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# Occurrence of Entomopathogenic Nematodes in Thailand

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## ABSTRACT

A soil survey of indigenous strains of entomopathogenic nematode was conducted in 42 provinces of Thailand between June 1996 to March 1998. A total of 306 soil samples were extracted using *Galleria* baiting technique. The nematodes were recovered from 9 out of 42 locations (21.4 %). The identification using morphological characteristics of infective-stage juveniles and adults revealed 8 isolates of Steinernematidae : *Steinernema* sp. and 1 isolate of Heterorhabditidae : *Heterorhabditis* sp. The nematodes were collected from central plains at the temperature range of 24-27°C (10-15 cm deep from soil surface) and 29-37°C (at soil surface) and they were heat-tolerant isolates from Thailand.

**Key words :** entomopathogenic nematodes, *Steinernema* sp., *Heterorhabditis* sp., heat-tolerant isolate

## INTRODUCTION

Insect nematodes have been discovered and studied for more than 70 years. Although nearly 40 nematode families have been isolated from soil inhabiting insects throughout the world and the association existing between nematodes and insects is variable; only two families, Steinernematidae and Heterorhabditidae, are commonly used as a biological control agent (Gaugler and Kaya, 1990) since they have a mutualistic relationship with the bacteria, *Xenorhabdus*, which could kill insect hosts Insect within 24-48 hours (Woodring and Kaya, 1988).

Surveys of entomopathogenic nematodes have been conducted in many parts of the world. So far, the family Steinernematidae is comprised of two genera, *Steinernema* Travassos, 1927 (Poinar, 1990) and *Neosteinernema* Nguyen and Smart, 1994 (Nguyen and Smart, 1994). The family Heterorhabditidae contains only one genus, *Heterorhabditis* Poinar, 1976 (Poinar, 1976). Currently, 18 species of *Steinernema*, 1 species of *Neosteinernema* and 7 species of *Heterorhabditis* have been described (Nguyen and Smart, 1996).

Thailand is situated in the tropical region of the world. Insects are of the most damaging pests causing losses in many crops. The control of insects is undertaken by using hazardous chemicals which adversely affect human and environment. However, an entomopathogenic nematode, *Steinernema carpocapsae* (All strain) imported from the US for more than 10 years, has proven highly effective in controlling several damaging insect pests in Thailand and its acceptance is increasing. *S. carpocapsae* could be produced by both *in vitro* and *in vivo* cultures, easy to be used and inexpensive compared with chemicals. Commercially, the nematodes are suspended in sponge and kept in packages of 4 million nematodes/package.


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The search for indigenous strains of entomopathogenic nematodes in Thailand has been commenced in 1996 under a financial support of The Thailand Research Fund (TRF). If Thai strains are obtained, they could serve as a resource for use in the tropics because they could be more effective against native insect pests and also present less risk to non-target organisms compared to exotic isolates.

Entomopathogenic nematodes at infective-stage juveniles are associated with disease inducing bacteria in insects. Approximately 250 bacterial cells were found in the foregut of one nematode (Ehlers, 1995). The rapidity in killing insects is not dependent solely upon bacteria, it also depends upon the search ability of nematodes for the prayed insects as well as on how quick the nematodes can carry the bacteria in insect blood content (Woodring and Kaya, 1988). The ability of nematodes which differ among species or strains, leads to the beginning of the bio-control era.

The objectives of this study were to discover new Thai strains of entomopathogenic nematodes for development and use in biological control programmes in Thailand and to document their occurence in different habitats and soil types.

## MATERIALS AND METHODS

### 1. Maintenance of *Galleria mellonella* cultures (Modification after Miduturi, 1997).

Greater wax moth larvae *G. mellonella* (Pyralidae : Lepidoptera), were used for culturing nematode isolates from Thailand. Insect cultures were maintained on an artificial diet containing 200 g rice powder, 100 ml honey, 100 ml glycerol and 50 g beer yeast in aerated plastic containers ($32.5 \times 17.6 \times 10$ cm) at 25-28°C. Approximately 200-300 eggs were placed on a piece of artificial diet in plastic containers and kept at 25-28°C. The eggs hatched in 3-4 days. After 2 weeks the larvae were given a new diet. After 3 weeks, late instar larvae were ready to be collected. Larvae could then be stored on paper shavings for 2-3 weeks at 10±1°C.

