Suppression of root-knot nematode *(Meloidogyne incognita*) activity in tomato using biocontrol agents

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ABSTRACT

A greenhouse experiment was conducted in the experimental farm station of Fac. Agric., Benha Univ., during 2012 season to evaluate the efficient antagonistic bacterial strains *Bacillus subtilis* B38, *Pseudomonas fluorescens* B103 and *Serratia marcescens* either individually or in a mixture for their activity against root-knot nematode *Meloidogyne incognita* on tomato compared with nemaless and chemical nematicide. Damping- off, survived plants, nematode galls number and populations, enzymatic activity, plant growth characters, phenol compounds, peroxidase activity and tomato yield were recorded. Data showed that mixture inoculations of bioagents were found to be more effective for significant reduction of damping- off and increased the survived plants of infested tomato with *M. incognita* and reduced number of galls and nematode larvae population compared with each individual. Moreover, the mixture of bioagents was found to be more efficient than nemaless application in controlling of root-knot nematode *M. incognita* but still less than chemical application with nematicide. Also, the highest values of phosphatase, chitinase and dehydrogenase activities were observed with the application of mixture bioagents compared to either individually or other treatments. Moreover, data revealed that treated tomato with mixed inoculation of *B. subtilis* B38, *P. fluorescens* B103 and *S. marcescens* recorded more significant values of growth characters and yield than nemaless treatment but still less than chemical nematicide treatment. Also, inoculation with nemaless and the tested bioagents either individually or in mixed form succeeded to increase the total and conjugated phenols as well as peroxidase activity in infected plants.

INTRODUCTION

Biological control employs natural enemies of pests or pathogens to eradicate or control their population. This can involve the introduction of exotic species, or it can be a matter of harnessing whatever form of biological control exists naturally in the ecosystem. The induction of plant resistance using non-pathogenic or incompatible microorganisms is also a form of biological control (Schouten *et al*, 2004).

Root-knot is caused by species of the genus *Meloidogyne,* which are obligate, sedentary parasites of vascular plants. The female deposits eggs in a gelatinous matrix within a root, from which they are released into the soil. The infective form is the second-stage juvenile hatching from the egg under warm and moist conditions, and rapidly penetrates the host roots and develops specialized feeding sites known as giant cells. These cells are multinucleate and continue to enlarge as the females expand to a pear shape and lay eggs (Sasser and Carter, 1985). Plant-parasitic nematodes are microscopic obligate biotrophic pathogens that feed on plant roots. They cause severe damage to a wide variety of crops and lead to significant yield losses of approximately 78 billion dollar worldwide annually (Caillaud *et al*, 2008). Ruanpanun *et al* (2010) reported that 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.

Control of root-knot nematode by bacteria was described by Spiegel *et al* (1991). 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). Padgham and Sikora (2007) found that biological control promises to be such an option. Application of microorganisms antagonistic to nematodes or compounds produced by these microbes could provide additional opportunity for managing the damage caused by root-knot nematodes. Such microorganisms can produce substances that may limit the damage caused by these nematodes, e.g. by producing antibiotics, siderophores and a variety of enzymes. These microorganisms can also function as competitors of nematodes for colonization sites and nutrients. The search for nematotoxic or antagonistic compounds in cultural filtrates has greatly intensified in recent years, due to the number of toxins, enzymes or compounds derivable from their metabolites (Liu *et al*, 2008).

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).

The aims of this work to study the impact role of the three potent strains (*B. subtilis* B38, *Ps. fluorescens* B103 and *S. marcescens*) as biological control agents either individually or in a mixture against root-knot nematode *M. incognita* were evaluated in comparison with nemaless and chemical nematicide.

MATERIALS AND METHODS

Experimental soil

Soil of the experiment was obtained from Qalyubeia Governorate. Representative soil samples were taken from the upper 15 cm layer. Experimental soil was subjected to mechanical and chemical analyses in Agricultural Consultancy and Analysis Center, Moshtohor Fac. Agric. according to the method described by Page *et al*. (1982) and obtained results are presented in Table 1.

