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Silver Nanoparticles-Chitosan Mixture Upregulates Xylanase Inhibitor (*Xip-I)* of *Triticum aestivum* Infected with *Puccinia graminis*

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ABSTRACT

Wheat is a staple crop globally, serving as a primary source of nutrition for millions and playing a pivotal role in agricultural economies. Stem rust, a disease caused by *Puccinia graminis*, is a major threat to wheat, leading to substantial yield reductions and diminished grain quality. This study investigated the effectiveness of two concentrations of silver nanoparticles (AgNPs) in a chitosan solution (CH-AgNPs-I and CH-AgNPs-II) against wheat stem rust. The treatments were applied to wheat plants both before and after rust infection. Subsequently, the gene expression of Xip-I was measured across all treatment groups. Results indicated that the application of 100 ppm AgNPs in 1% w/v chitosan (CH-AgNPs-I) prior to rust infection was the most efficacious, demonstrating a 4.2-fold increase in Xip-I expression in the resistant cultivar Giza 168. This treatment also showed visible reduction in infection symptoms, while the 250 ppm AgNPs treatment (CH-AgNPs-II) exhibited a lower 2.7-fold increase in Xip-I expression. For the susceptible cultivar Morocco, Xip-I upregulation following CH-AgNPs-I treatment reached 0.95-fold. Overall, applying CH-AgNPs-I before fungal infection provided the greatest protective effects. These findings suggest that an optimized concentration of silver nanoparticle-chitosan blend offers promise as a strategy for protecting wheat crops against stem rust attacks, potentially mitigating significant losses in wheat production.

Keywords: wheat, *Puccinia graminis*, chitosan, silver nanoparticles, *Xip-I*.

INTRODUCTION

Wheat (*Triticum aestivum* L.), is one of the most important food crops to human populations as it is consumed worldwide Igrejas and Branlard (2020) and is considered the major grain crop grown in Egypt (Atwa et al., 2025). It is grown all over the world, but the yield varies because of a cultivar of factors, including biotic and abiotic factors as well as the lack of effective ways for combating pandemic diseases (Nuttall et al., 2017). According to FAO, world wheat production has been lifted by 1.4 million tonnes and now stands at 788.5 million tonnes in 2023, albeit still 2.2 percent lower year on year, and wheat utilization in 2023/24 has been lifted by 2.9 million tonnes since December and indicates a 2.0 percent (15.4 million tonnes) increase from 2022/23.

Stem rust disease of wheat caused by *Puccinia graminis* Pers. f. sp. *tritici* is still the most dangerous biotic stress that threatens wheat production in Egypt and several wheat-growing areas worldwide. This is mainly due to the emergency and appearance of aggressive races of the causal pathogen (Singh et al., 2005). It could affect the entire wheat crop, especially during the late swing dates, leading to the blocking of

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the vascular system. Hence stunting and lodging of weak stalks eventually cause severe yield losses of even 100%, due to shriveled grain and damaged tillers (Abou-Zeid and Mourad, 2021; Mourad et al., 2021; Elshafei et al., 2022). In Egypt, yield losses due to stem rust ranged from 1.96% to 8.21% on the Egyptian wheat cultivars (Abou-Zeid and Mourad, 2021; Arab et al., 2021). In most cases, susceptible wheat cultivars were replaced by new resistant ones. Meanwhile, the strategy of introgression and deployment of stem rust resistance genes into commercial wheat cultivars throughout the 1950s greatly circumvented major disease epidemics and successfully controlled stem rust. Wheat farmers conventionally rely on chemicals to manage this fungal disease; however, the excessive use of chemical fungicides causes harmful environmental and human health consequences (Wightwick et al., 2010; Brauer et al., 2019). Furthermore, using the same fungicides regularly may raise the possibility of creating aggressive fungicide-resistant strains (Yang et al., 2019; Corkley et al., 2022). Also, researchers focused on finding economical and environmentally friendly strategies for integrated plant disease control. The discovery of new genes in plants is important for genetic engineering for several reasons as improving crop yield and quality, developing new products, and conservation and preservation of biodiversity. Overall, the discovery of new genes in plants is essential for advancing our understanding of genetics and improving the sustainability of agriculture and other industries (Altman and Hasegawa, 2011; Ronald, 2011; Ortiz et al., 2014; Anyshchenko, 2022).

