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## Efficacy of Silicon and Titanium Nanoparticles Biosynthesis by some Antagonistic Fungi and Bacteria for Controlling Powdery Mildew Disease of Wheat Plants

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**Abstract** Nanoparticles of silicon and titanium were biologically synthesized from different bacterial and fungal isolates. The most promising results were obtained with TEM microscopy revealed that *Pseudomonas putida* (PpFT<sub>1</sub>) produced the small size of both nano silicon (SNPs) ranged from 1.52 to 8.73 nm and nano titanium (TNPs) from 5.81 to 8.95 nm. While, SNPs and TNPs that synthesized by *Trichoderma harzianum* (ThFT<sub>1</sub>) ranged from 5.0 to 9.0 nm and 2.0 to 16.0 nm, respectively. The SNPs and TNPs were tested as seed soaking and subsequent foliar spray, treatment, to powdery mildew on wheat seedling under greenhouse conditions. The most effective treatments were SNPs at concentration of 100 and 150 ppm obtained by *P. putida* (PpFT<sub>1</sub>) and *Bacillus subtilis* (BsBN<sub>3</sub>) at concentration of 50, 100 and 150 ppm which reduced the powdery mildew severity by 83.3, 89.7, 84.6 and 91.0 % respectively. While, SNPs at concentration of 150 ppm obtained by *T. harzianum* (ThFT<sub>1</sub>) reduced the powdery mildew severity by 82.0 %. Meanwhile, other concentrations showed moderate effect. Nano titanium obtained by antagonistic fungi *i.e.* *T. harzianum* (ThFT<sub>1</sub>), *T. viride* (TvGK<sub>2</sub>) and *T. hamatum* (TmSA<sub>2</sub>) in addition to antagonistic bacteria *i.e.* *P. putida* (PpFT<sub>1</sub>), *P. fluorescens* (PfBN<sub>1</sub>), *B. subtilis* (BsBN<sub>3</sub>) were tested against powdery mildew disease under greenhouse conditions. The highest reduction was obtained with TNPs of *P. putida* (PpFT<sub>1</sub>) at concentration of 150 ppm which reduced the powdery mildew severity by 93.5 %. Followed by TNPs obtained by *P. putida* (PpFT<sub>1</sub>) at 100 ppm, *Bacillus subtilis* (BsBN<sub>3</sub>) at 150 ppm and *T. harzianum* (ThFT<sub>1</sub>) at 100 and 150 ppm which reduced the powdery mildew severity by 84.6, 80.7, 78.2 and 80.7 %. While, other concentrations showed moderate effect.

**Keywords:** Wheat plants- Nano silicon -Nano titanium -Biosynthesis - Powdery mildew

### Introduction

Wheat (*Triticum aestivum* L. em Thell.) is an important cereal crop in Egypt and all over the world. It is a staple food crop and also known as “king” of the cereals (Laghari *et al.*, 2010). Because of high bread consumption, Egypt is the largest wheat importer worldwide (Gazette, 2013).

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Powdery mildew caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal (Bgt), is one of the most devastating diseases of wheat worldwide. In Egypt, the disease appeared in the last few years with high disease severities on most of the common wheat cultivars (El-Shamy *et al.*, 2012), and can lead to reduction in yield about 10 to 18% in Egypt. Development of resistant varieties is the most effective, economically and environmentally friendly approach for disease control (Alam *et al.*, 2013). They added that the most common breeding strategy has been the use of major genes conferring hypersensitive types of resistance, but the effectiveness of this approach has commonly been ephemeral due to frequent changes in the pathogen population. However chemical fungicides have been the traditional weapons used to control wheat diseases, which are hazard and toxic to human, animals and leads to environmental pollution. There is a growing need to develop alternative approaches for controlling plant diseases and safe for human, animals and environment.

Nanotechnology is a promising field of interdisciplinary research and its practical applications into agriculture industry is receiving attention nowadays due to the potential benefits that nanomaterial's can guarantee in several respects such as pests management (Li *et al.*, 2011). Use of nanoparticles in plant disease management is a novel and fancy approach that may prove very effective in future with the progress of application aspects of nanotechnology. The nanomaterial's have potential prospects of use in plant disease management in different ways (Khan and Rizvi, 2014, Huang *et al.*, 2015). Some of the nano particles that have entered into the arena of controlling plant diseases are nano forms of carbon, silver, silica and alumino-silicates (Sharma *et al.*, 2012).