Table 1. Mechanical and chemical analyses of the used soil

Mechanical analysis				Chemical analysis			
Sand (%)	Silt (%)	Clay (%)	Textural class	EC (dsm^{-1})	pH	$CaCo_3$ (%)	Total N (%)
63.5	8.3	28.2	sandy	3.21	7.8	0.98	0.17

Preparation of pots

Hybrid yasmin-775 of tomato cultivar obtained from Technogreen Corporation for Agricultural projects, Cairo, Egypt was used as a host plant for *M. incognita*. This experiment was carried out in plastic pots 40 cm in diameter under greenhouse conditions. The pots were filled with 6kg of soil for nematode infestation.

Bacterial inocula

Three bacterial strains namely *Bacillus subtilis* (B38), *Pseudomonas fluorescens* and *S. marcescens* which recorded the highest values for nematicidal activities as well as maximum hydrolysis zone values of gelatinase, protease and chitinase (Bahloul, 2013). Bacterial inocula were prepared using poly broth medium (Bourgouin *et al*, 1984). The inocula suspension was approximately adjusted to 2.5×10^8 cfu/ml.

Nematode larvae

Meloidogyne incognita larvae were taken from Agricultural Zoology and Nematology Department, Faculty of Agriculture, Cairo University. Three thousands freshly hatched of second-stage juveniles (J2) were suspended and used to infect roots of the tomato seedlings after one week of transplanting.

Greenhouse experiment

Seedlings of tomato (yasmin-775) were washed with water and air dried. Seedlings roots were dipped into bacterial cell suspension (2.5×10^8 cfu/ml) of each tested bacterial

strains for 60 minutes before transplanting. Sucrose solution (30%) was added as an adhesive agent prior to inoculation. The bacterial inocula were added to the pots three times (every month) throughout the growing season at a ratio of 300 ml. /pot **(Khalifa, 2005).** In nematicide treatment the seedlings were immersed in Furadan (40kg/fed) and Nemaless at a ratio of 100 ml/L water.

Five tomato seedlings were planted in a pot containing infested soil with root-knot nematode. Three infested pots with root-knot nematode were planted with untreated seedlings (as control). Chemical fertilizers were supplemented with a full dose of inorganic nitrogen fertilizer (50 kg N/fed) as ammonium sulphate (20.5% N), inorganic phosphorus fertilizer (25 kg P_2O_5/fed) as super phosphate (15.5% P_2O_5) and potassium fertilizer (40 kg K_2O/fed) as potassium sulphate (48% K_2O) **(Khalifa, 2005).** Treatments were distributed in a randomized complete block design (RCBD) with three replicates.

Determinations

Damping-off and survival plants were counted and recorded after 120 days of transplanting with infested treatments with *M. incognita*. Also, galls number per plant and number of juveniles per 250 g soil were determined. While assessment of damping-off and survival plants were recorded after 60 days for *F. oxysporum* according method of **Hassan (1992)**. Also, alkaline phosphatase, chitinase and dehydrogenase activities of experimental soil were determined according to the method described by **Tabatabai (1982), Miller (1959)** and **Glathe' and Thalmann (1970)** respectively, after 30 and 60 days. Peroxidase activity and phenolic compounds were estimated according to the method described by **Allam and Hollis (1972)**, after 60 days transplanting.

A fresh and dry weight of shoots and number of flowers were determined at flowering stage (60 days) using three randomly selected plants. Plant height was also determined after 120 days of transplanting. Fruits were harvested at proper maturity stage (120 days), then counted, weighted and the following data were calculated; Number of fruits/plant, individual plant yield and weight of one fruit.

Statistical analysis

Statistical analysis was carried out according to **Snedecor and Cochran (1989)**. The differences between the mean values of various treatments were compared by Duncan's multiple range test (**Duncan's, 1955)**.

