

USAGE OF BIO-SILVER NANOPARTICLES AND BIO-AGENTS AGAINST *FUSARIUM SOLANI* AND THEIR EFFECTS ON BOTANICAL PARAMETERS OF TOMATO PLANT

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TOMATO
FUSARIUM WILT
AGNPS BIOSYNTHESIS
VITAVAX
BIOLOGICAL CONTROL

ABSTRACT. – Isolation trials from various tomato-growing sites in Kafrelsheikh governorate showing characteristic symptoms of wilt yielded one fungal isolate which was purified and identified as *Fusarium solani*. *In vitro* study, the synthetization of silver nanoparticles by *Mentha spicata* leaf extract was subjected to physical characterization using scanning electron microscopy (SEM) images, which confirmed that the particle diameters of silver nanoparticles (AgNPs) ranged from 57 to 97 nanometers. The antifungal activity of synthesized AgNPs with poisoned food technique was used against a pathogenic fungus with different concentrations (50, 100, 150, and 200 mg/l); furthermore, different concentrations of the fungicide Vitavax (0.5, 1.0, 1.5, and 2.0 g/l) and two biological control agents (*Trichoderma harzianum*, *Bacillus subtilis*) were also applied to investigate their antifungal activity against the fungus. The pots experiment was applied under greenhouse conditions during the 2020 season by soaking tomato seedlings in different treatments. The results indicated that the AgNPs treatment yielded the least disease severity among various treatments. All treatments were found effective at different rates, in particular, AgNPs to promote the vegetative growth parameters of tomato plants and controlling the root rot disease caused by pathogenic fungus. Treated plants with AgNPs recorded a low concentration of proline and the highest values of chlorophyll pigments. Furthermore, AgNPs improved the anatomical measurements compared with infected plants without treatment. This study demonstrated the biosynthesis of AgNPs by *Mentha spicata* leaves extract, which proved a distinct fungicidal activity against pathogenic fungus.

INTRODUCTION

One of the most valuable horticultural crops is the tomato plant (*Lycopersicon esculentum* Mill.), not only because of its economic importance but also for its nutritional value and sensory qualities. It is consumed in the form of fresh as well as processed products where 80 % of tomatoes grown all over the world are processed into products according to Viskelis *et al.* (2015).

Epidemiological studies have proved that the tomato plants have the ability to reduce various ailments because of containing a high number of antioxidants such as carotenoids, ascorbic acid, polyphenols, and many others (Perveen *et al.* 2015).

In Egypt, tomatoes have been produced in all governorates and are available all year round with a total production volume of 7.9 million tons (FAO 2016). Egypt is the fifth largest tomatoes producer worldwide.

The different fungi of soil-borne can attack the tomato plants by causing severe diseases such as wilt and root rot (Rashid *et al.* 2021). The most important of which are *Fusarium* species, which can live in organic materials and

nearly all soil types (Boyaci *et al.* 2010). This fungus can attack the vascular system in infected plants, block water transport through xylem by inducing vessel plugging, leading to foliage wilt and finally plant death (Portal *et al.* 2018).

The control of such diseases depends essentially on fungicide treatments (El-Mougy *et al.* 2004), whereas the application of various chemical pesticides (such as Vitavax) and their residual toxicity adversely affect the environment, human, and animal health. Consequently, several studies discussed the biological control of plant pathogens by microorganisms and concluded that the bio-control is considered a more natural, cost effective, and eco-friendly approach rather than other traditional chemical treatment strategies (El-kot & Belal 2006).

Trichoderma harzianum is well-known for its antagonistic activity against pathogenic fungi since it is considered the most functional bio-control agent that inhibits the growth of different *Fusarium* species. The antagonistic properties of the bio-control agent are based on the activation of various mechanisms, either directly through mycoparasitism and secreting antifungal metabolites,

or indirectly through the competition for nutrients and space, amending environmental conditions, promoting plant growth, induced resistance, and antibiosis (Silva *et al.* 2019).

Many microbial species such as *B. subtilis* have been found to efficiently control plant pathogens (Sivasakthi *et al.* 2014). The development of strategies and delivery methods of bio-control agent application by using antagonistic microorganisms to overwhelm the incidence of diseases caused by soil-borne pathogens is of great importance (Küçük & Kivanc 2005).

Recent developments of controlling plant diseases are relying on nanotechnology science, in particular, the preparation of arranged nanoparticles with specific size and shape, resulting in the formulation of a novel bio-synthesized nanomaterial with pesticidal activity. Because of the eco-friendly nature and the cost-effective features of the nanomaterials, they were considered as an alternative approach rather than the traditional chemical fungicides for the control of plant diseases (Kumar & Yadav 2009).

The popularity of nanoparticles (NPs) increased recently because of their significant properties. Along with the design of novel nanomaterials and considering their surface forms, their antimicrobial potential could be determined to alleviate or prohibit fungal growth for medical and agricultural purposes.

Silver nanoparticles are among nanoparticles of great concern owing to their antimicrobial activity. These particles were used for the control of microorganisms and the protection against prejudicial infections. The antifungal influences of silver nanoparticles were estimated against eighteen plant pathogenic fungi including the genus *Fusarium* (Kim *et al.* 2012).

Several studies discussed the antimicrobial activity of the silver nanoparticles, and the factors affecting its intensity. Hamouda *et al.* (2000) reported the presence of positive charges that qualify nanoparticles in reacting with the negatively charged proteins on the cell membranes due to the electrostatic attraction phenomena and accordingly contributing to their antimicrobial activity.

This study aimed to investigate the application of chemicals, bio-control agents, and AgNPs on tomato plants to improve its resistance against *F. solani* under greenhouse conditions and their impacts on vegetative growth parameters, physiological, and anatomical features.

MATERIALS & METHODS

Source of microorganisms: Microorganisms (*Trichoderma harizanum* and *Bacillus subtilis*) kindly obtained from Pr Dr Elsayed B Belal, Professor of Agricultural Microbiology, Agric. Botany Department, Faculty of Agriculture, Kafrelsheikh University according to Belal *et al.* (1996a, b).

