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RESEARCH ARTICLE

Potency Evaluation of Pseudomonas strains against root-knot nematode infecting Tomato.

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Abstract

The rhizobacteria strains such as Pseudomonas with known ability to induce systemic resistance against different soilborne pathogens were studied in split-root experiments for their ability to induce systemic resistance against the root-knot nematode *Meloidogyne incognita* in tomato. This study attempted to construct more efficient soil bacterial strains may be able to controlling nematodes, as well as, maintenance the soil fertility using different strains of *Pseudomonas* to improve the biological control against the nematodes number in infected fields, in order to reduce the use of nematocidial agents. Four Pseudomonas strains were used as biocontrol agents against *Meloidogyne* incognita greenhouse. Data of plant growth parameters such as fresh and shoot weight and nematode reproduction in term of egg masses per root system, galls per root system, one gm of root and females per root system were recorded. The plants treated with P. putida8 appeared significantly increased in suppressed females per root. 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. On the other hand, the results appeared higher accumulation of phenols plants tissue treated with Pseudomonas strains in response to invasion by root-knot nematodes collectively contribute to induced systemic resistance and decrease in nematode infection. The highest phenols accumulation was observed in plants treated byP.putida8 and PF- 23932 respectively**.**

Introduction

The tomato (*Lycopersicon esculentum* L.) is an important vegetable crop across the world. The fruits of tomato are popular throughout the world and are used in all kinds of vegetable and also are eaten as raw salad. Ripe tomato fruit has high nutritive value being a good source of vitamin A, B, C and minerals **(USDA, 2005).** Its versatility in fresh or processed form and adaptability has played a major role in its rapid spread. According to **FAO's report (2004**), the world tomato production and consumption rose dramatically in the past two decades and to more than 113 million metric tons in the year 2003.

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 billion per year in the United States (**Koenning *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).** In Nigeria a yield loss of between 28-68 % was reported in tomato fields (**Adesiyan *et al*., 1990).** Nematodes have widely differing nutritional behaviours and therefore occupy several

trophic levels in soil food webs. They can be grouped according to the type of food they consume, based on the morphology of their mouthparts. Nematodes are common soil pests that affect plants .The aboveground symptoms of disease caused by nematodes can be difficult to detect, and may be often confused with symptoms of nutrient deficiency. Typically, plants do not thrive, are paler than normal, and may wilt in the heat of the day. Affected plants are often dwarfed, with small leaves. Sometimes, when infected plants are growing in moist, fertile soil, or during cool weather, the aboveground parts can still appear healthy (**Hoffmann *et al*. 2006**).

Due to 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. 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**). Notable among biocontrol agents, Pseudomonas species are known to have a significant role in the suppression of some diseases **(Siddiqui *et al*.2001)** apparently via production of antifungal metabolites such as antibiotics, pyoverdine siderophores, cyanide and ammonia **(Raaijmakers and Weller 2001**).Pseudomonas fluorescent are known to inhibit plant-parasitic nematodes in the rhizosphere of a range of plants including tomato, soybean, mungbean, sugarbeet and potato (**Tian, *et al*.2007).**Members of *Pseudomonas aeruginosa* and Pseudomonas sp., have demonstrated in vitro antagonism towards various soil-borne root-infecting fungi and root-knot nematodes, but with great variability among the strains (**Davies, 2005**). Production of secondary metabolites such as 2, 4-diacetylphloroglucinol (DAPG) and cyanide is most often associated with nematode suppression by *Pseudomonas fluorescent* in the rhizosphere of potato and tomato (**Cronin, *et* al., 1997**). In addition, recently (**Siddiqui *et al*. 2005)** demonstrated the production of extracellular protease by *Pseudomonas fluorescens* as an antagonistic factor against root-knot nematode, M. incognita. The aim of the present investigation the biocontrol activity of Pseudomonas strains against the root-knot nematode *Meloidogyne* actions incognita infecting tomato, *Lycoperiscum esculentum*.

Materials and Methods

Organism and culture conditions:-

Four Pseudomonas 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.

Table 1. Pseudomonas strains used in this study.

Strains	Source or Reference	Designation
Pseudomonas putida (NRRL B-8)	National Center for Agriculture Utilization Research, , USA	P.putida8
Pseudomonas fluorescences (DSM50090)	Kindly provided by Hadi Asadi Rehamani (PHD), Soil microbiology lab. Soil and Water Reasearch Inst., Tahran, Iran	PF- 50090
Pseudomonas fluorescences (NRRL B-23932)	National Center for Agriculture Utilization Research, , USA	PF- 23932
Pseudomonas fluorescences	agricultural research center (ARC)	PF- 348

Preparation of bacterial inoculum

Pseudomonas strains were maintained on King's medium B agar (**King, *et al*., 1954).**Mass culture of strains was prepared in conical flasks containing King's B medium. The flasks were each inoculated under constant shaking at 150 rpm for 48 hrs at room temperature (25 ± 2°C).Strains were applied to soil at 2 mL pure culture/pot (1 × 1012 cfu mL-1).

