Pesticide Biochemistry and Physiology 157 (2019) 230–236

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Resistance induction in cucumber and direct antifungal activity of zirconium oxide nanoparticles against *Rhizoctonia solani*

Aly Derbalah[a,*], Mohsen Mohamed Elsharkawy[b], Amany Hamza[a], Abdelhamed El-Shaer[c]

[a] Pesticides Chemistry and Toxicology Department, Faculty of Agriculture, Kafrelsheikh University, 33516, Egypt
[b] Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University, 33516, Egypt
[c] Physics Department, Faculty of Science, Kafrelsheikh University, 33516, Egypt

ARTICLE INFO

Keywords:
Cucumber
Nanoparticles
Root rot
Resistance induction
Pathogensis related genes

ABSTRACT

Zirconium oxide nanoparticles (ZrONPs) were synthesized and evaluated for their ability to induce root rot resistance in cucumber and direct antifungal activity against *Rhizoctonia solani*. Resistance induction was investigated using real-time polymerase chain reaction (RT-PCR) and the effect of ZrONPs on the growth of cucumber plants was investigated. The results showed that ZrONPs at a concentration level of 100 µg/L significantly inhibited the growth of *Rhizoctonia solani* (86.6%) relative to untreated control under laboratory conditions. Cucumber plants treated with ZrONPs showed reduction in the severity of root rot disease under greenhouse (34–46%) and field conditions (52–56%) compared with non-treated control plants. Cucumber plants treated with ZrONPs expressed regulatory and defense genes involved in the salicylic acid (SA) and jasmonic acid (JA)/ethylene (ET) signaling pathways with 7–8 folds higher than the control. Treatment of cucumber with ZrONPs and carboxin + thiram significantly improved cucumber growth and yield characters. Therefore, using ZrONPs could be a new strategy to control this pathogen and considered the first report.

1. Introduction

Rhizoctonia solani is a fungus (Baker, 1970; Hassan et al., 2014) that has a significant effect on crops productivity, lives in the soil as sclerotia and does not generate asexual spores. *R. solani* as a causal fungus of widespread soil-borne diseases is responsible for causing significant economic losses in many important field and horticultural crops all over the world (Grosch et al., 2004; Elsharkawy et al., 2014). Previous studies have shown that this disease caused high rates of crop losses, including a 50% reduction in sugar beet (Kiewnick et al., 2001), 70% in lettuce (Davis et al., 1997), and about 20% in production of potato (Grosch et al., 2005). The strategies used to control *Rhizoctonia* diseases are somewhat limited due to the behavior of these pathogens. Moreover, *Rhizoctonia* diseases have a very wide range of hosts that can be infected and also *Rhizoctonia* fungi can tolerate various environmental conditions (Grosch et al., 2004). This is in addition to the lack of high resistance cultivars to these diseases (Li et al., 1995; Babadoost and Islam, 2003). Therefore, it became very important and urgent to find effective strategies to control the disease.

Induction of systemic resistance in the host plant becomes an important goal in order to reduce disease severity at low costs and without adverse effects on the environment (Abd-El-Moneem, 1996; Abdou et al., 2001; Elsharkawy et al., 2012). Nanomaterials, such as copper, silver, silica and titanium nanoparticles, could be new elicitors for resistance induction in plants and could enhance the antimicrobial activity against plant pathogens due to their properties such as their large surface to volume ratio and small size compared to the same substances in their normal size (Kim et al., 2012; Kanhed et al., 2014; Elsharkawy and Derbalah, 2018; Elsharkawy et al., 2018; Abdelmoteleb et al., 2018).

The use of nanoparticles has emerged as one of the most recent alternatives and trends in the control of plant pathogens (Kumar and Yadav, 2009; Jung et al., 2010; Morsy et al., 2018; Alam et al., 2019). The antimicrobial activity of nanoparticles is known to be proportional to surface area in contact with the microorganisms (Kim et al., 2012). The small size of nanoparticles and large surface area increases the chances of their interaction with plant pathogens, making them effective in controlling a wide range of these diseases (Morones et al., 2005; Martinez-Gutierrez et al., 2010; Lamsal et al., 2011; Jadhav et al., 2011; Alam et al., 2019). ZrO_2 nanoparticles have been of particular interest because they can be used in many different scientific and technological aspects due to their good mechanical and electrical properties, high electric constant and broadband gap (Gowri et al., 2014). Examples of applications of ZrO2 nanoparticles in different fields include gas

* Corresponding author.
E-mail address: aliderbalah@yahoo.com (A. Derbalah).

https://doi.org/10.1016/j.pestbp.2019.03.018
Received 23 January 2019; Received in revised form 28 March 2019; Accepted 31 March 2019
Available online 01 April 2019

sensors, solid fuel cells, high durability coatings, catalytic agents and others (Fergus, 2006; Rashad and Baioumy, 2008). However, its application in agriculture for control plant pathogens is rarely investigated (Gowri et al., 2014).

Thus, the objectives of this study were to produce zirconium oxide nanoparticles (ZrONPs) with unique size and shape, to investigate the direct antifungal activity of ZrONPs against *Rhizoctonia solani* under laboratory conditions, to evaluate its ability to induce systemic resistance against root rot disease in cucumber plants under greenhouse and field conditions, to identify the resistance induction mechanism by expression of regulatory and defense genes using qRT-PCR, and finally to investigate the effect of fabricated nanoparticles on some growth characters of cucumber.

2. Materials and methods

2.1. Chemicals

Zirconium tetrachloride (ZrCl4) with purity of 99.5% was obtained from Alfa Company, UK. The recommended fungicide used in this study for control root rot disease in cucumber is carboxin + thiram (carboxin 37.5% + thiram 37.5%) with a trade name of Vitavax 75% WP. This fungicide field rate is 200 g/100 L water and produced by Dhanuka Agritech Limited, India.

