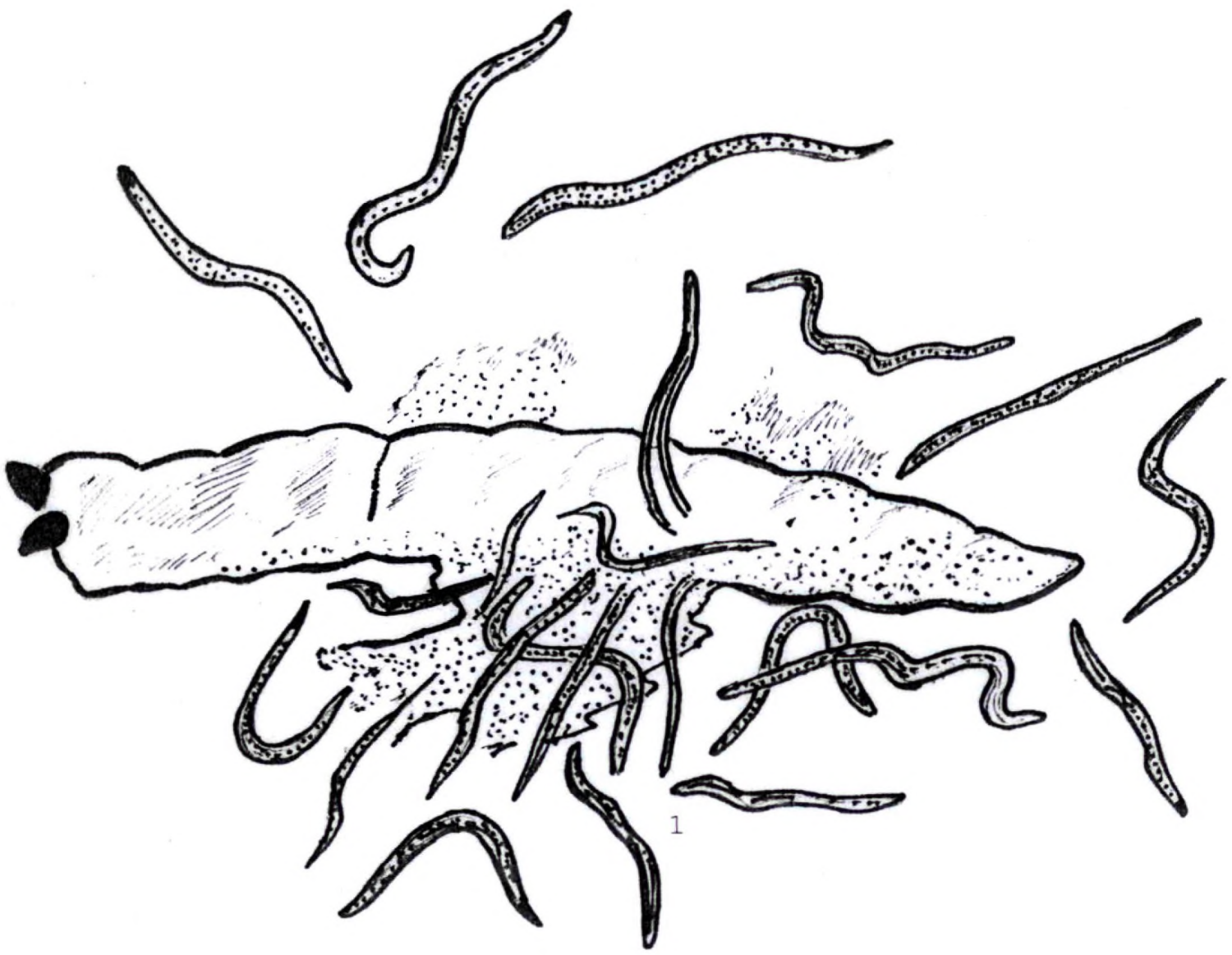
ISOLATION AND IDENTIFICATION OF ENTOMOPATHOGENIC NEMATODES ALONG THE SOUTHERN COASTAL BELT OF SRI LANKA.

FINAL REPORT

NSF GRANT NO : RG / 97 / B / 2



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ACKNOWLEGEMENT

At first, I wish to offer my deep gratitude to National Science Foundation, Sri Lanka which made it possible for me to carry out this research project by providing financial assistance. I express my sincere thanks to Dr (Mrs). M.G.V. Wickramasinghe, the main grantee of the project for the invaluable advice and guidance bestowed on me to conduct this research, successfully. I am also thankful Dr (Mrs). H.C.E. Wegiriya , the main grantee of the final part of this research project, for the suggestions and advice given to me in this regard.

I am grateful to Dr (Mrs) N.J.De.S. Amarasinghe, Head, Department of Zoology for the opportunity provided me to carry out this research work in the Department of Zoology, University of Ruhuna, Matara.

Grateful appreciation is also extended to Dr (Mrs). L.D Amarasinghe, Tea Research Institute, Talawakelle, for the invaluable guidance in the filed of entomopathogenic nematology. The work done by Dr. B. Briscoe, International Institute of Parasitology, is also acknowleged.

I express my sincere thanks to Dr (Miss) P.R.T. Cumaranatunga for the immense help extended to me in regard with photography work. Further, the assistance of Mr. H. W. Dharmasiri, technical officer, Department of Zoology, University of Ruhuna regarding the photography work, is greatly appreciated.

Last but not least, I wish to thank the technical assistants of this project for helping me both in field and laboratory work, to complete this research work.

W. T. S. Dammini Premachandra (Co-grantee)

Dept. of Zoology

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Matara.

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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:

Dr (Mrs) M.G.V. Wickramasinghe / Dr (Mrs) H.C.E. Wegiriya

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.

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FINAL REPORT

1. (a) **Contract No:**

RG/97/B/2

(b) Title of Project:

Identification and isolation of entomopathogenic nemato[illegible]e coastal belt of Southern Sri Lanka and its potential in the control of insect pests of rice and vegetables.

** However, this title was altered into " Identification and isolation of entomopathogenic nematodes found in the coastal belt of Southern Sri Lanka" later, as instructed by NSF.

(c) Institute where research is being carried out:

Dept. of Zoology, University of Ruhuna, Matara.

(d) Chief Scientific Investigator:

Dr (Mrs) M.G.V. Wickramasinghe

** Since Dr (Mrs) Wickramasinghe was offered Commonwealth fellowship for her sabbatical leave the responsibilities of the main investigator were handed over to Dr (Mrs) H.C.E. Wegiriya and she served as the main investigator of the project till the end.

(e) Date of award of the grant:

15.01.97

Though the date of award of the grant is 15.01.97 the cheque of Rs. 58, 590/= received in March 1997. As such, wax moth culture, which is a prerequisite to this research, was started to maintain in the laboratory, Dept. of Zoology, University of Ruhuna, Matara, in April 1997. However, soil sampling was started from June 1997.

(f) Date of completion

31.12.98

** The exact duration of the project was one year. However, this duration had to be extended (without additional funds) due to the difficulties arose in identification of isolated entomopathgenic nematodes at International Institute of Parasitology, UK.

(g) Total allocation

Rs. 1,44 360 / =

(h) Total spent

Rs. 1,21 685. 78

(i) No. of Research Assistants/Technical Assistants and period of service

One technical Assistant (T.A.) post

Service - 20 months (from April 1997 to December 1998)

However, three T.A s have been worked during these twenty month's period.

