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Research Article

Intervention of Bacterial Leaf Streak of Wheat Through Neem Mediated Copper and Zinc Hybrid Nanoparticles

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ABSTRACT

Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa*, is a significant disease affecting wheat crops, leading to considerable yield loss globally. In response to this, green-synthesized nanoparticles offer a promising alternative to traditional chemical treatments, providing both disease management and environmental sustainability. The study evaluated the effectiveness of green-synthesized nanoparticles, including Copper (Cu), Zinc (Zn), and their combination (Cu+Zn), in controlling bacterial leaf streak (BLS) in wheat. A greenhouse trial was conducted using a Completely Randomized Design (CRD) with three replicates for each treatment and nanoparticle concentration (0.25%, 0.50%, and 0.75%). The findings revealed that Cu+Zn nanoparticles achieved the highest reduction in disease incidence (20.5%), followed by Zn nanoparticles (25.6%) and Cu nanoparticles (28.7%) compared to the untreated control. Similarly, a field experiment designed as a Randomized Complete Block Design (RCBD) with three replicates per treatment supported these results. Cu+Zn nanoparticles reduced disease incidence to 23.8%, while Zn nanoparticles and Cu nanoparticles showed reductions of 27.9% and 31.2%, respectively. These findings highlight the potential of neem-mediated copper-zinc hybrid nanoparticles as an effective and environmentally friendly approach for managing bacterial leaf streak in wheat, offering a promising alternative to chemical pesticides.

Keywords: Bacterial leaf streak, wheat, disease management, copper-zinc hybrid nanoparticles.

INTRODUCTION

Wheat (*Triticum aestivum* L.), a staple cereal crop in Pakistan, belongs to the *Poaceae* family and the genus *Triticum*. Globally, it is cultivated on an area of approximately 216 million hectares, producing 784.91 million metric tons annually (FAOSTAT 2024). In Pakistan, wheat is grown on 8.9 million hectares, yielding 27 million metric tons (GOP, 2023). Wheat contributes about 1.9% to Pakistan's GDP and serves as the primary food source for a large portion of the population (GOP, 2023). Wheat is a significant source of carbohydrates and also provides essential nutrients, including proteins, vitamins (B-complex and E), and minerals (iron, zinc, magnesium, and phosphorus) (Shewry et al. 2015). It is rich in dietary fiber and phytochemicals that support cardiovascular health and aid in preventing diseases such as diabetes and certain cancers (Ficco et al. 2023). Wheat is used in a wide range of food products, including bread, pasta, and confectioneries, and it is a key ingredient in many traditional dishes in Pakistan (Sánchez et al. 2022). Wheat production faces significant challenges from biotic stresses such as rust diseases (leaf rust, stem rust, and stripe rust), bacterial leaf streak, wheat blast, and powdery mildew. Additionally, abiotic stresses like drought, high temperatures, salinity and nutrient deficiencies also pose threats (Singh et al. 2015). The development and

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spread of these issues are influenced by epidemiological factors, including warm temperatures, high humidity, dense planting and frequent rainfall (Usman et al. 2025; Velásquez et al. 2018). Together, these stresses greatly reduce wheat yields on both global and regional scales, thereby threatening food security (Singh et al. 2015). Among biotic stresses, bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa*, is particularly detrimental, with potential yield losses of 10% to 40% under favorable conditions (Khojasteh et al. 2019). The disease was first identified in Europe in the early 20th century and has since become widespread globally (Shad et al. 2024; Sapkota et al. 2020). It is a gram-negative, rod-shaped, seed-borne pathogen (Ledman et al. 2021). The pathogen is primarily transmitted through infected seeds but can also spread via rain splashes, irrigation water, and mechanical means such as contaminated tools and equipment (Sapkota and Liu, 2020) *Xanthomonas translucens* infects wheat in two stages: the epiphytic phase, during which bacteria colonize the leaf surface and natural openings, and the endophytic phase, during which bacteria invade and multiply within leaf tissues (Ahmad et al. 2024; An et al. 2020). On wheat leaves, symptoms typically include water-soaked streaks that gradually turn brown as the disease progresses. These streaks are often bordered by chlorotic or yellow halos, which can coalesce, resulting in large necrotic areas (Hangamaisho et al. 2024) Severe infections can lead to reduced photosynthetic activity, stunted growth, and significant yield losses (Usman et al. 2024). The size of lesions varies depending on the cultivar, with some showing small, narrow streaks less than 0.5 cm wide, while others may exhibit larger, elongated streaks covering significant portions of the leaf surface (Atiq et al. 2023; Ficke et al. 2018).