2.2. Fabrication of ZrONPs

Fabrication of ZrONPs was carried out by addition of 15 mmol of zirconium tetrachloride (ZrCl4, Alfa, 99.5%) gradually to 300 ml of deionized water and then stirred for two hours. In the next step, 1 M sodium hydroxide was added drop by drop under magnetic stirring conditions until the white hydrogel was formed. After aging for 30 min, hydrogel was washed several times with deionized water. The obtained hydrogel was then centrifuged at 6000 rpm for 10 min, washed with ethanol, centrifuged again, and at the last step calcined at 60 °C for 3 h; then the powder was calcined at 500 °C for 1 h (Lee et al., 2017).

2.3. Characterization of ZrO_2 nanoparticles

Physical characterizations of the synthesized ZrO_2 nanoparticles were carried out using X-ray diffraction (XRD), and scanning electron microscope (SEM) analysis. The Scherres equation was employed to calculate the particle size of ZrO_2:

$$D = K\lambda/\beta \cos \theta$$

where, θ is the angle between the incident and diffracted beams (degree), β is the full with half maximum (rad.), D is the sample particle size (nm) and λ is the X-ray wavelength.

2.4. Isolation, identification and culturing of R. solani

Samples of cucumber plants showing the symptoms of root rot disease were collected from different fields and stored with their soil in polyethylene bags and transported to the laboratory for further processing. The bottom parts of the stems and roots were then rinsed with tap water to remove all soil particles. Next, the plant material was cut into small pieces (about 2 cm). This was followed by cleaning the surface of these parts of the plant with 3% hypochlorite sodium for 3 min and washed several times in the distilled water and dried between sterilized filtration papers. Parts from the infected plant were transferred to the potato dextrose agar medium (PDA) and held at a temperature of 27–30 °C for 3–5 days. The fungal colonies were then purified using the hyphal tip technique (Dhingra and Sinclair, 1995).

The fungus was identified according to certain morphological and microscopic characteristics, based on the description given by Ainsworth and James (1971) and Alexopolous et al. (1996).

For the culturing and increasing the inoculum of *R. solani,* which has been purified and identified as mentioned above, the rice hull medium was used. This medium was prepared by sterilizing 500 ml flasks, containing 100 g of rice (straw grains) structure, 200 g of sand and 100 ml of water in an autoclave for 20 min at a pressure of 1.5 atm. The sterilized bottles were then inoculated with mycelia disks of the fungus previously grown on the PDA medium. In the end, the bottles were incubated at 20–28 °C ± 2 °C for 7 days.

2.5. Effect of ZrONPs on the growth of R. solani under laboratory conditions

Antifungal activity of ZrONPs against *R. solani* was evaluated using the method described by Fraternale et al. (2003) with some modifications. The autoclaved liquid PDA media spiked with ZrONPs at concentrations levels of 25, 50 and 100 μg L^{-1} and carboxin + thiram at a concentration level of 300 mg L^{-1} and then were poured into the Petri dishes (9 cm diameter). The concentrations of ZrONPs were selected based on primary screening test (data not shown). Moreover, the formed suspension of ZrONPs in water was stable. For control treatment, PDA medium without ZrONPs and the recommended fungicide were poured in Petri dishes. Then, 0.5 cm diameter disks were taken from a 7-days fungal growth of the examined fungus and placed in the center of each petri dish in different treatments. This was followed by incubation of inoculated Petri dishes at 25 ± 2 °C. Finally, after 7 days of incubation or when the fungal growth is completely covered in the control treatment, radial growth was measured in all treatments. Each treatment consists of three replicates. The degree of inhibition in the fungal growth was estimated using the equation described by Vincent (1947).

2.6. Efficacy of ZrONPs against root rot pathogen under greenhouse conditions

For this experiment, sand clay soil was sterilized using formalin with a concentration of 5% and air dried for 7 days (Wang et al., 2011). The sterilized soil was then transferred to a 25 cm diameter pot, each containing 4 kg soil. The dried artificial inoculation of soil with *R. solani* was carried out at a rate of 3% before one week of transplantation by using hull rice culture (w/w). The soil was maintained wet until the transplanting. Roots of cucumber seedlings (cv. DP-164), 14 days old, were treated by immersion for 2 h in a solution of ZrONPs (100 μg L^{-1}) and carboxin + thiram at a concentration level of 300 mg L^{-1} followed by foliar spray after transplanting. Soil drench treatments by ZrONPs or carboxin + thiram at given concentrations were carried out separately by injection of soil around cucumber roots (10 ml/plant). For untreated cucumber, the roots of cucumber seedlings were dipped in distilled water and after transplanting the foliage were sprayed with distilled water and then the soil was injected with distilled water. Each treatment was represented by three replicates. The disease incidence of post-emergence damping-off was recorded at 45 days after sowing as reported by Khalifa (1987).

$$\%\text{Post} - \text{emergence damping} - \text{off} = \text{No. of dead seedlings/No. of sown seeds} \times 100$$

2.7. Efficacy of ZrONPs against root rot pathogen under field conditions

This work was performed under field conditions at Sakha Research Station, Kafrelshiekh Governorate in two growing seasons (2017–2018 and 2018–2019). ZrONPs solution was prepared (100 μg L^{-1} + 0.2% tween 80) and used as a seed coating for 30 min before planting. However, the recommended fungicide (carboxin + thiram) was used at a concentration level of 2 g kg^{-1}. For control treatment, cucumber seeds were treated with water only. The experiment was designed

statistically as a randomized complete block with three replicates for each treatment with normal cultural practices. The block design with 3.5 m long, 1.5 m wide and had 14 plants. Cucumber seedlings (D.P 164 cultivar) with 21 days-old were transplanted with space of 50 cm. Cucumber seedlings were naturally infected by root rot fungus and need not for artificial inoculation. Each treatment was represented by three replicates.

The disease incidence of pre- and post-emergence damping-off was recorded at 15 and 45 days after sowing, respectively as reported by Khalifa (1987).

$$\%\text{Pre emergence damping off} = \text{No. of non}-\text{emerged seeds} / \text{No. of sown seeds} \times 100$$

$$\%\text{Post} - \text{emergence damping} - \text{off} = \text{No. of dead seedlings/No. of sown seeds} \times 100$$

Root rot severity was scored 90 days after transplanting based on Saldajeno and Hyakumachi (2011) with a scale rating (0–4) with 0: no superficial lesions and 4: dead plants.

