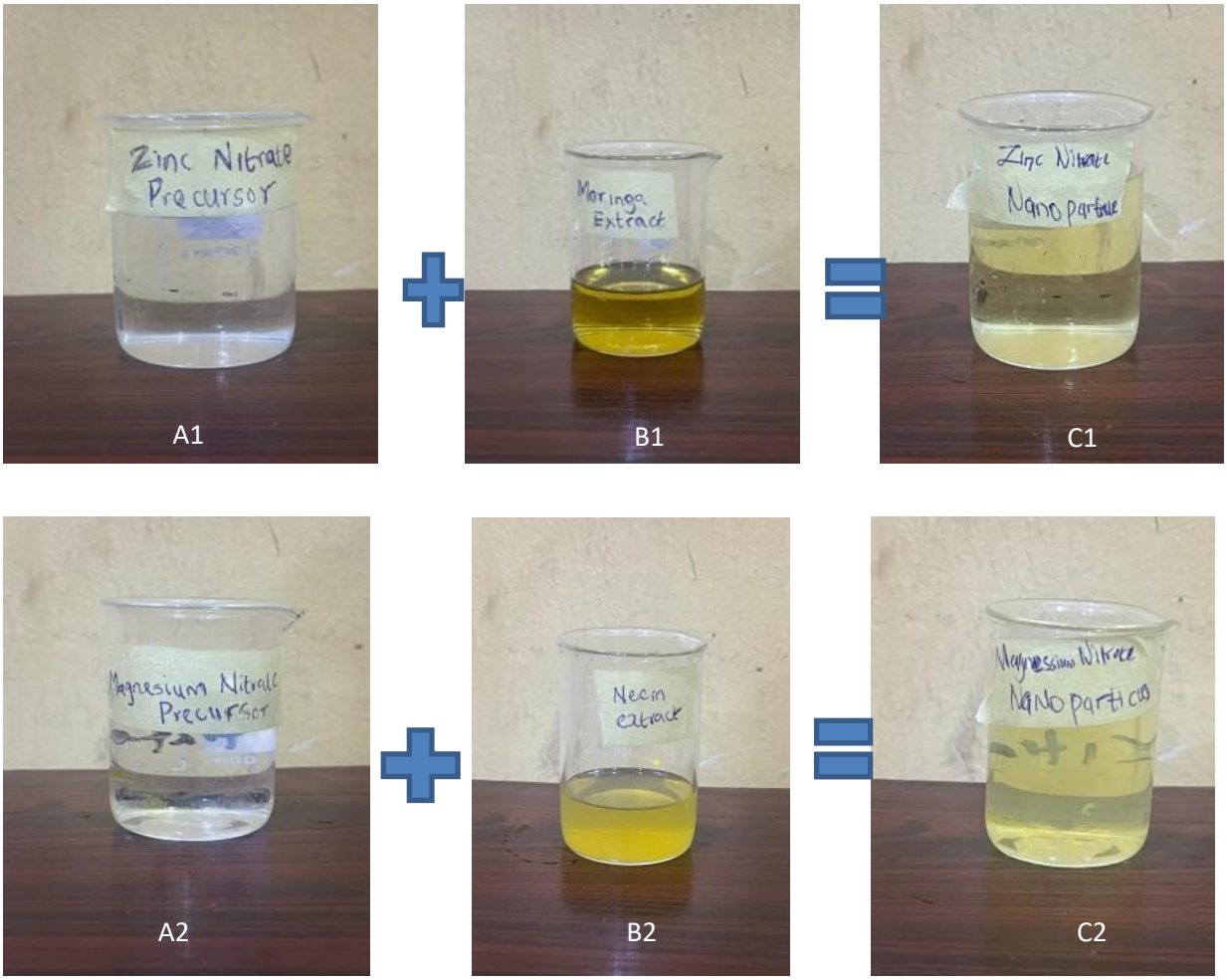

Plate 2.3: Two nanoparticles synthesized using two plant extracts and two precursor solutions.

Legend

A1: Zinc Nitrate precursor

B1: Moringa extract

C1: Zinc Oxide nanoparticles

A2:Magnesium Nitrate precursor

B2: Neem extract

C2: Magnesium Oxide nanoparticles

2.2.9 Bacteria broth adjustment

A preliminary test was carried out where by, bacteria broth was inoculated and left for 24 hours after which serial dilution was carried out. Then diluent 10^{-3} and 10^{-4} were plated using the pour plate method so as to determine which was more suitable for the experiment and easy to count. After 24 hours it was concluded that diluent 10^{-4} was easy to count having colonies. Then the rest of the bacteria isolates were also serially diluted to a 10^{-4} diluent which was then further used

2.2.10 Bacteria suspension preparation

In sterile test tubes, 1ml of the diluent 10^{-4} for each of the bacteria was put into the test tube using a micro pipette. Then the Nano particles were diluted into different concentrations 100, 75%, 50% and 25% with 0% as the control with just sterile water. Each of this concentration of Nano particles were added to the test tube containing the 1ml of bacteria broth. This procedure was done for each bacteria isolate and each nanoparticle making it 20 test tubes for 2 bacteria isolate and 2 nanoparticles. Each test tube was covered with aluminum foil and incubated in a mechanical shaker for 24 hours and then 100 µl was plated using the pour plate method.

