PATHOGENICITY AND CULTURE OF A *HETERORHABDITIS INDICA* ISOLATE FROM THAILAND

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ABSTRACT

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An isolate of the entomopathogenic nematode *Heterorhabditis indica*, designated "T2," was collected in Kanchanaburi Province, Thailand. The life cycle of T2 in *Galleria mellonella* larvae required 10 days and two generations at 25°C. The first generation was hermaphroditic and the second was amphimictic. Survival of infective-stage juveniles in water-filled sponges at 15°C was 81% after 1 month. In the laboratory, T2 was able to penetrate and kill *Aphis gossypii, Spodoptera exigua, Sitophilus zeamais, Tenebrio molitor, Bactrocera correcta*, and *Coptotermis gestroi* but reproduced poorly in all of these hosts except *S. exigua*. In *G. mellonella,* T2 reproduced best at 25°C, and the optimum soil moisture for infection was 7 to 9%. Isolate T2 reared on yeast salt broth caused 84% mortality 2 days after inoculation of diamondback moth (*Plutella xylostella* L.) third-instar larvae. The best artificial medium for mass-rearing *H. indica* T2 was modified yeast salt broth containing 2.0% soluble starch, 1.5% yeast extract, 2.5% nutrient broth, 1.0% lard, and 2.0% $MgCl_2 \cdot 6H_2O$.

Key Words: ecology, *Heterorhabditis indica,* pathogenicity, *Plutella xylostella*

RESUMEN

Maketon, M., V. Somsook, P. Rattanakorn, and D. Hotaka. 2011. Patogenicidad y cultivo de un aislamiento de *Heterorhabditis indica* de Tailandia. Nematropica 41:52-61.

Se colectó un aislamiento del nematodo entomopatógeno *Heterorhabditis indica*, designado "T2," en la Provincia de Kanchanaburi en Tailandia. El ciclo de vida de T2 en larvas de *Galleria mellonella* requirió 10 días y dos generaciones a 25°C. La primera generación fue hermafrodítica y la segunda anfimíctica. La supervivencia de los juveniles infectivos en esponjas con agua a 15°C fue del 81% después de un mes. En el laboratorio, T2 penetró y mató *Aphis gossypii, Spodoptera exigua, Sitophilus zeamais, Tenebrio molitor, Bactrocera correcta* y *Coptotermis gestroi*, pero no se reprodujo bien en ninguno de estos hospedantes, except en *S. exigua*. En *G. mellonella*, T2 se reprodujo mejor a 25°C, y la humedad de suelo óptima para infección fue de 7 a 9%. El aislamiento T2 cultivado en caldo de levadura causó mortalidad del 84% dos días después de la inoculación de larvas de tercer ínstar de *Plutella xylostella* L. El mejor medio artificial para el cultivo masivo de *H. indica* T2 fue el caldo de levadura modificado, con 2.0% de almidón soluble, 1.5% extracto de levadura, 2.5% caldo nutritivo, 1.0% grasa y 2.0% $MgCl_2 \cdot 6H_2O$.

Palabras clave: ecología, *Heterorhabditis indica*, patogenicidad, *Plutella xylostella*.

INTRODUCTION

Nematodes in the families Heterorhabditidae and Steinernematidae are obligate parasites of a large number of insect species, and there is much interest in their use as biological control agents (Kaya and Gaugler, 1993). The third-stage infective juveniles (IJ) carry mutualistic bacteria of either *Photorhabdus* sp. or *Xenorhabdus* sp. in the gutlumen. Both bacteria are released after the infective juveniles penetrate the haemocoel of the host and excrete both a toxin (Burnam, 1982) and an inhibitor of the insect humoral immune system (Gotz *et al*., 1981; Burnell and Stock, 2000). Artificial media for mass-rearing the nematodes have been extensively studied for commercial production (Somsook and Nanta, 1995).

The purpose of the current research was to survey soils in Thailand for *Heterorhabditis* spp. with potential as biological control agents of insect pests. One isolate of *Heterorhabditis indica* that exhibited sustantial activity against insects was selected for detailed study. The morphology, ecology, and host range of this isolate (designated the Thai isolate 2 or T2 of *H. indica*) were studied. Also studied were the effect of T2 on the diamondback moth (*Plutella xylostella* L.) and the rearing of T2 on artificial media.

MATERIALS AND METHODS

Isolation of entomopathogenic nematodes from soil in Thailand

A total of 60 soil samples were collected from the following provinces: Bangkok (coordinates: 13°45′8″N 100°29′38″E), Chantaburi (12°36'45"N 102°6'37"E), Chonburi (13°21'44"N 100°59'0"E), Kanchanaburi (14°0'15"N 99°32'57"E), Phetchaburi (13°6'43"N 99°56'45"E), Rayong (12°40'32"N 101°16'42"E), Chumporn (10°29'38"N 99°10'48"E), and Suratthani (9°8'24"N 99°19'50"E). Each sample consisted of 1 kg soil composed of three subsamples collected from each area. The subsamples were taken to a depth of at least 15 cm, and the three subsamples were bulked. For each sample, the following information was recorded: site location, weather temperature, soil type, associated vegetation, and date. The samples, which were labeled L1 to L60, were kept in a cooler (8-15°C) during transportation to the laboratory.