To evaluate the effects of ZrONPs and carboxin + thiram on growth characters of cucumber, plant height, leaf number, total chlorophyll, fresh and dry weights were measured in treated and non-treated cucumber plants. Average number and weight of fruits/plant were measured by harvesting fruits at the marketable size. Harvesting was repeated every day and extended for 90 days from transplanting.

2.8. Systemic expression of defense-related genes by RT-PCR analysis

For the RNA analysis, the leaves (two leaves of cucumber from each replicate) were collected and stored at 80 °C for further treatment. Total RNA was extracted following the technique described by Elsharkawy et al. (2012). RNA was quantified by nanodrop and cDNA was synthesized using the kit (Thermo Scientific, Fermentas, #EP0451). Quantitative RT-PCR was carried out using gene-specific primers (Elsharkawy, 2018) shown in Table 1. The normalization of the amount of the target genes (*β-1,3-Glucanase, CHIT1, PAL1, PR1* and *LOX1*) was obtained by standardization over the abundance of the constitutive *18SrRNA* gene (Table 1). Quantitative RT-PCR was done by utilizing 7500 real-time PCR system and the obtained data were analyzed using the ABI PRISM 7500 Software Tool (Applied Biosystems) that calculates the average expression ratio (standard curve method of relative gene quantification) and the *P*-values to estimate the statistical relevance of changes. Quantification of gene expression was carried out using the comparative 2-CT technique as characterized by Livak and Schmittgen (2001).

2.9. 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 $P \leq 0.05$.

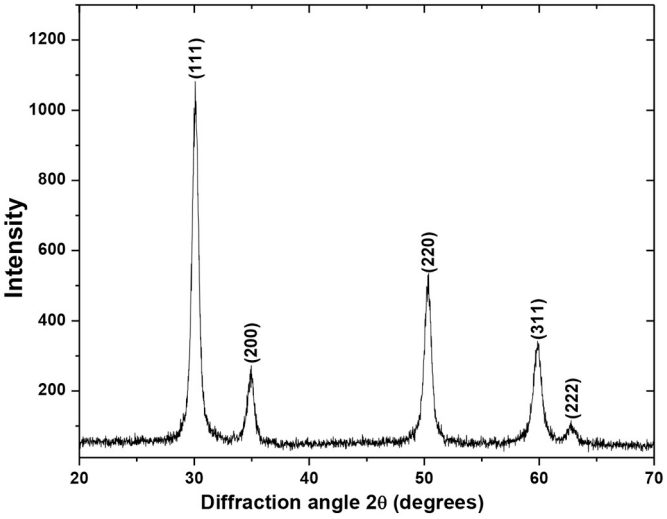

Fig. 1. XRD pattern of the ZrONPs nanoparticles.

3. Results

3.1. Characterization of fabricated nanoparticles

The crystalline structure of ZrO_2 nanoparticle was investigated using XRD and the result is shown in Fig. 1. The sharp diffraction peaks at 2θ value of 30, 50.4 and 60 correspond to the (111), (220) and (311) planes were presented in Fig. 1. Moreover, diffraction peaks at 2θ value of 35 and 62.8 correspond to the (200) and (222) planes were also illustrated (Fig. 1). The high intensity of (111), (220) and (311) confirm the formation of single phase and high purity of the tetragonal zirconium dioxide nanostructure (t-ZrO2) (Sathyaseelan et al., 2017).The calculated average particle size of the calcined ZrO_2 is found to be 17 nm.

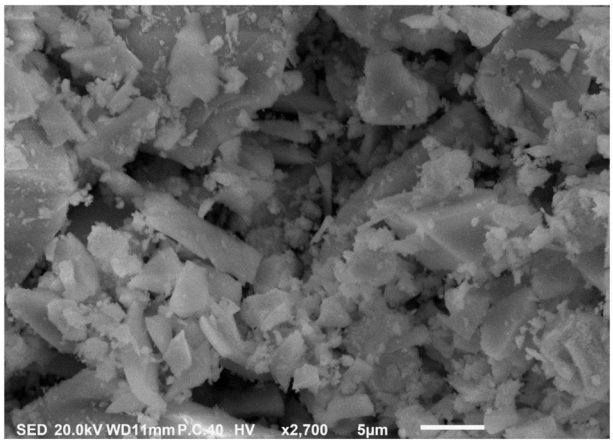

Fig. 2. Top view SEM image of calcined ZrONPs nanoparticles.

Table 1
Forward and reverse primers used in RT-PCR.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
EF 1-a	CTGTGCTGTCCTCATTATTG	AGGGTGAAAGCA AGAAGAGC
CHIT1	TGGTCACTGCAACCCTGACA	AGTGGCCTGGAATCCGACT
β-1,3-Glucanase	TCAATTATCAAAACTTGTTCGATGC	AACCGGTCTCGGATACAACAAC
PAL1	ATGGAGGCAACTTCCAAGGA	CCATGGCAATCTCAGCACCT
PR1	TGCTCAACAA ATGCGAACC	TCATCCACCCACAACTGAAC
LOX1	CTCTTGGGTGGTGGTGTTTC	TGGTGGGATTGAAGTTAGCC

Table 2
Growth inhibition percentages of the tested treatments against *R. solani* under laboratory conditions.

Treatments	Concentration	Growth inhibition Percentage
ZrONPs	25 (µg/L)	41.2 ± 0.45[c]
	50	58.3 ± 2.35[b]
	100	86.6 ± 1.42[a]
Carboxin + thiram	300 (mg L^{-1})	88.3 ± 2.47[a]
Control	0.00	0.00[d]

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 surface morphology of ZrO_2 nanoparticles was investigated employing scanning electron microscope as shown in Fig. 2. It is clear that the particles aggregate to each other to form tetragonal shapes. Further, ZrO_2 has mesopores where the single grains and boundaries are clearly visible. SEM results are in good agreement with the XRD result.

