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Effect of *Pasteuria penetrans* against *Meloidogyne* spp., on peanut cultivars

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Abstract

Twelve phytoparasitic nematode genera were found infected or associated with the rhizosphere of peanut plants. *Meloidogyne arenaria* was the most common nematode in all collected soil samples followed by *Tylenchorhynchus* spp., *Helicotylenchus* spp., *M. javanica* and *Pratylenchus penetrans*. All tested peanut cultivars were resistant to *M. incognita* race 2 and moderately resistant to *M. javanica*. Whereas, peanut cvs. Balady, Ismailia 1 and Giza 6 were found highly susceptible to *M. arenaria* race 1 and Giza 4 and Giza 5 cvs. were susceptible. The suppressive efficacy of using three isolates of *Pasteuria penetrans* (*Pp*) against *M. arenaria* race 1 infected peanut was tested through two microplot experiments during two growing seasons 2011-12. Treatments in 1st season with two concentrations of the three mixed *Pp* isolates caused the highest reduction of 74.8-86.9% in number of nematode root galls, egg-masses/root system and number of J_2/250 cm^3 soil compared with any single *Pp* isolate application. Meanwhile, all treatments in 2nd season with both concentrations of all *Pp* isolates either alone or as a mixed isolates resulted in 65.7-94.7% reduction in all nematode parameters. Treatments with high concentration of all *Pp* isolates either alone or as mixed resulted 61.4-72.2% encumbered J_2 in the 1st season and increased to 93.2-97.9% in the 2nd season. Treatments with two concentrations of mixed isolates and *Pp*T isolate resulted in a significant increase of 51.7-77.0% in dry weights of shoot and root systems and number of peanut pods/plant, followed by treatments with the same concentrations of *Pp*B and *Pp*G isolates, which showed 32.7-48.5% increase. Number of bacterial nodules/root system was significantly increased with *Pp* treatments in both seasons. However, numbers of adherent endospores on J_2 cuticle were ranged 3.6-9.4 and 6.2-11.4 endospore/J_2 in the 1st and 2nd season, respectively. High *Pp* concentrations resulted in the highest numbers of adherent endospores.

Keywords: *Pasteuria penetrans*, *Meloidogyne*, *Tylenchorhynchus*, *Helicotylenchus*, *Pratylenchus*, peanut

Peanut *Arachis hypogaea* L. is considered one of the important food crops produced in many subtropical and tropical countries and a high value cash crop for small and large growers alike. It was listed as one of the twenty crop plants that stand between man and starvation (Wittwer, 1981). In Egypt, cultivated area of peanut has increased especially in the new reclaimed sandy soil, as peanut area increased from 20000 hectare in year 2000 to about 70000 hectares in 2010 (Anonymous, 2011). Root-knot nematodes species are considered one of the most destructive pathogens attacking peanut worldwide. Root-knot nematode *Meloidogyne arenaria* (Neal) Chitwood was the most common species infecting peanut in many parts of the world (Dickson & De Waele, 2005; Korayem & Bondok, 2013). In addition, *M. javanica* reported attacking peanuts in different parts of the world (Ibrahim & El-Saedy, 1976; Cetintas *et al*., 2003). Infection with *M. arenaria* caused considerable yield losses in severely infested fields. Loss in peanut yield due to nematode pathogen was varied according to plant cultivar, nematode population density and environmental conditions. In Egypt it ranged from 20 to 90% (Korayem & Osman, 1994).

The gram-positive bacterium *Pasteuria penetrans* (Thorne) Sayre & Starr is an agent causes soil suppressive against root-knot nematodes (Kariuki & Dickson, 2007). *P. penetrans* is an endospore forming bacterium, which considered an important parasite of several *Meloidogyne*

species. The binding of a single endospore to a second-stage juvenile was sufficient to allow infection and propagation of the bacteria (Preston *et al*., 2003). Second-stage juveniles of the nematode acquired endospores of the bacterium as they move through soil (Timper, 2009). Within bacterial species existing strains were specific to species within the genus *Meloidogyne* even down to the host race level of the nematode (Chen & Dickson, 1998).

The objectives of this work were to i) identify phytoparasitic nematodes infected and associated with peanut plants in Alexandria, El-Behera and Giza governorates; ii) evaluate the reactions of five peanut cvs., Balady, Ismailia 1, Giza 4, Giza 5 and Giza 6 to root-knot nematodes; *M. arenaria* race 1, *M. javanica* and *M. incognita* race 2 under greenhouse condition and iii) study the effects of three isolates of *P. penetrans* and the nematicide, Nemacure 10G® on controlling *M. arenaria* race 1 infected peanut cv. Giza 6 through microplot experiments.

