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ORIGINAL PAPER

Effectiveness of Chitosan Nanoparticles in suppression of Late Blight in Potato

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ABSTRACT

This study investigates the use of chitosan nanoparticles (CHNPs) as an alternative method for managing late blight (LB) disease in potatoes, caused by *Phytophthora infestans*, which is considered to be a major disease in Egypt and worldwide. Therefore, reducing the reliance on pesticides and their associated risks. Five different concentrations of CHNPs (50, 100, 200, 300, and 400 mg/L) were tested *in vitro*, with 200 and 300 mg/L showing the highest inhibition (100%). These concentrations were then applied in the field, where 300 mg/L CHNPs significantly reduced disease severity (DS) in the Bellini and Jelly potato varieties, lowering DS from 40.3% and 37.3% (untreated) to 17.3% and 7.3%, respectively, compared to the control fungicide. Moreover, molecular markers (SCoT and SARP) revealed genetic changes in the treated plants, indicating that CHNPs induced new genes through interactions with the potato genome, potentially leading to point mutations. Overall, CHNPs showed promise for both managing LB and contributing to potato breeding efforts.

Keywords: CHNPs nanoparticles, potato varieties, *Phytophthora infestans*, molecular markers.

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INTRODUCTION

Potatoes are one of the most widely grown vegetable crops globally, ranking just after wheat and rice (Birch *et al.,* 2012). However, they are vulnerable to late blight (LB) disease caused by the oomycete *Phytophthora infestans*, which poses a serious threat to potato yield worldwide, including in Egypt (Lenman *et al,* 2016; Mahfouze and El-sayed, 2024). Genetic resistance to pathogens is considered the most effective method for controlling LB disease, offering an alternative to fungicides, which are costly for farmers and contribute to environmental pollution. Additionally, *P. infestans* can evolve resistance to fungicides, making chemical control less reliable (Jo *et al.*, 2015). Over-reliance on toxic fungicides can harm human and animal health and compromise food security (Hirooka and Ishii, 2013). In response, the emerging field of "green synthesis" in nano-biotechnology offers environmental and economic benefits. This approach, involving the use of safe, non-toxic, and eco-friendly substances, aims to create sustainable processes. Recent advancements in nanotechnology, particularly in the production of chitosan nanoparticles (CHNPs), have revolutionized fields within life sciences and high-tech industries, offering promising solutions to LB control (El-Naggar *et al.*, 2017). The application of nanotechnology, especially through the use of chitosan nanoparticles (CHNPs), has transformed many fields within the life sciences and high-tech industries (Oh *et al.,* 2019).

Chitosan (CH) is a cationic biopolymer derived from chitin found in the shells of crustaceans and fungi, has gained significant attention in various fields, especially in life sciences and high-tech industries, thanks to its biodegradability, biocompatibility, and non-toxic nature. As a resource for sustainable agriculture, CH is used as an antibacterial agent and plant

growth promoter. It influences plant physiological processes such as protein synthesis, cell division, nutrient uptake, and cell elongation, which can ultimately enhance crop yield. Additionally, CH acts as a catalyst, inhibiting microbial growth and triggering beneficial defense responses in plants through metabolic pathways (Le *et al*., 2019; Chakraborty *et al.,* 2020).

Despite these benefits, the exact mechanism by which chitosan nanoparticles (CHNPs) inhibit *Phytophthora infestans* (LB pathogen) remains unclear. This study aims to explore the direct inhibiting effect of CHNPs on LB *in vitro* and assess their ability to enhance the potato plant's resistance to *P. infestans* Moreover, the study evaluates changes in the genomic DNA of treated potato plants using SCOT and SRAP markers, providing insights into the genetic modifications triggered by CHNP treatment.

MATERIALS AND METHODS

2.1. Plant Materials

The two potato varieties Jelly and Bellini, used in this study were obtained from the Brown Rot Project, Dokki, Giza, Egypt.

2.2. Preparation of chitosan nanoparticles (CHNPs)

In this study, Chitosan nanoparticles (CHNPs) with a molecular weight of 50,000-190,000 Da, a degree of deacetylation 75-85%, and viscosity ranging from 20-300 cP were used. Additionally, acetic acid, and sodium tripolyphosphate (TPP) were also included in the experiment. All the chemicals were used without further purification and purchased from Sigma-Aldrich, USA chemical company. The CHNPs were prepared using the ionic gelation method as described by Calvo *et al*., (1997), with some modifications. The method involves the electrostatic interaction between the amine group of chitosan and a negatively charged group of polyanion like TPP. First, a 0.2% w/v chitosan solution is prepared by dissolving chitosan in a 1% v/v acetic acid solution at room temperature. Then, a 0.06% w/v TPP solution was added dropwise to the chitosan solution under vigorous stirring for 30 min. The resulting chitosan particle suspension was then centrifuged at 12000 g for 30 min. The pellet was suspended in deionized water. After the chitosan nanoparticles suspension was freeze-dried, it was prepared for further use or analysis. The size (Z-average mean) and zeta potential of nanoparticles were measured in triplicate using photon correlation spectroscopy and Laser Doppler Anemometry, with a Zetasizer 3000HS (Malvern Instruments, UK). The morphology and size of the nanoparticles were examined using transmission electron microscopy (TEM, Tecnai G20, FEI, Netherlands). The crystalline and phase structure of the synthesized chitosan was analyzed using an X-ray diffractometer (XRD, X'Pert Pro, PanAlytical, Netherlands). All preparation and characterization processes were carried out at the Nanotechnology and Advanced Materials Central Lab (NAMCL), Agricultural Research Center, Egypt.

