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Evaluation of *Pasteuria penetrans* application methods on controlling of root-knot nematode infecting eggplant

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ABSTRACT

Experiment was carried out to evaluate the effective application methods of *P. penetrans* isolates from Egypt (Pp EGY) and Japan (Pp JAP) on root-knot nematode (RKN*) Meloidogyne* spp. control on eggplant under greenhouse conditions. Two application methods, spore suspension and powder of Pp EGY and Pp Jap used during the experiment. Results indicated that Pp EGY and Pp JAP showed a high reduction of root-knot nematode gall numbers compared to the control. Infected females of RKN with Pp EGY were markedly significant compared to Pp JAP. Plant growth was greatly influenced when both Pp EGY and Pp JAP were used. fresh shoot root and shoot dry weights were increased markedly compared to the control. In addition, the results indicate that *P. penetrans* offers a satisfactory and environmentally friendly solution for controlling root-knot nematodes.

Key Words: Biological control, endoparasitic bacteria, *Meloidogyne* spp., root-knot nematodes.

INTRODUCTION

In Egypt Eggplant (*Solanum melongena* L.) is widely cultivated vegetable crops by Egyptian farmers due to highly demand by direct consumers or for export. Thus, the total cultivated area by eggplant reached 48411 ha as reported by the Food and Agriculture Organization (FAO,2016). Different plant pathogens reported attacking and affecting eggplant plants all over the world. Plant parasitic nematodes (PPNs) in addition to fungi, bacteria, viruses and other pathogens were the most destructive pathogens. Root-knot nematodes (*Meloidogyne* spp.) one of the most damaging genera of PPNs attacking vegetable crops throughout the worldwide. The annual losses causing by *Meloidogyne* spp. around USD$ 100 billion all over the world (Brand *et al*., 2010). Thus, it considered a main limiting factor for Egyptian vegetables cultivation (Ibrahim *et al*.2000) especially in the sandy new reclaimed land (Mousa, 1997; Haroon & Osman, 2003; Mokbel *et al*. 2006; Ibrahim *et al*. 2010; Bakr *et al*. 2011a). Earlier studies confirmed that Nematodes not only influence quantity and quality of vegetable marketable yields (Kingland,2001), but also vector for other plant pathogens (Nykyri *et al*.,2014). Plants infected by RKN with intensity galled roots are partially or totally unable to uptake water and nutrients from soil. Heavy infected plants give small yield, yield losses over 30% in eggplant, tomato, and melon (Sikora and Fernández ,2005). During summer season sometimes, plants production reached Zero when grown in sandy soils infested with high nematodes population (Bakr *et al*.,2015). Because of extensive use of highly toxic of nematicides to both human and the environment (Abawi and Widmer, 2000), development of alternative eco-friendly control strategies and long-term integrative approaches is urgently needed to replace chemical nematicides (Martin, 2003).

Biological control strategies using different fungi or bacteria are recently recommended for ecofriendly approaches for PPNs (Bakr *et al*.,2011b;2014). *Pasteuria penetrans* is a gram-positive, mycelial, endospore forming bacterium

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with septate mycelium, and is an obligate parasite bacterium on plant parasitic nematodes (Mankau and Imbriani, 1975). *Pasteuria penetrans* is widely distributed in agricultural soil and has been found attached to plant parasitic nematodes in many different climates and environmental conditions throughout the world (Sayre and Starr, 1988; Sturhan, 1988; Chen and Dickson, 1998). *Pasteuria* endospores (a persistent survival stage) attach to the cuticle of a host nematode as it moves through the soil. The bacterium then injects itself into the nematode where, which vegetatively grow inside the body and inhibit egg production, and then the bacteria sporulate, filling the female body with spores, then ruptures and releasing thousands of endospores into the soil, that can infect other nematodes (Hewlett *et al*., 2006).

Recently, *P. penetrans* reported as a promising biocontrol agent for suppressing field populations of several economic plant parasitic nematodes on different host plants throughout the world, especially *Meloidogyne* spp., (Chen and Dickson, 1998; Dube ,2001; Brito *et al*., 2004; Dabiré *et al*., 2006; Bakr *et al*.,2011b).