Nanotechnology provides a good platform to modify and develop the important properties of metal in the form of nanoparticles having promising applications in diagnostics, biomarkers, cell labeling, contrast agents for biological imaging, antimicrobial agents, drug delivery systems and nano drugs for treatment of various diseases (Singh and Singh, 2011). Due to these applications, many varieties of NPs in massive amounts are being industrially produced. The word nano technology is generally used when referring to materials with the size of 0.1 to 100 nanometers (Morones *et al.*, 2005). Use of nanoparticles in plant disease management is a novel and fancy approach that may prove very effective in future with the progress of application aspect of nanotechnology. The nanotechnology has potential prospects of use in plant disease management in different ways. Nanoparticles because of ultra-small size, even smaller than a virus particle and high reactivity, may affect the activity of microorganisms. The silver has been generally found non injurious to microorganisms (Khan and Rizvi, 2014).

This research work was designed to study the effect of nano silicon and titanium for controlling wheat powdery mildew under greenhouse conditions.

## **Materials and Methods**

### ***Biosynthesis of silicon and titanium nanoparticles***

The extracellular synthesis of silicon nano particles (SNPs) and titanium nano particles (TNPs) were anchored following the methods described by Singh *et al.*, (2008) and Thakker *et al.*, (2012) by the kind help of Prof. Dr. E.M. Hob Allah, Microbiology Dept. NRC. Nanoparticles were obtained using six fungal and bacterial isolates obtained from healthy wheat plants as follow: *Pseudomonas putida* (PpFT<sub>1</sub>), *Pseudomonas fluorescens* (PfBN<sub>1</sub>), *Bacillus subtilis* (BsBN<sub>3</sub>), *Trichoderma harzianum* (ThFT<sub>1</sub>), *T. viride* (TvGK<sub>2</sub>) and *T. hamatum* (TmSA<sub>2</sub>). This pre-inoculum from each fungi and bacterial isolates was added to 90 mL of potato dextrose broth (PDB), nutrient broth and King's B Medium (KBM), respectively, incubated for 72 h under shaking conditions (200 rpm) at 31°C. In its log phase of growth-cycle, the fungal and bacterial biomass were harvested and washed with autoclave-sterilized water under aseptic conditions. The harvested fungal and bacterial biomass (1 gm of wet weight) were then resuspended in 100 mL aqueous solution of 10<sup>-3</sup> M potassium hexafluorosilicate (K<sub>2</sub>SiF<sub>6</sub>) or 10<sup>-3</sup> M potassium hexafluorotitanate (K<sub>2</sub>TiF<sub>6</sub>) and kept on a shaker (200 rpm) at 31°C under anaerobic conditions. The reaction between the fungal and bacterial biomass with SiF<sub>6</sub><sup>2-</sup> or TiF<sub>6</sub><sup>2-</sup> was carried out for a period of 48 h. The reaction products were collected after separating the fungal and bacterial biomass from the reaction medium through centrifugation at 5000 rpm for 10 min. In a control experiment, the harvested bacterial biomass was resuspended in autoclaved deionized water in the absence of K<sub>2</sub>SiF<sub>6</sub> or K<sub>2</sub>TiF<sub>6</sub> and the product obtained was characterized for the presence of Si/SiO<sub>2</sub> or Ti/TiO<sub>2</sub> nanocomposite.

### ***Nanoparticles characterizations by TEM***

The obtained biotransformed products were analyzed and characterized by transmission electron microscopy (TEM). Nanoparticles were prepared by drop coating the isolated and resuspended solution on carbon-coated copper grids. TEM measurements were performed on a JEOL model 1200EX instrument operated at an accelerating voltage of 120 kV (Woehrle *et al.*, 2006; Jain *et al.*, 2011 and Prakasham *et al.*, 2012).

### ***Greenhouse experiments***

Wheat seeds of highly susceptible cultivar to powdery mildew disease (cv. Misr-1) were obtained from Department of Crop Research, Agricultural Research Centre, Giza.

Nano silicon and nano titanium synthesized by antagonistic fungi (*T. harzianum* (ThFT<sub>1</sub>), *T. viride* (TvGK<sub>2</sub>) and *T. hamatum* (TmSA<sub>2</sub>)) and antagonistic bacteria (*Pseudomonas fluorescens* (PfBN<sub>1</sub>), *P. putida* (PpFT<sub>1</sub>), *Bacillus subtilis* (BsBN<sub>3</sub>)) were tested against powdery mildew disease under greenhouse conditions.