Xylanase inhibitors (XIs) are a class of proteins that are found in various plants, including wheat. They are known to play a crucial role in the defense system of wheat against rust diseases, caused by *Puccinia* spp; *Puccinia triticina*, *Puccinia striiformis f.* sp. *tritici* and *Puccinia graminis* f. sp. *tritici*. XIs have been found to inhibit the activity of xylanases, which are enzymes produced by rust fungi to degrade the cell walls of wheat plants, allowing them to penetrate and infect the host cells (Elliott et al., 2009; Dornez, 2010; Omar et al., 2021).

In recent years, nanotechnology has become a vital approach in alleviating the effects of plant diseases through the development of alternative materials capable of controlling pathogens. There has been considerable attention directed towards two specific substances, chitosan and silver nanoparticles (AgNPs), due to their antifungal characteristics (Khan and Rizvi, 2017; Sanzari et al., 2019; Shang et al., 2019; Panpatte and Jhala, 2019; Omar et al., 2021). Chitosan has been employed to curb disease progression, limit their spread, and boost plant immunity. Additionally, chitosan treatment has been shown to affect multiple genes in plants, including those involved in the activation of defense pathways, leading to the accumulation of defense proteins (Kong et al., 2010; Saharan and Pal, 2016; Al-Dhabaan et al., 2018; Mohamed and Abdel-Hakeem, 2023; Mahmoud et al., 2025). AgNPs have been utilized to combat various fungal plant pathogens by impeding the structures and growth of fungi (Khot et al., 2012; Durán et al., 2016; Kumar et al., 2021). The research focuses on evaluating the efficacy of two different concentrations of AgNPs within a chitosan solution for managing stem rust disease in *T. aestivum* and assess how these concentrations of AgNPs, when combined with chitosan, impact the control and mitigation of stem rust disease in wheat crops.

MATERIAL AND METHODS

The experiment was carried out at the greenhouse of stem rust at Wheat Diseases Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt.

Plant materials

One stem rust resistant cultivar (Giza 168) and one susceptible cultivar (Morocco), in successful season 2023 [30] were provided by Wheat Diseases Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt.

Preparation of silver nanoparticles

Silver nanoparticles (AgNPs) were synthesized by the reduction of silver nitrate ($AgNO_3$) with sodium citrate, which acts as both a reducing agent and a capping agent. $AgNO_3$ was heated to 80°C to assist in the reduction reaction kinetics. As described previously Bacete et al. (2018), 50 mL of 1 mM $AgNO_3$ aqueous solution was heated to 80°C on a hot plate with constant stirring. Once 80°C was reached, 5 mL of 0.1 M trisodium citrate was added dropwise to the $AgNO_3$ solution. The sodium citrate reduces Ag+ ions to Ag0 atoms while the citrate ions adsorb to the newly formed AgNPs surfaces acting as a capping agent, preventing aggregation. The reaction was allowed to proceed for an additional 30 minutes after complete addition of trisodium citrate to ensure full reduction.

The reaction can be summarized as:

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2$$

Characterization of nanoparticles

The morphology and size of the synthesized silver nanoparticles (AgNPs) were characterized by transmission electron microscopy (TEM) using a JEOL JEM-2100 electron microscope operated at an accelerating voltage of 200 kV. AgNPs samples for TEM analysis were prepared by depositing a drop of appropriately diluted suspension onto a carbon-coated copper grid and allowed to dry completely. TEM micrographs were captured at magnifications ranging from 50,000X to 150,000X. The hydrodynamic diameter and zeta potential of AgNPs were measured by dynamic light scattering (DLS) using a Nicomp Nano Z3000 Zeta Potential Analyzer. DLS measurements were performed in triplicate at 25°C using a 632 nm He-Ne laser and detection angle of 90°. The concentration of AgNPs was adjusted to approximately 0.1 mg/mL in ultrapure water prior to measurements to avoid multiscattering effects. Zeta potential was measured via laser Doppler micro-electrophoresis using AgNPs suspensions diluted to 0.1 mg/mL in 1 mM KCl. The concentration of the final colloidal AgNPs suspension obtained after synthesis and purification was determined to be 1.5 mg/mL using inductively coupled plasma atomic emission spectroscopy (ICP-AES). This stock suspension was used for subsequent dilution to required concentrations for the various experiments.

