Evaluation of biotic and abiotic elicitors to control *Meloidogyne incognita* infecting tomato plants

Abd El-Monem M.A. Sharaf [1], Atef M. Kailla [2], Mohamed S. Attia [1]* and Mohamed M. Nofal [1]

[1.] Botany and Microbiology Department, Faculty of Science, Al-Azhar University, 11884 Nasr City, Cairo, Egypt
[2.] Nematology department plant pathology research institute, Agric. Research Center, Giza, Egypt
*drmohamedsalah.92.@yahoo.com

Abstract: A pot experiment was conducted in the experimental farm station of plant pathology research institute during 2015 season at three application times for biotic and a biotic elicitors, to evaluate the efficient antagonistic bacterial strains *Bacillus subtilis*, *Serratia marcescens*, Cyanobacterial strain *Spirulina platensis* and silver nanoparticles (AgNPs) against root-knot nematode *Meloidogyne incognita* on tomato plants. Second stage juveniles/250 g soil, Females/1 g root, and Developmental stages/1 g root, Photosynthetic pigments and phytochemicals as response to induction of SAR in tomato plants were recorded. The results demonstrated that root-knot nematode *Meloidogyne incognita* challenged plants emerged from *B. subtilis*, *S. marcescens*, *S. platensis* and AgNPs showed reduction in the level of second stage juveniles in soil, female and developmental stages of *Meloidogyne incognita*. Application of *B. subtilis* at the same time of inoculation of *M. incognita* recorded 90.48% reduction of average number of females. Significant improvements in tomato plants were obtained due to the used *B. subtilis*, *S. marcescens*, *S. platensis* and AgNPs. On contrary, considerable reductions in all tested parameters were occurred as a result of the *M. incognita* infection. Application of *S. platensis* one week before infection gave the most potent effect as regard the chlorophyll (a) as well as total chlorophyll (a+b) when being compared with other treatments as being compared with healthy ones. While, application of AgNPs (especially at the same time of infection or one week after infection) was more effective in increasing the contents of chlorophyll (a), (b) as well as total chlorophyll (a+b). The beneficial effects of the used treatments were extended to increase not only total phenol and free proline but also the activities of peroxidase and polyphenoloxidase enzymes in comparison with control plants. On the other hand, the results appeared that tomato plants treated with inducers show variability in number of polypeptide peroxidase and polyphenol oxidase isozymes in the leaves especially one week before infection or one week after infection according to the type of used elicitors.

[Sharaf AMA, Kailla AM, Attia MS and Nofal MM. **Evaluation of biotic and abiotic elicitors to control *Meloidogyne incognita* infecting tomato plants.** *Nat Sci* 2016;14(11):125-137]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 19. doi:10.7537/marsnsj141116.19.

Keywords: Cyanobacterial; *Bacillus subtilis*; Silver nanoparticles; Nematodes

1. Introduction

Root-knot nematodes are serious pathogens that severe damage to major crops. They damage plant root system which caused significant yield losses. Moreover, the predisposition of nematode-infected plants is secondary infection from fungal plant pathogen that additional adverse effects on plant growth (Ruanpanun *et al.,* 2010). They cause serious damage to many crops worldwide. Abou-Aly *et al.* (2015) reported that root-knot nematodes are serious pathogens that severe damage to major crops. Some specific bio-inoculants of *Bacillus spp.* and *Pseudomonas spp.* eliciting significant inhibition in the incidence or severity of various diseases on a diversity of hosts through plant defense activation (Chandra *et al.*, 2007). The induction of plant resistance using non-pathogenic or incompatible microorganisms is also a form of biological control (Schouten *et al.*, 2004). Plant treatments with various biotic and abiotic agents can induce resistance against subsequent pathogen attack (Walters *et al*., 2005). The bacterial strains *Paenibacillus polymyxa*, *Bacillus megaterium* and *Bacillus circulans* showed the highest protease, chitinase and gelatinase activities which might help to explain the way how the bacteria could act against the root-knot nematodes. It should be stated that the highest nematicidal activity exhibited by their strains against the second stage juveniles of *Meloidogyne incognita*. These strains proved to be the most efficient isolates as biofertilizers and nematicidal agents (El-Hadad *et al.*, 2010). Nanoparticles usually have better or different qualities than the bulk material of the same element. In the case of silver nanoparticles the antibacterial effect is greatly enhanced and because of their tiny size. Nanoparticles have immense surface area relative to volume. Therefore minuscule amounts of Silver Nanoparticles can lend antimicrobial effects to hundreds of square meters of its host material (Theivasanthi and Alagar, 2011).

The aims of this work to study the impact role of bacterial strains *Bacillus subtilis*, *Serratia marcescens*, Cyanobacterial strain *Spirulina platensis* and silver nanoparticles (AgNPs) against root-knot

nematodes, (*M. incognita*), which considered among the most difficult crop pests to control.

2. Material and Methods

2.1. Plant material

Four weeks-Tomato seedlings (*Solanum Lycopersicon* L. cv. Castle rock II PVP) were obtained from agricultural research center (ARC), ministry of agriculture, Giza, Egypt.

2.2. Root-knot nematodes (*Meloidogyne incognita*)

The nematode population used in this research originated from green house culture maintained at the plant pathology research, were *Meloidogyne incognita* chitwood was reared in a green house on tomato plants. Eggs of *M. incognita* were extracted from roots in 0.5 % sodium hypochlorite (Hussey and Barker, 1973) and caught on a 25 µm sieve. Second stage Juveniles (J2) were hatched from these eggs on Baermann funnels and only (J2) less than 2 days old were used for experimentation.

2.3. Source and application methods of inducers

Tow bacterial strains namely *Bacillus subtilis* and *Serratia marcescens* which recorded the highest values for nematicidal activities as well as maximum hydrolysis zone values of gelatinase, protease and chitinase (Bahloul, 2013) were selected. Bacterial inocula were prepared using poly broth medium (Bourgouin *et al.*, 1984). *B. subtilis* strain was isolated from Egyptian Soils and identified by Bio log Technique at Biofertelizer production unit, Soil, Water and Environment Research institute, Agricultural Research Center (ARC) Giza Egypt. The concentration of *B. subtilis* in suspension was counted by most probable number (MPN). *Serratia marcescens* strain isolated from Egyptian Soils. It was produced by Soils, Water and Environ. Res. Inst. Agriculture research center, and distributed on a commercial scale (trade name, Nemaless). The concentration of *B. subtilis* or *S. marcescens* in suspension was counted by most probable number (MPN). The inocula suspension was approximately adjusted to 10^9 CFU/ml culture (colony formation unit).

2.4. Alga strain source and growth conditions

Cyanobacterial strain *Spirulina platensis* was obtained from the Microbiology Department; Soils, Water and Environment Research Institute (SWERI), Agricultural Research, Center (ARC). The alga was grown on Zarrouk medium (Zarrouk, 1966) and was incubated in growth chamber under continuous illumination (2000 lux) at 35˚C± 2˚C for 30 days. The inocula suspension was approximately adjusted to 10^9 CFU/ml culture (colony formation unit).

2.5. Silver nanoparticles (AgNPs)

Biosynthesis of silver nanoparticles (AgNPs) of the *Streptomyces cyanoalbus*, (100ppm) was carried out according to method described by (Kalishwaralal *et al*., 2008; El-Batal *et al*., 2013) with slight modification.