Materials and Methods

Survey: Survey study carried out during the period of 2011-12 to determine the occurrence of plant-parasitic nematodes associated with peanuts in 3 Egyptian governorates, Alexandria, El-Behera and Giza. A total of 293 composite soil and root samples of 1 kg soil each were collected from the rhizosphere of peanut plants, at a depth of 20-35 cm, 93 samples from Alexandria, 177 from El-Behera and 23 from Giza governorates. Root samples were collected by lifting the plants carefully with a shovel. All samples were kept in polyethylene bags, labeled and transferred directly to the laboratory for nematode extraction and identification. Roots were tap washed free of soil and examined for root galls and root-knot nematode infection. Adult females of root-knot nematodes were isolated from infected gall roots and identified to species level using the perineal patterns (Taylor & Sasser, 1978; Hartman & Sasser, 1985). Each soil sample was thoroughly mixed and a volume of 250 cm^3 soil sample was washed for nematode extraction by means of Cobb's wet-sieving and centrifugal sugar flotation techniques (Ayoub, 1980). Nematode genera in the soil samples were identified and counted under the compound microscope using Peter's 1 ml eelworm counting slide. Identification of plant-parasitic nematodes was made according to the morphological characters of the adult and larval forms (Mai & Lyon, 1975). Frequency of occurrence of plant-parasitic nematodes and population densities/250 cm^3 soil were determined and recorded.

Reactions of five peanut cultivars: The reactions of five peanut cvs., Balady, Ismailia 1, Giza 4, Giza 5 and Giza 6 to root-knot nematodes; *M. arenaria* race 1, *M. javanica* and *M. incognita* race 2 were studied under greenhouse condition. Seeds of peanut cultivars were sown in 30 cm diam. clay pots filled with steam sterilized sandy clay soil (2: 1, v: v). A total of 75 pots were used for this test. After emergence seedlings were thinned to two seedlings/pot. A week later, pots were inoculated with 2500 eggs and 2nd stage juveniles (J_2)/pot of each root-knot nematode species. Treatments were replicated five times. Pots were arranged in a randomized complete block design. Plants were irrigated as per recommendations. The experiment was completed 60 days after nematode inoculation. Plants were harvested and roots were washed by running tap water. Galled roots were placed in an aqueous solution of phloxin B (0.15 g/l water) for 15 minutes to show nematode egg-masses. The behavior of each root-knot nematode species was recorded by number of galls and egg-masses. The gall and egg-masses range index was made according to Taylor & Sasser (1978) as 0 = resistant (R); 0-10 = moderately resistant (MR); 11-30 = moderately susceptible (MS); 31-100 = susceptible (S) and > 100 = highly susceptible (HS).

Root-knot nematodes culture: Egg-masses of root-knot nematodes *M. arenaria* race 1, *M. javanica* and *M. incognita* race 2 were obtained from the culture collection of Plant Pathology Department, Alexandria University (Alexandria, Egypt). Cultures of these root-knot nematode

species were established from single egg-masses of adult females, which identified using the morphological characteristics of the perineal patterns (Taylor & Sasser, 1978) and reared on tomato *Lycopersicon esculentum* Mill cv. Asala. The root-knot nematode eggs and J_2 were extracted from 8-10 wk-old infected tomato roots using sodium hypochlorite (NaOCl) solution as described by Hussey and Barker (1973).

Extraction and preparation of *P. penetrans* endospores concentration: Three Egyptian isolates of *Pasteuria penetrans* (*Pp*) were found parasitizing J_2 of root-knot nematode infected plants. The 1st and 2nd *Pp* isolates were obtained from encumbered J_2 infected banana (*Pp*B) and tomato (*Pp*T) roots, respectively at El-Behera governorate. The 3rd isolate (*Pp*G) was obtained from root-knot nematode infecting grapevine roots at Kafer El-Sheikh governorate. Isolates of *Pp* were separately cultured on juveniles of *M. incognita* infecting tomato plants cv. Asala in a greenhouse. Six weeks later, encumbered J_2 with *Pp* spores were collected by centrifugation attachment method (Hewlett & Dickson, 1994). Isolates were propagated on two-wk-old tomato seedlings, which inoculated with 1000 encumbered J_2. Two months later, infected root systems, which containing spores of each *Pp* isolate were air dried and ground in a laboratory grinder and pestle in order to release bacterial spores from root tissues. Resulting slurry passed through a 25-μm sieve to eliminate root debris and soil particles (Stirling & White, 1982). Number of spores/ml for each *Pp* isolate was assessed using the haemocytometer slide and adjusted to a concentration of 3×10^6 or 6×10^6 spores/ml. The three mixed isolates (Mixed *Pp*) with the same tested concentrations were prepared by using a concentration of 1×10^6 or 2×10^6 from each isolate and mixed together.

