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Interactions of *Heterodera daverti, H. goldeni* and *H. zeae* with *Meloidogyne incognita* on rice

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Abstract

The interactions of the cyst nematodes *Heterodera daverti*, *H. goldeni* and *H. zeae* with the root-knot nematode *Meloidogyne incognita* on rice (*Oryza sativa*) cultivars 'Giza 178' and Sakha 101' were studied in the greenhouse. Inoculation with *H. goldeni* alone or one week before inoculation with *M. incognita* on rice cv. Sakha 101 resulted in a significant increase in the number of cysts of *H. goldeni* as compared to plants inoculated with *M. incognita* concurrently or a week beforehand. When *H. daverti* or *H. zeae* were inoculated one week after inoculation with *M. incognita* on rice cultivars Giza 178 or Sakha 101, respectively, the final population of these cyst nematodes increased. Treatments with *M. incognita* alone or one week before inoculations with the tested cyst nematodes induced a significant increase in the numbers of *M. incognita* root galls and egg-masses as compared to other treatments.

Keywords: *Heterodera daverti*, *H. goldeni*, *H. zeae*, *Meloidogyne incognita*, *Oryza sativa*.

Introduction

In Egypt, plant-parasitic nematodes constitute to be 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 rice crops in various locations throughout Egypt (Ibrahim *et al*., 1986, 2010; Tarjan, 1964). Many of these phytoparasitic nematodes such as *Aphelenchoides besseyi*, *Ditylenchus angustus*, *Helicotylenchus* sp., *Heterodera* spp., *Hirschmanniella gracilis*, *H. oryzae*, *Longidorus* sp., *Meloidogyne incognita*, *Mesocriconema* sp., *Psilenchus hilarulus*, *Pratylenchus* sp., *Trichodorus* sp., *Tylenchorhynchus* sp., and *T. martini* may be considered as limiting factors in rice production in Egypt and other parts of the world (Bridge, 1988; Ibrahim & Rezk, 1972, 1973, 2010; Rao & Jayaprakash, 1978; Rezk & Ibrahim, 1978; Villanueva *et al*., 1992).

Previous studies indicated 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.*, 2012). However, investigations into the pathogenicity of *Heterodera daverti* and *H. zeae* on rice and the interactions of these cyst nematodes when combined with *M. incognita* on rice have not been thoroughly studied. The objective of this research was to study the interactions of the cyst nematodes *H. daverti*, *H. goldeni* and *H. zeae* when combined with *M. incognita* on rice cultivars Giza 178 and Sakha 101.

Materials and Methods

The cyst nematodes *Heterodera daverti* Wouts & Sturhan, 1979, *H. goldeni* Handoo & Ibrahim, 2002 and *H. zeae* Koshy, Swarup & Sethi, 1971 and the root-knot nematode *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, Race 1, were used in this study. Isolates of these nematodes were obtained from the culture collections of the Nematology Research Laboratory, Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Egypt.

Isolates of *H. daverti*, *H. goldeni* and *H. zeae* were cultured on rice (*Oryza sativa* 'Giza 178'), qasaba grass (*Panicum coloratum*) and corn (*Zea mays* 'Pioneer 3062'), respectively, in the greenhouse for 8 weeks, then mature cysts were collected from infected roots (Ayoub, 1980). Isolates of *M. incognita* were reared on eggplant (*Solanum melongena* 'Black Beauty') for 60 days in the greenhouse and then nematode eggs were extracted from the infected roots by immersing the roots in sodium hypochlorite (NaOCl) solution (Hussey & Barker, 1973).

The greenhouse experiments were conducted to study the interactions of *H. daverti* with *M. incognita* on rice cv. Giza 178 as well as *H. goldeni* and *H. zeae* with *M. incognita* on rice cv. Sakha101. Seeds of the tested rice cultivars were sown in 12 cm diameter plastic pots containing a mixture of equal volumes of steam sterilized sand and clay soil. After emergence, rice seedlings were thinned to five seedlings per pot. Two weeks after emergence, the soil of the treated pots were inoculated by making holes near the plants roots and then adding the initial population (Pi) of *H. daverti*, *H. goldeni* and *H. zeae* at the rate of 65, 70 and 50 crushed cysts/pot, respectively. Inocula of *M. incognita* were added at an initial population (Pi) of 5,000 eggs/pot. The applied combined treatments of *Heterodera* spp. and *M. incognita* included the following: 1) *Heterodera* spp. inoculated 7 days before *M. incognita*, 2) *Heterodera* spp. and 3) *M. incognita* at the same time, and *Heterodera* spp. inoculated 7 days after *M. incognita*. Uninfested pots served as controls. Treatments and controls were replicated five times. Pots were arranged in a randomized complete block design in a greenhouse at 20-26°C.

