

Using of plant growth promoting rhizobacteria as biocontrol agent for root-knot nematode under greenhouse¹EISayed I. A. and ²Nada.O. Edrees¹Microbiology Dept., Soil, Water and Environmental Inst., Agriculture Research Centre. Giza- Egypt² Department of Biology – Zoology- Faculty of science, King Abdulaziz University – Jeddah- SaudiEmail: Dr.Ahmedie@yahoo.com

Abstract: Biological control tactics have become an important approach to facilitating sustainable agriculture. Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize the rhizosphere and plant roots resulting in enhancement of plant growth or protection against certain plant pathogens. Studies were conducted to test the hypothesis that induction of soil suppressiveness against *Meloidogyne incognita* using rhizobacterial inoculants is related to soil microbial activity and rhizosphere bacterial populations. The potential of bacterial antagonists to control the root-knot nematode *Meloidogyne incognita* was investigated under greenhouse conditions. In the present investigation bacterial strains were screened for its chitinolytic activity. Within this study seven antagonistic bacteria with known antagonism towards fungal pathogens were selected and tested for their potential to control *M. incognita* on tomato. *Serratia marcescens* and B-762 strains showed high preference for chitinase activity on media supplemented with colloidal chitin than all other strains on agar plates. On the other hand the other strains (PF- 23932, PF- 348, 4Q1 and 4Q2) expressed low chitinase activity. Our results appeared significantly differed in their effect on plant growth during nematode exposure, in the length and weight of root and shoot, the number of leaves and number of flowers 60 days after nematode inoculation). All bacterial strains appeared significant increase in root length, shoot fresh and dry weight per plant above control plants infected with *Meloidogyne incognita*. No significant effect of the bacteria on the length of shoot, root fresh weight, number of leaves and number of flowers. Different bacterial strains affected to reduce the number of females per root and per soil, number of galls and number of egg masses formed by nematodes. This leading to increase biomasses production by nematodes plant treated with different bacterial strains. This was due to the decrease a number of larva in plants treated by bacterial strains in soil rhizosphere and plant roots. The results revealed appeared significant increase in total protein content at 60 days plant-old above uninoculated plants and plants infected by nematodes among the season. Other results appeared higher accumulation of phenolics in bacterized tomato challenge inoculated with nematodes. The highest accumulation was observed in plants treated by SM and PF-23932 respectively.

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Introduction

Root-knot nematodes *Meloidogyne* spp. are plant-parasitic nematodes. About 2000 plants are susceptible to their infection and they cause approximately 5% of global crop loss (**Hussey and Janssen, 2002**). Nematodes are the most abundant multicellular animals on earth. Numerically, between 80 and 90% of all multicellular animals on earth may be nematodes (**Bloemers et al., 1997**). Nematodes can be found in different environments, e.g. soil, sea or fresh water, as free-living, parasitic or predacious animals (**Yeates et al., 1984**). They cause serious damage to many crops worldwide. Their damages have exceeded \$10 billi on per year in the United States (**Koening et al., 1999**). Crops infected by nematodes especially vegetables such as tomato record yield losses of up to 80 % on heavily infested soils (**Kaskavalci, 2007**).

Biological control of soil-borne plant pathogens by application of specific microorganisms to seeds or planting material has been studied intensively over the past three decades. Micro-organisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front line defense for roots against attack by pathogens. Biological control of soil borne pathogens with antagonistic microorganism has been extensively investigated (**Deshwal et al. 2003**). Biological control tactics, including the use of chitin and chitinolytic organisms, are being evaluated as management options for plant-parasitic nematodes.

Chitin is a major constituent of the cell walls of many fungi, insect exoskeletons, and crustacean shells. Chitinolytic bacteria as biocontrol agents have showed potential antagonistic activity against pathogenic fungi by degrading the cell walls (**Someya,**

et al. 2011). Chitin is nitrogen containing polysaccharide consisting of b-1,4-linked N-acetyl-D-glucosamine which is chemically analogous to the cellulose, except that one of the hydroxyl groups of each glucoside residue is replaced by an acetylated or deacetylated amino group. Chitin is the second most abundant natural polymer and widely distributed as a structural component of crustaceans, insects, and other arthropods, as well as a component of the cell walls of most fungi and some algae. Approximately 75% of the total weight of shellfish, such as shrimp, crabs and krill are considered as waste, and chitin comprises 20–58% of the dry weight of the said waste (Wang and Chang 1997). About 1011 tons of chitin is alone produced annually in the aquatic biosphere (Patil, *et al.* 2000).

