Silver Nanoparticles Synthesis by Serratia Marcescens W2 Strain, Its Biocontrol Efficacy Against Fungal Phytopathogens, And Its Effect on Wheat Seeds

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Citation | Nafeh. A, Zahid. N, Aleem. S, Shahab. H, Afzal. D. I, "Silver Nanoparticles Synthesis by Serratia Marcescens W2 Strain, Its Biocontrol Efficacy Against Fungal Phytopathogens, And Its Effect on Wheat Seeds", IJIST, Vol. 07 Issue. 02 pp 871-880, May 2025

Received | April 13, 2025 **Revised |** May 15, 2025 **Accepted |** May 16, 2025 **Published |** May 17, 2025.

Due to its cost-effectiveness and eco-friendliness, the production of silver nanoparticles (AgNPs) utilizing biological agents has attracted a lot of attention. In this study, we investigate the potential of the Serratia marcescens W2 strain as a bio-factory for the production of silver nanoparticles and evaluate the biocontrol capability of this strain against fungal phytopathogens as well as its impact on wheat seeds. Using the extracellular enzymes and metabolites produced by Serratia marcescens W2, AgNPs were biosynthesised. The size, form, and composition of the AgNPs were determined using a variety of analytical techniques, such as X-ray diffraction (XRD), and scanning electron microscopy (SEM). AgNPs' impressive ability to inhibit fungal growth in vitro experiments demonstrates their robust biocontrol capabilities. Microscopic and biochemical investigations helped to better clarify the AgNPs' mode of action against these phytopathogens. Additionally, investigations on seed germination and seedling growth were used to evaluate the effect of the AgNPs on wheat seeds. As a result of the application of AgNPs, seed germination rates, and seedling vigor dramatically increased, according to the findings. Additionally, compared to the control group, the seedlings treated with AgNPs showed enhanced resistance to fungal infection. Overall, the results of this study demonstrate the potential of Serratia marcescens W2 strain in the green synthesis of AgNPs with improved antifungal activities. Furthermore, the use of these AgNPs promotes the germination and growth of wheat seedlings, indicating their potential use as a biocontrol agent and seed treatment to safeguard crops against fungus phytopathogens in sustainable agriculture. To completely understand the underlying mechanisms and determine the long-term impacts of AgNPs on the ecosystem and human health, more research is necessary.

Keywords: Green technology, Microbial Synthesis, silver nanoparticles, intracellular synthesis.

Introduction:

Nanoparticle synthesis involves a range of physical and chemical techniques, each tailored to achieve precise control over their size, shape, and composition [1]. Ultraviolet radiation spectrum analysis, aerosol technologies, lithography, photo-ablation, supersonic fields, and chemical reduction methods are all effective techniques for nanoparticle synthesis. However, these methods are expensive and employ hazardous synthetic compounds [2]. There is increasing concern about developing methods that are both feasible and suitable for practical applications. As the ability to mix nanoparticles of different configurations, sizes, shapes, and controlled properties is crucial to applied science, new, more efficient techniques are continually being developed. Microbic nanoparticle integration represents an innovative scientific method that merges applied science with microbial biotechnology, offering a promising new technique for nanoparticle synthesis and application [3]. Biogenesis of gold, silver, gold–silver composite, selenium, palladium, tellurium, platinum, tellurium, silica, titanium, zirconia, quantum spots, iron ore, and mineral nanoparticles has been documented through the microbial process. These processes involve both extracellular and intracellular mechanisms, utilizing plant and endophytic bacteria, actinomycetes, fungi, yeasts, and viruses. The production of organic nanoparticles primarily relies on biotechnology, requiring minimal technical expertise [4]. Despite their strength, organic nanoparticles often suffer from irregular sizes, rendering the rate of synthesis slow. To overcome these challenges, it is essential to optimize factors such as microbial growth methods and extraction systems. Additionally, combined approaches, such as photobiological techniques, can be utilized to enhance efficiency and improve consistency in nanoparticle production [5]. Given the vast diversity of microorganisms, their potential as organic materials for nanoparticle synthesis is however to be utterly investigated.