RESULTS AND DISCUSSION

Effect of bioagents on damping- off, survived plants, galls number and nematode population in infested tomato with *M. incognita*

Impact role of the three potent strains (*B. subtilis* B38, *Ps. fluorescens* B103 and *S. marcescens*) as biological control agents either individually or in a mixture against root-knot nematode *M. incognita* were evaluated compared with nemaless and chemical nematicide. Damping- off, survival plants, galls number and nematode population were also taken into consideration. Obtained data presented in **Table (2)** indicated that treatment of tomato plants with bioagents, nemaless and chemical nematicide significantly decreased the damping- off percentages, galls number and nematode population compared with un-treated, resulting an increase of survival plants.

In addition, the obtained data revealed that nematicide application recorded the highest significant reduction of damping- off and increased the survived plants of infested tomato with *M. incognita* followed by mixed culture of *B. subtilis* B38, *Ps. fluorescens* B103 and *S. marcescens*. Obtained data showed that the highest reduction in number of galls and nematode larvae population were recorded by the nematicide application, followed by the mixture of bioagents. Mixture inoculations of bioagents were found to be more effective for controlling of root-knot nematode (*M. incognita*) compared with each individual. Moreover, the mixture of bioagents was found to be more efficient than nemaless application in controlling of root-knot nematode *M. incognita*.

This result is in agreement with those of **Hashem and Abo-Elyousr (2011)** who found that application of *P. fluorescens, Paecilomyces lilacinus* and *Pichia guilliermondii* Moh10 was more effective against *M. incognita* in tomato compared to *Calothrix parietina* under greenhouse conditions. Moreover, **Khan *et al* (2007)** found that soil infestation with the nematode *M. incognita* caused severe galling on roots and decreased the yield of mungbean by 23.8%. Also, they reported that the application of *Ps. fluorescens* or *B. subtilis* suppressed the gall formation, reproduction and population of *M. incognita*. **Khalil *et al* (2012)** recorded some treatments such as abamectin, azadirachtin 0.15%, azadirachtin 0.03%, *Bacillus subtilis, Pseudomonas fluorescens, Paecilomyces lilacinus* and oxamyl as effective agents against root-knot nematode (*Meloidogyne incognita*) on the tomato cv. super strain B. This strain was the most investigated that obviously reduced root galls and egg masses on root system, and juvenile numbers in the soil and remarkably increased tomato plant growth characters.

Table 2. Effect of various treatments on damping-off percentage, survival plants, number of root galls and nematode population in infested tomato with *M. incognita* .

Treatments	Damping-off	Survived Plants (%)	No. of galls per root system	Nematode population/ 250 g soil
Control	80.27^{a}	19.73^{d}	253.3^{a}	2541.0^{a}
Nematicide	8.00^{d}	92.00^{a}	26.33^{d}	110.0^{f}
Nemaless	11.63^{bcd}	88.37^{abc}	65.67^{bc}	365.2^{def}
***B. subtilis* B38 (B)**	14.87^{b}	85.13^{c}	97.67^{b}	826.1^{b}
***Ps. fluorescens* B103 (Ps)**	10.43^{cd}	89.57^{ab}	88.67^{b}	707.4^{bc}
***S. marcescens* (SM)**	15.17^{b}	84.83^{c}	77.00^{bc}	638.1^{bc}
B + Ps	12.70^{bc}	87.30^{bc}	80.67^{bc}	461.4^{cde}
B + SM	11.57^{bcd}	88.43^{abc}	68.67^{bc}	503.0^{cd}
Ps + SM	13.93^{bc}	86.07^{bc}	65.00^{bc}	462.1^{cde}
B + Ps + SM	10.03^{cd}	89.97^{ab}	47.67^{cd}	219.3^{ef}

Enzymatic activities in rhizosphere of infested tomato with root-knot nematode

Phosphatase, chitinase and dehydrogenase activities were measured as indexes to detect changes in the microbial functioning in soil as affected by biocontrol agents against root-knot nematode, **(Table 3).** The obtained data showed that the alkaline phosphatase activity in rhizosphere of tomato treated with various treatments was higher at flowering stage than vegetative one. Also, results emphasized that the lowest values of phosphatase activity were observed with control treatment, untreated plants and nematicide application. This trend was observed at vegetative and flowering stages.