Experiments were terminated 90 days after soil inoculation. Roots of harvested plants were washed free of soil, and the numbers of mature intact nematode cysts on the roots and in the soil (Final population, Pf) were counted. Roots were stained with phloxine B solution (0.15g/liter of tap water) for 15 minutes to highlight nematode egg-masses (Taylor & Sasser, 1978). The number of root galls and egg-masses of *M. incognita* on infected rice roots were counted and the dry weights of the shoot and root systems of the harvested rice plants were determined.

Analysis of variance (ANOVA) was performed on the numbers of *H. daverti*, *H. goldeni* and *H. zeae* cysts, the reproduction factor (Rf), the numbers of root galls and egg-masses of *M. incognita*, and the dry weights of the shoot and root systems of the tested rice plants, using Statistical Analysis System (SAS) (SAS Institute, 1988).

Results and Discussion

The interactions of *H. daverti*, *H. goldeni* and *H. zeae* with *M. incognita* are presented in Tables 1-3. Inoculation with *H. daverti* after *M. incognita* showed a significant increase in the final population of *H. daverti* with Rf = 2.1 as compared with other treatments. Inoculations of *M. incognita* alone or one week before *H. daverti* showed a significant increase of *M. incognita* galls and egg-masses compared with other treatments. Nematode infections with *H. daverti* and/or *M. incognita* induced significant reduction of the shoot and root dry weights of the rice cv. Giza 178 (Table 1).

In experiments with *H. goldeni* and *M. incognita* on rice cv. Sakha 101(Table 2), inoculation with *H. goldeni* alone or one week before *M. incognita* gave a significant increase in the final population of *H. goldeni* with Rf = 4.9 and 3.3, respectively, as compared with other treatments. Also, inoculation with *M. incognita* alone or one week before *H. goldeni* showed a significant increase in numbers of *M. incognita* root galls and egg-masses compared to other treatments. The shoot and root dry weights of the tested rice cultivar were significantly reduced with nematode infections (Table 2).

In experiments with *H. zeae* and *M. incognita* on rice cv. Sakha 101 (Table 3), inoculation with *H. zeae* one week after *M. incognita* resulted in a significant increase in the final population of *H. zeae* with Rf = 3.1 as compared with other treatments. Inoculation with *M. incognita* alone or one week before *H. zeae* showed a significant increase in the numbers of *M. incognita* root galls and egg-masses compared with other treatments. The shoot and root dry weights of the tested rice cultivar were significantly reduced with the applied nematode treatments (Table 3).

Table 1. Interaction of the cyst nematode *Heterodera daverti* (HD) and root-knot nematode *Meloidogyne incognita* (MI) on rice cv. Giza 178.

Treatment	*H. daverti*		*M. incognita*		Dry weight (g)	
	No. of cysts/pot	Rf	No. of galls/pot	No. of egg-masses/pot	Shoot	Root
Control	-	-	-	-	2.26 a	1.82 a
H. daverti	99 c	1.5c	-	-	1.93 b	1.66 b
M. incognita	-	-	199 a	157 a	1.77 c	1.59 b
HD 1st + MI 7 d later	99 c	1.5c	139 c	99 c	1.61 d	1.36 c
HD+MI at the same time	109 b	1.7 b	146 c	103 c	1.51 d	1.27 c
MI 1st + HD 7 d later	138 a	2.1 a	172 b	125 b	1.61d	1.31 c

Data are average of 5 replicates.
Rf= Final population (Pf)/ initial population (Pi, 75 cysts/pot). Means with the same letter(s) in each column are not significantly different at *P* =0.05.

Table 2. Interaction of the cyst nematode *Heterodera goldeni* (HG) and root-knot nematode *Meloidogyne incognita* (MI) on rice cv. Sakha 101.

Treatment	*H. goldeni*		*M. incognita*		Dry weight (g)	
	No. of cysts/pot	Rf	No. of galls/pot	No. of egg-masses/pot	Shoot	Root
Control	-	-	-	-	2.98 a	1.93 a
H. goldeni	372 a	4.9 a	-	-	1.82 bc	1.58 b
M. incognita	-	-	295 a	199 a	1.97 b	1.57 b
HG 1st + MI 7 d later	246 b	3.3 b	126 d	103 d	1.65 c	1.39 b
HG+MI at the same time	153 d	2.0 d	137 c	120 c	1.62 c	1.49 b
MI 1st + HG7 d later	185 c	2.5 c	196 b	154 b	1.82 bc	1.62 b

Data are average of 5 replicates.
Rf= Final population (Pf)/ initial population (Pi, 75 cysts/pot). Means with the same letter(s) in each column are not significantly different at *P*=0.05.

Table 3. Interaction of the cyst nematode *Heterodera zeae* (HZ) and root-knot nematode *Meloidogyne incognita* (MI) on rice cv. Sakha 101.