As a result, there is a growing emphasis on developing aesthetically attractive and practical techniques for nanoparticle production. Because the number of nanoparticles with different courses of action, sizes, shapes, and controlled divergence might be massive. New reasonable methods, on the other hand, are being developed [6]. Microbic nanoparticle synthesis is an emerging innovative logical technique that connects technology with microbic biotechnology. Synthesis of gold, silver, gold–silver composite, selenium, palladium, tellurium, platinum, tellurium, silica, titanium, zirconia, quantum spots, magnetic iron-ore, and mineral nanoparticles by microorganisms, for instance, Microbes (extracellular even as intra-cell, plant life, and endophytic microorganisms), actinomycetes, growths, yeasts and infections led to the creation of natural nanoparticles, largely by emerging technologies. However, standard, natural nanoparticles do not appear to be systematically calculable, and the uniform movement is moderate [7]. To overcome these issues, several variables, including microbic improvement methodologies and extraction frameworks, need to be optimized. Additionally, combined approaches, such as photobiological processes, can also be employed to improve efficiency. Cell, organic chemicals, and sub-nuclear frameworks that mediate the synthesis of natural nanoparticles ought to be gathered well to maximize the synthesis and improve the properties of nanoparticles against pests and phytopathogens [8].

Research Objectives:

1. To biosynthesize silver nanoparticles using extracellular and intracellular metabolites of *Serratia marcescens* W2 strain.
2. To characterize the synthesized silver nanoparticles using techniques such as X-ray diffraction (XRD) and scanning electron microscopy (SEM).
3. To evaluate the antifungal activity of biosynthesized AgNPs against common fungal phytopathogens, specifically *Aspergillus flavus* and *Aspergillus niger*.
4. To assess the effect of AgNPs on wheat seed germination and seedling growth under controlled laboratory conditions.

5. To explore the potential of AgNPs as a dual-purpose agent for biocontrol and seed treatment in sustainable agriculture practices.

Statement of Novelty:

By using the *Serratia marcescens* W2 strain as a bio-factory for the environmentally friendly synthesis of silver nanoparticles (AgNPs), this study presents a fresh approach to sustainable agriculture. This microbial biosynthesis technique provides a green, economical, and biocompatible alternative to traditional chemical methods. It has two functions:

1. It has strong antifungal activity against major phytopathogens (Aspergillus flavus);
2. It promotes seed germination and seedling vigor in wheat. A unique addition to sustainable plant-microbe-nanoparticle interactions, the combination of antibacterial efficacy and plant growth improvement in a single biosynthesized nanomaterial represents a substantial leap in bio-nanotechnology and crop protection techniques.

Microbial Synthesis of Silver Nanoparticles:

Collection of Sample:

Serratia marcescens w2, a previously identified endophytic bacterium, was utilized in this work, provided by the Department of Biology, Lahore Garrison University, Lahore. This strain was selected due to its antifungal properties as well as its capacity to biosynthesize metal nanoparticles. The strain was isolated from glycerol stocks and maintained on Tryptic Soy Agar (TSA) as a growth medium.

Materials and Methods:

Preparation of Glycerol Stock:

Bacterial strains were preserved in glycerol stock for a long time. The medium for tryptic soy broth (TSB) was made and autoclaved and a pure culture was inoculated into broth and incubated at 30° C overnight. A 50% glycerol solution was prepared by mixing equal volumes of glycerol and water. To create the glycerol stock, 300 µL of this solution was combined with 700 µL of the bacterial culture, and the mixture was stored at -80°C for preservation [9][10].

Procedure I: For Intracellular Synthesis of Nanoparticles:

Tryptic Soy Broth was prepared by dissolving 30 g of tryptic soy broth in 1 liter of distilled water. After that, the medium was placed into dehydrogenated caped test tubes and autoclaved for 15 minutes at 121^0C under 1atm pressure. After autoclaving, a 3 mM silver nitrate salt solution was sterilized with 0.22 µL syringe filters and then added to the sterile media. The injected cultures were previously revived fresh cultures [11].

Extraction:

The intracellularly generated silver nanoparticles were extracted using lysis buffer solutions containing 0.05 mL Triton x 100, 0.1 mL Tris 0.1M, 10 mL EDTA, and up to 10 mL distilled water [12]. The fluid was centrifuged at 5000 rpm for 5 minutes at room temperature to collect pallets of nanoparticles, after 24 hours. Figure 1 below depicts a schematic diagram.