In the laboratory, the soil in each sample was mixed and 200 g was placed in a 13×18.0×7 cm^3 container (one container per sample) and baited with 10 fifth-instar *Galleria mellonella* (L.) larvae (weight 200-300 g each). After 2 days at 25°C, the cadavers were removed from the soil. The White trap method was used for obtaining infective juvenile (IJ) nematodes from *G. mellonella* larvae (White, 1927). The first and second generations of adult nematodes were obtained by dissecting cadavers on days 3-4 and on days 7-9, respectively.

Among the 60 soil samples there were only three IJ strains emerged from the host cadavers after trapping and reinfecting with *G. mellonella* and these soil samples were from Kanchanaburi province only. They were coded as T1, T2, and T3 (T = Thailand). Isolate T2 showed the highest vilulence and best IJ production from *G. mellonella* therefore it was chosen for further study.

Life cycle and morphology of T2

Infective juveniles of isolate T2 from *G. mellonella* were added to filter paper in one of 10, 9-cm diameter petri dishes (50 IJ per dish), and 20 healthy fifth-instar *G. mellonella* larvae were added to each dish. Dishes were maintained at 25°C. Every 24 hours, two larvae per dish were randomly selected, dissected, and rinsed with a 0.7% saline solution to remove nematodes. The nematodes within each larva were counted, and representative adults and juveniles were prepared for morphological study. Nematodes were placed in a watch glass and killed with heat in a water bath at 60°C for 2 minutes. Specimens were transferred to one drop of fixative solution (TAF: 2% triethanolamine, 8% formalin, and 90% deionized water) on a microscope slide. An equal amount of Ringer's solution (NaCl 0.9%, KCl 0.04%, $CaCl_2$ 0.04%, $NaHCO_3$ 0.02%, deionized water 99%) was added, and nematode body length and width were measured with a light microscope. Adult females, males, and juveniles were prepared for examination with scanning electron microscopy (Wagner and Lewis, 2000). The species and different stages of the life cycle were identified with standard keys (Kaya and Stock, 1997; Nguyen and Smart, 1996). All nematode samples were sent to Dr S. P. Stock for identification.

Survival of IJ in sponges as affected by temperature

Three different temperatures (15, 20, 25°C) were tested for survival capability, with 3 replications. A suspension of 5×10^5/ml IJ was prepared at and 8 ml was pipetted and transfered onto 24 pieces of sponges (1 cm^3 each), which were mixed thoroughly in a plastic bag (Somsook, 1990) for each replication. The number of IJ surviving in the sponges was determined after 1, 2, 3, and 4 weeks. At each sampling time, six sponges were removed from each bag and were rinsed four times with 0.1% formalin to release the nematodes. The nematodes released from each replication were counted.

Host range

Insect mortality and nematode reproduction were determined for several insect species after exposure T2 IJ: adult *Aphis gossypii* (Homoptera), second-instar *Spodoptera exigua* (Lepidoptera), adult *Sitophilus zeamais* (Coleoptera), second-instar *Tenebrio molitor* (Coleoptera), second-instar *Bactrocera correcta* (Diptera), and nymphal *Coptotermis gestroi* (Isoptera). The insects were exposed to the IJ in sterile 5.5-cm diameter petri dishes containing a 5.5-cm filter paper. Each filter paper held 0, 500, or 5000 IJ, and 10 larvae of each insect species were added to each dish to give 0, 50, or 500 IJ/host. Each dish also contained appropriate food for each type of insect host. After the insects were added, the dishes were covered, placed in plastic boxes to maintain moisture, and incubated at 25°C.

Each combination of IJ level and host insect was replicated four times. Insect mortality was recorded daily for 7 days. In addition, 20 cadavers from each combination of IJ level and host insect were placed on White traps for 10 additional days to determine whether the nematodes had reproduced. The emerging IJ were

counted. Cadavers without IJ emerging were dissected to determine if they contained any nematodes.

Effect of temperature on infection and reproduction

This experiment was conducted to determine the optimum temperature for infection and reproduction. Percentages of host mortality, time of IJ emergence, numbers of IJ emergence, and nematode developmental stages were determined. Tissue culture plates with 24 wells (14 cm^3 per well) were filled with sterile sand (0.5 g of sand per well) and incubated at 15, 20, 25, 30, or 35°C, with six plates at each temperature, respectively. After 1 hour, 50 IJ in 60 µl suspension was added to each well of four plates; the other two plates at each temperature did not receive nematodes and served as controls. One healthy *G. mellonella* larva (200-300 mg fresh weight) was added to each well. The plates were covered to prevent the larva from leaving the well. Larval mortality was recorded daily for 7 days. A total of 21 cadavers from two plates at each temperature were dissected to determine the number of nematodes in each larva. This procedure was performed 1 day after death at 25-35°C, 2 days after death at 20°C, and 3 days after death at 15°C. The cadavers from the other two plates at each temperature were placed on White traps, and the emerging IJ were counted daily for 20 days.

