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Biological Control of Root-Knot Nematodes (*Meloidogyne enterolobii*) in Guava by the Fungus *Trichoderma harzianum*

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In Thailand, Nakhon Pathom, Samut Sakhon, and Ratchaburi provinces, which are in the central region of the country, are the largest areas for guava cultivation. Kimju and Pansithong are the most extensively grown cultivars, and they have been seriously damaged by two species of root-knot nematodes, *Meloidogyne incognita* and *M. enterolobii* (or *M. mayaguensis*). The latter species was only recently reported in Thailand but is common in China, Brazil, and the United States. Substantial numbers of guava trees are in decline and show reduced fruit production. In this survey study of six guava orchards in central Thailand, we found that common aboveground symptoms of nematode infection included yellowing, stunting, folded leaves, blighted and wilted leaves (especially when water was lacking), and slow ripening of fruits. Underground symptoms included root galls, partially rotted roots, and, in some cases, prolific production of adventitious roots to compensate for damaged and nonfunctional roots. When we examined the morphometric characteristics of second-stage juveniles of *M. incognita* and *M. enterolobii* obtained from soil around guava plants, we observed no significant correlation between the characteristics of the two species or between the characteristics of populations within each species ($P > 0.05$). For the biological control of root-knot nematodes in guava, we evaluated a commercially available fungal agent, *Trichoderma harzianum*, which effectively controls several other soil-borne pathogens and has been shown to induce disease resistance and stimulate adventitious root growth in plants. We found that inoculation of the root zones of guava plants with *T. harzianum* reduced the number of nematodes in both soil and roots as compared to the number in untreated plants ($P < 0.05$). Moreover, inoculation of guava plants with *T. harzianum* arrested the development of the juvenile nematodes ($P < 0.05$). *Trichoderma harzianum* is less expensive than chemical control agents and poses no risk to the environment.

Key words: *Meloidogyne enterolobii*, *Trichoderma harzianum*, Guava, Biological Control

Introduction

Thailand exports agricultural commodities worth approximately 5.2 quadrillion baht (Centre of Agricultural Information, 2009). Tropical and subtropical fruits make up a large proportion of this revenue, and guava in particular is one of the most important fruits in Thailand. It is produced mainly in three central provinces: Nakhon Pathom, Ratchaburi, and Samut Sakhon. However, in these areas, root-knot nematodes, *Meloidogyne* spp., are a major constraint on guava production. In 1989, it was estimated that over 80% of guava trees in Thailand were damaged by the nematodes (Sontirat, 1989).

The first report of serious damage to guava by root-knot nematodes in Thailand was in 1987, in the Bangkruai district of Nonthaburi Province (Sukhakul, 2006). The symptoms are similar to those caused by nutrient deficiency, including stunted growth and yellow leaves. In 1992 and 1997, root-knot nematode infestations were reported to have expanded to Komsareewa and Pansithong guava cultivars grown in the Samphan district of Nakhon Pathom Province (Sukhakul, 2006). The infected guava plants showed a decline and reduced yields of fruit. In 1999, a decline in guava trees in the Cha-um district of Phetchaburi Province was attributed to root-knot nematodes (*M. incognita*). In 2003 and 2004, *M. incognita* infection

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severely damaged guava trees in a wide area of the Samphan district of Nakhon Pathom Province (Sukhakul, 2006). In 2012, an emergent and more aggressive species of root-knot nematode, *M. enterolobii,* was identified in guava roots (Kimju and Pansithong cultivars) collected from orchards in Nakhon Pathom Province (Jindapunnapat, 2012).