Earlier studies showed that *P. penetrans* decreased *M. incognita* race 1 on tomato under glasshouse conditions (Jonathan et al., 2000). *Pasteuria penetrans* infested root powder or infested soil markedly decreases reduction on root-knot nematodes *M. incognita* on tomato (El-Saedy and Mokbel, 2007; Mousa *et al*., 2008).

The present study was conducted to determine the effect of application method of spore suspension and the powder of *P. penetrans* on the control of *Meloidogyne* spp. infecting eggplant.

MATERIALS AND METHODS

Multiplication of *Meloidogyne* spp.:

Roo-knot nematodes *Meloidogyne* spp. was reared on tomato plants (*Lycopersicon esculentum*) CV. Beto-86. in plastic pots 50 cm in diameter containing sterilized clay–sand mixed soil (1:2 v/v) under the experimental glasshouse conditions at the farm, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt.

***Meloidogyne* spp. inoculum Preparation**:

Three months old tomato roots intensity infected with *Meloidogyne* spp. were used to prepare nematode inoculum using the sodium hypochlorite (NaOCl) technique to extract nematode eggs as described by Hussey and Barker (1973). Then Eggs incubated to obtained second stage juveniles. After that juvenile transferred to a flask containing tap water then number of juveniles/1ml counted under light microscope.

Source of *P. penetrans*:

Two isolates of *P. penetrans* were used during this study: Pp EGY and Pp JAP. Egyptian isolate was isolate by the author (Bakr *et al*., 2011b) while, Pp JAP was obtained from Prof. Dr. Simon R. Gowen, Department of Agriculture, Reading University, Early Gate Reading, RG6 2AT, United Kingdom.

Multiplication of *P. penetrans*:

Multiplication of *P. penetrans* was carried out in two phases: 1) *In vitro*: One thousand-second stage juveniles of *Meloidogyne* spp. were added to 1 ml of a *P. penetrans* spore suspension adjusted to 10^5 spores/ml and 4ml distilled water in 5 cm diameter Petri dishes and incubated for 24 hours at room temperature. After 24 hours the number of attached spores / juveniles was counted. Then the juveniles with attached spores were separated by pouring the suspension through a 20µm sieve to collect the juveniles with attached

spores on the sieve. 2) *In vivo*: Four weeks old tomato seedlings (CV. Beto 86) transplanting in plastic pots (15 cm in diameter) filled with a non-sterilized sand /clay soil mixture (2:1, v/v). Then, seedlings inoculated by One thousand juveniles encumbered with Pp spores by pipetting the solution in 3-4 holes around the hairy roots. Plants were watered and fertilized with a nutrient solution as previously described by Epestein (1972).

Eight weeks after nematode inoculation root systems were removed, washed and part of them were air-dried and then ground in a grinder until it became powder. Another part of the root systems was soaked in distilled water for 3-4 days or until the roots became soften (Ratnasoma and Gowen, 1996). Females infected with *P. penetrans* spores were then separated from the soften roots debris by sieving under vigorous tap water and collected in distilled water in small petri dishes and stored in a refrigerator at 10°C .The *Pasteuria* root powder and the *Pasteuria* infected females served as a source of *P. penetrans* inoculums according to Stirling and Wachtel (1980).Spore suspensions of the different *P. penetrans* isolates were prepared by crushing of *Pasteuria* infected female to small drops of tap water in a pestle and mortar and the concentration of the spore suspension measured and adjusted with haemacytometer slide.

Greenhouse Experiment

Four weeks old tomato seedlings were transplanted in plastic pots containing mixed sand / clay soil. Two types of *Pasteuria* were used:1) 2g of *Pasteuria* root powder containing 2×105 spores/g and 2) 2ml spore suspension containing 2×105 spores /ml were applied around the roots of tomato seedlings. Two types of nematodes inoculums were used: 1) 1500 eggs of *Meloidogyne* spp. 2)1500 fresh hatched second stage juveniles of *Meloidogyne* spp. Plants were watered as needed and weekly fertilized with a nutrient solution as described by Epestein (1972). After two months from nematode inoculation plants were removed; uprooted and their roots were carefully washed under running tap water to remove soil particles. Fresh shoot and root weight as well as number of galls/root system, egg masses/root system and developmental stages were recorded.