Microplot experiments

Effects of *P. penetrans* isolates and Nemacure against *M. arenaria*: A summer microplot study was conducted during the growing season of 2011 and repeated in season 2012 at the experimental microplot area, Dept. of Plant Pathology, Faculty of Agriculture, Alexandria University, Alexandria to evaluate the suppressive effect of the three *Pp* isolates; *Pp*B, *Pp*T and *Pp*G as compared with granular nematicide, Nemacure 10G® on peanut plants cv. Giza 6 cultivated in sandy loam soil with 2.5% organic matters, which naturally infested with *M. arenaria* race 1. Initial peanut root-knot nematode density (P_i) was estimated by collecting twenty five soil samples of 250 cm^3 each, before peanut planting. The P_i was 3500 J_2/kg soil. Peanut seeds cv. Giza 6 were surface-sterilized using the fungicide, Topsin® M 70% WP (3g/kg seeds) 24 hrs before cultivation. Prior to peanut cultivation, the soil was plowed to a depth of 20 to 25 cm and shaped into rows of 15 to 20 cm height, 3 m long and 85 cm wide with 50 cm gap between rows. All rows were irrigated to full water holding capacity and forty peanut seeds were cultivated in hills (8 hills/row) spaced 35-cm apart within the rows as five seeds/hill. Seedlings were thinned to two seedlings/hill after 10 days. Two days later, peanut seedlings were treated with *Pp* isolates at a concentration of 3×10^6 or 6×10^6 spores/seedling for each *Pp* isolate and with mixed *Pp* isolates at the same concentrations. The granular nematicide, Nemacure 10G® was applied at the rate of 2.5 g/seedling. The same treatments were applied in the 2nd experiment (2012). All recommended agricultural practices were adopted throughout both growing seasons. Separate rows that received only sterilized distilled water were served as a check treatment. All treatments were replicated four times (4 rows) and were laid out in a randomized complete block design (RCBD). Peanut seeds were cultivated on 1st of May and harvested at optimum maturity in mid of September.

At harvest time, numbers of nematode root galls, egg-masses/root system, J_2/250 cm^3 soil, number of encumbered J_2/250 cm^3 soil and % of encumbered J_2 to the total numbers of J_2/250 cm^3 soil, dry weights of shoot and root systems, number of peanut pods/plant and number of bacterial nodules/root system were determined. Also, one hundred encumbered J_2 of each treatment were selected to determine the average number of adhered spores/J_2 using a compound microscope at 400x magnification.

Statistical analysis: Data obtained were statistically analyzed using SAS software program (SAS Institute, 1997). Numbers of nematode root galls, egg-masses and J_2/250 cm^3 soil were transformed to $\sqrt{X+1}$ before statistical analysis. Moreover, means differences were compared using revised LSD test at 5% level of probability.

Results

Survey: Data presented in Table (1) indicated the presence of 12 phytoparasitic nematode genera in soil samples of the surveyed peanut fields of Alexandria, El-Behera and Giza governorates. The root-knot nematode, *M. arenaria* was the most prevalent nematode in all collected soil samples with frequency of occurrence (FO) 40.9-66.7% and population densities (PD) 205-304 J_2/250 cm^3 soil followed by the stunt nematode *Tylenchorhynchus* with 35.5-39.1% FO and PD 81-101 individuals/250 cm^3 soil. *Helicotylenchus, M. javanica* and *Pratylenchus penetrans* were found in peanut soil and root samples with FO 8.6-17.8 % and 19-55 individuals/250 cm^3 soil. The genera *Hemicycliophora, Heterodera* and *Trichodorus* were found in soil samples with 8.6-13.4% FO and PD 19-26 individuals/250 cm^3 soil. Meanwhile, *Criconema* spp., *Criconemella* spp., *Heterodera* spp., *Hoplolaimus* spp., *Trichodorus* spp., *Tylenchus* spp. and *Xiphinema* spp., were less common with FO 1.1-7.8% and PD 7-12 individuals/250 cm^3 soil.