2.6. Greenhouse experiment

Tomato seedlings were transplanted to 20 cm dian plastic pots filled with autoclaved sandy loam soil (1:1,V:V) each pot contained one tomato plant. Biotic and biotic elicitors (*Bacillus subtilis*, *Serratia marcescens*, *Spirulina platensis* and silver nanoparticles (AgNPs) were added at three times. The first treatment applied 7 days before inoculation with 2000 second stage juveniles of *M. incognita*, the second treatment applied at the same time of inoculation with 2000 *M. incognita* second stage juveniles and the third treatment applied 7 days after inoculation with 2000 second stage juveniles of *M. incognita*. Five plastic pots with tomato seedlings inoculated with 2000 second stage juveniles of *M. incognita* and left untreated with elicitors. Also, five plastic pots with tomato seedlings each left untreated with elicitors and uninoculated with nematode that served as control. Each treatment was replicated five times and all treatments were arranged in a complete randomized block design. Pots were kept in the greenhouse at 25±5 receiving water and ordinary nutrient solution as required. Tomato plants were harvested 60 days after nematode inoculation. Plants were removed from pots and roots were washed free of soil. Roots were stained in lactic acid fuchsin (Byrd *et al.,* 1983) and examined for the number of developmental stages and females in 1 g of root. Number of juveniles of *M. incognita* in 250 g of soil were extracted by sieves and Baermann funnels and counted. Reduction percentages of root knot nematode developmental stages, females and second stage juveniles number in comparison with nematode only.

2.7. Determination of pigments

The method used for the quantitative determination of pigments was that of Vernon and Selly (1966).

2.8. Determination of phytochemicals

Determination of phenolic compounds (mg/100g of fresh w.t.) was carried out according to that method described by Daniel and George (1972). Contents of free proline (mg/100g of dry w.t.) were determined according to the method described by Bates *et al.* (1973).

Peroxidase activity was assayed using solution containing 5.8 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of the enzyme extract and 2 ml of 20 mM H_2O_2 after addition of 2 ml of 20 mM pyrogallol, the rate of increase in absorbance as pyrogallol was determined spectrophotometric all by UV spectrophotometer (Jenway) within 60 second at 470 nm and 25^{o}C (Srivastava, 1987). The activity of polyphenol oxidase

enzyme was determined according to the method adopted by Matta and Dimond (1963).

2.9. Statistical analyses

Experimental data were subjected to one way analysis of variance (ANOVA) and the differences between means were separated by the least significant difference (L.S.D) at 5% level of probability using M state software (Snedecor and Cochran, 1982).

3. Results

3.1. Effect of tested elicitors and time of application on, Second stage juveniles/250 g soil, Females and Developmental stages/1 g root in infested tomato with *M. incognita*

The obtained results in table (1) revealed that the average numbers of Second stage juveniles/250 g soil were decreased according to the application of AgNPs or *S. marcescens* one week after the inoculation of *M. incognita* recording 86.66% and 120 average numbers of second stage juveniles, respectively. While, the application of AgNPs or *S. platensis* one week before the inoculation of *M. incognita* recording 113.33 average numbers of second stage juveniles. Also, application of *B. subtilis* at the same time with the inoculation of *M. incognita* recorded 100 average numbers of second stage juveniles.

Results in table (1) revealed that average numbers of females and development stages in 1g.root were decreased according the application of *B. subtilis* at the same time with inoculation of *M. incognita* recording 8 average numbers of females and development stages. While, the application of *S. platensis* one week before the inoculation of *M. incognita* recorded 15 and 11 average numbers of females and development stage, respectively.

Table 1. Impact of biotic and a biotic elicitors and time of application on second stage juveniles in soil, female and developmental stages of *Meloidogyne incognita* infecting tomato plants under greenhouse conditions

Treatment			Second stage juveniles/250 g soil		Females/1 g root		Developmental stages/1 g root	
Time of application	Material	Dose/pot	Average number	Red (%)	Average number	Red (%)	Average number	Red (%)
One week before infection	*B. subtilis*	2 ml	353.33 a	10.18	25.00 ef	70.24	28.00 c	50.00
	S. marcescens	2 ml	140.00 cd	64.38	48.00 c	42.86	41.00 b	26.79
	S. platensis	4 ml	113.33 d	71.25	15.00 gh	82.14	11.00 de	80.36
	AgNPs	2 ml	113.33 d	71.25	35.00 d	58.33	6.00 ef	89.29
At the same time of infection	*B. subtilis*	2 ml	100.00 d	74.55	8.00 hi	90.48	8.00 def	85.71
	S. marcescens	2 ml	240.00 b	38.93	21.00 efg	75.00	8.00 def	85.71
	S. platensis	4 ml	160.00 cd	59.29	17.00 fgh	79.76	9.00 def	83.93
	AgNPs	2 ml	133.33 cd	66.16	62.00 b	26.19	16.00 d	71.43
One week after infection	*B. subtilis*	2 ml	153.33 cd	61.07	30.00 de	64.29	11.00 de	80.36
	S.marcescens	2 ml	120.00 d	69.47	38.00 d	54.76	34.00 bc	39.29
	S. platensis	4 ml	206.66 bc	47.33	4.00 i	95.24	12.00 de	78.57
	AgNPs	2 ml	86.66 d	77.86	55.00 bc	34.52	11.00 de	80.36
Nematode only			393.33 a	0.00	84.00 a	0.00	56.00 a	0.00
L.S.D			67.208		9.995		9.051	

Results illustrated in Fig (1) show that using AgNPs one week after the inoculation of *Meloidogyne incognita* recorded 77.86% reduction of second stages juveniles. While, using *B. subtilis* at the same time inoculation of *Meloidogyne incognita* recorded 74.55% reduction of second stages juveniles. Also, application of *S. platensis* or AgNPs one week before the inoculation of *Meloidogyne incognita* recorded 71.25% reduction of second stages juveniles.

Data in Fig. (2) show that the application of *B. subtilis* at the same time inoculation of *M. incognita* recorded 90.48% reduction of average number of females. While, using *S. platensis* application one week before the inoculation of *M. incognita* recorded 82.14% reduction of number of females.

Data in Fig (3) show that the application of AgNPs one week before the inoculation of *M. incognita* recorded 89.29% reduction of average numbers of development stage. While, using *B. subtilis* or *S. marcescens* at the same time inoculation of *M. incognita* recorded 85.71% reduction of average numbers of development stage.

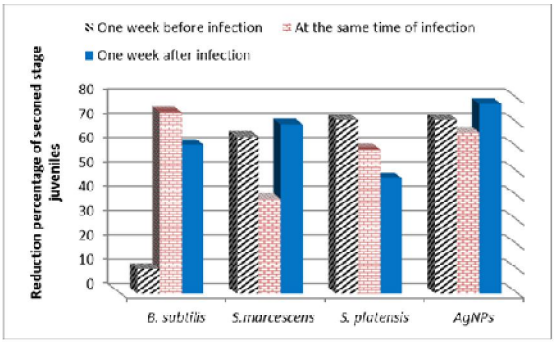

Figure 1. Effect of biotic and a biotic elicitors on percentage reduction of second stages juveniles

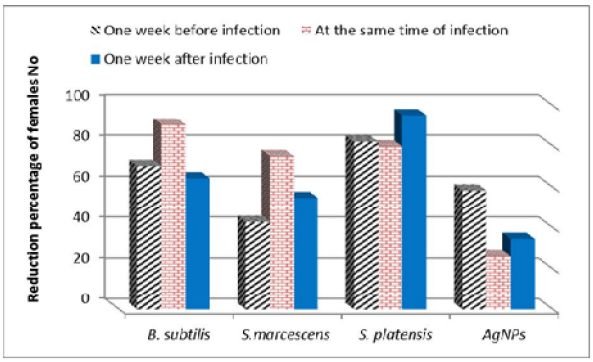

Figure 2. Effect of biotic and a biotic elicitors on percentage reduction of reduction of number of females

