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# Control of the Root-Knot Nematode, *Meloidogyne incognita* Using Two Egyptian Isolates of *Pasteuria penetrans*

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Two isolates of the parasite, *Pasteuria penetrans* (*Pp*) were evaluated for their potential against *Meloidogyne incognita* infected tomato plants. The first isolate was associated with root-knot nematode juveniles ($J_2$) infected banana roots at El-Behera governorate (*Pp*B) and the second isolate was associated with $J_2$ infected grapevine roots at Kafer EL-Sheikh governorate (*Pp*G). Treatment with the nematicide, Ragby 60% gave the highest reduction in nematode root galls and egg masses (96.3-97.3%). Treatment with spore suspension of *Pp*B ($5\times10^4$ and $1\times10^5$ spore/pot) from infested soil (IS) caused significant reduction in number of nematode root galls and egg masses, more than that of isolate *Pp*G. Treatments with spore suspension of *Pp*B ($2\times10^4$ or $4\times10^4$ spore/pot) from powdered roots (PR) caused considerable reduction, followed by the treatment with *Pp*G. Treatments with Ragby 60% and different IS spore suspension ($1\times10^5$ and $5\times10^4$ spore/pot) of *Pp* isolates caused significant increase in dry weight of shoot and root systems. The PR spore suspension of *Pp*B caused an increase in dry weight of shoots and roots. However, treatments with PR spore suspension of *Pp*G caused an increase in dry weights of shoot and root systems. Treatments with different numbers of *Pp*B spores attached to the cuticle of $J_2$ (5-10 and 15-20 spore/$J_2$) caused 37.6-46.4% reduction in the number of nematode root galls and egg masses, whereas the same treatments with the isolate *Pp*G caused 28.8-42.2% reduction in nematode root galls and egg masses. In addition, significant differences in the numbers of spores formed on juveniles released at experiment termination were detected. The effect of different numbers of juveniles encumbered with *Pp* isolates on *M. incognita* infected tomato plants revealed that treatments with high numbers (150 and 200 $J_2$/pot) encumbered with either *Pp* isolates caused significant reduction in number of nematode root galls and egg masses. Treatment with 100,150 and 200 $J_2$ encumbered with *Pp* isolates/pot caused a significant increase in dry weight of shoot and root systems. However, treatment with 50 $J_2$ encumbered with either *Pp* isolates/pot caused 12.5-35.6% increase in dry weight of shoot and root systems. Soil treated with IS of *Pp*B, containing $1\times10^5$ spores plus 150 or 200 $J_2$/pot encumbered with spores of *Pp*B to the infected tomato plants, previously inoculated with healthy $J_2$ of *M. incognita* showed significant reduction in number of nematode root galls; egg masses and number of $J_2$/250cc soil. Microscopic observations revealed the presence of bear structures of *Pp* spores adhering not only to lateral fields but also allover the $J_2$ cuticle. Scanning electron microscope examination indicated that spores measure about 2.7$\mu$m in diameter, the exsosporium membrane doesn't exist, and the spore can be resolved into two distinct components: a central spore, 0.9$\mu$m in diameter and a peripheral matrix, 1.8 $\mu$m in width.

**Keywords**: *Meloidogyne incognita*, nematicide, *Pasteuria penetrans*, Ragby, root-knot nematode and tomato.

Plant parasitic nematodes are considered serious world-wide pests of many cultivated crops (Davies *et al.*, 1991 and Akhter, 2000). Biological control of plant parasitic nematodes with soil organisms provides a safe control tactic receiving great interest among nematologists (Gowen and Ahmad, 1990 and Radwan *et al.*, 2004). *Pasteuria penetrans* (Thorne) Sayer & Starr, an obligate mycelial endospore-forming bacterial microparasite, has been found infecting a large number of nematode species, and it could have considerable potential as a biological control agent against plant parasitic nematodes (Stirling, 1985; Stiriling *et al.*, 1990; Chen *et al.*, 1996 and Chen and Dickson, 1998). *Pasteuria* spp. had long been present and are frequently observed on the juveniles cuticle, and it is the best known species against root-knot nematode that effectively suppress root-knot nematode populations in pot and microplot experiments (Sayre and Wergin, 1977; Sayre, 1980; Bird and Brisbane 1988; Channer and Gowen, 1988; Maqbool and Zaki 1990; Stiriling *et al.*, 1990; Bekal *et al.*, 2001 and Talavera *et al.*, 2002). The extended survival in soil, host specificity and tolerance to heat, desiccation and chemicals make *P. penetrans* one of the more promising biological control agents against the root-knot nematodes (Sturhan, 1988 and Stiriling *et al.*, 1990). The reliability of microbial control programs in general could be improved by a better quantitative understanding of host-parasite population dynamics (Pinnock and Brand, 1981).

The present research was carried out to study the effect of different concentrations of two local isolates of *Pasteuria penetrans* (*Pp*) spore suspension in two application form (infested soil and powdered roots) and the nematicide, Ragby 60%, different numbers of *Pp* spores on the encumbered $J_2$ cuticle, different numbers of $J_2$ encumbered with spores suspension of *Pp* isolates and IS spore suspension of *Pp*B isolate plus $J_2$ encumbered with *Pp*B spores on controlling root-knot nematode, *M. incognita*, infected tomato plants.