3.2. Antifungal activity of ZrONPs against R. solani under laboratory conditions

The growth of *R. solani* was significantly inhibited by ZrONPs at various concentrations compared with the untreated control as shown in Table 2. The growth inhibition percentage of *R. solani* treated with ZrONPs at a concentration level of 100 µg/l was very high (85.6%) as shown in Fig. 3. Moreover, there was no significant difference in growth inhibition percentage of *R. solani* between ZrONPs (100 µg L^{-1}) and the recommended fungicide (300 mg L^{-1}) spite of the recommended concentration of fungicide was higher than that of ZrONPs. The results showed that the degree of inhibition in *R. solani* growth positively correlated with ZrONPs concentration level.

3.3. Systemic protection against root rot disease in cucumber under greenhouse conditions

The results in Table 3 showed that post-emergence damping off and disease severity were significantly reduced in cucumber plants treated with ZrONPs and the recommended fungicide compared to untreated control. There were no significant differences between ZrONPs and the recommended fungicide in the reduction of post-emergence damping off and disease severity of treated cucumber plants in spite of the concentration of fungicide is much higher than that of ZrONPs. The reduction in the severity of root rot disease in cucumber treated by ZrONPs and carboxin + thiram using soil drench treatment was higher than foliar spray treatment.

3.4. Systemic protections against root rot disease in cucumber under field conditions

Disease severity, pre and post-emergence damping off were significantly reduced in cucumber plants treated with ZrONPs and the recommended fungicide under field conditions compared to the untreated control in both growing seasons (Table 4). The efficacy of the fungicide was significantly higher than ZrONPs against root rot of cucumber in both growing seasons. The reduction in the severity of root rot disease in cucumber treated with ZrONPs and carboxin + thiram under field conditions was higher in the first season than the second one.

3.5. Effect of ZrONPs application on cucumber growth characters

The application of ZrONPs and recommended fungicide positively

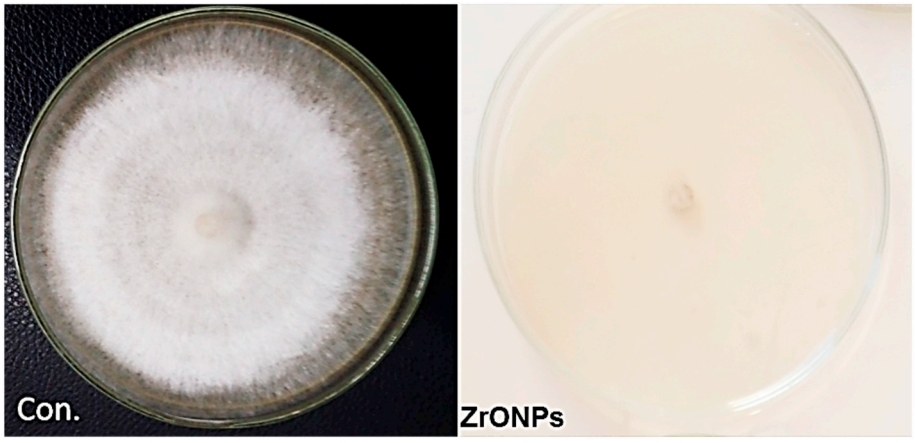

Fig. 3. Effect of ZrONPs (100 µg L^{-1}) on radial hyphal growth and number of colony formation of *R. solani* after 10 days (b) relative to untreated control.

Table 3
Efficacy of ZrONPs against root rot disease in cucumber under greenhouse conditions.

Treatments	Foliar spray		Soil Drench	
	Post-emergence damping off	Disease severity	Post-emergence damping off	Disease severity
ZrONPs	9.61 ± 0.62[b]	2.24 ± 0.10[b]	7.10 ± 0.41[b]	1.69 ± 0.11[b]
Carboxin + thiram	8.86 ± 0.35[b]	2.11 ± 0.13[b]	6.43 ± 0.21[b]	1.59 ± 0.13[b]
Control	24.64 ± 1.10[a]	3.41 ± 0.32[a]	23.92 ± 0.62[a]	3.16 ± 0.14[a]

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.

Table 4
Efficacy of ZrONPs against root rot disease in cucumber plants under field conditions in two growing seasons.

Treatments	Pre-emergence Damping off	Post-emergence Damping off	Disease severity
Season 2017–2018			
ZrONPs	13.99 ± 0.78[b]	10.84 ± 0.57[b]	1.53 ± 0.07[b]
Carboxin + thiram	9.94 ± 0.89[c]	8.32 ± 0.46[c]	1.04 ± 0.03[c]
Control	42.92 ± 1.25[a]	24.41 ± 1.36[a]	3.50 ± 0.21[a]
Season 2018–2019			
ZrONPs	14.59 ± 1.10[b]	11.64 ± 0.86[b]	1.74 ± 0.03[b]
Carboxin + thiram	10.34 ± 0.39[c]	8.92 ± 0.74[c]	1.13 ± 0.01[c]
Control	43.42 ± 2.14[a]	25.78 ± 1.37[a]	3.65 ± 0.05[a]

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.

affects the measured growth characters of treated cucumber plants. A marked increase in all measured growth parameters (plant height, total chlorophyll, fresh and dry weights, fruit number/plant, average weight/fruit, and yield/plant) in cucumber plants treated with ZrONPs and the recommended fungicide compared to non-treated cucumber plants in both growing seasons (Table 5). The measured growth characters of cucumber plants treated with ZrONPs were almost similar to the recommended fungicide in spite of the used concentration of fungicide is much higher than that of ZrONPs. Moreover, there was a slight increase in all measured growth parameters in the first season relative to the second one.

3.6. Effect of ZrONPs on the expression of defense-related genes in treated cucumber plants

The expression of pathogenesis-related genes (*β-1,3-Glucanase, CHIT1, PAL1, PR1* and *LOX1*) was significantly increased in cucumber plants treated with ZrONPs after one day of root rot disease inoculation (Fig. 4), while, the targeted responsive genes in untreated plants showed no stimulated expression after infection by root rot disease. Moreover, *CHIT1* was the highest expressed pathogenesis-related gene followed by *β-1,3-Glucanase, PR1, LOX1* and *PAL1*, respectively.

