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Bionomics of Entomopathogenic Nematode *Steinernema siamkayai* Stock, Somsook and Reid (n. sp.) and Its Efficacy Against *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae)

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ABSTRACT

The investigation on life cycle and development of *Steinernema siamkayai* in *Helicoverpa armigera* larva and its pathogenicity to insect comparing with other nematodes at various exposure times and concentrations was conducted under laboratory conditions. Life cycle of *S. siamkayai* in *H. armigera* was found to take 11 days to complete development at 30°C. Two generations were able to develop in the host. Only the infective juveniles (IJs) were used in the experiments. The symbiotic bacteria *Xenorhabdus* sp. associated with *S. siamkayai* isolated from hemolymph of *H. armigera* 18 h after infection was noticed to yield perfect colonies on NBTA media. The optimal temperature for entomopathogenic nematode *S. siamkayai* to control *H. armigera* larvae were 25 and 30°C, whereas 100 IJs per larva gave 91.66 and 89.58% larval mortalities, respectively, 72 h after infection. LC_{50} of *S. siamkayai*, *Steinernema carpocapsae* and *Steinernema riobrave* onto *H. armigera* and *Spodoptera litura* were found to be 22.5, 1.2 and 1.2 IJs per larva and 18.0, 0.2 and 1.8 IJs per larva, respectively. While LT_{50} of *S. siamkayai*, *S. carpocapsae* and *S. riobrave* with 5 IJs per larva used to penetrate into *H. armigera* were 89.9, 22 and 39.8 h, those of and *S. litura* were 72.5, 11.46 and 39.8 h, respectively.

Key words: entomopathogenic nematode, *Steinernema siamkayai*, *Melicoverpa armigera*

INTRODUCTION

Pest control in Thailand for the past 55 years has been based on the extensive use of chemical pesticides especially insecticides. Due to the adverse effects of insecticide, e.g. the development of insecticide resistant strains, the outbreak of unleashed secondary pests through the destination of their natural enemies by insecticides and insecticide hazards to mankind and their environment, there is an increasing trend toward the use of other means for the control of insect pests. The concept of insect pest management (IPM) has been introduced to the country since the early 1970s. From an ecological point of view, the use of biological agents in insect control as part of IPM will lead to ecological friendliness, economical profit ability, environmental safeness and sustainable agricultural development.

The most constraint in cotton production in Thailand is insect pests. Cotton plants are subject to insect attack from the seedling stage until the

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harvest time. The cotton bollworm, *Helicoverpa armigera* considered to be a minor pest of cotton in the early 1960s becomes a key pest warranting the application of insecticides. A switch to the use pyrethoids resulting in a new pest status such as cotton leafhopper, whitefly, aphid etc. remains constant threat to cotton production (Napompetch, 1994), hence alternative controlling methods are needed for all especially *H. armigera*.

Entomopathogenic nematodes are simple round worms which may be free-living, predaceous or parasitic. The species are beneficial in attacking insect pests, mostly sterilizing or otherwise debilitating their hosts. A very few cause insect death but these species tend to be difficult or expensive to be mass-produced, have narrow host specificity against pests of minor economic importance, or are poorly suited to exploit for pest control purposes. The only insect-parasitic nematodes possessing an optimal balance of biological control attributes are entomopathogenic or insecticidal nematodes in the genera *Steinernema* and *Heterorhabditis.*

Researches in nematode has begun in Thailand since 1986 by Somsook *et al*. and has been developed after that. In 1996 they found a new species which was identified and named *Steinernema siamkayai* by Stock *et al*. (1998). Although its morphology, hybridization and molecular structure have been investigated, the knowledges on this nematode are still inadequate for IPM program in the country. In order to use *S. siamkayai* to replace *S. carpocapsae* and other species of exotic in origins with economy budget in insect control, a basic information for developing nematode research in the future was required which led to the aim of this study.