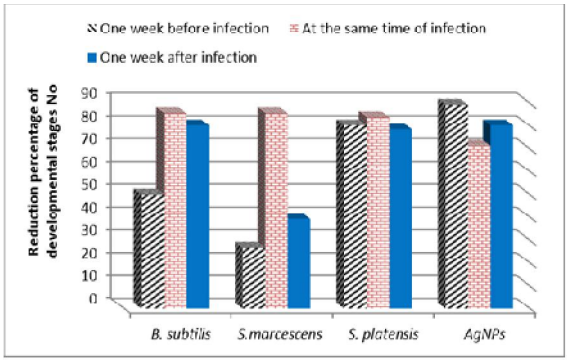

Figure 3. Effect of biotic and a biotic elicitors on percentage reduction of reduction of developmental stages

3.2. Photosynthetic pigments

Table 2. Effect of some elicitors and time of application and their interactions on chlorophyll (a), chlorophyll (b), total chlorophyll (a+b) and carotenoids of tomato plants, infected with *Meloidogyne javanica* under greenhouse conditions

Treatment			Chlorophyll (a) mg/g fresh weight	Chlorophyll (b) mg/g fresh weight	Chlorophyll (a+b) mg/g fresh weight	Carotenoids mg/g fresh weigh
Time of application	Material	Dose/pot				
One week before infection	*B. subtilis*	2 ml	9.82 i	4.23 g	14.05 h	2.92 h
	S. marcescens	2 ml	11.42 e	4.66 e	16.08 e	3.26 e
	S. platensis	4 ml	15.35 b	6.69 b	22.05 b	4.06 b
	AgNPs	2 ml	11.24 g	4.73 d	15.97 f	3.12 f
At the same time of infection	*B. subtilis*	2 ml	10.92 h	4.53 f	15.46 g	3.12 f
	S. marcescens	2 ml	6.75 l	2.73 k	9.48 k	2.12 j
	S. platensis	4 ml	8.24 j	3.74 h	11.99 i	2.32 i
	AgNPs	2 ml	11.34 f	4.76 d	16.1 e	3.05 g
One week After infection	*B. subtilis*	2 ml	13.81 d	5.64 c	19.45 d	3.7 d
	S.marcescens	2 ml	7.75 k	3.09 i	10.84 j	2.35 i
	S. platensis	4 ml	13.86 c	5.65 c	19.52 c	3.94 c
	AgNPs	2 ml	17.42 a	7.51 a	24.94 a	4.73 a
Nematode only			5.76 n	2.4 l	8.16 m	1.66 l
Control			6.33 m	2.9 j	9.24 l	1.85 k
LSD 5%			0.0426	0.0419	0.05388	0.04072

Results shown in table (2) revealed that contents of chlorophyll a, b as well as total chlorophyll a+b were highly significantly decreased in nematode-infected plants than that of healthy ones. At the same time, marked increases in the contents of carotenoids were observed in infected plants as being compared with healthy ones. On the other hand, the obtained results (Table 2) showed different responses as regards the photosynthetic pigments due to the application of different used elicitors. These results can be demonstrated as follows:

One week before infection it was noticed that (Table 2) application of *S. platensis* gave the most potent effect as regard the ch a as well as total chlorophyll a+b (15.35 & 22.05) in comparison with plants treated with *S. marcescens* (11.42 & 16.08), then followed by AgNPs (11.24 & 15.97) and *B. subtilis* (9.82 & 14.05), respectively. Also, it was found that nematode-infected plants pretreated with *S. platensis* gave the most potent effect as regard the ch b (6.69) in comparison with plants treated with AgNPs (4.73), then followed by *S. marcescens* (4.66) and *B. subtilis* (4 & 23), respectively. At the same time results in table (2) showed that application *S. platensis* gave the most potent effect as regard the contents of carotenoids (4.06) in comparison with plants treated with *S. marcescens* (3.26), then followed by AgNPs (3.12) and *B. subtilis* (2.92), respectively. Also, at the same time infection results in table (2) showed that application of AgNPs was more effective in increasing the contents of chlorophyll a, b as well as total chlorophyll a+b. Also, significant increases in the

contents of chlorophyll a, b as well as total chlorophyll a+b of nematode-infected plants were resulted in response to the treated with *B. subtilis* followed by *S. platensis*, then *S. marcescens*. Also, it was noticed that treatment with (AgNPs, *S. platensis, B. subtilis* and *S. marcescens*), respectively gave the most potent effect as regard the contents of carotenoids in nematode-infected plants as being compared with corresponding controls. While, one week after infection the obtained results (Table 2) revealed that treatment with AgNPs resulted in significant increase in the contents of chlorophyll a, b as well as total chlorophyll a+b of nematode-infected plants followed by treated with (*S. platensis*, *B. subtilis* and *S. marcescens*), respectively. At the same time results in table (2) showed that application AgNPs gave the most potent effect as regard the contents of carotenoids in comparison with plants treated with *S. platensis*, then followed by *B. subtilis* and *S. marcescens*, respectively.

3.3. Physiological and metabolic changes

Results in table (3) revealed that the changes in the activities of oxidative enzymes (POD, PPO), free proline content and total phenols in nematode-infected plants were significantly increased than that of non-infected ones (control). On the other hand, treatment with tested elicitors resulted in different responses as regards the oxidative enzyme activities, free proline content and total phenols of nematode-infected plants. These responses were varied according to the type of used elicitor and also to the time of application as follows:-

One week before infection it was noticed that (Table 3) application of AgNPs was more effective in stimulating POD activity before challenge followed by *B. subtilis* then *S. platensis* and *S. marcescens*. This stimulation was observed when compared with that of healthy (non-infected) plants. Also, at the same time infection results in table (3) showed that application of AgNPs was more effective in increasing POD activity followed by treated with (*B. subtilis, S. marcescens subtilis* and *S. platensis*, respectively. As being compared with corresponding controls.

Also, at the same time infection with *M. incognita* data describing increasing of free proline content of all treatments in relative to controls (Table3). It is quite evidence that, the greatest value was achieved by using *B. subtilis* on the infected plants followed by *S. marcescens* then AgNPs and *S. platensis*, respectively, more than on the healthy plants, indicating induction of systemic acquire resistant (SAR). Data generated in table (3) showed that treatment nematode infected plants with *S. marcescens* gave the highest amount of total phenol followed by (AgNPs, *S. platensis* and *B. subtilis*), respectively, more than on the healthy plants, and *S. platensis*), respectively. As being compared with healthy control. While, one week after infection the obtained results (Table 3) revealed that application of AgNPs was more effective in stimulating POD activity after challenge followed by *B. subtilis* then *S. platensis* and *S. marcescens*. This stimulation was observed when compared with that of healthy (control) plants.

For polyphenoloxidase (PPO) activities, one week before infection data describing increasing of PPO activity of all treatments in relative to controls (Table 3). It is quite evidence that, the greatest activities were achieved by using *B. subtilis* on the infected plants followed by AgNPs, then *S. marcescens* and *S. platensis* more than on the healthy plants, indicating induction of systemic acquire resistant (SAR). Also, at the same time infection data generated in table (3) showed the changes in the activities of PPO enzyme in tomato shoots in response to *M. incognita,* and tested elicitors. Treatment nematode infected plants with *S. platensis* gave the highest activities of PPO followed by (*S. marcescens*, *B. subtilis* and AgNPs), respectively. As being compared with healthy control. While, one week after infection data describing increasing of PPO activity of all treatments in relative to controls (Table 3). It is quite evidence that, the greatest activities were achieved by using *B. subtilis* on the infected plants followed by AgNPs, then *S. marcescens* and *S. platensis* more than on the healthy plants, indicating induction of systemic acquire resistant (SAR).