CHAPTER THREE

RESULTS

The isolation through serial dilution of rhizospheric soil from tomato plants successfully yielded four distinct mixed cultures. These cultures provide a foundation for further microbiological analysis and characterization, contributing to our understanding of the microbial dynamics in plant rhizospheres, labeled A, B, C, and D as shown in Plate 3.1.

Two pure cultures were obtained from the four mixed cultures of the rhizosphere of a tomato plant through sub-culturing. This involved the process by which a smaller number of distinct colonies from a mixed culture was transferred onto a sterilized petri dish that contains solidified Nutrient agar media through successful streaking which helps in identification and characterization. Plate 3.2 represents the pure culture of the two different bacteria colonies.

Table 3.1 provides the features and results of the morphological and biochemical identification of rhizosphere bacteria isolates; these characteristics are employed to help in the identification of the bacteria pure cultures. Morphological and Biochemical tests were carried out to aid the identification process, which includes Form, margin, elevation, pigmentation, optical activities, Gram staining, Citrate test, catalase test, and indole test.

The results of plant growth promoting test is represented on Table 3.2. The test included Ammonia test (NH3), Hydrogen cyanide test (HCN) and Indole acetic test (I.A.A), bacteria. Isolate one bacterium was positive to all of the three tests, while isolate two bacteria was positive to only Hydrogen cyanide while negative to indole acetic acid test and ammonia.

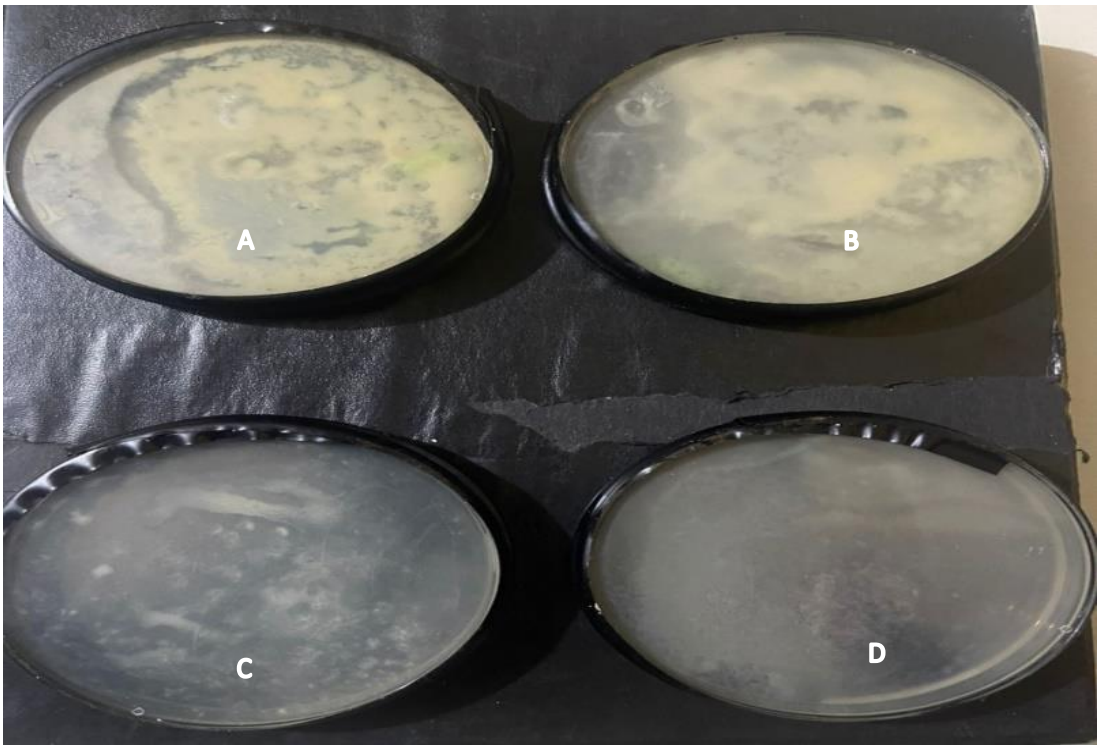

Plate 3.1: Mixed cultures of bacterial isolates from two tomato rhizospheric soil samples

