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Protection of *Pseudomonas fluorescens* against the Root-Knot Nematode, *Meloidogyne incognita*; Role of Enzyme-induced Resistance in Eggplant

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ABSTRACT

A *Pseudomonas fluorescens* culture was applied at different dilutions to attempt to induce resistance in eggplant (*Solanum melongena* L.) cv. 'Baladi' against the root-knot nematode, *Meloidogyne incognita*. The efficacy of this culture, when applied as a soil drench or root dip, was compared with inoculated non-treated plants under greenhouse conditions. *P. fluorescens* was able to reduce nematode parameters at all dilutions and in both types of application. The S/2 dilution (10^8 CFU/ml/2) was the most effective in reducing nematode reproduction as measured by the number of developmental stages, galls, egg masses, females and larvae/pot (percentage nematode reduction was 66, 68, 63, 74 and 72%, respectively) when treated as a soil drench compared to the untreated control inoculated with *M. incognita* only. This was followed by *P. fluorescens* at a concentration of S/10 (10^8 CFU/ml/10) which significantly reduced the same parameters by 61, 58, 55, 63 and 32%, respectively compared to control inoculated with *M. incognita* only. These treatments (S/2 and S/10) were higher than those treated by root dip in most cases. Also, plant growth criteria improved in treated plots compared to controls. The activity of three enzymes (peroxidase, polyphenol oxidase and chitinase) increased in treated plants exposed to S/2, S/10 and S/20 compared to the inoculated non-treated control. *P. fluorescens* thus induced resistance in eggplant against *M. incognita*.

Keywords: bacterial concentration, biotic-induced resistance, eggplant, nematode, seed soaking, soil drench

INTRODUCTION

Root-knot nematodes are obligate parasites and very damaging plant pests limiting agricultural productivity. Most cultivated plant species are susceptible to root-knot nematode infection (Sasser and Carter 1985). In Egypt, root-knot nematodes, *Meloidogyne* spp., are becoming a real threat to almost all vegetable crops, especially in newly reclaimed areas and they have been considered as limiting factors in crop production (Ibrahim *et al*. 2011). Due to environmental restrictions on nematicidal use for controlling plant parasitic nematodes, biological control and other eco-friendly disease control measures have recently gained increasing interest.

Certain strains of *Pseudomonas fluorescens* are able to suppress a variety of plant diseases caused by soil-borne plant pathogens, and hence are of considerable agricultural value (Kloepper 1993). Previous studies demonstrated that specific rhizobacteria reduce plant infection by various plant parasitic nematodes (Oostendrop and Sikora 1990; Muthulakshmi *et al*. 2010). A pot culture study was conducted by Jonathan and Umamaheswari (2006) to assess the biocontrol potential of endophytic bacteria, *Bacillus subtilis* (EBP 5, 22, 31 and EPC 16) prepared as a talc-base against the root-knot nematode, *Meloidogyne incognita*. A significant reduction in nematode population was observed in the combined treatment of EPB 5 + 31. Munif *et al.* (2001) reported that the endophytic bacterium *Pseudomonas pullida* Mt-19 was able to reduce *M. incognita* on tomato when applied as a seed treatment and/or soil drench. Siddiqui and Shaukat (2002) reported that two rhizobacteria, *Pseudomonas aeruginosa* strain IE-6S$^+$ and *P. fluorescens* strain CHA0, used as a bare root-dip treatment or as a soil drench, substantially reduced *M. javanica* juvenile penetration into tomato roots under glasshouse conditions.

The objective of this study was to investigate the protective effects of *P. fluorescens* against root-knot nematodes in eggplant through the enzyme-induced resistance pathway as these enzymes are considered to be indicators for inducing resistance in plants.