Chitinases (EC 3.2.1.14) are glycosyl hydrolases, which catalyze the degradation of chitin. These enzymes are present in a wide range of organisms such as bacteria, fungi, insects, plants, and animals. Chitinases belong to either family 18 or family 19 of glycosyl hydrolases (Davis and Henrissat 1995). The enzymes of the two families do not share similarities in amino acid sequence or three-dimensional structure. Family 18 encompasses chitinases found in bacteria, fungi, viruses, and animals, and class III or V of plant chitinases. Family 19 includes class I, II, or IV chitinases of plants and chitinases present in some *Streptomyces* and other *Actinobacteria* strains (Kawase *et al.* 2004).

The chitinases of the above-mentioned organisms play important physiological and ecological roles. Invertebrates require chitinases for partial degradation of their old exoskeletons (Ruiz-Herrera and Martínez-Espinosa 1999) and plants as a defense mechanism against fungal pathogens (Honee 1999). Chitinases are constituents of several bacterial species; some of the best known include the *Aeromonas*, *Serratia*, *Myxobacter*, *Vibrio*, *Streptomyces*, and *Bacillus* genera (Cody *et al.* 1990). Bacteria produce chitinases mainly to degrade chitin and utilize it as an energy source. In addition, some chitinases of chitinolytic bacteria, such as the chiA gene products from *Serratia marcescens* and

Enterobacter agglomerans, are potential agents for the biological control (Downing and Thomson 2000). Detection of chitin-degrading bacteria from natural sources such as rhizosphere soil is useful in the bacteria that produce antifungal or other novel compounds. A high correlation between chitinolysis and production of bioactive compounds has been reported (Hoster, *et al.* 2005). Microorganisms, which secrete a complex of mycolytic enzymes, are considered to be possible biological control agents of plant diseases (Chang *et al.*, 2003).

Chitinases play an important role in the decomposition of chitin and potentially in the utilization of chitin as a renewable resource. Production of chitinase is widespread in a variety of organisms such as bacteria, fungi, actinomycetes, yeasts, plants, protozoans, coelenterates, nematodes, arthropods and humans (Wang *et al.*, 2009).

At the present time, there is an increasing interest in using biological control agents as alternatives for chemical insecticides. Therefore, chitinases have been used directly or as improvers of virulence with many pathogenic agents (Otsu *et al.* 2003).

In this report, we used chitinolytic bacteria suitable for application as a biocontrol agent against root-knot nematode infecting Tomato.

Materials and methods

Bacterial strains and culture conditions:-

Bacterial strains (Table 1) were used in this study, which including their references, as well as, their origins. All strains used in this investigation are wild type strains. Bacterial cells were cultivated in a Luria-Bertani (LB) medium (Sambrook *et al.*, 1989). The medium was supplemented with erythromycin at 10 mg/ml. The cells were routinely grown at 30 °C in 100 ml Erlenmeyer flasks with a culture volume of 20 ml in a rotary shaker at 200 rpm. The cultures were inoculated from overnight cultures (1%). Glucose solution was autoclaved separately and added to the culture medium before the inoculation to the final concentration of 1%. The growth of bacterial cultures was monitored spectrophotometrically at a wavelength of 590nm (OD590).

Table 1. Bacterial strains used in this study.

Strains	Source or Reference	Designation
<i>Serratia marcescens</i>	Agricultural Research Center (ARC)	SM
<i>Bacillus amyloliquefaciens</i> (B-762)	National Center for Agriculture Utilization Research, USA	B-762
<i>Pseudomonas fluorescences</i> (NRRL B-23932)	National Center for Agriculture Utilization Research, USA	PF- 23932
<i>Pseudomonas fluorescences</i>	Agricultural Research Center (ARC)	PF- 348
<i>Bacillus thuringiensis</i>	Bacillus Genetics stock Center, Biochemistry Dept., Ohio University, Columbus, USA	4Q1
<i>Bacillus thuringiensis</i>	Bacillus Genetics stock Center, Biochemistry Dept., Ohio University, Columbus, USA	4Q2