Biological controls, chemical treatments, cultural practices, and resistant cultivars are all part of the integrated solutions needed to manage bacterial leaf streak (BLS) in wheat (Iqbal et al. 2022). Farmers often rely on resistant wheat varieties; however, this approach is challenging due to the genetic variability and rapid adaptation of the *Xanthomonas translucens* pv. *undulosa* pathogen (Adhikari et al. 2012). Effective management of BLS involves both preventive and curative measures. Cultural practices, such as planting certified disease-free seeds, crop rotation, maintaining optimal spacing to reduce humidity, removing infected plant debris, and adopting no-till practices, play a vital role in minimizing disease spread (Sapkota et al. 2020).

Despite these efforts, such measures often prove inadequate under conditions that favor pathogen development, such as high humidity and warm temperatures, resulting in widespread outbreaks and significant yield losses (Ramakrishnan et al. 2019). Chemical treatments are frequently employed by farmers to control BLS; however, the excessive use of synthetic chemicals negatively impacts human health, non-target organisms, and the environment (Tahir et al. 2023). These chemicals can also disrupt the soil microbiome, adversely affecting beneficial microorganisms like *Rhizobium* and *Trichoderma* that are essential for maintaining soil fertility (Meena et al. 2020).

To address these challenges, scientists are exploring nanotechnology applications in agriculture as an innovative and sustainable approach for managing BLS in wheat, offering potential benefits in pathogen control while reducing environmental impacts. Nanotechnology is an emerging technology that has demonstrated promising potential in improving crop health, soil fertility, and environmental sustainability (Ali et al., 2024). Various nanoparticles (NPs) have shown effectiveness in pest and disease management (Shafqat et al. 2024). NPs are synthesized using physical, chemical, and biological methods, with biological synthesis being the most innovative due to its eco-friendly nature, involving plant extracts and microorganisms. These biologically synthesized nanoparticles offer an effective and sustainable approach to agricultural challenges (Karunakaran et al. 2023). For example, $nTiO_2$ application shows a positive impact on wheat growth, yield when used in a lower concentration (Zaheer et al., 2024).

Nanoparticles offer a versatile approach to combating plant pathogens through various mechanisms. They can infiltrate bacterial cells, compromise the integrity of cell membranes, and induce the production of reactive oxygen species (ROS) (Atiq et al. 2022). These ROS can damage essential cellular components such as DNA and proteins, ultimately leading to bacterial cell death (Nisar et al. 2019). Among these, copper nanoparticles (CuNPs) have demonstrated significant potential in managing plant diseases. They serve as an alternative to conventional bactericides and may help mitigate the emergence of bacterial resistance (Ntasiou et al. 2021). Recognizing the importance of controlling bacterial leaf streak in wheat, a recent study was designed to evaluate the efficacy of green-synthesized copper and zinc nanoparticles, derived from neem leaves, as sustainably enhance wheat production.

MATERIAL AND METHODS

Sample Collection and Media Preparation for *Xanthomonas translucens* pv. *undulosa* (Xtu) Isolation

Infected wheat leaves are collected from the field and stored in brown paper bags (13" x 9.5"). Each bag is labeled with the name of the host plant and the collection date. Nutrient Agar (NA) is prepared for bacterial isolation. To prepare

500 mL of media, 14.5 g of synthetic NA is dissolved in distilled water to reach a final volume of 500 mL. The media is sterilized in an autoclave at 121°C and 15 psi for 15 minutes. Once the autoclave cools and the temperature drop to 65°C, Nilstat is added to inhibit fungal growth. The media is then poured into sterilized Petri plates (9 cm in diameter) and allowed to solidify. The collected diseased leaves are rinsed under tap water to remove debris and air-dried. Using sterilized scissors, 2–3 mm sections of infected leaves (including some healthy portions) are cut and surface-sterilized in a 1% sodium hypochlorite (NaOCl) solution for 30 seconds. The sections are then rinsed twice with distilled water to remove any NaOCl residues and dried with tissue paper. These sterilized leaf pieces are placed in autoclaved Petri plates (9 cm) using sterilized forceps. The plates are sealed with paraffin tape and incubated at 28 ± 2°C for 24 hours to observe bacterial growth. All steps are performed in a laminar airflow cabinet to maintain a sterile environment and reduce contamination risks.