Preparation of chitosan containing silver nanoparticles

Chitosan solution was prepared by dissolving high molecular weight chitosan powder (deacetylation degree ≥75%, Sigma-Aldrich) at a concentration of 1% w/v in 1% v/v acetic acid under constant magnetic stirring for 12 hours at 50°C. The completely dissolved chitosan was filtered through a 0.45 μm nylon membrane and used immediately for nanoparticle preparation.

The chitosan solution was then mixed with the silver nanoparticle suspension to obtain the following final concentrations:

Sol. 1: 100 ppm AgNPs in 1% w/v chitosan (CH-AgNPs-I),

Sol. 2: 250 ppm AgNPs in 1% w/v chitosan (CH-AgNPs-II).

Greenhouse experiment

Six wheat grains from each genotype were sown in plastic pots (6 cm in diameter) filled with a mixture (1:1 v/v) of peat moss and sand. After 7 days of planting, the procedures for inoculation and incubation were carried out. Inoculation of the tested wheat cultivars at the seedling stage was carried out using the freshly collected urediospores of *Puccinia graminis* race; TTKMC, where the leaves were rubbed gently between moist fingers with tap water. The inoculated seedlings were incubated at 18°C in a dark dew chamber for 14 h, then moved to the benches in the greenhouse and maintained at 20-24ºC and 70-80% relative humidity (RH) with 16 h light and 8 h dark at about 7600 lux B (Ohm and Shaner, 1976). The seedlings were observed daily until the development of rust pustules. Seedlings response was scored 10-12 days after inoculation based on the infection types (IT) expressed on each

genotype using a 0-4 scale. Infection types, i.e.; 0, 0, 1, and 2 represent low infection (IT), while 3 and 4 represent high infection (IT) (DL and Kolmer, 1989).

Wheat seedlings were treated as follows for each cultivar:

Negative control: Normal plants were sprayed with distilled water.

Positive control: Normal plants were infected with *Puccinia graminis.*

Treatment I: Plants were sprayed with CH-AgNPs-I

Treatment II: Plants were sprayed with CH-AgNPs-II

Treatment III: Plants were sprayed with CH-AgNPs-I before fungal infection.

Treatment IV: Plants were sprayed with CH-AgNPs-II before the fungal infection.

Treatment V: Plants were sprayed with CH-AgNPs-I after fungal infection.

Treatment VI: Plants were sprayed with CH-AgNPs-II after fungal infection.

All experiments were repeated three times.

Table 1. Primers of *Xip-I* and housekeeping β-Actin gene

Gene	Primer's sequence	Accession no.	Melting temp.
xylanase inhibitor gene (*Xip-I*)	F:5'GGCCTTATTCCTGACCCCG3' R:5'GGAGAGGTCGAGGTGGTACT3'	NM_001405501.1 https://ncbi.nlm.nih.gov/nuccore/NM_001405501.1	59.85 60.03
β-Actin	F:5'TGACGTGGATATCAGGAAGG3' R:5'GCTGAGTGAGGCTAGGATGG3'	XM_044591529 https://www.ncbi.nlm.nih.gov/nuccore/XM_044591529	56.43 59.61

Primer design

Primers were designed for wheat xylanase inhibitor gene (*Xip-I*) and actin gene by using primer BLAST at the National Center of Biotechnology Information program (NCBI) (Table 1).

RNA extraction and qPCR

Total RNA was extracted from wheat stem using the IQeasyTM Plus Plant RNA Extraction kit (iNtRON Technologies, Cat. No. 17491) according to manufactured protocol. RNA quality was assessed using A260/A280 ratio method, these measurements are typically performed using a spectrophotometer, which can quantify the amount of light absorbed by the sample at each wavelength. SensiFAST™ SYBR® No-ROX One-Step Kit (Bioline, Cat. No. BIO-98020) was used for realtime-PCR. qPCR was adjusted for 42 cycles; the first cycle was 45℃ for 10 minutes, followed by cycle at 95℃ for 2 minutes, the remaining 40 cycles involved denaturation for 5 seconds at 95℃, annealing for 10 seconds at 60℃ and 5 seconds at 72℃. The data obtained from RT-PCR were analyzed by the relative quantification $2^{-\Delta\Delta Ct}$ method Livak and Schmittgen (2001).