T.A. I	-	03 months (from 09th April 1997 - 09th July 1997)
TA II	-	07 months (from 09th July 1997 - 09th February 1998)
TA III	-	10 months (from 09th February 1998 - 09th December 1998)

** Even though, soil sampling was started in June 1997, the maintenance of wax moth culture was started to maintain in April 1997. Therefore, the service of TA was obtained with effect from April 1997.

(h) Whether RA has registered for, or obtained the post-graduate?

No.

2. Description of research carried out

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). In order to isolate the infective larvae, soil samples were collected from their natural habitats. *Galleria* baiting technique (Bedding & Akhurst, 1975) was applied to isolate these larvae from collected soil.

(I) Maintenance of wax moth culture

Final instar of the greater wax moth (*Galleria mellonella*) (L) (Lepidoptera : Pyralidae) larvae **(Plate 1)** were used as bait insects for the isolation of EPN from soil. In order to obtain these larvae continuously, a live wax moth culture was maintained in the laboratory at the Department of Zoology, University of Ruhuna, Matara since April 1997. These larvae were originally collected from infested bee hives in wild and they were reared in transparent polystyrene boxes (2.75 X 15.5 X 8.5 cm) at room temperature (28^0C) by providing an artificial diet which consisted of bread crumb (469g), yeast (Brewers) (137g), bee honey (125g), glycerin (139g) and bee wax (melted) 77g. (The bread crumbs and yeast were oven dried at 100^0C for 2 minutes and mixed with the other

ingredients.) The larvae were allowed to undergo further development up to pupal stage **(Plate 2)** which were then transferred into another separate plastic containers (10 X 23 cm). When adults **(Plate 3)** emerged from the pupae they were provided bee honey as a food. Eggs **(Plate 4)** were collected on double folded small pieces (5cm X 2cm) of oil papers which were then removed and placed in larval containers to hatch into larvae.

Plate 1: Final instar larvae of wax moth (*Galleria)*

Plate 2: Pupae (in cocoons) of wax moth (*Galleria*)

Plate 2: **Adults of wax moth (*Galleria)***

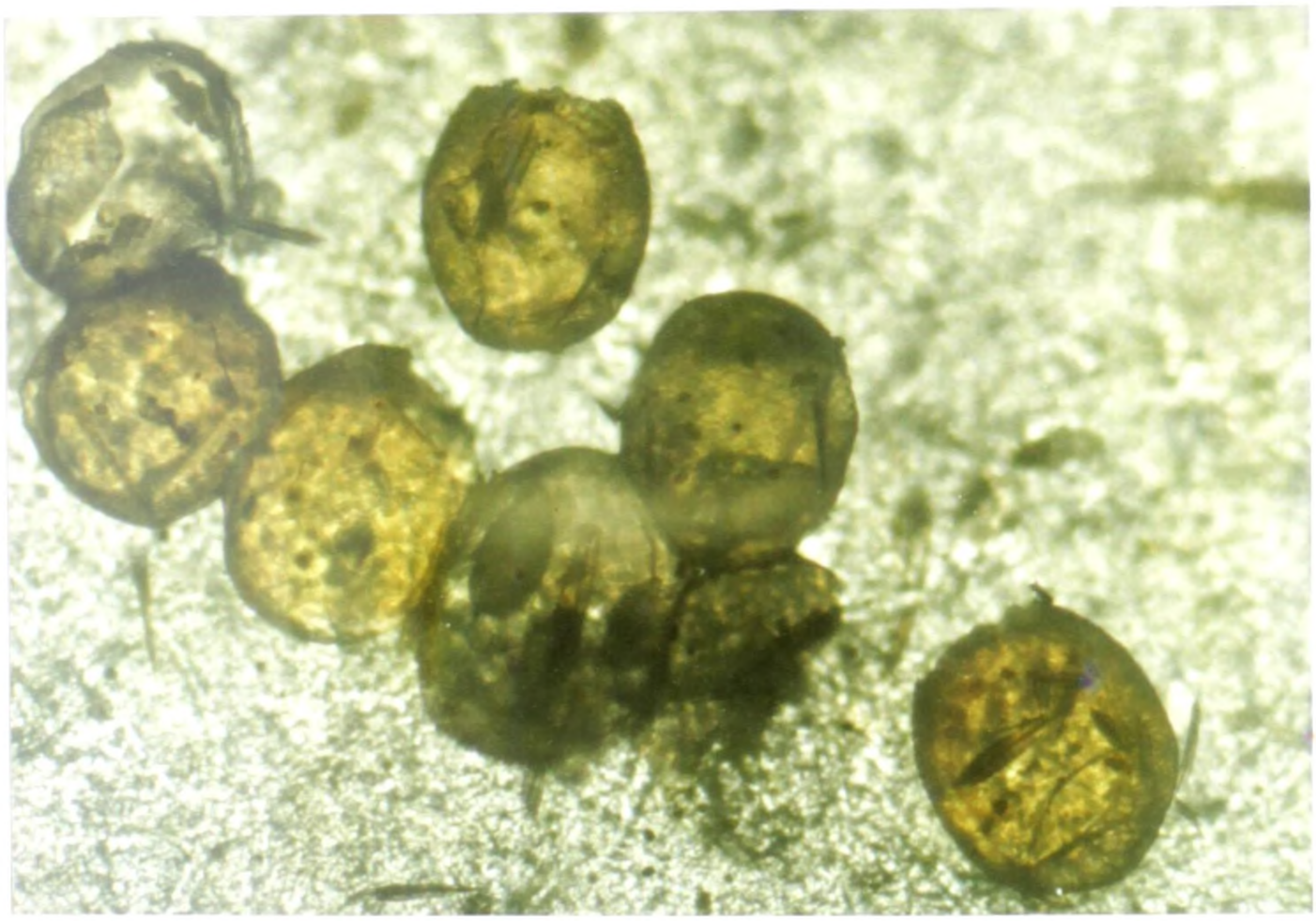


Plate 4: Eggs of wax moth (*Galleria)*

2.2 Isolation of EPN from their natural habitats

2.2.1 Soil sampling

The coastal region was selected to collect soil samples for the isolation of EPN. This is because most isolates of the EPN have been detected from coastal regions of the world. (Amarasinghe, *et al,* 1994 & Hara *et al,* 1991). Initially, six major sites were selected to represent the wet zone (Matara, Dondra and Weligama), intermediate zone (Tangalle) and dry zone (Hungama and Hambanthota) along the southern coast (Figure 1).