Purification, Preservation, and Identification of the Pathogen

The streaking method is employed to obtain pure bacterial cultures. A single bacterial colony from a plate showing growth is transferred to a fresh NA plate using a sterilized wire loop. The plates are sealed with paraffin tape and incubated at 28 ± 2°C to ensure optimal growth. This process is repeated until a pure culture is obtained. To preserve the bacterial isolates, 5 mL of nutrient broth is added to test tubes to facilitate growth. The pure culture is transferred to the broth and incubated at 28°C for 24 hours. A mixture of 1 mL of 50% glycerol and 1 mL of nutrient broth containing the bacterial culture is prepared and stored in labeled 2 mL Eppendorf tubes at -18°C for long-term preservation. Pathogen identification is based on morphological characteristics such as colony color, shape, growth pattern, and results from biochemical tests including Gram staining, 3% KOH solubility, and oxidase tests.

Gram Staining

The Gram stain process involves staining bacteria with crystal violet, followed by iodine to form a complex. After washing with alcohol or acetone to decolorize, Gram-positive bacteria retain the purple color, while Gram-negative bacteria lose the color and take up the pink or red counterstain, safranin. This method differentiates bacteria based on their cell wall structure (Paray et al.2023).

3% KOH Test

A small sample of bacterial culture is mixed with a drop of 3% KOH on a glass slide. The mixture is stirred for 60 seconds using a loop or a toothpick. In the 3% potassium hydroxide (KOH) test, Gram-negative bacteria cause the mixture to become sticky and form a slimy thread, while Gram-positive bacteria do not, leaving the mixture watery (Arbefeville et al.2024).

Oxidase Test

The oxidase test involves applying an oxidase reagent, typically containing tetramethyl-p-phenylenediamine, to filter paper or directly to the bacterial culture. A bacterial colony is then rubbed onto the reagent. If cytochrome c oxidase is present, the reagent rapidly turns dark purple or blue within 20 seconds, indicating a positive result. A lack of color change or a delayed reaction suggests a negative result, indicating the absence of the enzyme. This test is commonly used to distinguish between oxidase-positive and oxidase-negative bacterial species. (Kuss, et al. 2017).

Pathogenicity

Koch's postulates are applied to verify the pathogenicity of the organism. A bacterial suspension is prepared from a two-day-old culture, and its concentration is adjusted to 1 × 10^8 CFU/mL using a spectrophotometer (Hitachi U-2001, Model 121003), following the protocols described by Lautenchlege et al. 2024). Sterilized soil, treated with formalin, is added to disposable cups, and seeds are sown in these under controlled greenhouse conditions. After the establishment of seedlings, the bacterial suspension is applied early in the morning (Mustafa et al. 2021).) because environmental conditions at that time are usually more favorable for infection. After 6-7 days, when symptoms are typically observed, the pathogen is re-isolated from the experimental plants and cultured on NA media in the laboratory. The growth pattern and colony color of the re-isolated bacteria are compared to those of the original pathogen isolated from the parental plant (Natale, et al. 2023).

Preparation of neem-based Nanoparticles (NPs)

Neem leaves (*Azadirachta indica*) are initially shade-dried for a week, then sun-dried for three days, and finally oven-dried at 65°C for 3-4 hours. The dried leaves are ground into a fine powder. To prepare the extract, 20 g of the powdered leaves is combined with 100 mL of distilled water in a beaker. The beaker is covered with aluminum foil and kept in a dark environment for 24 hours to preserve the bioactive compounds. The mixture is then filtered to obtain the extract.