For egg-masses counting, infected roots were stained by dipping the root system in a solution of 0.015% Phloxine-B for 20 minutes following the technique confirmed by Daykin and Hussey (1985). Females of *Meloidogyne* spp. were collected by cutting the root system of each root in 2 cm pieces and soaking the roots in a beaker full of tap water for 4 days until they became soft (Ratnasoma and Gowen, 1996). The roots were then washed through stacked 75 mesh and 38 mesh sieves to separate the females from the root debris. The females of *Meloidogyne* spp. infected with Pp were distinguished by their opaque dull creamy white to amber colour compared to white, glistening females or crushed in a little drop of water on a glass slide, covered with a cover slip and then examined for the presence of *P. penetrans* endospores under a microscope at 400× magnification.

Statistical analysis

Obtained data were statistically analyzed using analysis of variance (ANOVA) and comparisons of means at the 5% level of significance using costat 6.3 version program according to Duncan's multiple range test.

RESULTS

Results of the present study showed clearly that related root-knot nematodes parameters and growth of tomato plants were affected by using *P. penetrans* as presented in table (1). The best result in reducing nematode parameters was obtained when tomato plants were treated by Pp EGY powder. This treatment reduced the nematode galling on root system and inhibited the nematode reproduction on tomato plants and increased number of infected females with *P. penetrans*.

After 14 weeks from transplanting of tomato seedlings, tomato root galling percentages were significantly lower in the *P. penetrans* treated pots. Examination of root system showed that the number of galls and egg masses/tomato root system were affected markedly by both application method of spore suspension and the powder of *P. penetrans* compared with the *P. penetrans* untreated control plants inoculated with nematode alone. Resultshowed that Females /root system were also decreased in treated plants. The highest reduction on number of females was found when Pp EGY used as a powder. Meanwhile, the lowest reduction was occurred with treatment of Pp JAP as a powder. Results showed that infected females /root system were also decreased in treated plants. The highest reduction on number of females was found when Pp EGY used as a powder. Meanwhile, the lowest reduction was occurred with treatment of Pp JAP as a powder. Results showed that developed stages/root system were also decreased in treated plants. The highest reduction on number of females was found when Pp EGY used as a powder. Meanwhile, the lowest reduction was occurred with treatment of Pp JAP as a powder.

Table (1): Effect of *Pasteuria penetrans* application methods on tomato root-knot nematode *Meloidogyne* spp. control.

Treatment	Nematode population/ root system					Reproduction Factor (RF)
	Galls	Egg masses	Females		Develop .Stages	
			Non-infected	Infected		
Eggs+P.p EGY +Powder	42.66 b	38.66 b	32.66 b	1.33 ab	5.00 b	0.0776
Eggs+P.p EGY +suspension	31.00 cd	27.00 cde	21.33 c	1.66 ab	6.00 b	0.0559
Eggs+P.p JAP +Powder	31.00 cd	25.33de	24.00 c	1.66 ab	4.66 b	0.0556
Eggs+P.p JAP +suspension	35.00 bcd	31.00 bcd	28.00 bc	1.66 ab	5.66 b	0.0663
Egg alone	105.66 a	112.66 a	113.66 a	0.00 b	107.00 a	0.3333
Larvae +P.p EGY +Powder	26.00 d	21.66 e	21.33 c	2.33 a	3.33 b	0.0499
Larvae +P.p EGY +suspension	36.33 bcd	30.66 bcd	24.00 c	1.33 ab	4.66 b	0.0606
Larvae +P.p JAP +Powder	37.33 bc	34.66 bc	20.00 c	1.66 ab	3.66 b	0.0599
Larvae +P.pJAP+suspension	34.00 bcd	30.66 bcd	22.66 c	2.00 ab	4.66 b	0.0599
Larvae alone	108.00 a	116.00 a	108.00 a	0.00 b	88.66 a	0.3166
Control	0.0 e	0.0 f	0.0 d	0.0 b	0.0 b	0.0

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test (P≤0.05).

Results presented in table (2) showed that *P. penetrans* enhanced plant growth was observed in all the bacteria-treated tomato plants as compared to the *Meloidogyne* spp. untreated control. Data showed the effect of *P. penetrans* application methods on tomato plant growth. Tomato plant growth parameters were greatly affected by treating the plant with *P. penetrans*. Adding Pp JAP as powder increased fresh root, fresh shoot, and dry shoot weight. The difference between treatments was not significant. Shoot, root fresh and dry weight of tomato plants were also affected by all polyethylene sheet colours compared with the non-covered one. Results also revealed that tomato plants grown on showed the highest fresh shoot, root weight and dry shoot weight.