2.3. In vitro antifungal activity of CHNPs

Potato dextrose agar (PDA) was prepared in Schott bottle and autoclaved at 121 °C for 15 min. After cooling the medium to 55°C, chitosan was prepared as described before, was added to achieve final concentrations of 50, 100, 200, 300, and 400 mg/L. The amended medium was then poured into Petri dishes (90 mm diameter). Mycelial discs (5 mm diameter) of *P. infestans* grown on PDA medium were placed on the chitosan nanoparticles (CHNPs) amended PDA plates. After seven days of incubation at 18 °C, the colony diameter was measured daily. Growth inhibition was calculated by the Abbott formula described by Chen *et al*., (2015) as follow:

$$\text{Growth Inhibition (\%)} = [(C - T)/C] \times 100$$

Where: C is the diameter of the colony on the non-amended control, and T is the diameter on the CHNPs amended medium. All experiments were performed in triplicate.

2.4. Efficacy of chitosan nanoparticles *in vivo*

Potato tubers of two varieties, Jelly and Bellini were grown in plastic pots (20 cm in diameter) with a sandy-clay soil mix (1/1, v/v), with one tuber per pot. After 45 days of sowing, a spore suspension of *P. infestans* ($1X10^5$ spores per ml) was sprayed on the potato plants After two hours of *P. infestans* infection, the plants were sprayed with two concentrations of CHNPs (200 and 300 mg/L). The inoculated plants were then covered with a polyethylene bag at 21/11 °C (day/night) for three days, after which the bags were removed, and the plants were watered daily. The control group had four treatments:

1. Water (dsH_2O)
2. Spore suspension of *P. infestans* (1×10^5 spores per ml)
3. CHNPs only (200 and 300 mg/L)
4. Potato varieties inoculated with *P. infestans* and sprayed with a fungicide (mancozeb).

Each treatment had three replicates for each treatment (three plants per replicate), and the pots were arranged in a completely randomized design. The potato plants were observed daily for disease assessment. Leaf disease incidence was evaluated after 10 days after inoculation, and disease severity (DS) was calculated using the formula:

Disease severity (DS)%= (Number of infected leaves/total number of leaves) X 100.

The disease severity (DS) scale (1–10) was 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%,7 = 61%70%, 8 = 71-80%, 9 = 81-90%, 10 = 91-100% leaf area covered with respective symptoms (CIB, 1989).

2.5. Extraction of total DNA

0.5 g of fresh potato leaves from each of the studied two varieties, Jelly and Bellini collected from potato plants treated and untreated after two weeks of treatment and were soaked in liquid nitrogen for DNA extraction using the DNeasy plant mini-prep kit (Qiagen, CA).

2.6. Evaluation of genotoxicity using SCoT and SARP markers

Six primers, including Start codon targeted (SCoT) markers (Collard and Mackill, 2009) and Sequence-related amplified polymorphism (SARP) markers (Li and Quiros, 2001) were used in the study (Tables 1 and 2). The total reaction mixture was 25 µl contained 10X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs mixed, 10 *p*mol primers, 1.25 U *Taq* polymerase and about 150 ng genomic DNA. An initial denaturing step was performed at 94°C for 5 min followed by 5 cycles at 94°C for 1 min, 35°C for 1 min and 72°C for 1 min, subsequently followed by 35 cycles at 94°C for 1 min, annealing temperature (Tables 1 and 2) for 1 min, and 72°C for 1 min with a final extension step at 72°C for 7 min.

2.7. Gel electrophoresis

Amplification products were separated on a 1.5% agarose gel containing 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid, and 2.5 mM EDTA, pH 8.3) and 0.5 µg/ml ethidium bromide at 90 V. Gels were analyzed by UVI Geltec version 12.4, 1999-2005 (USA).

2.8. Statistical analysis

The data was analyzed using MSTAT-C program and mean differences among the treatments were compared by Least Significant Difference (LSD) at 5% level of significance (Russell, 1994).

Table 1. The sequence of SCOT primers used in the present study.

SCOT marker no.	SCOT marker name	Primer seq. (3′–5′)	Annealing temperature (AT) °C
1	1	CAACAATGGCTACCACCA	50
2	11	AAGCAATGGCTACCACCA	50
3	12	ACGACATGGCGACCAACG	61
4	13	ACGACATGGCGACCATCG	61
5	16	ACCATGGCTACCACCGAC	56
6	36	GCAACAATGGCTACCACC	56

Table 2. Sequence of SRAP primers used in the present study.

SRAP marker no.	Forward primer	Sequence (5-3)	Reverse primer	Sequence (5-3)	Annealing temperature (AT) °C
1	Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT	50
2	Me3	TGAGTCCAAACCGGAAT	Em3	GACTGCGTACGAATTGAC	50
3	Me4	TGAGTCCAAACCGGACC	Em4	GACTGCGTACGAATTTGA	50
4	Me2	TGAGTCCAAACCGGAGC	Em3	GACTGCGTACGAATTGAC	50
5	Me5	TGAGTCCAAACCGGAAG	Em1	GACTGCGTACGAATTAAT	50
6	Me5	TGAGTCCAAACCGGAAG	Em2	GACTGCGTACGAATTTGC	50

RESULTS

3.1. Dynamic light scattering (DLS) analysis

Dynamic light scattering (DLS) was used to measure hydrodynamic diameter in the nanometer range. The size of CHNPs was 33 nm and the zeta potential was 44.5 mV (Figure 1).

3.2. Transmission electron microscope (TEM) analysis

The transmission electron microscope (TEM) gave information on the shape and size of particles. A typical TEM micrograph of the CHNPs is shown in Figure (2). The nanoparticles have a nearly spherical shape, a smooth surface, and a size range of about 29 nm.

3.3. X-ray diffraction (XRD) pattern of CHNPs

The X-ray powder diffraction patterns of CHNPs are presented in Figure 3. No distinct peaks were observed in the diffractograms, indicating an amorphous structure. The CHNPs consist of a dense network of interpenetrating polymer chains, cross-linked by TPP counter ions, as described by Tang *et al*., (2003). The XRD analysis suggests increased disorder in the chain alignment of the nanoparticles following cross-linking.