MATERIALS AND METHODS

1. Life cycle and measurement of various developmental stages of *S. siamkayai*

The life cycle of *S. siamkayai* was studied in the Nematode Labaratory of Entomology and Zoology Group, Plant Protection Research and Development Office, DOA, from 2003-2004, by exposing the 4th instar of *H. armigera* to 50 IJs per larva in multi-well plate with 0.39 g artificial diets incubated at 30°C. Every 24 h until the life cycle of the nematode completed, 5 cadavers were surface-sterilized by dipping them into 50 ml water in 100-ml beaker. They were then shaken well to remove all nematodes on the body surfaces (Glazer and Lewis, 2000). The cadavers were then dissected with needle in petri dish. The numbers of nematode were counted under stereomicroscope and were then killed (Kaya and Stock, 1997). All stages of nematode were fixed before measured using compound microscope, which equipped with an ocular micrometer and each stage of *S. siamkayai* was photoed. Between observation periods, cadavers were kept in moist condition at all times.

2. Symbiotic bacterium *Xenorhadus* sp. of *S. siamkayai* isolated from *H. armigera* Larva

The last instar of *H. armigera* larvae were placed in multi-well plate with artificial diets and were inoculated with 200 IJs per larva, incubated at 30°C 15, 18, 21 and 24 h after inoculation. Larval surfaces were sterilized by dipping into 0.1% hyamine and rinsed three times in sterile distilled water. Anterior proleg was cut with sterile scissors and a drop of exuded hemolymph was streaked onto NBTA with sterile loop and incubated at 28°C.

After 48 h, the colony, which occurred on NBTA plate at each time was observed. The isolated single colony of bacterium was reared to multiply in the culture medium, 150 ml YS broth in a 500-ml shake flask at 28°C in the shaking incubator set at 180 rpm for 24 h. The cells of bacterium were checked for gram's stain and were mounted on slides for checking of crystalline inclusion proteins of bacteria symbiont cells with *S. siamkayai* 15, 18, 21 and 24 h after inoculation.

3. Influence of temperature on the infection of *S. siamkayai* in *H. armigera* larvae

To study the effect of temperature on the infection of infective juveniles in *H. armigera* larvae, 48 (4th instar) larvae were used in replications and were kept at 20°C for 24 h in order to starving prior to the assay. The infective juveniles were cleaned with 0.1% hyamine 3 times and were rinsed with distilled water 3 times and kept at 20, 25, 30 and 35°C for 24 h prior to testing at each temperature.

Each larva was exposed to 2 concentrations, 50 and 100 IJs. Nematode suspension was then shaken and 10 µl was pipetted and put in each one of the multi-well plate onto the artificial diets (0.39 g) in well. A single larva was transferred into each well and the plate was covered with lid and sealed paraffin film.

The plates of each concentration were incubated at 20, 25, 30 and 35°C. After 24, 48 and 72 h, the insect mortalities were checked. The data were expressed as percentages of the total number of insect tested in each replicate (3 replications). Percentages of mortality were compared among treatments using analysis of variance (ANOVA) by factorial in CRD (4×3 factors) and compared means using Duncan's Multiple Range Test at 95% confidence.

4. Efficiencies of *S. siamkayai, S. carpocapsae* and *S. riobrave* Against *H. armigera* and *S. litura* Larvae

The larva of *H. armigera* and *S. litura* was individually placed in the multi-well plate fed with 0.39 g artificial diet. Each one was inoculated with IJs of *S. siamkayai, S. carpocapsae* and *S. riobrave* prepared in ten concentrations: 1, 5, 10, 15, 20, 30, 40, 50, 100 and 200 IJs per 10 µl per larva. The plates inoculated with *S. siamkayai, S. carpocapsae* and *S. riobrave* were incubated at 30, 25 and 30°C, respectively. The 4th instar larvae of *H. armigera* and *S. litura* were obtained from Biological Control Research Group, DOA. Ten insects were used for each treatment.

Twenty-four, 48 and 72 h after inoculation, the insect mortalities were checked, the cadavers were dissected and the numbers of nematode were counted.

Data on mortalities of both insects in all treatments at different concentrations of each nematode and time on LC_{50} and LT_{50} were calculated by probit analysis.

RESULTS AND DISCUSSION

1. Life cycle and measurement of various developmental stages of *S. siamkayai*

1.1 Life cycle of *S. siamkayai*

The life cycle of *S. siamkayai* in the 4th instar of *H. armigera* larva with concentrated 50 IJs per larva was found to be similar to that of *Steinernema* species. Those were described including egg, four juvenile stages and adult stages (male and female). It took 6 days to develop to IJ and 11 days to complete development at 30°C (Figure 1).