Colloidal chitin preparation

Colloidal chitin was prepared from chitin flakes by the method of **Mathivanan et al. (1997)**. The chitin flakes were ground to powder, added slowly to 10 N HCl and kept overnight at 4°C with vigorous stirring. The suspension was added to cold 50% ethanol with rapid stirring and kept overnight at 25°C. The precipitate was collected by centrifugation at 10,000 rpm for 20 min and washed with sterile distilled water until the colloidal chitin became neutral (pH 7.0). It was freeze dried to powder and stored at 4°C until further use.

Screening of chitinase producing bacteria

For enrichment of chitinase-producing microorganisms, a mineral medium containing colloidal chitin as sole carbon and energy source was used and incubated at 30°C. Screening was performed with bacterial strains on the colloidal chitin agar medium which containing 0.5% chitin, 0.03% peptone, 0.03% yeast extract, 0.07% K₂HPO₄, 0.03% KH₂PO₄, 0.05% MgSO₄·7H₂O, 1.5% Agar, 0.2% NH₄NO₃, 0.1% NaCl, (w/v) incubated at 37°C. Chitinolytic activity was measured by observing the size of the halo formed around the colonies after 7 days of incubation (**Someya et al., 2011**).

Plant material:

Tomato seeds (*Solanum Lycopersicon* L. cv. Castlerock II PVP) were obtained from agricultural research center (ARC), ministry of agriculture, Giza, Egypt. Seeds of tomato were surface disinfected for 1 min with 70% ethanol, rinsed five times with sterile distilled water and then disinfected again with 0.5% sodium hypochloride. The seeds were germinated as described by **Asaka and Shoda (1996)**. After four weeks the seedlings were utilized for greenhouse experiment.

Nematode inoculum:

The root-knot nematode culture was initiated by single egg mass of previously identified females (**Talyor et al., 1955**) and isolated from galled roots of highly infected tomatoes collected from Mansoura country, Dakahlia governorate, Egypt and propagated on coleus plants, (*Coleus blumei*) plants in the greenhouse of Nematology Research Unit, Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, where this work was done. Nematode inoculum of *M. incognita* eggs was then prepared according to the method recorded by **Hussey and Barker, (1973)**.

Greenhouse experiment:

The pots were placed in a growth chamber with 140 ± 14 μmol m⁻² s⁻¹ photon flux density, 20-25°C temperature. Pots were arranged in a randomized complete block design. Plants were fertilized with nutrient solution depending of the growth stage according to the manufacturer's specifications (Flora

Series, General Hydroponics Europe) and were watered daily to maintain field capacity. Plants received water and protected by conventional pesticides against mites and insects as needed. Plants were harvested after 60 days from nematode inoculation. Data dealing with plant length, fresh weights of shoot and root, shoot dry weight and number of leaves, and number of flower were determined and recorded. Infected tomato roots of each concentration per each treatment/replicate, (**Byrd, et al., 1983**) and examined with stereoscopic microscope for the number of galls, eggmasses, developmental stages and females of *M. incognita* and recorded. Then data on eggs/eggmasses, root galls, femals, eggmasses number per one gram of infected root/replicate of each treatment was calculated and recorded. *M. incognita* (J2) was extracted from soil/plastic bag in 100g/ replicate through sieving and modified Baermann technique (**Goodey, 1957**) counted by Hawksely counting under x10 magnification microscope, recorded and calculated for each bag (4.5 kg) soil.

Protein determination

Protein was extracted by dilute alkaline hydrolysis and proteins in the supernatants were quantified by the Coomassie Brilliant Blue procedure for protein determination (**Bradford, 1976**) was used to determine protein concentration, Bovine serum albumin ranging in concentrations from 0 to 100μg/ml was used as the standard from the standard curve.

Determination of total phenolic content

Total phenolics were determined using the Folin±Ciocalteau reagent (**Singleton & Rossi, 1965**). Samples (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged in cold at 10 000 g for 15 min and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residue was dissolved in 5 mL of distilled water. One-hundred microlitres of this extract was diluted to 3 mL with water and 0.5 mL of Folin±Ciocalteau reagent was added. After 3 min, 2 mL of 20% of sodium carbonate was added and the contents were mixed thoroughly. The colour was developed and absorbance measured at 650 nm after 60 min using catechol as a standard. The results were expressed as mg catechol/100 g of fresh weight material.