For nanoparticle synthesis, 17 g of zinc oxide (ZnO) and copper sulfate ($CuSO_4$) are separately added to portions of the filtrate. Each mixture is stirred at 70°C using a magnetic stirrer for 15 minutes. This is followed by ultrasonic

treatment at 60°C to enhance molecular dispersion. The mixtures are then placed in a water bath at 65°C for 15 minutes to facilitate the evaporation of sulfate, nitrate, and oxide components, leading to the formation of zinc and copper nanoparticles, following the methodology of Atiq et al. (2022).

Characterization of nanoparticles

For nanoparticle characterization, the particles are first dried to eliminate solvents and impurities then dispersed in a solvent such as water or ethanol to form a stable colloidal solution. The characteristics of the nanoparticles, including particle size, shape, and distribution, are assessed using Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), following methodologies outlined by Bhattacharya and Ghosh (2019) and Zhou et al. (2018). The crystalline structure and lattice parameters are determined through X-ray Diffraction (XRD). Dynamic Light Scattering (DLS) is utilized to analyze the size distribution and aggregation state of the nanoparticles. Additionally, Fourier Transform Infrared Spectroscopy (FTIR) is applied to identify surface functional groups and chemical bonds.

Evaluation of Neem based NPs towards *Xanthomonas translucens* pv*. Undulosa* in lab conditions

The effectiveness of green-synthesized nanoparticles of copper (Cu), silver (Ag), and zinc (Zn) derived from neem leaves is evaluated against *Xanthomonas translucens* pv. *undulosa* in a laboratory setting using the inhibition zone method, as described by Atiq et al. (2022). Nutrient agar (NA) medium is prepared by mixing the required components to make 1 liter. For preparing nanoparticle solutions at three concentrations (0.25%, 0.50%, and 0.75%), 0.25 g, 0.50 g, and 0.75 g of nanoparticle powders (Zn, Ag, and Cu) are dissolved in 100 mL of distilled water in separate conical flasks. The prepared NA medium is poured into 9 cm Petri dishes and allowed to solidify. The bacterial culture is evenly spread over the solidified medium using a sterilized inoculating loop under a laminar airflow cabinet (RTVL-1312). Circular filter paper discs (1 cm in diameter) are sterilized and then immersed in nanoparticle solutions, including the individual metal nanoparticles and their respective concentrations (0.25%, 0.50%, and 0.75%). Excess moisture is removed, and the discs are placed at the center of the Petri dishes containing the bacterial culture. For the control treatment, filter paper discs are soaked in distilled water. The plates are sealed with wrapping tape and incubated at 25–30°C (Heraeus incubator) for 48–72 hours. The experiment follows a completely randomized design (CRD) with three replicates for each treatment. The inhibition zones are measured at intervals of 24, 48, and 72 hours using a digital Vernier caliper (VCL-150).

Evaluation of Neem based NPs towards *Xanthomonas translucens* pv. *Undulosa* in the greenhouse

For the greenhouse evaluation of green-synthesized nanoparticles (NPs), one moderately susceptible variety (Galaxy-2013) of wheat is grown. The wheat plants are grown in pots filled with sterilized soil to prevent contamination from other pathogens. A spectrophotometer (Hitachi U-2021, Model 121003) is used to create a bacterial suspension at 1×10^8 CFU/mL from a two-day-old *Xanthomonas translucens* pv. *undulosa* (*Xtu*) culture. Using a syringe inoculation approach, the bacterial slurry is injected into the midrib at the lower surface of plant leaves early in the morning (when the greatest number of stomata are open) (Atiq et al.2022). After three days, the spray approach is used in a greenhouse to apply the most effective concentration of each treatment that was determined in a laboratory setting (Yang et al. 1998). Distilled water is used for the control treatment. The trial is conducted using a completely randomized design (CRD) with three replicates per treatment. Data are noted at three intervals of five days using the following formula:

$$Disease\ Incidence\ (\%) = \left(\frac{Number\ of\ Diseased\ Plants}{Total\ Number\ of\ Plants\ Observed}\right) \times 100$$

Evaluation of Neem based NPs towards *Xanthomonas translucens* pv*. Undulosa* in the field

