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Zinc oxide nanostructures as a control strategy of bacterial speck of tomato caused by *Pseudomonas syringae* in Egypt

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Abstract
This study was conducted to evaluate the ability of zinc oxide nanoparticles (ZONPs) with unique properties to protect tomato against the bacterial speck pathogen, caused by *Pseudomonas syringae* pv. tomato DC3000 (Pst). Protection of tomato against bacterial speck using ZONPS was evaluated by its direct antibacterial activity and its ability for inducing resistance in tomato plants. The results revealed that ZONPs showed significant direct antibacterial activity against *Pseudomonas syringae* pv. tomato under laboratory conditions. Moreover, tomato plants treated with ZONPs showed a significant reduction in disease severity and bacterial proliferation relative to non-treated plants. Furthermore, tomato plants treated with ZONPs showed higher self-defense enzyme activity relative to untreated plants. The regulatory and defense genes, *LePR-1a* and *Lipoxygenase* (*LOX*), involved in the salicylic acid (SA) and (JA) signaling pathways, respectively, were highly expressed in tomato plants treated with ZONPs compared to untreated plants. Growth characters of tomato plants treated with ZONPs were significantly enhanced relative to untreated plants. The control of bacterial speck pathogen of tomato using ZONPs through its direct antibacterial and by developing of systemic resistance in treated tomatoes against the pathogen is considered the first report.

Keywords Tomato · Bacterial speck · Nanoparticles · Systemic resistance induction · Control

Introduction

Tomato (*Lycopersicon esculentum*) is the second most important vegetable crop in the world as reported by the World Health Organization (WHO). Bacterial speck is a serious bacterial disease that affects tomatoes and its symptoms appear in a form of small, sunken, and black lesions on leaves, stems, and fruits (Bryan, 1933; Miller and Jones 2014). According to a previous study, tomato seedlings with these bacteria may lead to a reduction of up to 75% of the tomato production and reduce its quality as well (Yunis et al. 1980).

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Control of bacterial speck disease caused by Pst can be achieved through limited options of chemical control or through the available varieties resistant to this disease. Currently, the chemical control of this disease mainly depends on copper compounds such as copper hydroxide. However, the resistance strain of the pathogen to these compounds has been identified globally (e.g., Martin et al. 2004; Shenge et al. 2008), leading to an interest in alternative control strategies. Moreover, chemical control using pesticides causes a lot of damage to humans and the environment. As a result of the lack of access to effective bacterial pesticides and resistant cultivars to bacterial diseases in tomato crop, great efforts have been made to develop alternative strategies to control these diseases in tomato. Therefore, the use of nanoparticles is an alternative and effective way to control plant diseases, where the advantages of nanostructures are environmentally friendly and cheap (Elsharkawy and Mousa 2015). Nanoparticles also have a great ability to control plant pathogens compared to chemical pesticides (Park et al. 2006). The use of these nanoparticles to control plant pathogens via direct antibacterial activity against the pathogens and by stimulating induced resistance in treated plants against the pathogens considered a source of major concern. Recently, inorganic nano-

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biocides, such as silver (Morones et al. 2005; Salam et al. 2017) titanium dioxide (Sunada et al. 1998), and zinc oxide (Dimkpa et al. 2013), have paid great attention in the application for plant diseases control. Particular attention was paid to the sliver-based nanomaterials, as they showed a high inhibitory effect against bacteria.

Zinc oxide is a nutritional element for the plant and is used as fertilizer but is characterized by no adverse effects such as those produced from normal fertilizers. Since it has been used as a nanofertilizer, it plays an effective role in plant growth and productivity (Milani et al. 2015). It has been successfully used as fertilizer in many agricultural crops in low quantities and has also been used successfully as a pesticide in pest control (Raikova et al. 2006 and Batsmanova et al. 2013). Zinc oxide nanoparticles are also characterized by its ability to penetrate the plant and move within its tissues through cuticle, epidermis, stomata, hydathodes, stigma, root tips, rhizodermis, cortex lateral plants, root junctions, bark, and other several surfaces of plants (Eichert et al. 2008; Dietz and Herth 2011). The good physical properties of zinc oxide created by nanotechnology have introduced a strong candidate as an antibacterial agent that affects agricultural crops (Shah and Towkeer 2010). It can also be used as an antimicrobial agent to conserve food products (Huang et al. 2005; Aruoja et al. 2009; Sharma et al. 2009). The use of nanoparticles as pesticides has high acceptance because it is safer for plants and have less environmental pollution than chemical pesticides (Barik et al. 2008). Thus, the objectives of this study were to evaluate the ability of zinc oxide nanoparticles (ZONPs) to protect tomato against *Pseudomonas syringae* pv. tomato either by direct antibacterial activity or induction of systemic resistance in tomato plants. To explore the resistance induction mechanism, the activity of self-defense enzymes and the expression of regulatory and defense-related genes using RT-PCR were evaluated. Finally, the effect of the applied ZONPs on some growth characters of tomato was assessed.