Effect of soil moisture on infectivity

The effect of soil water potential on the ability of T2 IJ to seek and infect hosts was evaluated. A 24-well tissue culture plate (14 cm^3 per well) was used. One hundred IJ in 20 µl of water were placed on the bottom of each well, which was then filled with pre-moistened soil. The tested soil water potentials (% w/w soil moisture) were -500 kPA (3%), -300 kPA (4%), -100 kPA (5%), -30 kPA (7%), -10 kPA (9%), -5.5 kPA (13%), and -3 kPA (19%) (Halogen Moisture Analyzer, Model HR 73, Mettler-Toledo International, Switzerland).After the soil was compacted by tapping the plate, one healthy *G. mellonella* larva was placed on the surface of the soil in each well, and the plates were covered with a lid. Larval mortality was recorded daily. Dead larvae were rinsed with a 0.1% formalin solution and then with distilled water in order to avoid cuticle contamination from others nematodes or microbes. The larvae were dissected and the number of nematodes within each was determined.

Artificial media for mass production of Photorhabdus *sp.*

Three liquid media from Difco (IL, USA) were tested: yeast salt broth (YS broth), thioglucolate peptone glucose yeast extract medium (TPGY medium), and ATCC medium 1154 (L broth). Each medium (100 ml) was placed in a 250-ml Erlenmeyer flask and autoclaved at 121°C for 20 minutes. After cooling, each flask was inoculated with 10 colonies of *Photorhabdus* sp. isolated from luminescent *G. mellonella* hemolymph. Nematodes emerged from the *G. mellonella* cadavers were surface-sterilized, crushed individually in 100 µl uria broth with a motorized mortar and pestle, and plated on Luria broth and NBTA agar: nutrient agar supplemented with bromothymol blue and triphenyltetrazolium chloride) colonies. Flasks (three flasks per medium) were placed on a rotary shaker (IKA Labortechnik, Germany) at 250 rpm and 25°C in the dark. After 60 hours, an aliquot from each flask was diluted, and dilution plate counts were obtained using nutrient agar containing bromothymol blue and triphenyltetrazolium chloride.

Appropriate media for Heterorhabditis indica (T2)

Three artificial formulations (formula 1, 2, and 3) and *G. mellonella* larvae were tested for rearing the nematode. Formula 1 was based on Dunphy and Webster (1989) and contained 1.0% soluble starch, 0.5% yeast extract, 2.5% nutrient broth, 0.5% lard, and 2.0% $MgCl_2 \cdot 6H_2O$. Formula 2 was based on Somsook and Nanta (1995) and contained 30.0% dog food (Purina Corp., MO) and 1.0% lard. Formula 3 was based on ATCC medium 1366 and contained 1.0% peptone, 0.5% NaCl, 0.5% yeast extract, and 1.0% lard. The remaining ingredient in each formula was distilled water. All chemicals were from Sigma (St. Louis, MO, USA) unless indicated otherwise. Each formulation was mixed in a blender and then transferred into a 500-ml Erlenmeyer flask. The flasks were covered with cotton plugs and foil, and autoclaved at 121°C for 20 minutes. When cool, each medium was poured into a 1000-ml Erlenmeyer flask already half-filled with 1 cm^3 sterile sponges, after which 10 mL of the *Photorhabdus* sp. suspension (about 1×10^6 cfu/mL) obtained from YS broth at 28 hours was poured into each flask. The flasks were covered and swirled. After 24 hours, IJ (10,000 in 20 ml) were added to each flask. The flasks were shaken to disperse the nematodes and then kept at 25°C in the dark. Nematodes were counted from 3 sponges of each flask every day for 14 days. There were 10 replications for each treatment.

For the fourth treatment, 50 fifth-instar *G. mellonella* larvae were added to each of 10 plastic containers ($20\times30\times8$ cm^3). Fifty IJ were placed on each larva. All larvae were fed with artificial media and kept at 25°C in the dark.

Effect of T2 on diamondback moth (Plutella xylostella) larvae

Petri dishes (5 cm diameter) containing a Whatman #1 filter paper were autoclaved at 121°C for 20 minutes. Each dish then received 1 ml containing 500 IJ collected from the four nematode rearing treatments described above. Each treatment was replicated 10 times and an untreated control without nematode was included. Five

third-instar diamondback moth larvae obtained from the Department of Agriculture were added to each dish. The dishes were kept at 25°C, and diamondback moth mortality was recorded after 24 and 48 hours.

Effect of formula 1 nutrient level for culturing T2

Among the T2 obtained from the three formulas, those obtained from formula 1 showed the highest mortality of diamondback moth larvae (see Results). Formula 1 was studied further to determine how its performance was affected by nutrient level. The concentrations of soluble starch, yeast extract, nutrient broth, and lard were tested at 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0%. Only one nutrient was tested each time, i.e., the other nutrient levels remained the same in the original formula 1. The media and nematodes were added to flasks, incubated, and evaluated as described above. Each treatment was replicated 10 times.

SAS version 9.1.3 (SAS Institute Inc., NC, USA) was used for statistical analysis. Mortality data were normalized by log transformation and treatment effects were corrected for control mortality with Abbott's formula (1925). Analysis of variance (ANOVA) was used, and means were separated by Duncan's new multiple range test (DMRT) at the 5% level of significance.