Isolation, purification and identification of pathogenic fungus: Tomato plants showing root rot symptoms were collected from Kafrelsheikh governorate during the season of tomato plants in 2020. Diseased roots were washed with tap water, cut into small pieces and surface sterilized by 0.5 % sodium hypochlorite solution for three minutes then washed three times with sterilized distilled water. Samples were dried between two layers of sterilized filter papers to remove the excess water and placed on potato dextrose agar (PDA) medium in petri dishes. Inoculated dishes were incubated at 25 ± 3 °C for 4-5 days, and the developed fungal cultures were purified using hyphal tip isolation techniques according to El-kot & Belal (2006). The pure cultures were transferred on PDA. Cultural, morphological, microscopical and phyto-pathological properties were considered to identify the isolated pathogen. Stock cultures were maintained on PDA slants at 4 °C for further experiments.

Pathogenicity test: The pathogenicity test of *Fusarium* isolate was carried out on tomato plants (023 F1). Fungus was grown on glass bottles with a capacity of 50 ml, bottles were sterilized with 100 g of barley grains and 50 ml water and autoclaved at 121 °C for 20 min, then discs with 5 mm diameter were inoculated with a 10-day-old colony and incubated for two weeks at 28 °C to test their pathogenicity. Plastic pots (30 cm in diameter) containing sterilized sandy-clay (1:1 w/w) were infested with prepared inoculum at 2 % for ten days before transplanting according to El-kot & Belal (2006). All pots were irrigated before planting in order to achieve homogeneous distribution of the disease and good growth. Healthy seedlings 30-day-old of tomato plants were transplanted at the rate of three seedlings/pot. Three pots were used as replicates. A control experiment was prepared without the addition of the tested fungus. The pots were kept with careful observation under greenhouse condition.

Green synthesis of silver nanoparticles: The green synthesis of silver was carried out in the City of Scientific Research and Technological Applications, New Borg El Arab city, Alexandria, Egypt. A weight of 0.0169 g of AgNO₃ (Sigma Aldrich, USA) was dissolved in 100 mL of double distilled water to prepare a 1 mM aqueous silver nitrate solution. For the reduction of AgNO₃ into Ag⁺ ions, a volume of 10 ml of aqueous *Mentha spicata* leaves extract was added to 90 mL of freshly produced 1 mM AgNO₃ solution. The solution was thoroughly mixed and shaken until the color change was observed. The formation of AgNPs was indicated by the emergence of a reddish-brown color. A UV-Vis spectrophotometer was used to record the optical density at regular intervals until the value peaked (color changed to dark yellow). After centrifuging the solution for 20 minutes at 5000 rpm, the pellet (AgNPs) was washed three times with sterile distilled water and then rinsed with ethanol to remove any remaining contaminants. The AgNPs were then dried in an oven at 50 °C and subjected to further physical and biological evaluation.

In vitro evaluation of antifungal activity: *In vitro* preliminary antifungal assay was performed using Vitavax, AgNPs, *T. har-*

zianum and *B. subtilis*. The Vitavax treatment was added as a recommended dose with different concentrations (0.5, 1.0, 1.5, and 2.0 g/l) to the sterilized Potato dextrose agar medium (PDA) before solidification gently rotated and disbanded into sterilized Petri plates and inoculated at the middle with a 1 cm disc of 5-days-old fungal culture in the center. Different concentrations of AgNPs (50, 100, 150 and 200 mg/l) were added and allowed to solidify with (PDA) into each Petri dish and about 1 cm disc of 5-day-old culture of the test fungus was placed at the center of the Petri dish. The bacterial antagonism bioassay experiment regarding *B. subtilis* was performed in Petri dishes containing Nutrient Agar (NA) medium. NA medium was poured into Petri dishes (9 cm in diameter, 15 ml/dish), after solidification, a volume of 100 µl of *B. subtilis* liquid culture (10^8 cfu/ml) was transferred onto a nutrient agar plate and spread evenly with sterilized glass rods, eventually, Petri dishes were inoculated with a disc of pathogenic fungus (5 mm in diameter) in the center of the Petri dish. A control experiment for pathogenic fungus growth was designed and the treatment was replicated thrice. The plates were incubated at 28 °C until full-fungus growth of the control experiment. The bacterial antagonism was determined as an inhibition of the fungal growth according to Khan *et al.* (2018). The antagonistic activity of *T. harzianum* was evaluated using the dual culture technique according to Mosbah *et al.* (2017). Three Petri dishes were inoculated with pathogenic fungus and designed as a control. Each treatment was replicated thrice, and the inoculated Petri dishes were incubated at 28 °C for 7 days. The fungal toxicity of Vitavax, AgNPs and *T. harzianum* in terms of percentage inhibition of mycelial growth was calculated according to Singh *et al.* (1999).

Greenhouse experimental design: The experiment was carried out in randomized complete block design (RCBD) with twelve replicates for each treatment. The most effective treatments against pathogenic fungus were applied on tomato plants (023F1) under greenhouse conditions in Sakha, Agricultural Botany Department, Kafrelsheikh University.

Inoculum of pathogenic fungus and soil treatment: The greenhouse experiment was conducted to evaluate the effect of selected silver nanoparticles compared to Vitavax, *T. harzianum* and *B. subtilis* on the incidence of root rot disease on tomato plants. The pathogenic fungus of root rot mentioned above grew on barley sandy medium on glass bottles of 500 ml capacity for two weeks at 28 °C. Plastic pots (30 cm in diameter) containing sterilized sandy-clay soil (1:1 w/w) were infected with pathogenic fungus. Fungal inoculum was mixed with the sterilized soil surface of each pot at a rate of 2 % (w/w) potential inoculum. The soil was moistened with water for ten days before transplanting. The control experiment was prepared without the addition of the tested fungus.

Preparation of bio-agents inoculum: *Trichoderma harzianum* strain grew on Potato dextrose broth medium and incubated at 28 °C for ten days. Spore suspensions of strain were counted and adjusted at (1×10^6 spore/ml) by using a haemocytometer

slide. The biomass of *T. harzianum* was produced by inoculating 500 ml flasks containing 200 ml of PDB with disks of *T. harzianum*. Flasks were incubated on a rotary shaker at 250 rpm at 28 °C for ten days. The biomass of *T. harzianum* was collected by filtration through sterilized filter papers (Whatman No. 1), homogenized and suspended in distilled sterilized water and adjusted at 10^6 spore /ml, then stored in the refrigerator at $5 \pm 2^\circ$ C till use. The isolate of *B. subtilis* was separately grown on nutrient broth medium and incubated at 28 °C for 4-5 days in 250 ml flasks. The density of bacterial cell culture was adjusted at (1×10^8 cfu/ml) using a haemocytometer slide according to Morsy *et al.* (2021).