Materials and Methods

Synthesis of ZnO nanoparticles

ZnO nanostructures were fabricated by using a chemical bath deposition (CBD) method. The fabrication process was carried out by preparation of 0.25 M zinc nitrate hexahydrate (as a precursor of Zn^{+} ions) and 2.135 M of potassium hydroxide (as a catalyst) in 20 mL of deionized water. Each solution was separately stirred for 10 min and then added to each other and stirred again for 10 min. The final mixture solution was left in the oil path at 80 °C for 4 h. After collecting the precipitated nanopowder, it was washed several times with deionized water and ethanol and then dried for several hours to remove residual water vapor at 105 °C. The fabricated ZnO were characterized using XRD (Shimadzu 6000), UV-vis spectrophotometer (JASCO V-630), and scanning electron microscope (JSM-651OLV).

Antibacterial activity of ZONPs against Pst

To explore the antibacterial properties of ZONPs, an agar disk diffusion technique was used (Li et al. 2017). Streptomycin was used as a standard and sterile water served as control. This test was carried out by incubating the Pst cells in King's B broth medium with constant shaking at 25 °C and 180 rpm all night. Afterward, the bacterial suspension was diluted to a 1×10^{8} cfu mL^{-1} suspension and 100 mL of bacterial suspension were then filtered over a King's B agar plate. Before that, the disks of the filter papers were immersed in streptomycin and ZONPs at concentration levels of 100, 200, and 300 μg mL^{-1} and were placed on different plates. For control treatment, filter paper disks were immersed in sterilized water. After incubating at 25 °C for 48 h, the size of the inhibition zone around the filter papers was measured. All experiments were repeated three times.

Greenhouse experiments

Antibacterial effect of nanoscale zinc oxide against bacterial speck of tomato was evaluated under greenhouse conditions using tomato transplants (*Lycopersicon esculentum* cv. *Pantelosa*). Tomato plants were grown in plastic pots containing cultivated soil in a greenhouse. After growing to a five-leaf stage, tomato plants were treated with ZONPs (100 μg mL^{-1}) acibenzolar-*S*-methyl (ASM; 0.2 mg mL^{-1}), streptomycin sulfate (25 mg mL^{-1}), and sterile water (control) as spray treatment as well as benzothiadiazole (BTH) at a concentration level of 0.3 mM as a soil drench at 24 h before or after pathogen inoculation. Inoculation was carried out by spraying the Pst suspension containing 2.5×10^{7} c.f.u. mL^{-1} bacteria and 0.01% (*v*/*v*) Silwet L^{-77} (a surfactant used for spreading the spray solution on plant leaves). Each treatment consists of three replicates. After infection with Pst, all treated tomato plants were packed in transparent plastic bags for 48 h and then the bags were opened and all plants were placed in a greenhouse under 20–25 °C and 70% relative humidity.

Five days after the pathogen attack, disease severity in each plant was measured by recording the proportion of the number of leaves showing symptoms of the disease based on the scale: 0 = no symptoms to 100 = most severe with necrotic symptoms. The number of Pst on the infected leaves was estimated by collecting these leaves from each treatment. After that, these leaves were weighed and washed in a homogeneous manner in sterile water. Sequentially, a series of appropriate dilutions were added to KB medium containing 50 mg L^{-1} rifampicin for counting CFU of Pst without any contamination. After 48 h of incubation at 28 °C, the number of

Table 1 Forward and reverse primers sequence for pathogenesis-related genes

Gene	Forward primer	Reverse primer	Size	Accession number
*Le*β-tubulin	AACCTCCATTCAGGAGATGTTT	TCTGCTGTAGCATCCTGGTATT	180	DQ205342
LePR-1a	TCTTGTGAGGCCCAAAATTC	ATAGTCTGGCCTCTCGGACA	246	AJ011520
Lipoxygenase	CCTGAAATCTATGGCCCTCA	ATGGGCTTAAGTGTGCCAAC	227	U37840

rifampicin-resistant Pst (c.f.u. per each gram) of infected leaf tissues was estimated. Growth-promoting effects of the examined treatments (ZONPs, streptomycin, BTH, and ASM) on non-infected plants was evaluated by determining plant height (cm), as well as leaves' fresh and dry weight. This experiment was repeated three times.

