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Use of silver nanoparticles for controlling *Burkholderia glumae* in rice: Characterization and field results

Uso de nanopartículas de plata para el control de *Burkholderia glumae* en arroz: Caracterización y resultados de campo

Jeyson Adrian Gonzalez-Gonzalez[1]; Luz Yineth Ortiz-Rojas[2]; Giovanni Chaves-Bedoya[3]

[1] *Universidad Francisco de Paula Santander, Cucuta, Colombia, jeysonadriangg@ufps.edu.co, https://orcid.org/0009-0009-8247-6523*

[2] *Universidad Francisco de Paula Santander, Cucuta, Colombia, luzyintehor@ufps.edu.co, https://orcid.org/0000-0002-0818-3089*

[3] *Universidad Francisco de Paula Santander, Cucuta, Colombia, gchavesb@ufps.edu.co, https://orcid.org/0000-0003-1013-614X*

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ABSTRACT

Rice, *Oryza sativa* L., serves as a staple food for a substantial portion of the global population, making its protection against pathogens like *Burkholderia glumae* critically important. While recent studies have explored various nanotechnological approaches for phytopathogen control, the specific field application of silver nanoparticles against *B. glumae* remains poorly documented. This study evaluated the field efficacy of silver nanoparticles (AgNPs) as a treatment against *B. glumae*, comparing them with traditional methods such as Starner WP (a synthetic fungicide) application. AgNPs were synthesized electrochemically and characterized by UV/VIS spectrophotometry. *Burkholderiaglumae* detection was performed using Polymerase Chain Reaction (PCR). Employing a randomized block design, various growth parameters of rice seedlings were analyzed during five weeks post-treatment. Application of AgNPs improved yield parameters by reducing the incidence of grains affected by *B. glumae* by 45%, increasing the total grain number by 20%, and raising grain weight by 15%. In contrast, root volume decreased by about 10%, a finding consistent with prior greenhouse research. Treated seedlings exhibited more intense green foliage, suggesting improved plant health. These results were corroborated by morphological variations, indicating differential impact on growth and disease resistance. This study provides valuable insights into nanotechnology applications for sustainable agriculture and emphasizes the need for a multifaceted approach to disease management in rice. AgNPs represent a promising sustainable alternative to conventional bactericides.

Keywords: bacterial inhibition; crop protection; field trials; nanotechnology; phytopathology; sustainable agriculture

RESUMEN

El arroz (*Oryza sativa* L.) es un alimento básico para una parte sustancial de la población mundial, por lo que su protección contra patógenos como *Burkholderia glumae* (*B. glumae*) es de suma importancia. Aunque estudios recientes han explorado enfoques nanotecnológicos para el control de fitopatógenos, la aplicación específica de nanopartículas de plata en campo contra *B. glumae* aún es poco documentada. Este estudio evalúa la eficacia en campo de nanopartículas de plata (AgNPs) como tratamiento contra *B. glumae*, comparándolas con métodos tradicionales como Starner WP (un fungicida sintético). Las AgNPs fueron sintetizadas electroquímicamente y caracterizadas por UV/VIS. La detección de *B. glumae* se realizó mediante Reacción en Cadena de la Polimerasa (PCR). Empleando un diseño de bloques al azar, se analizaron diversos parámetros de crecimiento de plántulas de arroz durante cinco semanas posteriores al tratamiento. El ensayo confirmó que las AgNPs redujeron significativamente el número de granos afectados por *B. glumae* en 45% y aumentaron el conteo total de granos en 20% comparado con el control sin tratamiento. También se observó un aumento del 15% en el peso de los granos. Sin embargo, se notó una reducción del volumen radicular de aproximadamente 10% con el tratamiento de AgNPs, hallazgo consistente con investigaciones previas en invernadero. Las plántulas tratadas mostraron follaje verde más intenso, indicando mejora en salud vegetal. Estos resultados fueron corroborados por variaciones morfológicas, señalando un impacto diferencial en crecimiento y resistencia. Este estudio aporta información valiosa sobre la nanotecnología en agricultura sostenible y enfatiza la necesidad de un enfoque multifacético para manejo de enfermedades.

Palabras clave: agricultura sostenible; ensayos de campo; fitopatología; inhibición bacteriana; nanotecnología; protección de cultivos

INTRODUCTION

Rice serves as an essential dietary element worldwide and plays a pivotal role in food security and the economies of numerous countries. As the primary staple food for over half of the world›s population, its efficient and sustainable production is a global priority (Fukagawa & Ziska, 2019). Its cultivation spans across continents, feeding billions and underpinning the agricultural economies of numerous nations. The global significance of rice extends beyond simple nutrition; it is embedded in cultural practices, local cuisines, and the livelihoods of millions of farmers (Mohidem *et al.*, 2022). As climate change and population growth impose increasing pressures on agricultural systems, efficient and sustainable rice production becomes a critical challenge that demands innovative solutions (Benitez-Alfonso *et al.*, 2023).

In Colombia, rice cultivation is a cornerstone of the agricultural economy and a vital component of the national diet, contributing significantly to food security. This grain is an essential element in the daily diet of Colombians, highlighting its agricultural importance as the annual crop with the second-largest land area dedicated to its cultivation. In 2018, approximately 685,138 hectares were dedicated to rice cultivation, representing 30% of the total area for annual crops—a figure second only to corn. Despite ranking second in land area, rice leads in production value at the national level, establishing Colombia as the second-largest rice producer in Latin America and the Caribbean (Arango-Londoño *et al.*, 2020). However, despite the extensive land area dedicated to rice and the significant investment in its production, Colombian farmers consistently face numerous yield-limiting factors, among which crop diseases pose a particularly formidable threat.