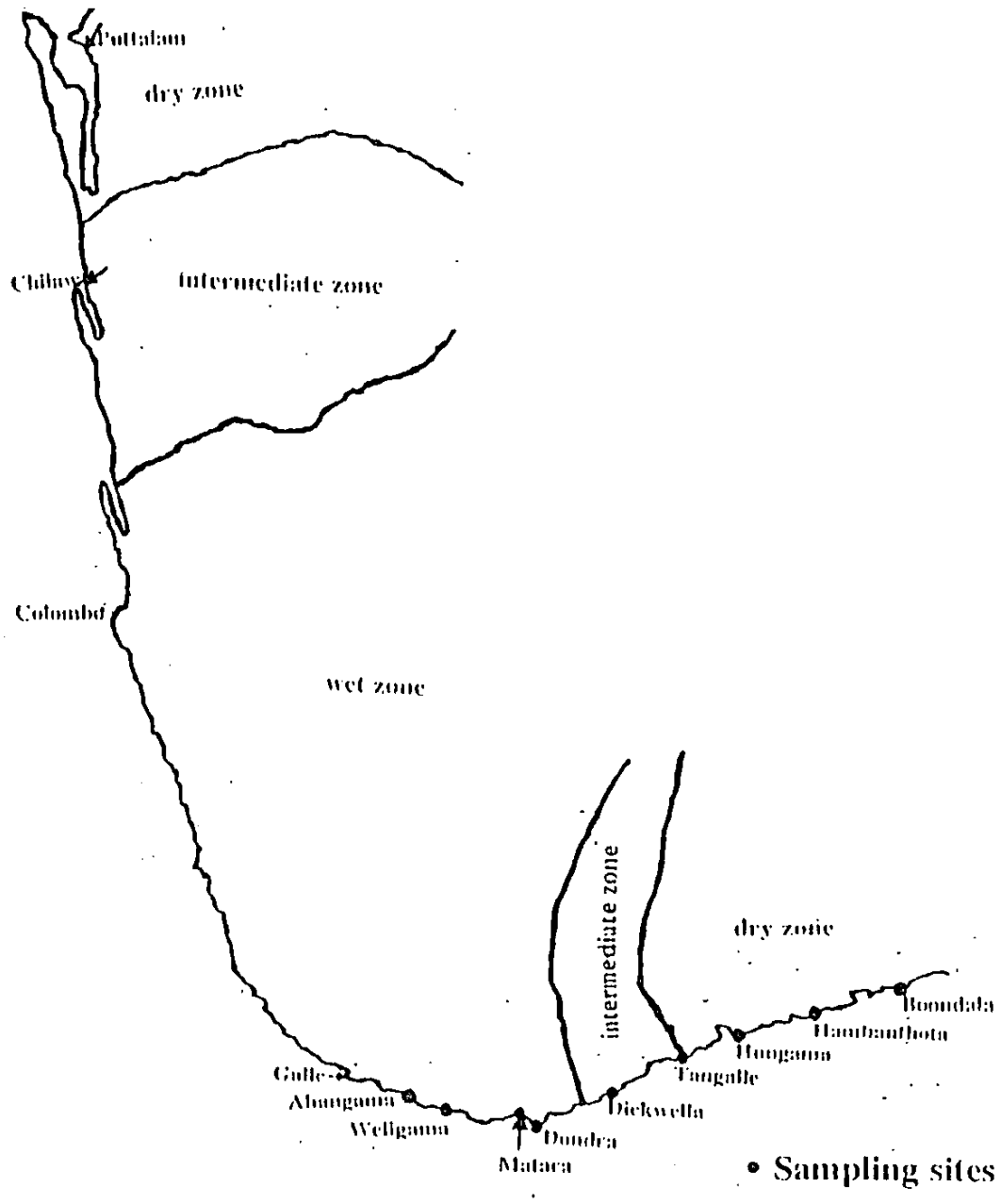


Figure 1: Sampling sites along the Southern coastal belt

All the six sites were sampled four times during a period of twleve months (from June 1997- June 1998). In addition, some selected sites Ahangama, Dickwella and Boondala representing wet zone, intermediate zone and dry zone **(Figure 1)** were sampled once to check the presence of indigenous EPN, during the extended time period. At each major site, four subsites **(Plates 5, 6, 7 & 8)** were selected and soil samples were drawn, at the distances of 0m (tidal zone), 10m, 20 m, 30 – 40 m from the tidal zone to the interior along a line transect to investigate the distribution pattern of EPN.

Plate 5: A sampling subsite representing wet zone (Matara)

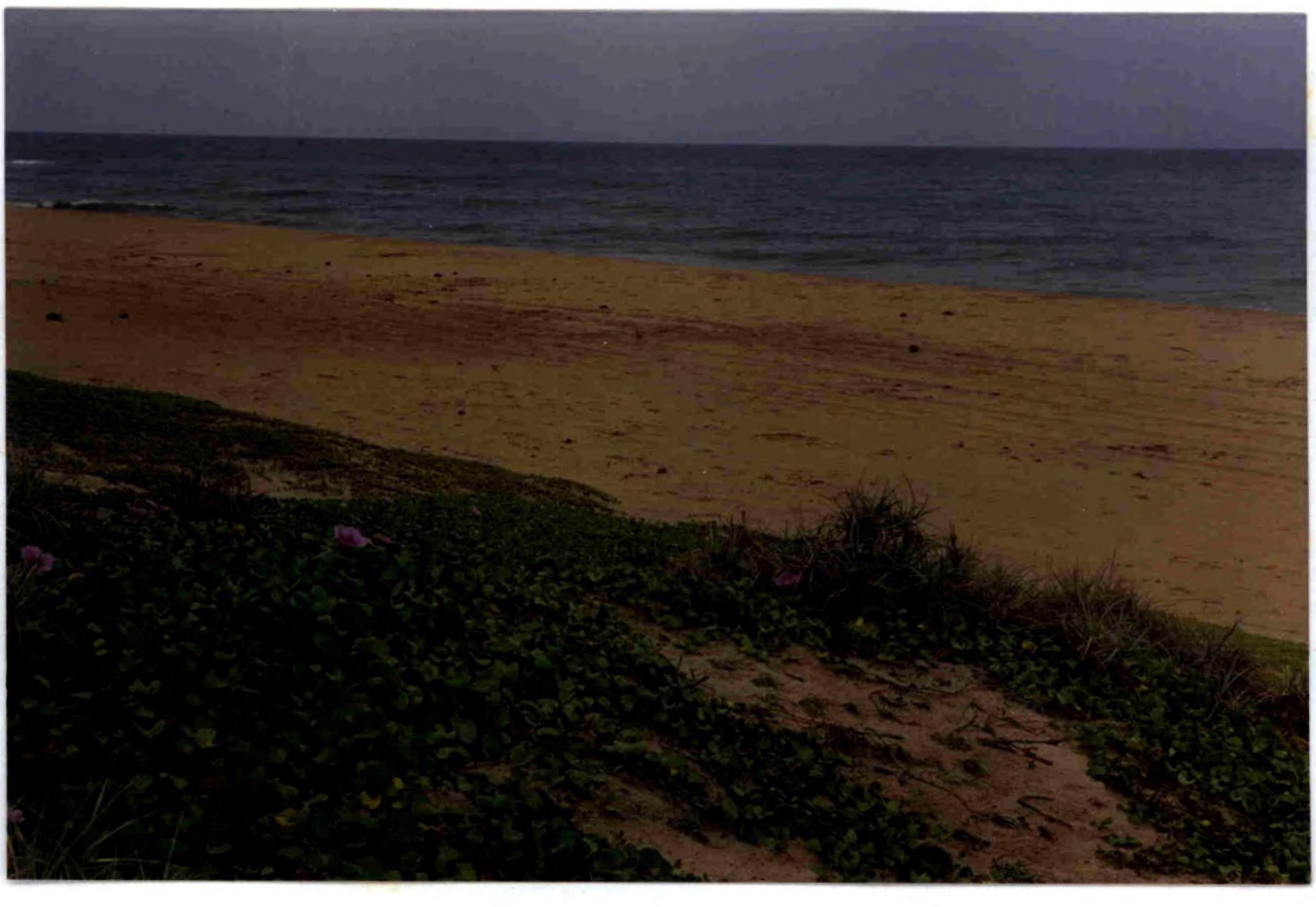


Plate 6: A sampling subsite representing intermediate zone (Tangalle)

Plate 7: A sampling subsite representing dry zone (Hungama)

Plate 8: A sampling subsite representing dry zone (Hambanthota)

Four random soil samples were drawn, at each distance over an area of $10m^2$ at a depth of 10cm using a soil corer (volume 200ml**) (Plate 9)** and pooled them into polythene bags, separately. The associated vegetation, elevation, soil temperature at 5cm depth, soil, P^H and insects present were recorded, at each subsite. The collected soil samples were transported to the laboratory for the isolation of existing EPN.