RESULTS

Isolation of entomopathogenic nematodes from soil in Thailand

T2 killed *G. mellonella* larvae within 2 days at 25°C. The infected larva became orange-red but did not decay. The mortality of *G. mellonella* larvae was highest and number of IJ produced per larva was greatest in soil sample containing T2. This soil sample was collected from the root zone of Siamese rough bush (*Streblue asper*) at Saiyoknoi Waterfall, Kanchanaburi Province, in western Thailand. The soil was a sandy loam with a pH of 6.9. The air temperature was 25-32°C, and the sample was collected in the afternoon during July. The IJ that emerged from parasitized larvae incubated in the T2 soil sample were designated as strain T2.

Life cycle and morphology of T2

The life cycle of T2 required 10 days at 25°C and consisted of two generations. The IJ fed on host haemolymph, released their symbiotic bacteria, and molted. These IJ developed into hermaphroditic females 3 days after inoculation. Females averaged 3.40 mm long and 0.17 mm wide, and began developing eggs within 6 hours of molting to the adult stage. The first-stage juveniles hatched from these eggs within 24 hours; 81% grew rapidly, and molted three times before becoming fourth-stage juveniles, while the other 19% developed into infective juveniles. The fourth-stage juveniles then molted and developed into males and females of the second generation. After mating, eggs from most females were laid outside the females' bodies, hatched and successfully developed into IJ. These females were 1.81 mm long and 0.11 mm wide. Eggs of smaller females hatched inside the bodies developed into IJ, and remained inside the female cadaver for 3 days before moving out into the decomposing fluids of the insect.

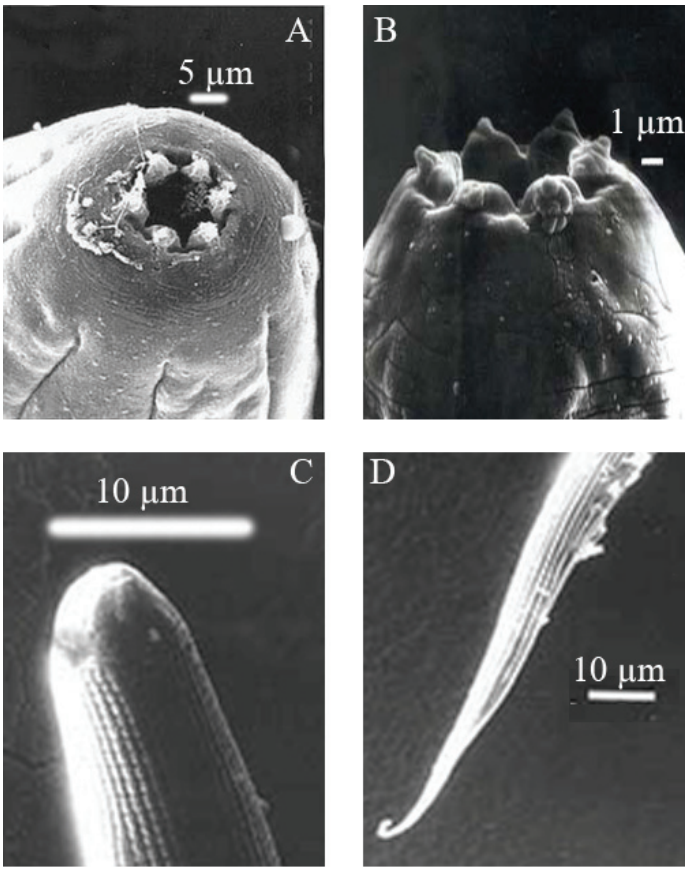


Fig. 1. SEM micrographs of *Heterorhabditis indica* T2 hermaphroditic female and juvenile. A. Head of hermaphroditic female; B. Head of amphimictic female; C. Head of infective juvenile; D. Tail of infective juvenile.

Both female types had six separated lips (Fig. 1A, B), didelphic reflexed ovary, a pointed tail with an anal swelling, and a vulva situated on a protuberance. The average body length and width of males were 0.92 mm and 0.06 mm, respectively.

The IJ was enclosed in the second-stage cuticle, and the cuticle had longitudinal lines (Fig. 1C, D). The mouth and anus were closed. The head had a prominent dorsal tooth. The pharynx and intestine were collapsed. The excretory pore was posterior to the nerve ring, and the tail was pointed. The IJ always became a hermaphroditic female. The average IJ body length and width were 0.53