MATERIALS AND METHODS

Seeds of eggplant (*Solanum melongena* L.) cv. 'Baladi', obtained from the Ministry of Agriculture and Land Reclamation, were sown in 50-cm diameter clay pots containing 10 kg of solarized sandy loam soil (1:1, w/w). Two-months-old seedlings were transplanted in 15-cm diameter earthen pots filled with one kg solarized sandy loam soil (1:1, w/w). Plants were fertilized three times weekly with N-P-K as recommended for eggplant production (Mahmoud 2002). Two weeks after transplanting, plants were treated as follows: *P. fluorescens* culture solution at a concentration of (10^8 CFU/ml) according to Siddiqui and Shaukat (2005) was considered as the standard concentration "S" and was diluted to S/2, S/10 and S/20 by adding distilled water. Plants were treated by pipetting 50 ml of each concentration into soil around the root system as a soil drench by making 4 holes around the root system or by dip roots for 1 h. One week later, each pot received 500 freshly hatched *M. incognita* juveniles. A pure culture of this nematode was reared on tomato plants in separate pots. Plants that received root-knot nematodes only (without treatment) served as the untreated control. Each treatment and control were replicated five times. For enzyme determination (peroxidase (POX), polyphenol oxidase (PPO) and chitinase), samples of infected roots were sampled

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Table 1 Effect of *Pseudomonas fluorescens* on the pathogenicity of *M. incognita* infecting eggplant.

Treatments	Method of application	Dev. stages		Galls		Egg masses		Females		Larvae/pot	
		No.	% Red.	No.	% Red.	No	% Red.	No.	% Red.	No.	% Red.
S/2	Soil drench	13 f	66	87 f	68	76 de	63	99 d	74	934 d	72
	Root dip	23 de	39	120 cd	57	106bc	48	147 c	62	1028 d	69
S/10	Soil drench	15 f	61	115 cd	58	92 cd	55	143 cd	63	2251 bc	32
	Root dip	26 cd	32	126 c	54	108 bc	47	177 bc	54	2119 c	36
S/20	Soil drench	30 bc	21	194 b	30	117 b	43	222 b	42	2545 b	23
	Root dip	33 b	13	108 de	61	101 bc	50	220 b	43	2048 c	38
Control inoculated with *M. incognita* only		38 a	-	276 a	-	204 a	-	384 a	-	3297 a	-

Each value represents mean of five replicates.
Red. = Reduction.
Dev. stages = Developmental stages
Means followed by the same letter(s) within a column are not significantly ($P \leq 0.05$) different according to Duncan's multiple range test.

Table 2 Effect of *Pseudomonas fluorescens* on growth parameters of eggplant infected with *M. incognita*.

Treatments	Method of application	Length (cm)				Fresh weight (g)				Dry weight (g)			
		Root	% Inc.	Shoot	% Inc.	Root	% Inc.	Shoot	% Inc.	Root	% Inc.	Shoot	% Inc.
S/2	Soil drench	28.00 a	69	27.20 abc	23	7.50 a	223	9.00 a	58	1.07 a	128	2.79 a	41
	Root dip	22.80 b	37	28.80 a	30	3.50 bcd	51	8.50 ab	50	0.77 b	64	2.84 a	43
S/10	Soil drench	21.80 b	31	27.80 ab	25	5.00 b	116	7.76 abc	37	0.56 c	19	2.61 ab	32
	Root dip	18.60 c	12	26.60 abc	20	4.20 bc	81	7.40 abc	30	0.50 c	03	2.10 bc	06
S/20	Soil drench	20.80 b	25	24.20 cde	09	5.00 b	116	6.50 bc	14	0.52 c	11	2.16 abc	09
	Root dip	18.20 c	10	21.20 e	00	3.50 bcd	51	5.80 c	02	0.32 d	00	1.89 cd	00
Control inoculated with *M. incognita* only		16.60 cd	-	22.20 e	-	2.32 d	-	5.68 c	-	0.47 c	-	1.98 bcd	-

Each value represents mean of five replicates. Inc. = Increase. Means followed by the same letter(s) within a column are not significantly ($P \leq 0.05$) different according to Duncan's multiple range test.