Legend

A: 10^{-2} dilution of inoculum of sample 1

B: 10^{-3} dilution of inoculum of sample 1

C: 10^{-2} dilution of inoculum of sample 2

D: 10^{-3} dilution of inoculum of sample 2

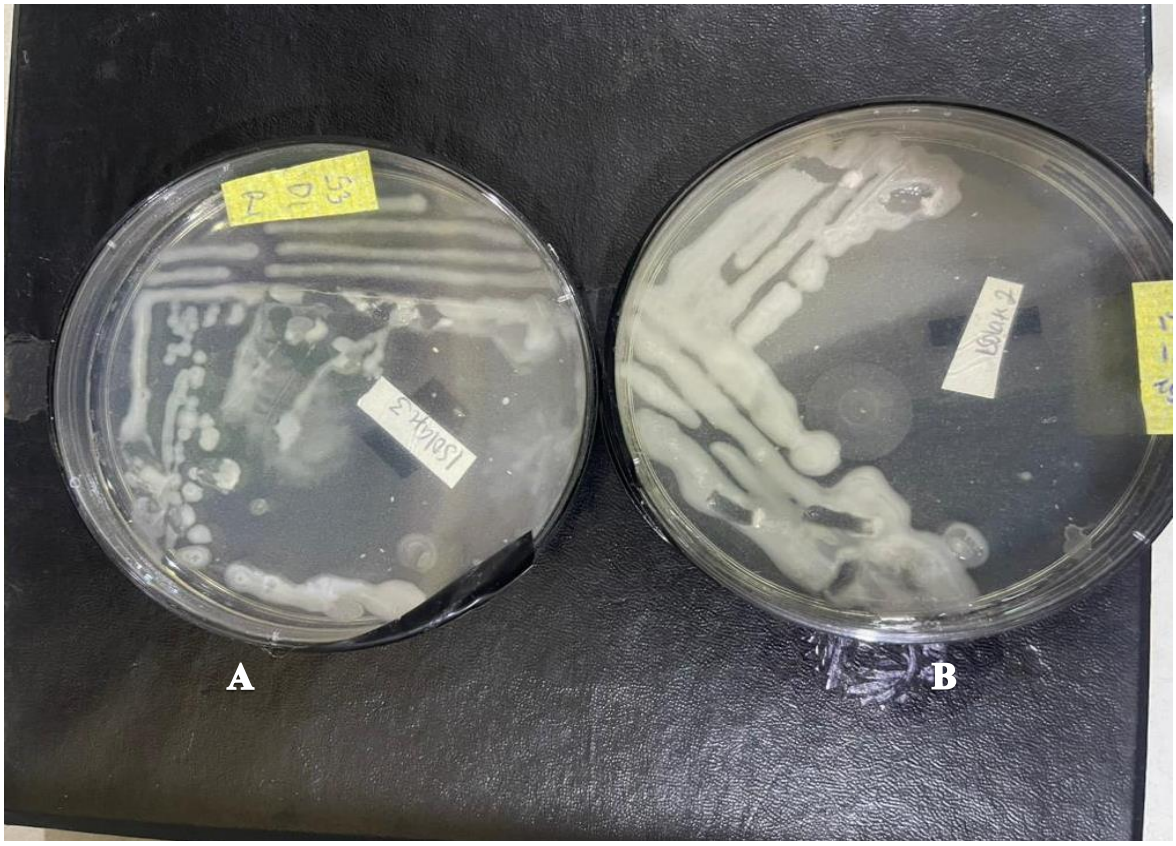

Plate 3.2: Pure culture of bacterial isolates from the mixed cultures

Legend

A: Isolate 1

B: Isolate 2

Table 3.1: Morphological and biochemical identification of bacterial isolates obtained from rhizospheric soils of tomato plants

Morphological characteristics	**1**	**2**
Shape	Irregular	Irregular
Surface	Undulate	Entire
Pigmentation	Cream	Cream
Elevation	Convex	Flat
Biochemical tests		
Gram stain	-	+
Indole	+	+
Catalase	+	+
Citrate	+	+
Probable organism	*Escherichia coli.*	*Bacillus* spp.