### 2. Collection of soil samples

A total of 306 soil samples were collected from 42 different locations of habitat types, including fruit crops, field crops, ornamental plants, woodland and beach (Table 1). Soil samples representing each site were collected to a depth of 10-15 cm using an auger. At each site, five subsamples were randomly taken from an area of approximately 10 $m^2$ and were placed in a plastic bucket in which they were mixed thoroughly. One kilogram of soil (one sample) was taken from the plastic bucket and placed in a plastic bag. Samples were placed in coolers (18-20°C) during transportation to the laboratory where they were stored at 10±1°C until further process. Site locations, soil temperatures, associated vegetations and insects present were recorded.

### 3. Baiting technique

The presence of entomopathogenic nematodes in soil samples was tested by baiting with wax moth *Galleria mellonella* larvae (Bedding and Akhurst, 1975). Ten last instars of *G. mellonella* were placed at the bottom of each plastic container (capacity 300 ml), soils were added and lids with ventilation holes were positioned. The insects in this trap bioassay were incubated in the dark at 25° C with 85% relative humidity. After 7 days, the larvae were removed from the soil and the dead larvae with characteristic signs and symptoms of entomopathogenic nematode infection were either dissected or placed on White traps (White, 1927), in which the emerging infective juveniles were then collected. These juveniles were re-exposed to *G. mellonella* in Petri dishes to confirm the pathogenicity test.

**Table 1** Locations, soil textures and associated habitats in which the nematodes were found in different regions of Thailand during June 1996 to March 1998.

| Location | No. of samples | Sampling time (month) | Soil texture | Habitat | Nematode code |
|---|---|---|---|---|---|
| Rayong | 15 | June/1996 | Sandy loam | Fruit crop | - |
| | | | Sand | Beach | - |
| Chantha Buri | 3 | June | Sand | Beach | - |
| Chon Buri | 5 | June | Sand | Beach | - |
| Chiang Mai | 5 | August | Sandy loam | Field crop | - |
| Lampang | 5 | September | Sandy loam | Woodland | - |
| Chiang Rai | 5 | October | Clay loam | Woodland | - |
| Phetchabun | 5 | October | Clay loam | Woodland | - |
| Ratcha Buri | 3 | November | Clay loam | Fruit crop | - |
| Ranong | 4 | November | Sandy clay loam | Fruit crop | - |
| Phangnga | 17 | November | Sandy loam | Fruit crop | - |
| Krabi | 3 | November | Sandy loam | Fruit crop | - |
| Chumphon | 16 | November | Sandy loam | Fruit crop | - |
| Kanchana Buri | 12 | December | Sandy loam | Fruit crop | KB |
| Chachoengsao | 7 | January/1997 | Sandy loam | Woodland | - |
| Buri Ram | 7 | January | Sandy loam | Field crop | - |
| Nakhon Ratchasima | 8 | January | Sandy loam | Field crop | - |
| Nakhon Sawan | 3 | February | Sandy loam | Field crop | - |
| Phichit | 9 | February | Sandy loam | Fruit crop | PC |
| Phitsanulok | 14 | February | Sandy loam | Field crop | - |
| | | | Sandy loam | Fruit crop | - |
| Tak | 4 | February | Sandy loam | Field crop | - |
| Phra Nakhon Si Ayutthaya | 5 | March | Sandy loam | Ornamental crop | AY |
| Chai Nat | 9 | April | Sandy loam | Field crop | - |
| Sin Buri | 8 | April | Sandy loam | Vegetable crop | - |
| Nakhon Nayok | 5 | April | Sandy loam | Woodland | - |
| Pathum Thani | 5 | April | Sandy loam | Vegetable crop | - |
| Kalasin | 15 | July | Sandy loam | Roadside verge | KS |
| Roi Et | 15 | July | Sandy loam | Roadside verge | RE |
| Maha Sarakham | 10 | July | Sandy loam | Roadside verge | MK |
| Khon Kaen | 10 | July | Sandy loam | Roadside verge | KK |
| Udon Thani | 5 | July | Sandy loam | Roadside verge | - |
| Nong Khai | 10 | July | Sandy loam | Roadside verge | NK |
| Nakhon Si Thammarat | 9 | August | Sandy loam | Vegetable crop | - |
| Nakhon Pathom | 10 | January/1998 | Sandy loam | Fruit crop | - |
| Supan Buri | 6 | January | Sandy loam | Field crop | - |
| Prachin Buri | 8 | February | Sandy loam | Roadside verge | - |
| Sakaeo | 5 | February | Sandy loam | Fruit crop | SK |
| Ang Thong | 6 | March | Sandy loam | Roadside verge | - |
| Kamphang Phet | 9 | March | Sandy loam | Roadside verge | - |
| Phare | 5 | March | Sandy loam | Roadside verge | - |
| Nan | 5 | March | Sandy loam | Roadside verge | - |
| Uttaradit | 8 | March | Sandy loam | Roadside verge | - |
| Lamphun | 10 | March | Sandy loam | Fruit crop | - |
| **Total** 42 locations | 306 samples | | | | recovered 9 nema samples |