4. Discussion

The application of nanotechnology in agriculture has become much needed worldwide. Nanoscience leads towards the development of low-cost nanotechnology applications for enhancing resistance against diseases (Shanmugaiah et al., 2015; Abdelmoteleb et al., 2018; Cumplido-Nájera et al., 2019). In our study, the data showed that ZrONPs exhibited significant antifungal activity against *R. solani* under laboratory conditions and this agree with findings of Gowri et al. (2014) who reported that nano-ZrO_2 showed potential antibacterial and antifungal effects against *Candida albicans* and *Aspergillus niger* (Gowri et al., 2014). The small particle size of ZrONPs (17 nm) compared to its equivalent bulk particle size of ZrO_2 might play an important role in its high antifungal activity against the tested fungus. This is may be due to the small particle size of fabricated ZrONPs that facilitates the penetration into fungal cell and induces its antifungal activity (Gowri et al., 2014). Kah and Hofmann (2014) also reported that the unique size and properties of NPs result in enhanced performance in biological systems when compared to traditional bulk of the same materials. Moreover, the antifungal action by the synthesized nanomaterials such as silver nanoparticles has been reported to have a significant role in disease suppression of *Rhizoctonia solani* (Shanmugaiah et al., 2015). Based on literature survey, the mechanism of ZrONPs antifungal activity against *R. solani* fungus has not been studied before. However, Gowri et al. (2014) evaluated the antifungal activity of zirconium oxide nanoparticles and reported that ZrO_2 nanoparticles may inhibit the growth of fungal strains effectively by interfering with cell function and leading to deformation of fungal hyphae (Gowri et al., 2014). Therefore, ZrONPs antifungal activity against *R. solani* fungus might be induced by the same mechanism.

Alternatives of chemical fungicides are urgently needed due to the high costs of using chemical fungicides especially in small areas and consumers prefer agricultural products free of pesticides. Additionally, fungicides were found to be more toxic to human cells than other pesticides (Mesnage et al., 2014). Therefore, in this study, we try to induce systemic resistance in cucumber plants against root rot pathogen using ZrONPs. This strategy has recently been seen as one of the trends in plant protection against disease infection (Heil and Bostock, 2002; Elsharkawy and Derbalah, 2018; Elsharkawy et al., 2018; Cumplido-Nájera et al., 2019). In this study, the protection against root rot in cucumber was evaluated by measuring pre and post-emergence damping off and disease severity. The results showed that the used nanoparticles suppressed *Rhizoctonia* root rot in cucumber plants and this is may be due to nano-size of fabricated ZrONPs (Servin et al., 2015; Cumplido-Nájera et al., 2019). The results also showed that the high efficacy of soil drench of ZrONPs against root rot relative to foliar spray treatment may be due to the direct and fast contact of ZrONPs in soil drench treatment with plant pathogen (soil-borne pathogen) compared to foliar spray treatment under greenhouse conditions. Finally, it can be said that the treatment of cucumber plants with ZrONPs activates or stimulates the induced resistance in the plants through genes responsible for self-defense within the plants. Furthermore, the expression of defense-related genes may be the key factor in the processing of plant stress signals such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Koike et al., 2001; Cai et al., 2009; Elsharkawy et al., 2012) that considered very important in plant defense response against the pathogen.

In the end, we can say, based on the results of this study, to achieve or obtain effective control of the disease of root rot in the cucumber, an

Table 5
Effect of ZrONPs on growth characters of cucumber under field conditions in the two growing seasons.

Treatments	Plant height (cm)	Total chlorophyll (SPAD)	Leaves number/ plant	Fresh weight/ plant (g)	Dry weight/ plant (g)	Fruit number/ plant	Average weight/ fruit (g)	Yield/plant (kg)
Season 2017–2018								
ZrONPs	259.1 ± 2.80[a]	42.1 ± 0.56[a]	35.6 ± 0.25[a]	40.9 ± 1.10[a]	5.6 ± 0.10[a]	34.5 ± 0.36[a]	94.2 ± 1.3[b]	3.6 ± 0.14[a]
Carboxin + thiram	260.8 ± 3.25[a]	40.6 ± 0.87[a]	36.0 ± 0.38[a]	42.4 ± 1.51[a]	5.8 ± 0.12[a]	35.3 ± 0.47[a]	99.4 ± 2.9[a]	3.9 ± 0.16[a]
Control	234.2 ± 4.12[b]	28.4 ± 0.32[b]	26.4 ± 0.17[b]	27.5 ± 0.97[b]	3.3 ± 0.09[b]	18.5 ± 0.81[b]	70.4 ± 1.51[c]	1.6 ± 0.03[b]
Season 2018–2019								
ZrONPs	257.2 ± 3.12[a]	41.1 ± 1.52[a]	33.8 ± 0.36[a]	39.3 ± 0.27[a]	5.5 ± 0.14[a]	33.8 ± 1.11[a]	92.8 ± 1.5[b]	3.4 ± 0.22[a]
Carboxin + thiram	258.3 ± 2.71[a]	39.4 ± 1.34[a]	34.4 ± 0.39[a]	40.9 ± 0.58[a]	5.7 ± 0.16[a]	34.7 ± 1.23[a]	97.8 ± 2.3[a]	3.7 ± 0.24[a]
Control	236.1 ± 4.17[b]	30.9 ± 1.38[b]	27.5 ± 0.83[b]	29.9 ± 0.47[b]	3.8 ± 0.18[b]	18.9 ± 0.98[b]	70.8 ± 1.6[c]	1.8 ± 0.08[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.