3.4. Inhibitory effect of CHNPs on the growth of P. infestans in vitro

As shown in Table 3 the 300 mg/L CHNPs treatment resulted in the highest reduction of inhibition zone, achieving 100%, followed by the 200 mg/L concentration, which reduced it by 52.5%. In contrast, the two lower concentrations of 50 and 100 mg/L exhibited minimal inhibition, with reduction percentages of 3.7 and 3.1%, respectively, compared to the untreated control (Figure 4). Based on these findings, the 200 and 300 mg/L CHNP concentrations were selected for in vivo application.

3.5. Inhibitory effect of CHNPs on the growth of P. infestans in vivo

The two potato varieties; Jelly and Bellini were treated with chitosan nanoparticles (CHNPs) at two concentrations: 200 and 300 mg/L. The results in Table (4) and Figure (5), reveal that treatment with 300 mg/L CHNPs significantly reduced disease severity (DS) compared to both the untreated control and fungicide. The lowest disease severity (DS) percentage in both potato varieties, Bellini and Jelly was recorded when treated with 300 mg/L CHNPs (17.3 and 7.3%, respectively), compared with the untreated control (40.3 and 37.3%) and fungicide (19.3 and 15%, respectively). Bellini seems to have responded more strongly to CHNPs than Jelly.

3.6. Assessment of DNA changes in potato varieties by SCoT and SRAP markers

Six SCoT and SRAP primers were used in this study to detect DNA damage in two potato varieties infected with *P. infestans* and treated with CHNPs at 200 and 300 mg/L (Figures 6 and 7). Among the six SCoT primers, specific DNA bands were detected in Bellini plants infected with *P. infestans* and treated with 300 mg/L CHNPs, including bands at 400 and 500 bp (using primer SCoT 1) and at 900 and 1300 bp (using primer SCoT 13). Additionally, a 1500 bp fragment was observed in Bellini plants infected with *P. infestans* and treated with 200 mg/L CHNPs, using primer SCoT 16 (Table 5). Furthermore, a 1500 bp band was detected

in Bellini plants both infected with *P. infestans* and treated with 200 mg/L CHNPs, as well as in plants treated with 200 mg/L CHNPs alone, using primer SCoT 13. For Jelly variety, several unique amplicons were observed in plants infected with *P. infestans* and treated with 300 mg/L CHNPs, including bands at 200 bp (SRAP 5), 300 bp (SCoT 12), 400 and 700 bp (SRAP 2-3), and 1300 bp (SCoT 13) (Table 5). These newly induced bands appeared only when plants were treated with high doses of CHNPs. Additionally, a 330 bp fragment was detected in Jelly plants infected with *P. infestans* and treated with both 200 and 300 mg/L CHNPs, using primer SCoT 12. This band was absent in all other treatments. Overall, CHNP treatment appears to restore plants to their original state before infection, facilitating rapid recovery, likely by directly affecting and potentially eliminating the pathogen. These findings confirm that both SCoT and SRAP markers are valuable tools for differentiating between potato plants treated with CHNPs and untreated ones (Table 5).

Table 3. Effect of chitosan nanoparticles on the growth of *P. infestans*.

Nanoparticle	Concentration (mg/L)	Mycelia linear growth (cm)	Growth reduction (%)
CHNPs	50	8.4[a]	3.7%[c]
	100	8.5[a]	3.1%[c]
	200	4.7[b]	52.5%[b]
	300	0[c]	100%[a]
	400	0[c]	100%[a]
L.S.D at 5%		0.83 %	9.5

Values followed by the same letter are not significantly different according to Duncan's multiple range test (P<0.05).

Table 4. The percentage of infection and disease severity of the two potato varieties infected with *P. infestans* and treated with CHNPs particles.

Potato cultivars	CHNPs Conc. (mg/L)	Percentage of infection	Disease severity %	Disease severity scale
Billini	200	28.8[b]	21.6[b]	3.3[b]
	300	15.0[d]	17.3[d]	3.1[b]
	Untreated control *(P. infestans* alone)	34.8[a]	40.3[a]	4.9[a]
	Fungicide (mancozeb)	21.67[c]	19.3[c]	2.3[c]
L.S.D at 5%		1.4	0.08	0.1
Jelly	200	17.7 [b]	14.5[b]	2.7[c]
	300	3.0 [d]	7.3[c]	1.6[d]
	Untreated control *(P. infestans* alone)	35.0 [a]	37.3[a]	4.3[a]
	Fungicide (mancozeb)	10.0 [c]	15.0[b]	2.8[b]
L.S.D at 5%		0.06	1.8	0.1

Values followed by the same letter are not significantly different according to Duncan's multiple range test (P<0.05).

Table 5. Summary of the effects of the two conc. 200 and 300 mg/L of CHNPs on two potato varieties infected with *P. infestans* using SCoT and SRAP markers.

Interpretation	Primers	Amplicons (bp)	Jelly						Bellini					
			*Cont.	*Infected	200 mg/L CHNPs	200 mg/L CHNPs +fungi	300 mg/L CHNPs	300 mg/L CHNPs +fungi	*Cont.	*Infected	200 mg/L CHNPs	200 mg/L CHNPs +fungi	300mg/L CHNPs	300mg/L CHNPs +fungi
Genetic variation between the two varieties	SCoT 11	1400							+	+	+	+	+	+
	SCoT 16	1100							+	+	+	+	+	+
	Srap 3	600							+	+	+	+	+	+
	Srap 5	1500				No amplicons exist			+	+	+	+	+	+
	Srap 5	700							+	+	+	+	+	+
	Srap 5	490							+	+	+	+	+	+
	Srap 5	350							+	+	+	+	+	+
	SCoT 16	280	+	+	+	+	+	+						
	SCoT 16	600	+	+	+	+	+	+						
	SCoT 13	420	+	+	+	+	+	+						
	Srap 1	900	+	+	+	+	+	+				No amplicons exist		
	Srap 1	600	+	+	+	+	+	+						
	Srap 5-2	600	+	+	+	+	+	+						
	Srap 5	300	+	+	+	+	+	+						
Effects of 300 mg/L CHNPs on P. infestans infection	SCoT 1	500												
	SCoT 1	400												+
	Srap 5	200						+						+
	SCoT 13	1300						+						+
	SCoT 13	900		+				+						+
	Srap 2-3	400						+		+				
	SCoT 36	1100	+	+			+	+					+	+
Effects of 200mg/L CHNPs on P. infestans infection	SCoT 16	+ 1500										+		
	SCoT 13	+ 1500				No amplicons exist					+	+		
	Srap 1	700								+	+			
Effects of 200 & 300 mg/L on P. infestans infection	Srap 3	500				+	+							
	Srap 2-3	700						+			+			
	SCoT 16	400				+	+			+	+			
	SCoT 12	300						+		+	+		+	
	SCoT 12	330				+		+		+	+		+	