Statistical analysis:

Data were subjected to the analysis of variance according to **Snedecor and Cochran (1955)**. Least significant difference (L.S.D.) was used to compare between means if the F-test was significant.

Results and Discussion

Determination of chitinase activity of bacterial strains on colloidal chitin supplemented medium:

Chitinase activity exhibited by six bacterial strains was determined by the diameter zone after 7 days of incubation in the colloidal chitin supplemented agar medium as a sole carbon and energy source. Variable preference for media supplemented with colloidal chitin was observed among the strains (Table 2). *Serratia marcescens* and B-762 strains showed high preference for chitinase activity on media supplemented with colloidal chitin than all other strains on agar plates (Table 2). On the other hand the other strains (PF- 23932, PF- 348, 4Q1 and 4Q2) expressed low chitinase activity. This results agreed with **Bahar et al. (2012)** who reported that the bacteria have been forced to produce chitinase, to be able to degrade complex chitin polymer, and to produce metabolites to support their growth in the media incorporated with chitin as the only carbon and energy sources without any nutrients. Whereas, **Brurberg et al. (2001)** reported that *Serratia marcescens* is an important microorganism with its strong chitinase mechanism, after *Bacillus thuringiensis* that has been widely used in biological control of hazardous insects and fungi species. **Kamil et al. (2007)** who found that only 5% of 400 isolates exhibited different clear zones sizes indicating chitinase activity. Out of these, four isolates designated MS1, MS2, MS3 and MS4 gave the widest clear zones.

TABLE 2. Diameters of clear-zones produced by chitinolytic bacteria in colloidal chitin medium.

Strains	Diameter of clear-zones (mm)
SM	7.2
B-762	5.3
PF- 23932	2.1
PF- 348	2.4
4Q1	1.3
4Q2	0.4

Evaluate of bacterial culture supernatants on plant growth of tomato infected with *M. incognita*.

Environmental concerns and increased regulation on use of chemical fumigants, more management, and strategies for control of root-knot nematodes (*Meloidogyne incognita*) are currently being investigated on biological control using microbial antagonists as a potential alternative to chemical nematicides. The importance of chitinases in biological control of fungi, nematodes, and insect pests has become an emerging field of research (**Ajit et al. 2006**). Chitinases of some insect pathogens have also been utilized for enhancing their pathogenicity (**Merzendorfer and Zimoch 2003**).

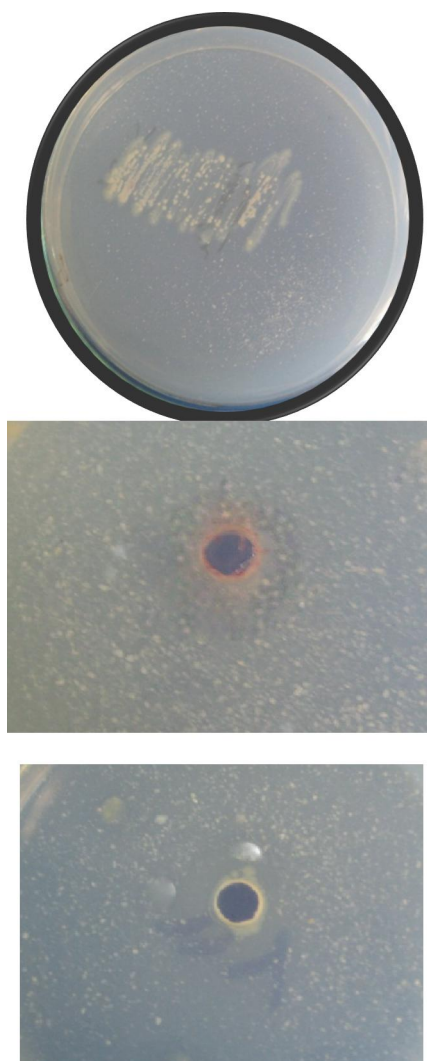


Fig. 1 Clear zone reactions produced by chitinolytic bacterial growth on colloidal chitin agar.