Meloidogyne enterolobii (or *M. mayaguensis*), an emerging species of root-knot nematode, is currently considered to be one of the most damaging root-knot nematode species in many countries because the nematode has wide geographical distribution, wide host ranges, and could overcome *Mi-1* gene resistance against *M. incognita*, *M. javanica*, and *M. arenaria* in many crops (Fargette, 1987; Fargette *et al*., 1994; Moens *et al*., 2009). *M. enterolobii* was first identified in aubergine in Puerto Rico (Rammah and Hirshmann, 1988) and was later discovered in Africa (Fargette *et al*., 1994; Duponnois *et al*., 1995; Willers, 1997; Trudgill *et al*., 2000; Moens *et al*., 2009), the United States (Brito *et al*., 2004), Europe (Blok *et al*., 2002), China (Zhuo *et al*., 2010), Vietnam (Iwahori *et al*., 2009), and Thailand (Jindapunnapat, 2012). In addition to *M. enterolobii*, nine other root-knot nematodes species have been reported in Thailand (Chinnasri *et al*., 2012): *Meloidogyne incognita, M. javanica, M. arenaria, M. hapla, M. exigua, M. graminicola, M. microcephala, M. naasi,* and *M. thailandica.* Root-knot nematodes, which are widely distributed and have a broad host range, are among the most damaging parasitic nematodes in ornamental plants, vegetables, fruit trees, and field crops in Thailand. *Meloidogyne enterolobii* was recently found in guava plants there (Chinnasri *et al*., 2012). This species may coexist in the same areas as *M. incognita*, which was previously reported as the main cause of yellowing, stunting, blighted leaves, and reduction in guava yields (Sukhakul, 2006; Sasnarukkit *et al*., 2010), and the coexistence of the two species may aggravate an already serious problem.

Nematicides are routinely used to control root-knot nematodes in guava. However, with the ban of effective nematicides from the market owing to concerns about risks to human health and the environment, safe and effective alternative control measures are highly sought after. Biological control methods are environmentally sound and effective for reducing or mitigating other crop diseases (Sahebani and Hadavi, 2008). For example, the fungus *Trichoderma harzianum* can be used to control a range of plant diseases because it competes for nutrients, produces compounds that are toxic to other microorganisms, and induces plant resistance to diseases (Chamswarng, 2004). *Trichoderma harzianum* has been reported to suppress *M. incognita, M. javanica,* and *M. arenaria* populations and to increase yields in vegetables and other crops in many countries (Sharon *et al*., 2001).

Therefore, in this study, we investigated the effects of a commercial formulation of *T. harzianum* on *M. enterolobii* in guava. In addition, we studied the symptoms caused by *M. enterolobii* infection in guava trees grown in six orchards in central Thailand, and we determined the morphometric characteristics of second-stage juveniles of *M. enterolobii* collected from the soil around the trees.

Materials and Methods

Soil collection and evaluation of symptoms of *M. enterolobii* infection in guava

Soil samples were randomly collected from six orchards infested with *M. enterolobii* in two provinces of central Thailand: Samut Sakhon Province (Khlongton orchard) and Nakhon Pathom Province (Thaveesak, Khlongjinda, Payoon, Apinya, and Namthan orchards). The total number of guava trees selected per orchard was two. Four composite soil cores were collected at a depth of 0.2 meter around the root zone of one guava tree. Subsequently, for each tree, all four cores were mixed together, and about one-fourth of the mixed soil was placed in a plastic bag. Soils samples were labeled and transferred to the laboratory in a foam box. The guava trees from which soil was collected were photographed, and above- and belowground symptoms of infestation were recorded. Interviews of guava growers of each orchard were performed to receive the information on the symptoms of the disease. A survey of the commercial plant nurseries which distributed guava seedlings to many orchards in central Thailand was also conducted to determine whether the nurseries were the source of nematode dissemination.

Nematode extraction and rearing of second-stage juveniles

Nematodes were extracted from 300-g soil samples by means of the Baermann funnel method (Baermann, 1917), as follows. The soil was mixed with water, and the supernatants of the resulting soil suspensions were poured successively through 60-, 100-, and 400-mesh

sieves. Nematodes suspended on the 100- and 400-mesh sieves were collected and placed on tissue papers in funnels, and 3 days later, the nematodes at the bottoms of the funnels were collected and counted under a microscope.

The remaining soil in each sample was then transferred into a 15-cm-diameter plastic pot in which a 1-month-old tomato seedling (*Solanum lycopersicum*) was planted. 30 days later, the single-egg-mass technique was used to pick up and transfer individual egg masses to 3.75-cm-diameter Syracuse watch glasses containing sterilized water. The second-stage juveniles that had emerged from each egg mass for 7 days were then used to inoculate a new set of 1-month-old tomato seedlings. The inoculation procedure was conducted by pouring 10 m*l* of nematode suspensions containing 1,000 juveniles into the holes made on the soil planted with tomato seedlings. After 2 months, females and egg masses were picked up from the tomato in each pot. Nematode species were identified using the esterase isozyme method (Esbenshade and Triantaphyllou, 1985) and DNA analysis (Brito *et al*., 2004).