* Cont.: healthy untreated control Infected: infected and untreated control

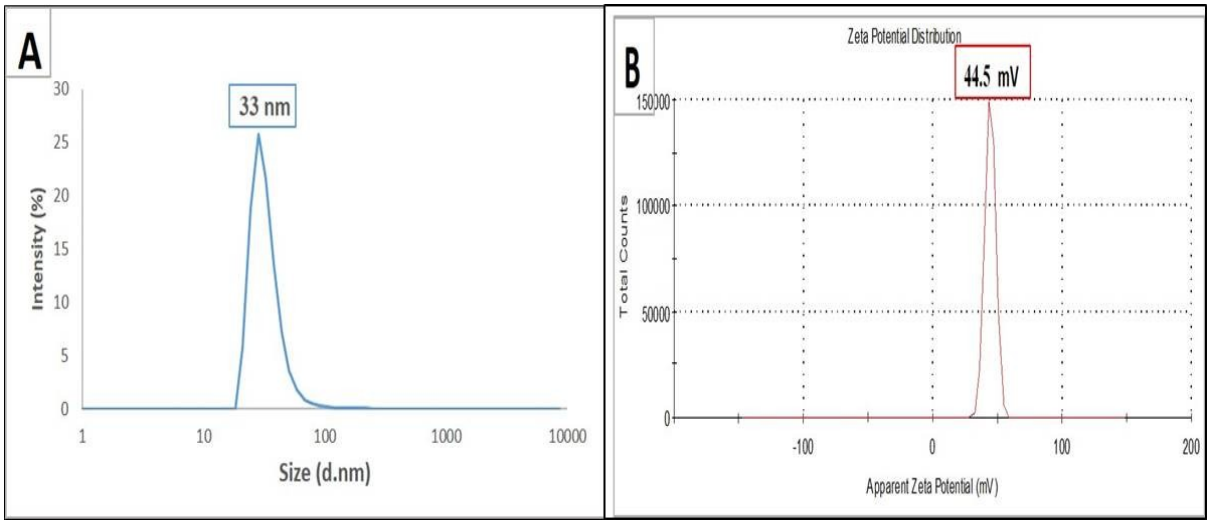

Figure 1. DLS analysis of CHNPs. Particle size (A), and Zeta potential (B).