### ***Effect of nano silicon and nano titanium on powdery mildew of wheat plants***

Wheat seeds were soaked in nano silicon (SNPs) and nano titanium (TNPs) at concentrations of 0.0, 50.0, 100.0 and 150.0 ppm for 12 hours and sown in 30 cm diameter plastic pots containing clay soil (10 seeds per pot) and grown under a 16 h photoperiod at 22 to 25<sup>0</sup>C at Research and Production Station of National Research Centre at Noubaria, Beheira governorate in 2014/2015 growing season. Seedlings (20 days old) were further sprayed with the same concentrations of (SNPs) and (TNPs). Wheat seedlings were fertilized once with NPK solution (20/ 20/ 20) and were watered periodically to maintain moisture at field capacity under greenhouse conditions. The greenhouse experiment was designed as a randomized complete block. Plants at 27 days of sowing were inoculated with the powdery mildew fungus (*Blumeria graminis* f. sp. *tritici*). Before infection, natural infected leaves were shaken to remove old spores. New spores were transferred by shaking conidia from infected leaves (small pieces) on healthy plants under greenhouse conditions as described by El-Shamy *et al.*, (2012). After inoculation, plants were incubated overnight in complete darkness at a relative humidity of 98%. Seedlings were then transferred to a growth chamber at 18–22<sup>0</sup>C and white fluorescent light (12 h light /12 h dark). Each treatment was replicated five times. Five pots without infection with pathogen were served as control (healthy plants). Seedlings were moved to natural light greenhouse chambers at 22-24<sup>0</sup>C, and disease severity were assessed at 15 days after inoculation (Feekes growth stage 3-4) (Leath and Heun, 1990).

### ***Powdery mildew assessment***

Assessment of powdery mildew was based on infection severity rate from 0 to 9 modified from the scale described by Leath and Heun, (1990). As follow:

0 = Healthy leaf , 1 = 1 to 10 , 2 = 11 to 20 , 3 = 21 to 30 , 4 = 31 to 40 , 5 = 41 to 50, 6 = 51 to 60, 7= 61 to 70 , 8= 71 to 80 , 9= 81 % up to complete leaf area infected.

### ***Statistical analysis***

Data were subjected to statistical analysis using analysis of variance (ANOVA) and means were compared by Duncan's multiple range test at  $P \leq 0.05$  levels (Duncan, 1955). Data were transformed to acquire the normal distribution necessary for statistical analysis to be carried out by Tukey test for multiple comparisons among means were utilized (Neler, *et al.*, 1985).

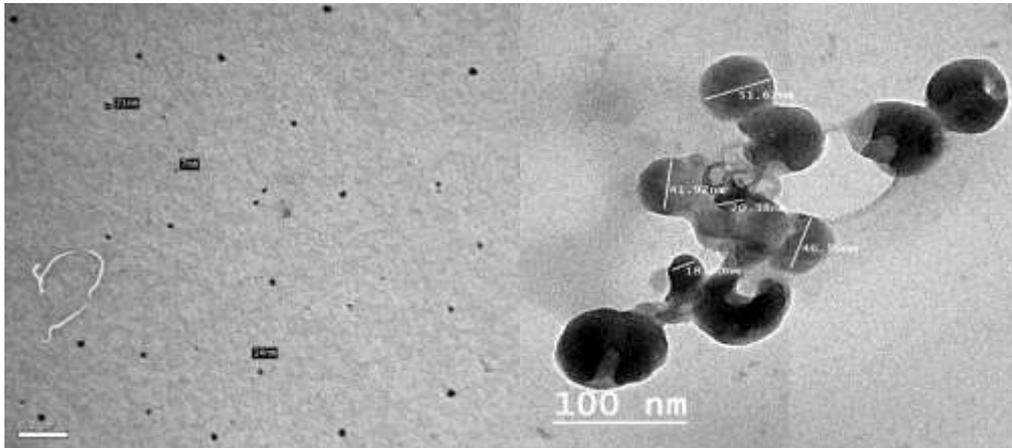
### **Results**

#### ***Transmission electron microscopy (TEM) image of silicon and titanium nanoparticles from some antagonistic fungi and bacteria***

Transmission electron microscopy (TEM) studies revealed the formation nature of particles synthesized from silicon and titanium metal from different fungal and bacteria Figs (1&2). Transmission electron microscopy (TEM) provided further insight into the morphology and size details of the synthesized silicon nanoparticles (SNPs) and titanium nanoparticles (TNPs). The TEM images at different magnifications are depicted in the Fig (1&2). Large variations in particle size were observed that TEM with average diameter ranged from 1.52– 51.67nm. The most promising results were obtained with *P. putida* (PpFT<sub>1</sub>) that produced the small size of both SNPs (1.52 to 8.73 nm) and TNPs (5.81to 8.95 nm). Moreover SNPs and TNPs that synthesized by *Bacillus subtilis* (BsBN<sub>3</sub>) produced average particles size (diameter) from 1.8 to 5.0 nm and from 3.0 to 14.0 nm, respectively. SNPs and TNPs that synthesized by *T. harzianum* (ThFT<sub>1</sub>) from 5.0 to 9.0 nm and 2.0 to 16.0 nm, respectively.