This life cycle was similar to the work of Stock *et al*. (1998) that found infective juveniles of *S. siamkayai* to be able to reach the adult stage in 24–32 h at 22°C. The second generation adults appeared in 5–6 days (120–144 h) and infective juveniles emerged 8–10 days (192–240 h) after infection with *G. mellonella*.

In the experiment, two generations of *S. siamkayai* were able to completely develop within *H. armigera*, from the infective juvenile to the infective juvenile stages again. This indicated the balance between the nematode density and nutrient obtained from the host. The result also agreed with that of Nguyen and Smart (1992) which described that, if the nutrient supply was sufficient and the population was not overcrowded, the IJ developed to adult males and females of first-generation. Then most eggs from these adult females hatched and the juveniles developed through each life stage to become adult males and females of the

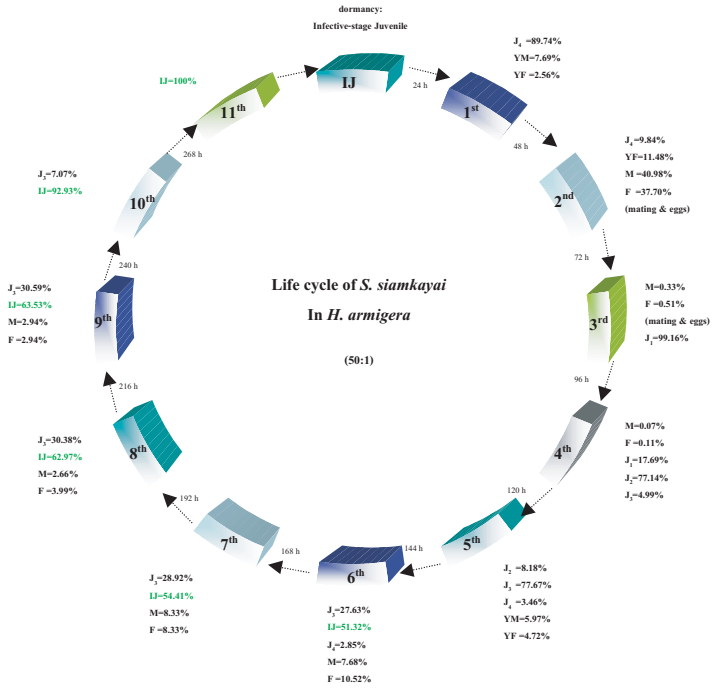

Figure 1 Life cycle of *Steinernema siamkayai* in *Helicoverpa armigera* (50:1) at 30°C.

second-generation. Generally, two complete generations occur in an insect host but smaller insects may permit only a single generation or perhaps only an incomplete generation to develop (Jackson, 1985).

1.2 Measurement of various developmental stages of *S. siamkayai*

The average measuring of the body length-width of 10 *S. siamkayai* in each stage was made every 24 h after infection (Table 1).

Stock *et al*. (1998) reported the most similarity of *S. siamkayai* n. sp. to *S. rarum* (De Doucet) *S. carpocapsae* (Weiser) and *S. monticolum* Stock, Choo and Kaya, in the general study of IJ morphology, whose average body lengths and the greatest widths of IJ were 446 / 21 µm, 511 / 23 µm, 558 / 25 µm and 706 / 37 µm, respectively. Furthermore, in the same species of entomopathogenic nematode, the infective juvenile body length showed the same trends, with the longer being at lower temperature and decreasing in length at higher temperature. The quality of food for the nematode and temperature influence the body length of the infective juvenile (Hazir *et al*., 2001).

2. Symbiotic bacterium *Xenorhabdus* sp. of *S. siamkayai* isolated from H. armigera larva

The symbiotic bacteria *Xenorhabdus* sp. of *S. siamkayai* (Stock *et al*., 1998) were isolated from a drop of hemolymph of infected 5th instar *H. armigera* larva.

Forty-eight hours after inoculation, the bacterial colonies on NBTA were checked and the isolation of bacteria after 15 h of infection was found to be negative. However, 18 h after infection, single bacterial colony of complete morphology which could be transferred for further study was noticed on NBTA. As for the isolations at 21 and 24 h after infection, the long stripe colonies were spotted. Yet, this type was found not to be suitable for the following tests.