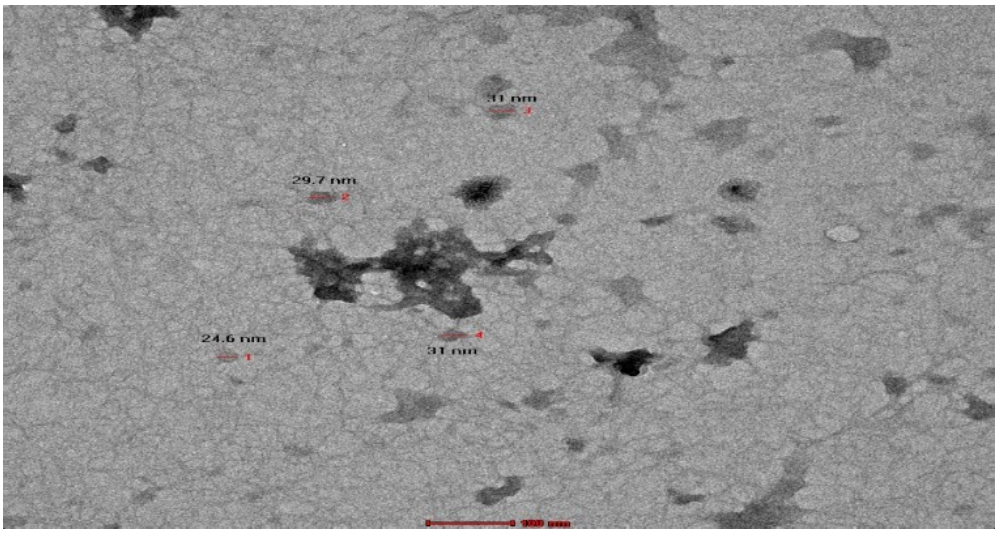

Figure 2. TEM image of CHNPs

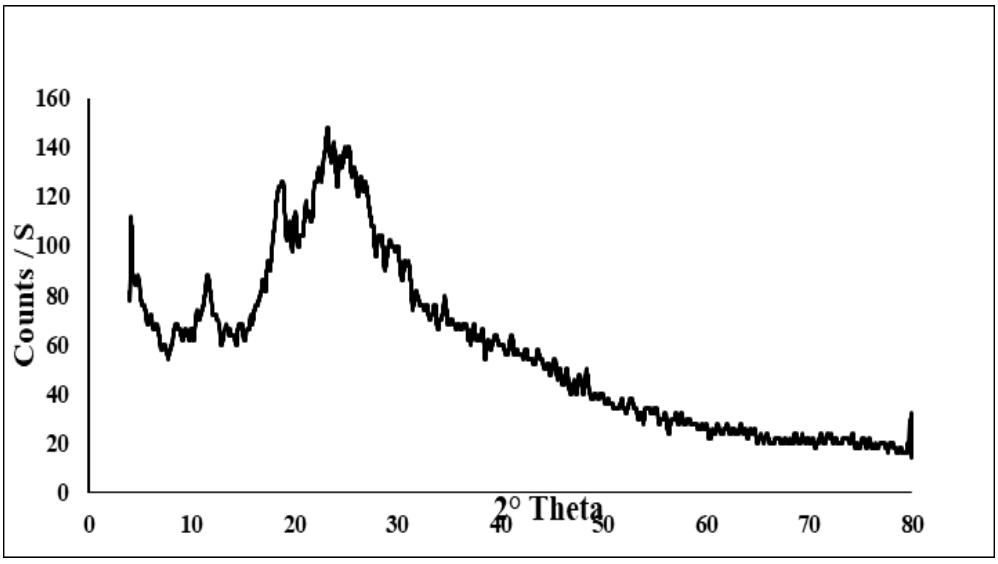

Figure 3. X-ray powder diffraction patterns of CHNPs.

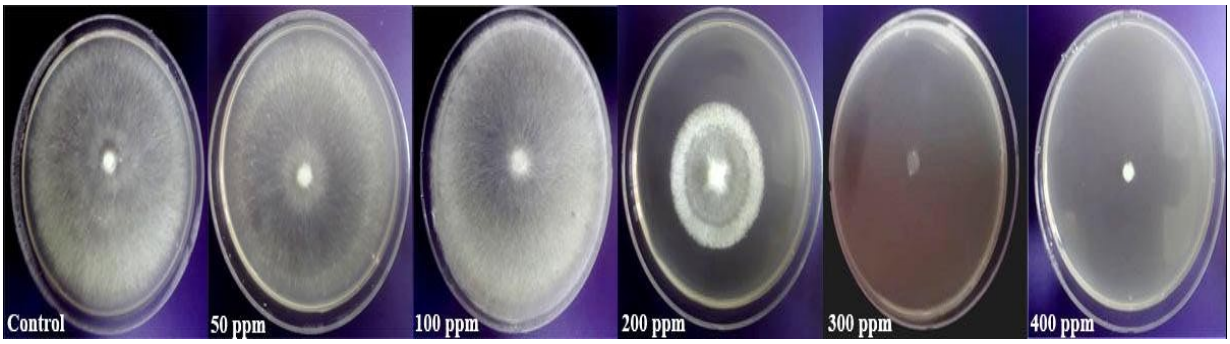

Figure 4. *In vitro* antifungal activity of CHNPs.