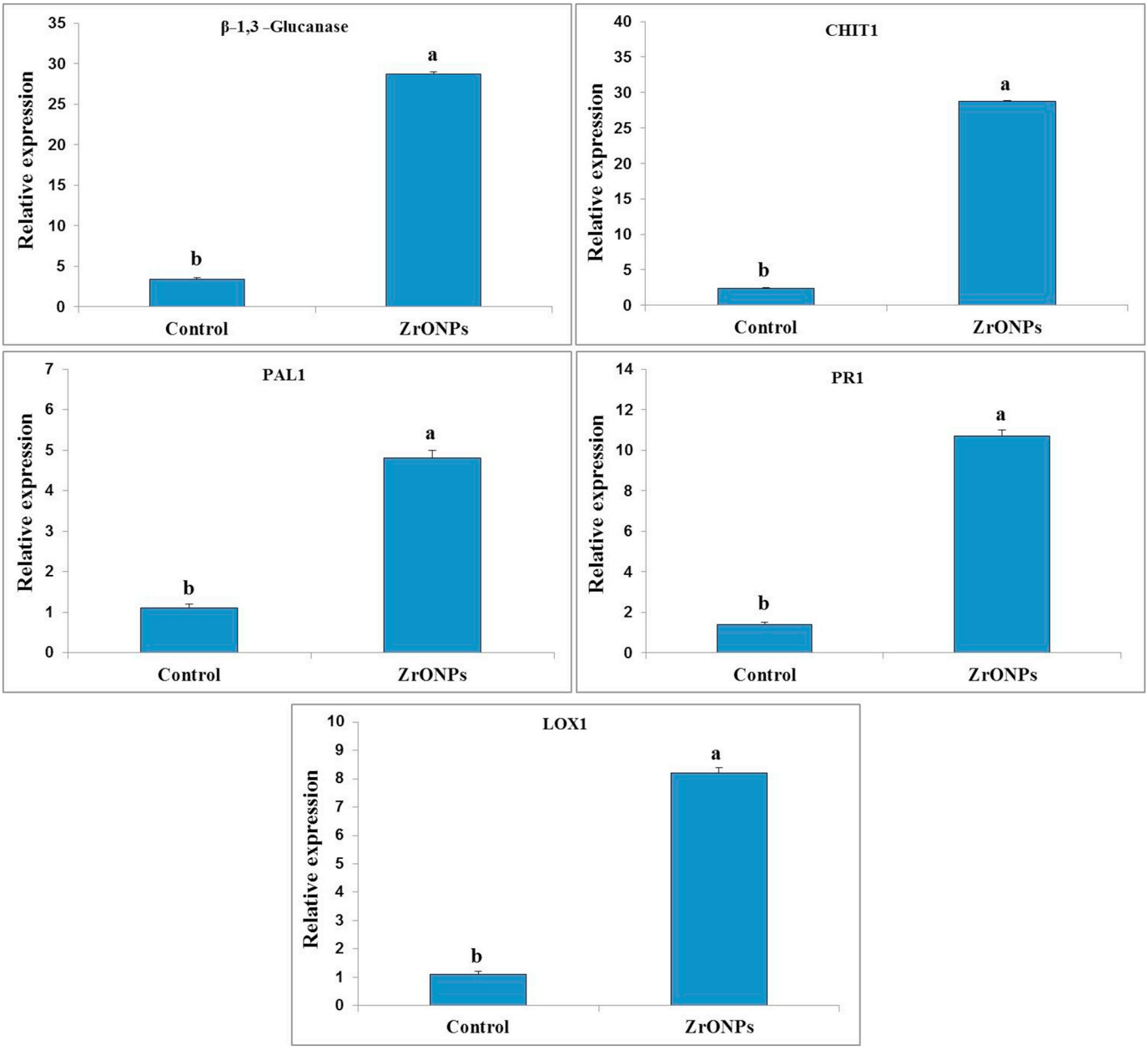

Fig. 4. Relative expression of *β-1,3-Glucanase, CHIT1, PAL1, PR1* and *LOX1* genes in leaves of cucumber plants treated with ZrONPs nanoparticles 1 day before challenge inoculation with *R. solani*.

early treatment of cucumber using ZrONPs before the emergence of disease symptoms is necessary to simulate the resistance induction in treated cucumber plants against the fungus causing the disease. The second step is to treat the cucumber plants with ZrONPs when symptoms of the disease appear in the future. These two steps may contribute to the effective control of this pathogen in the cucumber crop.

The results in this study also showed a significant improvement in the growth characters of cucumber plants treated with ZrONPs compared to untreated plants and this can be attributed to the application of ZrONPs activates the resistance induction in cucumber plant against the disease and also reduces the severity of the disease that positively affects the process of photosynthesis, which is reflected on plant growth (Brecht et al., 2003; El-Mougy and Abdel-Kader, 2009; Shenashan et al., 2017).

To control root rot disease, ZrONPs is applied at smaller amount ($100\,\mu g\,L^{-1}$) compared to recommended fungicide ($300\,mg\,L^{-1}$), thereby reducing concerns over NPs exposure to humans and the environment (Elmer and White, 2016). Moreover, the small amount of ZrONPs needed to control this pathogen might make the cost of synthesis of these nanoparticles more economical. Moreover, our wet chemical synthesis method is the easiest way for obtaining ZrO_2 powder at lower temperature, large mass production, and can be easy circulated for commercial uses.

5. Conclusions

Based on the results of disease severity assessment as well as the results of genetic and molecular analysis of treated cucumber plants, ZrONPs is a promising strategy to control root rot of cucumber by induction of systemic resistance in cucumber plants against the disease and also by direct antifungal activity against the causal fungus. In addition, the application of ZrONPs significantly improved the cucumber growth characteristics compared to the untreated cucumber plants. All this increase the possibility of using ZrONPs as a new effective strategy

to control root rot disease in cucumber plants.

References

Abd-El-Moneem, K.M., 1996. Effect of micronutrients on incidence of sesame charcoal root-rot and wilt disease complex. Assuit J. Agric. Sci. 27, 181–195.