Treatment	*H. zeae*		*M. incognita*		Dry weight (g)	
	No. of cysts/pot	Rf	No. of galls/pot	No. of egg-masses/pot	Shoot	Root
Control	-	-	-	-	2.79 a	1.94 a
H. zeae	108 b	2.2 b	-	-	1.96 b	1.61 b
M. incognita	-	-	289 a	195 a	1.85 bc	1.55 bc
HG 1st + MI 7 d later	116 b	2.3 b	220 c	118 c	1.77 bcd	1.38 cd
HG+MI at the same time	109 b	2.2 b	228 bc	106 c	1.59 d	1.41 cd
MI 1st + HG7 d later	156 a	3.1 a	238 b	163 b	1.63 cd	1.36 d

Data are average of 5 replicates.
Rf=Final population (Pf)/ initial population (Pi, 75 cysts/pot). Means with the same letter(s) in each column are not significantly different at *P*=0.05.

Our results show that all the tested rice cultivars were good hosts and highly susceptible to *M. incognita*. Infection with *M. incognita* reduced the root and shoot dry weights of the tested rice cultivars. These results are in agreement with those of other workers who indicated that *M. incognita* is a pathogen of major economic importance on rice and other small grain crops (Babatola, 1980; Ibrahim *et al.*, 1983, 1993; Johnson & Motsinger, 1989).

In nature and under field condition, rice plants are rarely exposed to the influence of a single nematode pathogen and most rice plants are parasitized by one or more nematode species simultaneously at any given time. Results of the interactions of *H. daverti* and *M. incognita* in roots of the rice cv. Giza 178 showed that inoculation with *H. daverti* after *M. incognita* gave a significant increase in the final population (Pf) and Rf of *H. daverti* as compared to other treatments. Inoculation of *M. incognita* alone or one week before *H. daverti* showed a significant increase in numbers of *M. incognita* galls and egg-masses compared to other treatments. Similar studies showed that *Meloidogyne* spp. may have no effect, inhibit or stimulate infection and development of *Heterodera* spp. on the same host plant (Jatala & Jensen, 1976 a,b, 1983; Ross, 1964).

The results indicate that inoculation of *H. goldeni* alone or one week before *M. incognita* on rice cv. Sakha 101 resulted in a significant increase in the final population (Pf) and Rf values of *H. goldeni* as compared to other treatments. Also, inoculation of *M. incognita* alone or one week before *H. goldeni* gave a significant increase of numbers of *M. incognita* galls and egg-masses (Table 2).

Sedentary endoparasitic nematodes such as cyst and root-knot nematodes are highly specialized parasites and have long lasting relationships with their host plants. Norton (1978) reported that competition between nematode species is generally mutually suppressive because they utilize the same sites for feeding and often cause drastic physiological changes in the host plant tissues. For example, several studies showed that *M. hapla* was inhibited by *H. schachtii* on sugar beet (Jatala & Jensen, 1976 a, 1983) and tomato (Griffin & Waite, 1982). Sharma & Sethi (1978) reported that *M. incognita* was detrimental to *H. cajani* on cowpea when *M. incognita* was established first. Moreover, showed that *H. oryzicola* was inhibited by the presence of *M. graminicola* on rice in simultaneous and sequential inoculation.

Inoculation with *H. zeae* after *M. incognita* resulted in a significant increase in the final population of *H. zeae* on roots of rice cv. Sakha 101 compared to other treatments and a significant increase in numbers of root galls and egg-masses (Table 3). Kaul & Sethi (1982) showed that inoculation of *H. zeae* before *M. incognita* suppressed reproduction of *M. incognita* on maize roots. Salawu (1978, 1992) studied the effects of *M. incognita* and *H. sacchari* singly and combined on the growth of rice and sugarcane and showed an increase in the numbers of *H. sacchari* and a decrease in the numbers of *M. incognita* in the combined inoculation of both nematode species. More research work is needed on the interaction of cyst nematodes (*Heterodera* sp.) and the root-knot nematode *M. incognita* on rice plants especially the effects of combined infections of these nematodes on the yield of rice crop under greenhouse and field conditions. It is evident that *H. goldeni* was more virulent and a high population reproduction with Rf = 4.9 on rice cv. Sakha 101 as compared to *H. zeae* and *H. daverti* which showed Rf = 2.2 and 1.5 on rice cvs Sakha 101 and Giza 178, respectively (Tables 1-3). These results confirm our early report of Ibrahim *et al*., (2012) on the pathogenicity and host preference of *H. goldeni* on rice and other poaceous crop cultivars. The importance of crop infection by different nematode pests was discussed by Eisenback & Griffin (1987) who indicated that host suitability is a key factor in nematode-nematode interactions and often is responsible for one species being dominant in these interactions. The amount of disease caused by two or more nematode species is usually additive and strong competitors cause less disease in combination with each other than from single nematode-plant associations. Next experiments to conduct in the near future would be on the biological and chemical control of *H. goldeni* and/or *M. incognita* on rice plants.

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