Measurement of second-stage juveniles

Second-stage juveniles were mounted on permanent slides by Sienhorst's method (Seinhorst, 1966), as follows: Egg masses obtained by means of the single-egg-mass technique were hatched in sterilized water over the course of 4 days. Second-stage juveniles emerging from the egg masses were killed with hot water (80℃), fixed with FA 4:1 fixative [10 parts formalin (40% formaldehyde), 1 part glacial acetic acid, and 89 parts distilled water], incubated for 48 h, and then transferred to Syracuse watch glasses filled with Seinhorst I solution (20 parts 95% ethanol, 1 part glycerin, and 79 parts water; Seinhorst, 1959). The watch glasses were subsequently placed in Petri dishes containing 95% ethanol and incubated at 35−40℃ for 12 h. Then Seinhorst II solution (95 parts 95% ethanol and 5 parts glycerin) was added to the watch glasses, which were subsequently stored at 40℃ until only glycerin remained in the watch glasses. Approximately, 20 nematodes from each watch glass were transferred into a drop of glycerin on a slide, which was then covered with a cover slip and sealed with nail polish.

The mounted second-stage juveniles were observed and measured under a well-calibrated microscope: (10×) 1 unit=10 μm, (20×) 1 unit=5 μm, (40×) 1 unit=2.5 μm, (100×) 1 unit=1 μm. Each sample consisted of 20 second-stage juveniles. The following nematode characteristics were recorded or calculated: mean length (L), body length/body width ratio (a), body length/tail length ratio (c), hyaline tail terminus (h), 100×h/tail length ratio (h%).

Biological control of *M. enterolobii* by *T. harzianum*

Preparation of Trichoderma inoculums

Commercial *T. harzianum*, obtained from the Department of Agriculture of Thailand, was mass produced by placing 2 g of the fungal inoculum into each of 25 plastic bags (20×30 cm) containing steamed jasmine rice seeds (250 g). The plastic bag was sealed and punctured by a needle (about 15 holes) to release air from the bag. The bags were incubated at room temperature (28−30℃) for 10 days. Fungal suspensions were prepared by flooding the bags with distilled water and vigorously shaking. Subsequently, the suspensions were settled down for one minute. The supernatants containing only fungal spores were poured into 1-L beakers. The spore densities were determined with a hemocytometer and then adjusted by adding distilled water until the concentrations reached 10^4−10^7 spores/ml.

Preparation of root-knot nematode inoculums

To prepare root-knot nematodes inoculums, we used *M. enterolobii* that had been reared on Cherry tomato cultivar (*Solanum lycopersicum*) for 30 days in the greenhouse at the Department of Plant Pathology of Kasetsart University (Bangkok, Thailand). Nematode eggs were extracted from plants by manually shaking tomato roots in 0.6% sodium hypochlorite (NaOCl) for 4 min. The resulting egg suspensions were poured through on a 500-mesh sieve, and the eggs on the sieve were thoroughly washed with water, transferred from the sieve to a beaker, and hatched into second-stage juveniles in sterilized water. The number of freshly hatched juveniles was counted under a stereo microscope on the fifth day after the eggs had been in the sterilized water. The nematode density was adjusted with sterilized water to the final concentration of 100 juveniles/ml.

Inoculation of guava plants with T. harzianum and M. enterolobii

Cleaned and nematode-free guava seedlings (Pansithong cultivar) were planted in 30-cm-diameter plastic pots filled with sterilized loamy soil. Guava plants were maintained in the greenhouse at the Department of Plant Pathology of Kasetsart University (Bangkhen Campus, Thailand) for 2 weeks. The roots of the guava seedlings were inoculated with *T. harzianum* at concentrations of 0, 10^4, 10^5, 10^6, and 10^7 spores/ml. Two weeks after fungal inoculation, the roots of the seedlings were inoculated with 2000 second-stage juveniles of *M. enterolobii* (in 20 ml of water). To maintain conditions favorable for the growth of *T. harzianum,* we covered the soil surfaces of the pots with the leaves of the rain tree (*Samanea saman*). The pots were arranged in a randomized completed block design with six treatments and 10 replicates (plastic pots). Means of the six treatments were subject to statistical analysis with the SPSS program package (ver. 16.0; SPSS, Inc., Chicago, IL, USA).