The experimental treatments: The treatments of AgNPs, Vitavax, *T. harzianum*, and *B. subtilis* were applied by dipping tomato seedlings (30-day-old) in Vitavax (2 g/l), AgNPs (200 mg/l) and suspension of *T. harzianum* (1×10^6 spore/ml), and *B. subtilis* (1×10^8 cfu/ml) as bio-control agents, each alone for 30 minutes, then seedlings were dried in the open air before transplanting in each treatment individually. Untreated seedling was dipped in sterilized water for 30 minutes and air dried before transplanting as uninfected control. On the other hand, a positive control experiment was designed according to each treatment where the seedlings were transplanted in uninfected soil (no pathogen) to study the effect of AgNPs, Vitavax, *T. harzianum*, and *B. subtilis* on the botanical parameters of tomato plants. The experiment was carried out in ten treatments, each treatment was comprised of twelve pots as replicates, and each pot contained three seedlings of tomato plants. The pots were kept in the greenhouse ($28 \pm 2^\circ$ C) and received the recommended dose of N, P and K fertilizers. Irrigation was carried out regularly. The pots were kept under careful observation and daily observed for the recording of symptom development. The parameters were collected at 45, 100 days from transplanting.

Disease assessment: Tomato plants were removed from the soil, washed thoroughly to remove soil debris and the root rot disease severity index was calculated according to Liu *et al.* (1995). Disease severity index was determined after 45, 100 days from transplanting.

Vegetative characteristics: The vegetative growth parameters of tomato plants including plant height (cm), leaves number, root volume (cm³), leaf area (cm²) and dry weight (g) were recorded in chosen samples of treatments and control after 45, 100 days from transplanting. The dry weight was determined on the roots, leaves and stems of tomato plants which were first separated and air-dried before being dried at 70 °C in the oven to constant weight before getting the dry weight.

Physiological and anatomical features: Physiological parameters of tomato plants including the chlorophyll pigments (chlorophyll a, b and total chlorophyll µg/cm²) concentration were recorded after 30, 45 and 60 days from transplanting. Estimation of chlorophyll pigments was done at a fresh area, where two cm² were taken from the terminal leaflet in the third leaf from the apical of the tomato stem. The extraction of photosynthetic pigments determined and calculated as described by Moran (1982).

The Proline concentration ($\mu\text{mol g}^{-1}$) was determined from leaves after 30 days from transplanting according to Bates *et al.* (1973).

The anatomical measurements for stems of tomato plants were carried out after 30 days from transplanting as described by Ruzin (1999), specimens of selected treatments were taken from the second internode of the stem from the apex, the killing and fixation of the chosen plant samples were carried out using the solution of Formalin acetic acid alcohol. The histological features were studied by microscopical examination of the chosen sections to detect the changes that occurred in the stems of tomato plants.

Statistical analysis: Data were analyzed by one-way analysis of variance (ANOVA), using Statistical Package (CO-STATE). Duncan's multiple range test at P value 0.05, was used to provide significance levels for the difference among the treatment means (Duncan, 1955).

RESULTS & DISCUSSION

Isolation and identification of the pathogenic fungus

Isolation trials, carried out on damping-off and root rot disease, collected from different locations at Kafrelsheikh Governorate, Egypt, resulted in the isolation of a fungus isolate belonging to the genus *Fusarium* as shown by preliminary microscopic examination. The isolated *Fusarium* isolate was identified as *Fusarium solani* according to El-kot & Belal (2006) as shown in Fig. 1, which represent infected vessels in the root of the tomato plant compared with an untreated plant. This fungus was previously reported to be associated with tomato damping-off and root rot diseases.

Scanning electron microscopy analysis

One of the most powerful tools for surface morphological evaluation with direct observation of nanoparticles

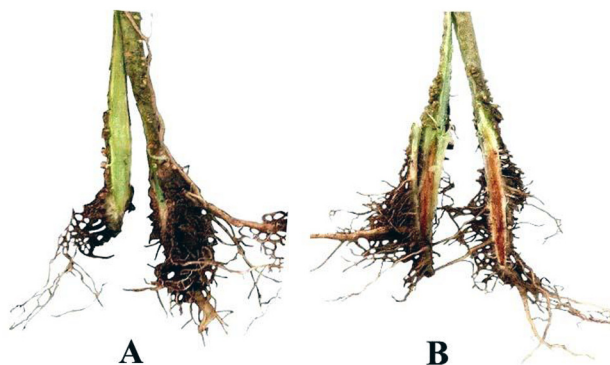


Fig. 1. – Longitudinal sector in tomato roots of plants infected by *F. solani* compared to uninfected plants. **A:** Untreated plant; **B:** Infected plant with *Fusarium solani*.

is scanning electron microscopy (SEM) Abdelkhalik & Al-Askar (2020). According to the obtained SEM data, the less miscible produced powders are made of pure AgNPs, which looking like spherical particles (Fig. 2). The agglomeration of AgNPs appeared in the SEM picture might be attributed to the free static charges on the surface of AgNPs. The particle diameter ranged from 57 to 97 nanometers. The form and size of the biosynthesized AgNPs were validated by the SEM examination as previously prepared (Datta *et al.* 2017, Heflish *et al.* 2021). The primary particles have a rather broad size distribution, according to SEM data, which agrees with the particle size distribution result.

In vitro evaluation of antifungal activity

In vitro antagonism assay showed varying levels of bioactivity and inhibition against the mycelial growth patterns of root-rot pathogen as demonstrated in Fig. 3, and Table I.

Vitavax treatment inhibited pathogenic fungi's mycelial growth. This result is in agreement with Muhanna *et al.* (2016) who reported that the chemical fungicide Vitavax had a direct effect on the pathogenic fungi's disease severity revealing the highest efficacy and the lowest disease severity percent.

AgNPs treatment inhibited pathogenic fungi's mycelial growth due to their several mechanisms of action, including interference with fungal cell membrane potential and triggering cell death (Mahdizadeh *et al.* 2015), meanwhile, the administration of Vitavax for treatment of several plant pathogenic fungus is relatively safe according to Park *et al.* (2006).