Legend

+; positive,

-; negative

Table 3.2: Plant growth-promoting test for two bacterial isolates from the rhizospheric soil of tomato plant

PGPT	**Isolate 1**	**Isolate 2**
HCN	+	+
IAA	+	-
NH_3	+	-

Legend

+; positive,

-; negative

HCN: Hydrgen Cyanide

IAA: Indole Acetic Acid

NH_3: Ammonia

PGPT: Plant growth-promoting test

Table 3.3 presents the treatment effects of magnesium oxide nanoparticles (MgONP) on *Escherichia coli* (isolate 2), indicating a significant increase in colony counts with higher concentrations of MgONP. The control group, which received no MgONP treatment, exhibited a baseline colony count of 300. In contrast, treatment with 100% MgONP resulted in a remarkable increase to 1550 colonies, suggesting a strong stimulatory effect on *E. coli* growth at this concentration. Intermediate treatments demonstrated varying degrees of efficacy, with the 25% MgONP treatment yielding 1450 colonies, indicating substantial growth promotion even at lower concentrations. The 50% and 75% MgONP treatments resulted in colony counts of 1250 and 850, respectively; notably, the 75% concentration showed a decrease in efficacy compared to the 25% and 100% treatments. These results suggest a complex relationship between MgONP concentration and *E. coli* growth, highlighting the potential for both beneficial and varying effects at different dosage levels. Visual representation of these findings is illustrated in Plate 3.3, which depicts the significant differences in colony growth across the treatments.

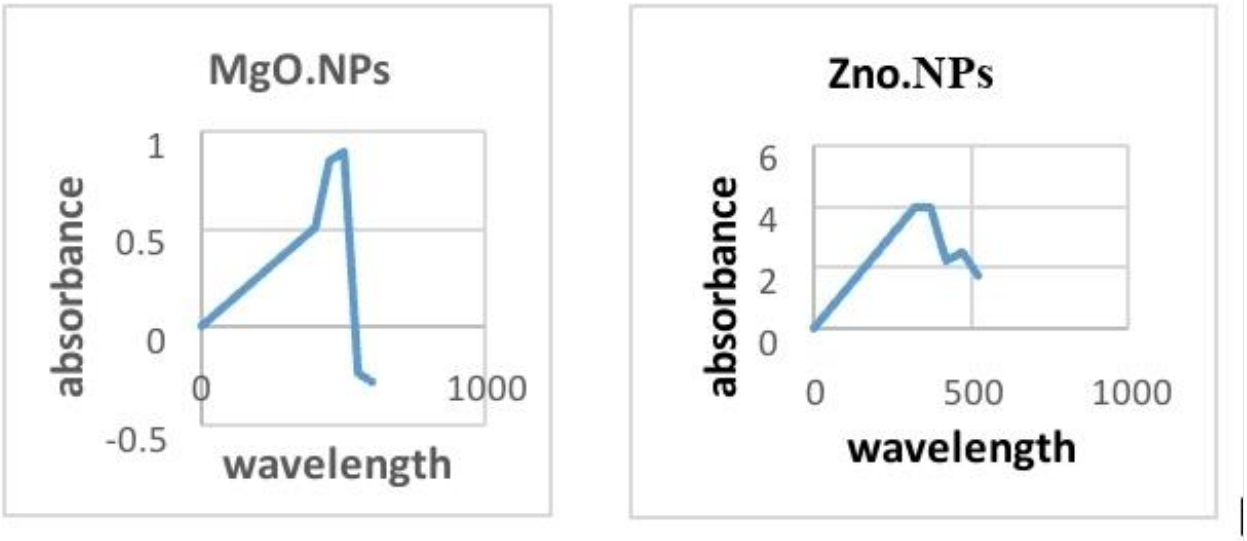

Figure 2.1: Absorbance value of 100% concentration of two different nanoparticles.

Legend

A: Absorbance value of 100% concentration of Magnesium Oxide nanoparticles

B: Absorbance value of 100% concentration of Zinc Oxide nanoparticles

Table 3.4 presents the effects of zinc oxide nanoparticles (ZnONP) on *Escherichia coli* (isolate 2), demonstrating a marked reduction in colony-forming units with increasing concentrations of ZnONP. The control group exhibited a baseline colony count of 300. In stark contrast, treatment with 100% ZnONP resulted in complete inhibition of colony growth, with no colonies detected, indicating a strong antimicrobial effect at this concentration. Lower concentrations of ZnONP also showed significant inhibitory effects, with the 75% and 50% treatments yielding minimal colony counts of only 4 and 3, respectively. Notably, the 25% treatment also resulted in no detectable colonies, reinforcing the potent antibacterial activity of ZnONP across all tested concentrations. These findings, illustrated in Plate 3.4, highlight the effectiveness of zinc oxide nanoparticles in suppressing *E. coli* growth, suggesting their potential application in antimicrobial strategies.

