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Pathogenic Variability among Eight Populations of *Meloidogyne javanica* Isolates on Tomato Plants

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Root-knot nematodes (*Meloidogyne* spp.) are the main pathogens of tomato plants (*Lycopersicon esculentum*) worldwide. Eight isolates of *Meloidogyne javanica* (Mj1, Mj2, Mj3, Mj4, Mj5, Mj6, Mj7 and Mj8) were collected from five Egyptian Governorates, *i.e.* Ismailiya, Beni-Suef, Beheira, Minya and Alexandria. Each isolate was identified by using perineal pattern and isozyme esterase assay. These isolates showed pathogenic variability when inoculated on the tomato cultivar (Super Marmand). Mj1, Mj7 and Mj8 significantly recorded high number of galls and number of juveniles per plant while, the isolates Mj3 and Mj4 had the lowest rate at the same parameters. However, the isolates Mj2, Mj5 and Mj6 were moderately pathogenic comparing with other isolates. In addition, the tomato growth parameters, significantly affected by all isolates compared to non-infected plants. Exclusively, Mj7 isolate reduced significantly the total fresh weight and shoot dry weight compared to the other isolates.

Keywords: *Lycopersicon esculentum*, *Meloidogyne javanica*, root-knot and tomato.

Root-knot nematodes (*Meloidogyne* spp.) are the most serious pests of agricultural crops. They are worldwide in their distribution and have been reported from more than 3000 species of plants. Tomato is an important economic annually crop in Egypt, and it is most frequently injured by root-knot nematodes especially in the newly reclaimed areas causing great damage (Ibrahim *et al*., 2010). The majority of Egyptian Governorates, tomato is planted at 242,851 feddan in the old and 272,374 feddan in the newly reclaimed land with average production of 17.152 and 16.175 tons/feddan, respectively, during 2012 (Anonymous, 2012). The tomato-cultivated areas damaged by *Meloidogyne* spp. estimated around 170,000 feddan. Three species, *i.e. Meloidogyne javanica*, *M. incognita* and *M. arinaria* were found in the surveyed agrarian area. The first two species are widely distributed (Ibrahim, 1983 and Mousa, 1997).

Morphology of the perineal pattern, located in the posterior body region of adult females, is the most frequently character involved for identifying *Meloidogyne* spp. The area of the perineal pattern comprises the vulva–anus area (perineum), tail terminus, phasmids, lateral lines and surrounding cuticular striate. *Meloidogyne javanica* distinguished by lateral ridges that divide the dorsal and ventral striate (Eisenback *et al*., 1980). The perineal pattern of female root-knot nematodes is usually examined with the light microscopic (Eisenback and Hunt, 2009), but resolution and depth of focus are extremely limited. The Scanning Electron Microscope (SEM) has a greater depth of focus and more resolving power to

produces images with increased resolution and enhanced surface morphology (Eisenback, 2010).

Isozyme electrophoretic profiles, often using esterase and malate dehydrogenase, have been used for identification of *M. incognita* and *M. javanica* (Esbenshade and Triantaphyllou, 1985).

The variability in virulence among populations of *M. javanica* is uncommon. The aim of this study to prove that, some certain population, possessing similar morphological characteristics, can produce different reactions on the same host. Therefore, this investigation was carried out to determine the variation of virulence among eight different Egyptian *M. javanica* isolates on tomato plants as the prefer host.

Materials and Methods

Isolation and purification of root-knot nematode isolates:

Root-knot nematode isolates were obtained from different naturally infected hosts and surrounding soil. Infected roots of tomato, egg-plant, banana, fig, grape, cowpea and cantaloupe plants were collected from five different Egyptian Governorates; Ismailiya, Beni-Suef, Beheira, Minya and Alexandria (Table 1). Each sample was included infected roots with root-knot nematodes and soil sample. Soil sample (500 g) was placed in a 20-cm-diam. pot to which one tomato seedling (*Lycopersicon esculentum* Mill. cv. Super Marmand), was transplanted. Pots were kept under greenhouse conditions during 45 days and irrigated as needed.

Table 1. *Meloidogyne* spp. isolates collected from naturally infected hosts among five different Governorates

Isolate code	Infected host	Governorate
M 1	Tomato	Ismailiya
M 2	Cowpea	Beni-Suef
M 3	Banana	Beheira
M 4	Fig	Beheira
M 5	Grape	Beheira
M 6	Egg-Plant	Ismailiya
M 7	Tomato	Minya
M 8	Cantaloupe	Alexandria

Nematode isolates were initiated from a single egg mass which collected from the infected roots and/or from the roots of tomato which grow in the soil samples. Each single egg mass was established on seedlings of tomato cv. Super Marmand singly when the seedlings were 30-day-old into 20-cm-diam. pots containing 1 kg autoclaved sandy loam soil (1:1v/v) under greenhouse conditions. Seedlings were watered as needed and fertilized once a week with 200 ml of the nutrient solution: Super Vit® (N:P:K, 19:19:19). The infected tomato seedlings were left for 45 days after inoculation with egg mass. Each root system per pot was contained one pure nematode isolate.