## Materials and Methods

*Nematode culture:*

A single egg-mass culture of root-knot nematode, *M. incognita*, was initiated and propagated on tomato plants (*Lycopersicon esculentum* Mill) cv. Marmand in Plant Nematology greenhouse, Department of Plant Pathology, Alexandria University. Whenever needed, egg suspension was prepared by extracting eggs from 8-10 wk-old tomato roots with sodium hypochlorite (Hussey and Barker, 1973). Eggs suspension was put on a sieve lined with tissue paper to collect freshly hatched juveniles after 48 hours of incubation.

*Pasteuria isolates:*

Two Egyptian isolates of *Pasteuria penetrans* (*Pp*) parasitizing $J_2$ of root-knot nematode (*Meloidogyne* spp.). An isolate was obtained from infected banana roots at El-Behera governorate (*Pp*B) and an isolate from $J_2$ infecting grapevine roots at Kafer EL-Sheikh governorate (*Pp*G). Each tested *Pp* isolate was separately cultured on *M. incognita* on tomato plants cv. Marmand in the greenhouse. Juveniles encumbered with *Pp* spores, 2-3 days old, collected by centrifugation attachment method (Hewlett and Dickson, 1994).

*Effect P. penetrans and the nematicide, Ragby 60% on M. incognita on tomato plants:*

Two-week-old tomato seedlings were inoculated with 500 $J_2$ encumbered with spores of each isolate of *Pp*. A month after inoculation, dosages of 10 and 20 grams of powdered roots (PR) and two doses of 15 and 30 grams of the infested soil (IS), containing spores of each *Pp* isolate, were grounded using a mortar and pestle in order to release the bacterial spores from plant root tissues and soil. Resulting slurry was passed through a 25-$\mu$m sieve to separate root debris and soil particles as described by Stirling and White (1982). Number of spores in the suspension was assessed using a haemocytometer slide. Two doses of spore concentration, resulted from IS, were applied ($5\times10^4$ and $1\times10^5$ spore/ml) and suspension resulted from PR were applied at ($2\times10^4$ and $4\times10^4$ spore/ml). Fifty ml of the nematicide, Ragby 60% at the rate of 4ml/l were used/pot.

Two seedlings of tomato plants cv. Marmand were transplanted in 15 cm diameter clay pots filled with autoclaved sandy clay soil. Two weeks later, seedlings were inoculated with 1000 fresh uninfected $J_2$ of *M. incognita*/pot, and treated with the previously mentioned biocontrol treatments at the same time of *M. incognita* inoculation. Treatments were replicated five times. Plants were harvested 60 days after bacterial inoculation. Numbers of nematode root galls, egg masses/plant and dry weight of root and shoot systems of tomato plants were recorded.

*Effect of number of P. penetrans spores attached to $J_2$ cuticle on controlling M. incognita on tomato plants:*

Fresh uninfected $J_2$ of *M. incognita* (300 $J_2$/pot) were applied to 15 cm diameter pot filled with autoclaved sandy clay soil (1 kg) and infested, at the same time, with 50 ($J_2$) encumbered with *Pasteuria* spores with different numbers attached to each $J_2$, (5-10; 15-20; 25-30 and 35-40 spore/$J_2$) of each *Pasteuria* isolate. Each treatment was replicated five times. Total nematode inoculum with both uninfected and encumbered $J_2$ was 350 $J_2$/pot.

Two seedlings of tomato plants two-wk-old/pot were transplanted 24 hours after nematode establishment. All pots received the recommended agricultural treatments for two months.

Plants harvested 60 days after bacterial inoculation. Numbers of nematode root galls, egg masses/plant and dry weight of root and shoot systems were recorded. Total number of $J_2$ extracted from soil by modified Bermann method (Schindler, 1961); was recorded and the number of *P. penetrans* spores attached to sample of (20) juveniles of each replicate was assessed using a microscope (x40).

*Effect of numbers of juveniles encumbered with spores of P. penetrans isolates on controlling M. incognita on tomato plants:*

Fresh uninfected $J_2$ of *M. incognita* were amended to 15 cm diameter pots filled with autoclaved sandy clay soil, and treated with different numbers of 50, 100, 150 and 200 of juveniles encumbered with 10-15 *Pp* spore/ $J_2$. Each treatment was replicated five times. Two seedlings of tomato plants two-wk-old/pot were transplanted 24 hours after nematode establishment. Final nematode inoculum was

adjusted to 900 $J_2$/pot, by adding uninfected $J_2$ to the number of encumbered $J_2$. Five untreated pots were left as a control. All replicates were arranged in a randomized complete block design in a greenhouse at 28±30°C. The experiment was terminated two months after the nematode inoculation. Plants were gently removed from pots, and roots were washed to be free of soil. Number of nematode root galls and egg-masses/plant, and dry weight of shoot and root systems were determined.