Table (2): Effect of *Pasteuria penetrans* application methods on tomato plant growth.

Treatment	Fresh root weight	% Efficacy*	Fresh shoot weight	% Efficacy	Dry shoot weight	% Efficacy
Eggs+P.pEGY+Powder	3.30 ab	54.92	11.53 abc	00.00	2.22ab	- 0.44
Eggs+P.pEGY+suspension	3.10 ab	45.53	11.23 abc	- 2.60	2.00 ab	- 10.31
Eggs+P.p JAP +Powder	3.63 ab	70.42	11.37 abc	- 1.38	1.93 ab	21.07
Eggs+P.p JAP +suspension	3.10 ab	45.53	10.46 abc	- 9.28	1.86 ab	- 16.59
Egg alone	3.16 ab	48.35	9.53 c	- 17.34	1.06 b	- 52.46
Larvae +P.pEGY+Powder	7.43 a	248.82	14.30 a	24.02	2.70 a	- 0.13
Larvae +P.pEGY+suspension	4.4 ab	106.57	12.07 abc	4.68	2.09 ab	- 6.27
Larvae +P.p JAP +Powder	7.20 a	230.02	13.96 ab	21.07	2.34 ab	4.93
Larvae +P.p JAP +suspension	3.76 ab	76.52	11.70 abc	1.47	2.66 a	19.28
Larvae alone	2.26 b	6.10	9.93 bc	- 13.87	1.33 ab	- 40.35
control	2.13 b	0.0	11.53 abc	0.0	2.23 ab	0.0

*** Efficacy = Treatment – Control / Control X 100.**

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test (P≤0.05).

DISCUSSION

Data showed that *P. penetrans* reduced highly all related nematode parameters compared to untreated control plants. These results agree with those obtained by Cho *et al*., (2000) and Gogoi and Neog (2005), who reported that *P. penetrans* controlled *M. incognita* and *M. arenaria* and reduced the number of galls, final population and increased plant growth parameters and yield. These results are also in agreement with those obtained by Chen and Dickson (1997); Jonathan *et al*., (1999); Mukhtar *et al*., (2005) and Ahmad and Mukhtar (2007), who reported that *P. penetrans* is effective against *M. arenaria* and *M. incognita* race1 in different crops.

Spores of Pasteuria can attach to the cuticles of the second stage juveniles and germinate after the juvenile has entered roots and begun feeding. The germ tubes can penetrate the cuticle, and vegetative micro-colonies then form and proliferate through the body of the developing female. Finally, the reproductive system of the female nematode degenerates and mature endospores are released into the soil (Mankau et al., 1976; Sayre & Wergin, 1977). Efficacy *P. penetrans* might occur through a lot of biological mechanisms. Reducing female fertility one of the most effective mechanisms in controlling *Meloidogyne* spp. as cited by Bird (1986); Bird and Brisbane (1988). Decreasing the movement, mobility of J2 and

their ability to locate host roots as showed by Mankau and Prasad (1977) and Davies *et al*. (1991). *P. penetrans* can reduce the number of J2 penetrating roots as suggested by Brown and Smart (1985); Davies *et al*., (1988), and Sekhar and Gill (1990). Less number of females in roots as observed by Davies *et al*., (1991). Reducing The number of J2 in soil by *P. penetrans* spores as confirmed by Chen *et al*., (1997) and Bakr(2008). The number of eggs on roots as reported by Ahmed and Gowen (1991); and Chen *et al*.,(1997). Dube (2001) found that *P. penetrans* suppressed populations of *M. javanica* in soil and reduced root galling as well as increased bean yield. Generally, it can be concluded that Pp was effective against *Meloidogyne* spp. and the efficacy may be referred to reducing one or more from the following: female fertility; the movement, mobility of J2 and their ability to locate host roots; the number of J2 penetrating roots; the number of females in roots; The number of J2 in soil and The number of eggs on roots.

For the wide application of the *P. penetrans* as an eco-friendly bio-control agent for the control of *Meloidogyne* spp. in crop production, future study should be focused on the development of economical and efficient mass production system of the endospores especially under Egyptian condition.

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