Moreover, inoculation of tomato plants with biocontrol agent *B. subtilis* B38, *Ps. fluorescens* B103 and *S. marcescens* individually or in a mixture significantly increased alkaline phosphatase activity compared to un-inoculated treatments. This trend was observed along the determination periods. Moreover, the mixture of bioagents *B. subtilis* B38, *Ps. fluorescens* B103 and *S. marcescens* recorded the highest values of alkaline phosphatase activity compared with other treatments. These results could be attributed to synergistic effect occurred in case of dual and mixed inoculation **(Abou-Aly, 2009)** as well

as to the higher ability of bioagent strains to colonize tomato roots. These results also coincide with those obtained by **Chebotar *et al* (2001)** who reported that the success of inoculated seeds or seedlings with beneficial bacteria usually depends on the colonization potential of the introduced strains.

Table 3. Effect of biocontrol agents on alkaline phosphatase, chitinase and dehydrogenase activities in rhizosphere of infested tomato plants with *Meloidogyne incognita*.

Treatments	Alkaline phosphatase activity (µg p-nitrophenol .g^{-1} .dm^{-1}. h^{-1})		chitinase activity (mM N-acetylglucose amine/ g dry soil /1 hrs)		Dehydrogenase activity (µgTPF/g dry soil/day)	
	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage
Control-1	11.97^{ijk}	16.29^{ij}	1.50^{i}	2.08^{i}	67.62^{jk}	99.82^{hi}
Control-2	4.20^{k}	4.40^{k}	1.98^{i}	2.55^{i}	41.73^{k}	63.73^{jk}
Nematicide	10.37^{ijk}	18.57^{hij}	2.16^{i}	3.16^{i}	50.37^{jk}	80.33^{ij}
Nemaless	22.40^{ghi}	39.37^{de}	10.40^{def}	14.03^{ab}	126.6^{fgh}	191.7^{bc}
B. subtilis B38 (B)	14.37^{ij}	33.77^{def}	6.23^{h}	9.40^{efg}	98.07^{hi}	162.1^{cdef}
Ps. fluorescens B103 (Ps)	26.17^{fgh}	49.20^{bc}	7.10^{gh}	12.03^{abcd}	112.1^{ghi}	180.5^{bcd}
S. marcescens (SM)	14.17^{ij}	28.57^{fg}	8.03^{fgh}	10.53^{def}	100.5^{hi}	162.5^{cde}
B + Ps	27.97^{fgh}	56.27^{ab}	9.50^{defg}	13.20^{abc}	140.9^{efg}	211.7^{b}
B + SM	16.57^{ij}	41.53^{cd}	9.13^{efg}	11.60^{bcde}	139.7^{efg}	212.6^{b}
Ps + SM	27.07^{fgh}	49.20^{bc}	8.03^{fgh}	13.57^{ab}	146.9^{defg}	199.3^{b}
B + Ps + SM	31.80^{ef}	60.40^{a}	10.93^{cde}	14.57^{a}	202.3^{b}	257.9^{a}

Control-1: healthy plants
Control-2: *M. incognita* infested plants

Ponmurgan and Gopi (2006) reported that phosphatase activity of phosphate dissolving bacteria *Pseudomonas* sp (strain GPO_2) which was isolated from groundnut rhizosphere has higher activity followed by the strain SPO_3 isolated from sorghum one. Also, there was a positive correlation between phosphate solubilizing bacteria and phosphatase activity.

The obtained data also revealed that chitinase activities in various treatments were in higher values at flowering stages than vegetative ones. Also, data indicated that inoculations of tomato with mixture of bioagent recorded the highest values of chitinase activity compared to individual inoculation.

Moreover, some antagonistic bacteria are capable of producing chitinase in soil which were involved in the biocontrol of fungal pathogens **(Pleban *et al*, 1997).** In addition, **Hallmann *et al* (1999)** suggested that endophytic bacteria which were exclusively promoted by chitin amendment soil might contribute to the observed suppressiveness of *M. incognita*.