Table 3 Effect of different concentrations of *P. fluorescens* on enzymes activities of eggplant (% of control values).

Treatments	Enzyme systems tested								
	Peroxidase activity			Polyphenol oxidase activity			Chitinase activity		
	Before inoculation	Days after inoculation		Before inoculation	Days after inoculation		Before inoculation	Days after inoculation	
		5	10		5	10		5	10
Soil drench									
S/2	118	328	735	205	152	154	114	186	600
S/10	131	138	947	127	114	262	186	121	1150
S/20	119	230	641	255	133	185	129	171	850
Root dip									
S/2	157	320	676	105	190	150	43	157	450
S/10	140	275	641	136	210	173	114	129	950
S/20	138	270	641	214	224	127	114	157	1100
Control received *M. incognita* only	100	100	100	100	100	100	100	100	100

before inoculation, and 5 and 10 days after inoculation. Enzymes were extracted by the method of Tuzun *et al*. (1989) and assayed according to the methods described by Lee (1973) for POX, Bashan *et al*. (1985) for PPO and Reid and Ogrydziak (1981) for chitinase. Two months after nematode inoculation, other replicates of plants were uprooted and the number of galls, females, egg masses, root developmental stages and soil juveniles were counted. The percentage reduction of the final nematode population and increment in plant growth parameters were recorded.

Data were analyzed statistically by LSD and Duncan's multiple range tests by using MSTAT version 4.

RESULTS

Data in **Table 1** indicates that the application of *P. fluorescens* as a soil drench at concentration S/2 produced the most significant ($P \leq 0.05$) reduction in root knot incidence, which was higher than when plants were treated by root dip as measured by the number of developmental stages, galls, egg masses, females and larvae/pot. The percentage nematode reduction was 66, 68, 63, 74 and 72%, respectively when treated as a soil drench compared to the control inoculated with *M. incognita* only, followed by *P. fluorescens* at a concentration of S/10 which significantly reduced the same parameters by 61, 58, 55, 63 and 32%, respectively compared to the control inoculated with *M. incognita* only. Moreover, *P. fluorescens* at a concentration of S/20 achieved the lowest percentage reduction in larvae/pot (23%) compared to the control. Also, the lowest percentage reduction was found when *P. fluorescens* was used as a root dip application at a concentration of S/20 as the percentage reduction in number of developmental stages was only 13% compared to control inoculated with *M. incognita* only.

As for plant growth, data in **Table 2** illustrates that the highest percentage increase in root length was 69% caused by S/2 as a soil drench, followed by 37% caused by S/2 as a root dip. However, the shoot length was significantly increased by 30, 25 and 23% by using S/2 as root dip, S/10 as a soil drench and S/2 as a soil drench, respectively compared to the control inoculated with *M. incognita* only. Moreover, treatments increased the root fresh weight by several fold at S/2, S/10 and S/20 concentrations as a soil drench, while the highest percentage increase in shoot fresh weight was 58 and 50% with S/2 as a soil drench and root dip, respectively compared to the control inoculated with *M. incognita* only. As for root dry weight, it increased by 128 and 64% as a soil drench and root dip, respectively while shoot dry weight increased by 43 and 41% as a root dip and soil drench, respectively.

For enzyme determination, data in **Table 3** shows a generalized increase in the activity of the three enzymes compared with the control inoculated with *M. incognita* only. POX activity increased after 5 and 10 days of nematode inoculation for all *P. fluorescens* concentrations com-

pared to control plants by soil drench or root dip in a time-dependent manner. The maximum increase in POX activity occurred after 10 days of nematode inoculation at S/10, S/2, and S/20 as a soil drench was 947, 735, and 641% higher than the control, respectively (**Table 3**).