*Effect of PpB spores plus $J_2$ encumbered with PpB spores on controlling root-knot nematode, M. incognita infected tomato plants:*

Two seedlings of tomato cv. Marmand two-wk-old were planted in 15 cm diameter clay pots filled with autoclaved sandy clay soil. Pots were inoculated with 1000 freshly uninfected $J_2$ of *M. incognita*/pot. Two weeks later, pots were treated with IS of isolate *Pp*B containing $1\times10^5$ spores plus 150 or 200 $J_2$ encumbered with isolate *Pp*B/pot. Each treatment was replicated five times. Experiment was terminated six weeks after the second treatment. Plants were harvested and number of nematode root galls and egg-masses per plant and number of juveniles/250 cc soil were assessed. Juveniles in soil of each treatment were extracted using modified sieving and centrifugal technique (Ayoub, 1980) and counted under a microscope (x40) using Peter's 1 ml counting slide.

*Light and scanning electron microscope examinations:*

Second stage juveniles encumbered with the bacterial spores of *Pp* were killed and fixed using the method of Seinhorest (1966) and photographed under compound microscope (x40). Moreover, similar juveniles were prepared for scanning electron microscopy, by fixing with 3% glutardehyde in 0.05 M phosphate buffer for 1.5 hours, dehydrating in an ethanol series to critical drying point onto the surface of an aluminium stub and air-drying. Aluminium stubs containing the dried specimens were coated with gold-palladium. Juveniles were examined and photographed with JSM-5300 scanning microscope operating at 25 kV (Sayre and Wergin, 1977; Spiegel *et al.*, 1996), at the Electron Microscopy Unit, Faculty of Science, Alexandria University.

*Statistical analysis:*

Data obtained were statistically analyzed according to SAS software program (SAS Institute, 1997). Numbers of nematode root galls and egg-masses were transformed to $\sqrt{X+1}$ before statistical analysis. Moreover, means were compared with revised LSD test at 5% level of probability.

## Results

*Effect of two Egyptian isolates of Pp and the nematicide, Ragby 60% on M. incognita on tomato plants:*

Data in Table (1) show that treatment with different spore concentrations of *Pasteuria* isolates extracted from powdered roots and soils provided various degrees of controlling *M. incognita* infecting tomato plants. Treatments with infested soil (IS) containing $5\times10^4$ and $1\times10^5$ spore/pot of the isolate *Pp*B caused reductions (73.1- 82.7%) more than that of isolate *Pp*G (54.3- 69.2%) in number of root galls

and nematode egg-masses. Also, treatment with powdered roots (PR) containing $2\times10^4$ and $4\times10^4$ spore/pot of the isolate *Pp*B caused considerable reduction (51-58.5%), followed by the treatment with the isolate *Pp*G (31.3-47.5%) in number of root galls and nematode egg-masses in comparison with control treatment (uninfected $J_2$). Treatment of Ragby 60% caused the highest reductions in numbers of nematode root galls and egg masses (96.3 and 97.3%), respectively (Table 1).

**Table 1. Effect of *P. penetrans* (*Pp*) isolates and the nematicide Ragby 60% on *M. incognita* development on tomato plants**

| Treatment | Mean No. of galls | Reduction (%) | Mean No. of egg masses | Reduction (%) |
|---|---|---|---|---|
| Uninfected *M. incognita* $J_2$ (control) | 791.8 a | - | 743 a | - |
| Ragby 60% 4ml/l | 29.6 g | 96.3 | 19.8 i | 97.3 |
| *P. penetrans* isolate *Pp*B from: | | | | |
| Powdered root | | | | |
| $2\times10^4$ spores/pot | 387.8 d | 51 | 341.4 ef | 54 |
| $4\times10^4$ spores/pot | 332.2 de | 58 | 305.6 de | 58.8 |
| Soil | | | | |
| $5\times10^4$ spores/pot | 213 f | 73.1 | 189 g | 74.5 |
| $1\times10^5$ spores/pot | 212.2 f | 73.2 | 128.2 h | 82.7 |
| *P. penetrans* isolate *Pp*G from: | | | | |
| Powdered root | | | | |
| $2\times10^4$ spores/pot | 543.6 b | 31.3 | 508.8 b | 31.5 |
| $4\times10^4$ spores/pot | 417.6 c | 47.3 | 389.8 c | 47.5 |
| Soil | | | | |
| $5\times10^4$ spores/pot | 379.8 cd | 54.4 | 339.8 d | 54.3 |
| $1\times10^5$ spores/pot | 265.6 ef | 66.3 | 228.8 gf | 69.2 |

(*Pp*B)= *P. Penetrans* originated from the infected 2$^{nd}$ stage juveniles on banana roots.
(*Pp*G)=*P. Penetrans* originated from the infected 2$^{nd}$ stage juveniles on grapevine roots.
Data are averages of 5 replicates.
Values in each column followed by the same letter(s) are not significantly different at $P= 0.05$ according to revised LSD test.

Treatment with the nematicide, Ragby 60% 4ml/l and the two spore concentrations of IS contained $5\times10^4$ and $1\times10^5$ spore/pot caused significant increase (87.6-97.9%) in dry weights of shoot and root systems (Table 2). However, PR treatments with *Pp*B spore concentration of $2\times10^4$ and $4\times10^4$ spore/pot caused (55.1-72.8%) increase in dry weights of shoot and root systems, followed by PR treatments of $2\times10^4$ and $4\times10^4$ spore/pot of the isolate *Pp*G, which caused (25.9-31.5%) increase in dry weight of shoot and root systems, compared to control treatment (Table 2).

