Vol. 24, No. 1, pp. 11-17 International Journal of Nematology June, 2014

# Life cycle and control of the cyst nematode *Heterodera goldeni* on rice in Egypt

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**Abstract.** The life cycle and methods for control of the cyst nematode *Heterodera goldeni* on rice (*Oryza sativa*) were examined in the greenhouse. Three tests were conducted to study the effects of soil treatments with some plant materials, stems of oyster mushroom (*Pleurotus ostreatus*), the biocontrol agent *Bacillus thuringiensis* (Bt), the bionematicide abamectin, and the nematicide fenamiphos on *H. goldeni* on rice. The results showed that the life cycle of this nematode was completed in 40 days on roots of rice. All the applied treatments were effective in reducing nematode infection on rice plants. Soil treatment with fenamiphos or rubber plant (*Ficus elastica*) leaves plus Bt supernatant resulted in the highest reductions (90-91%) in the number of nematode cysts, while treatments with orange peels, rubber plant leaves, mushroom stems and abamectin induced 80-86% reduction in the number of developed nematode cysts, and enhanced rice plant growth. Treatments with castor bean leaves, mallow weed (*Malva parviflora*) foliage, and Bt pellet showed only 55-59% reduction in numbers of *H. goldeni* cysts.

**Keywords.** Control, cyst nematode, *Heterodera goldeni,* life cycle, rice, *Oryza sativa.*

## INTRODUCTION

In Egypt, plant-parasitic nematodes, especially the root-knot and cyst nematodes, are among the most important pests of many economic crop plants (Ibrahim and Handoo, 2007; Ibrahim *et al.*, 2010). The cyst nematode *Heterodera goldeni* was described in 2002 by Handoo and Ibrahim (2002) attacking qasabagrass (*Panicum coloratum* L.) in Alexandria, Egypt. Subsequently, *H. goldeni* was found attacking common reed (*Phragmites australis)* and Dutch rush (*Juncus acutus)* in Iran, as well as a wild grass (*Pennisetum clandestinums)* in Israel (Tanha Maafi *et al.*, 2007). *H. goldeni* has never been reported from agricultural fields in northern Egypt, but its presence on grass plants makes it a potential parasite of major poaceous crop plants such as corn, rice, barley, sugarcane and wheat. Recently, Ibrahim *et al.* (2012) showed that this nematode infected and reproduced successfully on various cultivars of corn, sorghum and rice. Information on the life cycle and control of *H. goldeni* on rice plants is not existent in the literature. The objectives of the present investigation were to study the life cycle and control of *H. goldeni* on rice cultivars Giza 178 and Sakha 101.

## MATERIALS AND METHODS

An isolate of the cyst nematode, *H. goldeni,* was obtained from infected roots of qasabagrass (*Panicum coloratum* L.) in Maamoura, Alexandria, Egypt. It was increased on qasabagrass in the greenhouse at 22-28°C for 8 weeks and then mature cysts were collected from infected roots (Ayoub, 1980). Nematode eggs and second-stage juveniles ($J_2$) for experimental inoculation were obtained by crushing mature cysts. Nematode eggs were placed in water for 3-4 days at room temperature to obtain $J_2$s.

In a greenhouse test at 22-28°C, the life cycle of *H. goldeni* in the roots of rice cv. Giza 178 was studied. Rice seeds were sown in 15cm diameter plastic pots (1.0 liter) filled with a mixture of equal volumes of steam-sterilized sand and clay soil. Two weeks after sowing, rice seedlings were thinned to five seedlings/pot and soil was inoculated by creating holes near the plant roots and then adding 5,000 $J_2$ of *H. goldeni*/pot.