Identification of root-knot nematodes isolates:

1- Perineal pattern character:

To identify the morphological characters via perineal pattern using SEM, the adult females per pure isolate were fixed in 2.5% glutaraldehyde for 24 h at 4C, the post-fixed in 1% osmium tetroxide for 1h at room temperature. The females were then dehydrated with ascending concentrations of acetone to the critical point dried, and finally sputter coated with gold (Harley and Fergusen, 1990). The examination and photographing were done through a Jeol SEM (JSM – T330A) at the Central Laboratory of Fac. of Agric., Ain Shams Univ. The cuticle marking surrounding the vulva and anus (perineal pattern) of the adult females of the root-knot nematodes was used for identification (Taylor and Netscher, 1974).

2- Isozyme esterase:

To determine the esterase phenotype, adult females from each pure isolate were excised from tomato root tissues under stereomicroscope. Individually, one female per isolate macerated with sterilized needle in 0.1M phosphate extraction buffer (pH 7.4) with 20% sucrose, 2% Triton x-100, and 0.1% bromophenol blue dye. Electrophoresis of macerated individual females was accomplished with an automated apparatus (phastsystem Pharmacia, Uppsala, Sweden) on 10 to 15% gradient polyacrylamide gels. Esterase Phenotypes were determined by staining polyacrylamide gels for esterase (EST) enzyme activity (Esbenshade and Triantaphyllou, 1986).

Preparation of nematode inoculum in pure cultures:

Each root system per *M. javanica* isolate was washed under running tap water and the infective larvae (second larval stage L2) were extracted from the galled tomato roots by Mist Chamber technique (Reddy, 1983).

Pathogenicity test of M. javanica isolates:

The experiment was conducted using tomato seedlings cv. Super Marmand. Seedlings were sown in foam trays (84 well) filled with peat moss fertilized. After 30 days, the seedlings were transplanted singly into 20-cm-diam. pots contained 1 kg sterilized sandy loam soil (1:1 v/v) and watered every 2 days and fertilized with nutrient solution as mentioned before. The seedlings were inoculated with around 1000 second juvenile stages (J2) of each isolate per pot after 10 days from transplanting. Juveniles were placed in holes around the root of the plant by micropipette. Each isolate inoculated 9 replicates. After 60 days, the soil was gently washed from the roots per group. Number of galls was calculated per root system. To estimate nematode population, the infected roots with nematodes were staining with cold lacto-phenol plus acid fuchsine and stored 48 h. stained roots were then rinsed in tap water and cut into pieces 2-4 cm to facilitate counting of egg-masses per root system. In addition, the final population of nematodes was estimated based on the numbers of second juvenile stage per pot. Plant height, fresh weight and shoot dry weight were calculated per plant after 60 days old at the end of experiment.

Statistical analysis:

Data of experiment were statically computed using SAS ANOVA (Anonymous, 1992) with least significant difference (LSD) test at 5% probability level.

Results

Identification of Meloidogyne spp.:

1- Perineal patterns observation by SEM:

The perineal patterns that typical for *M. javanica* with a rounded to flattened dorsal arch and distinct lateral lines that clearly delineated the dorsal and ventral region of the patterns were used in this investigation (Fig. 1). It illustrated the presence lateral ridges that divided the dorsal and ventral striate. Generally, the ridges run the entire width of the pattern, but gradually disappear near the tail terminus. The dorsal arch is low and rounded to high, squares, and often contains a whorl in the tail terminal area. The striate are smooth to slightly wavy, and some striate may bend toward the vulvae edges.

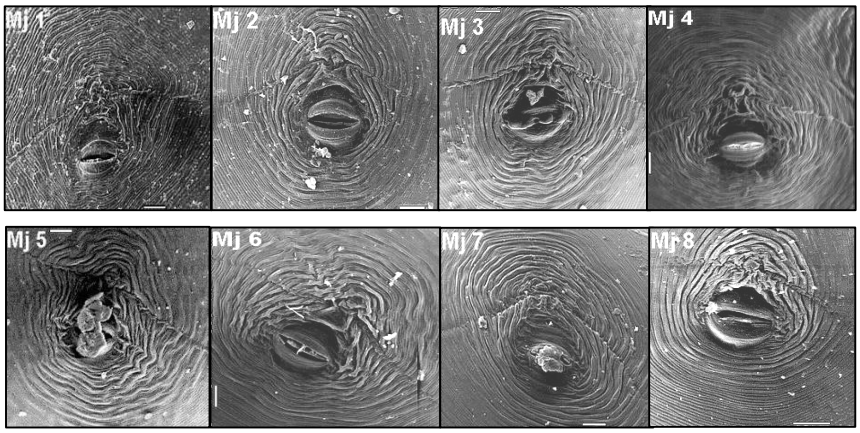

Fig.1. Scanning Electron Microscope photographs of *Meloidogyne javanica* perineal pattern among different eight populations (Mj1, Mj2, Mj3, Mj4, Mj5, Mj6, Mj7 and Mj8) (bar= 10 um).

