THE SUMMARY

Title of the project:

Identification and isolation of entomopathogenic nematodes (EPN) found in the coastal belt of Southern Sri Lanka.

Institute where research is being carried out:

Dept. of Zoology, University of Ruhuna, Matara.

Chief Scientific Investigator:

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Period of contract (date of award and completion)

One year. Date of award -15.01.97 Date of completion - 31.12.98

Scientific background and Scope/Objective of Project

Entomopathogenic nematodes (EPN) of the families Steinernematidae and Heterorhabditidae are obligate parasites of insects. The free living third stage juveniles carrying symbiotic bacterium, *Xenorhabdus* spp. enter the hosts via natural body openings. Inside the host, symbiont is released and the proliferation of it provides conditions for reproduction and growth of nematodes causing death of the host within 24 – 48 hours (Poinar, 1979). Therefore, they have a great potential in controlling of economically important pests and use of entomopathogenic nematodes can be adopted as a new tool in integrated pest management systems. This investigation was

carried out in en effort to isolate and identify the indigenous EPN along the southern coastal belt of Sri Lanka , in the scope of management of insect pests.

Experimental method

Soil samples were drawn from nine major sites representing wet zone (Matara, Dondra, Weligama and Ahangama), intermediate zone (Tangalle and Dickwella) and dry zone (Hungama, Boondala and Hambanthota) along the southern coast for the isolation of EPN. The coast was selected as most isolates of EPN were detected from the coastal regions of the world. At each major site, four subsites were selected to cover the ecologically diverse habitats and soil samples were drawn at the distances of 0m (tidal zone), 10m, 20m and 30-40m along a line transect, within the each subsite to investigate the distribution pattern of these nematodes with sea. Altogether, 432 soil samples (64 samples of each from Matara, Dondra, Weligama, Tangalle, Hungama and Hambanthota over a period of twelve months and 16 samples of each from Ahangama, Dickwella and Boondala , at only one sampling occasion over a period of three months.) were accessed for the presence of EPN using *Galleria* baiting technique (Bedding & Akhurst, 1975). Re-infection was done to confirm the pathogenicity of isolated EPN. Identification was carried out at International Institute of Parasitology, UK by using molecular biological techniques and locally by using taxomonical keys and life cycle studies.

Results obtained:

43 soil samples were positive for EPN (Dondra 16/24, Weligama 08/64, Matara 07/64, Tangalle 05/64, Hungama 02/64, Ahangama 03/16 and Dickwella 02/16) and successful re-infection of *Galleria* larvae by these nematodes confirmed that they were truly EPN. Nematodes recovered from Hambanthota and Boondala failed to re-infect *Galleria* larvae. The EPN recovered from each positive site are indicated in **Table I.**

Table I : Entomopathogenic nematodes recovered from seven sites along the Southern coastal belt of Sri Lanka.

Major site	**EPN found**
Matara	**Heterorhabditis indicus* & *Undescribed *Steinernema* species
Dondra	**Heterorhabditis indicus*
Weligama	*Undescribed *Steinernema* species
Tangalle	***Heterorhabditis indicus*
Hungama	*Undescribed *Steinernema* species
Ahangama	***Heterorhabditis indicus*
Dickwella	***Heterorhabditis indicus*

* Identification was done at International Institute of Parasitology, UK using molecular biological techniques.

** Identification was done locally.

Heterorhabditis populations of Ahangama, Dickwella and Tangalle that were identified locally using taxonomical key provided by Stock (1997) were most probally belonged to *H. indicus*. However, the dimensions taken from these *Heterorhabditis* isolates did not remarkably similar between each other. Many of their ranges are overlapping. Majority of EPN were recovered from the region of 30- 40 m from the tidal zone (0 m) to the interior.

However, the rate of recovery declines towards the tidal zone as well as towrds the dry zone. P^H of EPN positive soils were ranged from 7.5 -9.0 of sandy soil. Soil temperature was around 30^0C at the time of sampling. The associated vegetation included like *Ipomea*, *Cocos nucifera*, *Calotropis*, *Pandanus*, *Mimosa* and common grass etc. It was unable to discover insects associated with the EPN isolates from a single site at all the sampling occasions.

Conclusions:

The findings of this study indicated that the natural EPN population of Southern region comprised of both *Steinernema* and *Heterorhabditis* species. However, it is apparent that the *Heterorhabditis* species were dominated.

All the *Steinernema* populations detected in this survey were new to exiting pool of EPN. According to the IIP identification the undescribed *Steinernema* isolates detected from Matara and Hungama have close similarity to that of SSL 82 isolate which was recovered earlier from the Southern coast of Sri Lanka (Amarasinghe, *et al,* 1994). This *Steinernema* isolate showed a characteristic behavior of sudden coiling after a few shakes in water. However, the other undescribed *Steinernema* isolate did not show such a characteristic behavior. Identification of *Heterorhabditis*

species using morphological features alone is not possible. According to results obtained all the *Heterorhabditis* population discovered from the southern region were belonged to the *H. indicus*. However only taxonomical studies may lead to misidentification. Therefore, it is necessary to perform DNA based identification for further confirmation.

The *Heterorhabditis* isolates detected from the southern region did not show any similarity to *Heterorhabditis* isolates HSL 6, HSL 10 and HSL 105 which were previously detected from South-West region of Sri Lanka (Amarasinghe, *et al,* 1994) and *H. indicus* species which were detected from India (Poinar, 1990) ,morphologically. However, similarities may appear in DNA based studies.

During the study same individual subsites of the major sites have produced both positive and negative results for EPN. Homonick (1990) indicated that the populations of EPN may become extinct at certain sites and again the same sites may be re-established from nearest sites. In addition, mobility of EPN is favored by sandy soils (Bedding & Molyneux, 1984 & Kung, Gaugler & Kaya, 1990).

Papers published on work done under this contract:

1. W.T.S.D. Premachandra, L. D. Amarasinghe & M. G. V. Wickramasinghe(1997). Prevalence and distribution of entomopathogenic nematodes in the coastal belt of Dondra coastal belt of Sri Lanka. Abstract , Sri Lanka Association for the Advancement of Science 52nd Annual Session, 1997, pp 202 .

2. W.T.S.D. Premachandra, L. D. Amarasinghe & H.C.E. Wegiriya (1998) Occurrence of Entomopathogenic nematodes in six sites along the Southern coastal belt of Sri Lanka. Abstract, Institute of Biology, 18th Annual Session 1998, pp 14.