Table 3.3: Colony forming unit of PGPB (Isolate 1) *Escherichia coli* after magnesium oxide nanoparticle treatment

Treatment with MgO.NPs	**No. of colonies**	**CFU/mL**
Control	300	0.015
100%	1550	0.0775
75%	850	0.0425
50%	1250	0.0625
25%	1450	0.0725

Legend

MgONP: Magnesium Oixde nanoparticles

CFU: Colony forming unit

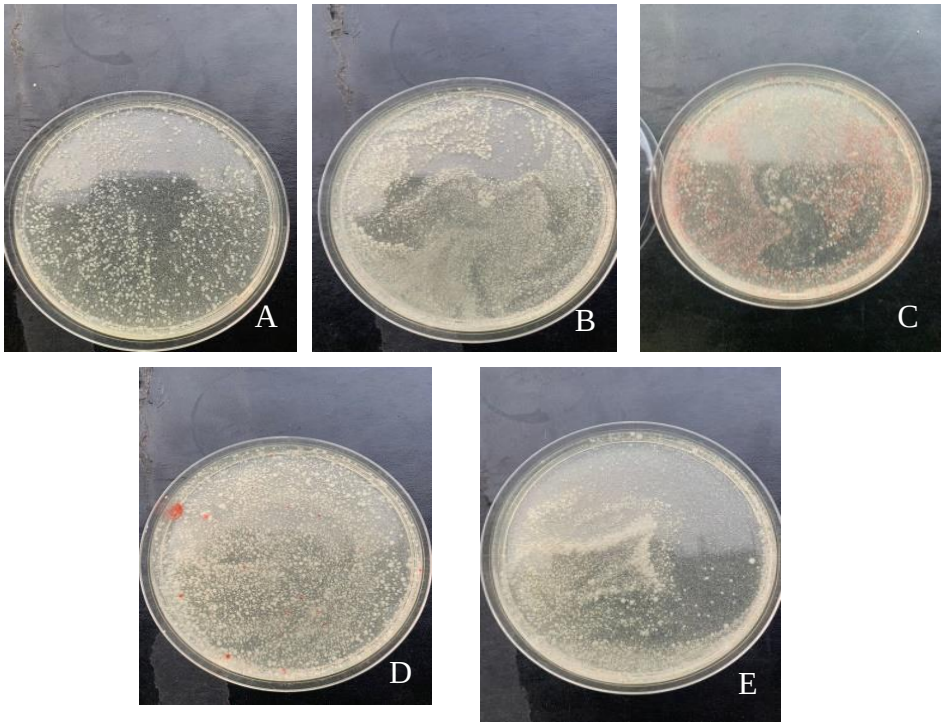

Plate 3.3: Cultures of Plant growth promoting bacteria Isolate 1 (*Escherichia coli*) treated with Magnesium oxide nanoparticle treatment

Legend

A: Control (treatment with sterile water)

B. Treatment with PGPB, *Escherichia coli* suspension + Magnesium Oxide nanoparticles 100%

C. Treatment with PGPB, *Escherichia coli* suspension + Magnesium Oxide nanoparticles 75%

D. Treatment with PGPB, *Escherichia coli* suspension + Magnesium Oxide nanoparticles 50%

E. Treatment with PGPB, *Escherichia coli* suspension + Magnesium Oxide nanoparticles 25%

PGPB: Plant growth-promoting bacteria.

Table 3.4: Colony forming unit of PGPB (Isolate 1) *Escherichia coli* after Zinc oxide nanoparticle treatment

Treatment with ZnO.NPs	**No. of colonies**	**CFU/mL**
Control	300	0.01500
100%	0	0.00000
75%	4	0.00020
50%	3	0.00015
25%	0	0

Legend

ZnO.NPs: Zinc Oxide nanoparticles

CFU: Colony forming unit

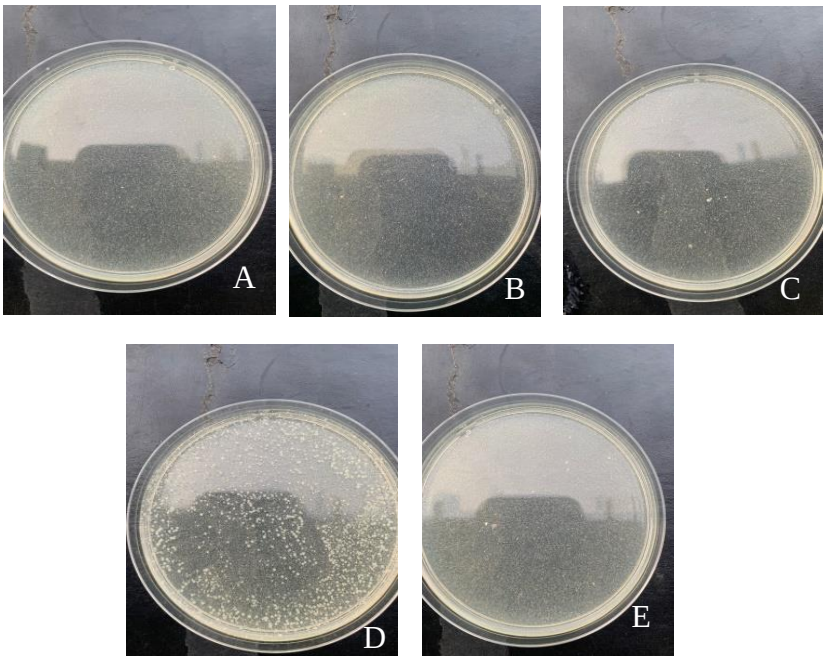

Plate 3.4: Cultures of Plant growth promoting bacteria Isolate 1 (*Escherichia coli*) treated with Zinc Oxide nanoparticle