Concerning the effect of tested elicitors on the challenged plants with nematode, it was found that application of *B. subtilis* one week before inoculation of *M. incognita* show significant increase in free proline content of tomato shoots compared to *S. marcescens*, AgNPs and *S. platensis*, respectively. As being compared with corresponding controls. While, application of AgNPs one week before inoculation of *M. incognita* show significant increase in total phenols of tomato shoots compared to *S. marcescens*, *B.* indicating induction of systemic acquire resistant (SAR).

While, application of tested elicitors one week after infection data generated in table (3) showed that treatment nematode infected plants with *S. platensis* gave the highest amount of free proline content followed by (*S. marcescens*, *B. subtilis* and AgNPs), respectively, more than on the healthy plants, indicating induction of systemic acquire resistant (SAR). One week after infection application of *S. platensis* was more effective in increasing amount of total phenol followed by (*S. marcescens,* AgNPs and *B. subtilis*), respectively. This stimulation was observed when compared with that of healthy (non-infected) plants.

Table 3. Effect of some elicitors and time of application and their interactions on activity of peroxidase, polyphenoloxidase, shoots proline and Shoot phenols in tomato plants, infected with *Meloidogyne javanica* under greenhouse conditions

Treatment			Peroxidase (unit/g fresh wt/hour)	Polyphenol-oxidase (unit/g fresh wt/hour)	Shoot prolin (mg/g dry weight)	Shoot phenols (mg/100g dry weight)
Time of application	Material	Dose/pot				
One week before infection	*B. subtilis*	2 ml	3.46 gh	1.31 bcd	1.15 f	0.36 f
	S. marcescens	2 ml	2.08 i	0.85 ef	1.34 ef	0.39 ef
	S. platensis	4 ml	3.21 h	0.63 f	1.51 ef	0.36 f
	AgNPs	2 ml	4.1 fg	0.9 ef	1.73 e	0.45 cde
At the same time of infection	*B. subtilis*	2 ml	5.91 bc	1.51 ab	2.75 c	0.51 bc
	S. marcescens	2 ml	5.36 cd	1.6 ab	2.55 cd	0.59 a
	S. platensis	4 ml	4.99 de	1.77 a	3.2 b	0.55 ab
	AgNPs	2 ml	6.41 b	1.36 bc	3.25 b	0.57 ab
One week After infection	*B. subtilis*	2 ml	5.57 cd	1.49 ab	3.37 b	0.36 f
	S. marcescens	2 ml	4.85 de	1.07 cde	2.76 c	0.50 ab
	S. platensis	4 ml	5.43 cd	1.04 cde	3.82 a	0.55 ab
	AgNPs	2 ml	7.51 a	1.45 ab	3.25b	0.47 cd
Nematode only			4.48 ef	0.99 de	2.27 d	0.41 def
Free of nematode			0.51 j	0.13 g	0.53 g	0.09 g
LSD 5%			0.7285		0.3398	0.3985

3.4. Oxidative isozymes

Tomato plants treated with inducers show variability in number, relative mobility of polypeptide peroxidase and polyphenol oxidase isozymes in the leaves. These responses were varied according to the type of used elicitor and also to the time of application as follows:-

3.4.1. Peroxidase isozyme

Table 4. Disc-PAGE banding patterns of peroxidase isozymes in infected tomato plants and treated with inducers one week before infection

RF	*B. subtilis*	*S. marcescens*	*S. platensis*	AgNPs	control	Nematode only	Polymorphism
0.228	+	+	+	+	+	+	Monomorphic
0.269	+	-	+	+	-	-	Polymorphic
0.427	+	+	+	+	+	+	Monomorphic
0.558	+	+	+	+	+	+	Monomorphic
0.610	+	-	+	+	+	+	Polymorphic
0.716	-	-	-	-	+	-	Unique
0.728	+	+	+	-	-	+	Polymorphic
0.784	+	+	+	+	+	+	Monomorphic
0.863	+	+	+	+	+	+	Monomorphic
0.892	-	-	-	-	-	+	Unique
0.937	+	-	+	+	+	+	Polymorphic
Total bands	9	6	9	8	8	9	

One week before the inoculation of *Meloidogyne javanica* data revealed that total numbers of peroxidase isozymes were 9 bands appeared in tomato healthy in table (4) and Fig. (4). The inducers and nematode-infection treatments showed variation in number polypeptide bands compared with healthy ones. Nematode only gave 8 Isozymes, while *B. subtilis* increased the number of isozymes (9 isozymes), *S. marcescens* gave (6 isozymes) and *S. platensis* gave (9 isozymes) as well as AgNPs gave (8 isozymes).

Also, at the same time the inoculation of *Meloidogyne javanica* results showed that total numbers of peroxidase isozymes were 6 bands appeared in tomato healthy in table (5) and Fig. (4). The inducers and nematode-infection treatments showed variation in number polypeptide bands compared with healthy ones. Nematode only gave 3 Isozymes, while *B. subtilis* increased the number of

isozymes (6 isozymes), *S. marcescens* gave (8 isozymes) and *S. platensis* gave (8 isozymes) as well as AgNPs gave (5 isozymes).

While, one week after the inoculation of *Meloidogyne javanica* data generated in table (6) showed that total numbers of peroxidase isozymes were 2 bands appeared in tomato healthy. The inducers and nematode-infection treatments showed variation in number polypeptide bands compared with healthy ones. Nematode only gave 2 Isozymes, while *B. subtilis* increased the number of isozymes (3 isozymes), *S. marcescens* gave (3 isozymes) and *S. platensis* gave (1 isozyme), while AgNPs gave (0 isozyme).

Table 5. Disc-PAGE banding patterns of peroxidase isozymes in infected tomato plants and treated with inducers at the same time of infection

RF	*B. subtilis*	*S. marcescens*	*S. platensis*	AgNPs	control	Nematode only	Polymorphism
0.217	+	+	+	+	-	+	Polymorphic
0.401	+	+	+	+	+	+	Monomorphic
0.529	+	+	+	+	+	+	Monomorphic
0.577	+	+	+	-	-	+	Polymorphic
0.730	+	+	+	+	+	+	Monomorphic
0.795	+	+	+	+	-	+	Polymorphic
0.837	-	+	+	-	-	-	Polymorphic
0.881	-	+	+	-	-	-	Polymorphic
Total bands	6	8	8	5	3	6	

Table 6. Disc-PAGE banding patterns of peroxidase isozymes in infected tomato plants and treated with inducers one week after infection

RF	*B. subtilis*	*S. marcescens*	*S. platensis*	AgNPs	Control	Nematode only	Polymorphism
0.449	+	+	-	-	-	-	Polymorphic
0.600	+	+	+	-	+	+	Polymorphic
0.781	+	+	-	-	+	+	Polymorphic
Total bands	3	3	1	0	2	2	

3.4.2. Polyphenol oxidase isozyme

One week before the inoculation of *Meloidogyne javanica* data revealed that total numbers of polyphenol oxidase isozymes were 3 bands appeared in tomato healthy in table (7) and Fig. (5). The inducers and nematode-infection treatments showed variation in number polypeptide bands compared with healthy ones. Nematode only gave 1 Isozyme, while *B. subtilis* increased the number of isozymes (4 isozymes), *S. marcescens* gave (4 isozymes) and *S. platensis* gave (4 isozymes) as well as AgNPs gave (3 isozymes).