Plate 9: The soil corer

2.2.2 Isolation of EPN from soil to wax moth larvae:

Three aliquots (approximately 200ml) were taken from the pooled soil and put into plastic containers (9.5 X 4.5cm). Five final instars of *Galleria* larvae were placed on the surface of the soil and the containers were once inverted. Then the covered containers with baited soil **(Plate 10)** were incubated at 25^0C temperature.

Plate 10: Containers with baited soil

After one week, dead insects (cadavers) were removed and examined for the mortality taking colour and the nature into account. The flaccid nature of cadaver is a sign for EPN infection. The brick red colour cadaver is an indication for the *Heterorhabditis* spp. Poinar, infection while brown or yellow colour indicates *Steinernema* spp. Travassos, infection. The cadavers suspected of having EPN infection were removed and cleaned in sterile distilled water for further processing.

2.2.3 Isolation of EPN from wax moth cadavers to distilled water

Cadavers that were suspected of having EPN infection were dissected in sterile distilled water to get the different stages of EPN out. The remainder were placed on White traps (White, 1927) **(Plate 11)** to collect emerging infectives.

Plate 11: Dead Galleria larvae on the White trap

The trap consisted of an inverted watch glass (6cm diameter) placed within a petri dish (9 X 1.8cm). *Galleria* cadavers were placed on a 55mm filter paper on the watch glass and the petri dish was filled with sterile distilled water just to touch the filter paper. Cadavers were kept on the trap which was covered by another petri dish (10 X 1cm) for one week to allow the emerging EPN larvae to migrate from the cadavers into water. These nematodes were harvested with aid of pasture pipette into a conical flask (100 ml) in which sterile distilled water was filled and they were stored at 10^0C temperature in refrigerator.

2.3. Confirmation of EPN

It is necessary to confirm that the harvested nematodes are truly EPN. The nematodes were re-exposed to fresh *Galleria* larvae to confirm pathogenicity. If the re-infection is successful it is an indication that they were truly EPN. Five fresh *Galleria* larvae were placed in a petri dish (5 X 5.8cm) which consisted of a moist filter paper. Nematodes suspension (50 nematodes per ml) was added on the surface of the *Galleria* and the surface of the damp filter paper, for inoculation.

The petri dish was closed by a petri dish cover (5.8 X 0.5 cm) and this was kept at 25^0C, for one week. The *Galleria* larvae were examined for the mortality daily and cadavers were dissected in sterile distilled water, for the presence of EPN.

2.4 Identification of EPN

At the beginning it was planned to send the isolated EPN to International Institute of Parasitology (IIP), UK for identification, by using molecular biological techniques (RFLP) as it is the best method for identification of EPN. It was possible to send the EPN isolates of Matara, Dondra, Weligama and Hungama to IIP, by storing them in 70% ethyl alcohol. However, it was failed to identify the EPN isolates of Dickwella, Ahangama and Tangalle from IIP due to lack of response. Under such circumstances, morphological studies (by using taxonomical keys) and life cycle studies were carried out as far as possible to identify the EPN isolates of Ahangama, Dickwella and Tangalle, as an alternative.

2.4.1. Life cycle studies

The life cycle of Heterorhabditidae and Steinernematidae nematodes consists of eggs, four larval stages, pupa and adult. Presence or absence of males in the first generation is a significant character to distinguish the individuals of Genus *Heterorhabditis* and *Steinernema*. In other words *Steinernema* produces both males and females in its first generation while *Heterorhabditis* produces only hermaphroditic females in their first generation (Woodring & Kaya, 1988). In order to study this aspect cadavers were dissected in saline from 24 hours after the death of the *Galleria* larvae to one week period The different stages of the EPN inside cadavers were identified morphologically with aid

of student's compound microscope. The time at which these different stages appeared was also recorded.

2.4.2 The colour and the nature of the cadavers

The colour of the cadavres gives an indication, type of the infection of EPN Genera, *Heterorhabditis* or *Steinernema*. The brick red colour cadaver is an indication for *Heterorhabditis* species while yellow cadaver is an indication for *Steinernema* species. (Woodring & Kaya, 1988).

2.4.3 Preparing EPN for observation under compound light microscope

The male and female nematodes dissected from infected wax moth larvae and the larcae collected from White traps were taken (by fishing using an eye hair attached to a needle) into 1ml of distilled water in a watch glass (6cm diameter) and 4ml of double strength fixative, TAF (normal strength TAF contains 7ml of 40% formaldehide, 2ml of triethanolamine and 91ml distilled water) which was heated to 100^{0}C was added. (Seinhorst, 1966). The nematodes were allowed to remain in the fixative for 12 hours. Then the nematodes were cleared in glycerine by using glycerol-ethanol evaporation method developed by Seinhorst, 1959.

Fixed nematodes were transferred to a watch glass containing 0.5ml of solution I (20 parts 95% ethanol, 1 part glycerin, 79 parts distilled water). The watch glass was then placed in a desicator and enough 95% ethanol added to the desicator to half fill the space below the holding shelf. The desicator was placed in an oven at 35^{0}C for about 12 hours to allow slow evaporation of the water from the solution I in the watch glass. After this, the watch glass was removed from the desicator and filled with solution II (5 parts glycerin 95 parts ethanol). The watch glass was placed in a petri dish which was partially covered to allow slow evaporation of the ethanol, and put back in an oven at 40^{0}C for 3 hours. The ethanol evaporates leaving the nematodes in pure glycerin. The processed nematodes were mounted on glass slides on which a drop of glycerin was placed and covered by a coverslip , using DPF.

Identification was done using taxonomical keys (Hominick, *et al.* 1997)

3. Results obtained

3.1 Prevalence of EPN

A total of 432 soil samples tested from nine major sites, only 43 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 sucessful 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.

3.2 Identification of isolated EPN

3. 2. 1 Identification at IIP

According to IIP identification based on DNA studies Matara yielded *H. indicus* and undescribed *Stienernema* species . Dondra also yielded *H. indicus* while Hungama also yielded an undescribed species of *Steinernema.*

3. 2. 2 Local identification

3. 2. 2. 1 Identification up to the Genus

EPN infected cadavers which were detected from soils of Matara, Dondra, Ahangama, Dickwella and Tangalle showed brick red in colour **(Plate 11)**.