Dehydrogenase (DHA) activity was determined as a criterion of respiration rate and total microbial activity in the soil. When infected tomato plants with root-knot nematode and treated with nematicide application a decrease in dehydrogenase activity were recorded because of enzymes inhibition could be caused by chemical pesticide. Concerning the tomato infested with root-knot nematode, the obtained data showed that the higher values of DHA were observed with the application of bioagents *B. subtilis* B38, *Ps. fluorescens* B103 and *S. marcescens* either individually or in a mixture compared to other treatments.

Also, obtained results indicated that inoculation of infested tomato with the mixed culture of bioagent strains caused significant increase of DHA as compared to individual inoculation.

Also, the higher values of phosphatase, chitinase and dehydrogenase activities in case of soil inoculated with the mixture of strains, may be attributed to the synergistic effect between the strains. This result is in agreement with those obtained by **Bopaiah and Shetty (1991)** who mentioned that the enzymatic activities of microflora and microbial biomass in the rhizosphere were higher than those of non rhizosphere. Dehydrogenase and phosphatase activities showed variable trends in the root regions and rhizosphere of the different crops.

Growth characters of infested tomato with root-knot nematode

Effect of biocontrol agents individually or in a mixture on growth characters, i.e. plant height, number of flowers, shoot fresh weight and shoot dry weight of infested tomato plants with root-knot nematode *M. incognita* as compared with chemical control is presented in **Table (4)**. The obtained data showed that soil infestation with root-knot nematode significantly decreased the growth characters of tomato as compared with control treatment. Generally, growth characters of tomato significantly increased when plants were treated with chemical nematicide, nemaless and bioagents (*B. subtilis* B38, *Ps. fluorescens* B103 and *S. marcescens*) compared to infested treatment. Moreover, data revealed that treated tomato with mixed inoculation of *B. subtilis* B38, *Ps. fluorescens* B103 and *S. marcescens* attained more significant values of growth characters than nemaless treatment but still less than chemical nematicide.

This result is in agreement with **Hashem and Abo-Elyousr (2011)** who found that fresh and dry weight of shoots and roots of plants were significantly reduced as a result of infection with *M. incognita*. However, application of biocontrol agents (*Ps. fluorescens, Paecilomyces lilacinus, Pichia guilliermondii* Moh10 and *Calothrix parietina*) singly or in mixture alleviate this reduction. Moreover, bioagents succeeded to enhance plant growth parameters compared with the control.

Table 4. Effect of bioagents on growth characters of infested tomato with *Meloidogyne incognita* under greenhouse conditions.

Treatments	Plant height (cm)	No. of flowers /plant	Shoots fresh weight g/plant	Shoots dry weight g/plant
Control-1	76.20^{e}	10.04^{ef}	86.28^{f}	16.31^{e}
Control-2	20.57^{f}	0.00^{g}	15.67^{g}	2.10^{f}
Nematicide	109.7^{a}	17.33^{a}	109.2^{a}	31.17^{a}
Nemaless	99.10^{bc}	10.00^{ef}	95.57^{d}	21.63^{d}
***B. subtilis* B38 (B)**	79.77^{e}	10.00^{ef}	88.80^{ef}	16.13^{e}
***Ps. fluorescens* B103 (Ps)**	88.13^{d}	11.33^{cde}	92.50^{de}	21.30^{d}
***S. marcescens* (SM)**	75.57^{e}	9.00^{f}	88.37^{ef}	17.30^{e}
B + Ps	97.50^{bc}	12.33^{bc}	100.4^{c}	26.17^{c}
B + SM	96.30^{c}	$12.00b^{cd}$	96.53^{cd}	25.17^{c}
Ps + SM	90.27^{d}	10.33^{def}	87.90^{f}	21.27^{d}
B + Ps + SM	101.3^{b}	13.33^{b}	104.7^{b}	28.10^{b}

