Pakistan Journal of Nematology (2018) 36 (2):123-129 ISSN 0255-7576 (Print)
ISSN 2313-1942 (Online)
www.pjn.com.pk
http://dx.doi.org/10.18681/pjn.v36.i02.p123-129

Pathogenicity and control of *Meloidogyne incognita* on rice in Egypt

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Abstract

The pathogenicity of root-knot nematode *Meloidogyne incognita* race 1(Mi1) and race 3 (Mi3) on rice (*Oryza sativa* L.) was studied in the greenhouse. Seventeen rice cultivars were tested for resistance to Mi1 and Mi3. The results showed that rice cvs Araby, Giza 159, Giza 170, Giza 171, Giza 172, Giza 177, Giza 178, Nahda, Sakha 101 and Sakha 102 were either susceptible or highly susceptible to Mi1. Rice cvs A95, IR1, IR22 and Japonica 47 were moderately susceptible to Mi1, whereas cvs IR28, IR459 and Philippini 24 were moderately resistant to Mi1. On the other hand, all the tested rice cultivars were either resistant or moderately resistant to Mi 3. Control of *M. incognita* race 1 on rice cv. Sakha 101 was studied in the greenhouse. Three tests were conducted to study the effects of soil treatment with some plant materials, stems of oyster mushroom (*Pleurotus ostreatus*), the biocontrol agent *Bacillus thuringiensis*, the bionematicide abamectin, and the nematicide fenamiphos on Mi 1 on rice cv. Sakha 101. All the applied control treatments were effective in reducing nematode infection on rice plants.

Keywords: Control, *Meloidogyne incognita*, pathogenicity, rice, *Oryza sativa*

In Egypt, plant-parasitic nematodes constitute one of the most important pest groups of many economically important agricultural crops (Ibrahim *et al*., 1986, 2010). Previous studies have shown the presence of large numbers of genera and species of phytoparasitic nematodes associated with the rice crops in various locations in Egypt (Ibrahim *et al*., 1986, 2010; Tarjan, 1964).

Many of these phytoparasitic nematodes, such as *Aphelenchoides besseyi*, *Heterodera* spp., *Hirschmanniella gracilis*, *H. oryzae*, *Meloidogyne incognita*, *Mesocriconema* spp., *Trichodorus* spp., *Tylenchorhynchus* spp. and *T. martini* may be considered limiting factors in rice production in Egypt and other parts of the world (Bridge *et al*., 1990; Hollis & Keoboonrueng, 1984; Ibrahim *et al*., 1972, 1986, 2010, 2016; Rao & Jayaprakash, 1978; Rezk & Ibrahim, 1978; Salawn, 1978;Villanueva *et al*., 1992). Previous studies indicated that the root-knot nematode *Meloidogyne incognita* is of widespread occurrence and adversely affects the production of rice and other field crops in Egypt (Ibrahim *et al*., 1972, 2010; Rezk & Ibrahim, 1978). However, investigation into the pathogenicity and control of *M. incognita* on rice have been limited (Babatola, 1980; Ibrahim *et al*., 1973).

The objectives of this research were to study the pathogenicity of *M. incognita* races 1 and 3 on certain rice cultivars and the biological and chemical control of *M. incognita* race 1 on rice cv. Sakha 101.

Published by Pakistan Society of Nematologists
Received: 20 Oct, 2017
Accepted:18 Dec, 2017

Materials and Methods

Inocula of the root-knot nematode *M. incognita* race 1 (Mi1) and race 3 (Mi3) used in this study were obtained from infected roots of tomato (*Lycopersicum esculentum*) cultivar Rutgers grown in the greenhouse in the Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

The reactions of 17 rice cultivars to *M. incognita* race 1 and race 3 were determined in greenhouse tests. Seeds of each of the tested rice cultivars were sown in 15-cm-diameter plastic pots filled with 1 kg of equal portions of sterilized sand and clay soil. After emergence, seedlings were thinned to 5 seedlings/pot. Egg-masses were collected from the stock plants and brought to a concentration of 5000 eggs/10ml.

Four weeks after emergence, soils of treated pots were infested by nematode eggs by creating holes near the plant roots and then adding initial population of 5000 eggs/pot of Mi1 or Mi3 whereas uninfected pots served as control. Nematode eggs were obtained from galled tomato roots using the method of Hussey and Barker (1973). Treatments and controls were replicated five times and the experiment was repeated one time. Pots were arranged in a randomized complete block design in a greenhouse at 20-28^{0}C.