Procedure II: For Extracellular Synthesis of Nanoparticles:

Tryptic Soy Broth (TSB) was prepared by dissolving 30 g in 1 liter of distilled water. This media was then poured into dehydrogenated caped test tubes and autoclaved for 15 minutes at 121^0 C at 1atm pressure [13]. Previously revived fresh cultures were then inoculated in these tubes under the laminar air flow cabinet and then incubated for 24-48 hours using a shaking incubator at 100 rpm and 30^0 C.

The broth containing the bacterial cells was centrifuged at room temperature for 15 minutes at 5000 rpm to get cell pallets. After that, 100 mg of silver nitrate salt was added to the broth and incubated for 24-48 hours in a shaking incubator at 30^0C.

Subsequently, the medium was centrifuged once more to collect the nanoparticles that had been produced. Extracellular synthesis is depicted schematically in **Figure 2.**

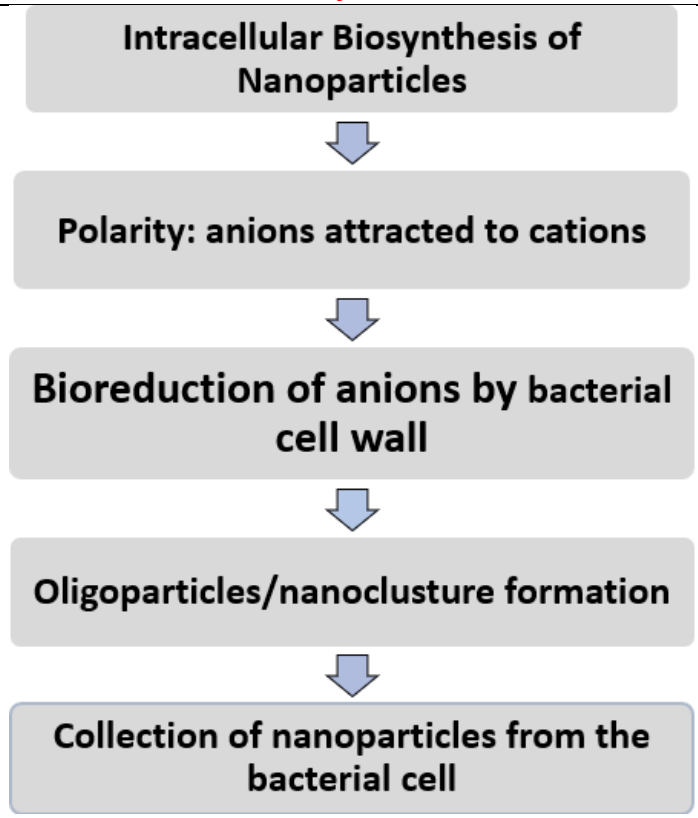

Figure 1. Generalized schematic diagram of intracellular biosynthesis of Nanoparticles