**Table 2. Effect of *P. penetrans* and the nematicide Ragby 60% on dry weight of tomato plants infected with *M. incognita***

| Treatment | Mean of shoot dry weight (g) | Increase (%) | Mean of root dry weight (g) | Increase (%) |
|---|---|---|---|---|
| Uninfected *M. incognita* $J_2$(control) | 9.8 d | - | 8.1 e | - |
| Ragby 60% 4ml/l | 19.3 a | 97.9 | 15.7 a | 93.8 |
| *P. penetrans* isolate *Pp*B from: | | | | |
| Powdered root | | | | |
| $2\times10^4$ spores/pot | 14.2 c | 30.9 | 10.2 d | 25.9 |
| $4\times10^4$ spores/pot | 14.3 c | 31.5 | 11.3 d | 39.5 |
| Soil | | | | |
| $5\times10^4$ spores/pot | 18.9 b | 92.9 | 15.2 a | 87.6 |
| $1\times10^5$ spores/pot | 18.7 b | 90.8 | 16.0 a | 97.5 |
| *P. penetrans* isolate *Pp*G from: | | | | |
| Powdered root | | | | |
| $2\times10^4$ spores/pot | 15.3 c | 56.1 | 13.8 c | 70.3 |
| $4\times10^4$ spores/pot | 15.2 c | 55.1 | 14.1 c | 72.8 |
| Soil | | | | |
| $5\times10^4$ spores/pot | 19.2 a | 95.9 | 15.8 a | 95.1 |
| $1\times10^5$ spores/pot | 19.0 a | 94.9 | 15.9 a | 96.3 |

(*Pp*B)= *P. penetrans* originated from the infected 2$^{nd}$ stage juveniles on banana roots.
(*Pp*G)=*P. penetrans* originated from the infected 2$^{nd}$ stage juveniles on grapevine roots.
Data are averages of 5 replicates.
Values in columns followed by the same letter(s) are not significantly different at $P$= 0.05 according to revised LSD test.

*Effect of number of Pp spores attached on the juvenile cuticles on controlling M. incognita on tomato plants:*

Data in Table (3) indicated that treatments with juveniles encumbered with (5-10 and 15-20 spores/$J_2$) of *Pasteuria* isolates *Pp*B and *Pp*G caused (37.6-46.4% and 28.8-42.2%) reduction in the number of nematode root galls and egg-masses, respectively. Whereas, treatments with juveniles encumbered with (25-30 and 35-40 spores/$J_2$) of *Pasteuria* isolates resulted in the lowest significant reductions (2.7-8.4%) and (4.7-8.7%) in number of nematode root galls and egg-masses, respectively.

Microscopic examination at the end of the experiment showed that there were significantly differences in number of bacterial spores formed on the released $J_2$ in soil among different treatments with *Pasteuria* isolates (Table 4). Data indicated that number of spores attached to the released $J_2$ is directly proportioned to the number of spores on the $J_2$ presented at the time of inoculation (Table 4).

**Table 3. Effect of number of *Pp* spores attached on the juvenile cuticles on *M. incognita* development on tomato plants**

| Treatment | *Pp*B | | | | *Pp*G | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean No. of galls / plant | Red. % | Mean No. of egg masses / plant | Red. % | Mean No. of galls / plant | Red. % | Mean No. of egg masses / plant | Red. % |
| Uninfected $J_2$ (control) | 140.8a | - | 130.2 a | - | 138.2 a | - | 129.2 a | - |
| $J_2$ encumbered with: | | | | | | | | |
| 5-10 spores | 77.2 b | 45.2 | 69.8 b | 46.4 | 81.6 b | 40.9 | 74.6 b | 42.2 |
| 15-20 spores | 87.8 b | 37.6 | 74.8 b | 42.7 | 98.4 b | 28.8 | 86.4 b | 33.1 |
| 25-30 spores | 129.2 a | 8.4 | 118.8 a | 8.7 | 134.2 a | 2.8 | 121.2 a | 6.1 |
| 35-40 spores | 137.0 a | 2.7 | 122.4 a | 5.9 | 132.0 a | 4.4 | 123.0 a | 4.7 |

(*Pp*B)= *P. penetrans* originated from the infected 2nd stage juveniles on banana roots.
(*Pp*G)= *P. penetrans* originated from the 2nd stage juveniles on grapevine roots.
Data are averages of 5 replicates.
Values in each column followed by the same letter are not significantly different at $P= 0.05$ according to revised LSD test.

**Table 4. Mean number of the attached *P. penetrans* spores of the two Egyptian isolates infecting the released $J_2$ of *M. incognita*, 60 days after nematode inoculation**

| Treatment | Mean number of spores /$J_2$ | |
|---|---|---|
| | *Pp*B | *Pp*G |
| Uninfected $J_2$ (control) | 0 a | 0 a |
| $J_2$ encumbered with: | | |
| 5-10 spores | 7 b | 6.2 b |
| 15-20 spores | 3.4 b | 3.2 b |
| 25-30 spores | 13.2 c | 12.4 c |
| 35-40 spores | 24.2 c | 22.4 c |

(*Pp*B)= *P. penetrans* originated from the infected 2nd stage juveniles on banana roots.
(*Pp*G)= *P. penetrans* originated from the infected 2nd stage juveniles on grapevine roots.
Data are averages of 20 juveniles/replicate.
Values in each column followed by the same letter are not significantly different at $P= 0.05$ according to revised LSD test.