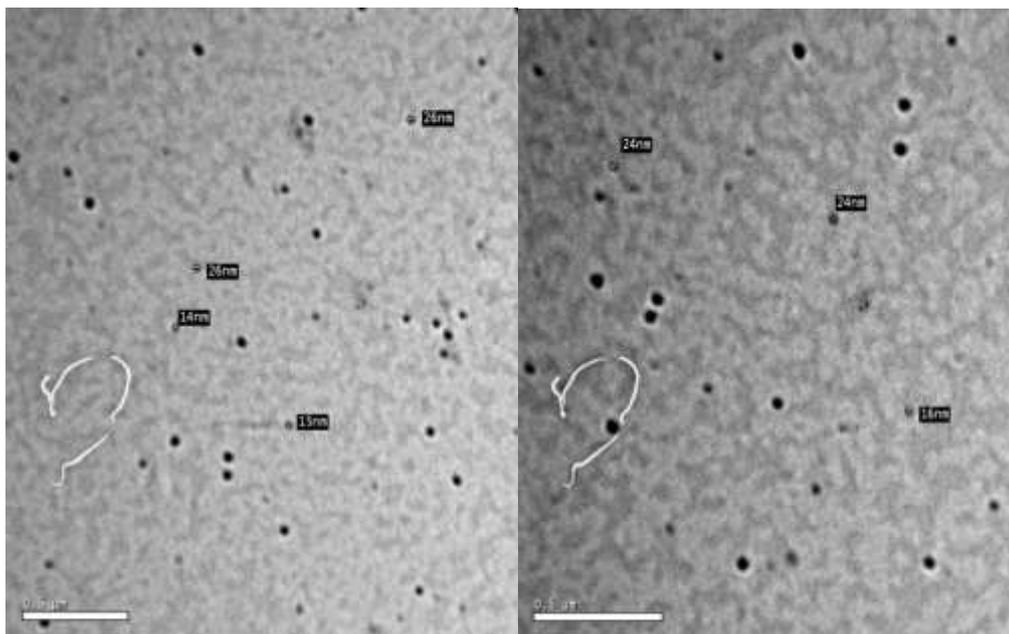
SNPs-*T. hamatum* (TmSA<sub>2</sub>)

TNPs-*T. hamatum* (TmSA<sub>2</sub>)



SNPs-*T. viride* (TvGK<sub>2</sub>)

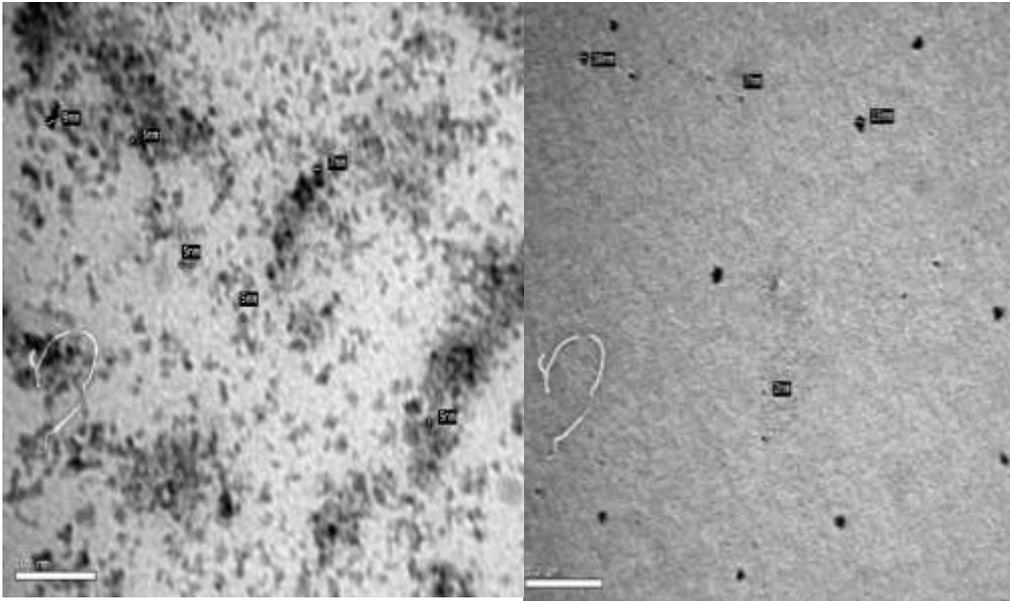
TNPs-*T. viride* (TvGK<sub>2</sub>)