Legend

A: Control (treatment with sterile water)

B. Treatment with PGPB, *Escherichia coli* suspension + Zinc Oxide nanoparticles 100%

C. Treatment with PGPB, *Escherichia coli* suspension + Zinc Oxide nanoparticles 75%

D. Treatment with PGPB, *Escherichia coli* suspension + Zinc Oxide nanoparticles 50%

E. Treatment with PGPB, *Escherichia coli* suspension + Zinc Oxide nanoparticles 25%

PGPB: Plant growth-promoting bacteria.

Table 3.5 and Plate 3.5 illustrate the treatment effects of magnesium oxide nanoparticles (MgONP) on *Bacillus* species (isolate 3), revealing a notable increase in colony-forming units with MgONP treatment. The control group recorded a baseline colony count of 450. In contrast, treatment with 100% MgONP resulted in a significantly higher colony count of 1700, indicating a strong stimulatory effect on *Bacillus* growth at this concentration. Additionally, lower concentrations of MgONP yielded varying colony counts, with 75%, 50%, and 25% treatments resulting in 850, 1000, and 1250 colonies, respectively. These results demonstrate that MgONP effectively promotes the proliferation of *Bacillus* species across all tested concentrations, underscoring its potential as a growth enhancer in microbial applications.

Table 3.6 and Plate 3.6 present the effects of zinc oxide nanoparticles (ZnONP) on *Bacillus* species (isolate 3), demonstrating a significant reduction in colony-forming units following treatment with ZnONP. The control group recorded a baseline colony count of 450. In stark contrast, treatment with 100% ZnONP resulted in a marked decrease to only 68 colonies, indicating a potent antimicrobial effect at this concentration. Further reductions were observed at lower concentrations, with the 75% and 50% treatments yielding colony counts of 2 and 4, respectively, and the 25% treatment also resulting in just 2 colonies. These findings highlight the effectiveness of zinc oxide nanoparticles in inhibiting the growth of *Bacillus* species, underscoring their potential utility in antimicrobial applications.

Table 3.5: Colony forming unit of PGPB (Isolate 2) *Bacillus* spp., after magnesium oxide nanoparticle treatment

Treatment with MgO.NPs	**No. of colonies**	**CFU/mL**
Control	450	0.0225
100%	1700	0.085
75%	850	0.0425
50%	1000	0.05
25%	1250	0.0625

Legend

MgONP: Magnesium Oxide nanoparticles

CFU: Colony forming unit

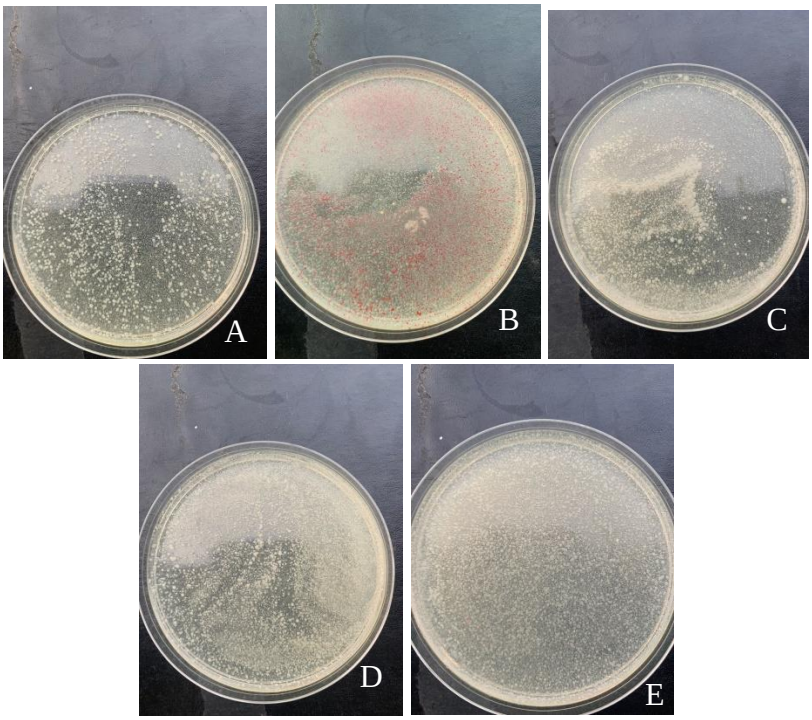

Plate 3.5 Cultures of Plant growth promoting bacteria Isolate 2 *Bacillus* spp., treated with Magnesium oxide nanoparticle treatment