Also, at the same time the inoculation of *Meloidogyne javanica* results showed that total numbers of polyphenol oxidase isozymes were 4 bands appeared in tomato healthy in table (8) and Fig. (5). The inducers and nematode-infection treatments showed no variation in number polypeptide bands compared with healthy ones. While, one week after the inoculation of *Meloidogyne javanica* data generated in table (9) and Fig. (5) show that total numbers of polyphenol oxidase isozymes were 6 bands appeared in tomato healthy (control). The inducers and nematode-infection treatments showed variation in number polypeptide bands compared with healthy ones. Nematode only gave 5 Isozymes, while *B. subtilis* increased the number of isozymes (6 isozymes), *S. marcescens* gave (7 isozymes) and *S. platensis* gave (4 isozymes) as well as AgNPs gave (3 isozymes).

Table 7. Disc-PAGE banding patterns of PPO isozymes in infected tomato plants and treated with inducers one week before infection

RF	*B. subtilis*	*S. marcescens*	*S. platensis*	AgNPs	Control	Nematode only	Polymorphism
0.415	+	+	+	+	-	+	Polymorphic
0.549	+	+	+	+	-	+	Polymorphic
0.761	+	+	+	+	+	+	Monomorphic
0.824	+	+	+	-	-	-	Polymorphic
Total bands	4	4	4	3	1	3	

Table 8. Disc-PAGE banding patterns of PPO isozymes in infected tomato plants and treated with inducers at the same time of infection

RF	*B. subtilis*	*S. marcescens*	*S. platensis*	AgNPs	Control	Nematode only	Polymorphism
0.402	+	+	+	+	+	+	Monomorphic
0.548	+	+	+	+	+	+	Monomorphic
0.774	+	+	+	+	+	+	Monomorphic
0.839	+	+	+	+	+	+	Monomorphic
Total bands	4	4	4	4	4	4	

Table 9. Disc-PAGE banding patterns of PPO isozymes in infected tomato plants and treated with inducers One week after infection

RF	*B. subtilis*	*S. marcescens*	*S. platensis*	AgNPs	Control	Nematode only	Polymorphism
0.160	-	+	-	-	+	+	Polymorphic
0.238	+	+	-	-	-	-	Polymorphic
0.456	+	+	+	+	+	+	Monomorphic
0.588	+	+	+	+	+	+	Monomorphic
0.776	+	+	+	+	+	+	Monomorphic
0.892	+	+	-	-	-	+	Polymorphic
0.821	+	+	+	-	+	+	Polymorphic
Total bands	6	7	4	3	5	6	

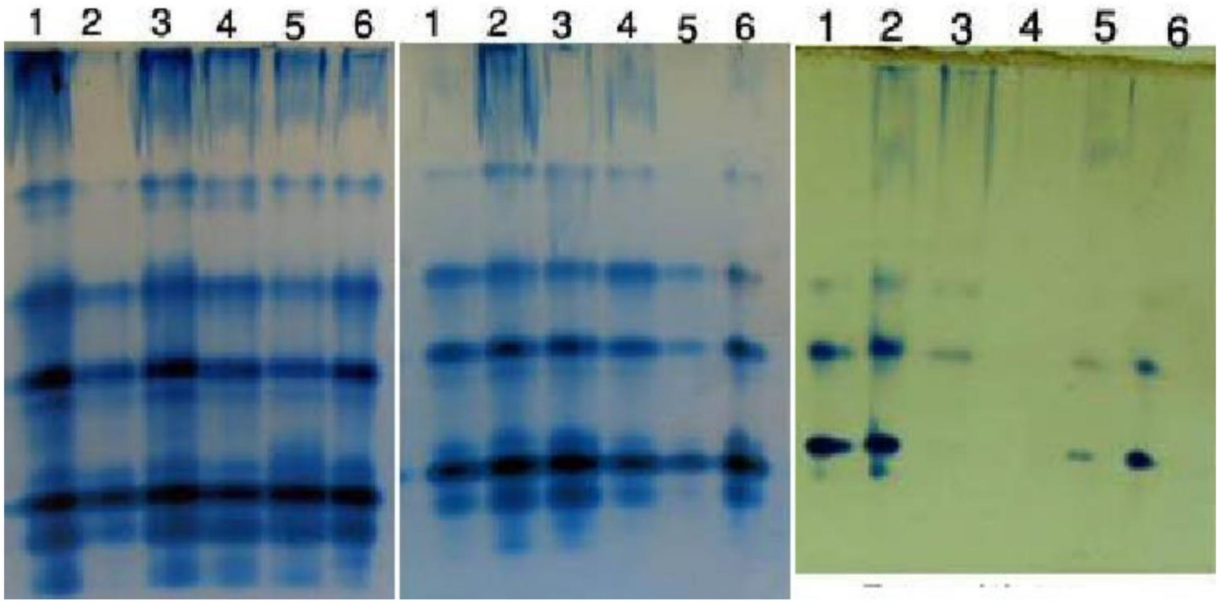

Figure 4. Native acrylamide gel (10%) electrophoresis of POD isozymes produced in infected tomato plants treated with inducers. 1: *B. subtilis*. 2: *S. marcescens.* 3: *S. platensis.* 4: AgNPs. 5: Nematode only. 6: control

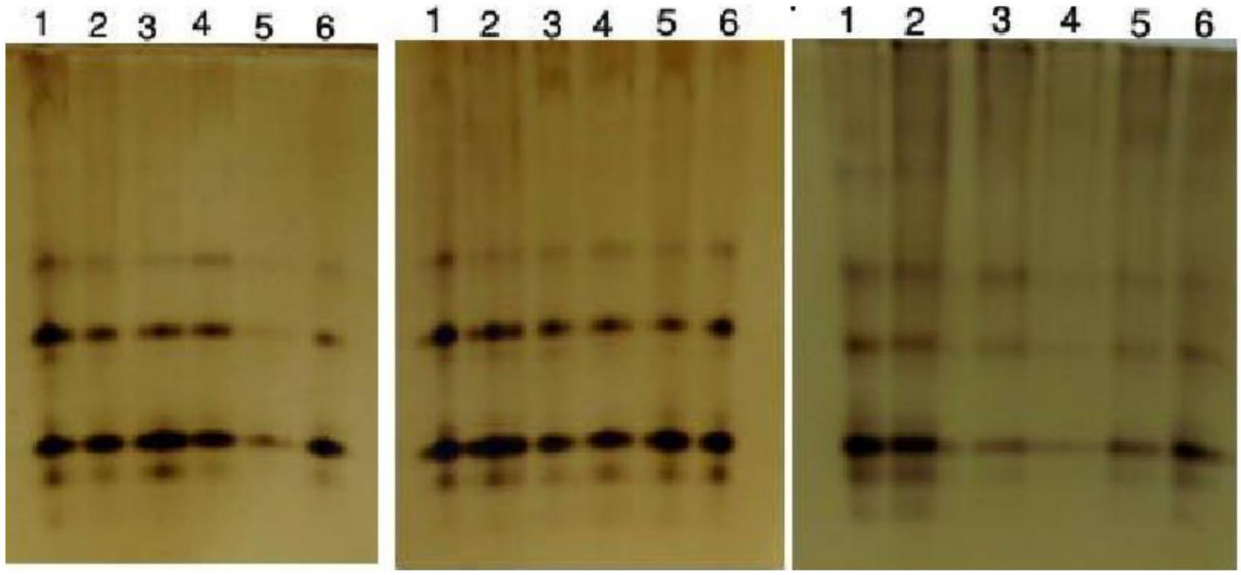

Figure 5. Native acrylamide gel (10%) electrophoresis of PPO isozymes produced in infected tomato plants treated with inducers. 1: *B. subtilis*. 2: *S. marcescens*. 3: *S. platensis*. 4: AgNPs. 5: Nematode only. 6: control

4. Discussion

The objectives of this study were induction of systemic resistance in tomato plants against root-knot nematode (*M. incognita*) infection. The first criterion to judge the occurrence of SAR in tomato plants treated with biotic or a biotic inducers. The reduction percentage of infection, tested inducers were able to reduction of second stages juveniles, reduction of number of females and reduction of average numbers of development stage infected tomato plants. The obtained results showed that biotic inducers or AgNPs reduce the level of second stages juveniles, number of females and reduction of average numbers of development stage of root-knot nematode (*M. incognita*) infection. Our results showed different abilities of PGPR (*B. subtilis* & *S. marcescens*), cyanpbacterial strain (*S. platensis*) and silver nanoparticle AgNPs according to the type of used elicitors and also the time of application in controlling the plant parasite nematodes *M. incognita*, which infect tomato plants.