In addition, only hermaphroditic females **(Plate 12)** could be found during the period of 3-4 days after initial infection (in first generation). Males **(Plate 13)** and amphimictic females **(Plate 14)** were found 6-9 days after initial infection and males posses a conspicuous bursa bearing bursal rays adjacent to the spicule **(Plate 15)**. All these features revealed that they belonged to the Genus - ***Heterorhabditis.***

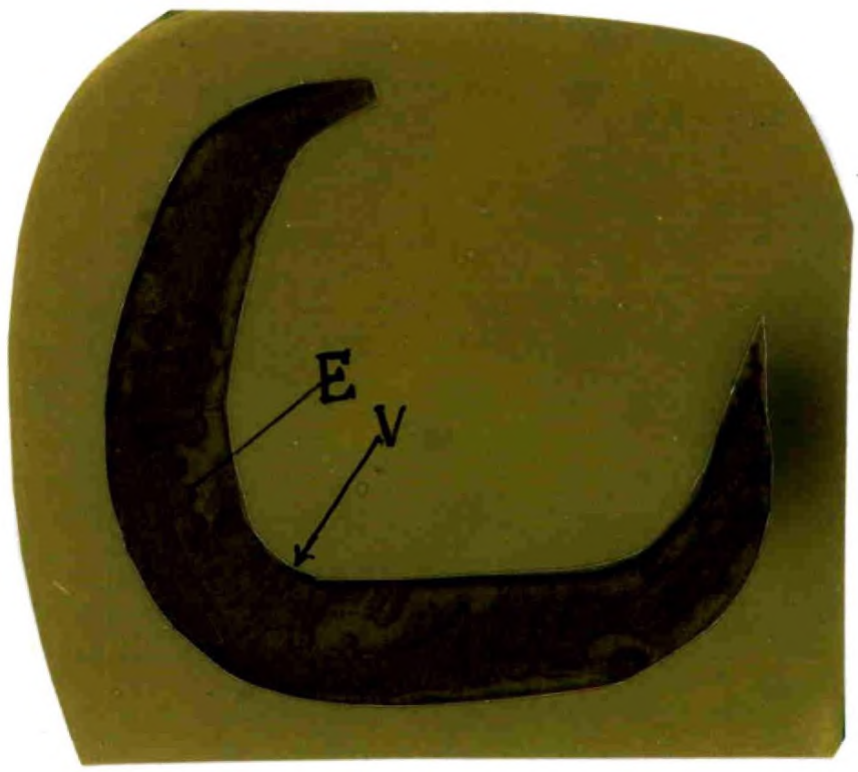


Plate 12: **Hermaphroditic female of *Heterorhabditis* spp. (isolated from Tangalle) (X 100)**

V - Vulva **E - Eggs**

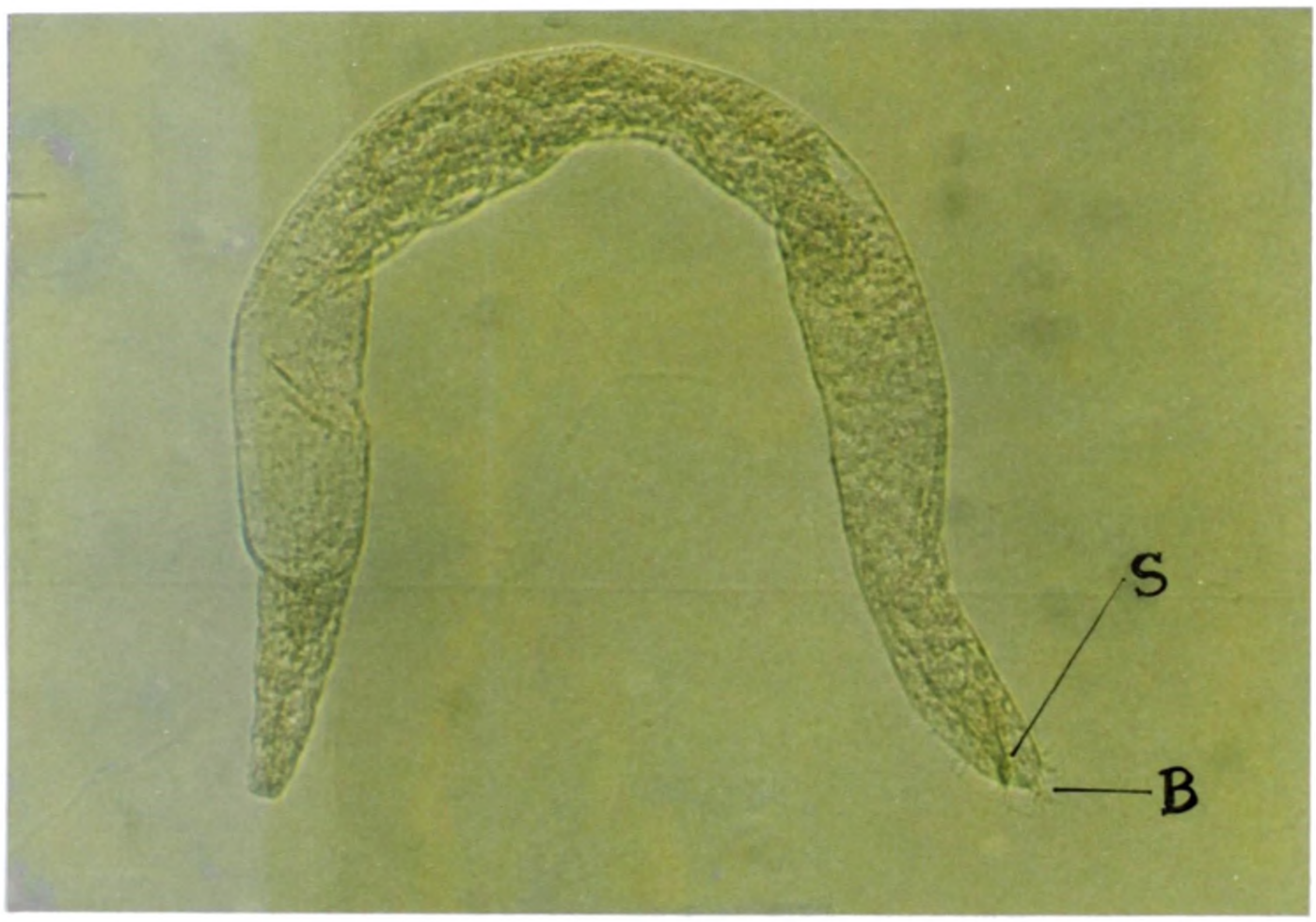


Plate 13: Male of *Heterorhabditis* spp. (isolated from Ahangama) (X 100)