It could then be seen that the complete and perfect symbiotic bacteria had the same characters

Table 1 Average morphometric characters of each stage of *Steinernema siamkayai* developed in *Helicoverpa armigera* larva at 30°C.

Stages of juvenile	Mean ± SD of *S. siamkayai*	
	Body length (μm)	The greatest width (μm)
J_1	214 ± 2.9 (210–220)	12.5 ± 0.9 (12–13.5)
J_2	261 ± 10 (245–284)	17 ± 1.2 (15–18.5)
J_3	329 ± 14 (305–351)	19 ± 0.8 (18–20)
IJ	405 ± 10 (392–419)	21 ± 0.8 (20–22)
J_4	516 ± 21 (489–558)	30 ± 3 (27–33)
YM	652 ± 32 (613–703)	55 ± 5 (50–63)
YF	1,058 ± 106 (880–1,238)	89 ± 5 (84–100)
M_1	1,146 ± 96 (1,050–1,288)	143 ± 9 (125–154)
F_1	4,688 ± 854 (3,830–6,300)	202 ± 17 (175–225)
M_2	853 ± 65 (713–920)	59 ± 8 (47–72)
F_2	2,127 ± 166 (1,820–2,320)	106 ± 7 (97–120)
Egg (diameter)	40.25 ± 1.23 (38.5–41.5)	

as the colonies isolated from NBTA 18 h after infection. They were granulate, convex, opaque and circular with irregular margins. In addition, there was absorption of bromthymol blue (BTB) from NBTA medium and clear zone around the colony was distinct. The characters were similar to the primary colony form. They supported nematode propagation very well in comparison to secondary form. Akhurst (1993) reported that the colony type of primary form was obtained from IJs while secondary form appeared from *in vitro* cultures of primary form.

YS broth was used to multiply the cells. Afterward, the cells were treated with gram's staining which expressed the rod-shape and gram negative and the synthesized crystalline inclusion on cells appeared 18 h post–incubation. Primary form variants produce protoplasmic crystalline inclusions in stationary period cultures (Couche and Gregson, 1987; Bowen and Ensign, 2001), whereas secondary form bacterial cells are usually long and inclusion bodies are rarely found.

The symbiotic bacteria primary form are used to rear nematode only because the primary form variant produces the crystalline inclusion proteins that support nematode reproduction, whereas secondary form lack antibiotic activity and can inhibit nematode growth (Bintrim and Ensign, 1998; Boemare *et al*., 1992, 1993).

3. Influence of temperature on the infection of *S. siamkayai* in *H. armigera* larvae

50:1

Twenty-four hours after infection, percent mortalities of *H. armigera* larva at 25 and 30°C were found not to be significantly differ from each other while significantly differed from those at 20 and 35°C. There were also no significant differences between percent mortalities at 20 and 35°C and between those at 25 and 35°C.

Forty-eight and 72 h after infection, no significant differences were found among percent mortalities of *H. armigera* larva at 20, 25 and 30°C, while at 35°C there was significant difference from those at 25 and 30°C.

100:1

Twenty-four and 48 h after infection, it was also found that percent mortalities of *H. armigera* larva at 25 and 30°C were not significantly different from each other while significantly differed from

Table 2 Mean mortality percentages of *Helicoverpa armigera* larva infected with *Steinernema siamkayai* at 50 and 100 infective juveniles per larva at different temperatures.

Temperatures (°C)	Mean mortality percentages at the concentration of					
	50:1 of exposure time[1/ 2/ 3/]			100:1 of exposure time[1/ 2/ 3/]		
	24 h	48 h	72 h	24 h	48 h	72 h
20	2.8 c B	36.8 ab A	45.8 ab A	15.3 c B	70.1 b A	72.9 b A
25	54.9 ab A	63.2 a A	77.8 a A	79.9 a A	89.6 a A	91.7 a A
30	65.3 a A	67.4 a A	70.1 a A	83.3 a A	88.9 a A	89.6 ab A
35	24.3 bc A	28.5 b A	29.2 b A	50.0 b A	52.8 c A	52.8 c A

1/ In a column, means followed by the same letters are not significantly different at the 5% level by DMRT.