Table 1. Frequency of occurrence and population densities of phytoparasitic nematodes associated with peanut plants in Alexandria, El-Behera and Giza governorates.

Nematode genera and species	Governorate and locality			
	Alexandria	EL-Behera		Giza
	Borg El-Arab (93)	EL-Nobaria (87)	North EL-Tahrir (90)	Embaba (23)
Criconema	1.1 , 9	- , -	- , -	1.4 , 6
Criconemella	2.2 , 7	- , -	- , -	-,-
Helicotylenchus	14.0 , 55	-,-	17.8 , 41	-,-
Hemicycliophora	- , -	8.0 , 19	- , -	7.4 , 17
Heterodera	4.3 , 12	- , -	- , -	3.2 , 11
Hoplolaimus	- , -	2.3 , 11	- , -	- , -
Meloidogyne arenaria	55.9 , 228	66.7 , 304	46.7 , 205	40.9 , 246
Meloidogyne javanica	- , -	- , -	12.2 , 26	13.4 , 23
Pratylenchus penetrans	8.6 , 23	- , -	10.0 , 19	- , -
Trichodorus	6.5 , 13	- , -	- , -	4.6 , 10
Tylenchorhynchus	35.5 , 101	39.1 , 81	- , -	- , -
Tylenchus	4.3 , 10	5.7 , 12	7.8 , 8	6.2 , 11
Xiphinema	- , -	2.3 , 7	- , -	- , -

Reactions of five peanut cultivars: Data presented in Table (2) showed that all tested peanut cvs. were resistant to *M. incognita* race 2 and moderately resistant to *M. javanica*. On the other hand, Balady, Ismailia 1 and Giza 6 were found highly susceptible to *M. arenaria* race 1, while Giza 4 and Giza 5 cvs. were found susceptible.

Table 2. Reactions of peanut cultivars to root-knot nematodes; *Meloidogyne arenaria* race 1, *M. javanica* and *M. incognita* race 2.

Cultivar	Root-knot nematode species	No. of galls/root	No. of egg-masses/root	Reaction
Balady	*M. arenaria*	371	356	HS
	M. javanica	6	2	MR
	M. incognita	0	0	R
Ismailia 1	*M. arenaria*	236	225	HS
	M. javanica	5	2	MR
	M. incognita	0	0	R
Giza 4	*M. arenaria*	75	82	S
	M. javanica	4	8	MR
	M. incognita	0	0	R
Giza 5	*M. arenaria*	84	92	S
	M. javanica	2	1	MR
	M. incognita	0	0	R
Giza 6	*M. arenaria*	351	335	HS
	M. javanica	7	3	MR
	M. incognita	0	0	R

Effects of *P. penetrans* isolates and Nemacure: Data presented in Table (3) showed that treatment with Nemacure 10G® resulted in the highest reduction of 96.2-99.9% in number of nematode root galls, egg-masses/root system and number of J_2/250 cm^3 soil in both 1st and 2nd seasons. Also, results indicated that all *Pp* isolate treatments and the mixed *Pp* isolates with both tested concentrations reduced number of root galls, egg-masses/root system and number of J_2/250 cm^3 soil in both microplot experiments of 2011 and 2012 with 44.9-94.7 % compared with the check treatment. Nematode parameters were significantly reduced with increasing *Pp* concentrations. Treatments of the 1st season with both tested concentrations of mixed *Pp* isolates caused reductions of 74.8-86.9% in number of nematode root galls, egg-masses/root system and number of J_2/250 cm^3 soil. Meanwhile, treatments with both doses of *Pp*T and *Pp*G isolates and 6×10^6 spore/seedling of *Pp*B isolates showed 50.0-73.7% reduction in number of nematode root galls, egg-masses/root system and number of J_2/250 cm^3 soil, followed by treatment with 3×10^6 spore/seedling of *Pp*B isolate, which caused a considerable reduction of 44.9-45.5%. In the 2nd season, all treatments with both tested doses of all *Pp* isolates either alone or as mixed isolates resulted in 65.7-94.7% reductions in number of root galls, egg-masses/root system and number of J_2/250 cm^3 soil compared to the check treatment. Also, there is a significant difference between treatments in percentage of encumbered J_2. Treatments with 6×10^6 spore/seedling of all *Pp* isolates either alone or as mixed isolates resulted in 61.4-72.2% encumbered J_2 in the 1st season and increased to 93.2-97.9% in the 2nd season. Meanwhile, treatment with 3×10^6 spore/seedling of all *Pp* isolates either alone or as mixed isolates showed 39.3-63.9% and 66.6-95.4% encumbered J_2 in the 1st and 2nd seasons, respectively (Table 3).