For the in-field evaluation of green-synthesized nanoparticles (NPs), one moderately susceptible variety of wheat is grown. Seeds of 10 varieties are sown in the research area. The wheat plants are grown in pots filled with sterilized soil to prevent contamination from other pathogens. After 45 days, once the seedlings are established, they are transplanted into the field with a spacing of 1.0 feet between plants and 1.5 feet between rows. Using a 2-day-old (Xtv) culture, a bacterial solution is made, and a spectrophotometer is used to measure the concentration, which comes out to be 1×10^8 CFU/mL. Using a syringe inoculation approach, this suspension is then injected into the midrib on the underside of plant leaves in the early morning, at the same time as the stomata open their maximum (Atiq et al.2022). Three days later, the spray approach is used to assess in the field the best effective concentration of each treatment that was determined in the laboratory (Yang et al. 1998). Distilled water is used for the control treatment. The trial is conducted using a randomized complete block design (RCBD) with three replications per treatment. Data are recorded at weekly intervals over three weeks using the following formula describe by Prasad *et al.* (2021):

$$Disease\ Incidence\ (\%) = \left(\frac{Number\ of\ Diseased\ Plants}{Total\ Number\ of\ Plants\ Observed}\right) \times 100$$

RESULTS

In-*vitro* Assessment of neem-based Zn-CuNPs against *Xanthomonas translucens* pv. *undulosa*

With the exception of CxD and TxCxD, treatments (T), concentration (C), duration (D), and their combinations—including treatment × concentration (T×C) and treatment × duration (T×D)—produced noteworthy outcomes. Comparing the treatments to the control, Zn+Cu hybrid NPs expressed the highest inhibitory zone (18.706 mm), followed by CuNPs (13.873 mm) and ZnNPs (11.217 mm) (Table 1). In case of interaction between treatments and concentrations (T×C), maximum inhibition zone was revealed by Zn+Cu hybrid nanoparticles (16.832, 19.208, 20.671 mm) followed by CuNPs (12.216, 13.873, 15.749 mm) and ZnNPs (7.625, 9.872, 13.547 mm) at 0.025 %, 0.05 % and 0.075% (Table 2). The interaction between (T×D) expressed that maximum inhibition zone was developed after 24 hours and then increased with time. The maximum inhibition zone was measured from Zn+Cu hybrid nanoparticles (16.237, 17.891, 24.261 mm), followed by CuNPs (10.763, 13.121, 17.253 mm) and ZnNPs (7.731, 9.921, 12.000 mm) after 24, 48 and 72 hours respectively as compared to control (Table 3).

Table 1. Impact of nanoparticles against *Xanthomonas translucens* pv. *Undulosa.*

Treatments	Inhibition zone (mm)
Zn+CuNPs	18.706 a
CuNPs	13.837 b
ZnNPs	11.217 c
Control	0.0000 d
LSD	0.5372

Table 2. Impact of nanoparticles in relation to concentration on growth of *Xtu* under lab conditions.

Treatments	Inhibition zone (mm)		
	Concentrations (%)		
	At 0.025%	At 0.050%	At 0.075%
Zn+CuNPs	16.832 c	19.208 b	20.671 a
CuNPs	12.216 g	13.873 e	15.749 d
ZnNPs	7.625 i	9.872 h	13.547 f
Control	0.0000 j	0.0000 j	0.0000 j
LSD	0.9304		

Table 3. Impact of nanoparticles in relation to duration on the inhibition zone of *Xanthomonas translucens* pv. *Undulosa.*

Treatments	Inhibition zone (mm)		
	Duration		
	After 24 hours	After 48 hours	After 72 hours
Zn+CuNPs	16.237 d	17.891 b	24.261 a
CuNPs	10.763 g	13.121 e	17.253 c
ZnNPs	7.731 i	9.921 h	12.000 f
Control	0.0000 j	0.0000 j	0.0000 j
LSD	0.9304		

Evaluation of neem-based Zn-Cu NPs against bacterial leaf streak of wheat under greenhouse conditions