Root samples of infected plants were collected at 24 and 48 hours and then at 2 day intervals up to 40 days after nematode inoculation. The collected root samples were

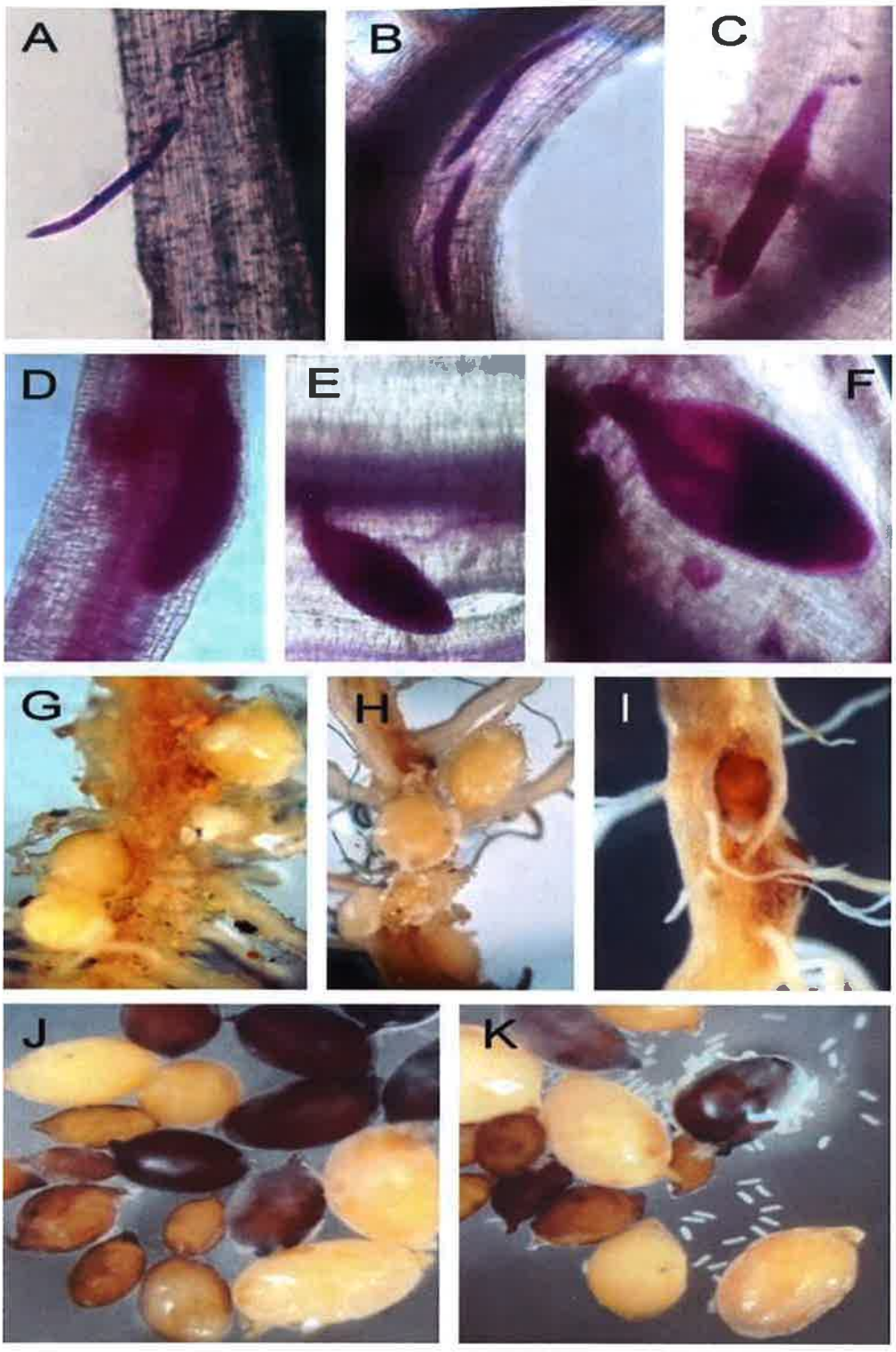


**Fig. 1.** Development of *Heterodera goldeni* in roots of rice cv. Giza 178. A: Penetrating $J_2$; B: Second-stage juvenile; C: Third-stage juvenile; D: Fourth-stage juvenile; E, F: Young female; G, H: White females on root; I: Cysts on root; J, K: Light and dark brown cysts.