Statistical analysis

The study conducted gene expression experiments in three independent replicates, and the statistical significance was determined through two-way analysis of variance (ANOVA) by using IBM SPSS Statistics 23.0, the variables tested were genotypes and treatments. Multiple comparisons between various treatments were performed using Student's t-test, with a significance level (α) of 0.05. Any P-value less than or equal to 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Characterization of silver nanoparticles

The obtained AgNPs revealed a spherical shape with an average diameter of 16.88 ± 6.11 nm (Figure 1A). Furthermore, AgNPs showed hydrodynamic sizes ranging from 10 to 45 nm, with the main peak at 15 nm and a polydispersity index (PDI) of 0.68 (Figure 1B). Finally, the zeta potential was – 25 mV (Figure 1C).

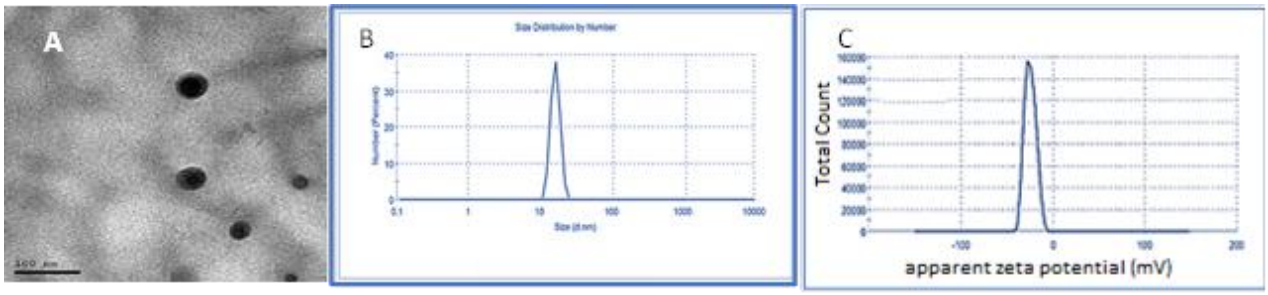

Figure 1. Characterization of silver nanoparticles (AgNPs): A - TEM image, B - hydrodynamic size, C - zeta potential

Field observations

While comparing with the stem rust infected plants, the plants of the Morocco cultivar that were treated with CH-AgNPs prior to being infected with stem rust showed a complete absence of infection symptoms when using CH-AgNPs-I. This indicates that the addition of CH-AgNPs with such concentration conferred a protective effect against stem rust disease, even in the susceptible Morocco genotype.

After being infected with stem rust and subsequently sprayed with CH-AgNPs, the plants were observed and compared to the plants only infected with stem rust after 12 days of inoculation. It was discovered that there were no differences in the infection symptoms as both sets of plants displayed spots of infection. However, the plants that were sprayed with CH-AgNPs did not exhibit an increase in infection.

Table 2. Effect of spraying CH-AgNPs on infection type before and after infection wheat seedlings of Morocco with *Puccinia graminis*

	Morocco	Giza168
Infection with rust only	4	1
Chitosan-Silver nanoparticles before infection	1	0
Chitosan-Silver nanoparticles after infection	+1	1

0-4 scale of infection. Infection types: 0, 1, and 2 represent low infection (IT), while 3 and 4 represent high infection (IT).

Data in Table 2 showed the effect of CH-AgNPs on infection type. Data indicated that application of CH-AgNPs before infection and CH-AgNPs after infection showed low infection type (1 and +1, respectively) compared to the control one of the wheat cultivar Morocco. In Giza168, the infection type was low in control and treatments.

Gene expression

Comparing fold change values between different treatments and cultivars provides insights into the effectiveness of treatments and the genetic response of different wheat cultivars to stem rust infection. Understanding the dynamics of gene expression changes over time and across different treatments can provide valuable information about the plant's adaptive responses to pathogen pressure. In our study, the gene expression of *Xip-I* gene was examined in all treatments (Figure 2), the obtained results showed an upregulation in the expression of *Xip-I* gene before and after treatments. In resistant cultivar Giza168, the expression of *Xip-I* was upregulated by 2.5-fold change in rust infected plants (Positive control), while it was increased by 4.2-fold change in the plants which sprayed by concentration 1 of chitosan-silver nanoparticles (Treatment I) and 2.7-fold change in concentration 2 (Treatment II). In treatment III, where plants were sprayed with CH-AgNPs-I before fungal infection, the expression of *Xip-I* was upregulated by 3.9-fold change, this upregulation was higher than the expression of the gene in Treatment IV, where plants were sprayed with the