The bacterial strains *Paenibacillus polymyxa*, *Bacillus megaterium, Bacillus circulans, Bacillus subtilis*, *Serratia marcescens* showed the highest protease, chitinase and gelatinase activities which might help to explain the way how the bacteria could act against the root-knot nematodes. It should be stated that the highest nematicidal activity exhibited by their strains against the second stage juveniles of *Meloidogyne incognita*. These strains proved to be the most efficient isolates as biofertilizers and nematicidal agents (El-Hadad *et al.*, 2010; Bahloul, 2013). The obtained results showed that all tested elicitors application at (One week before infection, at the same time infection and one week after infection) recorded significant reduction number of females and developmental stages/g of roots as well as second stage/250g of soil. Kloepper and Ryu (2006) showed that damage of root knot nematode was reduced by using PGPR, a single strain or two strains or complex mixtures of PGPR. The plant growth promoting rhizobacteria significantly reduced number of females and developmental stage of roots by root-knot nematodes in tomato crops and resulted in increased yield (Kokalis-Burelle and Dickson, 2003). Under greenhouse conditions, cell suspensions of different *Pseudomonas fluorescens* and *Serratia spp* strains have been found to be effective in suppressing populations of *Meloidogyne incognita* (Ashoub and Amara, 2010). Whereas, Vagelas *et al*. (2003) stated that PGPR has been reported acting as a biological agent against plant-parasitic nematodes. Our results demonstrated that using *S. platensis* application one week before the inoculation of *M. incognita* recorded 82.14% reduction of number of females. It Microalgal metabolites have attracted attention, because they are a resource for toxins, and potential new drugs (Shimizu, 2003). Data obtained in table (1) raveled that application of AgNPs *or S. marcescens* one week after the inoculation of *M. incognita* recording 86.66% and 120 average numbers of second stage juveniles, respectively. While, the application of AgNPs or *S. platensis* one week before the inoculation of *M. incognita* recording 113.33 average numbers of

second stage juveniles. Biosynthesis of silver nanoparticles (AgNPs), a new class of material with remarkably different physicochemical and biological characteristics from convenient silver-containing substances, has been shown to have antibacterial, antifungal, antiviral and nematicidal effects and it can reduce damage and losses caused by diseases (Choi *et al*., 2009; Eo and Lee, 2009).

Data in table (2) showed that application of AgNPs, either (one week before infection or one week after infection) was more effective in increasing the contents of chlorophyll a, b as well as total chlorophyll a+b. Enhanced photosynthetic pigment contents were recorded in leaves of *Pelargonium zonale* treated with silver nanoparticles when compared to the control (Hatami, *et al.,* 2013).

Photosynthetic pigments content were positive markedly affected as result to using the bacterial strains (*Bacillus subtilis*, *Serratia marcescens*), Cyanobacterial strain (*Spirulina platensis*) as biotic elicitors specially one week before infection and became one of visible evidence of sufficient of treatments. In this study, chlorophyll degradation was produced in nematode-infected plants than that of healthy ones (Table 2). The decrease in chlorophyll is considered to be a symptom of oxidative stress condition this decrease after infection might be due to the generation of reactive oxygen species (ROS) causing damage to chlorophyll *a* that is mean the plant failed to capture the light and so photosynthesis will decrease or stopped (Ali *et al.,* 2006). This reduction may be due to chlorophyll degradation, reduced chlorophyll synthesis and stability of thylakoid membrane. In addition, it may be associated with the increased activity of chlorophyll degrading enzyme, chlorophyllase (El-Shanhory *et al*., 2014).

At the same time, marked increases in the contents of carotenoids were observed in infected plants as being compared with healthy ones. On the other hand, the obtained results (Table 2) showed different responses as regards the Photosynthetic pigments due to the application of different used elicitors. So, results obtained indicate that the harmful effect of root-knot nematode (*M. incognita*) infection on photosynthetic pigments could be reduced via exploiting the role of *Bacillus subtilis*, *Serratia marcescens* and *Spirulina platensis* inoculation that can enrich the plant and soil with N_2 element. According to our results, *S. platensis* reduced the harmful effect of nematode on leaf chlorophyll content and these findings are supported by Belnap *et al.* (2003) who reported that biological soil crust likely affects nematodes in several ways. First, the increased nitrogen fixation, primary production, and chelation of metals of cyanobacteria, lichens, and mosses in a well developed, late stage crust increases the amount and availability of nutrient sources that can support secondary and tertiary consumers. Second, the greater biomass and diversity of late-stage crust organisms directly provide more abundant and more diverse food for nematodes. Third, soil moisture retention can be greater in well-developed crusts (George *et al.,* 2003), which may increase the period of time in which nematodes can be active and feeding. Finally, the crust forms a boundary between the atmosphere and mineral soil (Belnap *et al.,* 2003) that increases the stability of the soil environment and may allow the persistence of more nematodes that are sensitive to disturbance. Similar results were reported by Abd El-Baky *et al*. (2010) who mentioned that chlorophylls in photosynthetic membranes could be protected the photosynthetic apparatus from excessive ROS by quenching of single toxygen and other radicals. Application of algal extracts increased the levels of phenolic compounds, ascorbic acid and α-tocopherol, in plants irrigated sea water to protect the membrane by preventing or reducing oxidative damage by ROS. However, it is hypothesized a cycle where H_2O_2 scavenged by phenolic compounds to produce phenoxyl radicals, this radical reduces the ascorbic acid into mono (OH)-dehydroascorbate (Mostafa *et al*., 2013). Application of algal extracts increased the levels of phenolic compounds and ascorbic acid in plants irrigated sea water to protect the membrane by preventing or reducing oxidative damage by ROS. Plants have endogenous defense mechanisms that can be induced in response to attack by plant parasitic nematodes. It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed to activate them. Inducing the plant's own defense mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy (Ramamoorthy *et al*., 2001). The amino acid proline may act as a potent scavenger of ROS and this property of proline might prevent the induction of programmed cell death by ROS (Chen and Dickman, 2005). The data shown in table (3) appeared that application of *B. subtilis* one week before inoculation of *M. incognita* show significant increase in free proline content of tomato shoots compared to *S. marcescens*, AgNPs and *S. platensis*, respectively. As being compared with corresponding controls. The highest accumulation was observed in plants treated by *B. subtilis* on the infected plants followed by *S. marcescens*, and then AgNPs and *S. platensis*, respectively, more than on the healthy plants, indicating induction of systemic acquire resistant (SAR). Phenolic compounds are known to play a major role in the defense mechanism of plants against various external infectious agents. *Pseudomonas fluorescens* releases antimicrobial factors including lytic enzymes which lead to the

accumulation of phenolics (Meena *et al*., 2000) by secretion of indole acetic acid that induced phenol metabolism in plants (Shabaev *et al*., 1999; Patricia *et al*., 2009). In addition to some species of *Pseudomonas, Bacillus* reported to induce systemic resistance in plants against invading pathogens and antagonists to root-knot nematodes of *Meloidogyne spp*. (Kloepper and Ryu, 2006). Zaghloul *et al.* (2007) reported that the values of total phenols increased in tomato plants treated with *Bacillus subtilis*. Also, Gamil (1995) proved that the inoculation with *Bacillus polymyxa* (*Paenbacillus polymyxa*) increased peroxidase and polyphenol oxidase activities of squash leaves. Moreover, biocontrol agents via *Pseudomonas sp.* and *Bacillus sp.* have been used for induced systematic resistance such as phenolic compounds and peroxidase activity in plants against plant diseases (Saravanakumar *et al.*, 2007).