**Table 1.** Effects of some plant materials on the infection of the cyst nematode *Heterodera goldeni* (Hg) on rice cv. Sakha 101.

| Treatment | No. of cysts/pot | Reduction % | Rf[x] | Shoot Dry Weight (g) | Root Dry Weight (g) |
|---|---|---|---|---|---|
| *Heterodera goldeni* | 220[w] | - | 2.93 | 1.91 | 1.65 |
| Rubber plant + Hg | 36a[y] | 84 | 0.48ab | 4.08a | 2.10a |
| Castorbean + Hg | 99b | 55 | 1.32c | 3.06b | 1.97b |
| Mallow weed + Hg | 90bc | 59 | 1.20c | 3.19b | 1.96b |
| Milk Thistle + Hg | 71c | 68 | 0.95 | 3.83a | 2.08a |
| Orange Peels + Hg | 45a | 80 | 0.60a | 3.24b | 2.09a |
| Mushroom stems + Hg | 30a | 86 | 0.40b | 4.97 | 2.70 |

[w]Means are average of 5 replicates.
[x]Rf= Final nematode population / initial nematode population (Pf/Pi). Pi= 75 cysts/pot.
[y]Means with the same letter in each column are not significantly different at P=0.05.

washed with tap water, cut into small pieces, and fixed in FAA solution. Fixed roots were stained for 3 minutes by boiling in a 0.1% acid fuchsin-lactophenol solution, then washed in tap water, and preserved in clear lactophenol. Stained roots were mounted in clear lactophenol on glass slides and examined under a compound light microscope (Ayoub, 1980). In three greenhouse tests, the effects of various dried plant materials, the biocontrol agent *Bacillus thuringiensis* (Bt) Berliner, the bioproduct Vertemic (1.8 EC abamectin), and the nematicide Nemacur (fenamiphos) on *H. goldeni* on rice plants cv. Sakha 101 were determined. Two Egyptian isolates of *B. thuringiensis*, 7N and Soto, were cultured on $T_3$ broth liquid medium for 72 hrs at 30°C (Travers *et al.*, 1987). The culture fluid suspension of Bt was placed in sterilized Eppendorf tubes and centrifuged at 13,000 rpm for 15 minutes to obtain cell-free supernatant. The supernatant of Bt was transferred to another glass tube and used for soil treatments. The pellet was washed with sterilized distilled water, re-centrifuged and dissolved (suspended) in sterilized distilled water to reach a standard concentration solution of 1mg/ml (Mohammed *et al.*, 2008). In all applied 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 the treated pots was inoculated by creating holes near the plant roots and then adding an initial population (Pi) of 75 crushed *H. goldeni* cysts/pot. Treatments and control were replicated five times and each experiment was performed once. Pots were arranged in a randomized complete block design in a greenhouse at 22-28°C.

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*), peels of orange fruits (*Citrus sinensis* (L.) Osbeck) cv. Baladi, and stems of oyster mushroom (*Pleurotus ostreatus*) on the infection of *H. goldeni* on rice plants cv. Sakha 101 was studied. The tested plant materials were collected from The Agricultural Experiment Station of Alexandria University, Abees, Alexandria and oven dried at 60°C for 48 hrs, ground to a fine powder, and incorporated in the soil of the treated pots at the rate of 2% (w/w), 1 day before sowing rice seeds.

In the second test, the effects of the supernatant of Bt, dried rubber plant leaves (RPL), and the nematicide Nemacur on the infection of *H. goldeni* on rice plants cv. Sakha 101 were determined. Dried RPL were added and mixed with the soil of the treated pots 24 hrs before sowing rice seeds. Two weeks after seedling emergence, soil was inoculated with *H. goldeni* and 24 hrs later Bt supernatant and Nemacur were applied to treated pots. Bt supernatant solution was added at the rate of 10ml/pot in two doses, 24 hrs and 7 days after nematode inoculation. Nemacur was applied at the rate of 0.25g/pot. The applied treatments included the following: *H. goldeni* alone or plus Bt, RPL, Bt+RPL, and Nemacur.