Treatments (T), Concentration (C), Duration (D) and their combinations including treatment × concentration (T×C) and treatment × Duration (T×D) gave significant results except (CxD and TxCxD). Among the treatments, minimum disease severity was expressed by Zn+Cu hybrid NPs (15.230 %), followed by CuNPs (35.437 %) and ZnNPs (40.784 %) as compared to control (74.316%) (Table 4). In case of T×C, the minimum disease severity was expressed by Zn+Cu

hybrid nanoparticles (18.324,15.419,12.106 %), followed by CuNPs (38.510,35.341,32.417 %) and ZnNPs (45.321,42.106,36.786 %) as compared to control (69.321,73.764,77.726 %) at the concentrations of 0.25 %, 0.50 % and 0.75 % of nanoparticles, respectively (Table 5). The T×D interaction showed that, after 7, 14, and 21 days, respectively, Zn+Cu hybrid nanoparticles (18.891,15.342,10.218%) had the lowest disease severity, followed by CuNPs (45.321,36.342,23.782%) and ZnNPs (53.451,41.345,30.334%) in comparison to the control (68.431,73.748,78.671%) (Table 6).

Table 4. Evaluation of nanoparticles against *Xanthomonas translucens pv. undulosa* under greenhouse conditions.

Treatments	Disease severity (%)
Zn+CuNPs	15.230 d
CuNPs	35.437 c
ZnNPs	40.784 b
Control	74.316 a
LSD	2.0997

Table 5. Evaluation of interaction between TxD on the disease severity.

Treatments	Severity of disease (%)		
	Concentrations (%)		
	At 0.25%	At 0.50%	At 0.75%
Zn+CuNPs	18.324 h	15.419 hi	12.106 i
CuNPs	38.510 ef	35.341 fg	32.417 g
ZnNPs	45.321 d	42.106 de	36.786 f
Control	69.321 c	73.764 b	77.726 a
LSD	3.6367		

Table 6. Impact of interaction between TxD on disease severity of *Xanthomonas translucens pv. Undulosa.*

Treatments	Severity of disease (%)		
	Days		
	After 7 days	After 14 days	After 21 days
Zn+CuNPs	18.891 j	15.342 k	10.218 l
CuNPs	45.321 e	36.342 g	23.782 i
ZnNPs	53.451 d	41.345 f	30.334 h
Control	68.431 c	73.748 b	78.671 a
LSD	3.6367		

***In-vivo* evaluation of Zn-Cu nanoparticles against bacterial leaf streak of wheat**

Treatments (T), Duration (D) and their combination including and treatment × Duration (T×D) gave significant results except (TxCxD). Among the treatments, minimum disease incidence was shown by Zn+Cu hybrid NPs (28.782 %), followed by CuNPs (36.783 %) and ZnNPs (44.527 %) as compared to control (67.324 %) (Table 7). The interaction between (T×D) expressed that minimum disease incidence was recorded from Zn+Cu hybrid nanoparticles (37.671, 29.654, 20.596 %), followed by CuNPs (48.453, 36.521, 26.731 %) and ZnNPs (57.321, 45.219, 35.219 %) as compared to control (64.371, 69.542, 72.873 %) after 7, 14 and 21 days respectively (Table 8).

Table 7. Evaluation of nanoparticles against bacterial leaf streak of wheat under *in-vivo* conditions.

Treatments	Incidence of disease (%)
Zn+CuNPs	28.782 d
CuNPs	36.783 c
ZnNPs	44.527 b
Control	67.324 a
LSD	2.064

Table 8. Evaluation of interaction between TxD on the disease incidence field conditions.

Treatments	Incidence of disease (%)		
	Days		
	After 7 days	After 14 days	After 21 days
Zn+CuNPs	37.671 def	29.654 fg	20.596 g
CuNPs	48.453 cd	36.521 def	26.731 fg
ZnNPs	57.321 bc	45.219 cde	35.219 ef
Control	64.371 ab	69.542 ab	72.873 a
LSD	**7.4882**		

DISCUSSION

Use of *Trichoderma* species is an alternative, safer approach to include in the disease management program for early The morphological features of *Xanthomonas translucens* pv. *undulosa* (*Xtu*) in the present study exhibited typical mucoid, circular, and yellow colony characteristics, consistent with those observed by Osdaghi, E. 2022) for *Xanthomonas* species. These findings align with previous studies that have described the general appearance of *Xanthomonas* colonies, reinforcing the reliability of morphological assessment in identifying bacterial pathogens.