Among the many challenges confronting rice production, the bacterium *B. glumae* emerges as a significant threat. It is the causative agent of bacterial panicle blight, a disease notorious for precipitating substantial reductions in yield and degrading grain quality (Ortega & Rojas, 2021). *B. glumae* infects the panicles of rice plants, leading to premature drying and discoloration of the grains, which severely impacts the marketability and usability of the harvested rice. This pathogen is especially problematic in regions with warm and humid climates, conditions that are prevalent in many of the world›s primary rice-producing areas, including Colombia (Morales-Becerra *et al*., 2023).

The lifecycle and proliferation of *B. glumae* are tightly linked to environmental factors. Bacterial panicle blight (BPB) is particularly influenced by the simultaneous occurrence of high daily minimum temperatures (~22˚C) and high relative humidity (~77%), conditions that may become increasingly common due to global warming (Shew *et al*., 2019). This climate dependency complicates efforts to manage the bacterium›s presence in rice fields, as *B. glumae* can be transmitted through various means, primarily through seeds (Sayler *et al*., 2006). Once established, the pathogen can inflict considerable damage before symptoms become visibly detectable, making early intervention difficult. The increasing threat of global warming exacerbates these challenges, highlighting the urgent need for developing adaptable management strategies for *B. glumae* in rice cultivation.

The economic repercussions of outbreaks caused by *B. glumae*, the causative agent of bacterial panicle blight, are significant, with affected regions reporting substantial losses of up to 75% in both yield and quality (Zhou, 2019). This disease not only reduces yield but also compromises grain quality, posing a threat to food security and farmers› incomes (Ortega & Rojas, 2021; Shew *et al*., 2019). The control of *B. glumae* presents additional challenges. While bactericides can provide a degree of management, their efficacy is often limited, and their usage can contribute to environmental and health concerns. The development of resistant rice varieties offers a promising long-term solution, but this approach requires extensive research and time to be implemented effectively (Sundin *et al*., 2016).

Despite various traditional control methods—including the use of bactericides, the selection of resistant varieties, and the implementation of integrated pest management (IPM) measures—the persistent challenge of panicle rot in rice cultivation remains significant (Ortega & Rojas, 2021). These strategies have not been fully effective in combating the disease, highlighting the urgent need for more effective solutions (Peñaloza Atuesta *et al*., 2020). In this context, nanotechnology, specifically the application of silver nanoparticles (AgNPs), emerges as a promising approach to disease management in agriculture. AgNPs offer a novel and potentially more sustainable method to mitigate the impacts of bacterial diseases like *B. glumae*. By targeting bacterial infections at a microscopic level, this approach could be less harmful to the environment and safer for human health compared to traditional practices (Lee & Jun, 2019).

Recognized for their potent antimicrobial properties, silver nanoparticles (AgNPs) are increasingly noted for their effectiveness in combating pathogens, presenting a significant advancement in the quest for sustainable agricultural practices (Chaves-Bedoya *et al*., 2023; Jahan *et al*., 2024). This shift towards nanotechnology highlights the critical need for innovative approaches that not only precisely target pathogens but also align with the goals of environmental sustainability and human health safety (Mann *et al*., 2021).

The antimicrobial properties of AgNPs are attributed to their ability to disrupt bacterial cell membranes, interfere with essential cellular functions, and induce oxidative stress, which collectively contributes to their efficacy against a wide range of bacteria (Khan *et al*., 2023) Their nanoscale size allows for targeted action against pathogens like *B. glumae*, potentially reducing the need for broad-spectrum chemical applications that can adversely affect non-target species and the environment. Moreover, the versatility of AgNPs in delivery methods offers opportunities for precise application, minimizing waste and maximizing efficacy (Partila, 2019). This approach represents a convergence of technological innovation and sustainable practice, providing a solution that could reduce the chemical load on ecosystems while addressing the challenges posed by crop diseases (Wang *et al*., 2022).

The primary objective of this study was to assess the effectiveness of silver nanoparticles (AgNPs) in combating *B. glumae* in rice, applied specifically on the FEDEARROZ 2020 variety cultivated in Norte de Santander, Colombia. AgNPs were evaluated as a viable and sustainable alternative to traditional bactericides, aiming to effectively control the pathogen while also exploring their broader agronomic impacts on plant health, growth, and yield in field conditions. The findings of this research open the doors for further exploration into the benefits of nanotechnology for enhancing plant defenses and improving resource efficiency in crops. The insights gained here could also guide policies and best practices for safely and effectively integrating nanotechnology into agricultural frameworks.

MATERIALS AND METHODS

Electrochemical Synthesis of Silver Nanoparticles (AgNPs)

The synthesis of silver nanoparticles (AgNPs) was achieved using an electrochemical process. High-purity silver rods (99.99%, Aldrich® brand), each 10 cm in length and 2 mm in diameter, served as electrodes. These rods were aligned parallel to each other with a 2-centimeter gap and connected to a power source that maintained a consistent potential difference of 24 volts. The electrolytic medium comprised 200 mL of sterile distilled water, ensuring a contamination-free environment for synthesis. The entire synthesis procedure was conducted at ambient temperature for one hour, optimizing conditions for nanoparticle formation (Padilla-Sierra *et al*., 2021).

To quantify the synthesized AgNPs, the total dissolved solids (TDS) within the electrolyte were measured using a TDS/PPM meter, which provided an accurate determination of nanoparticle concentration. Furthermore, the spectral properties of the synthesized AgNPs were analyzed using a Genesis 10S spectrophotometer (Thermo Scientific®). This analysis identified the maximum absorption wavelength, which was indicative of the AgNPs' presence and concentration within the solution. By employing these measurements, a calibration curve was established to correlate AgNP concentration with absorbance, in accordance with the Beer-Lambert Law (Swinehart, 1962). This procedure facilitated a precise characterization of the nanoparticle synthesis outcome.