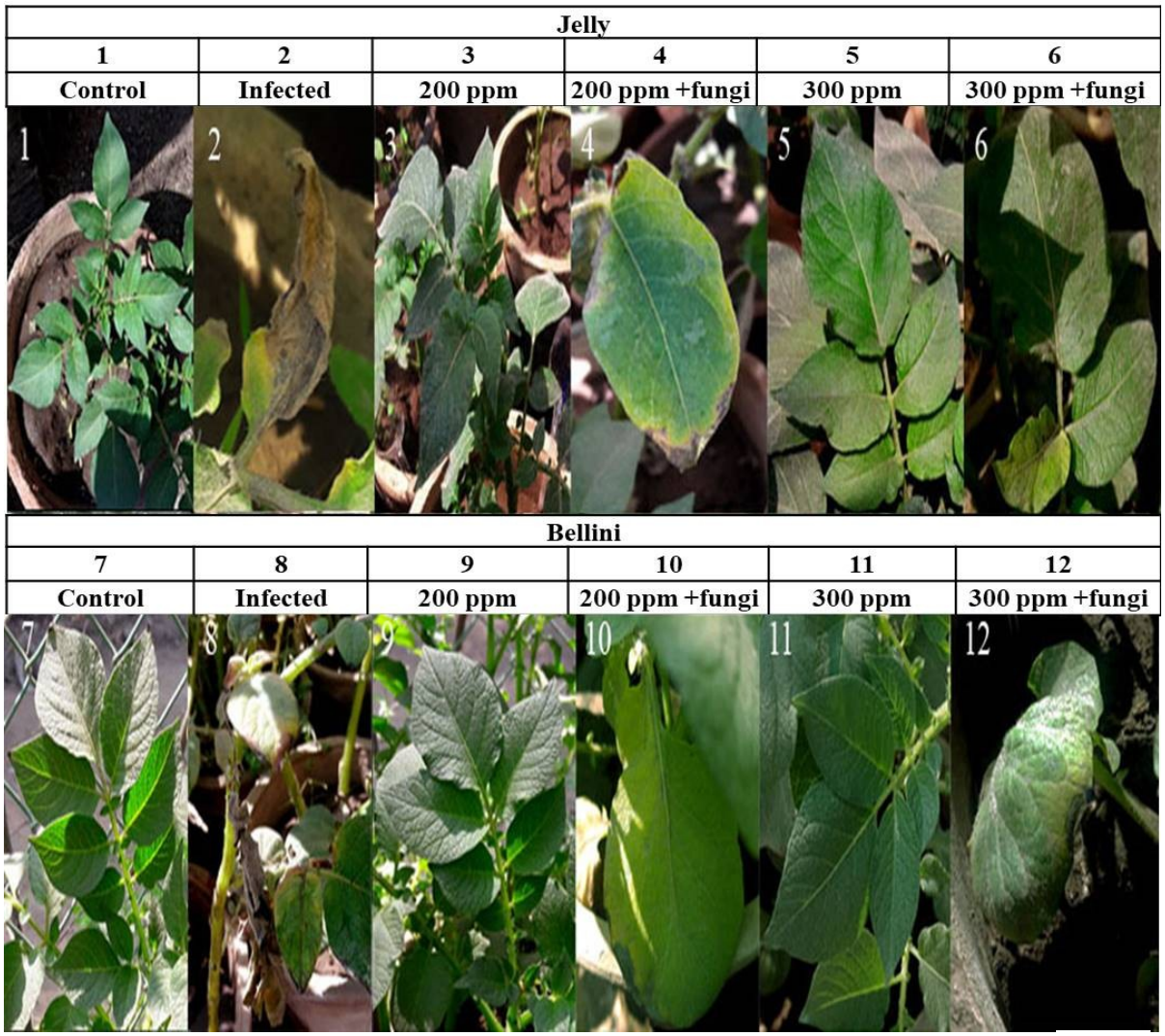

Figure 5. Symptoms on leaves of two potato varieties; Jelly and Bellini treated with CHNPs and untreated control. The infected plants of potato variety Jelly show necrotic brown spots on the edge and Bellini variety shows dark brown blotches surrounded by yellowish-green rings as well as dead leaves, potato plants treated with 200 and 300 mg/L CHNPs showed no disease symptoms, and potato plants infected with fungi and treated with 200 and 300 mg/L CHNPs displayed mild symptoms. Cont.: Un-infected and untreated control Infected: infected and untreated control

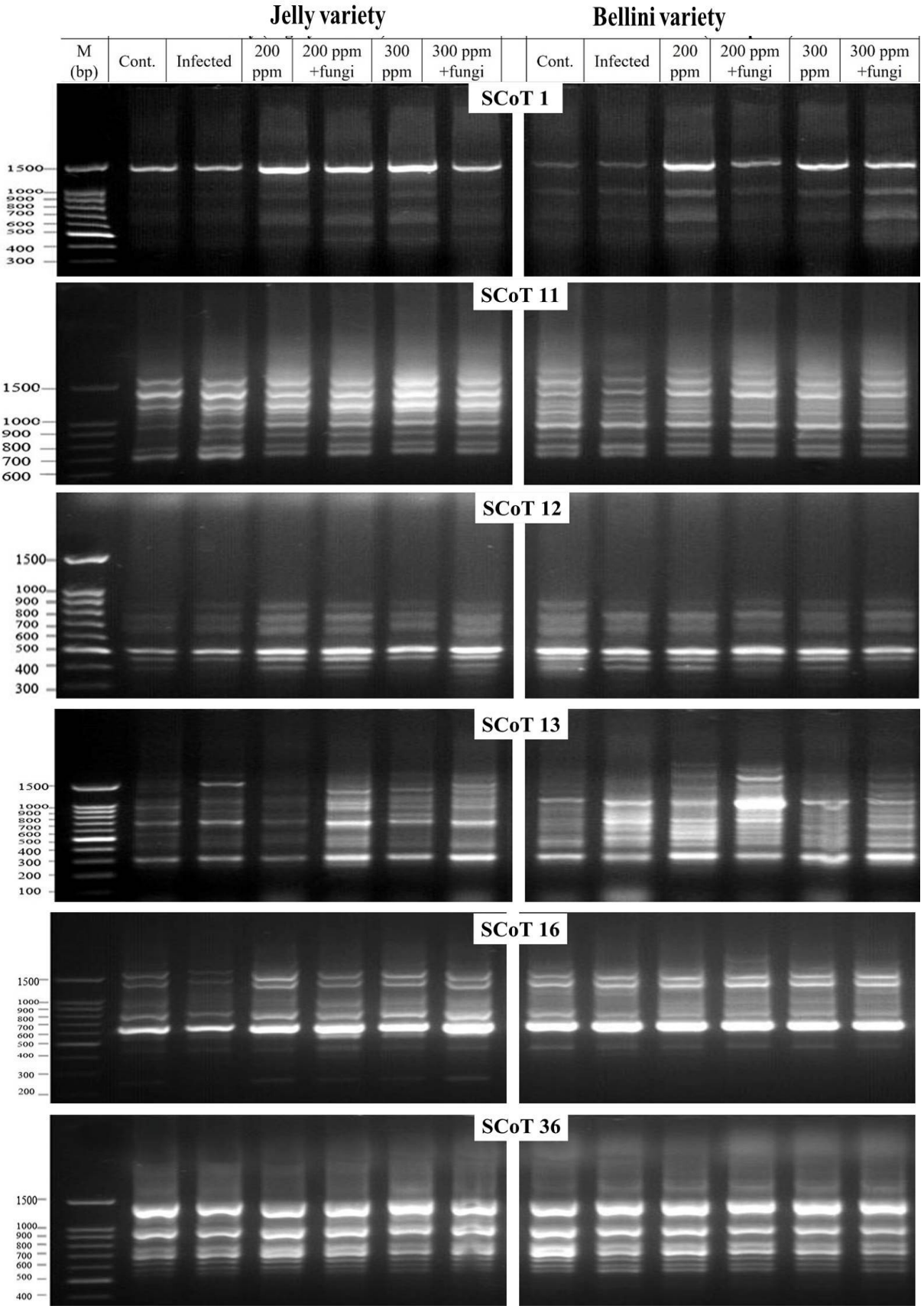

Figure 6. SCoT markers to detect the effects of two concentrations of CHNPs against *P. infestans* infection in two potato varieties. Cont.: Un-infected and untreated control Infected: infected and untreated control