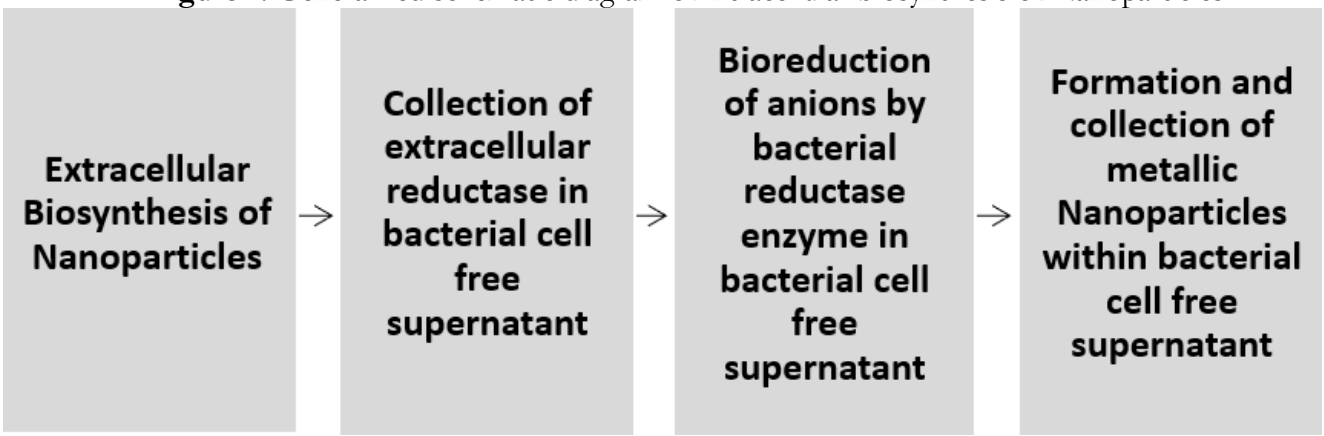

Figure 2. Generalized schematic diagram of extracellular biosynthesis of Nanoparticles

Results:

The antifungal effectiveness and plant growth-promoting qualities of silver nanoparticles (AgNPs) made with the *Serratia marcescens* W2 strain were investigated in this study. Both intracellular and extracellular methods were used to create the nanoparticles, and XRD and SEM methods were used to characterize them. The following subsections are used to arrange the results:

Characterization of Metal Nanoparticles:

X-Rays Diffraction of Nanoparticles synthesized by Serratia Marcescens w2 Strain:

The pattern generated by nanoparticles is seen in the *S. marcescens* w2 X-Ray Diffraction analysis graph, with bigger peaks at (2) 27.98, 32.31, and 46.23, respectively, corresponding to (111), (200), and (220) in Figure 3. Furthermore, Figure 3 below reveals that numerous additional peaks are not as distinct due to the synthesis of bio-organic compounds other than nanoparticles [14].

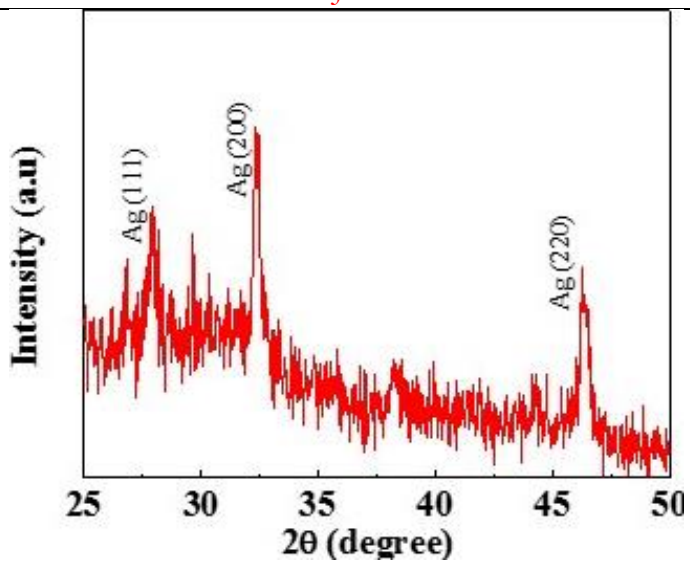

Figure 3. X-ray diffraction analysis of w2 shows the pattern formed by nanoparticles, indicating greater peaks at (2θ) 27.98, 32.31, and 46.23 corresponding to (111), (200), and (220) respectively. The peak (200) shows particle size as 23 nm.

Scanning Electron Microscope (SEM) Analysis of Nanoparticles Synthesized by Serratia Marcescens W2 strain:

Scanning Electron Microscope (SEM) analysis of nanoparticles synthesized by Serratia marcescens W2 reveals that the nanoparticles produced are nonporous. [15] non-uniformity was observed as shown in Figure.4. below due to no use of a stabilizing agent.