2/ In a row, means followed by the same capital letters are not significantly different at the 5% level by DMRT.

3/ The area of one hole in the multi-well plate was 1.76 cm^2.

those at 20 and 35°C.

After 72 h of infection, similar results were obtained as those 48 h after infection at 25 and 30° C. However there was no significant difference between percent mortalities at 20 and 30°C, while percent mortality at 35°C was significantly different from those at 20, 25 and 30°C.

Comparison of percent mortalities of *H. armigera* larva between the 2 concentrations (50:1 and 100:1) at 20, 25, 30 and 35°C, 24, 48 and 72 h after infection was made. No significant differences between percent mortalities of both concentrations at each temperature at all times were noticed except those of each concentration at 20°C, 24 h after infection.

The results, thus, indicated that the optimal temperature for infection of entomopathogenic nematode *S. siamkayai* in *H. armigera* larvae were 25 and 30°C at each concentration but with the greatest percent mortalities of both concentrations at 25°C, 72 h after infection. It could be seen that some *H. armigera* larvae were able to either tolerate the infection of nematode or to build up the immunity. The results fell in similar ranges of 25–28°C suggested by Kaya (1977) and Molyneux (1986) to be the optimal temperatures for growth and reproductive of entomopathogenic nematodes. Temperatures higher than 32°C have an adverse effect on reproduction, growth and survival (Wharton, 1986).

The larval mortalities could also be compared with the experimental result of Somsook and Somsook (2003) which indicated that *S. siamkayai* caused 24, 8 and 4% larval mortalities of *H. armigera* at 30, 25 and 35°C, respectively. Accordingly, Sasnarukkit (2003) reported pathogenicity of *S. siamkayai* against diamondback moth to be greater at higher temperatures between 25–35°C than lower temperatures between 15–20°C and the optimal pathogenicity temperatures for three nematode species, *S. siamkayai*, *S. carpocapsae* and *H. bacteriophora* were 30, 25 and 25°C, respectively. The mortalities of diamondback moth larva at optimal temperatures for each nematode species were 57.5, 85 and 55%, respectively. Hazir *et al*. (2001) presented temperature to have some effects on the time of death and penetration rate of nematode species.

4. Efficiencies of *S. siamkayai*, *S. carpocapsae* and *S. riobrave* against *H. armigera* and *S. litura* larvae

Hominick and Reid (1990) and Glazer *et al*. (1991) proposed an alternative to LC_{50} estimation as an assessment of nematode efficacy.

LC_{50} and LT_{50} of *S. siamkayai*, *S. carpocapsae* and *S. riobrave* required to cause mortality to *H. armigera* and *S. litura* were then determined. It was found that LC_{50} of *S. siamkayai*, *S. carpocapsae* and *S. riobrave* to *H. armigera*

larvae were 22.5, 1.2 and 1.2 IJs per larva, respectively. As for *S. litura* larvae, LC_{50} of *S. siamkayai*, *S. carpocapsae* and *S. riobrave* were 18.0, 0.2 and 1.8 IJs per larva, respectively, at 72 h after infection.

While LT_{50} of *S. siamkayai*, *S. carpocapsae* and *S. riobrave* with 5 IJs per larva to *H. armigera* were 89.9, 22.0 and 39.8 h, those of *S. litura* larvae were 72.5, 11.5 and 39.8 h, respectively (Table 3).

The results obtained on invasion efficacy of *S. carpocapsae* and *S. riobrave* to *H. armigera* and *S. litura* agreed with Sasnarukkit (2003) who reported *S. carpocapsae* to have LC_{50} of 1.8 IJs against dimondback moth larva 60 h after infection, but did not agree with the results of Malee (2003) which suggested LC_{50} of *S. carpocapsae* to *Ostrinia furnacalis* and *H. armigera* to be 4.7 and 3.2 IJs under laboratory condition. LC_{50} of *S. siamkayai* to diamondback moth larvae in the experiment of Sasnarukkit (2003) was not also similar to those of *S. siamkayai* to *H. armigera* and *S. litura* in this study. The differences in both cases should be caused by different insect used along with different infectivity of each nematode. Glazer and Navon (1990) also reported the pathogenicity of entomopathogenic nematodes against *H. armigera* larvae to be tested under laboratory conditions. Complete mortality was achieved with 200 IJs per 3rd instar *H. armigera* larva. The LD_{50} was 54 IJs per larva at 48 h within similar ranges as those of the 'All' strains were obtained with *S. feltiae* 'Mexican' and 'Pye' strain, *S. glaseri*, *Steinernema* sp. and *Heterorhabditis* sp. 'HP88'.