Control-1: healthy plants

Control-2: *M. incognita* infested plants

Chemical nematicide viz. carbofuran showed a significant increase of growth parameters and suppression of *Meloidogyne incognita* population. Chemical carbofuran could be replaced with microbial antagonist (*Serratia marcescens* and *Trichoderma harzianum*) isolates to comply with environmental issues **(Abd-Elgawad and Kabeil, 2010).**

Phenolic compounds and peroxidase activity in tomato leaves

Concerning formation of phenolic compounds in infested tomato with root-knot nematode, the obtained data in Table (5) indicated that the lowest values of phenols and peroxidase activity were detected in healthy plants followed by root-knot nematodes infestation plants. Generally, inoculation of tomato with nemaless and the tested bioagents (*B. subtilis* B38*, Ps. fluorescens* B103 and *S. marcescens* SM) either individually or in mixture form succeeded to increase the total and conjugated phenols as well as peroxidase activity in infected plants, while high values were observed with mixed culture of bioagents compared with individual application. Increase the activities of peroxidase and polyphenol oxidase can limit disease development through the formation of polymerized phenolic barriers around the sites of infection (Smit and Dubery, 1997).

Table 5. Values of phenols and peroxidase activity in infested tomato with *M. incognita*.

Treatments	Phenols, mg / g fresh weight			Peroxidase as absorbance/g fresh leaves
	Total	Free	combined	
Control-1	6.08^{e}	3.26^{g}	2.82^{f}	1.32^{g}
Control-2	8.96^{d}	4.36^{f}	4.60^{e}	1.03^{g}
Nematicide	11.73^{c}	6.10^{e}	5.63^{cd}	2.30^{f}
Nemaless	15.03^{b}	6.76^{d}	8.27^{a}	4.23^{b}
B. subtilis B38 (B)	16.44^{a}	9.31^{b}	7.13^{b}	3.10^{e}
Ps. fluorescens B103 (Ps)	12.57^{c}	6.46^{de}	6.11^{c}	3.80^{d}
S. marcescens (SM)	9.18^{d}	4.40^{f}	4.78^{e}	3.00^{e}
B + Ps	14.63^{b}	9.13^{b}	5.50^{d}	4.10^{c}
B + SM	14.27^{b}	8.93^{b}	5.34^{d}	3.80^{d}
Ps + SM	17.37^{a}	9.95^{a}	7.42^{b}	4.00^{c}
B + Ps + SM	16.23^{a}	7.70^{c}	8.53^{a}	4.50^{a}

Control-1: healthy plants
Control-2: *M. incognita* infested plants

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).

Recently, Protein content, chitinase and peroxidase activities in leaves and roots of the two tomato cultivars (Super Strain B and Alisa) were 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 *Meloidogyne incognita* (Abd-Elgawad and Kabeil, 2010).

Effect of various treatments on the yield of tomato plants

Obtained data in Tables (6) clearly indicated that soil infestation with root-knot nematode significantly decreased number of fruits, fruit weight and fruits yield per plant of tomato as compared with control-1 plants.

Table 6. Effect of various treatments on yield and yield components of infested tomato plants with *M. incognita* .

Treatments	Number of fruits/plant	Average of fruit weight (g)	Fruits yield/plant (kg)
Control-1	5.33^{e}	72.9^{de}	0.830^{g}
Control-2	0.00^{f}	0.00^{f}	0.000^{h}
Nematicide	14.33^{a}	98.37^{a}	1.500^{a}
Nemaless	8.33^{cd}	87.50^{b}	1.133^{bc}
B. subtilis B38 (B)	8.00^{cd}	79.33^{c}	0.900^{f}
Ps. fluorescens B103 (Ps)	9.33^{c}	77.17^{cd}	0.900^{f}
S. marcescens (SM)	6.00^{d}	71.97^{e}	1.000^{de}
B + Ps	9.66^{bc}	91.13^{b}	0.966^{ef}
B + SM	10.33^{bc}	80.73^{c}	0.900^{f}
Ps + SM	9.33^{c}	73.83^{de}	1.067^{cd}
B + Ps + SM	12.00^{ab}	89.27^{b}	1.167^{b}

Control-1: healthy plants
Control-2: *M. incognita* infested plants

The obtained results showed that the infested plant (control-2) didn't record any yield or yield component. Generally, except control treatment tomato cultivated in soil treated with nematicide, nemaless and bioagents (*B. subtilis* B38*, Ps. fluorescens* B103 and *S. marcescens*) either individually, dual or triple inoculation were significantly increased number of fruits per plant, average of one fruit weight and fruits yield weight while, nematicide application resulted in high values, followed by the mixture of bioagent.