**Figure 1.** Transmission electron microscopy (TEM) image of silicon and titanium nanoparticles from three *Trichoderma* species.

SNPs-*T. harzianum* (ThFT<sub>1</sub>)

TNPs-*T. harzianum* (ThFT<sub>1</sub>)

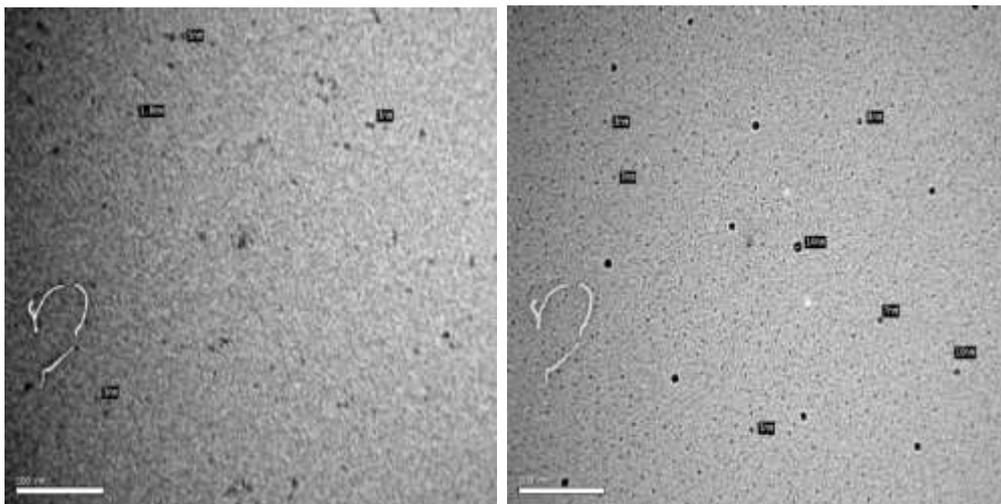


**Figure 1. (Cont.)** Transmission electron microscopy (TEM) image of silicon and titanium nanoparticles from three *Trichoderma* species.

- (1) Transmission electron microscopy (TEM) image of silicon and titanium nanoparticles from three *Trichoderma* species.
- (2) Magnification 100-500 nm $\times$
- (3) Silicon nanoparticles average size from 5 to 26 nm
- (4) Titanium nanoparticles average size from 2 to 51.67 nm

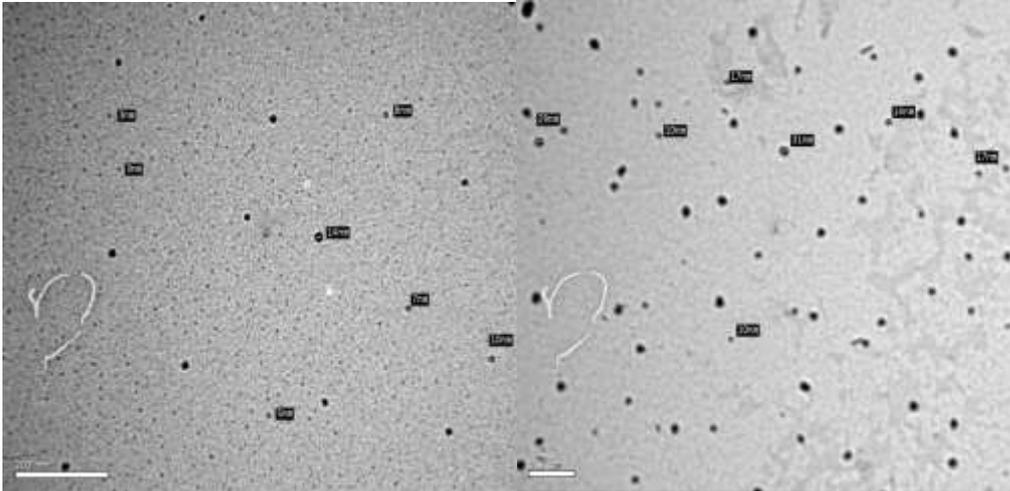
SNPs-*B. subtilis* (BsBN<sub>3</sub>)