Legend

A: Control (treatment with sterile water)

B. Treatment with PGPB, *Bacillus* spp., suspension + Magnesium Oxide nanoparticles 100%

C. Treatment with PGPB, *Bacillus* spp., suspension + Magnesium Oxide nanoparticles 75%

D. Treatment with PGPB, *Bacillus* spp., suspension + Magnesium Oxide nanoparticles 50%

E. Treatment with PGPB, *Bacillus* spp., suspension + Magnesium Oxide nanoparticles 25%

PGPB: Plant growth-promoting bacteria.

Table 3.6: Colony forming unit of PGPB (Isolate 2) *Bacillus* spp., after Zinc oxide nanoparticle treatment

Treatment with ZnO.NPs	**No. of colonies**	**CFU/Ml**
Isolate 3(Control)	450	0.0225
100%	68	0.0034
75%	2	0.0001
50%	4	0.0002
25%	2	0.0001

Legend

ZnONP: Zinc Oxide nanoparticles

CFU: Colony forming unit

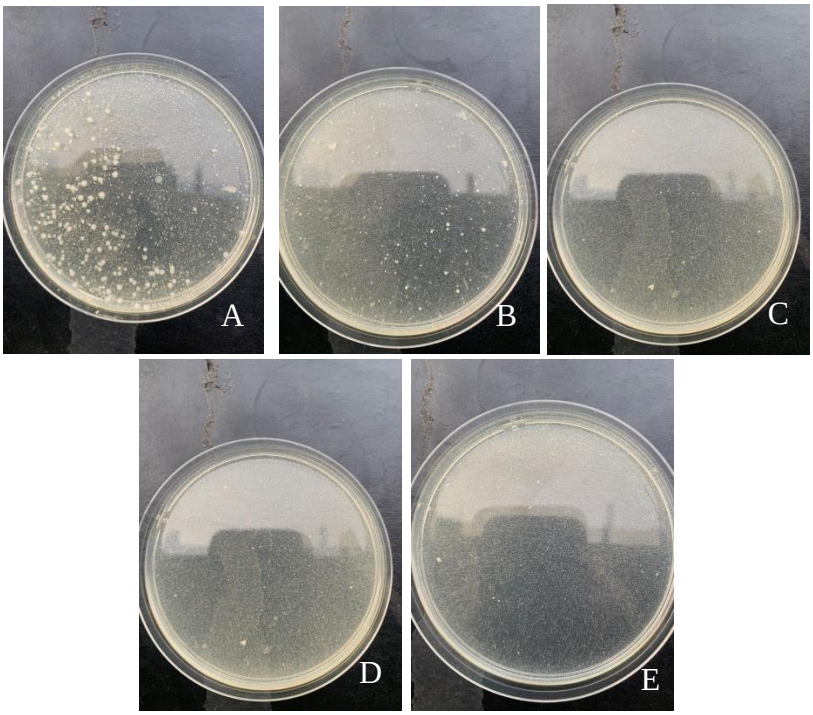

Plate 3.6 Cultures of Plant growth promoting bacteria Isolate 2 *Bacillus* spp., treated with Zinc oxide nanoparticle treatment

Legend

A: Control (treatment with sterile water)

B. Treatment with PGPB, *Bacillus* spp., suspension + Zinc Oxide nanoparticles 100%

C. Treatment with PGPB, *Bacillus* spp., suspension + Zinc Oxide nanoparticles 75%

D. Treatment with PGPB, *Bacillus* spp., suspension + Zinc Oxide nanoparticles 50%

E. Treatment with PGPB, *Bacillus* spp., suspension + Zinc Oxide nanoparticles 25%

PGPB: Plant growth-promoting bacteria.

DISSCUSSION

Globally, food security has become a critical concern due to the rise in human population and the current climate change crisis. Usage of conventional agrochemicals to maximize crop yields has resulted in the degradation of fertile soil, environmental pollution as well as human and agro-ecosystem health risks. Nanotechnology in agriculture is a fast-emerging and new area of research explored to improve crop productivity and nutrient-use efficiency using Nano-sized agrochemicals at lower doses than conventional agrochemicals. One innovative way to increase the yield, quality and efficiency of crops is through the use of Nanotechnology (Moodley, *et al*., 2018). Due to their extraordinarily smaller size, high surface are to volume ratio, catalytic reactivity, and form, nanoparticles are of tremendous interest. They result in physical and chemical changes compared to their bulk equivalents (Iravani, 2011). It is possible to create nanoparticles through physical, chemical or biological methods, however physical and chemical processes are now being substituted for the biological ones. In this study, magnesium nitrate nanoparticles and Zinc nitrate nanoparticles were being synthesized using Moringa and neem plant extract respectively; and were used to test the antimicrobial effect on the different bacteria isolate at different concentrations, (100, 75,50 and 25%) with 0% as their control.