In the third test, the effects of the supernatant and pellet

**Table 2.** Effects of rubber plant leaves (RPL), *Bacillus thuringiensis* (Bt) isolates 7N and Soto and Nemacur® 10 G on the infection of the cyst nematode *Heterodera goldeni* (Hg) on rice cv. Sakha 101.

| Treatment | No. of cysts/pot | Reduction % | Rf[x] | Shoot Dry Weight (g) | Root Dry Weight (g) |
|---|---|---|---|---|---|
| *Heterodera goldeni* | 197[w] | - | 2.63 | 1.99 | 1.69 |
| RPL + Hg | 30 a | 85 | 0.84a | 3.10a | 1.90a |
| Bt 7N + Hg | 51b | 74 | 0.68 | 2.23b | 1.91a |
| Bt Soto + Hg | 59b | 70 | 0.79a | 2.12b | 1.79b |
| Bt 7N + RPL + Hg | 18a | 91 | 0.24b | 3.52 | 2.05 |
| Bt Soto + RPL + Hg | 23a | 88 | 0.31b | 3.07a | 1.96a |
| Nemacur® | 20a | 90 | 0.27b | 2.20b | 1.80b |

[w]Means are average of 5 replicates.
[x]Rf= Final nematode population / initial nematode population (Pf/Pi). Pi= 75 cysts/pot.
[y]Means with the same letter in each column are not significantly different at P=0.05.

**Table 3.** Effects of *Bacillus thuringiensis* (Bt), abamectin (Vertemic®)and Nemacur® on the infection of the cyst nematode *Heterodera goldeni* (Hg) on rice cv. Sakha 101.

| Treatment | No. of cysts/pot | Reduction % | Rf[x] | Shoot Dry Weight (g) | Root Dry Weight (g) |
|---|---|---|---|---|---|
| *Heterodera goldeni* | 212[w] | - | 2.83 | 1.98 | 1.67 |
| Bt supernatant + Hg | 53 | 75 | 0.71 | 2.22a | 1.89a |
| Bt pellet + Hg | 93 | 56 | 1.24 | 2.11 | 1.76b |
| Vertemic® + Hg | 29ab[y] | 86 | 0.39a | 2.21a | 1.84a |
| Nemacur® + Hg | 22b | 90 | 0.29 | 2.17a | 1.78b |

[w]Means are average of 5 replicates.
[x]Rf= Final nematode population / initial nematode population (Pf/Pi). Pi= 75 cysts/pot.
[y]Means with the same letter in each column are not significantly different at P=0.05.

of Bt, the bioproduct Vertemic (abamectin 1.8% EC), and the nematicide Nemacur (fenamiphos) 10G on the infection of *H. goldeni* on rice plants cv. Sakha 101 were determined. Two weeks after seedling emergence, pots were inoculated with *H. goldeni* 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. Tests were terminated 60 days after soil inoculation. Roots were washed free of soil. Numbers of mature intact nematode cysts on roots and in soil (final population, Pf) were counted and the reproduction factor (Rf), Rf= Pf/Pi was determined.

Analysis of variance (ANOVA) was carried out with SAS version 7 (SAS Institute, 1988) on the final population (Pf) of *H. goldeni*, the reproduction factor (Rf) and the dry weights of the shoot and root systems of the tested rice plants.

## RESULTS AND DISCUSSION

Figure 1 shows the developmental stages of the cyst nematode *H. goldeni* in the roots of rice cv. Giza 178. Second stage juveniles ($J_2$s) penetrated the rice roots within 24 hrs after nematode inoculation. Nematode penetration occurred near the root tips and $J_2$s were found oriented along the root axis 48 hrs after nematode inoculation. Third and fourth-stage juveniles were observed in infected roots after 8 and 14 days following nematode inoculation, respectively. White nematode females and yellow colored cysts were seen attached to infected roots 25 and 30 days after nematode inoculation. Light and dark brown colored cysts were observed on infected roots 36 days after nematode inoculation. Second generation $J_2$s were found in the soil 40 days following nematode inoculation.

The results show that the development and life cycle of *H. goldeni* on rice roots of cultivar Giza 178 were completed after 36-40 days following nematode inoculation. This finding is considered a first report of the development and life cycle of *H. goldeni* on rice plants. In a similar study, Hutzell and Krusberg (1990) reported that the life cycle of *H. zeae* on maize cultivar Pioneer 3184 was completed after 15-18 days at 33°C and 42 days at 25°C. Moreover, in similar studies Hung (1963) showed that $J_2$s of *H. lespedeza* invaded the host roots within 2 days after inoculation and mature cysts and egg deposition occurred between 36 and 38 days. Endo (1964) reported that $J_2$s of *H. glycines* penetrated soybean roots (var. Lee) within 1 day after inoculation. Salawu (1994) reported that $J_2$s of *H. sacchari* entered sugarcane roots within 24 hr after inoculation and the life cycle from $J_2$s to adult females was completed within 30-35 days at a soil temperature of 24-35°C.