*Effect of number of juveniles encumbered with spores of Pp isolates on controlling M. incognita on tomato plants:*

Data in Table (5) indicated that increasing numbers of $J_2$ encumbered with *Pp*B isolate resulted in more significant decrease in nematode infection parameters than that of isolate *Pp*G, compared to control. Treatment with 200 $J_2$ encumbered with *Pasteuria* isolate *Pp*B/pot caused significant reduction (86.7% and 87.1%), followed by treatment with 150 $J_2$ encumbered with *Pp*B/pot (73.4% and 75.4%) reduction in number of root galls and nematode egg-masses. Treatment with 200 and 150 $J_2$ encumbered with isolate *Pp*G/pot caused significant reduction (69.9-83.3%) in number of root galls and nematode egg-masses compared to control treatment. Treatment with 50 and 100 $J_2$ encumbered with either two *Pasteuria* isolates/pot caused 52.4-54.9% reduction in number of root galls and nematode egg-masses in comparison with the control treatment.

**Table 5. Effect of number of $J_2$ encumbered with spores of *Pp* isolates on *M. incognita* development on tomato plants**

| Treatment | *Pp*B | | | | *Pp*G | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean No. of galls | Red. % | Mean No. of egg masses | Red. % | Mean No. of galls | Red. % | Mean No. of egg masses | Red. % |
| Uninfected $J_2$ (control) | 892.8 a | - | 881.4 a | - | 843.2 a | - | 819.4 a | - |
| Encumbered $J_2$ | | | | | | | | |
| 50 $J_2$ /pot | 416.8 b | 53.3 | 418.6 b | 52.5 | 394.8 b | 52.6 | 389.8 b | 52.4 |
| 100 $J_2$ /pot | 409.6 b | 54.1 | 397.2 b | 54.9 | 387.2 b | 53.6 | 371.2 b | 54.6 |
| 150 $J_2$ /pot | 237.4 c | 73.4 | 216.8 c | 75.4 | 253.8 c | 69.9 | 247 c | 69.9 |
| 200 $J_2$ /pot | 118.8 d | 86.7 | 113.6 d | 87.1 | 141.8 d | 83 | 136.6 d | 83.3 |

*Pp*B= *P. Penetrans* originated from the infected $J_2$ of *Meloidogyne* spp. on banana roots.
*Pp*G=*P. Penetrans* originated from the infected $J_2$ of *Meloidogyne* spp. on grapevine roots.
Data are averages of 5 replicates.
Values in each column followed by the same letter are not significantly different at $P= 0.05$ of LSD test.

Data in Table (6) indicated that treatments with 100,150 and 200 $J_2$ encumbered with either *Pasteuria* isolates/pot caused significant increment ranged from 31.1% to 66.5% in dry weights of shoot and root systems. However, treatments with 50 $J_2$ encumbered with either *Pasteuria* isolates, caused (12.5-35.6%) compared to the control treatment with uninfected juveniles (Table 6).

**Table 6. Effect of number of $J_2$ encumbered with spores of *Pp* isolates on the dry weight of tomato plants infected with *M. incognita***

| Treatment | *Pp*B | | | | *Pp*G | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean of Shoot dry weight (g) | Increase (%) | Mean of Root dry weight (g) | Increase (%) | Mean of Shoot dry weight (g) | Increase (%) | Mean of Root dry weight (g) | Increase (%) |
| Uninfected *M. incognita* $J_2$ (control) | 7.3 d | - | 6.2 d | - | 8.4 cd | - | 5.6 c | - |
| Encumbered $J_2$: | | | | | | | | |
| 50 $J_2$ /pot | 9.5 cd | 23.1 | 8.1 b | 23.5 | 9.6 bc | 12.5 | 8.7 b | 35.6 |
| 100 $J_2$ /pot | 13.7 b | 46.7 | 12.9 b | 51.9 | 12.2 b | 31.1 | 10.5 ab | 45.7 |
| 150 $J_2$ /pot | 16.8 a | 56.5 | 15.8 a | 60.7 | 15.3 a | 45.1 | 13.2 a | 57.8 |
| 200 $J_2$ /pot | 17.6 a | 58.5 | 16.8 a | 63.1 | 18.9 a | 55.6 | 15.7 a | 66.5 |

*Pp*B= *P. Penetrans* originated from the infected $J_2$ of *Meloidogyne* spp. on banana roots.
*Pp*G=*P. Penetrans* originated from the infected $J_2$ of *Meloidogyne* spp. on grapevine roots.
Data are averages of 5 replicates.
Values in each column followed by the same letter are not significantly different at $P= 0.05$ of LSD test.

*Effect of P. penetrans isolate PpB on M. incognita infected tomato plants:*

Data in Table (7) indicated that galled tomato plants treated with *Pasteuria* isolate *Pp*B showed a significant decrease in nematode infection parameters compared to *Pp* untreated galled plants. Treatments with IS contained $1\times10^5$ *Pp* spores plus 150 and 200 encumbered $J_2$/pot caused 83% and 84.7% reductions in number nematode egg-masses, 69.2% and 77.6% reductions in number of nematode root galls and 70.1% and 75.5% reductions in number of juveniles/250 cc soil, respectively in comparison to *Pp* untreated galled tomato plants.