Experiments were terminated 7 weeks after soil inoculation, and roots were washed free of soil. Roots were stained in aqueous solution of phloxin B 0.15g/1L water) for 15 minutes to detect the nematode egg-masses. The numbers of root-knot nematode galls and egg-masses were counted. Plants were rated according to the numbers of egg-masses observed on their roots. Plants with 0-2 egg-masses/plant were considered resistant, 3-10 egg-masses/plant moderately resistant, 11-30 egg-masses/plant moderately susceptible, 31-100 egg-masses/plant susceptible, and > 100 egg masses/plant highly susceptible (Taylor & Sasser, 1978).

Two Egyptian isolates (7N and Soto) of the bio control agent *Bacillus thuringiensis* (Bt) Berliner were obtained from the Agricultural Genetic Engineering Research Institute (AGERI). The two isolates of *B. thuringiensis* (Bt 7N and Soto) were used before in previous work by Ibrahim *et al*., 2013, 2014. Bt isolates were grown on T3 liquid medium for 72 hrs at 30°C (Travers *et al*., 1987). The culture fluid suspensions of Bt isolates 7N and Soto were used to prepare the supernatant and pellet preparations used in the control tests of Mi1 on rice cv. Sakha101 (Ibrahim *et al*., 2014). Three greenhouse tests examined the effects of various dried plant materials, the biocontrol agent Bt, the bioproduct Vertemic (1.8 EC abamectin) and the nematicide Nemacur (fenamiphos) on Mi1 on rice plants cv. Sakha 101. In all these tests, seeds of rice cv. Sakha 101 were sown in 15-cm-diameter plastic pots (1.0 liter) filled with a mixture of equal volumes of steam-sterilized sand and clay soil. After emergence, seedlings were thinned to 5 seedlings/pot.

Two weeks after emergence, the soil of treated pots was inoculated by creating holes near the plant roots and then adding an initial population of 5,000 Mi1 eggs/pot. Treatments and control were replicated five times while the experiment was repeated one time. Pots were arranged in a randomized complete block design in a green house at 22-28^0 C.

The first test investigated the effects of leaves of rubber plant (*Ficus elastica* Roxb.) and castor bean *(Ricinus communis L.*), foliage of mallow weed (*Malva parviflora* L.) and milk thistle (*Silybum marianum L.*), peels of orange fruits (*Citrus sinensis* L. Osbeck) cv. Baladi and stems of oyster mushroom (*Pleurotus ostreatus*) on the infection of Mi1 on rice plants cv, Sakha 101, as they showed good control effects against *Heterodera goldeni* on rice plants (Ibrahim *et al*., 2014). The tested plant materials were collected from the Agricultural Experiment

Station of Alexandria University, Abees, Alexandria and oven dried at 60^0 C for 48 hrs, ground to fine powder by using an electric grinder, and incorporated in the soil of treated pots at the rate of 2%, one day before sowing rice seeds.The second test examined the effects of the supernatant and pellet (prepared as described by Ibrahim *et al.*, 2014) of the Bt isolates 7N and Soto, the bioproduct Vertemic (abamectin 1.8% EC), and the nematicide Nemacur (fenamiphos) 10G on the infection of Mi1 on rice plants cv. Sakha 101. Two weeks after seedling emergence, pots were inoculated with Mi.1 and the tested materials were added to the soil 24 hrs after nematode inoculation. The applied treatments included Bt supernatant 10 ml/pot, Bt pellet 10 ml/pot, Vertemic 10 ml/pot, and Nemacur 0.25 g/pot. The Bt treatments were applied again 7 days after nematode inoculation (Ibrahim *et al*., 2014; Mohammed *et al*., 2008). In the third test, the effects of peels of orange fruits (POF) and/or the supernatant of Bt isolates 7N and Soto on the infection of Mi1 on rice plants cv. Sakha 101 were determined. Dried POF were added and mixed with the soil of treated pots at the rate of 2%, 24 hrs before sowing rice seeds. Two weeks after seedling emergence, soil was inoculated with Mi1 and 24 hrs later Bt supernatant was applies to treated pots. Bt supernatant solution was added at the rate of 10 ml/pot in two doses, 24 hrs and 7 days after nematode inoculation. The applied treatments included Mi1 alone or plus POF, Bt 7N, Bt Soto, and Bt + POF. Tests were terminated 60 days after nematode inoculation. Roots were washed to make free of soil. Numbers of root galls and egg masses of Mi1 on roots were counted, and the dry weights of the shoot and root systems were determined. Analysis of variance (ANOVA) was performed with SAS version 7 (SAS Institute, 1988) on numbers of root galls and egg-masses and the dry weights of the shoot and root systems of the tested rice plants.