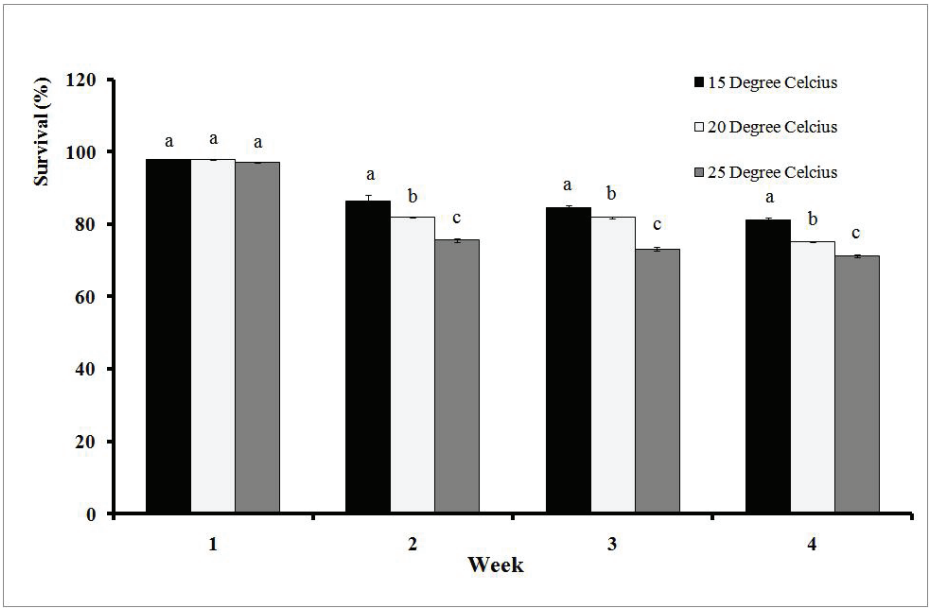


Fig. 2. In vitro survival of *Heterorhabditis indica* T2 as affected by temperature.

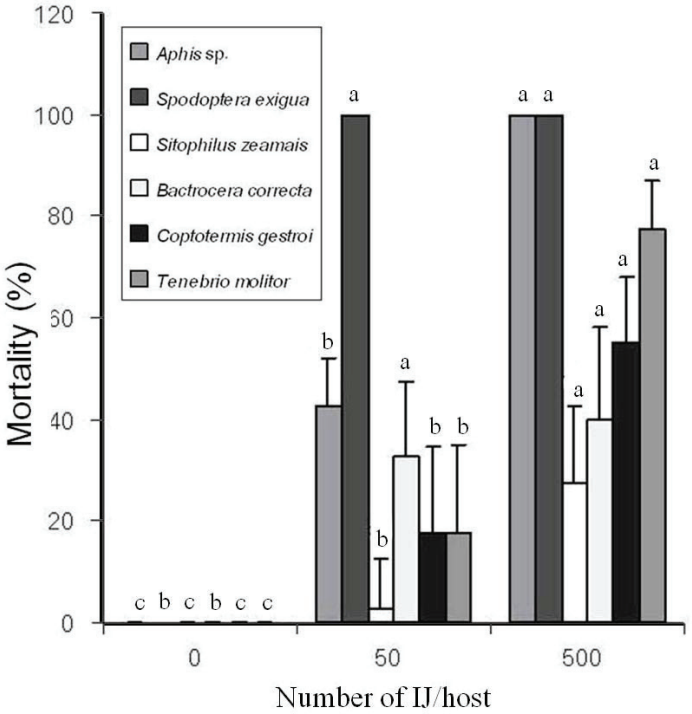


Fig. 3. Mortality of insects inoculated with three levels of *Heterorhabditis indica* T2.

mm and 0.03 mm, the tail length was 0.1 mm, and the c ratio was 5.26.

The life cycle of T2 was similar to that of *Heterorhabditis indica*, and T2 was identified molecularly as *H. indica* by means of PCR (S.P. Stock, unpublished data). T2 specimen was deposited at the Department of Agriculture, Ministry of Agriculture and Cooperative, Bangkok, Thailand.

Survival of IJ in sponges as affected by temperature

Survival exceeded 70% at all three tested temperatures, but was greatest at 15°C followed by 20°C and 25°C ($F = 43.34$, $df = 2$, $p < 0.05$) (Fig. 2). Beginning

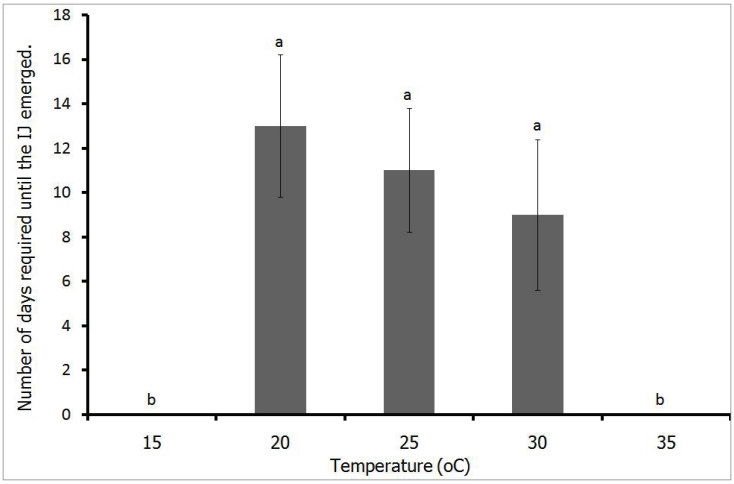
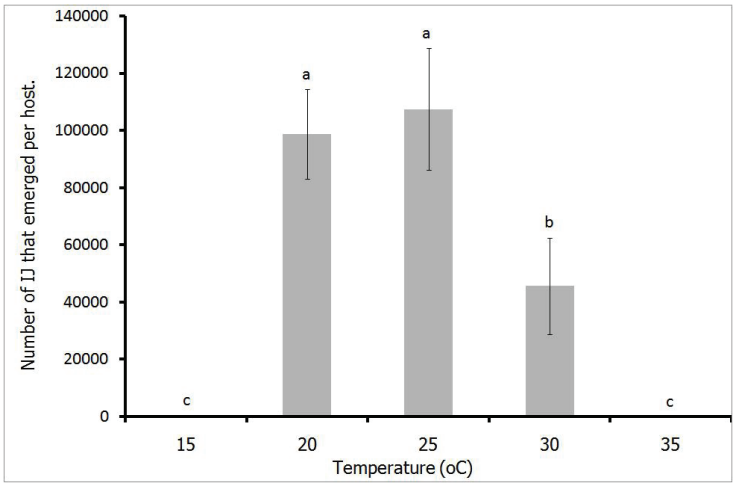