Table 3. Effects of *P. penetrans* (*Pp*) isolates and Nemacure 10G® on *M. arenaria* infecting peanut plants cv. Giza 6 and number of encumbered J_2.

Pp isolates & concentration (Spore/seedling)	No. of galls/root	Reduction (%)	No. of egg-masses/root	Reduction (%)	Number of J_2/250 cm^3 soil	Reduction (%)	No. of encumbered J_2/250 cm^3 soil	Reduction (%)
				1st season (2011)				
Check	966.4 a	-	959.4 a	-	2377.8 a	-	0.0	-
*Pp*B								
3×10^6	532.4 b	44.9	517.8 b	46.0	1297.0 b	45.5	509.2 d	39.3
6×10^6	312.6 d	67.7	304.4 de	68.3	1006.2 d	57.7	617.6 b	61.4
*Pp*G								
3×10^6	378.4 c	60.8	357.6 c	62.7	1189.4 bc	50.0	565.8 c	47.6
6×10^6	308.4 d	68.1	301.6 d	68.6	1073.0 cd	54.9	673.6 a	62.8
*Pp*T								
3×10^6	333.6 cd	65.5	316.2 d	67.0	1136.6 c	52.2	595.6 bc	52.4
6×10^6	265.4 e	72.5	252.0 e	73.7	878.8 e	63.0	601.0 b	68.4
Mixed *Pp* isolates								
3×10^6	176.2 f	81.8	164.2 f	82.9	598.4 f	74.8	382.2 e	63.9
6×10^6	150.4 g	84.4	125.6 g	86.9	500.2 g	79.0	361.0 f	72.2
Nemacure 10G® (2.5 g/seedling)								
	36.6 h	96.2	21.4 h	97.8	67.8 h	97.1	0.0	-
				2nd season (2012)				
Check	1369.6 a	-	1357.8 a	-	3285.8 a	-	0.0	-
*Pp*B								
3×10^6	307.4 b	77.6	284.0 b	79.1	1116.0 b	66.0	742.8 ab	66.6
6×10^6	217.4 d	84.1	208.0 cd	84.7	761.8 c	76.8	710.0 b	93.2
*Pp*G								
3×10^6	260.8 c	81.0	231.8 c	82.9	1128.6 b	65.7	769.6 a	68.2
6×10^6	221.4 d	83.8	204.8 d	84.9	811.4 c	75.3	779.0 a	96.0
*Pp*T								
3×10^6	173.8 e	87.3	141.6 e	89.6	761.8 c	76.8	591.0 c	77.6
6×10^6	97.6 fg	92.9	65.6 g	95.2	476.8 d	85.5	456.8 cd	95.8
Mixed *Pp* isolates								
3×10^6	120.0 f	91.2	109.2 f	92.0	445.8 d	86.4	425.2 d	95.4
6×10^6	84.2 g	93.9	71.6 g	94.7	256.2 e	92.2	250.8 e	97.9
Nemacure 10G® (2.5 g/seedling)								
	4.8 h	99.6	1.6 h	99.9	12.0 f	99.5	0.0	-

Values of each column followed by the same letter(s) are not significantly different at $P = 0.05$.

Data presented in Table (4) showed a significant increase in peanut growth parameters during both growing seasons. During the 1st and 2nd seasons, treatments with Nemacure 10G®, two doses of 3×10^6 and 6×10^6 spore/seedling of mixed *Pp* isolates and *Pp*T isolate resulted in significant increases of 51.7-77.0% in dry weights of shoot and root systems and number of pods/plant, followed by treatments with the same two doses of both *Pp*B and *Pp*G isolates showed 32.7-48.5% increase compared with check treatment.

Table 4. Effects of three isolates of *P. penetrans* on growth parameters of peanut plants cv. Giza 6 infected with *M. arenaria* race 1.