The culture supernatants of the strains significantly differed in their effect on plant growth during nematode exposure, as revealed by ANOVA of the length and weight of root and shoot, the number of leaves and number of flowers 60 days after nematode inoculation ($P= 0.005$, Table 3). All bacterial strains appeared significant increase in root length, shoot fresh and dry weight per plant above control plants infected with *Meloidogyne incognita*. No significant effect of the bacteria on the length of shoot, root fresh weight, number of leaves and number of flowers. These results agreed with **Zeinat, et al., (2009)** who found that *Pseudomonas fluorescens* and *Serratia marcescens* treatments significantly increased all growth parameters in the presence or absence of the pathogen and confirmed that *Serratia marcescens* and *Pseudomonas fluorescens* were potent as bio-control

agents for root-knot nematodes. **Burkett-Cadena (2008)** reported that suppression of nematodes and nematode damage was induced by various PGPR-based formulations, including ones with a single PGPR strain (FZB42), two strains (Bio-Yield), complex bacterial mixtures (Equity), and microbial metabolites formed during batch fermentation (AgBlend). Whereas, **Aballay, et al. (2013)** who found that the strains of *Serratia marcescens*, *Comamonas acidovorans*, *Pantoea agglomerans*, *Sphingobacterium spiritivorum*, *Bacillus mycoides*, *Alcaligenes piechaudii* and *Serratia plymuthica*. A further three strains, of *Bacillus megaterium*, *P. agglomerans* and *Pseudomonas savastanoi*, significantly increased root weight, but did not decrease nematode damage or population density. **Eklund (1970)** confirmed that *Pseudomonads*, are natural inhabitants on the root surface and primary consumers of root exudates rich in amino acids which

are converted to ammonia along the root to maintain a micro-zone around the growing roots that would be suppressive to pathogens. Under greenhouse conditions, cell suspensions of different *Pseudomonas fluorescens* strains have been found to be effective in suppressing populations of *Meloidogyne incognita* (**Ashoub and Amara, 2010**). Whereas, **Vagelas et al., (2003)** stated that *Pseudomonas oryzae* has been reported acting as a biological agent against plant-parasitic nematodes. The effect of rhizobacteria, or bacteria living in the soil under the influence of roots, on plant-parasitic nematodes has been investigated poorly (**Serratosa et al. 1994**). Among these rhizobacteria, *Pseudomonas fluorescens* constitute a major bacterial group. Certain strains of FP have been demonstrated to act positively on plants either by promoting their growth or by inhibiting fungal root pathogens (**Lemanceau 1992**).

Table (3): Nematocidal effect of bacterial strains on growth parameters in plants infected with *Meloidogyne incognita*.

Treatments	Plant growth parameters						
	Plant length (cm)		Plant fresh Weight (g)		Shoot D.W (g)	No. of leaves	No. of flowers
	Shoot (cm)	Root	Shoot (g)	Root(g)			
Uni. control	48	12.6	16.4	2.2	6.3	6.9	2.7
N- control	32	7.8	6.8	1.6	1.6	3.5	0.0
SM	75	20.1	31.1	2.9	4.9	8.5	3.2
B-762	66	18.1	21.7	3.9	5.3	8.7	3.2
PF- 23932	79	19.5	25.3	4.3	4.3	7.0	2.0
PF- 348	73	21.0	22.2	3.9	4.9	8.0	2.1
4Q1	72	13.5	24.3	2.6	3.6	8.4	2.7
4Q2	73	12.8	21.0	3.0	4.3	6.4	2.2
F-test	NS	*	*	NS	*	NS	NS
LSD 5%		3.16	2.26		0.47		

Uni. Control= Plants grown in autoclaved soil, whereas, N = plants grown in soil infected with *M. incognita*.

NS, *= Insignificant and significant at 0.05 probability levels, respectively

*Each value presented the mean of three replicates

The suppression of root-knot nematode by PGPR inoculants, as found in our study, agrees with previous reports with BioYield in greenhouse and field trials (**Kokalis Burelle et al., 2002**). BioYield, a product that contains spores of *B. subtilis* strain GB03, and *B. amyloliquefaciens* strain GB99 on a chitosan carrier, has been shown to induce growth promotion in tomato seedlings and reduce severity of diseases cause by several pathogens (**Klopper and Ryu, 2006**).