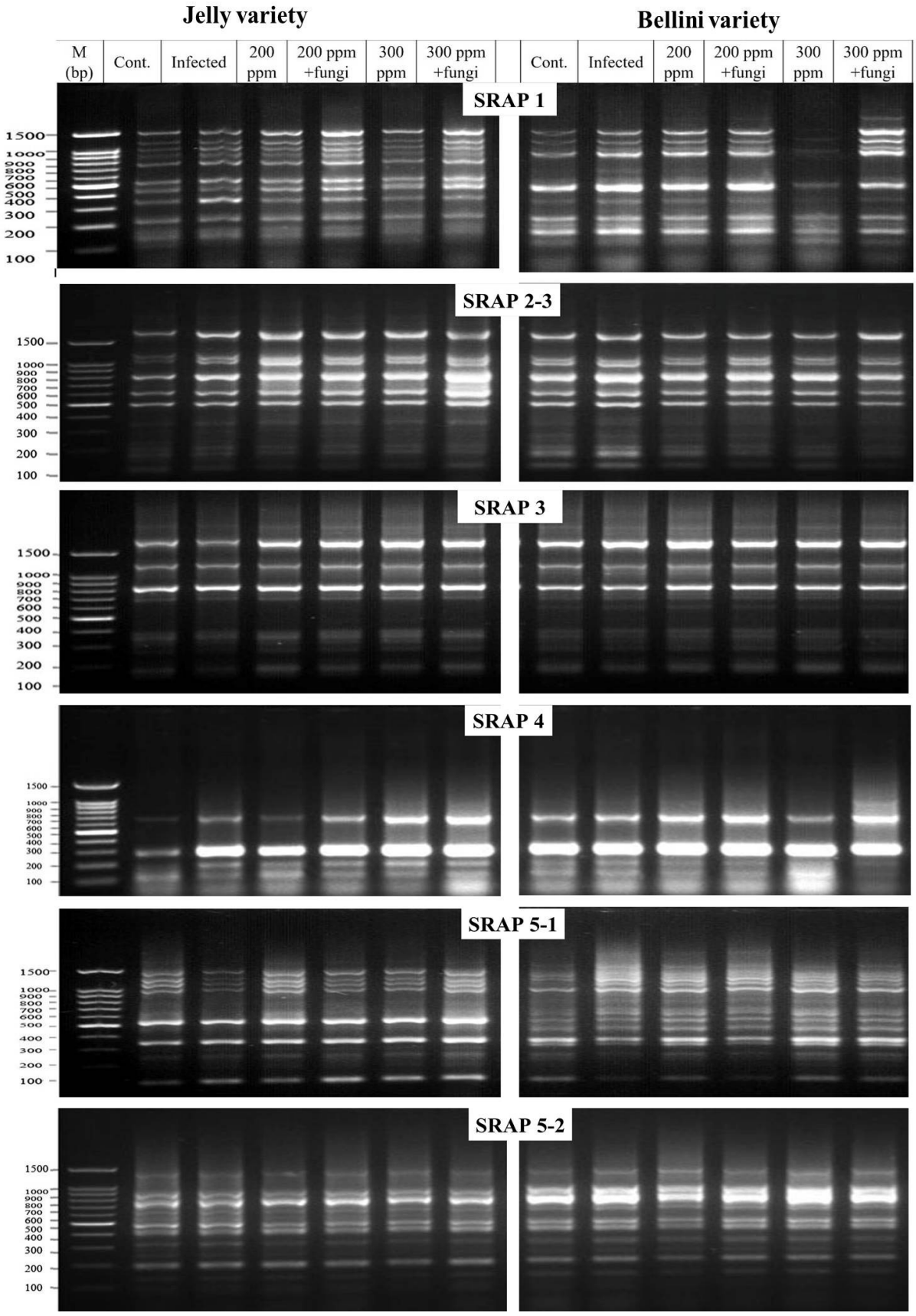

Figure 7. SRAP markers to detect the effects of two conc. of CHNPs against *P. infestans* infection in two potato varieties. Cont.: Un-infected and untreated control Infected: infected and untreated control

DISCUSSION

Chitosan (CH) is a biological fungicide that enhances plant defense mechanisms against pathogens, thereby boosting the immune response in plants, fruits, and vegetables. *P. infestans*, the causative agent of late blight (LB), is one of the most significant pathogens affecting potatoes in Egypt and worldwide (Birch *et al.,* 2012; Mahfouze *et al*., 2023). In this study, the two potato varieties, Jelly and Bellini, were treated with CHNP concentrations of 200 and 300 mg/L after infection with *P. infestans*. The highest growth reduction percentage was observed in both potato varieties Jelly and Bellini when treated with 200 and 300 mg/L CHNPs (100%). Therefore, CHNPs effectively inhibited *P. infestans* at concentrations of 200 and 300 mg/L. additionally, the lowest disease severity (DS) percentages were observed in both potato varieties Bellini and Jelly treated with 300 mg/L CHNPs measuring 17.3 and 7.3%, respectively. In comparison, DS percentages in the untreated control were 40.3 and 37.3%, while the fungicide recorded 19.3 and 15% for Bellini and Jelly, respectively.

Chitosan nanoparticles can be utilized at lower concentrations, specifically between 0.001% and 0.01% w/v, while the applied range of normal-sized chitosan is around 0.01–1% w/v, additionally, chitosan micro/nanoparticles possess the capability to encapsulate other substances within their core, effectively simulating drug delivery systems for the targeted release of active compounds to target sites. The nanostructure of chitosan safeguards these incorporated compounds from pH fluctuations, enzymatic degradation, and various adverse environmental factors as described by (Asgari-Targhi *et al.,* 2018, Imam *et al*., 2021; Riseh *et al*., 2022). As well as, following transformation, chitosan micro/nanoparticles retain their ability to elicit plant defense responses and exhibit antimicrobial properties, while demonstrating enhanced effectiveness in controlling pests and diseases. (Kumaraswamy *et al*., 2018; Sravani *et al.,* 2023). Chitosan is used extensively in the formulation of nanoparticles for various purposes. Its advantages include being biodegradable, biocompatible, cost-effective, non-allergenic, degradable by both specific and nonspecific enzymes, and exhibiting low toxicity to humans. (Park and Kim, 2010; Keawchaoon and Yoksan, 2011; Ing *et al*., 2012; El-Naggar *et al.,* 2024).