Fig. 4. Effect of temperature on emergence time of infective juveniles from the larvae of *Galleria mellonella* larvae inoculated with *Heterorhabditis indica* T2.

Fig. 5. Effect of temperature on number of infective juveniles emerging from the larvae of *Galleria mellonella* larvae inoculated with *Heterorhabditis indica* T2.



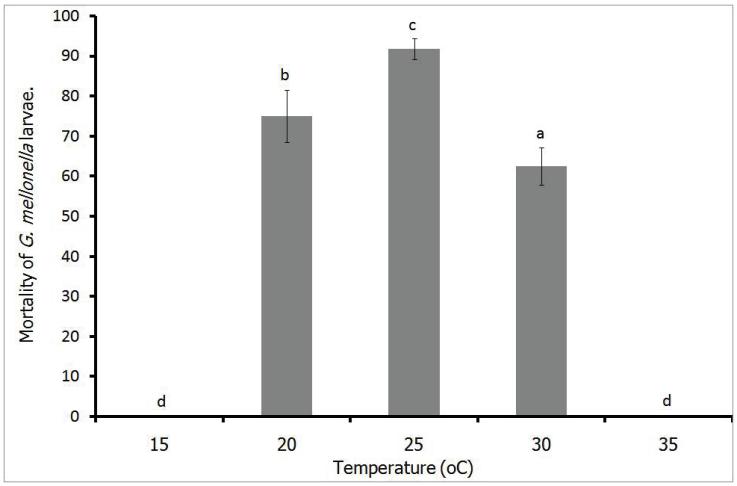


Fig. 6. Effect of temperature on mortality of *Galleria mellonella* larvae inoculated with *Heterorhabditis indica* T2.

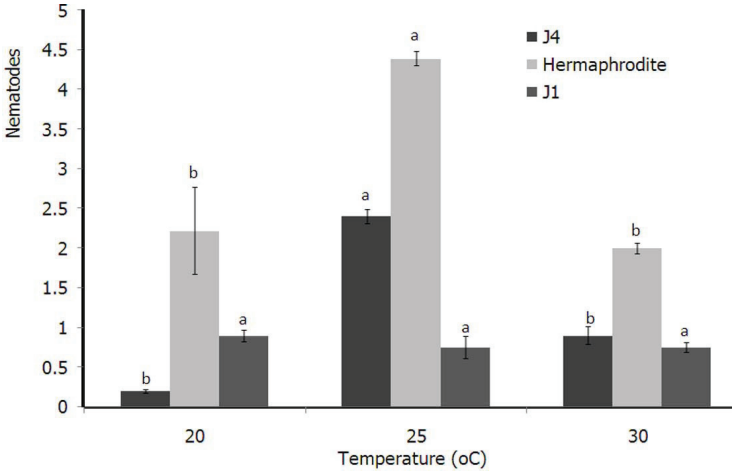
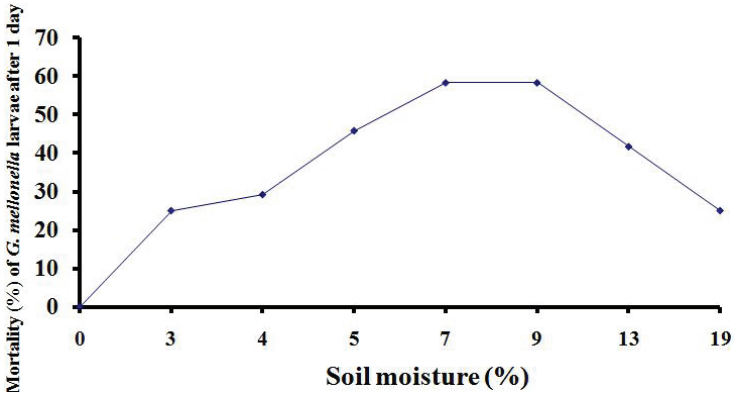


Fig. 7. Effect of temperature on development of *Heterorhabditis indica* T2 within *Galleria mellonella* larvae. *Galleria mellonella* larvae infected by *H. indica* T2 were dissected 1 day after death at 25-35°C, 2 days after death at 20°C, and 3 days after death at 15°C.