***Pp* isolates & concentration (spore/seedling)**	**Root dry weight (g)**	**Increase (%)**	**Shoot dry weight (g)**	**Increase (%)**	**No. of pods/plant**	**Increase (%)**
1st season (2011)						
Check	5.4 f	--	6.6 e	--	7.4 e	--
*Pp*B						
3×10^6	8.4 de	35.7	11.0 d	40.0	13.5 cd	45.2
6×10^6	9.1 de	40.7	12.1 d	45.5	14.2 cd	47.9
*Pp*G						
3×10^6	8.1 e	33.3	11.8 d	44.1	12.1 d	38.8
6×10^6	9.8 d	44.9	12.7 d	48.0	12.7 cd	41.7
*Pp*T						
3×10^6	11.6 c	53.4	16.6 c	60.2	15.9 c	53.5
6×10^6	14.3 b	62.2	19.7 ab	66.5	18.7 bc	60.4
Mixed *Pp* isolates						
3×10^6	14.3 b	62.2	18.6 bc	64.5	22.1 b	66.5
6×10^6	14.9 b	63.8	20.3 ab	67.5	26.9 a	72.5
Nemacure 10G® (2.5 g/seedling)						
	17.5 a	69.1	21.2 a	68.9	26.0 a	71.5
2nd season (2012)						
Check	7.0 f	-	8.6 g	-	9.3 g	-
*Pp*B						
3×10^6	10.4 d	32.7	12.9 f	33.3	17.5 e	46.9
6×10^6	11.4 cd	38.6	14.8 ef	42.9	15.9 f	41.5
*Pp*G						
3×10^6	10.9 d	35.8	14.1 ef	39.0	16.6 ef	44.0
6×10^6	12.7 c	44.9	16.7 e	48.5	17.6 e	47.2
*Pp*T						
3×10^6	14.5 bc	51.7	19.1 d	55.0	24.9 d	62.7
6×10^6	19.4 a	63.9	23.0 b	62.6	28.1 c	66.9
Mixed *Pp* isolates						
3×10^6	16.3 b	57.1	20.9 c	58.9	31.6 bc	70.6
6×10^6	18.9 ab	63.0	26.1 a	67.0	40.4 a	77.0
Nemacure 10G® (2.5 g/seedling)						
	20.2 a	65.3	23.4 b	63.2	37.2 b	75.0

Data presented in Table (5) showed significant increases in number of bacterial nodules on peanut roots during both growing seasons. Treatments with both doses of 3×10^6 and 6×10^6 spore/seedling of mixed *Pp* isolates and *Pp*T alone showed significant increases of 90-92.8% in bacterial nodules/root system, followed by treatments with both doses of *Pp*B and *Pp*G, which showed 81.4-88.2% in the 1st season. In the 2nd season, treatments with both doses of all *Pp* isolates showed 90.8-94.8% increase in numbers of bacterial nodules/ root system. On the

other hand, treatment with Nemacure 10G® resulted in lowest increases of 6.9-10.6% in number of bacterial nodules/root system in the 1st and 2nd seasons compared with the check treatment. The laboratory examinations for encumbered J_2 recovered from different treatments showed that doses of all *Pp* isolates resulted in a significant differences in number of endospores adhered to J_2 during both seasons (Table 6). Numbers of adherent endospores on J_2 cuticle were ranged from 3.6-9.4 and 6.2-11.4 endospore/J_2 in the 1st and 2nd seasons, respectively. The highest numbers of adherent endospores were recorded with mixed *Pp* isolates in both seasons. High *Pp* concentrations resulted in the highest numbers of adherent endospores.

Table 5. Effects of *P. penetrans* isolates on number of bacterial nodules on peanut roots cv. Giza 6.

***Pp* isolates & concentration (spore/seedling)**	**Number of bacterial nodules/root system**			
	1st season (2011)		**2nd season (2012)**	
	Total number	**Increase (%)**	**Total number**	**Increase (%)**
Check	33.5 g	0.0	29.5 f	0.0
*Pp*B				
3×10^6	180.5 f	81.4	320.2 e	90.8
6×10^6	232.8 d	85.6	331.7 e	91.1
*Pp*G				
3×10^6	218.7 e	84.7	342.0 de	91.4
6×10^6	284.3 cd	88.2	377.1 d	92.2
*Pp*T				
3×10^6	304.4 cd	90.0	456.8 c	93.5
6×10^6	338.0 c	90.1	497.1 b	94.1
Mixed *Pp* isolates				
3×10^6	413.9 b	91.9	511.0 b	94.2
6×10^6	470.0 a	92.8	569.5 a	94.8
Nemacure 10G® (2.5 g/seedling)				
	36.0 g	6.9	33.0 f	10.6

Table 6. Effects of *P. penetrans* isolates on number of adherent endospores to encumbered J_2.