Results and Discussion

The reactions of 17 rice cultivars to *M. incognita* race 1 (Mi1) and race 3 (Mi3) are presented in Table 1. Rice cvs Araby, Giza 170, Giza 172, Giza 177, Giza 178 were susceptible to Mi 1, whereas rice cvs Giza 159, Giza 171, Nahda, Sakha 101 and Sakha 102 were highly susceptible to Mi1. Rice cvs A95, IR1, IR22 and Japonica 47 were highly susceptible to Mi1 while rice cvs. IR 22, IR 28 and Philippini 24 were moderately resistant to Mi1. In contrast, all tested rice cultivars were either resistant or moderately resistant to Mi3 (Table 1). Compared to the treatment with Mi1 alone, the other treatments with dried plant and fungal material suppressed nematode infection and significantly reduced the numbers of Mi1 root galls and egg-masses on infected rice roots (Table 2) by 59-73%.

Treatments with dried rubber plant leaves, orange fruit peels and oyster mushroom stems induced the highest (66-76%) reduction in the numbers of nematode galls and egg- masses. Treatments with castor bean leaves, mallow weed foliage and milk thistle gave 59-65% reduction in the number of Mi1 egg- masses. In a similar study, Tsai (2008) reported that extracts of fresh peels of lemon, orange and grapefruit showed significant nematostatic effect against second stage juveniles of *M. incognita*. The present results are in agreement with those of other authors who indicated the effective use of organic soil amendments to control plant parasitic nematodes (Ibrahim *et al*., 2013, 2014; Radwan *et al.*, 2004; Saifullah *et al.*, 1990; Tsai, 2008).

Soil treatments with bionematicides suppressed nematode infection on rice plants and significantly reduced the numbers of galls and egg-masses of Mi1 on infected rice roots compared to the control (Table 3). The highest reductions of nematode egg masses (86-89%) occurred with treatments of Vertemic and the synthetic chemical nematicide Nemacur. Treatments with Bt Soto or 7N supernatants reduced the number of Mi1 egg-masses by 66% and 70%, respectively. In contrast, treatment with the corresponding pellets was less effective and reduced Mi1 egg-masses by 34-40% compared to other control treatments.

Table 1. Reaction of some rice cultivars to the root-knot nematode *Meloidogyne incognita* race 1 and race 3.

Cultivar	*Meloidogyne incognita* race 1			*Meloidogyne incognita* race 3		
	No. of galls/ plant	No. of egg-masses/ plant	Reaction	No. of galls/ plant	No. of egg-masses/ plant	Reaction
A95	22	18	MS	0	0	R
Araby	108	94	S	6	4	MR
Giza 159	210	172	HS	7	4	MR
Giza 170	124	92	S	8	5	MR
Giza 171	228	153	HS	8	4	MR
Giza 172	92	84	S	7	4	MR
Giza 177	120	80	S	9	5	MR
Giza 178	142	94	S	8	3	MR
IR 1	18	16	MS	5	3	MR
IR 22	20	17	MS	8	6	MR
IR 28	6	4	MR	6	4	MR
IR 459	8	4	MR	7	5	MR
Japonica 47	16	12	MS	6	3	MR
Nahda	238	178	HS	8	4	MR
Philippini 24	6	4	MR	0	0	R
Sakha 101	230	170	HS	9	6	MR
Sakha	190	134	HS	8	5	MR

*S = Susceptible. HS = Highly susceptible.
MS = Moderately susceptible. MR = Moderately resistant.
R = Resistant.

Table 2. Effect of some plant materials and mushroom stems on the infection of the root-knot nematode *Meloidogyne incongnita* race 1 (Mil) on rice ev. Sakha 101.