Fig. 8. Effect of soil moisture on mortality of *Galleria mellonella* larvae 1 day after placement in soil containing *Heterorhabditis indica* T2.



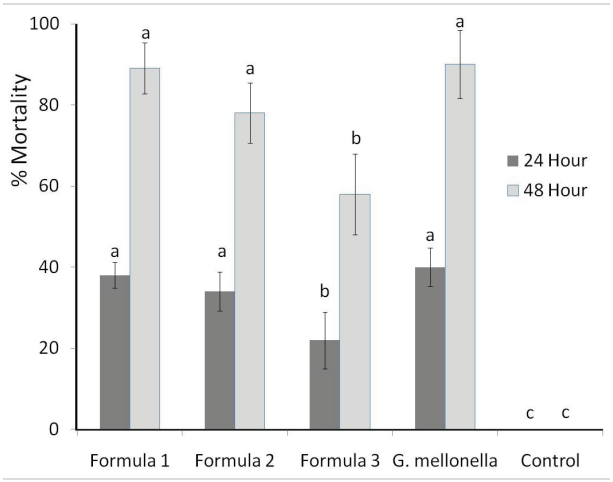


Fig. 9. *Heterorhabditis indica* T2 induced mortality of diamondback moth (*Plutella xylostela*) larvae as affected by the medium on which the nematode was reared.

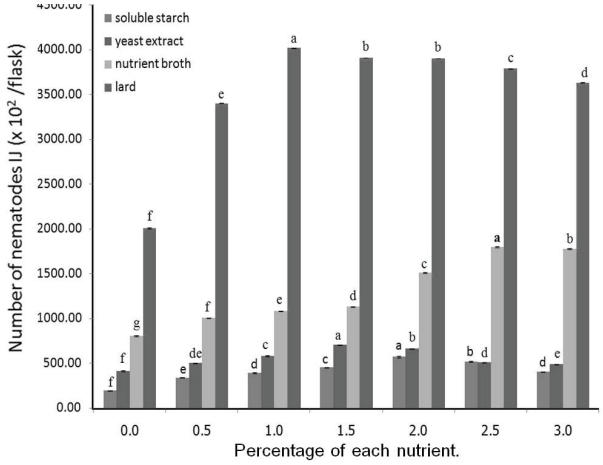


Fig. 10. Effect of different ingredient concentrations in formula 1 versus numbers of *Heterorhabditis indica* T2 (IJ) produced.

in week 2, IJ survival remained nearly the same for each treatment, with almost no further mortality.

Host range

At the lower density of IJ (50/host), *S. exigua* had the greatest mortality and *Stilophilus zeamais* had the least (Fig. 3). Mortality generally increased when IJ density was increased from 50 to 500/host in every insect host except *S. exigua* ($F = 173.59$, $df = 2$, $p < 0.05$). Of the tested hosts, only *S. exigua* supported reproduction of *H. indica* T2. When exposed to 50 IJ/host and 500 IJ/host, each second-instar *S. exigua* produced 234 IJ and 261 IJ, respectively. In contrast, the other insects produced only a few IJ and upon dissection mostly contained fourth-stage juveniles and hermaphrodites.

Effect of temperature on infection and reproduction

Emergence of IJ from *S. exigua* occurred earlier at the intermediate temperatures (20, 25, 30°C) than at 15°C or 35°C. (Fig. 4). The number of IJ that emerged per host was greater at 25 and 20°C than at 30°C ($F = 39.22$, $df = 4$, $p < 0.05$); no IJ emerged at 15 and 35°C (Fig. 5). Host mortality was greatest at 25°C. No hosts died at 15 and 35°C (Fig. 6).

Figure 7 illustrated the appropriated temperature for nematodes developing stages. For J4 and hermaphrodite, 25°C was the optimum temperature which was statistically difference from 20 and 30°C, respectively (for J4; $F = 9.57$ for hermaphrodite; $F = 3.5$, $df = 2$, $p < 0.05$). However, there was no statistically difference among the three temperatures for J1 stage ($F=1.55$, $df = 2$, $p > 0.05$).

Effect of the soil moisture on infectivity

After 1 day, mortality of *G. mellonella* larvae caused by *H. indica* T2 was greater at 7 and 9% soil moisture than at higher or lower soil moistures ($F = 5.41$, $df = 7$, $p < 0.05$), Fig. 8). After 2 days, however, mortality increased to 75% at all soil moisture levels except at 19%. After 3 days, the cadavers were dissected to determine the number of established nematodes. The highest number of nematodes was found in *G. mellonella* larvae in soil at 9% soil moisture.

Artificial media for mass production of Photorhabdus sp.

Bacterial cell concentration was consistently higher in YS broth than in TPGY medium and L broth at 36 ($F = 19.58$, $df = 2$, $p < 0.05$) 48 ($F = 112.74$, $df = 2$, $p < 0.05$) and 60 hours ($F = 520.31$, $df = 2$, $p < 0.05$).

Appropriate media for Heterorhabditis indica (T2)

Nematode size was larger when IJ were grown in formula 1, formula 2, and *G. mellonella* larvae than in formula 3 (Table 1).