Determination of fungal survival in soil

Thirty days after fungal inoculation, the survival of *T. harzianum* in soil was determined by the soil dilution plate method as follows: Ten grams of soil were sampled from each pot, placed in a 500 ml beaker, and added with 90 ml of sterilized water to make a soil suspension of 10^{-1} concentration. Subsequently, soil suspensions were successively diluted with distilled water to a final concentration of 10^{-5}. Each of the suspensions was then transferred to a Petri dish containing potato dextrose agar and cultured for 7−14 days. The survival of *T. harzianum* was determined in each Petri dish by recording as positive (+) if there was at least one *T. harzianum* colony on the dish and as negative (−) if no *T. harzianum* colonies were present.

Determination of fungal effect on guava plants

Guava growths were determined by fresh shoot weight, dry shoot weight and fresh root weight. The procedures were as follows: the shoots and the roots of guava plants were cut into 5-cm-long pieces and freshly weighed on a weighing apparatus. The shoots were placed in an oven of 60℃ for 3 days. Dry shoot weights were recorded and analyzed with the SPSS program package (ver. 16.0; SPSS, Inc., Chicago, IL, USA).

Determination of nematode penetration and development

Nematode penetration and development in guava roots were determined by the acid fuchsin staining method as follows: Thirty days after nematode inoculation, guava plants were gently uprooted, and the roots were thoroughly washed with water and cut into 1-cm-long pieces. Staining solution was prepared from 1 ml of stock solution (10 g of acid fuchsin＋1 L of water) and 30 ml of tap water and heated to boiling. The guava roots were immediately transferred to the staining solution, boiled for 30 sec, rinsed with tap water, destained with glycerol containing a few drops of hydrochloric acid, and incubated at 60℃ for 7 days. The numbers of nematodes in the roots was counted under a stereo microscope, and their life stages were recorded.

Results

Survey of nematode-infection symptoms in guava

The guava plants surveyed in Nakhon Pathom and Samut Sakhon provinces were heavily infested with root-knot nematodes. Aboveground symptoms of infection included undeveloped fruits (Fig. 1A), yellowing and folded leaves (Fig. 1B), blighted and wilted leaves (especially when soil water was depleted), and brittle branches and stunting (Fig. 1C). Belowground symptoms included galled and partially rotted roots (Fig. 1D). In some cases, we observed prolific production of adventitious roots to compensate for damaged and nonfunctional roots.

The infestation of root-knot nematodes in guava in central Thailand may stem from the planting of nematode-infected guava seedlings brought at local nurseries. In a survey of the local nurseries, we found a number of nematode-infected guavas for sale.

Measurement of second-stage juveniles

Measurements of second-stage juveniles obtained with an ocular meter mounted on a microscope revealed no significant difference in the values between the juveniles isolated from the six orchards ($P > 0.05$; Table 1).

Nematode species were identified by DNA analysis and determination of nematode esterase isozyme phenotype. Using a previously reported polymerase chain reaction method for discrimination between the five major *Meloidogyne* species (Powers and Harris, 1993), we determined that the nematodes isolated from the