Treatment	No of galls/plant	No of egg-masses/plant	Reduction%		Dry weight (g)	
			Galls	Egg-masses	Shoot	Root
M.incognita	232a*	150 a	--	--	1.93 e	1.32 d
Rubber plant + Mil	67 d **	51 c	71	66	2.97 d	2.10be
Caster bean + Mil	72c	56 bc	69	63	4.52 c	2.87 a
Mallow weed + Mil	72 c	53 c	69	65	2.83 d	1.99 c
Milk thistle + Mil	90 b	62 b	61	59	6.75 a	3.13 a
Orange peels + Mil	56 e	41 d	76	73	4.92 c	2.43b
Mushroom stem + Mil	61 de	49 cd	73	67	5.93 b	2.88 a

*Means are average of 5 relicates.
**Means with the same letter in each column are not significantly different at P = 0.05.

Table 3.Effect of *Bacillus thuringiensis* (Bt) isolates 7N and Soto, Vertemie and Nemacur^{-R} - 10 G on the infection of *Meloidogyne incognita* (Mil) on rice ev. Sakha 101.

Treatment	No of galls/plant	No of egg-masses/plant	Reduction%		Dry weight (g)	
			Galls	Egg-masses	Shoot	Root
M.incognita race 1	213 a*	131 a	--	--	1.94g	1.58g
Bt 7Nsupernatant + Mil	53**	39e	75	70	2.18 a	1.93a
Bt 7N pellet + Mil	111 c	78c	48	40	2.0ef	1.78cd
Bt Soto supernatant+Mil	66 d	45d	69	66	2.09 cd	1.87b
Bt soto pellet + Mil	122 b	87b	43	34	1.99 f	1.72ef
Vertemic + Mil	35f	18f	84	86	2.12 bc	1.83bc
Nemacur + Mil	25g	15f	88	89	2.05de	1.81c

*Means are average of 5 replicates.
**Means with the same letter (s)+ in each column are not significantly different at P=0.05.

Table 4. Effect of peels of orange fruits supernatant and/or the supernatant of *Bacillus thuringiensis* (Bt) isolates 7N and Soto, on the infection of *Meloidogyne incongnita* (Mil) on rice ev. Sakha 101.

Treatment	No. of galls/plant	No. of egg-masses/plant	Reduction%		Dry weight (g)	
			Galls	Egg-masses	Shoot	Root
M.incognita race 1	228a	146 a	--	--	1.94 e	1.52 d
Orange peels +Mil	62c**	45 c	73	69	3.48 b	2.06 a
Bt 7N + Mil	53 d	39 d	77	73	2.18 c	1.93 b
Bt Soto + Mil	66 e	45 e	71	59	2.19 c	1.87bc
Orange peels+Bt7N+Mil	23 e	12 e	90	92	3.96 a	2.03 a
Orange peels+Bt soto+Mil	25 e	16e	89	89	3.93 a	2.04 a

*Means are average of 5 replicates.
**Mean with the same letter (s) in each colum are not significantly different at p = 0.05.

Soil treatment with peels of orange fruit and/or Bt suppressed *M. incognita* race 1 infection on rice plants and significantly reduced numbers of nematode galls and egg-masses on infected rice roots compared to the control (Table 4). The highest reductions of nematode galls and egg-masses (89-92%) were recorded with treatments of Bt 7N or Soto supernatant plus peels of orange fruit. Treatments with either Bt 7N, Bt Soto or orange fruit peels reduced the numbers of *M. incognita* galls and egg masses by 69-77% on infected rice roots. In a similar study, Radwan *et al.,* (2004) show that the integration of *B. thuringiensis* with an organic soil amendment was more effective in controlling *M. incognita* than the treatment with *B. thuringiensis* alone. Also, Ibrahim *et al.,* (2014) reported that treatment of *B. thuringiensis* plus rubber plant leaves was very effective in reducing the infection of the cyst nematode *Heterodera goldeni* on rice plants. The present results also agree with those of other studies on the use of *B. thuringiensis* as a biocontrol against plant parasitic nematodes (Devidas and Rehberger, 1992; Sharma, 1994; Zuckerman *et al.*, 1995; Tian *et al.*, 2007; Ravari and Moghaddam, 2015; Yu *et al.*, 2015).

Acknowledgements

The authors are grateful to Dr. D. J. Chitwood for review of the manuscript and Dr. M. Kantor for technical assistance. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer002E.

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