All treatments suppressed nematode infection and significantly reduced the reproduction factor (Rf) and number of nematode cysts on infected rice roots compared to the control (Table 1). The treatments reduced the number of developed *H. goldeni* cysts by 55-86%. Treatments with dried rubber plant leaves, orange fruit peels and oyster mushroom stems induced the highest (80-86%) reductions in the number of nematode cysts. On the other hand, treatments with castorbean leaves and mallow weed foliage gave 55% and 59% reduction in the numbers of nematode cysts, respectively. In a similar study, Tsai (2008) showed that peels of lemon, orange, and grapefruit were effective against *Meloidogyne incognita*. Moreover, the present results are in agreement with those of other authors who described the effective use of organic amendments to control root-knot nematodes (Radwan *et al.,* 2004; Saifullah *et al.,* 1990).

The applied soil treatments suppressed nematode infection on rice plants and significantly reduced *H. goldeni* reproduction factor and number of cysts on infected rice roots compared to the control (Table 2). The highest reductions of nematode cysts (90-91%) were recorded with treatments of Bt supernatant of 7N isolate plus rubber plant leaves and the nematicide Nemacur. Treatment with either Bt supernatant of 7N and Soto isolates reduced the number of *H. goldeni* cysts by 74% and 70%, respectively.

In a similar study, Radwan *et al.* (2004) showed that the integration of *B. thuringiensis* with organic soil amendment was more effective in controlling the root-knot nematode *Meloidogyne incognita*. Moreover, the present results are in agreement with those of other studies on the use of Bt as a biocontrol agent against plant-parasitic nematodes (Devidas and Rehberger, 1992; Ingnoffo and Dropkin, 1977; Sharma, 1994; Zuckerman, 1993, 1995).

Treatments with the biocontrol agent Bt supernatant and pellet, Vertemic and the nematicide Nemacur significantly reduced Rf and the numbers of developed *H. goldeni* cysts on infected rice roots compared to the control (Table 3). The treatments reduced the numbers of nematode cysts by 56-90%. Treatment with the nematicide Nemacur induced the highest reduction (90%) in the nematode cysts followed by Vertemic with 86%. Treatment with Bt supernatant resulted in a 75% reduction in numbers of nematodes while treatment with Bt pellet gave only 56% reduction in nematode cysts.

All soil treatments significantly increased the amount of dry weights of the shoots and roots of *H. goldeni* infected rice plants compared to control (Tables 1, 2, 3). The tested rice cultivars Giza 178 and Sakha 101 were good hosts for *H. goldeni*, as this nematode reproduced successfully on their roots. A previous study by Ibrahim *et al.* (2012) showed that rice cultivars Giza 171, Giza 177, Giza 178, Sakha 101 and Sakha 102 were either susceptible or highly susceptible to *H. goldeni*.

Treatments with the supernatant of Bt were effective in reducing the infection and developed cysts of *H. goldeni* on rice roots (Tables 2, 3). Studies by Mohammed *et al.* (2008)

on the nematicidal effects of some isolates of Bt against *M. incognita* on tomato plants showed that both crude culture suspension and cell free supernatant of Bt isolate 7N greatly reduced the numbers of nematode egg masses on infected plants.

The present results indicate that the tested rice cultivars Giza 178 and Sakha 101 were good hosts for *H. goldeni* as this nematode reproduced successfully on their roots. These results are in agreement with our recent study on the host suitability of some rice cultivars for *H. goldeni* (Ibrahim et al., 2012). Previous studies showed that rice plants were attacked by certain species of the cyst nematodes, i.e., *Heterodera oryzae* (Luc and Brizuella, 1961), *H. oryzicola* (Rao and Jayaprakash, 1978), *H. sacchari* (Babatola, 1983; Vovlas *et al.*, 1986), *H. elachista* (Shimizu, 1976), and *H. goldeni* (Ibrahim *et al.*, 2012).

**Acknowledgements.** The authors thank Joseph Mowery and Mariah Cutler 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.

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