Estimation of peroxidase activity

The activity of peroxidase(POD) enzyme in collected leaves (3 and 6 days after pathogen inoculation) was estimated by using spectrophotometric analysis. Sampling time was selected based on a screening test showing that 3 and 6 days were the best periods for getting high enzyme activity. Guaiacol is a substrate metabolized by using a big range of peroxidases (PODs), so it is used as a marker for the POD in our analyses. Samples of plant leaves weighing 1 g were taken after 3 and 6 days of inoculations, then cursed in a mortar using phosphate buffer (0.1 M; pH 7) in a ratio of 1 g of plant leaves in 4 mL of phosphate buffer. The supernatant needed for enzyme activity estimation was obtained by centrifugation of collected samples at 10.000 rpm for 10 min (Maxwell and Betman 1967). Phosphate buffer with a volume of 2.5 mL (pH 7.0) and enzyme extract (0.2 mL) was added in all test tubes (2 sets). In the experimental set, 0.2 mL of 1% guaiacol solution was added and mixed. After that, both sets were left at room temperature for 15–20 min. Finally, 0.1 mL of H_2O_2 (0.3%) was added to all the test tubes and mixed well. For the blank, 0.2 mL of distilled water, 2.5 mL of phosphate buffer (pH 7.0), and 0.1 mL of H_2O_2 (0.3%) were added and mixed. The absorbance of samples was measured at 460 nm using a spectrophotometer (L-5000, Germany). To achieve the greatest accuracy in the obtained results, each sample was measured three times. The enzyme activity was measured as described by Ippolito et al. (2000).

Estimation of polyphenoloxidase activity

Samples of plant leaves were collected after 3 and 6 days of pathogen inoculation and were ground. Sampling time was selected based on a screening test showing that 3 and 6 days were the best periods for getting high enzyme activity. The extraction process (Valero et al. 1988) was then carried out by addition of 100 mL of phosphate buffer (pH 7.3) containing 10 mM sodium ascorbate to 1 g of crushed leaves and homogenized in a blender for 15 s. After that, the homogenate was filtered and centrifuged at 10.000 rpm for 30 min. Then, 5 mL of Triton-X-100 (1.5%), prepared in 100 mM phosphate