**4. Preparation of nematodes for identification**

Nematodes recovered from *Galleria* traps were identified to families and genera for this description. Males and females were obtained by dissecting cadavers of *G. mellonella* larvae 4 days after infection but infective juveniles were collected from the watery trap 10 days after infection. The nematodes were mounted in water mounts under a coverglass. The examination of morphology and microphotography was performed with an Olympus microscope equipped with differential interference contrast optics.

## RESULTS

Many kinds of nematodes were isolated from soil samples collected from the survey. Entomopathogenic nematodes were recovered from 9 of 42 locations (21.4%) and mainly from sandy loam soil with a temperature zone at neil surface and at a depth of 10-15 cm of 27-37°C and 24-29° C respectively (Figure 1). The nematodes were recovered from 2.4%, 7.1% and 11.9% of the samples taken in ornamental plants, fruit crops and roadside verges respectively while soil samples from beach, field crops, woodland and vegetable crops did not yield any entomopathogenic nematodes.

Identification was initially conducted to family and genus level by morphological characteristics of infective-stage juveniles, females and males. All isolates were compared with a key to 2 major families and genera of entomopathogenic nematodes which was compiled by Kaya and Stock (1988). Eight nematode isolates from family Steinernematidae, genus *Steinernema* (KB, PC, AY, KS, MK, KK, NK, and SK code) and 1 isolate from family Heterorhabditidae, genus *Heterorhabditis* (RE code) were recovered (Table 2).

**Family Steinernematidae**

*Diagnosis :* Order Rhabditida, superfamily Alloionematoidea, family Steinernematidae, Type genus : *Steinernema* Travassos, 1927. Obligate insect parasites. Infectives carry symbiotic bacteria in the bacterial chamber of the intestine. Both males and females are necessary for reproduction.

*Infective-stage juvenile :* Body slender. Mouth and anus closed. Excretory pore anterior to nerve ring. Mouth region not armed with a dorsal hook (Figure 2 A).

*Female :* Large nematode. Amphimictic with opposed reflexed ovaries. Vulva with lips, located in midbody region. Older females become ovoviviparous, the young juveniles consuming female body contents and eventually killing the females.

*Male :* Body smaller than female. Testis single, reflexed at tip. Spicules paired and separate. Gubernaculum long. Bursa absent. Tail tip with mucron (Figure 2 C).

**Family Heterorhabditidae**

*Diagnosis :* Order Rhabditida, superfamily Rhabditoidea, family Heterorhabditidae, Type genus : *Heterorhabditis* Poinar, 1976. Obligate insect parasites. Infectives carry symbiotic bacteria in the bacterial chamber of the intestine. Both hermaphroditic and amphimictic females present.

*Infective-stage juvenile :* Body slender. Mouth and anus closed. Excretory pore posterior to nerve ring. (Figure 2 B).

*Female :* Large nematode. Hermaphoroditic in first generation and amphimictic in second generation.

*Male :* Body smaller than female. Testis single, reflexed at tip. Spicules paired and separate. Gubernaculum long. Bursa present. (Figure 2 D).