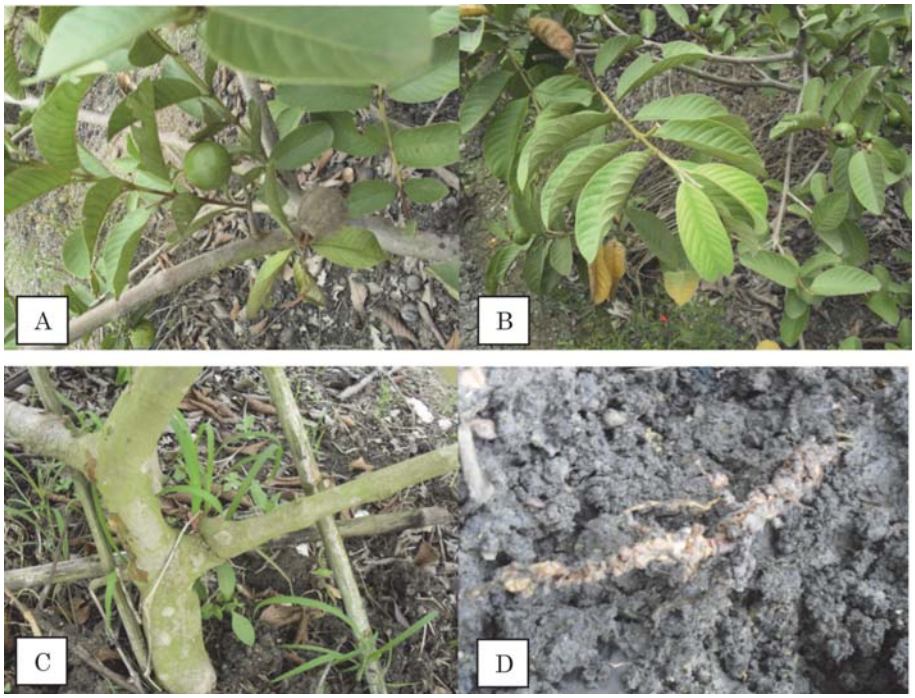

Fig. 1. Guava trees infected with *Meloidogyne* in central Thailand showed (A) undeveloped fruits, (B) folded leaves, (C) brittle branches and stunting, and (D) galled roots.

Table 1. Average values (n=20), standard deviations, and ranges of morphometric characteristics of *Meloidogyne* second-stage juveniles collected from six guava orchards in central Thailand.

Orchard	Species[a]	Morphometric characteristics of second-stage juveniles[b]				
		L	a	c	h	h%
Apinya	I	368.00±35.64a (340.0-430.0)	31.53±5.07b (24.0-36.0)	6.68±0.72b (5.76-7.58)	8.00±2.74a,b (5.0-12.5)	14.63±5.90a (10.53-25.0)
Thaveesak	I	381.05±27.05a (320.0-445.0)	31.48±6.13b (19.5-45.33)	6.78±0.91b (3.12-9.25)	7.14±2.58a (5.0-15.0)	12.48±4.14a (4.55-25.0)
Khlongjinda	I	384.50±35.17a (320.0-430.0)	31.03±6.30b (21.3-43.0)	4.57±0.91a (3.29-7.0)	10.50±2.31c (7.5-13.75)	17.84±3.66b (13.33-25.0)
Namthan	E	366.50±23.63a (335.0-410.0)	25.79±4.69a (20.1-39.0)	8.66±1.16c (6.96-11.2)	8.80±2.50a,b (5.0-15.0)	20.53±5.17b,c (11.63-30.0)
Payoon	E	376.25±26.70a (330.0-420.0)	32.37±5.85b (23.0-41.0)	6.48±0.77b (5.3-8.0)	7.63±1.90a,b (5.0-12.5)	12.88±2.30a (8.7-16.7)
Khlongton	E	367.50±26.33a (310.0-400.0)	24.32±4.49a (18.1-35.0)	8.97±1.41c (6.61-13.3)	9.65±2.18b,c (5.0-13.0)	23.45±5.66c (11.11-35.14)

[a] I=*M. incognita*, E=*M. enterolobii*.
[b] L=length, a=body length/width ratio, c=body/tail length ratio, h=hyaline tail terminus, h%=100×h/tail length ratio.

Apinya, Thaveesak, and Khlongjinda orchard samples were *M. incognita*, whereas those isolated from the Namthan, Payoon, and Khlongton orchard samples were *M. enterolobii* (Table 1).

Table 2. Survival of *T. harzianum* and effects of *T. harzianum* and *M. enterolobii* inoculation on mean (n=10) fresh shoot weight (FSW), dry shoot weight (DSW), and fresh root weight (FRW) of guava.

Treatment	Percentage of Petri dishes positive for fungal growth and development (%)	Guava shoot[a]		Guava root[a]
		FSW (g)	DSW (g)	FRW (g)
No nematodes+ no fungi	0	40a	16a	52.5ab
Nematodes only	0	37.5a	15a	38.5a
Nematodes+fungi at 10^4 spores/ml	0	44a	17.2a	53.4ab
Nematodes+fungi at 10^5 spores/ml	20	45a	21ab	58.8abc
Nematodes+fungi at 10^6 spores/ml	40	60.67ab	25.67ab	94.34c
Nematodes+fungi at 10^7 spores/ml	100	82b	36.4b	79bc

[a] In each column, the values labeled with the same lowercase letter are not significantly different (P>0.05), as indicated by Duncan's multiple range test.

Biological control of *M. enterolobii* by *T. harzianum*

Determination of fungal survival in soil

Trichoderma harzianum survived in the soil around guava plants. Thirty days after fungal inoculation, the percentages of Petri dishes that were positive for fungal growth and development (as indicated by the presence of a *T. harzianum* colony in the dish) were 0%, 0%, 0%, 20%, 40%, and 100% for the following treatments: untreated, inoculation with nematodes only, inoculation with nematodes and fungal spores at 10^4 spores/ml, inoculation with nematodes and fungal spores at 10^5 spores/ml, inoculation with nematodes and fungal spores at 10^6 spores/ml, and inoculation with nematodes and fungal spores at 10^7 spores/ml, respectively (Table 2). Inoculation of the guava root zone with *T. harzianum*, especially at concentrations higher than 10^6 spores/ml, enhanced fresh and dry shoot weights.