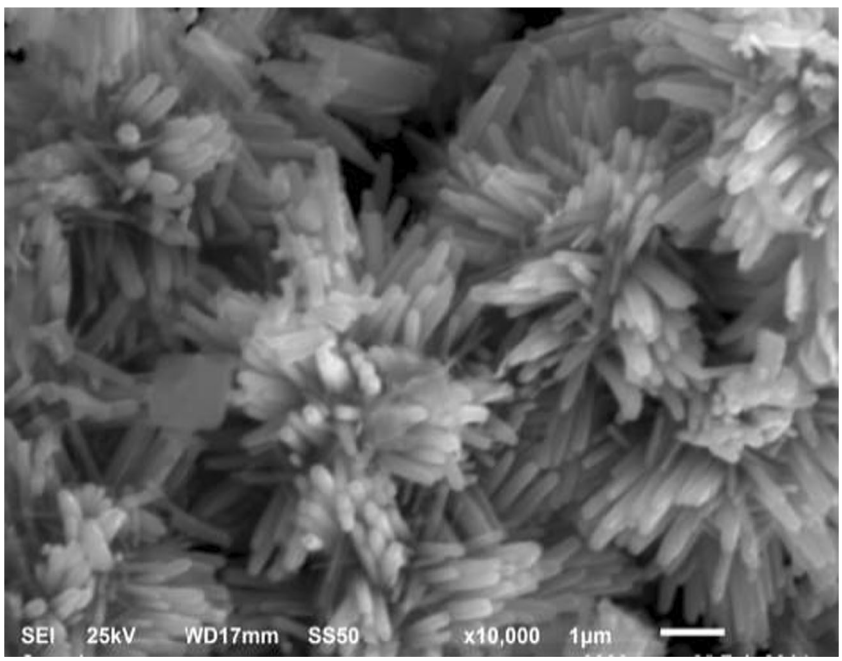

Fig. 1 SEM image of ZnO nanostructures prepared by the CBD method

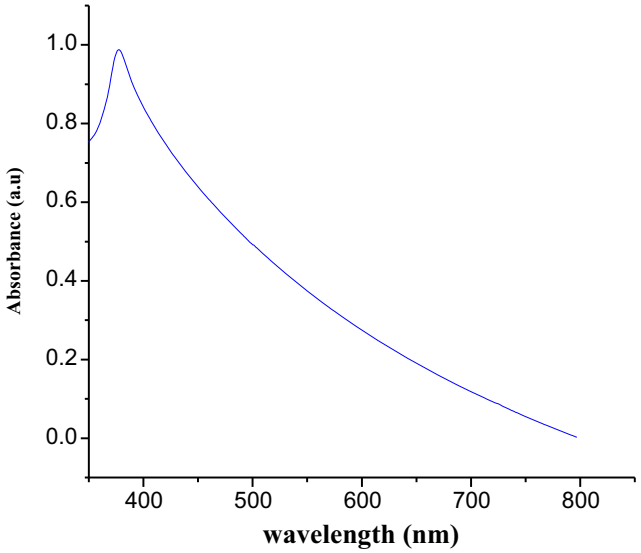

Fig. 2 UV-Vis absorption spectrum of ZnO nanostructures

buffer (pH 7.3), were used for re-extraction of the precipitate for 15 min. The extract solution was completed to 25 mL with phosphate buffer (pH 7.3) and the filtrate was centrifuged (15.000 rpm) for 1 h. The precipitate was collected by fractionation between 45 and 95% saturation using ammonium sulfate. Then, the precipitate fraction was gathered, re-dissolved in phosphate buffer and dialyzed at 4 °C in cellulose dialysis tubing. The solution after dialysis was used as an enzyme source. 4-Methyl catechol and 4-methyl phenol (p-cresol) were used as the substrates for measuring catecholase and cresolase activity (Sanchez-Ferrer et al. 1988). The final step was carried out by addition of 3 ml of phosphate buffer (pH 7.3), 1 mL crude enzyme extract, and 1 mL of the substrate. The change in absorbance of the prepared solution at 398 nm was recorded using an Utech-5300UVspectrophotometer, USA.

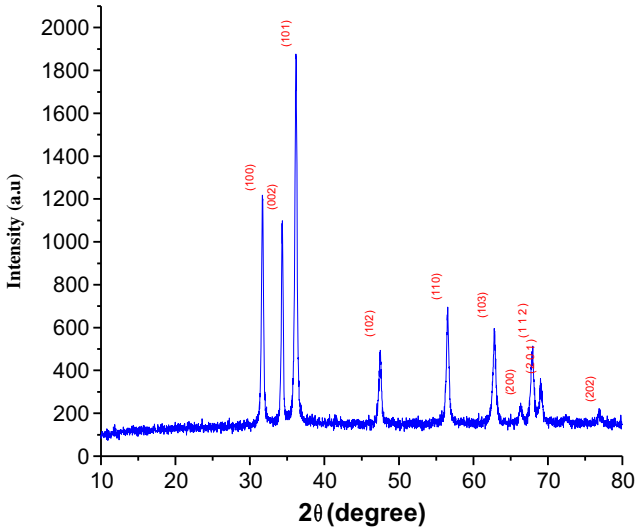

Fig. 3 XRD of ZnO nanostructures prepared by CBD method

Fig. 4 Growth inhibition of Pst by ZONPs at a concentration level of 300 μg mL^{-1}

RT-PCR analysis

Three leaves of tomato plant were collected randomly after 2 days of infection and stored under – 80 °C until the next steps were taken. An RNA extraction process was performed as described by Elsharkawy et al. (2012). Samples were ground in liquid nitrogen and then homogenized by the following extraction buffer:100 mM Tris-HCl (pH 9…5), 10 mM EDTA (pH 8.0), 2% lithium dodecyl sulfate, 0.6 M NaCl, 0.4 M tri-sodium citrate, and 5% 2-mercaptoethanol. Then, the homogenate was extracted again by a chloroform/isoamyl alcohol (24:1) mixture and centrifuged under room temperature. The obtained supernatant was re-extracted with water-saturated phenol, guanidium thiocyanate, sodium acetate (pH 4.0), and chloroform. The upper aquifer produced during extraction and containing RNA was precipitated by isopropanol and then collected, washed, dried by air, and eventually dissolved in RNase-free water. Then, samples were treated with RNase-free DNase and then DNase was inactivated following the steps described by the manufacturer (Takara Bio Inc.).

Table 2 In vitro effect of ZONPs and streptomycin on Pst DC3000

Treatments	The diameter of the zone of inhibition (mm)		
	100 μg mL^{-1}	200 μg mL^{-1}	300 μg mL^{-1}
ZONPs	9.0 ± 0.09^{a}	11.0 ± 0.05^{b}	17.0 ± 0.12^{a}
Streptomycin	12.0 ± 0.11^{a}	17.0 ± 0.15^{a}	22.0 ± 0.46^{a}
Control	0.0 ± 0.00^{b}	0.0 ± 0.00^{c}	0.0 ± 0.00^{b}

Statistical comparisons were made among treatments within a single column. The different letters represent significant differences using Fisher's LSD test at $P \leq 0.05$.