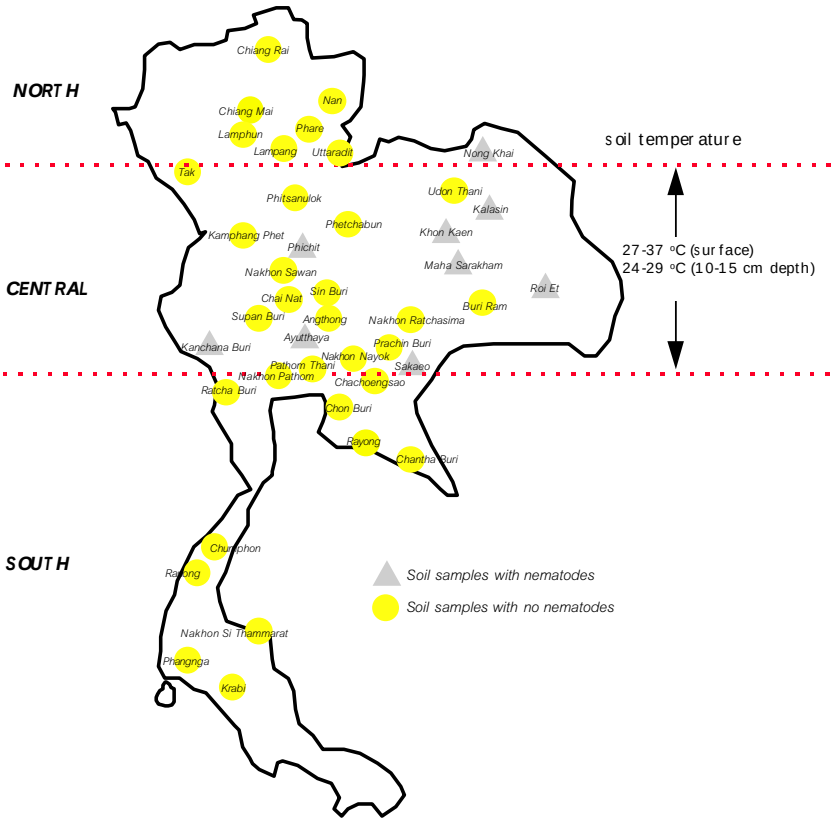


**Figure 1** Map of soil sampling areas in Thailand showing the locations (42 provinces) and temperatures where nematodes were found.

**Table 2** Key to 2 major families and genera of entomopathogenic nematodes (Kaya and Stock, 1988).

| Definition | Family Steinernematidae<br>Genus *Steinernema* | Family Heterorhabditidae<br>Genus *Heterorhabitis* |
|---|---|---|
| 1. Pathogenicity to insect | obligate pathogen | obligate pathogen |
| 2. Reproduction in the cadaver | 2-3 amphimictic generations | 2 generations : Hermaphroditic in the first generation, and amphimictic in the second generation |
| 3. Position of excretory pore | anterior to nerve ring | posterior to nerve ring |
| 4. With and without bursa in male | bursa absent | bursa present |
| 5. Color of cadavers | yellow-brown or black with no luminescence in the dark | red, brick-red, purple,orange or sometimes green with luminescence in the dark |