Assessment of AgNP Antimicrobial Activity Against B. glumae

In our endeavor to explore innovative solutions for the management of *B. glumae* infections in rice crops, the study utilized the certified bacterial strain ATCC 33617, graciously provided by the "Federation Nacional de Arroceros" (FEDEARROZ). The primary cultivation of the bacteria was conducted in a nutrient-rich broth within a controlled environment chamber, which was maintained at a steady temperature of 32°C with constant agitation. This setup was designed to simulate optimal growth conditions and facilitate the accurate assessment of colony-forming units (CFUs). Spectrophotometry was employed to measure absorbance after 24 hours, targeting a benchmark of 1X108 CFUs. This density corresponded to an absorbance reading of 0.2 ± 0.05 at 600 nm, thereby establishing a baseline for bacterial density.

To pinpoint the inhibitory concentration of silver nanoparticles (AgNPs) effective against *B. glumae*, a series of carefully designed experiments was conducted. These experiments involved preparing various concentrations of AgNPs, ranging from 1 to 10 mg/L, with each concentration being systematically doubled to cover the entire spectrum effectively. The experimental units consisted of tubes containing 1 mL of AgNP solution mixed with 1 mL of nutrient broth and 100 µL of the bacterial suspension, the latter adjusted to reflect a concentration of 2x108 bacteria at 625 nm wavelength. This rigorous approach allowed for the establishment of a concentration gradient, facilitating a comprehensive evaluation of the AgNPs› antimicrobial properties.

For the laboratory-based control experiments, two distinct control setups were established. The negative control consisted of a solution without any nanoparticles or antibiotics, allowing for the unimpeded growth of *B. glumae*. The positive control utilized ciprofloxacin at a concentration of 1 mg/mL, selected for its known antimicrobial efficacy in a controlled environment. This was in contrast to field experiments, where the commercial product Starner was used as a practical control in agricultural settings. After a 24-hour incubation to facilitate bacterial growth, aliquots were taken from each test tube and plated on King B agar. The plates, including the controls, were then incubated for an additional 24 to 48 hours to assess the inhibitory effect of the different concentrations of AgNPs on bacterial growth.

Determination of Growth Inhibition Percentage

The growth of *B. glumae* was assessed by measuring the optical density at a wavelength of 625 nm using a Thermo Scientific® Genesis 10S spectrophotometer. Three separate measurements were taken to precisely monitor bacterial growth under various experimental conditions. The percentage of bacterial growth inhibition achieved by the silver nanoparticles (AgNPs) was then calculated using the following equation 1:

$$\text{Percentage of Inhibition} = ((OD_{control} - OD_{sample}) / OD\_control) \times 100$$

Where: OD_control: Optical density of the control sample (bacterial culture without treatment) OD_sample: Optical density of the treated sample (bacterial culture with AgNPs treatment).

Plant Material

The plant material for this study was sourced from La Llana Farm, a rural property owned by the Gelvez business group. Located in the municipality of Tibú, Norte de Santander (coordinates: 8°19’58.1”N 72°35’37.6” W), this farm cultivates the FEDEARROZ 2020 rice variety. Rice plant samples were collected in the morning and transported to the FITOBIOMOL phytopathology laboratory at the Universidad Francisco de Paula Santander. The samples were placed in properly labeled resealable plastic bags and stored in insulated foam coolers with ice to preserve their integrity during transit. This procedure was conducted to facilitate the early detection of *Burkholderia* spp. Sampling was timed to coincide with specific phenological stages, including seedling, tillering, and flowering, to capture the pathogen’s presence throughout the plant’s development.

DNA extraction and PCR amplification for Bacterial Blight Detection

To facilitate early detection of bacterial blight, DNA extraction was performed on the collected plant samples using the CTAB method (Doyle & Doyle, 1987).

Subsequently, primers specifically designed to detect *B. glumae* were applied using the PCR technique. The primers 2BglF (5'-ACGTTCAGGGATRCTGAGCAG-3') and 2BglR (5'-AGTCTGTCTCGCTCTCCCGA-3') targeted the 16S-23S spacer regions of the ribosomal DNA and were capable of amplifying a 286 base pair band (R. J. Sayler et al., 2006). The PCR protocol was carried out using a ProFlex thermal cycler (Applied Biosystems). The program was initiated at 94°C for 30 seconds (initial denaturation), followed by 35 cycles of 94°C for 1 minute (denaturation), 65°C for 1 minute (annealing), and 68°C for 2 minutes (extension). A final extension step was performed at 68 °C for five minutes, and the program concluded by holding the temperature at 4 °C. The PCR results were analyzed on 1.5% agarose gels through electrophoresis in a chamber with 1X TAE buffer, followed by ethidium bromide staining. A 100 bp marker was used as a reference to determine the size of the expected product, which was subsequently visualized under a UV transilluminator (UPV).

Application of AgNPs

Sampling and Variable Evaluation. Following the confirmation of *B. glumae* presence through early molecular detection techniques, silver nanoparticles (AgNPs) were applied during the pre-flowering phase, which typically occurs between 45 and 55 days after planting. This timing was chosen to ensure that the treatment was administered after the pathogen, thereby maximizing the efficacy of the intervention. The experiment was conducted over two consecutive years, with the first trial in 2021 and the second in 2022, to ensure the consistency of the results. A randomized block design was implemented, consisting of 9 m2 plots with three replicates per treatment (Figure 1). The image on the left shows the randomized block design setup, while the image on the right depicts the treatment application. Treatments included a positive control (Starner WP), a negative control (untreated), and AgNPs at 10 mg/L.