Results in table (3) revealed that the changes in the activities of oxidative enzymes (POD, PPO) in nematode-infected plants were significantly increased than that of non-infected ones (healthy plants). On the other hand, treatment with tested elicitors resulted in different responses as regards the oxidative enzyme activities of nematode-infected plants, indicating induction of systemic acquire resistant (SAR). These are in accordance with Saravanakumar *et al*. (2007), who stated that induction of peroxidase activity was significantly higher of about two-fold increase in enzyme activity in tea plants treated with *Pseudomonas fluorescens* Pf1 compared to the untreated control. It was noticed that (Table3) application of AgNPs was more effective in stimulating POD activity before challenge followed by *B. subtilis*, then S. *platensis* and *S. marcescens*. This stimulation was observed when compared with that of healthy (control) plants. SAR in cucumber is correlated with increasing in peroxidase activity, as well as polyphenol oxidase (PPO) in *N. glutinosa* (Ali *et al.,* 2006). In addition, proteins and isozymes polymorphisms are good indicators of response to biotic and abiotic stresses (Doebley, 1989; Sofy *et al.,* 2013; 2014; El-Dougdoug *et al.,* 2014). Variation in isozyme reveals the information in biochemistry entity of resistant genes to physiological changes, genetic characteristics and development of different organisms (Sosa and Garcia-Reina, 1992; Wang, 1998; Sofy *et al.,* 2014; El-Dougdoug *et al.,* 2014). Moreover, their relative contents and activities could be used as biochemical indicator to identify whether a strain had resistant ability to an external stress (Luo *et al*, 1999). In this study, clear differences existed not only in enzymatic activity but also in enzymatic composition between challenged plants without tested inducers and challenged plants treated with tested inducers. In general, activities of tested isozymes in challenged plants treated with all tested inducers were higher than that in challenged plants without inducers, which might be the potential factor for induction of SAR against nematode according to previous findings. Also, biotic inducers increased many PR-proteins such as isozymes of peroxidase, β-1, 3 glucanase and chitinase, (Neetu *et al*., 2008 and Anand *et al*., 2009).

Recently, chitinase and peroxidase activities in leaves of tomato plants was significantly increased by inoculation of tomato plants with bioagents (*Serratia marcescens*, and *Trichoderma harzianum*) compared with nematicide may play either a direct or indirect role in the suppression of root-knot nematode *M. incognita* (Abd-Elgawad and Kabeil, 2010).

Finally, this study demonstrated that all tested elicitors significantly reduced root-knot nematode numbers. Specially, application of *B. subtilis* or AgNPs one week before infection or one week after infection was the best elicitors that showed the highest antagonistic effect on the root-knot nematode infecting tomato plants and also the best treatment to enhance plants health than the other used of tested plants which are toxic to root-knot nematode. Therefore, the results imply that it should focus on using biological agents and nanotechnology as a safety method for human and environment to management the root-knot nematode in Egypt.

Corresponding Author:
Dr. Mohamed S. Attia
Botany and Microbiology Department
Faculty of Science
Al-Azhar University, 11884 Nasr City, Cairo, Egypt
E-mail: drmohamedsalah.92.@yahoo.com

References

1. Abd El Baky, H.H. F.K. El-Baz and G.S. El-Baroty, (2010). Enhancing antioxidant availability in wheat grains from plants grown under seawater stress in response to microalgae extract treatments. J. Sci. Food Agric., 90: 299-303. DOI: 10.1002/jsfa.3815.
2. Abd-Elgawad, M. M. M. and Kabeil, Sanaa, S. A. (2010). Management of the root-knot nematode, *Meloidogyne incognita* on tomato in Egypt. J. of Amer. Science, 6(8): 256- 262.
3. Abou-Aly, H. E.; R. A. Zaghloul; N. A. Neweigy; S. A. El-Sayed and A. M. Bahloul,(2015). Suppression of root-knot nematode (*Meloidogyne incognita*) activity in tomato using biocontrol agents. The 2nd Minia International Conference "Agriculture and Irrigation in Nile Basin Countries"32-25.Minia,Egypt.
4. Ali, S.H.; Eisa, S.S. and El-Dougdoug, Kh.A. (2006): Role of reactive oxygen species and anti-oxidants in hypersensitive local virus infected

plants. J. Agric. Sci. Mansoura Univ. 31: 6465-6480.
5. Anand, R.; Kulothungan, S.; Karthika, S.; Sentila, R. and Bhuvaneswari, K. (2009). Assay of chitinase and beta -1,3 glucanase in *Gossypium hirsutum* seedlings by *Trichoderma* spp. against *Fusarium oxysporum*. International J. Plant Sci. 4: 255-258.
6. Ashoub, A.H. and M.T. Amara, (2010):Biocontrol Activity of Some Bacterial Genera Against Root-Knot nematode, *Meloidogyne incognita*. J. American Sci., 6: 321-328.
7. Bahloul, A. M.E (2013). Biocides production for using in quality improvement of some vegetable crops. Msc. Thesis, Fac. Agric., Benha Univ., Egypt.
8. Bates LS, Waldren RP, Teare ID (1973). Rapid Determination of Free Proline for Water Stress Studies. Plant Soil; 39: 205-207.
9. Belnap, J.; Hawkes, C.V. and Firestone, M.K. (2003). Boundaries in miniature: two examples from soil, Bioscience, 53: 739-749.
10. Bourgouin, C.; Larget, T. I. and de-Barjac, H. (1984). Efficacy of dry powder from B. sphaericus RB80, a potent reference preparation for biological titration. J. Invertebrate. Pathol., 44: 146-150.
11. Byrd, D. W., Jr. T. Kirkpatrick, and K. R. Barker. (1983). An improved technique for clearing and staining plant tissue for detection of nematodes. Journal of Nematology 15:142-143.
12. Chandra, A.; Saxena, R.; Dubey, A. and Saxena, P. (2007). Change in phenylalanine ammonia lyase activity and isozyme patterns of polyphenol oxidase and peroxidase by salicylic acid leading to enhance resistance in cowpea against Rhizoctonia solani. Acta Physiol. Plant 29: 361–367.
13. Chen, C. and Dickman, M.B. (2005). Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*. Plant Pathology. 102:3459-3464.
14. Choi O., Clevenger T.E., Deng B., Surampalli R.Y., Ross L., Hu Z.(2009). Role of sulfide and ligand strength in controlling nanosilver toxicity. *Water Res*. 43: 1879–1886.
15. Daniel HD, George CM (1972). Peach seed dormancy in relation to indogenous inhibitors and applied growth substances. J. Amer. Soc. Hort. Sci.; 97(5): 651-654.
16. Doebley, J. (1989). Isozymic evidence and the evolution of crop plants, In: Isozymes in Plant Biology, D. Soltis, and P. Soltis, eds. (Portl and, Oregon: Dioscorides Press). 165-191.
17. El-Batal, A. I.; Hashem, A.M. and Abdelbaky, N. M., (2013): Gamma radiation mediated green synthesis of gold nanoparticles using fermented soybean-garlic aqueous extract and their antimicrobial activity. Springer Plus 2:129.
18. El-Dougdoug KA, Sofy AR, Mousa AA and Refaey EE (2014). Monitoring variability responses of cultivated potato varieties infected with *Potato virus Y* pepper isolate. Egyptian Journal of Virology, 11(2): 82-101.
19. El-Hadad ME, (2010). *In vitro* evaluation of some bacterial isolates as biofertilizers and biocontrol agents against the second stage juveniles of *Meloidogyne incognita*. World J. Microbiol. Biotechnol.,;26: 2249–2256.
20. El-Shanhorey, N. A., M. Z. M. Salem, and N. H. Mohamed, (2014). Effect of salinity and humic acid treatments on growth and chemical of Jotropha curcas plants. Bull. Fac. Agric., Cairo Univ., 65:474-488.
21. Eo J., Lee B.Y. (2009). Effects of ethylene, abscisic acid and auxin on fruit abscission in water dropwort (*Oenanthe stolonifera* DC.). *Sci. Hortic.* 123: 224–227.
22. Gamil, N. A. M. (1995). Induced resistance in squash plants against powdery mildew by cobalt and phosphate sprays. Annals Agric. Sci., Moshtohor, 33: 183-194.
23. George, D.B.; Roundy, B.A.; Clair, L.L.; Johansen, J.R.; Schaalje, G.B. and Webb, B.L. (2003). The effects of microbiotic soil crusts on soil water loss. Arid Land Research and Management, 17: 113-125.
24. Hatami. M, Ghorbanpou. M, (2013). Effect of nanosilver on physiological performance of pelargonium plants exposed to dark storage. Journal of Horticultural Research 2013, vol. 21(1): 15-20.
25. Hussey, R.S. and Barker, K.R. (1973): Acomparison of methods of collecting inocula of Meloidogyne spp. including a new technique for separating nematodes from the soil. Plant Dis. Rep. 57. 1025-1028.
26. Kalishwaralal, K.; Deepak, V.; Ramkumarpandian, S.; Nellaiah, H. and Sangiliyandi, G. (2008): Extracellular biosynthesis of silver nanoparticles by the culture supernatant ofBacillus licheniformis. Materials Letters, 62: 4411–4413.
27. Kloepper, J.W. and Ryu, C.M., (2006). Bacterial endophytes as elicitors of induced systemic resistance. In: Microbial root endophytes (eds. B. Schulz, C. Boyle, T. Siebern), Springer-Verlag, Heildelberg, pp. 33–51.
28. KoKalis-Burelle N, Dickson DW (2003). Effects of soil fumigants and bioyield on rootknot nematode incidence and yield of Tomato. Proceeding of international Research conference on Methyl Bromide Alternatives and Emissions Reductions:501-503.
29. Luo, L. X.; Cui, K. M. and Li, Z. L. (1999). Periodicity of cambial activity and variability of peroxidase and esterase isozymes in *Eucommia ulmoides* Oliv. *Acta Sci. Nat. Univ.Pek.* 35: 209-216.
30. Meena, B., Radhajeyalakshmi, R., Marimuthu, T., Vidhyasekaran, P., Sabitha Doraiswamy and Velazhahan, R (2000). Induction of pathogenesis