TNPs-*B. subtilis* (BsBN<sub>3</sub>)



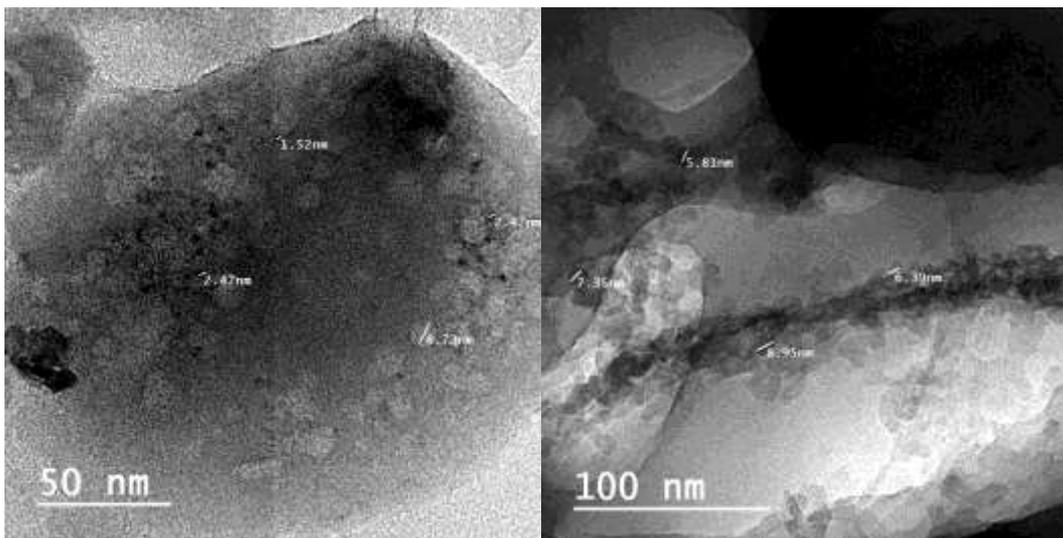
SNPs -*P. fluorescens* (PfBN<sub>1</sub>)

TNPs -*P. fluorescens* (PfBN<sub>1</sub>)



SNPs-*P. putida* (PpFT<sub>1</sub>)

TNPs-*P. putida* (PpFT<sub>1</sub>)



**Figure 2.** Transmission electron microscopy (TEM) image of silicon and titanium nanoparticles from three antagonistic bacteria

- (1) Transmission electron microscopy (TEM) image of silicon and titanium nanoparticles from three antagonistic bacteria
- (2) Magnification 50-200 nm ×
- (3) Silicon nanoparticles average size from 1.52 to 14.0 nm
- (4) Titanium nanoparticles average size from 3.0 to 31.0 nm

## Greenhouse experiments

### Efficacy of nanoparticles to control wheat powdery mildew

Three concentrations of SNPs and TNPs obtained by three fungal bioagents *i.e.* *Trichoderma harzianum* (ThFT<sub>1</sub>), *T. viride* (TvGK<sub>2</sub>) and *T. hamatum* (TmSA<sub>2</sub>) and three bacterial isolates *i.e.* *Pseudomonas fluorescens* (PfBN<sub>1</sub>), *P. putida* (PpFT<sub>1</sub>) and *Bacillus subtilis* (BsBN<sub>3</sub>) were tested against powdery mildew disease under greenhouse conditions.

### Effect of SNPs on disease severity of powdery mildew of wheat plants

Results in Table (1) indicate that all tested concentrations of SNPs significantly reduced powdery mildew disease of wheat plants. The most effective treatments are SNPs at concentration of 100 and 150 ppm obtained by *P. putida* (PpFT<sub>1</sub>) and *B. subtilis* (BsBN<sub>3</sub>) at concentration of 50, 100 and 150 ppm which reduced the powdery mildew severity by 83.3, 89.7, 84.6 and 91.0 % respectively. While, SNPs at concentration of 150 obtained by *T. harzianum* (ThFT<sub>1</sub>) reduced the powdery mildew severity by 82.0 %. Meanwhile, other concentrations showed moderate effect.

**Table 1.** Effect of nano silicon (SNPs) obtained by six fungal and bacterial bioagents on disease severity of powdery mildew in wheat plants (cv.Misr-1) under greenhouse conditions.