second concentration of CH-AgNPs mixture before the fungal infection. After infection with rust, the gene expression was decreased in treatment V and VI (2.6- and 2.4-fold change, respectively) comparing with Treatments III and IV. In the susceptible cultivar (Morocco) the gene expression of *Xip-I* in rust infected plants was 0.26-fold change. After applying CH-AgNPs-I, the gene expression was increased compared with rust infected plants (0.95-fold change). While, in the CH-AgNPs-II the fold change was 0.78. In treatments III and IV, the gene expression was 0.73- and 0.60-fold change, respectively. In treatments V and VI, where nanoparticles were applied after infection, the gene expression was 0.71- and 0.22-fold change, respectively.

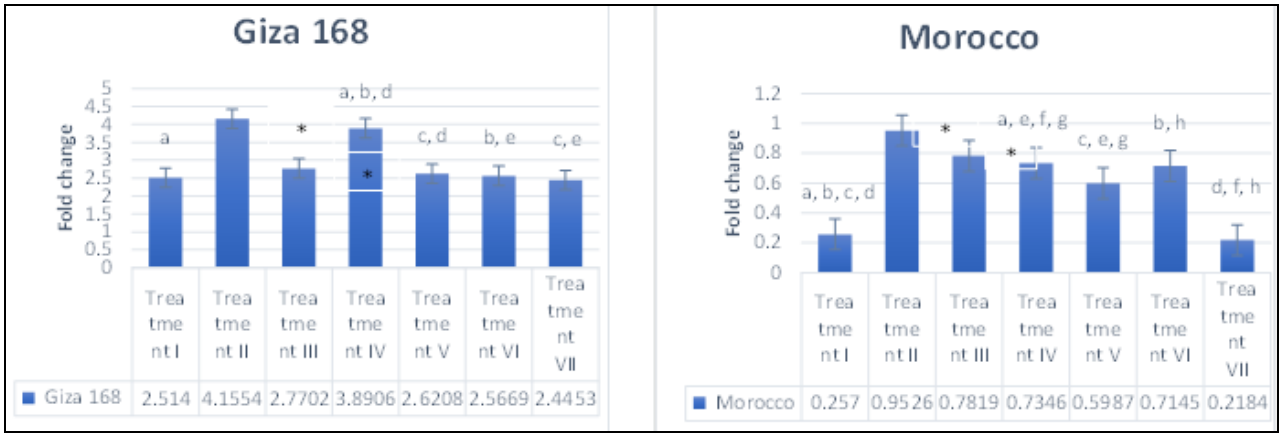

Figure 2. A fold change of *Xip-I* gene in wheat cultivars; Giza 168 and Morocco in response to rust and application of the two concentrations of CH-AgNPs, each bar represents treatment. The same letter means a significant difference at $p \leq 0.05$. (*) means a significant difference between the two concentrations of CH-AgNPs.

Around 20-40% of agricultural crop yield losses occur globally due to various diseases caused by phytopathogenic bacteria, phytopathogenic fungi, pests, and weeds Omran and Baek (2022). It is estimated that in 2050 the world's human population will reach around 10 billion, around 800 million people in the world will be hungry, and around 653 million people in the world will be undernourished in 2030; thus, fulfilling the food demand will remain a huge challenge. The current research progress and disease management strategies are insufficient to fulfil the food demand by 2050.

Recently, researchers aimed to achieve one of the sustainable development goals, which is to eradicate hunger Salvia et al. (2019). As part of this effort, they are exploring new technologies to safeguard crops against both biotic and abiotic stresses. The stem rust fungus specifically attacks the aerial parts of the plant, forming pustules on green wheat plants that penetrate the outer layers of the stalk (Abdulridha et al., 2023). Infected plants produce fewer tillers and set fewer seeds, and in severe cases, the plant may not survive (Rodriguez-Algaba et al., 2020). The infection can cause an apparently healthy crop to turn into a tangled mess of broken stems and shrunken grains, which may occur up to three weeks before harvest time.