CONCLUSION

The life cycle of 2 generations of *S. siamkayai* in the 4th instar of *H. armigera* larva took 6 days for development to IJ and 11 days to complete development at 30°C. The symbiotic bacteria *Xenorhabdus* sp. associated with *S. siamkayai* isolated from hemolymph of *H. armigera* larva took 18 h after infection to complete perfect colonies on NBTA media. The optimal temperature for entomopathogenic nematode *S. siamkayai* at 50 and 100 IJs per larva to penetrate into *H. armigera* larvae were 25–30°C. LC_{50} and LT_{50} of *S. siamkayai* were higher than those of *S. carpocapsae* and *S. riobrave* onto *H. armigera* and *S. litura*.

Table 3 LC_{50} and LT_{50} of *Steinernema siamkayai*, *Steinernema carpocapsae* and *Steinernema riobrave* against *Helicoverpa armigera* and *Spodoptera litura* larvae 72 h after infection.

Helicoverpa armigera

Nematode species	LC_{50}	Slope ± SE	LT_{50}	Slope ± SE
S. siamkayai	22.5 (12.7-38.8)	1.2 ± 0.3	89.9	3.2 ± 1.8
S. carpocapsae	1.2 (0.2-2.7)	1.4 ± 0.4	22.0	0.6 ± 1.7
S. riobrave	1.2 (0.1-3.0)	1.1 ± 0.4	39.8	7.6 ± 4.3

Spodoptera litura

Nematode species	LC_{50}	Slope ± SE	LT_{50}	Slope ± SE
S. siamkayai	18.0 (9.0-32.2)	1.1 ± 0.3	72.5	3.7 ± 1.7
S. carpocapsae	0.2 (-)	1.1 ± 0.8	11.5	1.8 ± 1.4
S. riobrave	1.8 (0.3-7.8)	1.7 ± 0.6	39.8	7.6 ± 4.3

According to the results of this study, the investigation should be further conducted in order to develop increasing efficacy of *S. siamkayai* and symbiotic bacteria. In addition, specificity of *S. siamkayai* to the insects should also be investigated.

LITERATURE CITED

Akhurst, R.J. 1993. Bacterial symbionts of entomopathogenic nematodes-the power behind the throne, pp. 127-135. *In* R.A. Bedding, R.J. Akhurst and H.K. Kaya (eds.). **Nematodes and the Biological Control of Insect Pests**. CSIRO Publications, East Melbourne, Victoria.

Bintrim, S.B. and J.C. Ensign. 1998. Insertional inactivation of genes encoding the crystalline inclusion proteins of *Photorhabdus luminescens* results in mutants with pleiotropic phenotypes. **J. Bacteriol**. 180:1261-1269.

Boemare, N.E., M.H. Boyer-Giglio, J.O. Thaler, R.J. Akhurst and M. Brehelin. 1992. Lysogeny and bacteriocinogeny in *Xenorhabdus nematophilus* and other *Xenorhabdus* spp. **Appl. Environ. Microbiol**. 58:3032-3037.

Boemare, N.E., M.H. Boyer-Giglio, J.O. Thaler and R.J. Akhurst. 1993. The phages and bacteriocins of *Xenorhabdus* spp., symbiont of the nematodes *Steinernema* spp. and *Heterorhabditis* spp, pp. 137-14. *In* R.A. Bedding, R.J. Akhurst and H.K. Kaya (eds.). **Nematodes and the Biological Control of Insect Pests**. CSIRO Publications, East Melbourne, Victoria.

Bowen, D.J. and J.C. Ensign. 2001. Isolation and characterization of protein inclusion produced by the entomopathogenic bacterium *Photorhabdus luminescens*. **Appl. Environ. Microbiol**. 67:4834-4841.

Couche, G.A. and R.P. Gregson. 1987. Protein inclusions produced by the entomopathogenic bacterium *Xenorhabdus nematophilus* subsp. *nematophilus*. **J. Bacteriol**. 169:5279-5288.