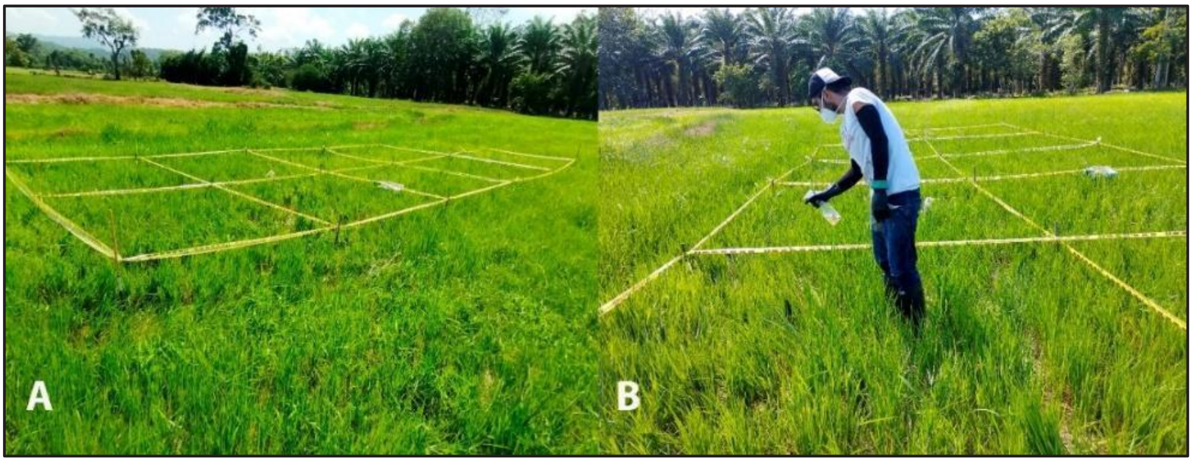


Figure 1. *Field trial layout and treatment application*

Statistical Analysis

Statistical analyses were conducted using SAS for Windows, version 9.0, utilizing the PROC GLM procedure for the ANOVA and the means statement with the Tukey option for the post-hoc analysis. The assumptions of ANOVA, including normality of residuals and homogeneity of variances, were assessed through diagnostic plots and tests. The experimental design was a randomized complete block design (RCBD) with three treatments, replicated five times. The treatments included 1) a positive control treated with Starner WP at a dosage of 160 mL per block, 2) a negative control where plants were maintained under normal conditions, and 3) plants treated with silver nanoparticles (AgNPs) at a concentration of 10 mg/L. Variables assessed five weeks after application included chlorotic leaves, plant height, root size, number of affected grains, total grain count, grain weight, and plant weight. For variables where significant treatment effects or interactions were detected, additional analyses were conducted to explore the nature of these effects. The significance level for all tests was set at $\alpha = 0.05$. Post-hoc comparisons among treatment means were performed using Tukey›s Honestly Significant Difference (HSD) test to control for Type I error across multiple comparisons. This test was applied to identify significant differences between treatment groups for each measured variable.

RESULTS

Synthesis and Concentration Dynamics of Silver Nanoparticles (AgNPs)

In the examination of the synthesis kinetics of silver nanoparticles (AgNPs), a strong linear relationship was established between time and nanoparticle concentration. As shown in Figure 2A, the experimental data were well-fitted by a linear model, exhibiting an R-squared value of 0.9634. The synthesis rate, determined from the slope, was 0.2764 mg/L min-1, signifying a consistent and efficient production of AgNPs within the electrolytic solution.

A two-hour synthesis period yielded a final concentration of 32±4 mg/L of silver nanoparticles in the aqueous solution. The UV-Vis absorption spectrum of the AgNPs at a concentration of 26 mg/L, as depicted in Figure. 2B, displayed a maximum absorbance peak at a wavelength of 413 nm. This specific wavelength corresponded to the surface plasmon resonance of the silver nanoparticles, which confirmed the successful synthesis and nanoparticle characteristics.

A calibration curve at $\lambda = 413$ nm was constructed to establish the relationship between absorbance and varying concentrations of AgNPs (Figure 2C). In accordance with the Beer-Lambert law, the curve demonstrated excellent linearity, exhibiting a coefficient of determination (R2) of 0.9764. The consistent linear response, as depicted by the continuous line in the graph, indicated the potential for accurately estimating the concentration of AgNPs in a given solution using UV-Vis spectroscopy focused at the particular wavelength of 413 nm, where the surface plasmon resonance occurred.