2- Esterase phenotype:

To ensure identification, adult females of *M. javanica* were identified based on its characteristic esterase phenotypes. Thus, all eight isolates of *M. javanica* had an esterase phenotype designated as J3 with 3 bands (Fig. 2). The J3 esterase phenotype is highly species-specific and can be used a very identification of *M. javanica*.

Pathogenicity test of M. javanica isolates:

All isolates of *M. javanica* were pathogenic to tomato plants cv. Super Marmand; they produced galls and egg masses on tomato roots (Table 2). Data indicated that, the highly pathogenic isolates were Mj1, Mj7 and Mj8, respectively. It was recorded high number of galls and number of juveniles per pot significantly. On the other hand, the isolates Mj3 and Mj4 had the lowest rate at the same parameters. However, isolates Mj2, Mj5 and Mj6 were moderately pathogenic comparing with other isolates.

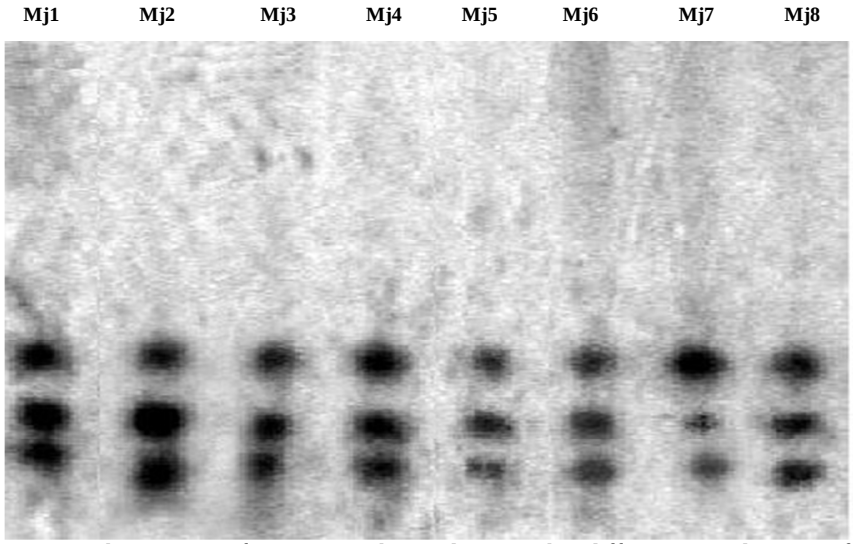

Fig 2. Phenotypes of esterase observed in eight different populations of *Meloidogyne javanica* (Mj1, Mj2, Mj3, Mj4, Mj5, Mj6, Mj7 and Mj8).

Table 2. Infection parameters of different *M. javanica* isolates on tomato plant (Super Marmand) cv. under greenhouse conditions

Nematode isolate	No. of galls	No. of egg-masses	No. of juveniles /pot*
Mj 1	166	147	16630
Mj 2	155	107	12940
Mj 3	129	81	8100
Mj 4	143	100	12100
Mj 5	142	97	11480
Mj 6	133	85	9000
Mj 7	183	150	17920
Mj 8	174	149	16020
Check	0	0	0
LSD (0.05)	17.59	17.18	3430

* Number of juveniles per pot at the end of the experiment.

Effect of different M. javanica isolates on growth parameters of tomato plants:

Results in Table (3) indicated that all *M. javanica* isolates decreased plant height, plant fresh weight and shoot dry weight significantly compared with the check plants. In contrast, the isolate Mj7 decreased the plant fresh weight and shoot dry weight significantly compared by isolates Mj1, Mj2, Mj3 and Mj4 but not significantly with Mj5, Mj6 and Mj8.