This study aimed to explore the effects of magnesium oxide nanoparticles (MgONP) and zinc oxide nanoparticles (ZnONP) on the growth of plant growth-promoting bacteria, specifically *Escherichia coli* and *Bacillus* species. The results reveal significant trends that highlight the potential of these nanoparticles to enhance bacterial growth, which could have implications for agricultural practices. For *E. coli* (isolate 2), the data clearly show that MgONP treatment resulted in a substantial increase in colony forming units (CFU). The control group, which was not treated with nanoparticles, had a baseline of 300 colonies. However, as the concentration of

MgONP increased, the colony counts rose dramatically, with the highest concentration (100% MgONP) yielding 1550 colonies. This trend suggests that MgONP may create a more favorable environment for *E. coli*, enhancing its growth and potentially its plant growth-promoting activities, such as nitrogen fixation or hormone production. Interestingly, the lower concentrations of MgONP also resulted in increased CFU, indicating that even minimal exposure to these nanoparticles can stimulate bacterial growth. This is a crucial finding, as it suggests that MgONP could be used in lower quantities while still achieving beneficial effects, thereby reducing potential environmental impacts. The results for ZnONP treatment on *E. coli* were equally compelling. Treatment with 100% ZnONP resulted in a complete inhibition of colony growth, with no colonies detected. This sharp contrast to the positive effects of MgONP indicates that ZnONP may exert a more complex influence on bacterial growth. While this could suggest that ZnONP is detrimental to *E. coli*, it also opens up avenues for further research into its potential role as a selective agent for specific bacterial strains or its ability to induce stress responses that might enhance certain beneficial traits. When examining the effects on *Bacillus* species (isolate 3), the results varied significantly compared to *E. coli*. The control group began with 450 colonies, but the application of 100% MgONP led to an unexpected increase in colony count to 1700. This suggests that MgONP may not only support growth but might also enhance the proliferation of *Bacillus*, which is known for its plant growth-promoting capabilities, including the production of plant hormones and antagonistic effects against plant pathogens. In terms of ZnONP, treatment resulted in a notable reduction in *Bacillus* growth, with colony counts decreasing from 450 to 68 at 100% concentration. This suggests that while ZnONP negatively affects *Bacillus*, it does not necessarily inhibit its plant growth-promoting properties completely. Further research could investigate the mechanisms behind this interaction and

whether lower concentrations of ZnONP might yield different results. Overall, the findings from this experiment underscore the potential of nanoparticles, particularly MgONP, to enhance the growth of plant growth-promoting bacteria. These results suggest that MgONP could be a valuable tool in agricultural practices to boost beneficial bacterial populations, potentially improving plant health and yield. Conversely, the complex interactions observed with ZnONP highlight the need for further investigation to fully understand its effects on different bacterial species and its possible applications in plant growth promotion. This research contributes to the growing body of knowledge on the use of nanoparticles in agriculture and sets the stage for future studies aimed at optimizing nanoparticle formulations for enhanced plant growth and sustainability.

CONCLUSION AND RECOMMENDATION

In conclusion, this study demonstrates the significant potential of magnesium oxide nanoparticles (MgONP) and zinc oxide nanoparticles (ZnONP) in enhancing the growth of plant growth-promoting bacteria such as *Escherichia coli* and *Bacillus* species. The results indicate that MgONP can substantially increase bacterial populations, suggesting its efficacy as a beneficial agent in agricultural practices. Conversely, ZnONP exhibits a more complex relationship with bacterial growth, potentially inhibiting *E. coli* while affecting *Bacillus* differently. These findings highlight the importance of understanding the varying effects of different nanoparticles on microbial communities, which could have significant implications for sustainable agriculture.

For future work, it is recommended to further investigate the mechanisms behind the interactions between nanoparticles and specific bacterial strains. Research should focus on optimizing nanoparticle concentrations to maximize their growth-promoting effects while minimizing any potential negative impacts. Additionally, exploring the effects of these nanoparticles in real agricultural settings, including soil and plant interactions, will be crucial for validating their efficacy and safety. Long-term studies on the environmental impacts of using nanoparticles in agriculture are also necessary to ensure sustainable practices. Ultimately, this research opens new avenues for developing innovative strategies to enhance plant growth and improve agricultural productivity through the use of nanoparticles.

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