The treatment of *B. subtilis* inhibited the growth of fungus due to its antagonistic activity against the pathogenic fungus according to Ashwini & Srividya (2014) who reported that *B. subtilis* might have a role in fungal growth control through the degradation of the fungal cell wall. Additionally, *Bacillus* bacteria have the ability to

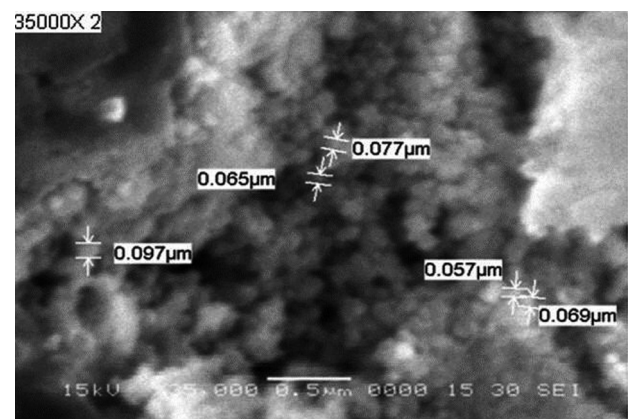


Fig. 2. – SEM images of silver nanoparticles biosynthesized by *Mentha spicata* leaf extract as reducing agents, (Bar = 0.5 μm at $\times 35000$).

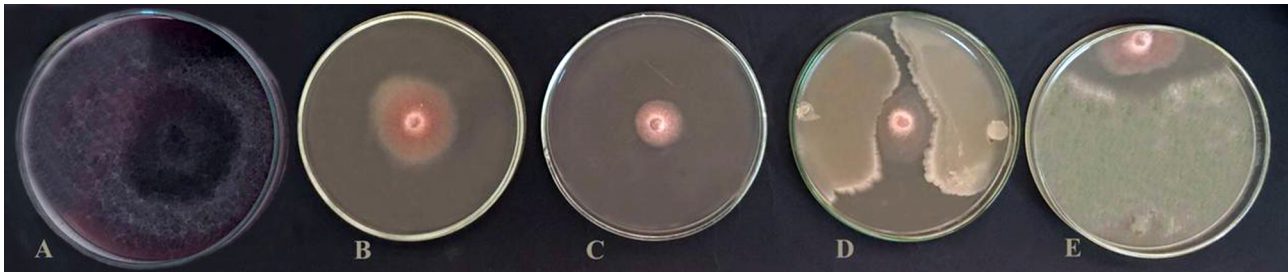


Fig. 3. – Effect of Vitavax, AgNPs, *Bacillus subtilis* and *Trichoderma harzianum* on the linear growth of *Fusarium solani*. **A:** *F. solani* (un-inoculated); **B:** *F. solani* + Vitavax 2g/L; **C:** *F. solani* + AgNPs 200 mg/L; **D:** *F. solani* + *B. subtilis*; **E:** *F. solani* + *T. harzianum*.

Table I. – Inhibitory effect of Vitavax, AgNPs, *Bacillus subtilis* and *Trichoderma harzianum* on the linear growth of *Fusarium solani* *in vitro*.

Treatments	Concentrations	Inhibition%	Treatments	Concentrations	Inhibition%
Vitavax	0.5 g/l	51.48 h	AgNPs	50.0 mg/L	67.77 d
	1.0 g/l	57.77 g		100.0 mg/L	72.96 c
	1.5 g/l	63.70 ef		150.0 mg/L	76.66 b
	2.0 g/l	72.22 c		200.0 mg/L	81.48 a
B. subtilis		65.55 de	T. harzianum		61.48 f
LSD 0.05	2.71				

Table II. – The effect of tested treatments on the inhibition of *Fusarium solani* disease severity of tomato plants in pots experiment.

Treatments	% Disease severity	
	After 45 days	After 100 days
Untreated plants	0.00 d	0.00 e
<i>F. solani</i>	40.70 a	81.43 a
AgNPs	0.00 d	0.00 e
AgNPs + <i>F. solani</i>	16.60 c	29.60 d
Vitavax	0.00 d	0.00 e
Vitavax + <i>F. solani</i>	20.30 bc	37.00 cd
<i>T. harzianum</i>	0.00 d	0.00 e
<i>T. harzianum</i> + <i>F. solani</i>	27.73 b	46.26 b
<i>B. subtilis</i>	0.00 d	0.00 e
<i>B. subtilis</i> + <i>F. solani</i>	24.03 bc	44.40 bc
LSD 0.05	8.47	8.67

produce a large number of antibiotics (polymyxin, diffidin, subtilin, mycobacillin, and bacitracin) according to Belal *et al.* (2013).

The treatment of *T. harzianum* inhibited the growth of pathogenic fungus. Similar findings were reported by Ghoniem & Belal (2013) who demonstrated that several modes of action of *Trichoderma* were proposed to explain its antifungal activity through attack many fungi by coiling around and penetrating into the hyphae and lysing the fungal mycelia by producing β (1,3) glucanase and chitinase.

Greenhouse experimental design

The greenhouse experiment showed that treated plants with AgNPs, Vitavax, *T. harzianum* and *B. subtilis* have the efficacy of controlling the disease on tomato plants under greenhouse conditions. All of the treatments dramatically decreased damping-off and root rot symptoms.

Disease assessment

The obtained results in Table II revealed that the application of AgNPs, Vitavax, *T. harzianum* and *B. subtilis* were effective in reducing disease severity compared with infected plants with *F. solani*.

Vegetative characteristics

Data presented in Table III and Table IV showed that inoculation of *F. solani* on tomato plants had a negative impact on these plants, resulting in a decrease of all vegetative growth parameters including plant height, dry weight, leaf area, roots volume and leaves number.

In this respect, the application of AgNPs yielded a positive impact on the treated plants, which led to increasing the vegetative growth parameters significantly compared to untreated plants. Similar findings were reported by (Singhi *et al.* 2017) who stated that the nanoparticles provided different metabolic reactions due to the increased surface area available to the plant host, which increased the rate of photosynthesis; furthermore, increased production of dry matter, crop yield and also protection of the plant host from different biotic stress. The application of bio-control agent treatments, *B. subtilis* and *T. harzianum*, promoted the vegetative growth compared to untreated plants, these results were confirmed by Khan *et al.* (2020) who studied the role of the bio-agent inoculants and their effects on growth parameters involving stimulation of cell division, elongation, formation of dried tissues in plants, and production of plant growth regulators.

Table III. – The control of *Fusarium solani* by different treatments and their effects on vegetative growth parameters in tomato plants after 45 days from transplanting under greenhouse conditions.