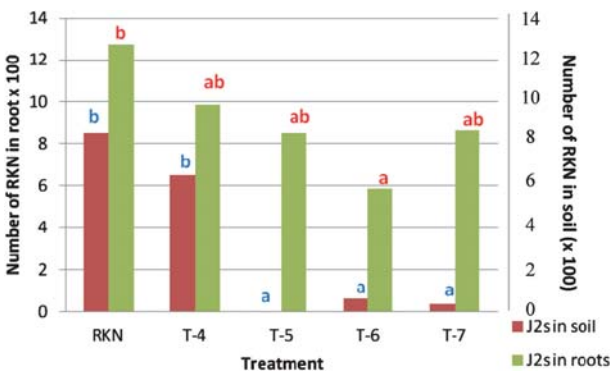

Fig. 2. Effect of *T. harzianum* concentration on average (n=10) number of *M. enterolobii* (RKN) second-stage juveniles (J2s) in soil and guava roots. Bars labeled with the same lowercase letter indicate data that are not significantly different (P>0.05), as indicated by Duncan's multiple range test. RKN=only root-knot nematodes inoculated, T-4=nematodes+fungi at 10^4 spores/ml, T-5=nematodes+fungi at 10^5 spores/ml, T-6=nematodes+fungi at 10^6 spores/ml, T-7=nematodes+fungi at 10^7 spores/ml.

Determination of nematode penetration and development

On average, 1275 and 850 nematodes were retrieved from the roots (green) and from soil (red), respectively, in the absence of fungal inoculation (that is, inoculation with nematodes only). In contrast, in the presence of *T. harzianum* at all concentrations, the number of nematodes in the roots and in soil ranged from 588 to 988 and 0 to 6.5, respectively (P<0.05; Fig. 2). However, only inoculation at a concentration of 10^6 spores/ml resulted in a statistically significant decrease in the number of nematodes in the roots. In contrast,

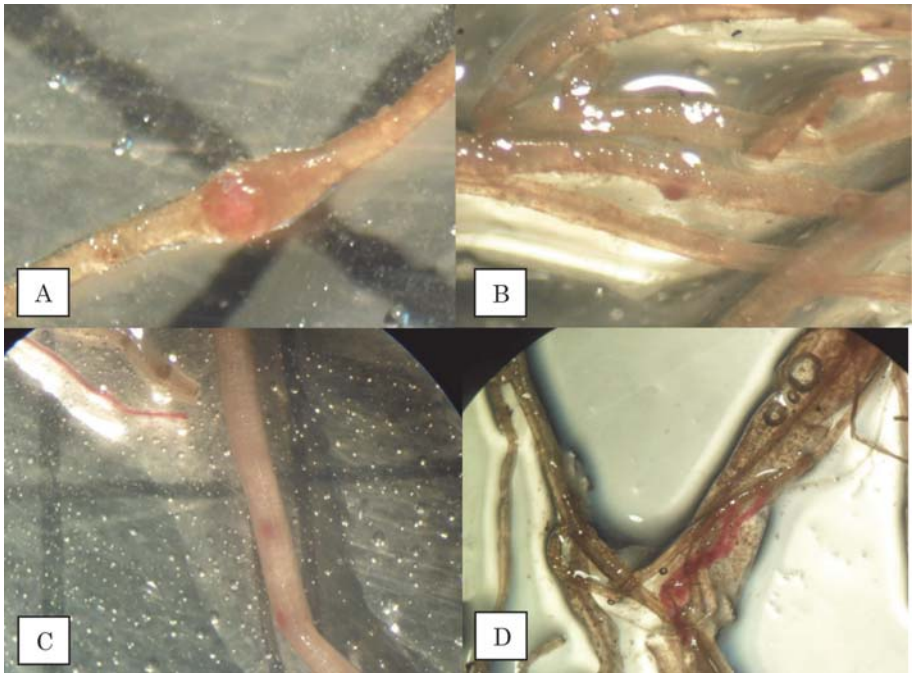

Fig. 3. Development of root-knot nematodes in guava roots 30 days after infection: (A) egg mass produced by a root-knot nematode female within a small root gall, (B) swelling female within a small root knot, (C) swelling female within a normal root, and (D) vermiform juveniles within roots.