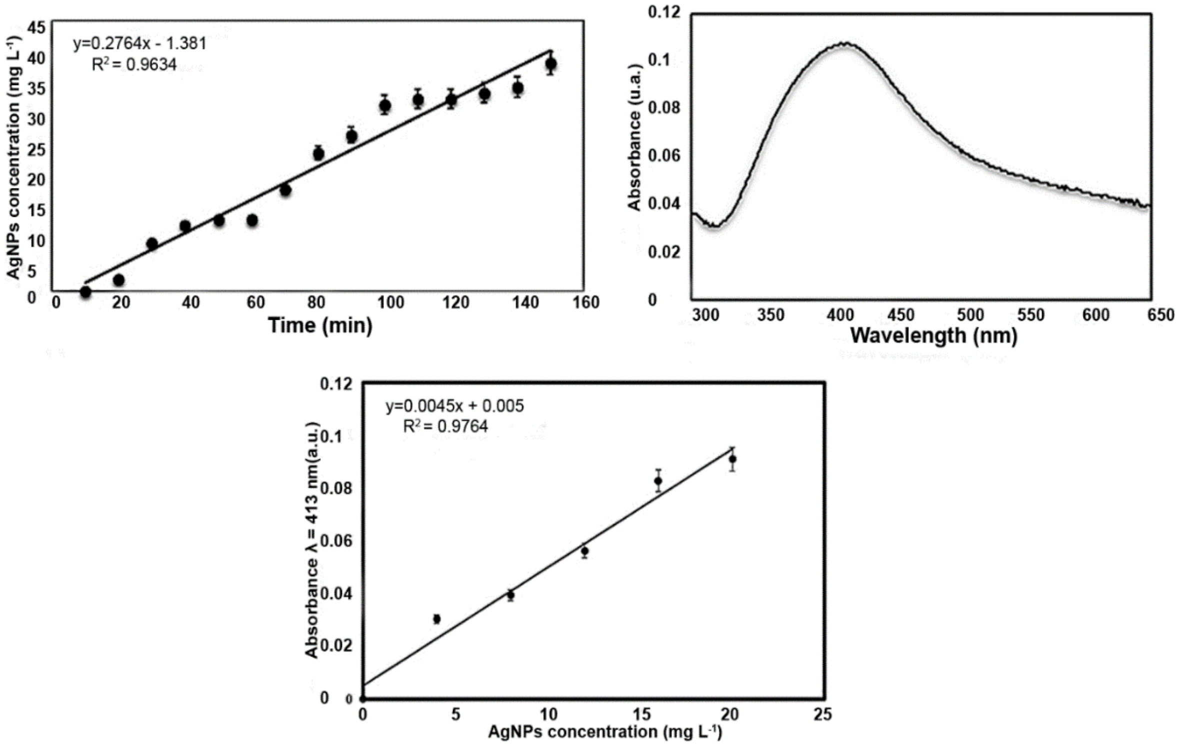


Figure 2. *Characterization of silver nanoparticles (AgNPs) synthesis and concentration*

Determination of Inhibitory Concentration

To determine the bactericidal impact of silver nanoparticles (AgNPs) on *B. glumae*, a series of tests was performed using concentrations of AgNPs ranging from 1 to 10 mg/L. After 24 hours of incubation at 32°C with constant agitation at 150 rpm, the absence of turbidity was observed in all test tubes across the concentration gradient (Figure 3).

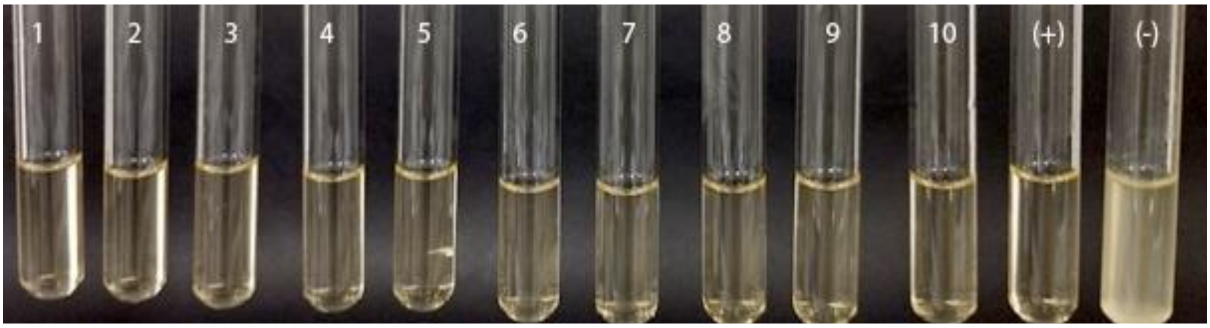


Figure 3. *Test tubes showing the reaction of Burkholderia glumae to increasing concentrations of silver nanoparticles (AgNPs)*

The subsequent culturing of the samples on Petri dishes provided a more granular insight into the bacterium›s response to the AgNPs. At concentrations ranging from 1 to 5 mg/L, significant colony growth was observed, indicating that these AgNPs levels were insufficient to prevent the proliferation of *B. glumae* (Figure 4). However, a marked reduction in colony-forming units was evident at concentrations from 6 mg/L onwards, indicating a substantial inhibitory effect by the AgNPs.

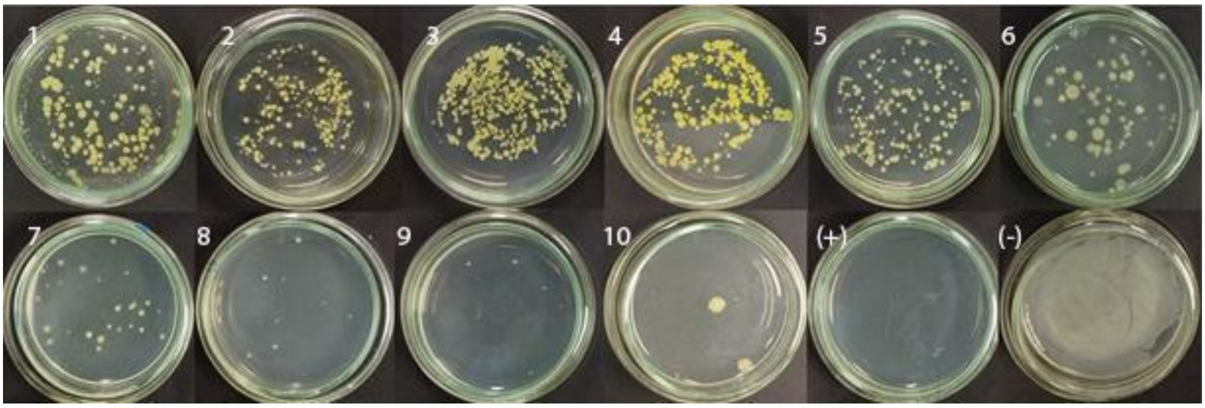


Figure 4. *Agar plates illustrating B. glumae colony formation in response to increasing concentrations of AgNPs*