Treatments	Vegetative growth parameters after 45 days						
	Plant height (cm)	Dry weight (g)			Leaf area (cm ²)	Roots volume (cm ³)	Leaves number
		root	stem	leaves			
Untreated plants	49.26 d	1.72 d	5.17 d	4.09 d	903.67 d	4.70 d	11.33 b
<i>F. solani</i>	30.06 j	1.02 j	4.02 j	3.33 j	735.29 j	1.40 j	9.00 f
AgNPs	61.36 a	2.09 a	5.51 a	4.43 a	878.67 a	6.50 a	13.00 a
AgNPs + <i>F. solani</i>	42.26 f	1.42 f	4.90 f	3.86 f	852.42 f	3.33 f	10.66 bcd
Vitavax	45.66 e	1.59 e	5.02 e	3.98 e	878.67 e	4.33 e	11.00 bc
Vitavax + <i>F. solani</i>	39.03 g	1.33 g	4.79 g	3.72 g	821.32 g	3.00 g	10.00 de
<i>T. harzianum</i>	52.50 c	1.85 c	5.31 c	4.18 c	923.52 c	5.20 c	12.33 a
<i>T. harzianum</i> + <i>F. solani</i>	33.60 i	1.11 i	4.56 i	3.57 i	788.23 i	2.00 i	9.66 ef
<i>B. subtilis</i>	56.56 b	1.97 b	5.41 b	4.32 b	954.40 b	5.90 b	12.33 a
<i>B. subtilis</i> + <i>F. solani</i>	35.13 h	1.22 h	4.69 h	3.65 h	806.42 h	2.50 h	10.33 cde
LSD 0.05	1.35	0.03	0.26	0.27	6.13	0.19	0.76

Table IV. – The control of *Fusarium solani* by different treatments and their effects on vegetative growth parameters in tomato plants after 100 days from transplanting under greenhouse conditions.

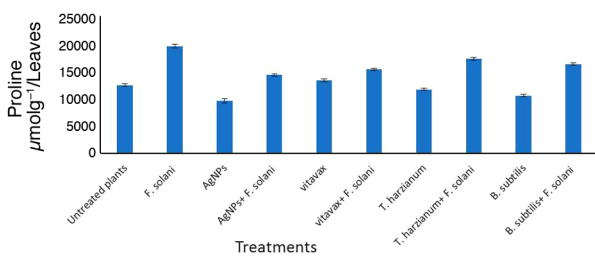
Treatments	Vegetative growth parameters after 100 days						
	Plant height (cm)	Dry weight(g)			Leaf area (cm ²)	Roots volume (cm ³)	Leaves number
		root	stem	leaves			
Untreated plant	66.36 d	2.70 d	6.97 d	6.96 d	1536.02 d	5.70 d	20.66 b
<i>F. solani</i>	40.86 j	2.07 j	5.93 j	5.68 j	1254.40 j	2.00 j	16 .00 e
AgNPs	76.83 a	3.01 a	7.30 a	7.22 a	1594.11 a	7.63 a	23.00 a
AgNPs + <i>F. solani</i>	59.06 f	2.50 f	6.50 f	6.31 f	1392.64 f	4.10 f	19.33 cd
Vitavax	63.33 e	2.63 e	6.86 e	6.82 e	1504.41 e	5.26 e	20.33 bc
Vitavax + <i>F. solani</i>	55.00 g	2.38 g	6.40 g	6.20 g	1369.11 g	3.80 g	19.33 cd
<i>T. harzianum</i>	69.63c	2.79 c	7.09 c	7.07 c	1560.29 c	6.40 c	22.33 a
<i>T. harzianum</i> + <i>F. solani</i>	47.90 i	2.19 i	6.19 i	5.98 i	1319.11 i	2.88 i	19.00 d
<i>B. subtilis</i>	73.26 b	2.90 b	7.22 b	7.16 b	1580.14 b	6.90 b	22.66 a
<i>B. subtilis</i> + <i>F. solani</i>	51.10 h	2.26 h	6.30 h	6.09 h	1343.38 h	3.23 h	19.00 d
LSD 0.05	1.07	0.02	0.40	0.32	7.05	0.23	1.07

Table V. – Chlorophyll pigments concentration ($\mu\text{g}/\text{cm}^2$) of tomato plants after (30, 45, and 60) days from transplanting showing the effect of by *Fusarium solani* and other treatments.

Treatments	Chlorophyll a $\mu\text{g} / \text{cm}^2$			Chlorophyll b $\mu\text{g} / \text{cm}^2$			Total chlorophyll $\mu\text{g} / \text{cm}^2$		
	30 days	45 days	60 days	30 days	45 days	60 days	30 days	45 days	60 days
Untreated plants	14.30 d	22.50 d	27.46 d	6.09 c	7.84 c	13.85 c	20.39 d	30.34 d	41.32 d
<i>F. solani</i>	8.10 j	16.41 h	16.05 i	1.94 f	3.67 g	8.02 g	10.04 i	20.09 h	24.07 i
AgNPs	18.18 a	29.11 a	32.51 a	9.12 a	13.87 a	17.74 a	27.30 a	42.99 a	50.26 a
AgNPs + <i>F. solani</i>	11.80 f	20.87 ef	24.59 f	5.67 c	6.63 d	11.76 e	17.47 e	27.51 ef	36.35 f
Vitavax	12.70 e	21.85 de	25.77 e	5.69 c	6.57 de	12.85 d	18.39 e	28.42 e	38.62 e
Vitavax + <i>F. solani</i>	11.16 g	21.09 e	23.78 fg	3.89 d	5.64 ef	11.40 e	15.08 f	26.73 f	35.18 f
<i>T.harzianum</i>	15.31 c	25.61 c	29.23 c	7.18 b	8.07 c	14.14 c	22.50 c	33.36 c	43.37 c
<i>T. harzianum</i> + <i>F. solani</i>	9.26 i	19.06 g	22.13 h	2.93 e	4.93 f	9.10 f	12.19 h	24.00 g	31.33 h
<i>B. subtilis</i>	16.43 b	26.61 b	30.55 b	7.95 b	10.14 b	16.69 b	24.39 b	36.76 b	47.24 b
<i>B. subtilis</i> + <i>F. solani</i>	10.22 h	19.66 fg	23.42 g	3.15 de	5.27 f	9.64 f	13.42 g	24.93 g	33.07 g
LSD 0.05	0.39	1.23	0.90	0.81	0.98	0.73	1.07	1.49	1.35