Table 3. Effect of different *M. javanica* isolates on growth parameters of tomato plant (Super Marmand) cv. under greenhouse conditions

Nematode isolate	Plant height (cm)	Plant fresh weight (gm)			Shoot dry weight (gm)
		Shoot	Root	Total	
Mj 1	16.0	10.6	2.2	12.8	2.07
Mj 2	15.7	10.1	2.1	12.2	1.32
Mj 3	16.5	9.2	2.2	11.4	1.01
Mj 4	15.5	8.5	2.3	10.8	1.01
Mj 5	14.5	7.5	1.9	9.4	0.83
Mj 6	14.3	7.4	1.8	9.2	0.82
Mj 7	14.0	6.9	1.8	8.7	0.76
Mj 8	14.7	7.9	2.2	10.1	0.90
Check	26.5	12.7	3.8	16.5	2.23
LSD (0.05)	3.07	2.42	0.71	2.44	0.51

Discussion

A high frequency of *M. javanica* populations on different plant hosts in Egypt is a major concern for tomato production areas. Given that soil infested with virulent isolates could significantly reduce the production of tomato yield. This study determined that, eight isolates of *M. javanica* isolated from different hosts were distributed among five Egyptian Governorates. Early study, Taylor and Sasser (1978) recorded a highest frequency of *M. javanica* in Egypt among 172 root-knot nematodes isolates collected from 18 countries of the Middle East and Mediterranean region. Characters of perineal pattern via SEM in present examination illustrated that, no different morphological characters among the eight isolates of *M. javanica* (Mj1, Mj2, Mj3, Mj4, Mj5, Mj6, Mj7 and Mj8) collected from five different Egyptian Governorates (Ismailiya, Beni-Suef, Beheira, Minya, and Alexandria) however these populations differed in their pathogenicity. The previous studies have showed that no variation in perineal pattern characters has been observed among 20 populations of *M. javanica* collected from different regions (Eisenback *et al*., 1981). A comparative study of morphological characters of different populations of *M. javanica* collected from the United States, Morocco, and Egypt has performed by Rammah and Hirschmann (1990) did not found major morphological differences, but the populations differed in their pathogenicity. In addition, the present investigation revealed that, the isozyme phenotypes of all isolates originated from different new reclaimed soil in Egypt have typical esterase patterns of *M. javanica* according to Esbenshade and Triantaphyllou (1985). Same results were obtained early by several investigations (Esbenshade and Triantaphyllou, 1985 & 1990 and Carneiro *et al.*, 2000). Consequently, the current results confirmed that, the population of *M. javanica* in Egypt is highly frequented and has no morphological and molecular differences.

The pathogenicity test of this investigation showed that the highly virulence and reproductive *M. javanica* isolates were Mj1, Mj7 and Mj8. Both Mj1and Mj7 were isolated from tomato plants that grown in Ismailiya and Minya Governorates,

respectively, while, Mj8 was isolated from cantaloupe plants grown in Alexandria governorate. The successful pathogenicity of root-knot nematode to host plant depends on their ability to search and penetrate roots, establish feeding sites, develop into mature stages and reproduce progeny (Jones, 1981 and Hussey, 1989). The present results showed that *M. javanica* isolates were infested on tomato and cantaloupe plants could be highly virulent compared to the other isolates.

In all cases, nematodes affect plant growth by manipulate their plant host cells structurally and physiologically to satisfy the nutritional requirements. Genetically, root-knot nematodes modified the expression genes of their plant host (Sidhu and Webster, 1981).

This study allowed identification of the most widespread root-knot nematode specie in Egypt affecting tomato plants. Thus, the resistant genes in root-knot nematode resistant plants should be investigated and these plants adapted in crop rotation with susceptible plants. Subsequently, it could be useful in developing cropping pattern strategies for the management of root-knot nematodes.

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اختلاف القدرة المرضية لثمانية عزلات لنيماتودا *Meloidogyne javanica* على نباتات الطماطم

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تعتبر نيماتودا تعقد الجذور من أهم الآفات التي تصيب الطماطم على مستوى العالم. من خلال الدراسات التي أجريت في هذا البحث على ثمانية عزلات لنيماتودا تعقد الجذور تم عزلها من خمس محافظات مصرية مختلفة هى الاسماعيلية و بني سويف والبحيرة والمنيا والاسكندرية، أوضحت النتائج أن جميعها *Meloidogyne javanica* وهى Mj1،Mj2، Mj3، Mj4، Mj5، Mj6 ، Mj7،Mj8 حيث تم تعريفها كلاً على حده من خلال المواصفات الظاهرية للأناث الناضجة (Perineal pattern) ، وكذلك باستخدام Isozyme esterase. وباختبار القدرة المرضية لهذه العزلات على نباتات الطماطم صنف سوبر مارمند وجد أن أكثرها قدرة مرضية مع تزايد في التعداد هي Mj1، Mj7، Mj8 في حين كانت العزلات Mj2 ، Mj5 ، Mj6 ذات قدرة مرضية متوسطة ، في حين كانت العزلتي Mj3 ، Mj4 الأقل قدرة مرضية على نباتات الطماطم. كما أوضحت النتائج أن جميع العزلات لها القدرة على اختزال أطوال النباتات وأوزانها الغضة والجافة وخاصة العزلة Mj7 والتي اختزلت الوزن الجاف بشكل معنوي مقارنة بالعزلات الاخرى والنباتات السليمة.