Effects of bacterial strains on certain pathogenicity of *Meloidogyne incognita* infecting tomato.

Biological control of plant-parasitic nematodes by microorganisms such as *Serratia marcescens* and *Trichoderma harzianum* has been considered a more natural and environmentally acceptable alternative to such chemicals (**Suarez et al., 2004**). Thus, the overall

goal of such biocontrol agents is the identification and deployment of highly effective strains against several plant pathogenic fungi and/or nematode pests before their development into registered, ready-for-sale plant protection products.

The data presented in Table 4. showed that the effect of bacterial strains that have ability to produced chitinase on the development of root-knot nematode (*Meloidogyne incognita*) infecting tomato under greenhouse conditions. Different bacterial strains affected to reduce the number of females per root and per soil, number of galls and number of egg masses formed by nematodes. This leading to increase biomasses production by nematodes plant treated with different bacterial strains. This was due to the decrease a number of larva in plants treated by

bacterial strains in soil rhizosphere and plant roots. These results agreed with **Adam et al. (2014)** who found that the treatment of tomato seeds with several strains significantly reduced the numbers of galls and egg masses compared with the untreated control. Whereas, **Khan et al. (2012)** who found that the controls seed inoculation of the bacterial isolates CD 38 or CD 62 inhibited ($P < 0.05$) galling due to *M. incognita* by 44% on mungbean roots, respectively compared to controls. However, none of the bacterial isolates were able to influence the reproductive potential (egg production) of nematode. **Andreoglou et al. (2003)**, who found that *Pseudomans anyzbatitans* culture filtrates contain compounds that inhibit hatching of root knot nematodes in vitro, Aslo, *P. orgzhabitans* cells decrease the number of female nematodes and egg masses when applied to soil at the time of nematode inoculation further demonstrating that *P. argzhabitans* produces metabolites used as a

biological agent against plant- parasite nematodes. **Becker et al. (1988)** showed a reduced galling by the rootknot nematode *M. incognita* on tomato, cucumber and clover following applications of *Ps. fluorescens* biovar I and IV and *Bacillus sp.* strains isolated from plant rhizosphere. Also, **Klopper 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 galling and egg masses on the roots by root-knot nematodes in tomato crops and resulted in increased yield (**Kokalis-Burelle and Dickson, 2003**). The plant growth promoting rhizobacteria have been reported to improve plant growth either through direct stimulation by the synthesis of phytohormones (**Xie et al., 1996**) or by decreasing the effect of pathogens (**Weller et al., 2002**).

Table (4): Impact of bacterial strains on the development and reproduction of *Meloidogyne incognita* infecting tomato.

Treatments	Average number of nematode in				No. of galls 1g/root	Red%	RGI	No. of egg masses 1g/root	Red%	E.I	No. of egg/1g root	Red%
	One gram root		250g/soil	Red%								
	females	Red %										
N- control	276	----	423	----	458	----	5	229	----	5	2143	---
SM	47	83	155	64	88	87	4	29	0.86	4	735	66
B-762	55	80	149	66	91	85	4	35	0.84	4	1172	45
PF- 23932	76	72	125	70	72	89	4	25	0.88	3	414	81
PF- 348	86	69	118	72	83	84	4	36	0.83	4	943	56
4Q1	42	0.84	133	0.68	76	0.82	4	26	0.88	3	1633	0.23
4Q2	104	0.62	41	0.91	62	0.85	4	17	0.92	3	285	0.86

*Root gall index (RGI) or egg-masses index (EI) was determined according to the scale given by **Taylor & Sasser (1978)** as follows : 0= no galls or eggmasses, 1= 1-2 galls or eggmasses, 2= 3-10 galls eggmasses, 3= 11-30 galls or eggmasses, 4= 31-100 galls or eggmasses and 5= more than 100 galls or eggmasses. **, N = plants grown in soil infected with *M. incognita*. ***NS, *= Insignificant and significant at 0.05 probability levels, respectively. ****Each value presented the mean of three replicates

Reduction % = $\frac{\text{Non- infected plant} - \text{infected plant}}{\text{Non - infected plant}} \times 100$