S - Spicule B - Bursa

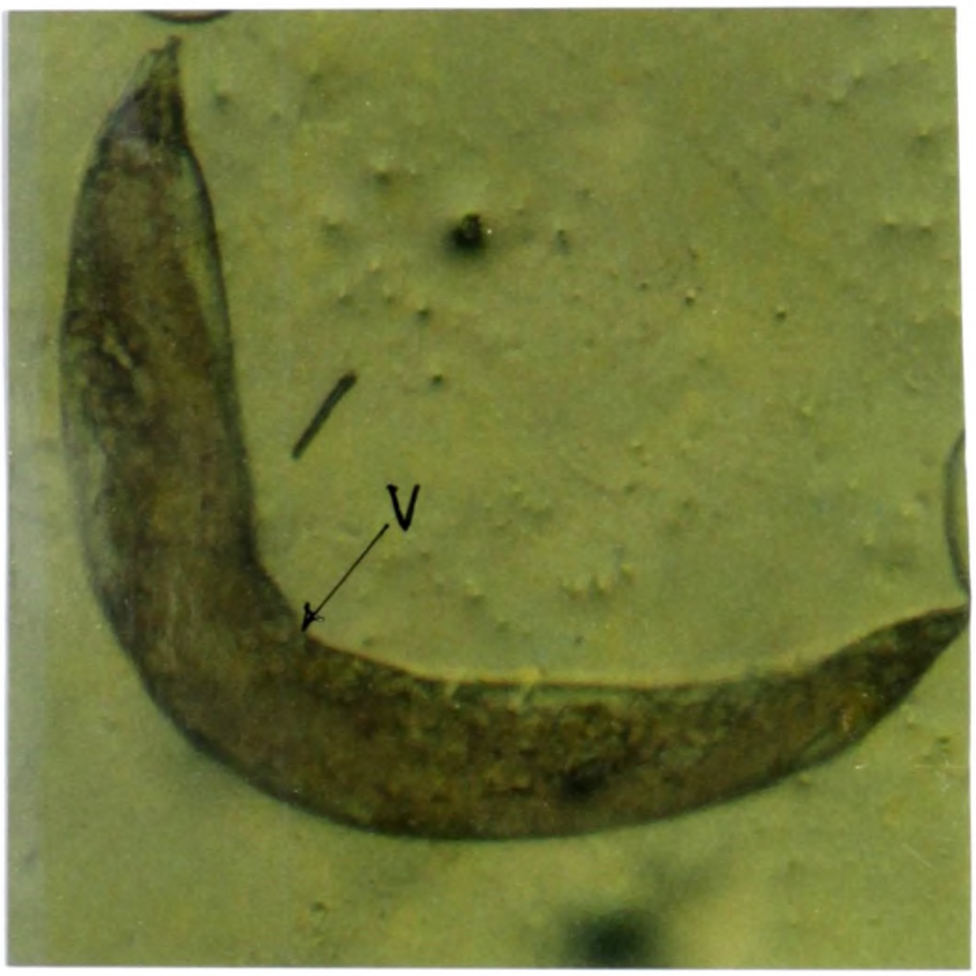


Plate 14: Amphimictic female of *Heterorhabditis* spp. (isolated from Dickwella) (X 100)

V - Vulva

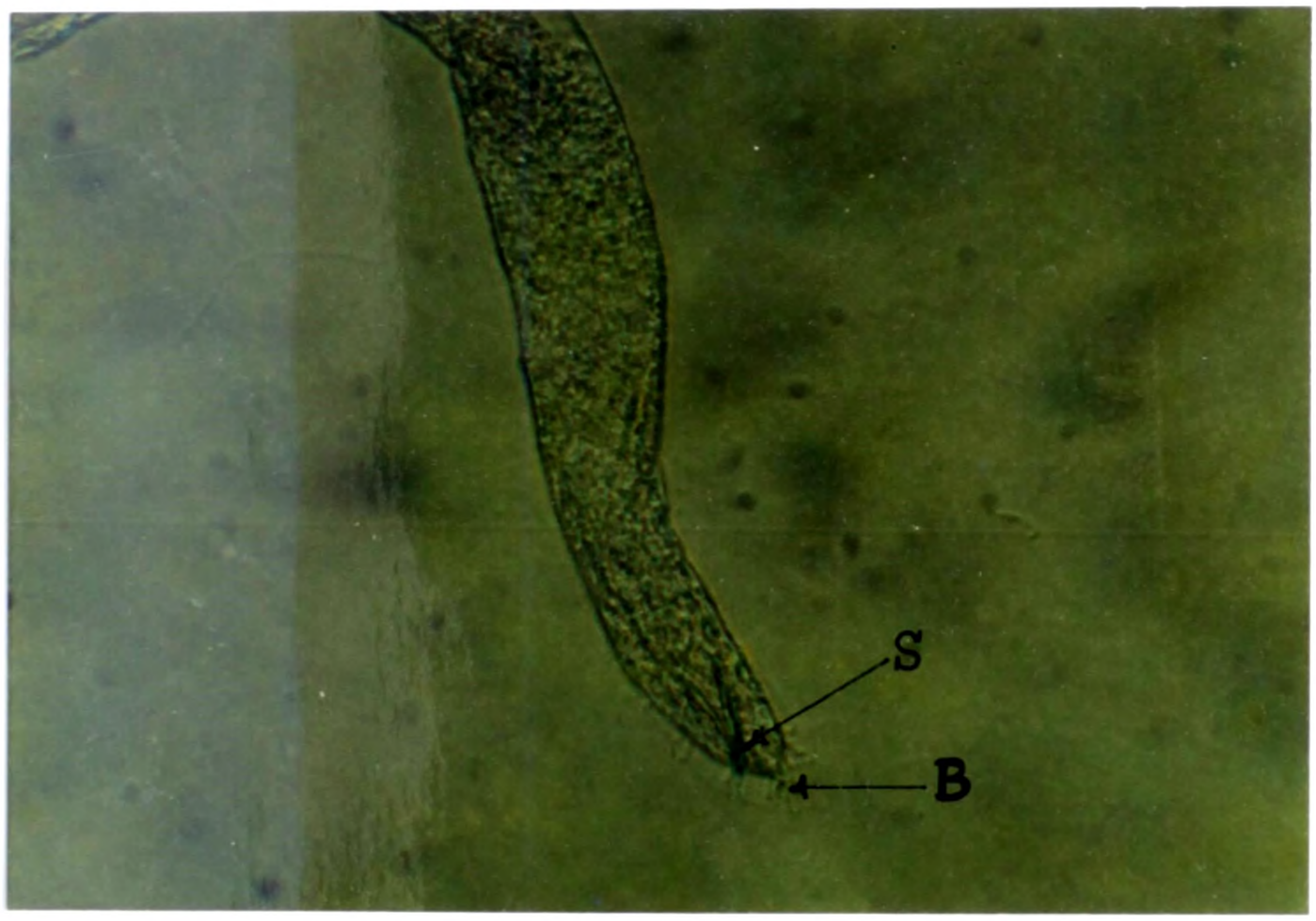


Plate 15: Posterior end of the male of *Heterorhabditis* spp. (isolated from Ahangama) (X 100)

S - Spicule B - Bursa

Some of the cadavers detected from Matara and all the cadavers detected from Weligama and Hungama appeared brown in colour **(Plate 16).** Both females **(Plate 17)** and males **(Plate 18)** of these nematodes could be found during the period of 2-4 days (in first generation) after initial infection. However, the males of these nematodes did not possess a bursa adjacent to the spicule **(Plate 19)**. These features confirmed that they belonged to the Genus - ***Steinernema.***

Plate 16: ***Steinernema*** **infected wax moth larvae**

Plate 17: Female of *Steinernema* spp. (isolated from Matara) (X 100)