Abdelmoteleb, A., Gonzalez-Mendoza, D., Valdez-Salas, B., Grimaldo-Juarez, O., Ceceña-Duran, C., 2018. Inhibition of *Fusarium solani* in transgenic insect-resistant cotton plants treated with silver nanoparticles from *Prosopis glandulosa* and *Pluchea sericea*. Egypt. J. Biol. Pest Control 28. https://doi.org/10.1186/s41938-017-0005-0.

Abdou, E., Abd-Alla, H.M., Galal, A.A., 2001. Survey of sesame root rot/wilt disease in Minia and their possible control by ascorbic and salicylic acids. Assuit J. Agric. Sci. 32, 135–152.

Ainsworth, G.C., James, P.W., 1971. Ainsworth and Bishy's Dictionary of fungi, 6th ed. Commonwealth Mycological Institute, Kew, Surrey, UK, pp. 663.

Alam, T., Asad, R., Khan, A., Ali, A., Sher, H., Ali, M., 2019. Biogenic synthesis of iron oxide nanoparticles via *Skimmia laureola* and their antibacterial efficacy against bacterial wilt pathogen *Ralstonia solanacearum*. Mater. Sci. Eng.C 98, 101–108.

Alexopolous, C.J., Mims, C.W., Blackwell, M.M., 1996. Introductory to Mycology, 6th ed. John Wiley.

Babadoost, M., Islam, S.Z., 2003. Fungicide seed treatment effects on seedling damping-off of pumpkin caused by *Phytophthora capsici*. Plant Dis. 87, 63–68.

Baker, K.F., 1970. Types of Rhizoctonia diseases and their occurrence. In: Parmeter Jr.J.R. (Ed.), *Rhizoctonia* solani: Biology and Pathology, pp. 125–148.

Brecht, M., Datnof, L., Nagata, R., Kucharek, T., 2003. The Role of Silicon in Suppressing Tray Leaf Spot Development in St Augustine Grass. University of Florida, Gainsville, FL, pp. 1–4.

Cai, K., Gao, D., Chen, J., Luo, S., 2009. Probing the mechanisms of silicon-mediated pathogen resistance. Plant Signal. Behav. 4, 1–3.

Cumplido-Nájera, C.F., González-Morales, S., Ortega-Ortíz, H., Cadenas-Pliego,G., Juárez-Maldonado, A., 2019. The application of copper nanoparticles and potassium silicate stimulate the tolerance to *Clavibacter michiganensis* in tomato plants. Scientia Horticult. 245, 82–89.

Davis, R.M., Subbarao, K.V., Raid, R.N., Kurtz, E.A., 1997. Compendium of Lettuce Diseases. APS Press, pp. 15–16.

Dhingra, O.D., Sinclair, J.B., 1995. Basic Plant Pathology Methods, Second edition. CRC Press, Inc, pp. 434.

Elmer, W.H., White, J.C., 2016. The use of metallic oxide nanoparticles to enhance growth of tomatoes and eggplants in disease infested soil or soilless medium. Environ. Sci. Nano 3, 1072–1079.

El-Mougy, N.S., Abdel-Kader, M.M., 2009. Salts application for suppressing potato early blight. J. Plant Prot. Res. 49, 353–361.

Elsharkawy, M.M., 2018. Induced systemic resistance against *Cucumber mosaic virus* by *Phoma* sp. GS8–2 stimulates transcription of pathogenesis-related genes in *Arabidopsis*. Pest Manag. Sci. 75, 859–866.

Elsharkawy, M.M., Derbalah, A., 2018. Antiviral activity of titanium dioxide nanostructures as a control strategy for *Broad bean strain virus* in faba bean. Pest Manag. Sci. https://doi.org/10.1002/ps.5185.

Elsharkawy, M.M., Shimizu, M., Takahashi, H., Hyakumachi, M., 2012. Induction of systemic resistance against *Cucumber mosaic virus* by *Penicillium simplicissimum* GP17-2 in *Arabidopsis* and tobacco. Plant Pathol. 61, 964976.

Elsharkawy, M.M., Hassan, N., Villajuan-Abgona, R., Hyakumachi, M., 2014. Mechanism of biological control of *Rhizoctonia* damping-off of cucumber by a non-pathogenic isolate of binucleate *Rhizoctonia*. Afr. J. Biotechnol. 13, 640–650.

Elsharkawy, M., Derbalah, A., Hamza, A., El-Shaer, A., 2018. Zinc oxide nanostructures as a control strategy of bacterial speck of tomato caused by *Pseudomonas syringae* in Egypt. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-018-3806-0.

Fergus, J.W. 2006. Electrolytes for solid oxide fuel cells. J. Power Sources 162, (1) 8 30–40.

Fraternale, D., Giamperi, L., Ricci, D., 2003. Chemical composition and antifungal activity of essential oil obtained from in vitro plants of *Thymus mastichina* L. J. Essent. Oil Res. 15, 278–281.

Gowri, S., Gandhi, R.R., Sundrarajan, M., 2014. Structural, optical, antibacterial and antifungal properties of zirconia nanoparticles by biobased protocol. J. Mater Sci. Technol. 30 (8), 782–790.

Grosch, R., Koch, T., Kofoet, A., 2004. Control of bottom rot on lettuce caused by *Rhizoctonia solani* with commercial biocontrol agents and a novel fungicide. J. Plant Dis. Protect. 111 (6), 572–582.

Grosch, R., Faltin, F., Lottmann, J., Kofoet, A., Berg, G., 2005. Effectiveness of three antagonistic bacterial isolates to suppress *Rhizoctonia solani* Kuhn on lettuce and potato. Can. J. Microbiol. 51, 345–353.

Hassan, N., Elsharkawy, M.M., Villajuan-Abgona, R., Hyakumachi, M., 2014. A non-pathogenic species of binucleate Rhizoctonia inhibits the formation of infection structures caused by *Rhizoctonia solani* on cucumber. Acta Agr. Scand. B. S. P. 65, 208–214.

Heil, M., Bostock, R., 2002. Induced systemic resistance (ISR) against pathogens in the context of induced plant defenses. Ann. Bot. 89, 503–512.