Non - infected plant

Response of total protein and phenolic compounds in tomato plants treated with bacterial strains to suppression *Meloidogyne incognita*:

The Data shown in Fig. 2 appeared the effect of different bacterial strains that have ability of produce chitinase on the total protein and total phenolic compounds in tomato plants infected by root-knot nematode (*Meloidogyne incognita*). The results revealed appeared significant increase in total protein content at 60 days plant-old above uninoculated plants and plants infected by nematodes among the season. These results agreed with **Sannazzaro et al., (2006)** who reported that rhizobacteria enhanced protein concentrations in plants. **Stefan et al. (2010)** found that *Bacillus pumilus* Rs3 inoculation increased with

66 % the total amount of seed soluble protein, probably due to stimulation of protein biosynthesis processes in soybean plants, providing in this way soybean seeds with higher nutritional value. *Bacillus pumilus* Rs3 treatment does not induce any qualitative changes of seed protein content.

Other results appeared higher accumulation of phenolics in bacterized tomato challenge inoculated with nematodes. The highest accumulation was observed in plants treated by SM and PF- 23932 respectively. This results in agree with **Akram et al. (2013)** who found that a significant increase in total phenolic contents was observed in bacterial-treated plants.

Phenolic compounds are known to play a major role in the defense mechanism of plants against various external infectious agents. Phenolics are the compounds whose quantity is raised when a plant comes under attack by a pathogen (Waterman and Mole 1994). Systemic induction of phenolic compounds under influence of bacterial strains was first reported by Van Peer *et al.* (1991). *Pseudomonas fluorescens* releases antimicrobial factors including lytic enzymes which leads 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). The use of *Pseudomonas fluorescens* for inducing systemic resistance against phytonematodes has been well documented (Patricia *et al.*, 2009). Some rhizobacteria (*Bacillus* spp.) have been found to produce lipopeptides, surfactins, bacillomycin D, and fengycins which are secondary metabolites mainly with inhabitant pathogen activity (Chen, *et al.* 2006). 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).

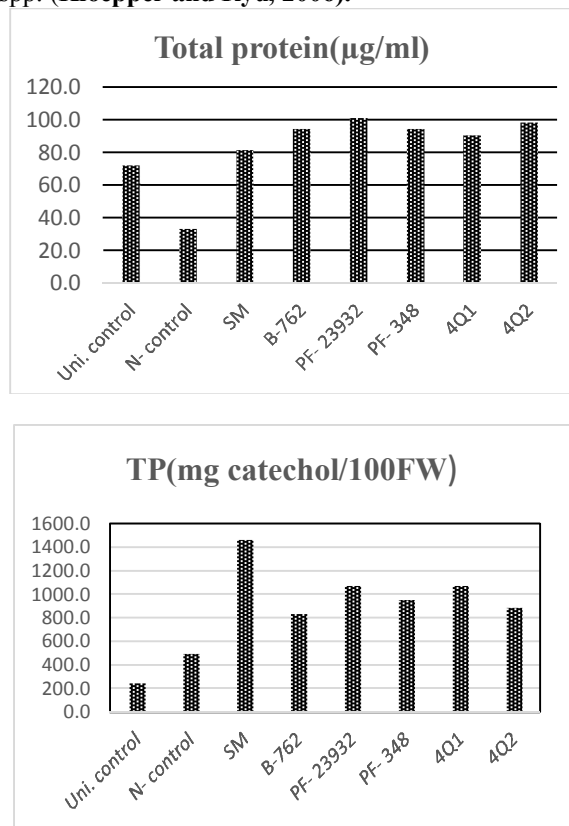


Fig 2. Total protein and phenolic content of tomato plants infected by *Meloidogyne incognita* grown in pots and inoculated with bacterial strains.

Conclusion

Chitinase plays an important role in the decomposition of chitin and potentially in the utilization of chitin as a renewable resource. The result concluded that bacterial strains that have the ability to produce a huge amount of chitinase in short time. So it may be applicable to field condition against plant pathogenic parasitism as nematode which is the major problem for agricultural food production. Present study demonstrated that bacterial strains have ability to produce chitinase enzyme were more effective in reducing the nematode infestation.

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