inoculation with *T. harzianum* at concentrations from 10^5 to 10^7 spores/ml significantly reduced the number of nematodes in the soil compared to the number in the absence of fungus and in the presence of fungus at 10^4 spores/ml (Fig. 2). All the nematodes were sausage shaped at all *T. harzianum* concentrations except 10^7 spores/ml (Fig. 3A−C); at this concentration, 10% of the root-knot nematodes were vermiform (Fig. 3D).

Discussion

Biological control of *M. enterolobii* on guava is a potential alternative to chemical nematicides. *Trichoderma harzianum,* a free-living fungus that flourishes in soil and root ecosystems, has long been employed for the biological control of an array of plant diseases. This fungus reportedly enhances root growth and induces plant resistance to diseases by activating ethylene production, hypersensitive responses, and plant defense mechanisms (Ranasingh *et al*., 2006). In this study, we evaluated the effectiveness of *T. harzianum* in controlling *M. enterolobii* in guava. We found that inoculation with *T. harzianum* at concentrations between 10^5 and 10^7 spores/ml reduced the number of root-knot nematodes both in the soil around guava plants and in guava roots. Our results suggest that *T. harzianum* produced chemical compounds detrimental to nematodes in soil or that the fungus stimulated some defensive mechanisms in guava to inhibit nematode infection of the roots or to delay the development of nematodes that entered the guava roots. Systemic acquired resistance or induced systemic resistance may have been induced in guava in response to *T. harzianum* infection, leading to suppression of nematode infection and development. This possibility will have to be verified by determination of the marker genes for these types of resistance.

Our results were consistent with those of Sahebani and Hadavi (2008) and Affokpon *et al*. (2011), who reported that inoculation with *T. harzianum* at ~10^6 spores/ml controlled root-knot nematodes in West African vegetable production systems. Our observation that guava plant health was improved by inoculation with *T. harzianum* also agreed with the results of Golzari *et al*. (2011), who reported that treatment of tomato with *T. harzianum* increased top fresh weight and dry weight. *Trichoderma* isolates have also been shown to stimulate tomato growth in both sterilized and unsterilized soil (Affokpon *et al*., 2011).

Because effective nematicides have been banned from the market, it is necessary to look for alternatives to minimize the damage to guava caused by root-knot nematodes. The commercial formulation of *T. harzianum* may serve as such an alternative because the fungus is effective against nematodes and is not dangerous to human health or the environment. In addition, the commercial formation is ready to use and widely available. However, studies to determine the optimum concentration and the long-term efficacy of the fungus under real-world conditions will be necessary before this method can be used in practice.

Conclusion

In this study, *Meloidgyne enterolobii* or *Meloidogyne mayaguensis* was found to infect guava plants in central provinces of Thailand. Nakhon Pathom and Samut Sakhon provinces which are the important guava cultivating areas of Thailand were infested with root-knot nematodes (*Meloidogyne* spp.). Guava production from these provinces constitutes approximately 52.61% of the whole country production.

The soil samples of guava plants were randomly collected from 6 orchards in Nakhon Pathom and Samut Sakhon provinces. Aboveground symptoms of infection exhibit undeveloped fruits, yellowing and folded leaves, brittle branches and stunting. Belowground symptoms of infection exhibit galled and partially rotted roots.

The measurement of the size of *Meloidogyne*'*s* second-stage juveniles revealed no significant differences in value between the second-stage juveniles of two species of root-knot nematodes found in six orchards.

The Bio-control of *Meloidogyne enterolobii* by *Trichoderma harzianum* was investigated and revealed promising results. At the fungal concentration higher than 10^6 spores/ml, fresh and dry shoot weights of guava were enhanced. Additionally, at these concentrations, the number of second-stage juveniles in soil and roots were reduced as compared to no fungal inoculation and at the fungal concentration lower than 10^6 spores/ml. The development of root-knot nematodes in guava roots was retarded when the fungal concentration of 10^7 spores/ml was applied.

The commercial product of *Trichoderma harzianum* was effective to control root-knot nematodes in guava plants. The results reiterated the effects of *Trichoderma harzianum* on root-knot nematodes as the fungi were able to induce guava growth and delay nematode development.

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