Each mean value came from three replicates

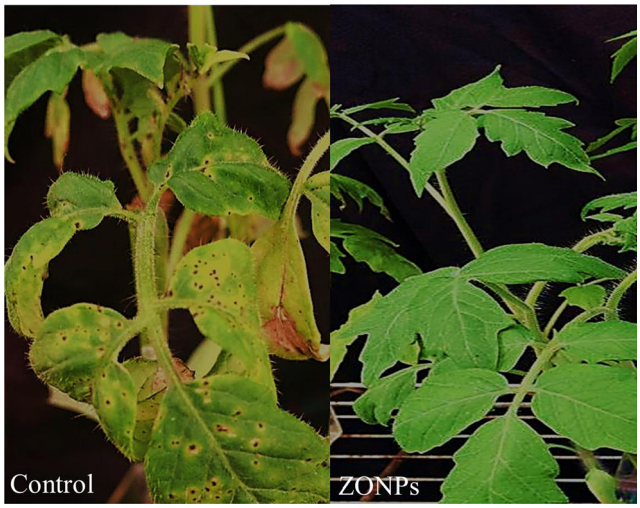

Fig. 5 Disease symptoms caused by Pst infection in water-treated tomato plants (control) and tomato treated with ZONPs by foliar spray after 1 week of Pst challenge inoculation

Approximately 1 μg of total RNA was converted to a single-stranded cDNA via reverse-transcription using a mixture of oligodt primer, RNase inhibitor (20 units μl^{-1}), and RTase (50 units μl^{-1}) according to the steps described by the manufacturer (Toyobo). The amplification of cDNA was done following the method of Elsharkawy et al. (2012), to monitor the expression of a set of well-characterized defense-related genes, such as *PR-1a* and *Lipoxygenase* (*LOX*), and (the internal control) *Leβ-tubulin* (Aimé et al. 2013). The gene-specific primers used in this experiment are listed in Table 1. This experiment was replicated three times for getting accurate data.

Data analysis

For analysis of variance (ANOVA) of obtained data, XLSTAT PRO statistical analysis software (Addinsoft) was used. Fisher's least significant difference (LSD) test was used to separate the mean of each treatment. All analyses were performed at a significance value of $P \leq 0.05$.

Results

Characterization of fabricated ZnO nanoparticles

As presented in Fig. 1, the ZnO nanostructures are formed as nanorods with a hexagonal wurtzite crystal structure. The grain-like flower morphology came from an accumulation of ZnO nanorods. As shown in Fig. 2, the UV light emission at 376 nm may be due to the transition of an electron between the conduction and the valence band, that indicates a calculated band gap value of 3.23 eV which agrees with the value reported by Lin et al. (2014). The XRD pattern of ZnO nanorods as presented in Fig. 3 showed that the diffraction peaks at 31.8°, 34.4°, 36.3°, 47.5°, 57°, 62.9°, 66.4°, 67.9°, 69°, and 77° correspond to the (100), (002), (101), (102), (110), (103), (200), (112), (201), and (202) lattice planes, respectively. The high intensity of (101), (100), and (002) direction planes indicate the hexagonal wurtzite structure of ZnO (in the space group *P63mc*, with lattice constants of $a = b = 0.323$ (nm), $c = 0.521$ (nm)) (JCPDS 36-1451) (Ibupoto et al. 2013; Soni et al. 2013;Lin et al. 2014).

Antibacterial activity of ZONPs against Pst under laboratory conditions

The growth of Pst was significantly inhibited by ZONPs compared with the untreated control as shown in Fig. 4. The disk dipped in ZONPs showed a clear zone free of Pst due to the antibacterial activity of ZONPs Fig.4. However, the disk dipped in water showed no clear zone around and the growth of Pst Fig. 4. The data in Table 2 showed that the diameter of the inhibition zone of Pst increased with the increasing concentration of the tested treatments (streptomycin and ZONPs).