Efficacy of Silver Nanoparticles in Bacterial Growth Inhibition

The interaction between silver nanoparticles (AgNPs) and *B. glumae* was quantified through absorbance measurements. The study found that even at a low concentration of 1 mg/L, AgNPs achieved significant inhibition of bacterial growth. This finding was consistent across five separate experimental repetitions, reinforcing the reliability of the observed effect (Figure 5).

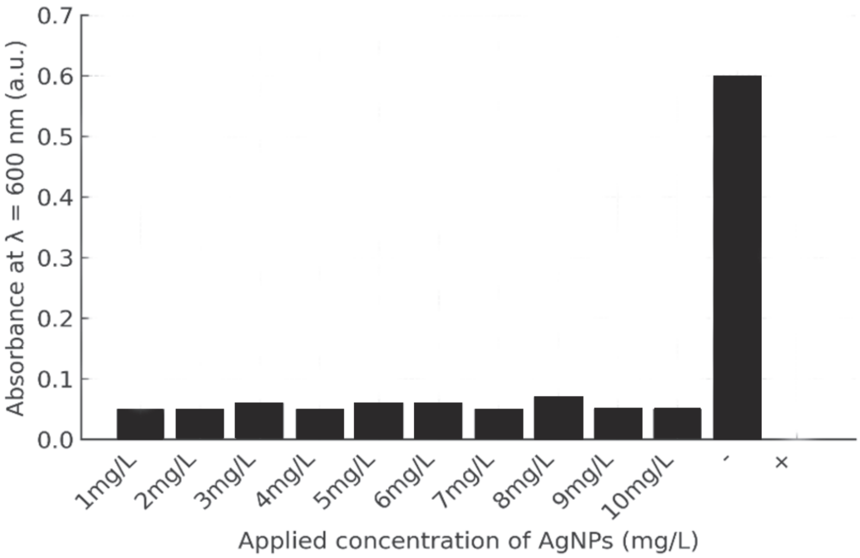
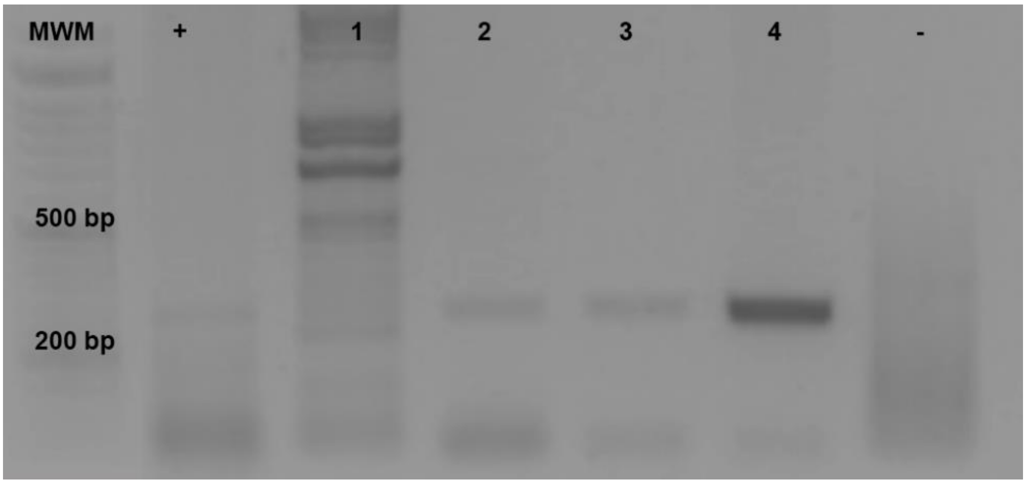


Figure 5. *Bar graph depicting absorbance at various AgNP concentrations applied to cultures of B. glumae. Lower absorbance bars across all concentrations indicate effective inhibition of bacterial growth.*

The bar graph with shorter bars illustrates this inhibition clearly; each bar represents the absorbance level corresponding to the respective AgNP concentration. The data indicate that high levels of inhibition were consistently maintained across the range, beginning as low as 1 mg/L. The positive control, treated with ciprofloxacin, confirmed the maximum inhibition efficacy expected of an antibiotic.

Molecular Detection of Bacterial Blight

DNA was successfully extracted from the leaf samples, yielding high-quality genetic material suitable for further analysis. Direct extraction from leaf tissues ensured the integrity of the DNA, which is crucial for precise molecular detection. Subsequent PCR amplification revealed a distinct 286-base pair (bp) band, confirming the presence of *B. glumae*, as shown in Figure 6.



MWM: molecular weight marker; (+): positive control; lanes 1–4: rice samples (amplification observed in samples 2–4); (–): negative control.

Figure 6. *Gel electrophoresis showing PCR amplification of B. glumae.*

Field Trial Results

The field trial assessed the impact of silver nanoparticles (AgNPs) at a 10 ppm concentration on the incidence of *B. glumae* in rice plants. The results were compared to both a positive control treated with Starner WP and an untreated negative control. Statistical analyses of the field data, performed using SAS, demonstrated that while AgNP treatment did not significantly alter the plant weight, the incidence of chlorotic leaves, or total grain count, led to a marked improvement in root length and a significant decrease in the number of affected grains.