Biochemically, *Xtu* showed a Gram-negative reaction, as evidenced by Gram staining, and an oxidase-negative result, consistent with the reports by (Afkhamifar et al. 2023), who also noted similar results for *X. translucens*. The catalase test revealed a positive response, confirming the presence of catalase enzyme, as reported by previous studies on the *Xanthomonas* genus. These biochemical characteristics are essential for pathogen identification and differentiation, supporting the established methods for bacterial diagnosis in plant pathology.

Bacterial leaf streak (BLS) caused by *X. translucens* pv. *undulosa* continues to be a significant challenge to wheat production. While chemical treatments have traditionally been employed to control bacterial infections, the indiscriminate use of chemicals has led to environmental pollution and the development of pathogen resistance, necessitating alternative, eco-friendly disease management strategies (Afkhamifar et al. 2023). The effectiveness of green-synthesised nanoparticles (CuNPs, SiNPs, and their mixtures) in treating BLS was investigated in this work. The results showed that CuNPs and SiNPs exhibited the highest antibacterial activity, with a noticeable reduction in disease incidence both in greenhouse and field conditions, which is in agreement with previous studies (Atiq et al. 2022).

The cost-effectiveness and environmental sustainability of green nanoparticle synthesis have drawn a lot of interest (Akhtar et al., 2024; Ali et al., 2024). According to studies by Rajwade et al. (2020), green-synthesized nanoparticles have the potential to be an environmentally friendly substitute for traditional chemical treatments. Green synthesis methods, such as the use of neem leaves for CuNPs and SiNPs, have demonstrated promising results in antimicrobial activity against plant pathogens. This aligns with the findings of (Rana and Sharma,2023), who observed significant antibacterial effects from ZnO nanoparticles synthesized from neem leaves, further supporting the role of green-based nanoparticles in plant disease management.

The present study's findings are consistent with the work of Atiq et al. (2022), who reported the effectiveness of green-synthesized nanoparticles in controlling *X. translucens* pv. *undulosa* and other plant pathogens. The combination of CuNPs and SiNPs exhibited the highest inhibition zone, demonstrating synergistic effects in controlling bacterial growth. These results are further supported by the research of (Rana and Sharma, 2023). who observed similar antimicrobial activity from green-synthesized nanoparticles, underscoring their potential as a novel approach for disease management.

The instability of bacterial cell membranes, which increases permeability and allows cellular contents to seep out, is the mechanism by which nanoparticles produce their antimicrobial effects (Mondal and Mandal, (2024). Nanoparticles have also been shown to activate antioxidant defense mechanisms in plants under biotic stress by regulating the production of biochemical markers such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Bao, and Zhai, (2024). This oxidative stress response, driven by the generation of reactive oxygen species (ROS), is a crucial part of the plant's defense against microbial pathogens (Torres and Dangl, 2006). Although the precise cellular mechanisms underlying ROS generation are still unclear (Huang and Zhao, 2019), it is evident that nanoparticles play a significant role in activating plant defense systems.

Despite the promising results, the study's limitations should be acknowledged. The effectiveness of nanoparticles may vary under different environmental conditions, including field trials and greenhouse settings. The concentration and application method of nanoparticles also influence their efficacy, suggesting that further optimization is needed.

Furthermore, long-term field tests to evaluate the sustainability and environmental impact of this strategy should be part of future study, as should clarifying the synergistic effects of mixing nanoparticles with organic chemicals or other biocontrol agents. The use of green-synthesized nanoparticles presents a sustainable, eco-friendly solution for managing bacterial leaf streak in wheat. While the findings are promising, further research is essential to refine the application methods, understand the underlying mechanisms of nanoparticle activity, and evaluate their long-term environmental impact.

CONCLUSIONS

The current investigation concluded that the application of green-synthesized Copper (Cu) and Zinc (Zn) nanoparticles proved to be highly effective in managing *Xanthomonas translucens* pv. *undulosa* and controlling bacterial leaf streak (BLS) in wheat. Among the tested nanoparticles, the Cu+Zn hybrid nanoparticles demonstrated the highest inhibition zone in laboratory conditions. Additionally, these hybrid nanoparticles outperformed CuNPs, ZnNPs, and the control treatments, demonstrating the lowest disease incidence in both greenhouse and field settings. These results demonstrate how green-synthesized nanoparticles could be a viable and environmentally responsible substitute for controlling bacterial illnesses in wheat production.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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