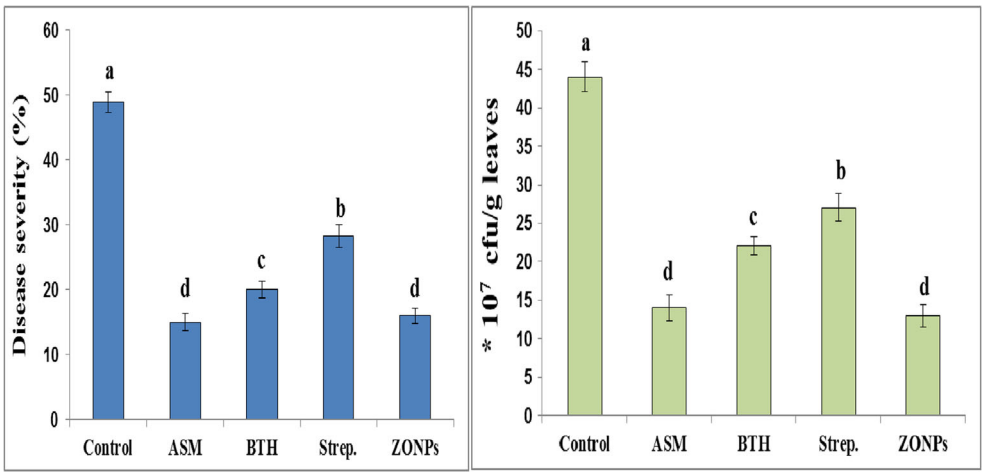

Fig. 6 Induced suppression of disease symptoms and number of (Pst) bacteria in tomato plants in response to treatments with ASM, BTH, streptomycin, and ZONPs before one day of Pst inoculation. Columns represent mean values. Bars indicate standard errors. Different letters above columns indicate significant differences by Fisher's LSD test for tomato ($P \leq 0.05$)

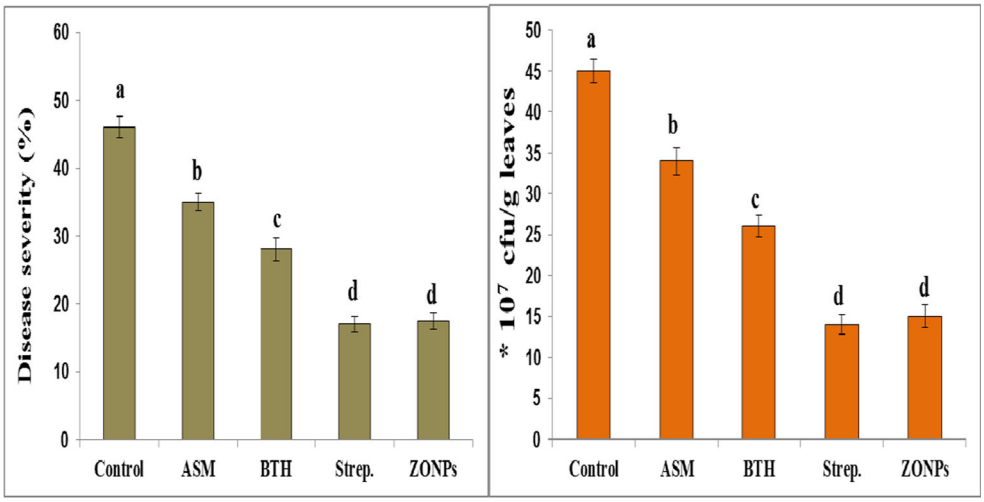

Fig. 7 Induced suppression of disease symptoms and the number of (Pst) bacteria in tomato plants in response to treatments with ASM, BTH, streptomycin, and ZONPs after 1 day of Pst inoculation. Columns represent mean values. Bars indicate standard errors. Different letters above columns indicate significant differences by Fisher's LSD test for tomato ($P \leq 0.05$)

Moreover, the inhibition of Pst growth by streptomycin was higher than that of ZONPs (Table 2).

Systemic protection of tomato by ZONPs under greenhouse conditions

Disease symptoms in tomato plants treated with ZONPs (as foliar spray) after one week of Pst inoculation were significantly reduced compared to untreated tomato plants (control) (Fig. 5). The resistance induction against bacterial speck in plants treated by ZONPs compared to standard inducers (ASM, BTH) and streptomycin was evaluated by the reduction in disease symptoms and the number of Pst bacteria (colony-forming unit, c.f.u) in tomato plants (Figs. 6 and 7). The results showed that the disease symptoms and number of Pst bacteria were markedly suppressed in treated plants relative to untreated one (Figs. 6 and 7). ASM and ZONPs were the most effective treatments with no significant differences followed by BTH and streptomycin, respectively, in tomato plants treated 1 day before Pst inoculation (Fig. 6). However, for tomato plants treated 1 day after inoculation, streptomycin and ZONPs were the most effective treatments with no significant differences followed by BTH and ASM, respectively (Fig. 7).