**Table 7. Effect of *Pp*B spores plus $J_2$ encumbered with *Pp*B spores on controlling root-knot nematode, *M. incognita* infected tomato plants**

| Treatment | Mean No. of Galls | Reduction (%) | Mean No. of Egg masses | Reduction (%) | Mean No. of $J_2$/ 250 cc soil | Reduction (%) |
|---|---|---|---|---|---|---|
| 1st treatment | | | | | | |
| Untreated | 639 a | - | 597 a | - | 960 a | - |
| Treated | 196.8 b | 69.2 | 101.6 b | 83 | 287 b | 70.1 |
| 2nd treatment | | | | | | |
| Untreated | 622 a | - | 603 a | - | 865 a | - |
| Treated | 139.5 b | 77.6 | 92.4 b | 84.7 | 212 b | 75.5 |

1st treatment= IS contained $1\times10^5$ spores +150 $J_2$ encumbered with *Pp*B spore/pot.
2nd treatment= IS contained $1\times10^5$ spores +200 $J_2$ encumbered with *Pp*B spore/pot.
Data are averages of 5 replicates.
Values in each column followed by the same letter are not significantly different at $P= 0.05$.

*Light and scanning electron microscope examinations:*

Microscopic observations revealed the presence of bear structures of the parasite *Pp* spores adhering to the $J_2$ cuticle. The spores were adhering not only to lateral fields but also allover the $J_2$ cuticle. Light and scanning electron microscope examination showed that $J_2$ are highly encumbered with bacterial spores which apparently attached along the juveniles cuticle (Fig.1, A, C, D, E). Scanning electron microscope examination indicated that spores measure about 2.7$\mu$m in diameter, adhering to the surface of root-knot juveniles, considered mature. The exsosporium membrane doesn't exist, and the spore can be resolved into two distinct components: a central spore, 0.9 $\mu$m in diameter, that is spherical, and a peripheral matrix, 1.8 $\mu$m in width, that surround the spore. The smooth central surface of spore was easily distinguished from its peripheral matrix, which forms an encircling ring with a particulate surface (Fig.1, B).

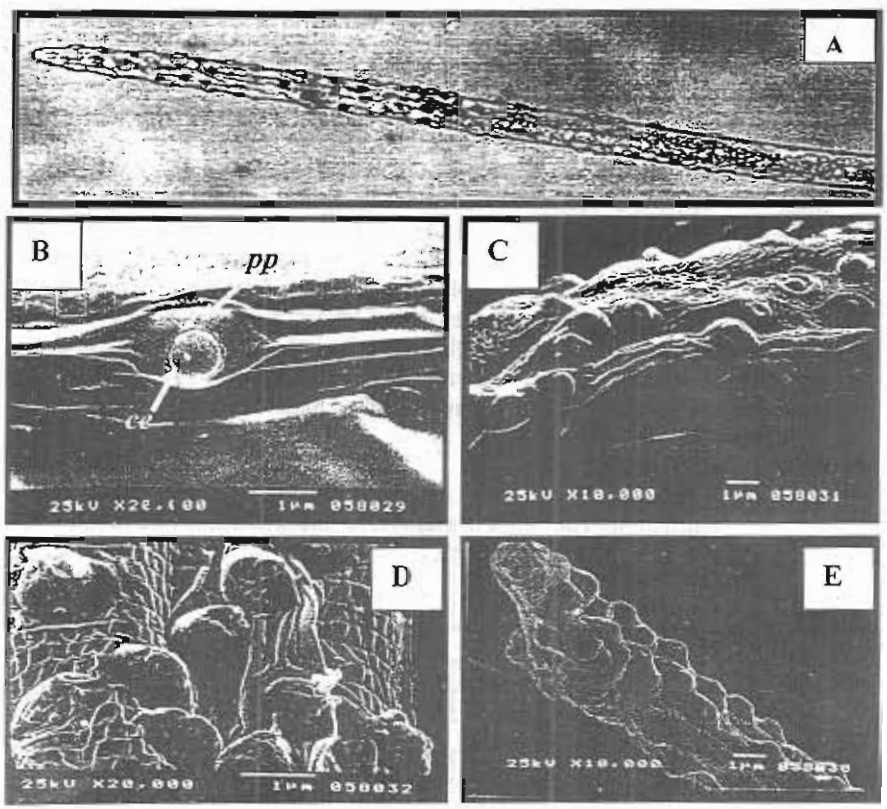


Fig. 1: Light microscope micrograph showed encumbered *M. incognita* $J_2$ highly infected with bacterial spores, which apparently attached along the juvenile cuticle (A). Scanning electron micrographs of spores associated with juveniles, the central spore [*ce*] can be distinguished from the peripheral parasporal matrix [*pp*] (B). The spores apparently attached along the juvenile cuticle C, D& E.