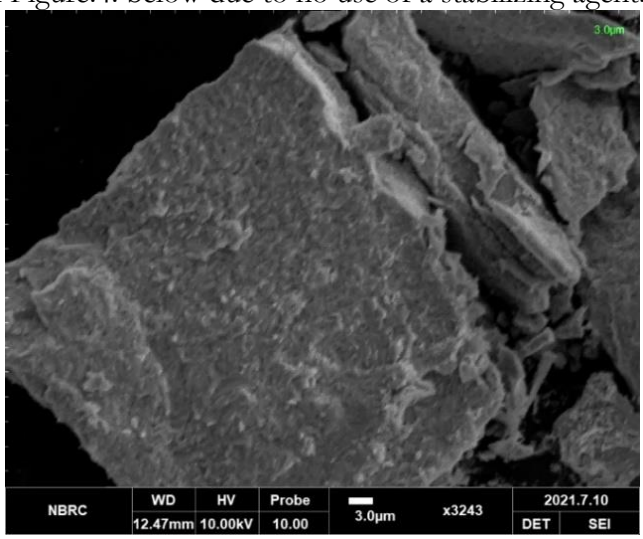

Figure 4. Scanning electron microscope (SEM) analysis nanoparticles synthesized by *Serratia marcescens* w2 strain showing the obtained nanoparticles are nonporous and non-uniform.

Antifungal Activity:

The antifungal activity of the nanoparticles was tested using the well diffusion technique. Fresh slants of fungal cultures were prepared using Potato Dextrose Agar (PDA) for this purpose. After inoculating the fungal plugs, the slants were incubated at 30°C for 72 hours. About 2 mL of 0.9% normal saline and 2 mL of distilled autoclaved water were added after 72 hours of development.

The spore count was calculated using the formula 0.1 OD = 1x 10^6 spores/ mL after the samples were examined for optical density (OD) at 700 nm. The exact quantity of spores injected per 20 mL of medium was determined [16]. Microbiologically synthesized nanoparticles exhibit strong inhibitory efficacy against the fungal phytopathogens *Aspergillus flavus*.

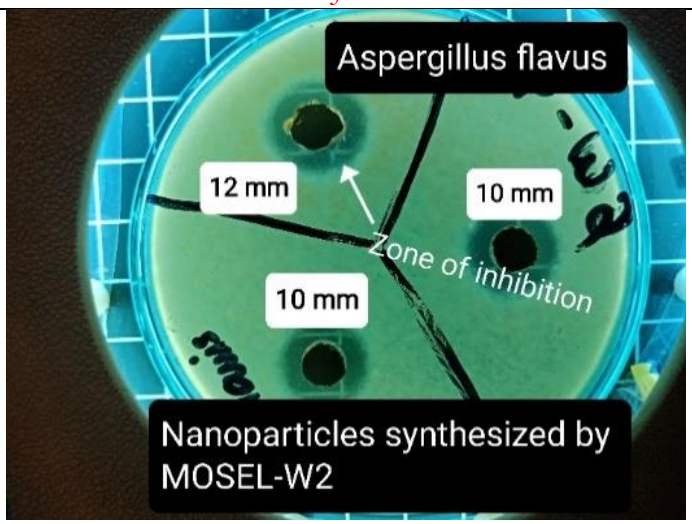

Figure 5. Nanoparticles biologically synthesized by *S. marscenes* w2 show anti-fungal activity against *A. flavus*, using a good diffusion method. Zones of inhibitions were measured to be 12 mm, 10 mm, and 10 mm.

Impact of Nanoparticles on the Growth and Germination of Wheat Seeds and Seedlings:

Improved germination rates and seedling vigor were seen in wheat seeds treated with biosynthesized AgNPs. Three test groups were formed:

1. Control group: seeds that have not been treated.
2. Seeds treated with nanoparticles; seeds treated with both AgNPs and fungal spores.
3. In comparison to the control group, the shoot length of the seeds treated with nanoparticles increased by 12.9%, whilst the dual treatment group improved by 3.3%, as seen in Figure 6 & Figure 7.

The Greenhouse Test: In vivo experiments showed that wheat plants treated with AgNPs and cultivated in fungal-infested soil had much longer roots and shoots than untreated controls. Fungal colonization on root surfaces was decreased, demonstrating AgNPs' capacity for biocontrol.

Synthesized bio-nanoparticles when applied to test seeds, showed enhanced growth as well.

Figure 6. Synthesized bio-nanoparticles when applied to test seeds, showed enhanced growth.

Wheat seeds when treated with fungal spores as well as biosynthesized nanoparticles, showed inhibition of fungal spores as well as a slight increase in growth of seed shoots.