Mechanism of resistance induction by ZONPs in tomato

To identify the resistance induction mechanism of tomato plants treated with ZONPs against Pst, the expression of pathogenesis-related genes (*LePR-1a* and *Lipoxygenase*) in tomato plants treated with ZONPs, BTH, streptomycin, and ASM using RT-PCR was evaluated as shown Fig. 8. Le*β-tubulin* gene was used as internal control. Furthermore, the activity of self-defense enzymes (peroxidase and polyphenoloxidase (PPO)) in treated tomato plants was also estimated to clarify the resistance induction mechanism against Pst (Table 3). The results showed that expression of the pathogenesis-related gene (*LePR-1a*) was highly detected in tomato plants treated with ZONPs and BTH after inoculation of tomato plants with Pst (Fig. 8). On the other hand, the same pathogenesis-related gene (*LePR-1a*) was detected with low density in tomato plants treated with ASM and not detected in streptomycin treatment (Fig. 8). However, the expression of *Lipoxygenase* gene was slightly detected in tomato plants treated with ZONPs, streptomycin, BTH, and ASM, respectively. Moreover, the target-responsive genes did not show a stimulated expression after Pst infection in untreated plants

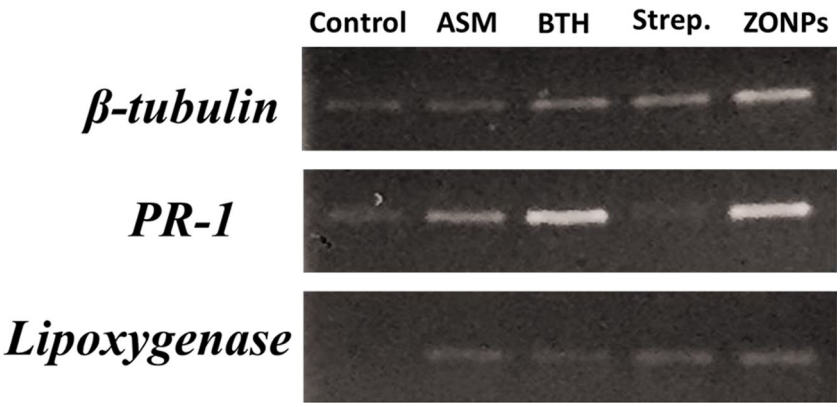

Fig. 8 Expression of genes in leaves of tomato plants treated with ZONPs before 1 day of challenge inoculation with Pst

Table 3 Effects of ASM, BTH, streptomycin, and ZONPs on defense enzymes in tomato (POD) and (PPO) at 1 day after Pst challenge inoculation

Treatment	POD activity (Units μg^{-1} FW)	PPO activity (Units μg^{-1} FW)
ASM	1287 ± 21.3a	698 ± 5.7a
BTH	843 ± 17.5c	553 ± 4.7c
Streptomycin	580 ± 14.8d	333 ± 3.5d
ZONPs	1130 ± 22.4b	631 ± 4.9b
Control	340 ± 4.2e	215 ± 2.8e

Statistical comparisons were made among treatments within a single column. The different letters represent significant differences using Fisher's LSD test at $P \leq 0.05$

Each mean value came from three replicates

compared to treated plants. The results in Table 3 showed a significant increase in peroxidase and polyphenoloxidase activities in treated plants compared to untreated Table 3. In addition, the highest enzymes activity was registered in tomato plants treated with ASM followed by ZONPs, BTH, and streptomycin, respectively.

Effect of ZONPs and other inducers on growth characters of tomato

The applied treatments (ZONPs, streptomycin, ASM, and BTH) showed a significant increase in tomato plants growth characters (plant height as well as fresh and dry weight) compared with the untreated plants (Table 4). The highest growth characteristics were recorded for tomato plants treated with ZONPs followed by streptomycin, ASM, and BTH, respectively.

Discussion

The results of our study showed that the fabricated ZnO nanostructures are a superior candidate for controlling bacterial speck of tomato by its direct antibacterial activity and indirectly by enhancing induced resistance within the plant against the disease. Also, it is considered a safe and environmentally friendly strategy to control Pst compared to chemical pesticides. This is probably due to those inorganic compounds such as ZnO in the size of nanostructures represent antibacterial substances at low concentrations due to their high surface area and good chemical and physical properties (Rai et al. 2009). Moreover, it also has high stability under drastic environmental conditions such as high temperature and pressures (Sawai 2003). Furthermore, some of the fabricated nanoparticles such as ZnO and silica are non-toxic and contain some important elements for the human body. Furthermore, most of the inorganic substances with bactericidal activity are metals and metal oxide nanoparticles (Cioffi et al. 2005; Chaudhry et al. 2008; Bradley et al. 2011). Referring to the effect of nanomaterials as an antibacterial agent, Jung et al. (2010) reported that nano-silver could be used to control the plant pathogenic bacteria. The high antimicrobial impact and low cost of zinc oxide nanoparticles contributed to its application in food preservation technology to reduce bacterial contamination (Pandey et al. 2010). However, until now, there is no research literature addressing the utilization of ZnO nanoparticles as antibacterial agent towards the plant pathogenic bacteria by induction of systemic resistance in treated plants.