As for PPO, the results showed a different pattern of enzyme activity than POX. A maximum increase in activity was observed after 10 days of inoculation by using S/10 concentration (262% over the control) following the application of a soil drench, while by application of bacteria at S/10 and S/2 as a root dip resulted in maximum activity after 5 days of nematode inoculation (210 and 190% of control, respectively; **Table 3**).

The maximum increase in chitinase enzyme activity after 10 days was observed by using S/10 and S/20 concentrations as a soil drench (1150 and 850% more than the control, respectively) while, when using as a root dip, maximum enzyme activity after 10 days was obtained by using S/20 and S/10 concentrations (1100 and 950% more than the control, respectively; **Table 3**).

DISCUSSION

The present data indicates that the application of *P. fluorescens* as a soil drench at a concentration of S/2 produced the highest reduction in larvae/pot followed by root dip application. These results agree with those of Siddiqui and Shaukat (2002, 2003) and resemble those of Kempester *et al*. (2001) who found that the application of pectinolytic *P. fluorescens* strains P 29 and P 80 as a soil drench reduced the fecundity of *Heterodera trifolii* infecting white clover (*Trifolium repens*) and increased the proportion of distorted females (females with an abnormal shape) and females with few eggs compared to the water-treated control. These effects might be attributed to induced systemic resistance, or ISR (Siddiqui and Shaukat 2005) which may be explained by the fact that enzymes induced by systemic resistance cannot directly induce nematode mortality; rather, they cause abnormal females and as a result lower nematode fecundity.

Plant growth-promoting rhizobacteria (PGPR) antagonize soil pathogens by competing for resources such as iron, or by the production of antibiotics or lytic enzymes (Van Loon *et al*. 1998). Work on the mode of action of PGPR with biological control activity suggests that some PGPR strains can induce physiological changes throughout the plant, making it more resistant to pathogens. ISR has been demonstrated for various rhizobacteria in several plants (Muller *et al*. 1998). Certain studies demonstrated that specific rhizobacteria reduced plant infection by various plant parasitic nematodes (Sikora and Hoffmann-Hergarten 1993; Siddiqui *et al*. 2009). The mechanisms which mediate these effects include the production of metabolites (Siddiqui and Shaukat 2003) which reduce hatch and attraction and/or degradation of specific root exudates which control nematode behavior. Other studies indicated that the mode of action of bacteria that induces resistance is mediated through systemic resistance pathways (Jonathan and Umamaheswari 2006).

Enzymes in host plants play an important role in the mechanisms of resistance to nematodes. Many enzymes have been reported to be involved. For instance, the activities of PPO and POX in nematode-infected plants (whether susceptible or resistant) are known (Mohamed and Hammad 2003). In the present work, determination of certain enzymatic activities of eggplant infected by *M. incognita* treated by *P. fluorescens* revealed a generalized increase in the activity of three enzymes (POX, PPO and chitinase) compared to control non-treated plants irrespective of the concentration and method of application. Showing the same trend, Mohamed and Hassabo (2005) also indicated maximum induction of chitinase and POX in cotton roots of a resistant cultivar after *M. incognita* inoculation. The high activities of chitinase and POX in the resistant cotton cultivar lowered the final population of *M. incognita* indicating the role of these enzymes in the resistance mechanism of the host against nematode infection (Collinge *et al*. 1993). Although the author could not directly link increased chitinase activity and nematode response, he concluded that since chitinase is a pathogenesis-related protein, it may have some effect on the resistance mechanism of plants leading to a reduced nematode population. Andress *et al*. (2008) found that POX increased in the roots of a resistant line of wheat (H-93-8) compared with the susceptible line in response to cereal cyst nematode (*Heterodera avenae*). The Cre 2 gene resistance gene in this line inhibited reproduction of this nematode. POX catalyzes the formation of lignin through polymerization of phenols. The onset of systemic acquired resistance (SAR) is characterized by expression of genes for pathogenesis-related proteins such as chitinase and peroxidase (Ramamamurthy *et al*. 2001; Jeunn *et al*. 2004).

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