Table VI. – Anatomical features of the tomato stems of infected & uninfected plants showing the effect of Vitavax, AgNPs, *B. subtilis* and *T. harzianum* treatments compared to the untreated plants

Diameter (μm)		Thickness (μm)				Treatments
Stem	Xylem vessels	Xylem tissue	Phloem tissue	Cortex tissue	Epidermis	
4980.59 d	78.51 d	459.22 d	54.75 d	367.66 d	25.51 d	Untreated plants
4857.85 j	29.87 j	381.30 j	22.22 j	280.57 j	7.62 j	<i>F. solani</i>
5022.07 a	97.37 a	491.38 a	71.04 a	392.40 a	39.35 a	AgNPs
4940.42 f	57.12 f	460.50 f	46.43 f	348.83 f	19.20 f	AgNPs + <i>F. solani</i>
4958.40 e	72.76 e	449.49 e	50.47 e	356.80 e	21.42 e	Vitavax
4922.97 g	51.70 g	417.45 g	43.14 g	339.05 g	16.48 g	Vitavax + <i>F. solani</i>
4998.28 c	83.59 c	468.91 c	60.43 c	375.12 c	30.66 c	<i>T. harzianum</i>
4888.81 i	37.61 i	398.22 i	33.42 i	306.83 i	10.67 i	<i>T. harzianum</i> + <i>F. solani</i>
5013.52 b	90.39 b	478.23 b	65.46 b	382.94 b	35.40 b	<i>B. subtilis</i>
4908.46 h	45.93 h	409.00 h	38.51 h	321.54 h	13.29 h	<i>B. subtilis</i> + <i>F. solani</i>
4.40	2.51	3.87	2.90	5.30	1.59	LSD 0.05

Fig. 4. – Effect of Vitavax, AgNPs, *Bacillus subtilis* and *Trichoderma harzianum* on proline content in tomato leaves in the presence or absence of *Fusarium solani*.

On the other hand, the application of AgNPs on plants infected by *F. solani* enhanced vegetative growth parameter compared to the infected/untreated experiment. The obtained results came in agreement with Mahdizadeh *et al.* (2015) who indicated that silver nanoparticles caused cell death of pathogenic fungi. The positive effects of AgNPs were attributed to its toxicity caused by the discharge of Ag^+ according to Piccapietra *et al.* (2012). In addition, silver nanoparticles impaired cell division and induced cell disintegration according to Yin *et al.* (2012), as well as its ability to function as a catalyst forming reactive oxygen species (ROS) due to their physiochemical features as stated by Choi *et al.* (2008). Moreover, Kim *et al.* (2007) reported that free radicals have been shown to develop on the surface of AgNPs. Other potential toxicity pathways including interactions with thiol-groups in essential proteins, as well as enzymes involved in cellular respiration and ion transport as reported by Levard *et al.* (2012).

Vitavax treatment of plants infected by *F. solani* showed a significant increase in growth parameters compared to the infected/untreated experiment which came in accordance with Abu-Taleb & Al-Mousa (2008) who stated that fungicides inhibit the pathogenic fungi by changing and inhibiting cell metabolism. Carboxin has been shown to lower glucose oxidation by 50 percent, acetate

oxidation by 70-90 percent, and DNA, RNA, and protein production by up to 60-90 percent. Approximately, 90 % of the fungal growth is inhibited by these effects. Moreover, in the respiratory chain, certain fungicides prevent electron transport between cytochrome b and cytochrome c, which drastically reduces the production of aerobic energy and prevents fungal growth.

The treatment of *B. subtilis* on plants infected by *F. solani* showed inhibition of fungus growth and increase of growth parameters compared to the infected/untreated experiment. *B. subtilis* can secrete hydrolytic enzymes and antifungal metabolites such as subtilin, bacitracin, bacillin, and bacillomycin, all of which have the ability to suppress fungal growth as reported by Montealegre *et al.* (2003).

The application of *T. harzianum* plants infected by *F. solani* increased the vegetative growth parameters compared to the infected/untreated experiment, similar results were reported by Elkhwaga *et al.* (2018) who informed that *Trichoderma* species can be used as a bio-control agent in controlling a number of plant diseases with high efficacy.

Physiological and anatomical features

The presented data in Table V indicated that *F. solani* had a negative impact on photosynthetic pigments as chlorophyll pigments (chlorophyll a, b and total chlorophyll) tend to decrease in the infected plants. On the other hand, chlorophyll pigments increased in treated plants with AgNPs compared to infected/untreated plants as a result of AgNPs induction of significant stimulation in pigment fractions contents of tomato plant according to Pandey *et al.* (2014) who noticed that chlorophyll content increased with the application of AgNPs, which could significantly promote photosynthesis. The application of *B. subtilis* followed by *T. harzianum* and Vitavax treatments, respectively, increased chlorophyll pigments (chlorophyll a, b and total chlorophyll) compared with untreated plants.