Table 4 Effect of ZONPs and other inducers on some tomato growth characters

Treatments	Plant height (cm)	Fresh weight (g)	Dry weight (g)
ZONPs	40.9 ± 2.5[a]	211.4 ± 5.3a	33.4 ± 1.7a
Streptomycin	33.4 ± 1.9[b]	147.7 ± 4.9b	26.7 ± 1.5b
ASM	32.2 ± 1.8[b]	142.9 ± 5.1b	25.3 ± 1.4b
BTH	25.7 ± 1.5[c]	118.1 ± 3.6c	21.8 ± 1.2c
Control	24.9 ± 1.2[c]	114.9 ± 3.8c	20.4 ± 1.0c

Statistical comparisons were made among treatments within a single column. The different letters represent significant differences using Fisher's LSD test at $P \leq 0.05$

Each mean value came from three replicates

The antibacterial activity of ZONPs against Pst could be due to two mechanisms, the first mechanism via the direct antibacterial activity of ZONPs against Pst. This direct bactericidal effect may be due to the disruption of the function of the bacterial cell membrane via producing free oxygen radicals such as superoxide and hydroxyl radicals as a result of its photocatalytic activity (Akhtar et al. 2012; Sirelkhatim et al. 2015). Thus, these free radicals could oxidize glutathione (GSH) that modulates physiological levels of ROS and is involved in the cell's oxidative stress response (Navale et al. 2015). Therefore, the oxidation of GSH destroys the cell membrane and induces deformation of the contents of the cytoplasm, leading eventually to cell death (Divyapriya et al. 2014). Previous studies on the antibacterial effect of zinc oxide showed that the particle size and the oxidative stress are responsible for its antibacterial effect (Sourabh et al. 2014; Navale et al. 2015). Since the small size of the ZnO nanoparticles facilitates the process of entering the cell membranes which gives the opportunity for ZnO inhibitory effect to occur inside the cell. Furthermore, the small size of ZnO nanoparticles is proportional to the high surface area that may increase the oxidative stress of ZnO nanoparticles.

The second mechanism of ZONPs protection against Pst involves the induction of systemic resistance in treated tomato plants by increasing the expression of the defense-related gene (*PR-1a*) (Idrees et al. 2011).

The results exhibited increased levels of defense-related enzymes (POX and PPO) as well as the expression of

pathogenesis-related genes (*PR-1a* and *LOX*) which supported the mechanism of induced systemic resistance against Pst by ZONPs. This mechanism is in agreement with what has been mentioned by Stangarlin et al. (2011) who explained that the resistance induction within the plant includes the improvement of the activity of self-defense enzymes within the plant as a result of plant treatment with inducers to protect against pathogens.

The result of this study showed that the application of ZONPs increased tomato growth characters in comparison to the untreated plants. Several studies suggested that ZONPs improved the growth and development of treated plants (Sedghi et al. 2013; Ramesh et al. 2014; Taheri et al. 2015). Also, Elizabath et al. (2017) reported that applications of ZnO and FeO nanoparticles increased the yield and growth characters of carrot compared to untreated control. Furthermore, in corn, Taheri et al. (2015) reported that shoot dry matter and leaf area index was increased by 63.8% and 69.7% by addition of ZnO particles in irrigation water.

Finally, to obtain effective control of bacterial speck pathogen in tomato, early treatment of tomato with ZONPs is required before the appearance of the disease symptoms to induce systemic resistance in tomato plants against the disease. The second step is curative by treating tomato plants with ZONPs via direct antibacterial effect when the symptoms of the disease appear in the future. The results of this study are considered to be very important in the application of the nanoparticles against this plant disease under the field conditions. This study showed that the use of low amounts of zinc oxide could help in reducing side effects of pesticides on humans and the environment. It also reduces the costs of the control process.

Conclusions

Based on disease severity estimation and molecular and biochemical analysis, ZONPs are promising to control Pst infection in tomato via inducing systemic resistance and direct antibacterial activity. Furthermore, the use of ZONPs in the control of the bacterial speck disease in tomatoes led to an improvement in the growth characteristics of the treated plants as compared to untreated control. Therefore, the use of zinc oxide nanoparticles can be considered as a new strategy to control this disease in the tomato crop.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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