SNPs Source	Powdery mildew severity rate			Reduction %	
	Si Conc. (ppm)	Exp.1	Exp.2		
<b>SNPs-<i>Trichoderma harzianum</i></b> (ThFT <sub>1</sub> )	50	3.0b	2.7bc	2.9b	62.8
	100	2.5bc	2.6bcd	2.6bcd	66.6
	150	1.2efg	1.6efgh	1.4fgh	82.0
<b>SNPs-<i>T. viride</i></b> (TvGK <sub>2</sub> )	50	2.2c	2.7bc	2.5bcd	67.9
	100	2.0cd	2.0def	2.0def	74.3
	150	1.6de	2.0def	1.8efg	76.9
<b>SNPs-<i>T. hamatum</i></b> (TmSA <sub>2</sub> )	50	2.6b	2.8b	2.7bc	65.4
	100	2.0cd	2.2bcde	2.1cde	73.1
	150	1.5def	1.8efg	1.7efg	78.2
<b>SNPs-<i>P. fluorescens</i></b> (PfBN <sub>1</sub> )	50	2.0cd	2.2bcde	2.1cde	73.1
	100	1.5def	1.7efg	1.6efg	79.5
	150	1.2efg	2.1cdef	1.7efg	78.2
<b>SNPs - <i>P. putida</i></b> (PpFT <sub>1</sub> )	50	1.4ef	1.9efg	1.7efg	78.2
	100	1.0fgh	1.5fgh	1.3ghi	83.3
	150	0.6hi	1.0h	0.8hi	89.7
<b>SNPs- <i>B. subtilis</i></b> (BsBN <sub>3</sub> )	50	1.0fgh	1.3gh	1.2ghi	84.6
	100	0.8ghi	1.5fgh	1.2ghi	84.6
	150	0.4i	1.0h	0.7i	91.0
<b>Control</b>		7.4a	8.1a	7.8a	

(1) Figures with the same letters in the same column are not significantly different, (P=0.05). (2) Powdery mildew scale from 0 to 9 according (Leath and Heun, 1990).(3).

Powdery mildew severity rate was scored after 15 days of inoculation. (4).Seeds were treated with each concentration of nano silicon and seedlings at 20 days were sprayed with similar one.

### Effect of TNPs on disease severity

Results in Table (2) indicate that, all tested concentrations of TNPs significantly reduced powdery mildew disease of wheat plants. The highest reduction was obtained with TNPs of *P. putida* (PpFT<sub>1</sub>) at concentration of 150 ppm which reduced the powdery mildew severity by 93.5 %. Followed by TNPs obtained by *P. putida* (PpFT<sub>1</sub>) at 100 ppm, *Bacillus subtilis* (BsBN<sub>3</sub>) at 150 ppm and *T. harzianum* (ThFT<sub>1</sub>) at 100 and 150 ppm which reduced the powdery mildew severity by 84.6, 80.7, 78.2 and 80.7 % respectively. While, other concentrations showed moderate effect.

**Table 2.** Effect of nano titanium (TNPs) obtained by six fungal and bacterial bioagents on disease severity of powdery mildew in wheat plants (cv.Misr-1) under greenhouse conditions.

TNPs Source	Powdery mildew severity rate				Reduction %
	Ti Conc. (ppm)	Exp.1	Exp.2	Mean	
TNPs <i>T. harzianum</i> (ThFT <sub>1</sub> )	50	2.6bc	2.9bc	2.8bc	64.1
	100	1.4fg	1.9ef	1.7efg	78.2
	150	1.3fg	1.6f	1.5fg	80.7
TNPs <i>T. viride</i> (TvGK <sub>2</sub> )	50	1.8def	2.4cde	2.1de	73.1
	100	2.0de	2.5bcd	2.3cde	70.5
	150	1.7def	2.1de	1.9ef	75.6
TNPs <i>T. hamatum</i> (TmSA <sub>2</sub> )	50	2.2cd	3.0b	2.6bcd	66.6
	100	1.7def	2.5bcd	2.1de	73.1
	150	1.5efg	2.0def	1.8ef	76.9
TNPs <i>P. fluorescens</i> (PfBN <sub>1</sub> )	50	2.8b	3.1b	3.0b	61.5
	100	2.1cd	2.6bcd	2.4bcd	69.2
	150	1.8def	2.1de	2.0de	74.3
TNPs <i>P. putida</i> (PpFT <sub>1</sub> )	50	1.6ef	1.9ef	1.8ef	76.9
	100	1.0g	1.4f	1.2g	84.6
	150	0.4h	0.5g	0.5h	93.5
TNPs <i>B. subtilis</i> (BsBN <sub>3</sub> )	50	1.9de	2.5bcd	2.2cde	71.8
	100	1.5efg	2.0def	1.8ef	76.9
	150	1.3fg	1.6f	1.5fg	80.7
Control		7.4a	8.1a	7.8a	