Statistical analyses of the field data, performed using SAS, showed significant variations in several parameters. The AgNP-treated plants exhibited a reduction in root growth compared to the negative control, a finding supported by the statistical output showing a marked difference in root length. However, these treatments also led to a significant reduction in empty grains and the gain in individual grain weight, illustrating the potential of AgNPs to enhance rice plant health and yield. As expected, the positive control, treated with a well-established bactericide, displayed the highest level of disease inhibition. Figure 7 shows rice seedlings subjected to different treatments: the untreated negative control, Starner for seedlings treated with Starner WP, and AgNPs for those treated with silver nanoparticles at a concentration of 10 mg/L. A visual assessment reveals notable differences in root volume and plant coloration across the treatments. The negative control exhibited a greater abundance of roots, while the root volume in the AgNP-treated plants appeared less dense. Furthermore, the negative control exhibited a lighter green hue, whereas both the Starner WP and AgNP-treated plants showed deeper green tones.

Figure 7. *Rice seedling responses to treatment with starner wp and silver nanoparticles*

Plants subjected to the various treatments displayed symptomatic spikelets, as shown in Figure 8. Grains with a straw-colored tint and a significant proportion of empty grains were particularly evident. The image depicts the symptomatic grains of rice affected by *B. glumae*, with different treatments indicated by A for the negative control, B for Starner-treated grains, and C for grains treated with AgNPs. Symptomatic grains with straw-colored hues within yellow frames were indicative of possible pathogen impact. The green frames denoted empty grains, while the white frames pointed to full grains exhibiting a healthier appearance.

Figure 8. *Comparison of grain symptoms in rice affected by B. glumae under different treatments*

As presented in Table 1, the negative control group exhibited an increased incidence of affected grains. Conversely, both the positive control and the AgNP-treated groups showed a decrease in this incidence. Furthermore, the AgNP-treated group also presented a higher grain weight and a greater plant weight when compared to the control groups. Although the AgNP-treated plants showed a reduction in root size, they maintained stable plant health metrics, including the percentage of chlorotic leaves, plant height, and root size.

Table 1. *Impact of AgNP treatment on various growth parameters in rice (Oryza sativa L.) infected with Burkholderia glumae. Values represent the average of 15 observations per treatment.*

Treatment	% Affected Grains	Grain Weight (g) (avg)	Plant Weight (g) (avg)	Root Length (cm) (avg)
Starner 20 WP	18	1.82	8.3	6.6
Negative	38	1.72	9.3	6.2
AgNPs	23	2.00	9.8	4.9

DISCUSSION

The synthesis kinetics of silver nanoparticles (AgNPs) demonstrated high efficiency and predictability. The linear relationship between time and concentration (R^2 = 0.9634) underscored the effectiveness of the electrochemical approach employed. This finding provides a predictable and quantifiable method for AgNP synthesis, which is particularly advantageous for large-scale agricultural applications where precise dosages of nanoparticle formulations are essential. The characteristic absorbance peak at 413 nm reflects the unique light-absorption properties of silver nanoparticles, which are critical for their antimicrobial function. Furthermore, the high degree of linearity in the calibration curve (R^2= 0.9764) confirmed the reliability of the experimental data and validated the use of absorbance as a measure for accurately quantifying nanoparticle concentration.

The observed absence of turbidity in all test tubes across the concentration gradient typically signifies bacterial growth inhibition. This suggests that AgNPs could be effective against *B. glumae*, potentially even at the lowest concentration examined. The stark difference in bacterial growth between the lower (1-5 mg/L) and higher (6-10 mg/L) AgNP concentrations reveals a clear concentration threshold at which AgNPs become effective in hindering bacterial proliferation. This threshold is crucial for understanding the minimal effective concentration needed for the AgNPs to serve as a viable alternative to traditional bactericides. The presence of abundant bacterial colonies in the negative control and the absence of growth in the positive control validate the reliability of the experimental approach and the specificity of AgNPs› antibacterial action.

Despite expectations of a dose-dependent relationship, the data indicate that high levels of inhibition were maintained consistently across the range, beginning as low as 1 mg/L. This finding suggests that even at low concentrations, AgNPs possess strong antibacterial properties against *B. glumae*. The molecular detection results, with the distinct band at 286 base pairs, correspond to the expected size for *B. glumae*, aligning with the band size reported in previous studies (Sayler *et al.*, 2006), confirming the molecular detection of bacterial blight within the sampled plant material.

The field trial concentration of 10 ppm was selected based on previous laboratory experiments, which demonstrated significant inhibition of bacterial growth even at concentrations as low as 1 ppm. These properties, which involve the disruption of bacterial cell membranes and interference with DNA replication, appear to translate effectively to field conditions, positioning them as a promising alternative to conventional treatments. While these findings affirm the antimicrobial efficacy of silver nanoparticles (AgNPs) against *B. glumae* and suggest their viability as an alternative to traditional pesticides, it is crucial to also acknowledge the broader environmental implications.

Extensive studies are necessary to evaluate the long-term effects of AgNPs on soil health, microbial communities, and overall ecosystem dynamics. This holistic examination is crucial for ensuring the sustainable integration of nanotechnology in agriculture, where phytoremediation strategies could play a pivotal role in mitigating potential nanoparticle accumulation and safeguarding ecological balance and public health (Ihtisham *et al*., 2021).