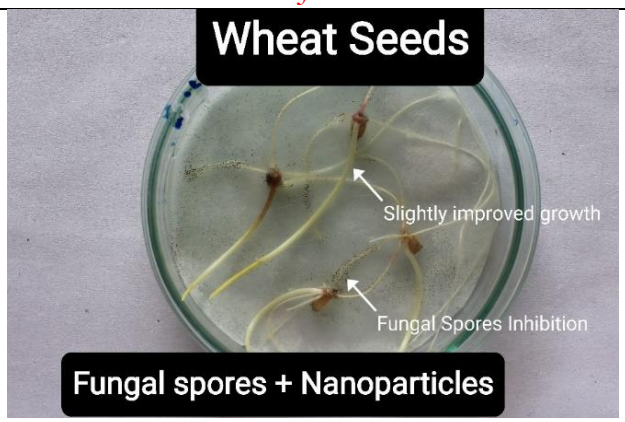

Figure 7. Wheat seeds when treated with fungal spores and biosynthesized nanoparticles at the same time, showed inhibition of fungal spores and a slight increase in growth of seed shoots.

Antimicrobial studies demonstrate that even a small concentration of 10 mg/mL yields significant results, indicating the high efficacy of the biosynthesized nanoparticles. Table.1.

Table 1. Shoot lengths of wheat seeds treated with synthesized nanoparticles and seeds treated with fungal spores and nanoparticles at the same time.

Control (shoots in mm)	Nanoparticles treated seeds (shoots in mm)	Fungal spores + nanoparticles treated seeds (shoots in mm)
8.2	8.6	7.1
6.9	8.11	7.7
6.8	9.9	9.9
8.1	8.7	7.9
8.1	8.4	7.9
7.4	7.6	7.1
7.2	8.3	7.3
7.9	8.8	7.7
8	8	8
7.575	8.55125	7.825
0.570087713	0.664517817	0.900396738
0.201556444	0.234942527	0.31833832

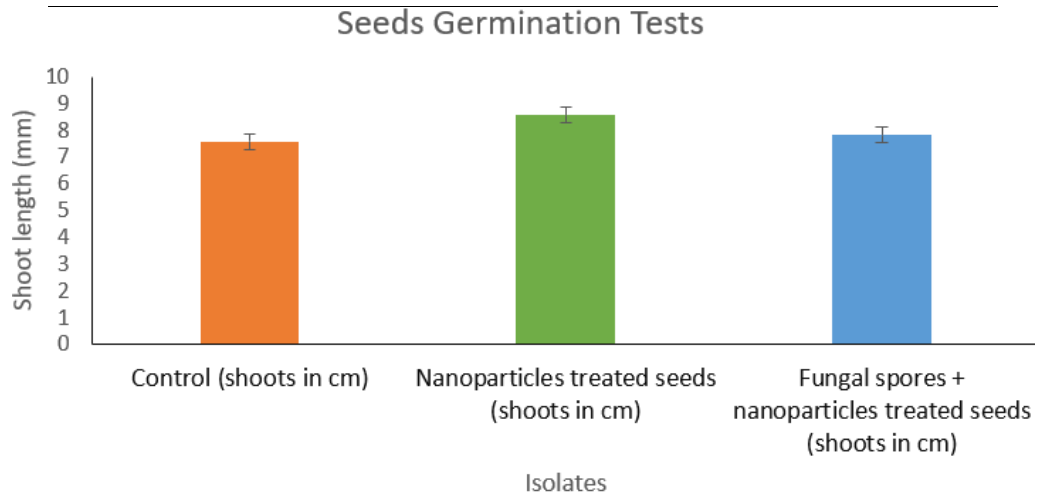

Figure 8. Graphical representation of a comparison of seed germination in different conditions. The orange bar represents seed shoots grown without anything applied to it. The green bar represents an improvement in growth after applying nanoparticles to seed shoots.

The blue bar represents the inhibition of fungal spores by nanoparticles and also a slight increase in the shoot growth.