Jadhav, S., Gaikwad, S., Nimse, M., Rajbhoj, A., 2011. Copper oxide nanoparticles: synthesis, characterization and their antibacterial activity. J. Clust. Sci. 22, 121–129.

Jung, J.H., Kim, S.W., Min, J.S., Kim, Y.J., Lamsal, K., Kim, K.S., Lee, Y.S., 2010. The effect of nano-silver liquid against the white rot of the green onion caused by *Sclerotium cepivorum*. Mycobiology 38, 39–45.

Kah, M., Hofmann, T., 2014. Nanopesticide research: current trends and future priorities. Environ. Inter. 63, 224–235.

Kanhed, P., Birla, S., Gaikwad, S., Gade, A., Seabra, A.B., Rubilar, O., Duran, N., Rai, M., 2014. In vitro antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. Mater. Lett. 115, 13–17.

Khalifa, E.Z., 1987. Further Studies on Some Soil-Borne Fungi Affecting Soybean and Their Control. PhD thesis. Faculty of Agriculture, Menoufiya University, Al Minufya, Egypt, pp. 148.

Kiewnick, S., Jacobsen, B.J., Braun-Kiewnick, A., Eckhoff, J.L.A., Bergman, J.W., 2001. Integrated control of *Rhizoctonia* crown and root rot of sugar beet with fungicides and antagonistic bacteria. Plant Dis. 85, 718–722.

Kim, S.W., Jung, J.H., Lamsal, K., Kim, Y.S., Min, Y.S., Lee, S., 2012. Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. Mycobiology 40, 53–58.

Koike, N., Hyakumachi, M., Kageyama, M., Tsuyumu, K., Doke, N., 2001. Induction of systemic resistance in cucumber against several diseases by plant growth-promoting fungi: lignification and superoxide generation. Eur. J. Plant Pathol. 107, 523–533.

Kumar, V., Yadav, S.K., 2009. Plant-mediated synthesis of silver and gold nanoparticles and their applications. J. Chem. Technol. Biotechnol. 84, 151–157.

Lamsal, K., Kim, S.W., Jung, J.H., Kim, Y.S., Kim, K.S., Lee, Y.S., 2011. Application of silver nanoparticles for the control of *Colletotrichum* species in vitro and pepper anthracnose disease in field. Mycobiology 39, 194–199.

Lee, H.J., Kang, D.C., Pyen, S.H., Shin, M., Suh, Y.W., Han, H., Shin, C.H., 2017. Production of H_2-free CO by decomposition of formic acid over ZrO_2 catalysts. Appl. Catal. A Gen. 531, 13–20.

Li, Z.K., Pinson, S.R.M., Marchetti, M.A., Stansel, J.W., Park, W.D., 1995. Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (*Rizoctonia solani*). Theor. Appl. Genet. 91, 382–388.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-CT} method. Method 25, 402–408.

Martinez-Gutierrez, F., Olive, P.L., Banuelos, A., Orrantia, E., Nino, N., Sanchez, E.M., Ruiz, F., Bach, H., Av-Gay, Y., 2010. Synthesis, characterization and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. Nanomed. Nanotechnol. Biol. Med. 6, 681–688.

Mesnage, R., Defarge, N., de Vendômois, J.S., Séralini, G.E., 2014. Major pesticides are more toxic to human cells than their declared active principles. Biomed. Res. Int. 2014, 179691.

Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramirez, J.T., Yacaman, M.J., 2005. The bactericidal effect of silver nanoparticles. Nanotechnology 16, 2346–2353.

Morsy, K.M., Elsabagh, R., Trinetta, V., 2018. Evaluation of novel synergistic antimicrobial activity of nisin, lysozyme, EDTA nanoparticles, and/or ZnO nanoparticles to control foodborne pathogens on minced beef. Food Cont. 92, 249–254.

Rashad, M.M., Baioumy, H.M., 2008. Effect of thermal treatment on the crystal structure and morphology of zirconia nanopowders produced by three different routes. J. Mater. Processing Technol. 195 (1–3), 178–185.

Saldajeno, M.G.B., Hyakumachi, M., 2011. The plant growth-promoting fungus *Fusarium equiseti* and the arbuscular mycorrhizal fungus *Glomus mosseae* stimulate plant growth and reduce severity of anthracnose and damping-off diseases in cucumber (*Cucumis sativus*) seedlings. Ann. Appl. Biol. 159, 28–40.

Sathyaseelan, B., Manikandan, E., Baskaran, I., Senthilnathan, K., Sivakumar, K., Moodley, M.K., Ladchumananandasivam, R., Maaza, M., 2017. Studies on structural and optical properties of ZrO_2 nanopowder for opto-electronic applications. J. Alloys Comp. 694, 556–559.

Servin, A., Elmer Mukherjee, W., Torre-Roche, R.D., Hamdi, H., White, J.C., Bindraban, P., Dimkpa, C., 2015. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. J. Nano Res. 17 (92), 1–21.

Shanmugaiah, V.H., Harikrishnan, N.S., Al-Harbi, K., Shine, J.M., Khaled, N., Balasubramanian, R., Kumar, S., 2015. Facile synthesis of silver nanoparticles using streptomyces sp. vsmgt1014 and their antimicrobial efficiency. Digest J. Nanomater. Biostruct. 10 (1), 179–187.

Shenashan, M., Derbalah, A., Hamza, A., Mohamed, A., El Safty, S., 2017. Antifungal activity of fabricated mesoporous alumina nanoparticles against root rot disease of tomato caused by *Fusarium oxysporium*. Pest Manag. J. 73 (6), 1121–1126.

Vincent, J.H., 1947. Distortion of fungal hyphae in presence of certain inhibitor. Nature 15, 850.

Wang, Q., Xiong, D., Zhao, P., Yu, X., Tu, B., Wang, G., 2011. Effect of applying an arsenic-resistant and plant growth–promoting rhizobacterium to enhance soil arsenic phytoremediation by *Populus deltoids* LH05-17. J. Appl. Microbiol. 111, 1065–1074.