V - Vulva

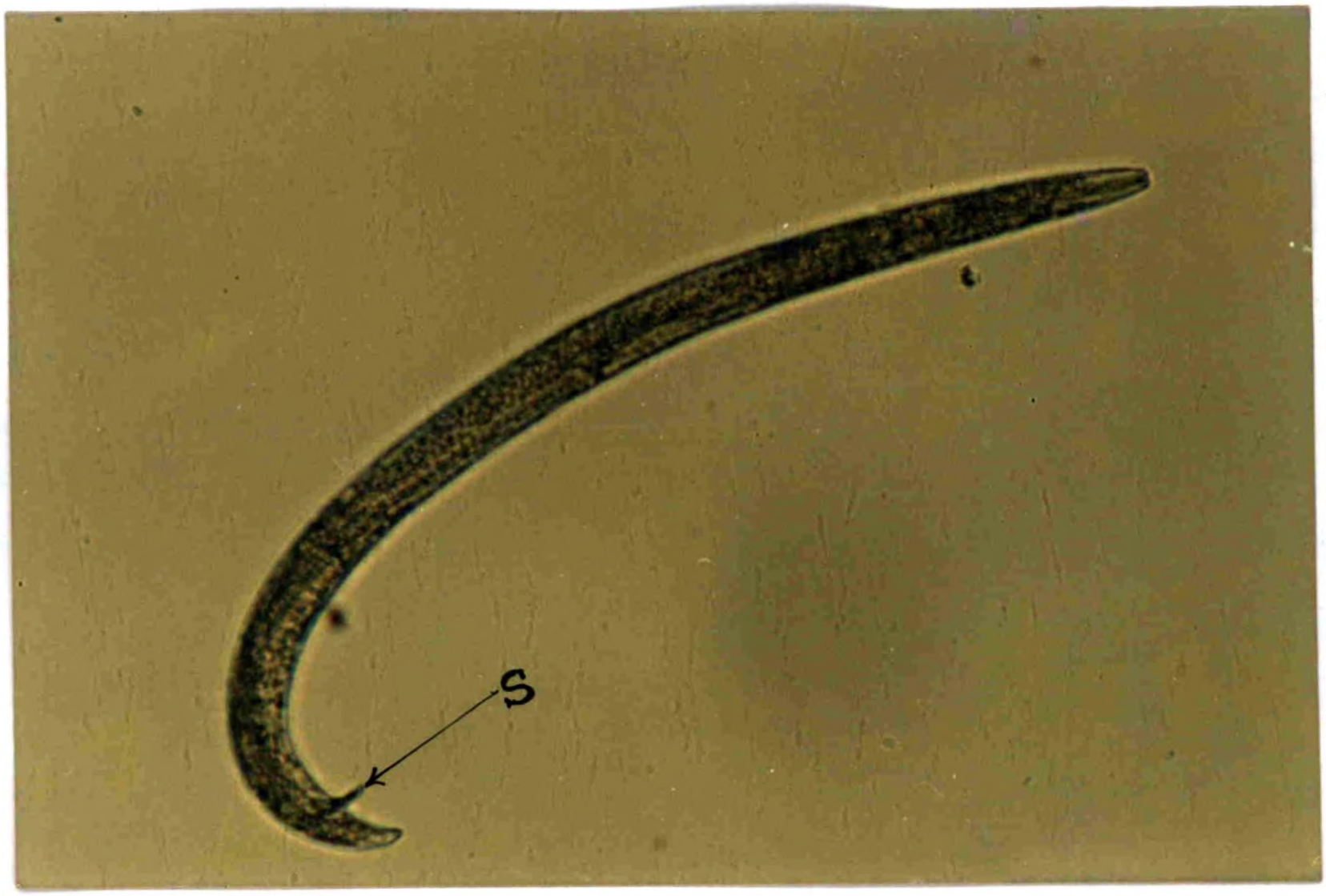
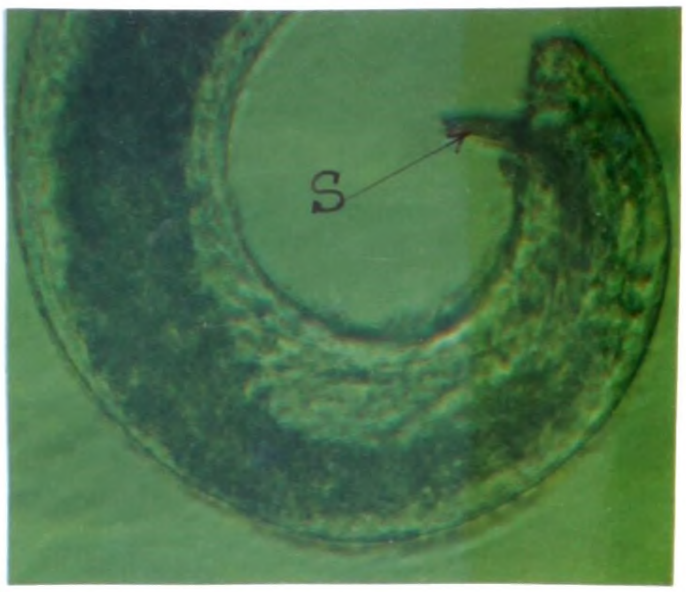


Plate 18: Male of *Steinernema* spp. (isolated from Hungama)(X 100)

S - Spicule



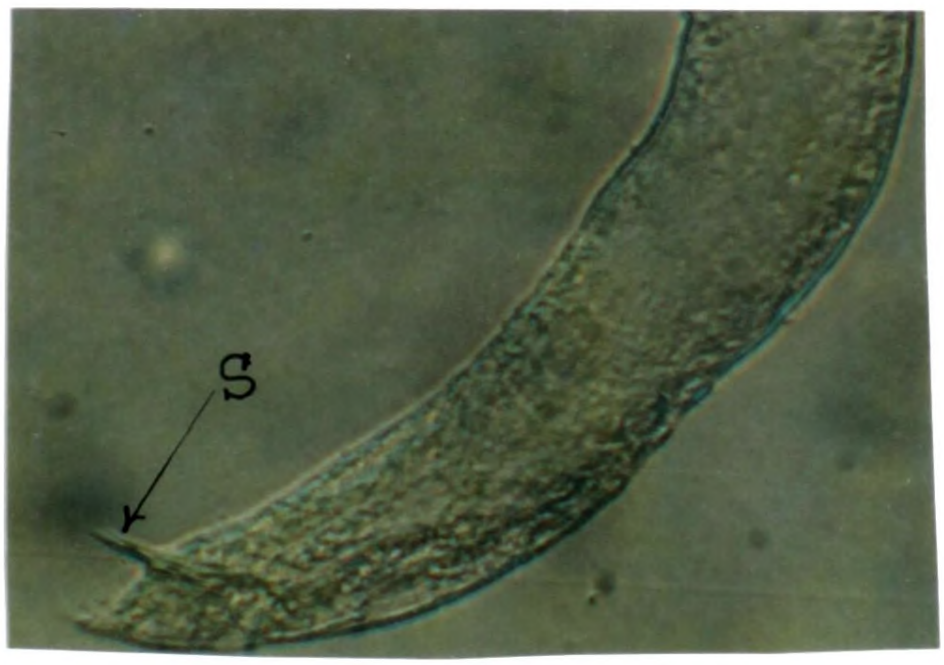


Plate 19: Posterior end of *Steinernema* spp. (isolated from Matara) (X 400)

S - Spicule

The third stage infective juveniles of both *Steinernema* and *Heterorhabditis* did not show conspicuous morphological differences **(Plates 20 & 21).**

Plate 20: Third stage juvenile of *Heterorhabditis* spp. (isolated from Tangalle) (X 200)