## Discussion

*Pasteuria penetrans* (*Pp*) has a great potential as economical and environmental friendly biological control agent (Nishizawa, 1984; Chen and Dickson, 1998). In the present work, treatment with the nematicide, Ragby 60% 4ml/l decreased significantly *M. incognita* reproduction. In addition, treatments with different spore concentrations of the Egyptian isolates of *Pp*, extracted from infested powdered roots and soil showed varying degrees of *M. incognita* control. In addition, health of tomato plants, grown in pots treated with different spore concentrations of *Pp* isolates, was enhanced. These findings are in agreement with those of other workers (Tzortzakakis *et al.*, 1997; Talavera *et al.*, 2002 and Zareen *et al.*, 2002 a & b). These results were in agreement with those of other workers (Alan and Brisbane, 1988; Kumari and Sivakumar, 2005).

Alan and Brisbane (1988) reported that, treatment of *M. incognita* on tomato plants with soil collected from grapevine rhizosphere, has shown a significant suppressive effect to nematode reproduction and caused significant increase in plant health. In addition, Kumari and Sivakumar (2005) showed that, treatment with air dried root powder of grapevine plants containing *P. penetrans* showed a great reduction in *M. incognita* reproduction on grapevine plants.

Incorporating of dried root powder or soil could result in increasing spore numbers in soil and provide a simple means by which growers could gain more lasting suppression and could improve plant health (Gowen and Ahmad, 1990).

The results suggest that small numbers of 5-10 spore/$J_2$ of *Pasteuria* were a suitable way for effectively introducing the parasite into uninfested soil and reducing nematode parameters, and increasing spore numbers to 20-45 spore/$J_2$ showed no significant differences in reducing nematode reproduction in comparison with the control treatment with uninfected juveniles. These findings are in agreement with those of other workers (Minton and Sayre, 1989; Espanol *et al.*, 1997; Chand and Gill, 2003 and Darban *et al.*, 2005).

Striling and White (1982) reported that a juvenile carrying larger number of *Pasteuria* spores become less mobile in the rhizosphere and can not easily invade plant tissues. In addition, Zareen *et al.* (2002 b) found that *M. javanica* invasion was suppressed in treatments, receiving juveniles encumbered with heavy spore load.

It was also found that tomato plants treated with increased numbers of juveniles encumbered with low spore numbers 10-15/$J_2$ gave significant decrease in *M. incognita* reproduction and enhanced plant growth. These findings are in agreement with those of Gowen and Ahmad (1990) and Zareen *et al.* (2002 a).

The present data indicated that tomato plants, infected by *M. incognita*, treated with IS contained $1\times10^5$spore/ml of isolate *Pp*B plus 150 and 200 $J_2$ encumbered with the same isolate/pot showed a significant decrease in gall formation, egg mass production and number of juveniles/250cc soil. These findings are in agreement with those of Tzortzakakis *et al.* (1997) and Zareen (2002 a&b).

Microscopic studies indicated that *M. incognita* juveniles were highly encumbered with *P. penetrans* spores which were attached allover the cuticle. This finding is in agreement with those of Bekal *et al.* (2001) and Atibalentja *et al.* (2004) and disagreement with those of Bird and Brisbane (1988); Afolabi *et al.* (1995) and Spiegel *et al.* (1996). Sayre and Wergin (1977) indicated that *Pasteuria* spores are attached only to juveniles cuticle along the lateral fields.

Interest in the use of *P. penetrans* will increase in the future, particularly if chemical soil treatments are restricted. However, the parasite may be effective only as a part of a package of other treatments that stress nematodes and make them more susceptible to attack by biocontrol agents. Additional test treatments and practices that favour the biocontrol agent *P. penetrans* are necessary to develop techniques to monitor its incidence in soil and then correlate it with enhanced plant growth and decreased nematode reproduction.

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# مقاومة نيماتودا تعقد الجذور *Meloidogyne incognita* بإستخدام عزلتين مصريتين من البكتريا *Pasteuria penetrans*

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درس قدرة عزلتين من البكتريا *Pasteuria penetrans* على مقاومة نيماتودا تعقد الجذور *Meloidogyne incognita* على نباتات الطماطم في أربعة تجارب منفصلة. جراثيم العزلة الأولى *Pp*B وجدت ملتصقة بكيوتيكل يرقات الطور الثاني للنيماتودا المصاحبة لعينات تربة وجذور نباتات موز من محافظة البحيرة وجراثيم العزلة الثانية *Pp*G وجدت ملتصقة بكيوتيكل يرقات الطور الثاني لنيماتودا تعقد الجذور المصاحبة لعينات تربة وجذور نباتات عنب من محافظة كفر الشيخ .