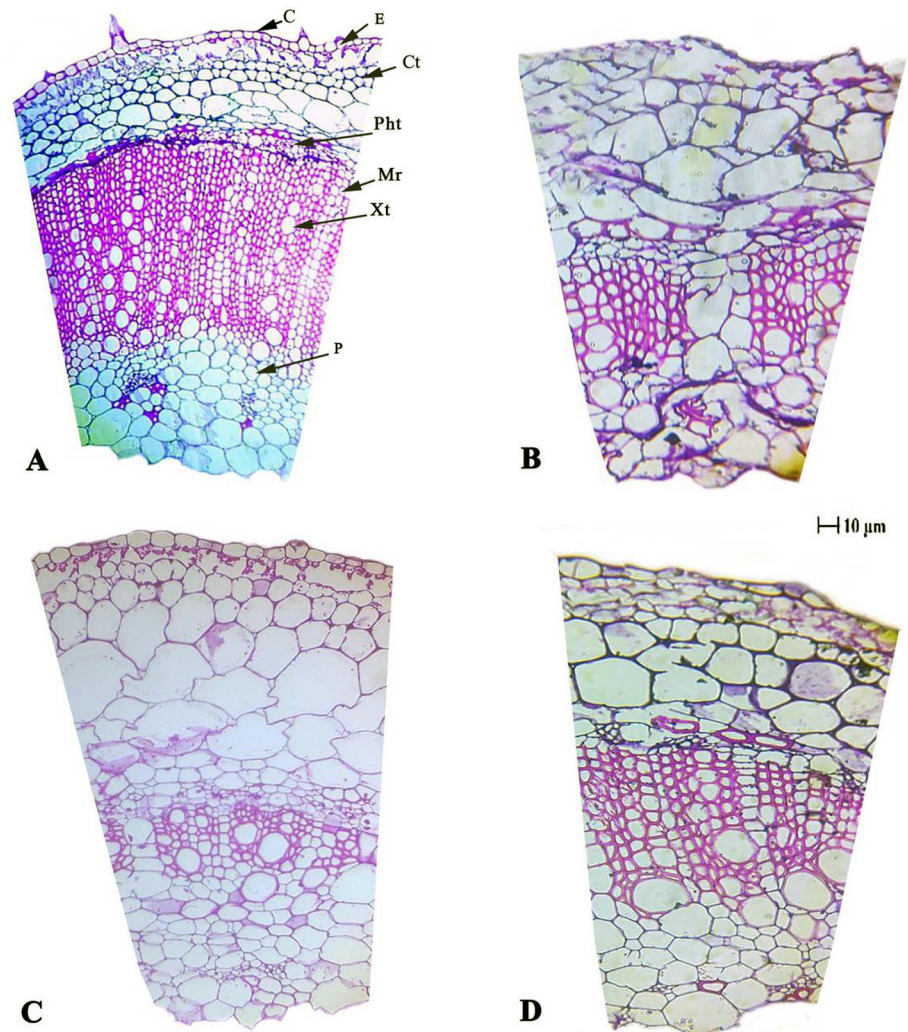


Fig. 5. – Transverse sections through the tomato stems of infected & uninfected plants showing the effect of Vitavax treatment compared to the untreated plants. **A:** Untreated plants; **B:** *Fusarium solani*; **C:** Vitavax; **D:** *Fusarium solani* + Vitavax. C, cuticle, E, epidermis, Ct, cortex tissue, Pht, phloem tissue, Xt, xylem tissue, MR, myeloid rays, P, pith.

The obtained data in Fig. 4 showed that the highest values of proline concentration were recorded in infected plants with *F. solani* compared with untreated plants. Abdelsalam & Kandil (2016) reported similar findings and stated that proline is produced from glutamate due to a loss of feedback control in the proline biosynthesis pathway.

The application of AgNPs on plants infected by *F. solani* decreased proline concentration compared to the infected/untreated experiment, these results agreed with (Sondi & Salopek-Sondi 2004) who reported that the cell membrane could be deteriorated due to the formation of “pits” in the cell wall and the accumulation of AgNPs in the cell membrane, with such morphology, the membrane exhibits a significant increase in permeability and irregular transport mechanism resulting in the cell death eventually, as plant recovered the fungal stress after the death of fungal cells, the concentration of proline is decreased consequently as its accumulation is considered a plant resistance to stress factor.

The treatment of *B. subtilis* on plants infected by *F. solani* reduced proline concentration compared to the

infected/untreated experiment, similar findings were showed by Ashwini & Srividya (2014), who reported that *Bacillus* sp. secretes hydrolytic enzymes such as chitinase and glucanase, which play an important role in fungal growth control by degrading the fungal cell wall.

The treatment of *T. harzianum* on plants infected by *F. solani* decreased the concentration of proline compared to the infected/untreated experiment whereas the ability of *T. harzianum* as bio-agent to control the wilt pathogen reduces the stress on the plant, wherefore the proline level decreased according to Sain & Pandey (2016). The lowest concentration of proline was recorded in the treated plants with AgNPs compared to untreated plants.

Results indicated in Table VI and Fig. 5 showed that *Fusarium solani* had a negative impact on the anatomical measurements of the infected plants including reduction of the epidermis thickness, cortex tissue, phloem tissue and xylem tissue, as well as the diameter of xylem vessels and stem compared to the healthy plants. On the other hand, the application of AgNPs treatment improved the anatomical measurements of tomato stems compared to the untreated plants, these improvements might be

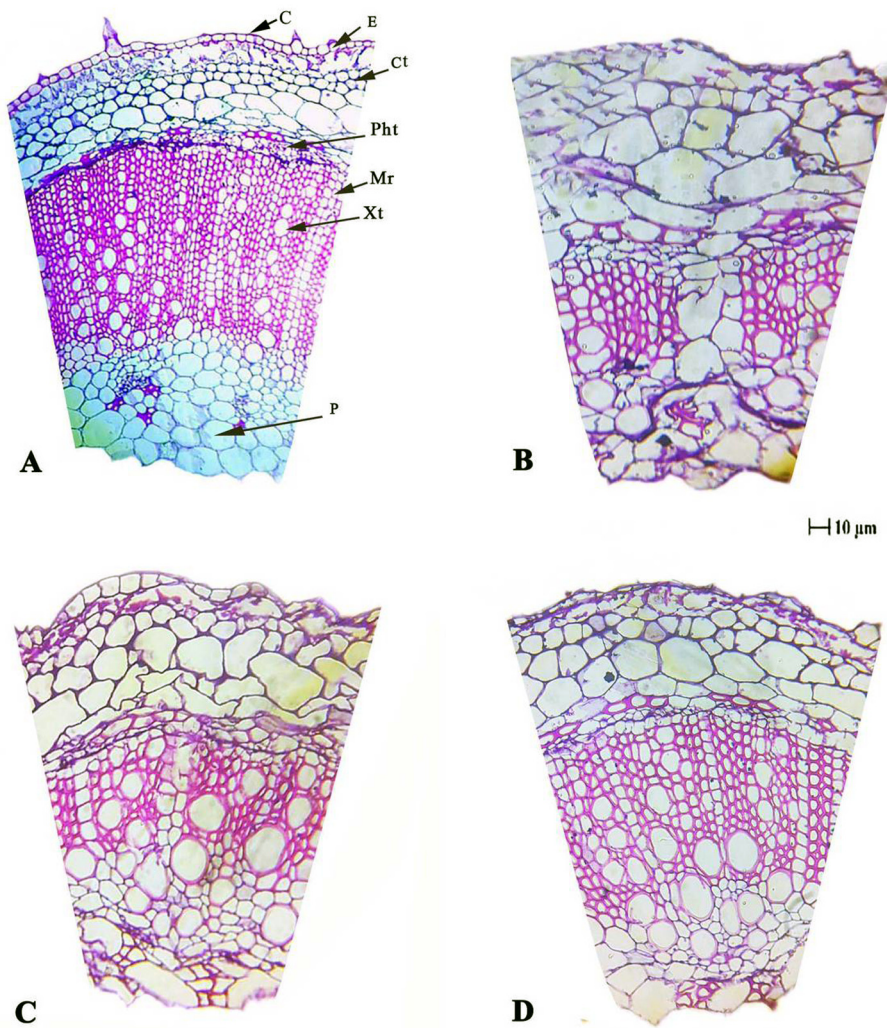


Fig. 6. – Transverse sections through the tomato stems of infected & uninfected plants showing the effect of AgNPs treatment compared to the untreated plants. **A:** Untreated plants; **B:** *Fusarium solani*; **C:** AgNPs; **D:** *Fusarium solani* + AgNPs.

attributed to the effect of AgNPs on the physiological processes in plants such as ions uptake, cell elongation, and cell division according to Vishwakarma *et al.* (2017), as shown in Fig. 6.