***Pp* isolates and concentration (spore/seedling)**	**Number of endospores/J_2**	
	1st season (2011)	**2nd season (2012)**
Check	0.0	0.0
*Pp*B		
3×10^6	3.6 f	6.2 e
6×10^6	4.7 e	6.6 e
*Pp*G		
3×10^6	4.4 e	6.8 de
6×10^6	5.7 d	7.5 d
*Pp*T		
3×10^6	6.1 d	9.1 c
6×10^6	7.5 c	9.9 b
Mixed *Pp* isolates		
3×10^6	8.3 b	10.2 b
6×10^6	9.4 a	11.4 a

Discussion

The present results indicated that *M. arenaria* race1 was widespread occurrence in all surveyed peanut areas followed by *Tylenchorhynchus*, *Helicotylenchus*, *M. javanica* and *Pratylenchus penetrans*. The genera *Hemicycliophora*, *Heterodera* and *Trichodorus* were found in soil samples but with low FO and PD. Meanwhile, *Criconema*, *Criconemella*, *Heterodera*, *Hoplolaimus*, *Trichodorus*, *Tylenchus* and *Xiphinema* were less common. These findings were agreed with El-Saedy (1975), Cetintas *et al*., 2003). However, Minton *et al*., (1969) stated that both *M. arenaria* and *M. javanica* cause similar symptoms on peanut. This may resulted in the false assumption that all heavily galled peanut roots, pods and pegs are caused by *M. arenaria*.

Susceptibility of peanut cultivars findings were agreed with Ibrahim & El-Saedy (1976), Hirunsalee *et al*., (1995), Abdel-Momen & Starr (1997) and Korayam & Bondok (2013). Ibrahim & El-Saedy (1976) tested 9 peanut cvs. to *M. javanica* infection and showed variable degrees of susceptibility. Abdel-Momen & Starr (1997) found that *M. arenaria* produced greater numbers of eggs than *M. javanica* on Florunner peanut. However, biocontrol of using three Egyptian isolates findings were in agreement with Chen & Dickson (1998), Kariuki & Dickson (2007) and Timper (2009). Kariuki & Dickson (2007) used dried roots from an infested field site to transfer *P. penetrans* to another field site. In Egypt, El-Saedy & Mokbel (2007) reported two isolates of *P. penetrans* parasitizing J_2 of *M. incognita* and reduced nematode parameters. The bacterium is an obligate parasite of root-knot nematode and not successfully cultivated on artificial media (Chen & Dickson, 1998) and efforts being continued (Hewlett *et al*., 2004).

The formation and development of nitrogen-fixing root nodules is the result of a symbiotic relationship between leguminous plants and soil bacteria collectively called rhizobia, but including more specifically the genera *Azorhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* (Kinkema *et al*., 2006). The importance of nodulation and nitrogen fixation to agriculture, natural ecosystems and the global nitrogen cycle are indisputable (Graham & Vance 2003; Oldroyd *et al*., 2011).

The present data showed a significant increase in number of bacterial nodules formed on peanut root system as a result of *Pp* isolate treatments, which attack root-knot nematode juveniles. However, numbers of adherent endospores were increased with *Pp* concentration increased. Root-knot nematodes *Meloidogyne* spp. reduced nodulation on peanuts (Taha & Raski, 1969; Barker & Hussey, 1976). Masefield (1958) reported that nematode galls on the roots may affect nodulation by causing nutrient deficiency in host plants and by occupying spaces on root system, a reason which was supported later by Malek & Jenkins (1964). Root-knot nematodes, *Meloidogyne* spp., and other species of plant-parasitic nematodes inhibit nodulation and nitrogen fixation in certain legumes (Epps & Chambers, 1962; Malek & Jenkins, 1964). Nodule formation took place either before or after the addition of nematode larvae. However, nematode infection affected number of nodules per plant indirectly by reducing the size of the root system (Taha & Raski, 1969). Robinson (1961) suggested the production of nodules or galls depended on early nodulation started with attack of nematodes.

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