These results are in agreement with Yu *et al* (2011) who reported that *B. subtilis* CAS15 has great potential for plant growth promotion and biological control which increased the fruit weight and the yield. Infestation with *M. incognita* caused severe galling on roots and decreased the yield of mungbean. On the other hand, Khan *et al* (2007) reported that application of *Ps. fluorescens* suppressed the nematode pathogenesis and increased the yield of mungbean by 30.9% that was greater than the nematicide treatment by 16.7%.

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الملخص العربى

إخماد نشاط نيماتودا تعقد الجذور فى الطماطم بإستخدام عوامل المقاومة الحيوية

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أقيمت هذه التجربة تحت ظروف الصوبة بمركز البحوث والتجارب الزراعية بكلية الزراعة بمشتهر جامعة بنها خلال موسم 2012م ، لدراسة التأثير التضادى لبكتريا *Bacillus subtilis B38 , Pseudomonas fluorescence and Serratia marcescens* B103 على نيماتودا تعقد الجذور فى الطماطم ومقارنة ذلك بالنيمالس ومبيدات النيماتودا الكيميائيية .

ولقد اوضحت النتائج أن التلقيح المختلط ببكتريا المقاومة الحيوية أدى الى انخفاض معنوى فى نسبة سقوط الشتلات فى الطماطم وزيادة فى نسبة النباتات السليمة وذلك عند عدوى الطماطم بنيماتودا تعقد الجذور . كذلك أدى التلقيح المختلط ببكتريا المقاومة الحيوية الى انخفاض معنوى فى كل من عدد يرقات النيماتودا وتدرنات النيماتودا على جذور الطماطم مقارنة بالتلقيح الفردى بكل ميكروب عل حده .

كذلك أظهرت النتائج ان تلقيح نباتات الطماطم ببكتريا المقاومة الحيوية كان اكثر فعالية فى إخماد نشاط نيماتودا تعقد الجذور مقارنة بالنيمالس ومبيدات النيماتودا الكيميائية، وعند تلقيح الطماطم ببكتريا المقاومة الحيوية أدى الى زيادة نشاط إنزيمات الفوسفاتيز ، الشيتينيز ، الدىهيدروجينيز فى منطقة الريزوسفير مقارنة بمعاملة الطماطم بالنيمالس ومبيدات النيماتودا الكيميائية .

كذلك اوضحت النتائج أن صفات النمو والمحصول لنباتات الطماطم قد إزدادت معنويا وخصوصا عند التلقيح المختلط بكل من بكتريا *Bacillus subtilis B38* و بكتريا B103 *Pseudomonas fluorescence* وبكتريا *Serratia marcescens*مقارنة بمعاملة الطماطم بالنيمالس ومبيدات النيماتودا الكيميائية ،. وعند تلقيح نباتات الطماطم ببكتريا المقاومة الحيوية لوحظ زيادة معنوية فى محتوى أوراق نباتات الطماطم من الفينولات الكلية والحرة والمرتبطة وكذلك فى نشاط إنزيم البيروكسيديز مقارنة بمعاملة الطماطم بالنيمالس ومبيدات النيماتودا الكيميائية.

وفى ضوء النتائج المتحصل عليها من هذه الدراسة يمكن القول بأنه يمكن إستخدام بكتريا المقاومة الحيوية السابقة الذكر فى مقاومة نيماتودا تعقد الجذور فى الطماطم كطرق بديلة و أمنة وصديقة للبيئة بدلاً من إستخدام مبيدات النيماتودا الكيميائية والتى تسبب تلوث خطير للبيئة وتؤثر على صحة الإنسان.