(1). Figures with the same letters in the same column are not significantly different, (P=0.05). (2) Powdery mildew scale from 0 to 9 according (Leath and Heun, 1990). (3). Powdery mildew severity rate was scored after 15 days of inoculation. (4). Seeds were treated with each concentration of nano titanium and seedlings at 20 days were sprayed with similar one

## Discussion

For improving effectiveness of bioagents against disease, plant application of different concentrations of nano silicon and nano titanium obtained by antagonistic microorganisms were tested against powdery mildew disease under greenhouse conditions. In the present study transmission electron microscopy (TEM) revealed that different antagonistic microorganisms synthesized silicon nanoparticle (NSPs) and titanium nanoparticles (TNPs). TEM Technique used to visualize size and shapes of biosynthesized silicon and titanium nanoparticles have predominantly shown spherical shape structures. TEM with average diameter ranging 1.52–51.67 nm. Received results indicate differences between microorganisms belonging to two groups. *P. putida* (PpFT<sub>1</sub>) that produced the small size of both nano silicon and nano titanium forward by SNPs and TNPs synthesized by *Bacillus subtilis* (BsBN<sub>3</sub>) and *T. harzianum* (ThFT<sub>1</sub>). This result is parallel with the results by Priyadarshinia *et al.*, (2013) and Zawadzkal *et al.*, (2016) where TEM micrograph of silver nanoparticles obtained after 24 h of incubation showed nanoparticles with variable shape, and the size of the particle ranged from 5 to 25 nm. Saifuddin *et al.* (2009) referred that, the size is within the size range reported earlier for AgNPs from bacteria which varied from 5 to 50 nm in *B. subtilis*. Tian *et al.* (2014) investigated the antibacterial activity of TiO<sub>2</sub> coatings modified by iron NPs. Sensitivity of tested pathogens to nano particles depends on the crystalline form of carrier, particle size and surface area metals. The obtained results point for characterization of NPs and there pathways of the antibacterial and antifungal activity of tested treatments.

Significant reductions in powdery mildew infection and development were obtained in all treatments. Disease severity was significantly reduced using nano silicon followed by nano titanium, under greenhouse in compared with untreated control plants. The most effective treatments are nano silicon and titanium obtained by *P. putida* (PpFT<sub>1</sub>). There are many examples of both plants and microorganisms that produce nanomaterials in nature (Raliya *et al.*, 2013 and Pantidos and Horsfall, 2014). Nanotechnology has been reported as an additional technology which could help in meeting the global demands for sustainable agriculture and prevention of crop losses. Several scientists have concentrated their efforts on the development of non-target, biodegradable and ecofriendly nano-formulations showing strong biological activities against plant pathogens (Oluwaseun and Sarin, 2017).

Silicon is regarded as an essential element for many plant species and Si application has been demonstrated to inhibit various plant pathogens, e.g. *Blumeria graminis* sp. *tritici* infecting wheat (Bédanger *et al.*, 2003; Rémus-Borel *et al.*, 2005) and *Podosphaera fuliginea* infecting cucumber (Menzies *et al.*, 1991). There are several hypotheses concerning the role of

Si in inhibiting fungal infection. Si has been thought to protect through mechanical strengthening of the plant (Fauteux *et al.*, 2005). In accordance with this, the enhanced resistance of Si-treated host plants to pathogenic fungi has been suggested to result from more efficient resistance to pathogen penetration of host tissue, e.g. due to the specific deposition of Si compounds in cell walls as suggested for rice blast (Kim *et al.*, 2002) and may play a more active role in plant–pathogen interactions by stimulating other plant defense responses such as deposition of phenolic compounds/phytoalexins with antifungal properties and enhanced production of defence-related enzymes (Rémus-Borel *et al.*, 2005).

TiO<sub>2</sub> photocatalyst technique has great potential in various agricultural applications, including plant protection since it does not form toxic and dangerous compounds and possesses great pathogen disinfection efficiency. Scientists have been trying to improve the phytopathogenic disinfection efficiency of TiO<sub>2</sub> thin films by dye doping and other suitable methods (Yao *et al.*, 2007).

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