related proteins, phenolics and phenylalanine ammonia lyase in groundnut by Pseudomonas fluorescens. Zeitschrift fir Pflanzenkrantheiten and Pflanzeschutz, 107: 514- 527.
31. Mostafa, Soha S.M., Abou Elkhair, W. Amal and Shhata, Heba Sh., (2013). Effect of plant growth promoting substances from rhizo- and cyano-bacteria on sugar beet growth, yield and yield quality in saline soil. Intr. J. of Acad. Res., 5(6): 58-65.
32. Neetu, S.; Piyush, P.; Dubey, R.C. and Maheshwari, D.K. (2008). Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BN1. World J. Microbiol. Biotechnol. 24:1669- 1679.
33. Patricia, T., Daouda, K., Jingfang, Y., Pingsheng, J., Brian, B. and Mcspadden, G. (2009). Evaluation of an Antibioticproducing strain of Pseudomonas fluorescens for Suppression of Plant-Parasitic Nematodes. Journal of Nematology, 41(3): 234–240.
34. Ramamoorthy, V.; Viswanathan, R.; Raguchander, T.; Prakasam, V. and Samiyappan, R. (2001). Induction of systemic rhizobacteria in crop plants against pests and diseases by plant growth promoting.20:1–11.
35. Ruanpanun, P.; Tangchitsomkid, N.; Hyde, Kevin, D. and Lumyong. S. (2010). Actinomycetes and fungi isolated from plant-parasitic nematode infested soils: screening of the effective biocontrol potential, indole-3-acetic acid and siderophore production. World J. Microbiol. Biotechnol., 26: 1569–1578.
36. Saravanakumar, D. C.; Vijayakumar, N.; Kumar, R.; Samiyappan (2007): SERRATIA SP-induced defense responses in the tea plant against blister blight disease. Crop Prot., 26: 556-565.
37. Schouten, A. G.; van den Berg, V.; Edel-Hermann, C. Steinberg and Gautheron, N. (2004). Defense responses of Fusarium oxysporum to 2, 4-DAPG, a broad spectrum antibiotic produced by Pseudomonas fluorescens. Mol. Plant-Microbe. Interact., 17: 1201-1211.
38. Shabaev, V. P., Olyunina, L. N. and Smolin, Y. Y (1999). Functional activity of maize roots after inoculation with growth promoting rhizosphere bacteria, Pseudomonas. Biological Bulletin of Russian Academic Science, 26: 30-35.
39. Shimizu Y (2003) Microalgal metabolites. Current Opinion in Microbiology. 6:236–242.
40. Snedecor,G.W. and W.G.Chochran. (1982): Statistical Methods. 7th ed. Iowa State Univ. Press.Iowa,USA.
41. Sofy AR, Attia MS, Sharaf AMA and El-Dougdoug KA (2014). Potential impacts of seed bacterization or salix extract in faba bean for enhancing protection against bean yellow mosaic disease. Nature and Science, 12(10): 67-82.
42. Sofy AR, Mahfouze SA and El-Enany MAM (2013). Isozyme Markers for Response of Wild Potato Species to *Potato Spindle Tuber Viroid* Egyptian Isolate. World Applied Sciences Journal, 27(8): 1010-1022.
43. Sosa, P. A. and Garcia-Reina, G. (1992). Genetic variability and differentiation of sporophytes and gametophytes in populations of *Gelidium arbuscula* determined by isozyme eletrophoresis. *Mar. Biol.* 113: 679-688.
44. Theivasanthi, T. and Alagar, M. (2011): Antibacterial studies of silver nanoparticles, 5. In arXiv 1101(0348).
45. Vagelas, I.K., Gravanis, F.T. & Gowen, S.R. (2003). Control of *Fusarium oxysporum* and *Meloidogyne spp.* With Pseudomonas oryzihabitans. In: Proceedings of the BCPC International Congress – Crop Science and Technology, Vol. 1. Glasgow, UK, pp. 419-424.
46. Vernon LP, Selly GR (1966). The chlorophylls. Academic press.New York and London.
47. Walters D.R, Newton A.C. and Lyon G.D., (2005). Induced resistance: helping plants to help themselves. Biologist, 52: 28-33.
48. Wang, Z. R. (1998). Vegetable Allozyme Analysis. Science Press. Beijing (in Chinese).
49. Zaghloul, R. A.; Hanafy, Ehsan, A.; Neweigy, N. A. and Khalifa, Neamat, A. (2007). Application of biofertilization and biological control for tomato production. Proccedings of the 12th Microbiology Conf., Cairo, Egypt, 18-20 March.
50. Zarrouk, C., (1966). Contribution á l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthése de Spirulina maxima (Setch. Et Gardner) Geitler. PhD. Thesis, University of Paris, France.

10/25/2016