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, egg masses, developmental stages and females of M. incognita and recorded. Then data on eggs/egg masses, root galls, females, and egg masses 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 with80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residue was dissolved in5 mL of distilled water. One-hundred microliters of this extract was diluted to 3 mL with water and0.5 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 color 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

Biological control is considered as new efficient method that becomes widely used for controlling plant parasitic nematodes, as aim to decrease the extent of environment degradation and the effect of the excessive toxic nematicides. The results obtained from specificity tests using different *Pseudomonas* strains against the infection of tomato plants with nematode larva grown in pots under greenhouse condition were presented in Table (2). By direct inoculation with a constant number from nematodes larve juvenile, the results showed different abilities of bacterial agents in controlling the plant parasite nematodes *Meloidogyne*, which infect tomato. The results appeared a significant increase in plant fresh weight, shoot dry weight and number of leave between all treatments. The PGPR strains increased the root and shoot length in a variable range. The maximum root and shoot length was observed after treatment with PF- 23932, PF- 50090, PF- 348 and P.putida8 showed moderate effect on root and shoot growth., whereas, The minimum root and shoot length was observed in nematode alone (N- control).

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,

The highest number of shoot dry weight/g (9.63g) was detected in the plant treated with PF- 23932 ; then PF-50090 (7.30g) and P. putida8 (6.7 g), whereas, the lower number of shoot dry weight was observed in nematode alone (N- control) 1 g/plant. **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 oryzihabitans has been reported acting as a biological agent against plant-parasitic nematodes.

Table (2): Nematocidial effect of *Pseudomonas* 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	55.0	12.0	13.2	3.0	3.00	6	2
N- control	32.1	9.3	3.6	1.4	1.00	5	0
P.putida8	71.3	17.6	23.7	3.9	6.70	8	3
PF- 50090	81.3	19.2	25.9	4.4	7.30	8	3
PF- 23932	82.6	24.7	34.6	4.8	9.63	11	4
PF- 348	78.5	19.7	15.2	4.8	4.17	10	3
F-test	NS	NS	*	NS	*	*	*
LSD 5%			1.63		0.47	0.76	0.85

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 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**). The data presented in Table 3.showed that the effect of *Pseudomnas* strains 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 **Andreoglou *et al*. (2003),** who found that *Pseudomans* anyzbatitans culture filtrates contain compounds that inhibit hatching of root knot nematodes in vitro, Also, *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 root knot 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, **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 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 (3): Impact of *Pseudomonas* 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	---
P.putida8	47	83	155	64	88	87	4	29	0.86	4	735	66
PF- 50090	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
F-test	*		*		*			**			**	
L.S.D. 0.05	5.17	----	33.19	----	4.17	----	---	2.41	----	----	3.06	----
0.01								3.5			4.45	

*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 egg masses, 1= 1-2 galls or egg masses , 2= 3-10 galls egg masses, 3= 11-30 galls or egg masses, 4= 31-100 galls or egg masses and 5= more than 100 galls or egg masses.**, 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 % =Non- infected plant – infected plant R% ×100

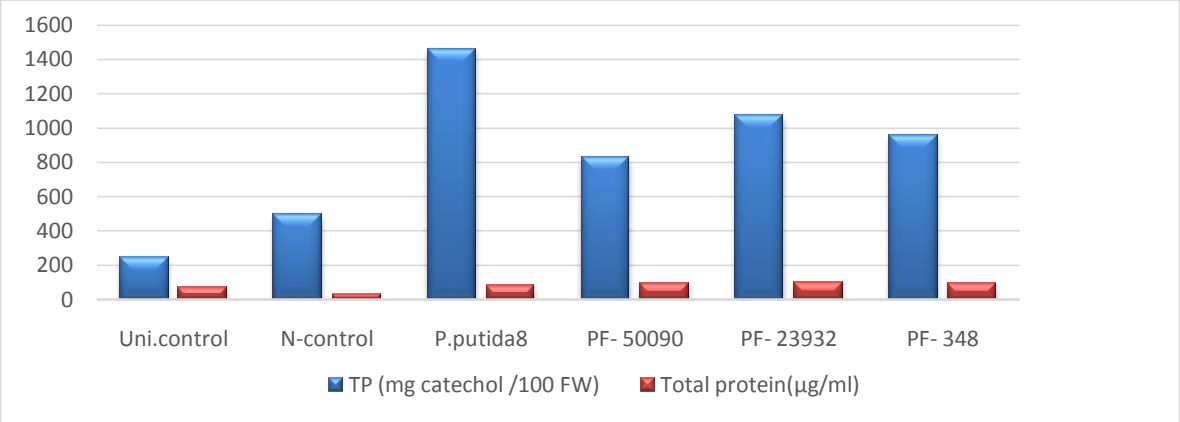
Non - infected plant

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 Data shown in Table 4 and Figure appeared the effect of different Pseudomonas strains 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. On the other higher accumulation of phenolics in bacterized tomato challenge inoculated with nematodes. The highest accumulation was observed in plants treated byP.putida8 and PF-23932 respectively**.** 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 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. (**Kloepperand Ryu, 2006).**

Table 4. Total protein and activity of phenols in tomato plants treated with *Pseudomonas fluorescens* challenge inoculated with *Meloidogyne*.

Treatment	TP(mg catechol /100 FW)	Total protein(µg/ml)
Uni.control	242	72
N-control	494	33
P.putida8	1460	81
PF- 50090	829	95
PF- 23932	1072	101
PF- 348	953	94
F- Test	*	*
LSD 5%	147.59	5.62



CONCLUSION

This study evaluated nematicidal effect of tomato infect with root-knot nematode, *Meloidogyne incognita* which is one of major pest in many vegetables plants. Present study demonstrated that *Pseudomonas* strains were more effective in reducing the nematode infestation. Our results have shown that the impact of *Meloidogyne incognita* can be greatly reduced by used *Pseudomonas* strains.

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