Discussion:

In current research, *Serratia marcescens* w2, strain was used to synthesize metallic silver nanoparticles. Their antifungal activity was checked against fungal phytopathogens *A. flavus* (17) [17] Biologically synthesized nanoparticles also showed some positive effects on seed germination. For such purpose biosynthesized nanoparticles have not been used previously. Metal nanoparticles have already been synthesized using *Serratia marcescens* w2, (18). However, these nanoparticles have not yet been evaluated as nano-pesticides, even though this study was performed to do so.

The use of green technology to create nano-bodies known as nanoparticles has become a popular approach in sectors such as health and agriculture. Bismuth nanoparticles synthesized by *S. marcescens* showed antibacterial as well as antifungal activity. This synthesis was done by reacting bismuth sulfate with the extracellular metabolites of the bacteria used (19). Titanium nanoparticles synthesized by *Lactobacillus spp.* Showed antimicrobial activity. This synthesis was done by reacting dihydroxy titanium with extracellular metabolites of the bacteria (20). The antimicrobial activity was evaluated using the good diffusion method.

Silver nanoparticles were synthesized utilizing *Syzygium cumini* and *Centella Asiatic* (21). The antimicrobial activity of these silver nanoparticles was evaluated using the good diffusion technique against *Staph. Aureus* and *Pseudomonas aeruginosa*. At varied concentrations, inhibitory zones were identified after a 24-hour incubation at 350^0C temperature.

Magnetic nanoparticles have been utilized as medication-delivery vehicles for decades (22). Metallic nanoparticles are attached to the concerned drug and delivered into the body, where the metallic particles in the medication are controlled while the drug is in the bloodstream. Nanoparticles as catalysts are a newly developing aspect of nanoparticles (23). Palladium nanoparticles supported on carbonaceous materials have shown encouraging results in hydrogen sorption, producing more stable and efficient fuel cells, and are expected to be employed in vehicle batteries shortly.

Glycerol is a well-known substance. It is now widely generated as a byproduct of the biodiesel manufacturing process. Palladium, platinum, and gold metal nanoparticles are currently being used to oxidize glycerol on carbonaceous surfaces, as well as to promote environmental cleanup (24).

While transporting DNA, RNA, and proteins across the cell membrane, nanoparticles can prevent them from being destroyed. Because of the safe delivery of these macromolecules, gene therapy, and protein-based therapeutic techniques are now accessible. To guarantee successful delivery, carriers must I form condensed complexes with biomolecules, (ii) allow cell membrane penetration following complexation, and (iii) release their payloads inside cells (25). gave an example of gene delivery.

One of the most essential aims of delivery systems is to target only diseased tissue with their payloads. This may be accomplished in two ways.

In vitro and in vivo biological specimens have been imaged using optical imaging (OI), magnetic resonance imaging (MRI), ultrasound imaging (USI), positron emission tomography (PET), and other molecular imaging techniques. Bioimaging technology is progressing thanks to the creation of brilliant and magnetic nanoparticles. Luminescent nanoprobes for OI and magnetic nanoparticles for MRI have both been widely utilized in imaging. For simultaneous optical imaging (OI) and magnetic resonance imaging (MRI), dual-mode nanoparticles are also available (26).

Conclusion:

From the present studies, it may be concluded that the endophytic bacteria *S. marcescens* W2, can produce nanoparticles that show prominent antifungal activities. These metal

nanoparticles are prospective nano pesticides that can biocontrol fungal phytopathogens and may be employed in the area of pharmacology as they are prospective sources of valuable drugs.

- From the above studies, it was observed that metal nanoparticles specifically synthesized using microbes show prominent antifungal activity specifically against phytopathogens *Aspergillus flavus*.
- Scanning Electron Microscope (SEM) analysis of the synthesized nanoparticles showed the formation of nanostructures with trigonal, tetragonal, and pentagonal shapes with nonporous surfaces and nonuniform sizes.
- Synthesized bio-nanoparticles when applied to test seeds, showed enhanced growth.
- Wheat seeds when treated with fungal spores as well as biosynthesized nanoparticles, showed inhibition of fungal spores as well as a slight increase in growth of seed shoots.
- Antimicrobial studies show that even a small amount as 10 mg/ mL gives results, indicating the high efficacy of biosynthesized nanoparticles.

Acknowledgement:
All the mentioned authors have contributed to the article and all authors are in agreement of all the content of the manuscript.

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