Effect of T2 on diamondback moth (Plutella xylostella) larvae

Mortality of diamondback moth larvae was significantly greater when larvae were exposed to IJ grown in formula 1, formula 2, and *G. mellonella* larvae than in formula 3 ($F = 695.19$, $df = 4$, $p < 0.05$) (Fig. 9). Mortality of wax moth was greater at 48 hr than 24 hr in all treatments ($F = 109.88$, $df = 1$, $p < 0.05$).

Effect of formula 1 nutrient level for culturing

Figure 10 illustrated the population of IJ obtained from four ingredients. There were statistically difference among types of the ingredients ($F = 1914.36$, $df = 3$, $p < 0.05$) and the concentrations of each ingredient used ($F = 43.60$, $df = 6$, $p < 0.05$). However, the maximum IJ population did not occurred from the highest nutrient concentration.

DISCUSSION

Based on the morphological data presented here and unpublished molecular data, the nematode designated as strain T2 in this study is *Heterorhabditis indica*. Survival in storage tended to be greater at 15°C than at higher temperatures, although this was not statistically evaluated.

The IJ of *H. indica* T2 were able to kill insects belonging to several different orders, including *Aphis gossypii, Spodoptera exigua, Sitophilus zeamais,*

Table 1. Body width (µm) of *Heterorhabditis indica* (T2) reared on different media.

Medium	J2	J3	IJ	J4		H	F	M
				1°	2°			
Formula 1	13.5 ± 0.04a	20.0 ± 10.7ab	24.4 ± 0.04b	25.3 ± 0.04b	24.7 ± 0.09b	153.3 ± 4.21a	112.5 ± 0.73a	53.3 ± 0.34ab
Formula 2	13.4 ± 0.04a	20.0 ± 0.04b	24.2 ± 0.04b	25.2 ± 0.03b	24.7 ± 0.09b	151.3 ± 3.72a	111.0 ± 0.67a	52.3 ± 0.33ab
Formula 3	13.3 ± 0.04a	19.6 ± 0.06b	23.6 ± 0.08c	24.7 ± 0.06c	24.4 ± 0.30b	144.8 ± 3.33a	107.0 ± 0.71a	51.8 ± 0.26b
Galleria mellonella	13.5 ± 0.04a	20.6 ± 0.06a	25.5 ± 0.10a	25.8 ± 0.05a	25.5 ± 0.48a	182.0 ± 4.60a	114.0 ± 0.68a	56.0 ± 0.41a

Remark: means in a column followed by the same letter are not significantly difference at p>0.05 by DMRT. J: juvenile stage, H: hermaphrodite, F: adult female, M: adult male, 1°: first generation, 2°: second generation

Table 2. Body length (µm) of *Heterorhabditis indica* (T2) reared on different media.

Medium	J2	J3	IJ	J4		H	F	M
				1°	2°			
Formula 1	13.5 ± 0.04a	20.0 ± 10.7ab	24.4 ± 0.04b	25.3 ± 0.04b	24.7 ± 0.09b	153.3 ± 4.21a	112.5 ± 0.73a	53.3 ± 0.34ab
Formula 2	13.4 ± 0.04a	20.0 ± 0.04b	24.2 ± 0.04b	25.2 ± 0.03b	24.7 ± 0.09b	151.3 ± 3.72a	111.0 ± 0.67a	52.3 ± 0.33ab
Formula 3	13.3 ± 0.04a	19.6 ± 0.06b	23.6 ± 0.08c	24.7 ± 0.06c	24.4 ± 0.30b	144.8 ± 3.33a	107.0 ± 0.71a	51.8 ± 0.26b
Galleria mellonella	13.5 ± 0.04a	20.6 ± 0.06a	25.5 ± 0.10a	25.8 ± 0.05a	25.5 ± 0.48a	182.0 ± 4.60a	114.0 ± 0.68a	56.0 ± 0.41a

Remark: means in a column followed by the same letter are not significantly difference at p>0.05 by DMRT. J: juvenile stage, H: hermaphrodite, F: adult female, M: adult male, 1°: first generation, 2°: second generation

Tenebrio molitor, Bactrocera correcta, and *Coptotermis gestroi*; of these insects, however, only *S. exigua* supported nematode reproduction. The nematode was also able to kill the diamondback moth, which is an important agricultural pest that rapidly develops resistance to most chemical insecticides (Maketon *et al.*, 2008). Therefore, this pest may be a good candidate for an integrated pest management program that includes *H. indica* T2. With *G. mellonella* larvae, *H. indica* T2 caused the most mortality and achieved the highest reproduction at 25°C, which is the typical night temperature in the tropics; temperature during the day often exceeds 30°C year-round. It follows that if the nematode is to be used as a biological control agent, it should be applied in the late afternoon or evening rather than in the morning. This nematode did not control household cockroaches, including American cockroach (*Periplaneta americana* L.) and German cockroach (*Blatella germanica* L.) (Maketon *et al*., 2009). The knowledge gained from this research should be useful for future research on the use of *H. indica* T2 as a biological control agent against the diamondback moth and other insect pests.

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