Chitosan nanoparticles exhibit a wide range of biological activities due to their modified physicochemical properties, such as surface area, size, cationic characteristics and enhanced encapsulation efficiency, either independently or in combination with other components. Saharan *et al*., 2013. Therefore, treatment with CHNPs significantly enhanced resistance to late blight (LB) pathogen in both potato varieties. Moreover, CHNPs reduced infection and helped restored certain genes affected by the infection to their original genetic state. This is consistent with several studies reporting fungicidal activity against a broad range of fungi, including, *Penicillium* sp., *Rhizopus* sp., *P. infestans*, and *Alternaria* sp., (Badawy and Rabea, 2011; El-Mohamedy *et al.*, 2019; Huang *et al*., 2021).

CHNPs have been shown to inhibit mycelial growth and affect spore germination, spore viability, linear growth, and hyphal growth. Oerke and Dehne, (2004) stated that the antifungal effect of CHNPs on fungi relies on multiple mechanisms. One key factor is the positive charge of CHNPs which enables electrostatic interactions with the negatively charged surface of pathogens. Additionally, CHNPs can penetrate microbial cells and interact with DNA/RNA, leading to the inhibition of mRNA synthesis and microbial reproduction. Furthermore, CHNPs accumulate on the pathogen's surface, disrupting its cellular functions (Aktar *et al*., 2009). Finally, the accumulation of CHNPs on the surface of microorganisms acts as a barrier, preventing the entry of nutrients and metabolites into the cell, thereby inhibiting infections (Maluin and Hussein, 2020).

While chitosan (CH) functions as a defense elicitor that stimulates innate immunity in potato plants against fungal infection, its specific mode of action and effectiveness, against late blight (LB) remain unclear (Zheng *et al.*, 2021). However, CH application has been shown to significantly enhance *Solanum tuberosum* L. resistance, reducing LB disease incidence in both potted and field-grown potatoes. Transcriptomic analysis revealed that CH activates multiple defense-related pathways in plant hosts. Katiyar *et al*., (2015) reported that CH induces systemic acquired resistance (SAR) and stimulates various disease-related enzymes, including peroxidase, catalase, polyphenoloxidase, phenylalanine ammonia-lyase, β-1,3-glucanase, and chitinase. Additionally, CH enhances salicylic acid accumulation, increases phenylalanine ammonia-lyase activity, and reduces malondialdehyde content, ultimately boosting the biological activity of plants. Atia *et al.*, (2005) demonstrated that CH exerts dual effects in tomato plants infected with LB: (a) direct interference in the developmental stages of *P. infestans* and (b) lesion formation, leading to enhanced disease resistance strategies.

The identification of DNA markers related to resistance traits enables efficient and precise screening of potato lines, thereby saving both time and resources. Markers linked to diseases like late blight allow breeders to test seedlings early without field observation. Genetic modifications near resistance (R) genes validate their functions and facilitate their incorporation into elite varieties through advanced methodologies as described by Torres, (2009) and Islam, *et al.,* (2024). Collard and Mackill (2008) mentioned that SCAR and SRAP markers help identify DNA regions associated with traits, such as disease resistance. In this context, if certain markers are consistently found in late blight-resistant genotypes, they can be linked to resistance genes (e.g., *R* genes). In addition, SRAP/SCAR markers are linked to resistance genes from wild *Solanum* species; breeders can track and incorporate these genes into cultivated varieties. Gebhardt and Valkonen (2001) reported that markers linked to resistance are validated; they can be used in marker-assisted selection by screening early-generation seedlings for presence of resistance-associated markers. In this study, two types of molecular markers, SCoT and SRAP markers, were used to assess DNA changes in the two potato varieties, Jelly and Bellini, treated with CHNPs at concentrations of 200 and 300 mg/L compared to the controls. The appearance of new bands in potato plants treated with higher CHNPs doses, as detected by SCoT and SRAP assays, findings align with those of An and Jin (2012), who reported that nanoparticles can interact with nucleic acids, leading to significant alterations in DNA helix conformation, and changes in the orientation of nitrogenous bases within the DNA strand. The SCoT marker is a novel, simple, and reliable gene-targeted marker based on the translation start codon (Xiong *et al.*, 2009). SCoT polymorphisms are dominant and reproducible, relying on the short-conserved regions of plant genes that flanked by the ATG translation start codon (Collard and Mackill, 2009). In contrast, the SRAP marker is designed to amplify open reading frames (ORFs) (Li and Quiros, 2001; Xiong *et al.*, 2009; Collard and Mackill, 2009). Both SCoT and SRAP assays effectively detected polymorphisms in the DNA coding sequences, between treatments and control. The observed polymorphism was attributed to the appearance or disappearance of DNA fragments, likely due to DNA damage caused by CHNP interactions, leading to point mutations (Li *et al*., 2013). Kumar *et al*., (2020) reported that while lower concentration of nanoparticles (NPs) benefit plant growth, higher concentrations can induce chromosomal aberrations. The effect of NPs depends on their mode of application, size, and concentrations. Therefore, in the future NPs may serve as a valuable tool for farmers to enhance crop productivity, provided the optimum concentration is applied.

CONCLUSIONS

In this study, the evaluation of potato varieties for late blight (LB) resistance under glasshouse conditions provides insights into their potential field performance. Our results demonstrated that treating *P. infestans*-infected Jelly and Bellini potato varieties with CHNPs at concentrations of 200 and 300 mg/L both in *in vitro* and *in vivo* effectively inhibited pathogen growth and enhanced potato resistance. Furthermore, CHNPs induced genomic DNA modifications, as evidenced by the appearance of new bands detected using SCOT and SRAP markers. Given that CHNPs are safe, non-toxic, and eco-friendly, we recommend their use as an alternative to conventional fungicides, which are highly toxic, contribute to environmental pollution, and pose risks to human health. However, their widespread implementation necessitates further research to address unanswered questions regarding long-term effects, environmental impact, and field applicability. Future investigations, including large-scale field trials and transcriptomic studies, will be pivotal in understanding their efficacy and molecular mechanisms. This strategy holds promise for integration into potato breeding programs for sustainable LB management.

Author's contribution

Majority contribution for the whole article belongs to the author. The author read and approved the final manuscript.

Competing interests

The author declares that he has no competing interests.

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