The observation that AgNPs may stimulate root growth while simultaneously reducing root volume presents an interesting paradox that requires further investigation. This suggests that while AgNPs are effective in controlling bacterial blight, they may also present a trade-off in terms of root development. This finding aligns with earlier greenhouse experiments, where a similar reduction in root growth was noted in rice treated with AgNPs (Morales-Becerra *et al*., 2023). Such consistent findings across different settings underscore the need for careful consideration of the potential impacts on root development when using AgNPs as a treatment. This is particularly relevant as root infections can seriously compromise a plant›s ability to absorb water and nutrients, negatively impacting its growth and development (Fadiji *et al*., 2023).

Results of this study show that AgNP treatment reduces the incidence of grains affected by *Burkholderia glumae* compared to the negative control, though its efficacy is not as high as the traditional bactericide, Starner WP. The observed increased grain weight in the AgNP-treated plants suggests that the nanoparticles not only reduce pathogen incidence but also promote better reproductive development. However, the reduction in root length is consistent with previous greenhouse findings and raises questions about the broader effects of AgNPs on plant physiology. This trade-off between pathogen control and root development emphasizes the need for optimization of AgNP application concentrations to balance antimicrobial efficacy with overall plant health.

The results obtained in this study reveal a complex interaction between silver nanoparticles (AgNPs) and rice plants, characterized by both beneficial and potentially limiting effects. The 10% reduction in root volume observed in AgNP-treated plants is consistent with the phytotoxicity mechanisms. As reported by Tripathi *et al.* (2024), AgNPs can affect plant growth and development by inducing oxidative stress and compromising cellular membrane integrity. This effect is primarily due to the ability of AgNPs to be absorbed and accumulated in plant tissues, where they can interfere with fundamental cellular processes, including mitochondrial function and protein synthesis (Tripathi *et al*., 2024).

However, it is important to highlight that, despite this reduction in root volume, AgNP-treated plants showed more intense green foliage and significant improvement in productive parameters. This suggests that AgNPs can exert growth-promoting effects when applied at appropriate concentrations. This duality in AgNP effects has been documented by Alfosea-Simón *et al.* (2025), who reported that silver nanoparticles can promote plant growth, alleviate stress, and fight against pathogens when used under controlled conditions and at optimized concentrations. The authors emphasize that AgNPs promote growth and alleviate biotic and abiotic stresses through different application methods, being particularly effective in integrated disease management. In this study, the 10 mg/L concentration of AgNPs proved effective for *B. glumae* control, significantly reducing the number of affected grains by 45% while maintaining overall plant viability. Such information advocates that this concentration falls within the optimal range to maximize antimicrobial benefits while minimizing adverse phytotoxic effects.

The visual assessment reveals that while previous discussions have indicated that AgNP treatments led to longer roots, the contrast in volume may suggest that root thickness and the proliferation of finer root structures could be compromised. This is a critical observation, as root density can significantly influence water and nutrient uptake, ultimately affecting plant health and productivity (Fadiji *et al*., 2023). The coloration of the seedlings provides additional insights, with the deeper green tones in treated plants potentially indicative of better overall plant health. The balance between root length and volume suggests that while AgNPs can effectively inhibit the pathogen, they might also influence root architecture differently than chemical treatments such as Starner WP. Further studies are therefore warranted to explore these nuances and optimize treatment concentrations and methods for the best agronomic outcomes.

Prior studies have established that *B. glumae* thrives under specific environmental conditions, such as temperatures exceeding 40°C and high humidity levels, which correlate

with the onset of disease in rice crops (Ortega & Rojas, 2021). Furthermore, these conditions are exacerbated by the local environmental factors at our cultivation site, which is characterized by high humidity and pronounced elevated temperatures during the flowering phase, especially at night. The convergence of these environmental factors plays a significant role in the disease development and spread (Velásquez *et al*., 2018). The demonstrated efficacy of AgNPs in disease mitigation under these challenging conditions supports their potential as a protective agent in rice cultivation against *B. glumae*.

Environmental and Economic Considerations

The application of silver nanoparticles (AgNPs) in agricultural fields raises important environmental and economic concerns (Khan *et al*., 2023). From an environmental perspective, while AgNPs are known for their antimicrobial properties, their long-term effects on soil health, microbial communities, and non-target organisms remain under-researched (Wang *et al*., 2017). It is therefore crucial to assess the potential accumulation of silver in the soil and its impact on the broader ecosystem. This will ensure that AgNPs can be applied safely without disrupting local biodiversity. Future studies should aim to quantify these effects over multiple growing seasons and in different environmental conditions.

From an economic perspective, the large-scale adoption of AgNPs in rice cultivation may be constrained by the cost of nanoparticle synthesis and application. Although AgNPs present a promising alternative to traditional bactericides, the financial feasibility of adopting these materials at the commercial level requires careful evaluation. Comparing the cost-effectiveness of AgNPs with conventional treatments such as Starner WP will be essential to determine whether AgNPs can be a viable solution for farmers. Optimizing the concentration and application method could potentially reduce costs while maintaining their efficacy in controlling *B. glumae*.

CONCLUSIONS

The use of silver nanoparticles (AgNPs) demonstrated efficacy against *Burkholderia glumae* in rice crops under field conditions. The treatment significantly reduced the incidence of affected grains by 45% and improved grain weight by 15% compared to the untreated control. However, AgNP treatment also showed side effects on root development, evidenced by a 10% decrease in root volume, a finding consistent with previous greenhouse studies. These results highlight the potential of AgNPs as effective antimicrobial agents while also underscoring the need to optimize their concentration and application method to maximize benefits without compromising overall plant health. This study provides valuable evidence on the specific application of AgNPs against *B. glumae* under field conditions, thereby contributing to the development of integrated disease management strategies in rice cultivation that more effectively and sustainably incorporate established antimicrobial technologies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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