The most well-known methods to manage fungal diseases are utilization of chemical fertilizers and production of resistant wheat cultivars. Nevertheless, the fungicides proved to be effective against destructive fungal pathogens, but they cause collateral damage and are toxic to human health (Brauer et al., 2019). Plant pathogens, particularly *P. striiformis*, vigorously generate new races that lead to epidemics in varieties that were regarded as pathogen or disease resistant (Hovmøller et al., 2016). The plant cell wall encompasses plant cells and is an intricate and dynamic structure that participates in various metabolic processes (Ganie and Ahammed, 2021). In interactions between plants and microbes, the cell wall serves multiple vital functions. It acts as the primary

pre-existing physical obstacle that invading pathogens must breach to invade and inhabit the host tissue, provides nutrients to intruders, and can produce signaling molecules that serve as damage-associated molecular patterns when it is partially broken-down (Cosgrove, 2005; Underwood, 2012). The plant cell wall is mainly composed of polysaccharides such as cellulose, xyloglucan,-1,3:1,4-glucan, xylan, mannan, pectin, and callose (Cosgrove, 2005; Voragen et al., 2009). To overcome the plant cell wall, most pathogens, during pathogenesis, as well as many saprophytic microbes, secrete a wide array of cell wall-degrading enzymes (CWDEs) with different substrate specificity, which provide a source of nutrients and can facilitate the penetration and/or colonization of the microorganisms into the host tissues (Tonukari, 2000; Dickinson, 2003; Voragen et al., 2009; Quoc and Bao Chau, 2017).

The potential involvement of Xylan degradation in the cell wall of plants is a mechanism for fungal pathogens to cause disease. However, xylanases also can induce cell death in host tissues, regardless of their enzymatic activity, contributing to pathogenesis. Despite this, plants have developed a defense mechanism against xylanase through the evolution of xylanase inhibitors (Beliën et al., 2006; Couturier et al., 2012; Bacete et al., 2018). Evidence supporting this hypothesis includes studies showing that XIs inhibit microbial xylanases but not those derived from plants. Additionally, studies have shown that the expression of XIs in plants enhances resistance against fungal pathogens such as *Fusarium graminearum* (Brutus et al., 2005; Brito et al., 2006; Kikot et al., 2009; Sella et al., 2013), *Botrytis cinerea* (Frías et al., 2019) and *Puccinia graminis* (Bekhit, 2018).

Plants can trigger defense responses when exposed to chitosan and its derivatives, with the effects and specificity being influenced by physical and chemical factors such as molecular weight and acetylation degree. Furthermore, wheat (*Triticum aestivum*) has been found to undergo chitosan-induced biochemical and enzymatic reactions (Zhang et al., 2017; Díaz-Martínez et al., 2018). The activation of plants' antioxidant defense system has been reported because of metallic nanoparticles (NPs), with a particular emphasis on AgNPs. There have been studies indicating that AgNPs may play a role in managing disease through their antifungal and antioxidant properties (Ghazy et al., 2021; Khan et al., 2021; Tariq et al., 2022).

Chemical reduction is the most common method for preparation of silver nanoparticles. The formation of AgNPs can be observed by the change in solution color following sodium citrate addition. This color change occurs due to the surface plasmon resonance of AgNPs. Heating accelerates the reaction kinetics and speeds up AgNPs formation. Sodium citrate plays a dual role - it controls nanoparticle growth and stabilizes the synthesized AgNPs.

In the current work, the AgNPs synthesized exhibited a moderately broad size distribution, however our formula demonstrated high potential for managing wheat stem rust disease. Although the polydispersity could be improved in future optimizations.

In the same framework, the current study aimed to explore the antifungal properties of CH-AgNPs against *P. graminis* and examine their impact on *Xip-I* gene expression in wheat. The addition of chitosan to silver nanoparticles provides several advantages. First, chitosan prevents aggregation and maintains a uniform nanoparticle size distribution. The amino and hydroxyl groups of chitosan bind to the nanoparticle surface, facilitating dispersion and enhancing silver nanoparticle solubility in water. This allows for preparation of homogeneous nanoparticle solutions/suspensions. Second, chitosan extends the release of silver ions from the nanoparticles over time, providing sustained antimicrobial activity. Finally, chitosan and silver nanoparticles synergistically inhibit fungal growth. Chitosan disrupts fungal membranes, while silver ions inhibit intracellular processes.