Glazer, I. and A. Navon. 1990. Activity and persistence of entomopathogenic nematodes tested against *Heliothis armigera* (Lepidoptera: Noctuidae). **J. Econ. Entomol**. 83:1795-1800.

Glazer, I. and E.E. Lewis. 2000. Bioassays for entomopathogenic nematode, pp. 229-247. *In* A. Navon and K.R.S. Ascher (eds.). **Bioassays of Entomopathogenic Microbes and Nematodes**. CAB International. London.

Glazer, I., R. Gaugler and D. Segal. 1991. Genetics of the nematode *Heterorhabditis bacteriophora* strain HP88: The diversity of beneficial traits. **J. Nematol**. 23:324-333.

Hazir, S., S.P. Stock, H.K. Kaya, A.M. Koppenh? fer and N. Keskin. 2001. Developmental temperature effects on five geographic isolates of the entomopathogenic nematode *Steinernema feltiae* (Nematoda: Steinernematidae). **J. Invertebr. Pathol**. 77:243-250.

Hominick, W.M. and A.P. Reid. 1990. Perspectives on entomopathogenic nematology, pp. 327-345. *In* R. Gaugler and H.K. Kaya (eds.). **Entomopathogenic Nematodes in Biological Control**. CRC Press, Boca Raton, Florida.

Jackson, J.J. 1985. **Parasitism of the Western Corn Rootworm with the Nematode *Steinernema feltiae***. Ph.D. Thesis, University of Minnesota, Minneapolis, Minnesota.

Kaya. H.K. 1977. Development of the DD-136 strain of *Neoaplectana carpocapsae* at constant temperature. **J. Nematol**. 16:346-349.

Kaya, H.K. and S.P. Stock. 1997. Techniques in insect nematology, pp. 281-324. *In* L.A. Lacey (ed.). **Manual of Techniques in Insect Pathology**. Biological Techniques Series, Academic Press, San Diego.

Malee, S. 2003. **Use of Entomopathogenic Nematode, *Steinernema carpocapsae* (Weiser) for Sweet Corn Lepidopterous Larvae Pests Management**. M.S. Thesis,

Kasetsart University. Bangkok. (in Thai)

Molyneux, A.S. 1986. *Heterorhabditis* spp. and *Steinernema* (= *Neoaplectana*) spp.: temperature and aspects of behavior and infectivity. **Exp. Parasitol**. 62:169-180.

Napompetch, B. 1994. Technical aspects of research in Thailand with emphasis on integrated pest management approach, pp. 37-50. *In* **Cotton Cedors in Continental Southeast Asia. Regional Conference Proceedings, Vientiane Laa PDR. Oct 26-28, 1994**. French Ministry of Foreign Affairs/ CCl/CIRAD-DORAS Project.

Nguyen, K.B. and G.C. Smart, Jr. 1992. Life cycle of *Steinernema scapterisci*. **J. Nematol**. 24:160-169.

Sasnarukkit, A. 2003. **Efficacy of an Entomopathogenic Nematode, *Steinernema siamkayai* Stock, Somsook and Reid on Controlling Diamondback Moth, *Plutella xylostella* (Linnaeus)**. Ph.D. Thesis, Kasetsart University, Bangkok.

Somsook, V. and S. Somsook. 2003. Study on the efficacy of entomopathogenic nematodes *Steinernema carpocapsae* (Weiser), *S. riobrave* Cabanillas, Poinar and Raulston and *S. siamkayai* Stock, Somsook and Reid for controlling lepidopterous larvae at different temperatures. In Thai with English abstract. **Ent–Zoo–Gaz**. 25:244-257.

Somsook, V., A. Tantichodok and U. Katenud. 1986. Control of *Cossus* sp. with entomopathogenic nematode, *Steinernema carpocapsae*. In Thai with English abstract. **Ent–Zoo–Gaz**. 8:115-119.

Stock, S.P., V. Somsook and A.P. Reid. 1998. *Steinernema siamkayai* n. sp. (Rhabditida: Steinernematidae), an entomopathogenic nematode from Thailand. **Syst. Parasitol**. 41:105-113.

Wharton, D.A. 1986. **A Functional Biology of Nematodes**. Croom Helm, London, 192 p.