أتضح من النتائج أن استخدام المبيد النيماتودي راجبي ٦٠% بمعدل(٤مل/لتر) أدى الى حدوث أعلى نسبة خفض في أعداد العقد الجذرية وأكياس بيض النيماتودا ، وأدت المعاملة بمجفف الجذور المحتوى على الجراثيم البكتيرية للعزلة *Pp*B بتركيزات ( ١×١٠$^{٥}$ أو ٥×١٠$^{٤}$ جرثومة/أصيص) إلى خفض معنوى في أعداد العقد الجذرية وأكياس بيض النيماتودا ، فى حين أن إستخدام نفس التركيز للعزلة *Pp*G نتج عنه خفض في أعداد العقد الجذرية وأكياس بيض النيماتودا (٥٤,٣-٦٩,٢%). أما إستخدام التربة الملوثة بالجراثيم البكتيرية بتركيز (٢×١٠$^{٤}$ أو ٤×١٠$^{٤}$ جرثومة/أصيص) للعزلة *Pp*B فنتج عنه خفض معنوى في أعداد العقد الجذرية وأكياس بيض النيماتودا (٥١- ٥٨,٨%)، يليه إستخدام نفس التركيز للعزلة *Pp*G (٣١,٣-٤٧,٥%). وقد أدت المعاملة بمجفف الجذور لأى من العزلتين *Pp*G و*Pp*B بتركيز( ١×١٠$^{٥}$ أو ٥×١٠$^{٤}$ جرثومة/أصيص) وكذا المبيد النيماتودي راجبي ٦٠% الى حدوث زيادة معنوية فى الوزن الجاف لكل من المجموع الخضرى والجذرى للنباتات المعاملة (٨٧,٦-٩٧,٩%) ونتج عن المعاملة بالتربة الملوثة بجراثيم العزلة *Pp*B بتركيز (٢×١٠$^{٤}$ أو ٤×١٠$^{٤}$ جرثومة/أصيص) حدوث زيادة معنوية فى الوزن الجاف لكل من المجموع الخضري والجذري للنباتات المعاملة (٥٥,١-٧٢,٨%). ونتج عن المعاملة بالتربة الملوثة بجراثيم العزلة *Pp*G بنفس التركيز السابق زيادة فى الوزن الجاف لكل من المجموع الخضرى والجذرى للنباتات المعاملة (٢٥,٩-٣٩,٥%) مقارنة بالكنترول.

وقد أدى استخدام أعداد مختلفة من الجراثيم البكتيرية ملتصقة بكيوتيكل يرقات الطور الثاني (٥-١٠ جرثومة/يرقة أو ١٥-٢٠ جرثومة/يرقة) لكل من العزلتين *Pp*G و*Pp*B إلى حدوث خفض معنوي فى أعداد العقد الجذرية وأكياس البيض النيماتودية. كما لوحظ وجود اختلافات معنوية في أعداد الجراثيم المتكونة على كيوتيكل يرقات الطور الثاني المستخلصة في نهاية التجربة. أدت المعاملة باستخدام أعداد مختلفة من يرقات الطور الثاني لنيماتودا تعقد الجذور المصابة بأى من العزلات البكتيرية بمعدل( ١٥٠ أو ٢٠٠ يرقة مصابة/أصيص) إلى خفض معنوى فى عدد العقد الجذرية وأكياس البيض النيماتودية. كما أدى استخدام (٥٠ أو ١٠٠ يرقة مصابة بأى من عزلات البكتيريا *P. penetrans* / أصيص) حدوث خفض فى عدد العقد الجذرية وأكياس البيض للنباتات المعاملة. كذلك تسببت المعاملة بأعداد مختلفة (١٠٠، ١٥٠، ٢٠٠ يرقة/أصيص) من يرقات الطور الثاني لنيماتودا تعقد الجذور والحاملة لجراثيم أى من العزلتين البكتيريتين السابقتين فى حدوث زيادة معنوية فى الوزن الجاف لكل من المجموع الخضرى والجذرى للنباتات المعاملة. فى حين نتج عن استخدام ٥٠ يرقة حاملة لجراثيم أى من العزلتين البكتيريتين/أصيص إلى حدوث زيادة فى الوزن الجاف لكل من المجموع الخضرى والجذرى للنباتات المعاملة مقارنة بالكنترول.

بدراسة تأثير إضافة مجفف الجذور المحتوى لجراثيم العزلة *Pp*B بتركيز ١×١٠$^{٥}$ جرثومة/مل وكذلك إضافة يرقات الطور الثاني الحاملة لجراثيم نفس العزلة بمعدل( ١٥٠أو ٢٠٠ يرقة مصابة/أصيص) لنباتات الطماطم المصابة بنيماتودا تعقد الجذور *M. incognita* ، أتضح حدوث خفض معنوى فى أعداد أكياس البيض/نبات (٨٣-٨٤,٧%) وأعداد العقد الجذرية النيماتودية/نبات (٦٩,٢-٧٧,٦%) وأعداد يرقات الطور الثانى المستخلصة فى نهاية التجربة (٧٠,١-٧٥,٥%) /٢٥٠ سم$^{٣}$ من التربة.

أظهر تصوير يرقات الطور الثانى لنيماتودا تعقد الجذور الحاملة والمصابة بالبكتريا *P. penetrans* باستخدام الميكروسكوب الضوئي بقوة تكبير X٤٠ ، أن جراثيم البكتريا منتشرة على كل السطح الخارجى لكيوتيكل اليرقات. وباستخدام الميكروسكوب الالكتروني الماسح وجد أن قطر الجرثومة ٢,٧ ميكروميتر وهى تعتبر جرثومة ناضجة ، وأن طبقة الاكسوسبوريم الخارجية غائبة. والجرثومة تتكون من جزئين طبقة الالياف المحيطة بالجرثومة بقطر ١,٨ ميكروميتر، ويمكن تميزها بسهولة، ومركز الجرثومة الداخلى الذى يشكل حلقة بقطر ٠,٩ ميكروميتر.