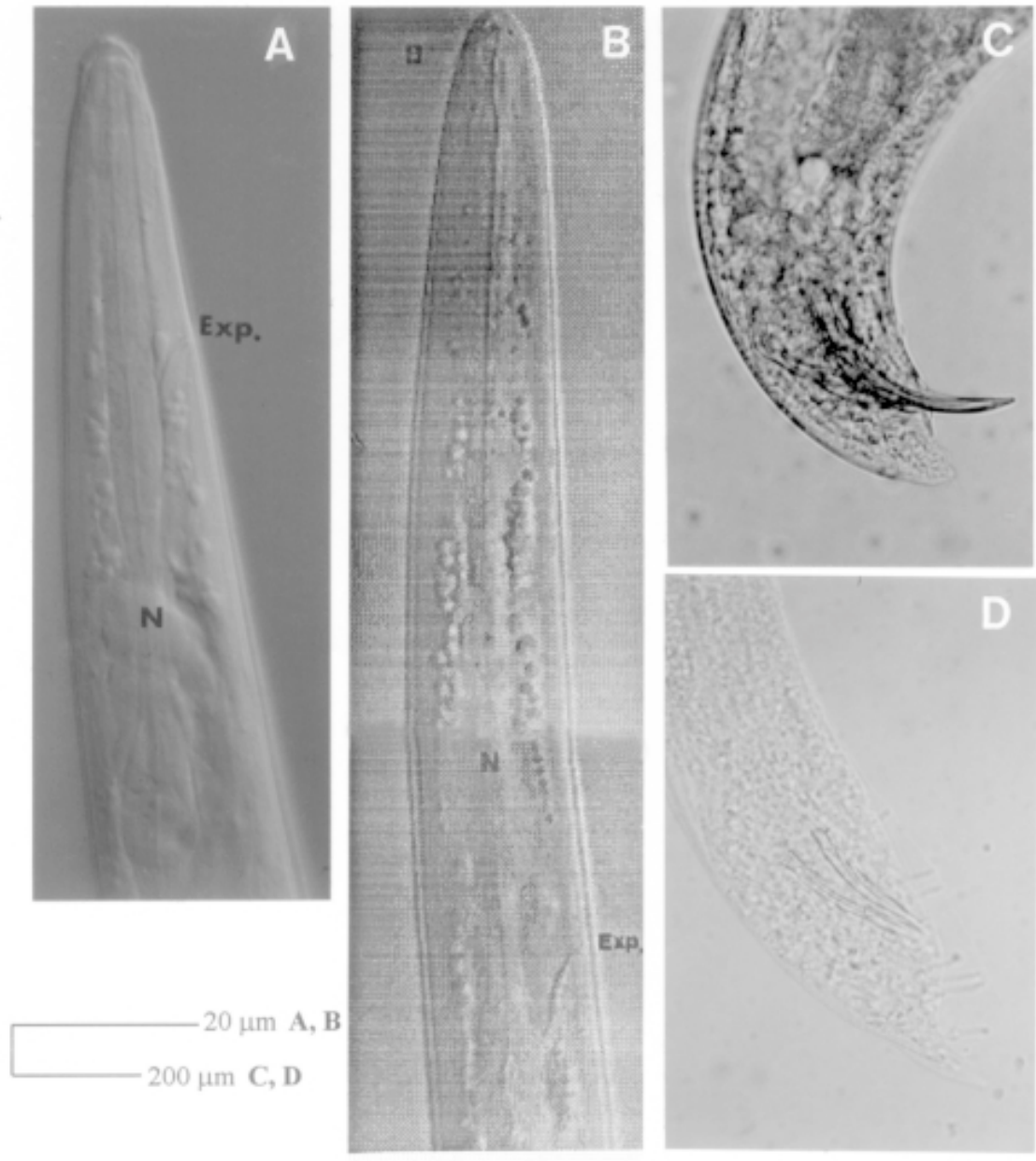


**Figure 2** Light micrographs of steinernematid and heterorhabditid. Infective-stage juveniles of steinernematid (A) and heterorhabditid (B) showing excretory pore (Exp.), anterior and posterior to nerve ring (N). Males of steinernematid (C) and heterorhabditid (D) with and without bursa.

## DISCUSSIONS

Occurrence of entomopathogenic nematodes was low and restricted only to fruit trees, ornamental plants, roadside verges and in sandy loam soils. The present survey in forty-two provinces of Thailand yielded 8 steinernematid and 1 heterorhabditid populations. All isolates were recovered only from the central area of the country while the samples from the north and south did not yield any entomopathogenic nematodes. The recovery frequency of entomopathogenic nematodes in the present survey was 21.4% and steinernematids were predominant. The recovery rate of *Steinernema* spp. was less than that from surveys conducted in the temperate zone, 37-48% in Europe (Hominick *et al.*, 1995).

Soil temperature is one of the important factors determining the abundance of nematodes in soil. Due to being recovered from high temperature soils (27-37°C at soil surface and 24-29°C at 10-15 cm soil depth), all collected isolates may be considered as heat tolerant strains like *Steinernema riobrave* (Cabanillas *et al.*, 1994).

The present surveys are important because they document the occurrence of entomopathogenic nematodes in various habitats and localities and also yield of new species and isolates of the nematodes (Miduturi, 1997). At the same time, the advantage of using native isolates of entomopathogenic nematodes for biocontrol is likely to be an enhanced ecological compatibility and also in the reduction of singnificant impact on non-target organisms when compared with exotic isolates.

Accordingly, the new steinernematid and heterorhabditid isolates will be evaluated in the future for their control potentials against natural hosts under local conditions.

The taxonomic studies to identify of their species by using morphometric and DNA analysis are being conducted.

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