The findings demonstrated that *Xip-I* gene expression was upregulated across all treatments. In the case of the resistant wheat cultivar Giza 168, *Xip-I* gene expression increased by 2.5-fold after being infected with *Puccinia graminis* (Positive control). The observed upregulation of the *Xip-I* gene before treatment suggests that wheat plants, particularly the resistant cultivar Giza168, may have constitutive defense mechanisms primed and ready to respond to potential threats such as stem rust. These results are consistent with prior research (Shehab-Eldeen and Abou-Zeid, 2020; Atia et al., 2021; Khalil et al., 2024). Which reported that resistant varieties of wheat as Giza 168 have different mechanisms, such as upregulating some genes to resist rusts. In another investigation, for the first time, the activity of Cu-chitosam in foliar fertilizer applications was studied and shown to have the potential to inhibit stem and leaf rust in studied Egyptian wheat genotypes, resulting in increasing plant immunity and defensive response activities (Omar et al., 2021). In a similar manner, our results indicated that the application of CH-AgNPs has an effective role in increasing the expression of *Xip-I* gene. The expression of *Xip-I* gene was affected not only by the concentration of CH-AgNPs but also by the time when CH-AgNPs were applied to the plants. This is clear in the obtained results from the resistant cultivar (Giza 168), where the gene expression was increased in plants which were sprayed with CH-AgNPs-I before fungal infection (treatment III) more than in plants which were sprayed with CH-AgNPs-II before the fungal infection (treatment IV). It means that the time of CH-AgNPs application is an important factor which must be considered. Also, the gene expression of treatments III and IV was increased more than groups where the application of CH-AgNPs was after infection with *P. graminis* (treatments V and VI), which indicates the application of CH-AgNPs before infection has a protective role against stem rust disease, it is consistent with Sabir et al. (2022), where foliar application of silver nanoparticles protected wheat crop from stripe rust. Also, in *Solanum lycopersicum*, AgNPs protect the plants from early blight disease Kumari et al. (2017).

In the susceptible cultivar Morocco, the expression of the *Xip-I* gene exhibited fluctuations in comparison to the rust-infected plants (Positive control), where the fold change was 0.26. Following the application of CH-AgNPs-I, the gene expression increased when compared to rust-infected plants, with a fold change of 0.95. In contrast, in CH-AgNPs-II, the fold change was 0.78. This indicates that CH-AgNPs has a positive effect on the gene expression of *Xip-I*, leading to an increase in its expression. When plants were sprayed with CH-AgNPs-I before fungal infection (treatment III) and CH-AgNPs-II before fungal infection (treatment IV), the gene expression was 0.73-fold change and 0.60-fold change, respectively. In contrast, when plants were sprayed with CH-AgNPs-I after fungal infection (treatment V) and CH-AgNPs-II after fungal infection (treatment VI), the gene expression decreased to 0.71-fold change and 0.22-fold change, respectively, in comparison to treatments III and IV. This implies that treating wheat crops with CH-AgNPs can stimulate gene expression and offer protection against stem rust attacks. There are no similar experiments specifically conducted on the susceptible cultivar of wheat to test the efficacy of CH-AgNPs in increasing the cultivar's resistance to the disease. The significant increase in *Xip-I* gene expression following treatment with CH-AgNPs, particularly at concentration 1 and 2, suggests that these nanoparticles may trigger or amplify defense responses in wheat plants. The observed absence or reduction of stem rust infection symptoms in plants treated with CH-AgNPs, especially when applied before infection, correlates with the upregulation of the *Xip-I* gene. Although, the differential expression patterns of the Xip-I gene between the resistant cultivar Giza168 and the susceptible cultivar Morocco highlight the genetic basis of defense responses against stem rust. Giza168 exhibits more pronounced upregulation of *Xip-I* gene expression, reflecting its inherently stronger defense mechanisms compared to Morocco.

CONCLUSIONS

This study highlights the substantial global impact of agricultural crop losses caused by diseases and emphasizing the urgent need for innovative strategies to manage them amid rising food demands. Our research focused on exploring the antifungal properties of silver nanoparticle-chitosan mixtures (CH-AgNPs) against *Puccinia graminis* in wheat, particularly examining their influence on the expression of the Xip-I gene. The results demonstrated that applying CH-AgNPs before infection notably upregulated *Xip-I* gene expression, offering effective protection against stem rust. This protective effect varied depending on the timing of application and the concentration of CH-AgNPs, with earlier applications showing more pronounced benefits. The study also revealed that CH-AgNPs could potentially enhance resistance in both resistant and susceptible wheat cultivars. These findings contribute to the broader efforts to combat hunger by safeguarding crop yields against fungal diseases, suggesting that CH-AgNPs could be a valuable addition to current agricultural disease management practices.

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