The treated plants with *B. subtilis* showed an improvement in the anatomical features compared to the untreated plants as shown in Fig. 7, this improvement is attributed to the plant-growth-promoting substances including IAA, gibberellins, cytokinins and spermidines synthesized by *Bacillus* spp. which play an important role in increasing root and shoot cell division and elongation as indicated by Radhakrishnan & Lee (2016). Results indicated that the treatment of *T. harzianum* promoted the anatomical aspects of tomato stems compared to the untreated plants as shown in Fig. 7, these findings are consistent with the influence of *T. harzianum* inoculation on the anatomical measurements of tomato stems according to Hermosa *et al.* (2012) who found that the beneficial effects of *Trichoderma* spp. can be divided into direct and indirect impacts whereas the immediate benefits comprised the stimulation of plant development as well as promoting and improving the plant root growth and structure.

Results presented in Fig. 6 showed that the application of AgNPs on infected plants by *F. solani* resulted in lowering the risk of tissues degradation in tomato stems compared to infected/untreated plants, these results were consistent with the findings of Song *et al.* (2012) who indicated that nanoparticles exhibited a variety of improvements on the translocation in various regions of plants as well as enhancing the morphological and physiological traits where the morphological features have been directly connected with the physiological attributes of plants.

Vitavax treatment of infected plants by *F. solani* reduced the severity of fungal infection and improved the anatomical features of the plant compared to infected/untreated plants as reported by Abu-Taleb & Al-Mousa (2008). Furthermore, *B. subtilis* and *T. harzianum* treatments of infected plants by *F. solani* increased the thickness of epidermis, cortex tissue, phloem tissue, xylem tissue and diameter of xylem vessels and stem compared to infected/untreated plants as showed in Fig. 7.

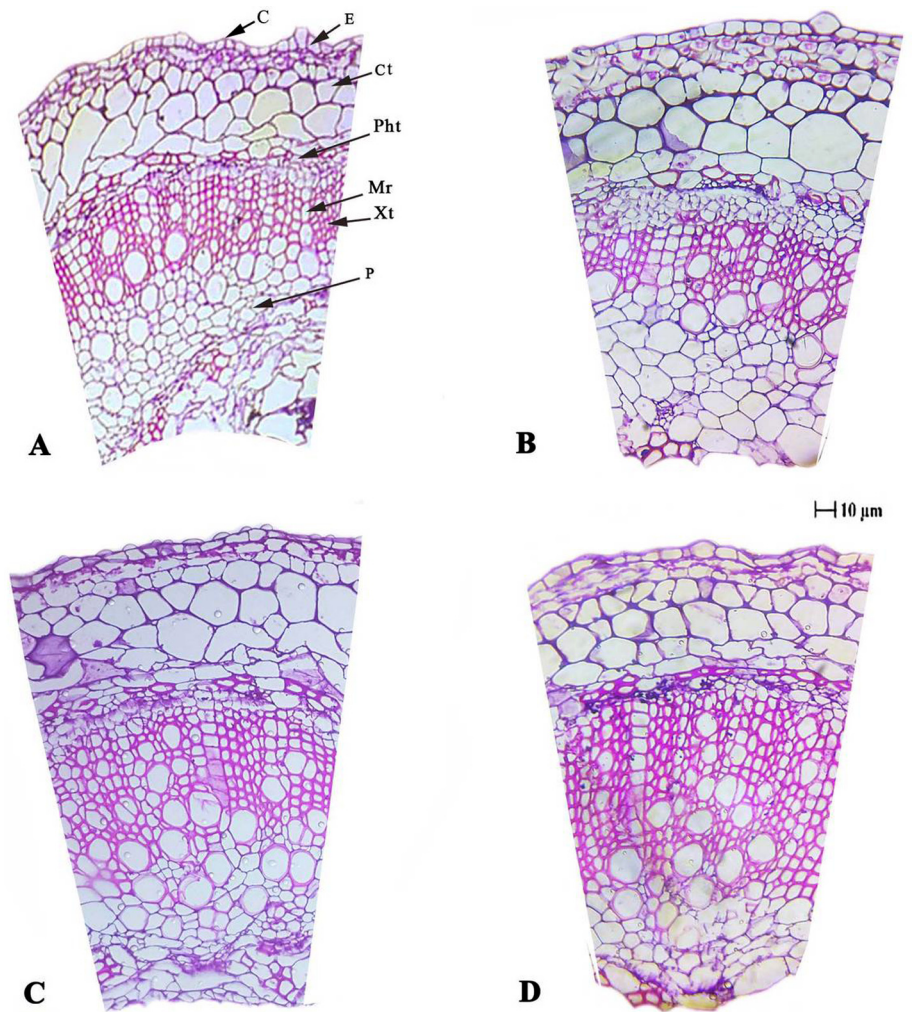


Fig. 7. – Transverse sections through the tomato stems of infected & uninfected plants showing the effect of *B. subtilis* and *T. harzianum* treatment. **A:** *Trichoderma harzianum*; **B:** *Fusarium solani* + *Trichoderma harzianum*; **C:** *Bacillus subtilis*; **D:** *Fusarium solani* + *Bacillus subtilis*.

CONCLUSION

In comparison to fungicides, which pose a serious threat to the environment and human health, biosynthetic nanosilver might be an optimal strategy for the control of *Fusarium solani* infection of tomato plants, since AgNPs achieved the highest vegetative growth parameters, plant productivity in addition to its capability of inhibiting fungal infection as an effective treatment. Furthermore, the biological treatments including *Trichoderma harzianum* and *Bacillus subtilis* modeled a functional approach for the fungal infection control.

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