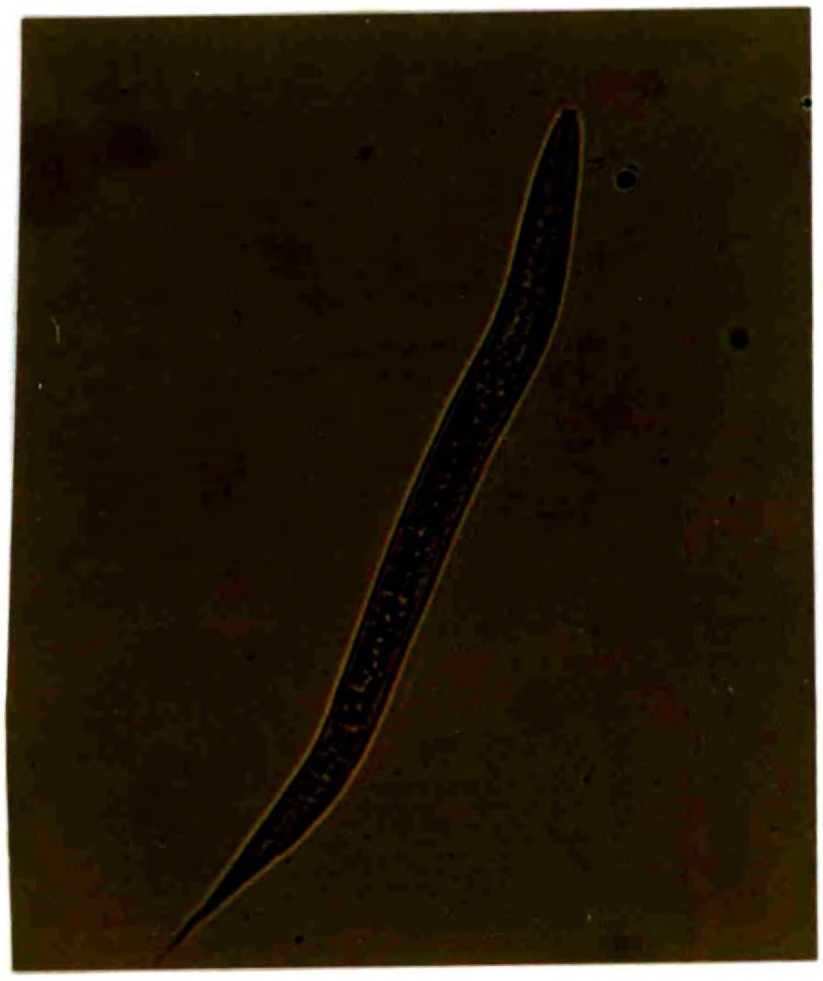


Plate 21: Third stage juvenile of *Steinernema* spp. (isolated from Matara) (X 100)

3.2.2.2 Identification up to species level

The *Heterorhabditis* populations detected from Tangalle, Ahangama and Dickwella were identified using the taxomomical key provided by Stock 1997 (Hominick, *et al* 1997). The dimensions of third stage juveniles of these *Heterorhabditis* population are given in **Table 1.**

Table 1: Dimensions of the *Heterorhabditis* infective juveniles detected from Tangalle, Ahangama and Dickwella (n= 20).

Site	Body length	Tail length	Width	Ratio a	Ratio c	Ratio f
Dickwella	538.67 ± 25.74 (480-586.67)	95.17± 5.87 (86.67 - 110.0)	20.40 ± 4.84 (16-24)	27.75 ± 6.21 (16.67-36.67)	5.68 ± 0.45 (4.80-6.37)	0.21± 0.05 (0.17-0.27)
Ahangama	574.67 ± 19.30 (533.33-600)	98.33 ± 6.62 (83.33-106.67)	24.80 ± 2.46 (24-32)	23.35 ± 2.0 (17.08-25.0)	5.86 ± 0.51 (5.0-7.04)	0.25 ± 0.02 (0.23-0.30)
Tangalle	549.33 ± 40.26 (466.67-640)	100.83 ± 4.82 (90-106.67)	22.80 ± 2.93 (16-24)	24.63 ± 4.71 (19.44-36.67)	5.45 ± 0.44 (4.50-6.40)	4.52 ± 0.78 (3.75-6.46)

Notes:

1. All measurements in microns.
2. Mean ± SE followed by range in parenthesis
3. Ratio a = Total length / Body width
4. Ratio c = Total length /Tail length
5. Ratio f = Body width /Tail length

According to these dimensions of third stage juveniles, all the three *Heterorhabditis* populations could be identified as *H. indicus*. The parameters that lead to this conclusion are as follows:

1. Infective juveniles average total body length > 700 microns
2. Infective juveniles average tail length > 80 microns
3. Male spicules without a rostrum
4. Infective juveniles mean ratio c < 6 microns

However, it is apparent that these dimensions did not remarkably similar between each *Heterorhabditis* population. Many of their ranges are overlapping. The total body length of the Ahangama population is statistically different with that of Tangalle and Dickwella population while there is no statistical difference in total body lengths of Dickwella and Tangalle population.

However, the tail length of Dickwella and Tangalle populations were satistically different. Tail length of Ahangama population is statistically different with that of Tangalle and Dickwella population.

The body width of all the three populations showed statistical difference. Using Students T test the "t" values and significant levels for total body length, tail length and body width of the *Heterorhabditis* population of Tanglle (HT) , Ahangama (HA) and Dickwella (HD) are illustrated in **Table 2.**

Table 2 : **"t" values and significant levels for total body length, tail length and body width of the *Heterorhabditis* populations of HT, HA & HD.**

Feature	Population / Isolate	"t" value	Significant level
Total body length	HA vs HT	2.54	P<0.05(S)
	HA vs HD	5.0	P<0.05(S)
	HD vs HT	1.00	P>0.05(NS)[1]
Tail length	HA vs HT	1.64	P>0.05(NS)[1]
	HA vs HD	1.60	P>0.05(NS)[1]
	HD vs HT	3.52	P<0.05(S)
Body width	HA vs HT	3.05	P<0.05(S)
	HA vs HD	4.07	P<0.05(S)
	HD vs HT	3.66	P<0.05(S)

1 - not significant at 5% level

It is important to compare these dimensions with that of other Sri Lankan *Heterorhabditis* isolates (HSL 6 (ii), HSL 10 & HSL 105) which were recovered from South -West coast (Amarasinghe, *et al,* 1994) as well as with *H. indicus* which was detected from India (Poinar, *et al* ,1992).

The total body length, tail legth and body width of third stage juveniles of **HSL 6, HSL 10, HSL 105** and *H. indicus* (isolated from India) are shown in **Table 3.**

Table 3: **Total body length, tail length & body width of 3rd stage juveniles of HSL 6, HSL 10, HSL 105 and *H. indicus* (isolated from India).**

Isolate/Species	Total body length	Tail length	Body width
HSL 6*	541 ± 3	87 ± 3	18.7 ± 1
	(516-576)	(56 - 97)	(18 -22)
HSL 10*	541 ± 6	92 ± 2	17 ± 1
	(504 - 576)	(70 - 106)	(16 - 19)
HSL 105*	539 ± 5	67 ± 4	20 ± 1
	(516 - 572)	(56 - 82)	(18 -24)
*H. indicus***	528 ± 26	101 ± 6	20 ± 6
	(479 - 573)	(93 - 109)	(19 - 22)

"t " values and significant levels for total body length, tail length and body width of HSL 6, HSL 10, HSL 105 and *H. indicus* with that of HT, HD and HA are shown in **Table 4.**

Table 4: "t " values and significant levels relating to the comparison of total body length, tail length & body width of HSL 6, HSL 10, HSL 105 & *H. indicus* with that of HT, HD& HA.

Feature	Population / Isolate	"t" value	Significant level
Total body length	HA vs HSL 6	7.80	P < 0.05 (S)
	vs HSL 10	7.80	P < 0.05 (S)
	vs HSL 105	8.27	P < 0.05 (S)
	vs *H. indicus*	10.81	P < 0.05 (S)
	HD vs HSL 6	0.41	P > 0.05 (NS)[1]
	vs HSL 10	0.41	P > 0.05 (NS)[1]
	vs HSL 105	0.06	P > 0.05 (NS)[1]
	vs *H. indicus*	1.85	P < 0.05 (NS)[1]
	HT vs HSL 6	0.93	P > 0.05 (NS)[1]
	vs HSL 10	0.93	P > 0.05 (NS)[1]
	vs HSL 105	1.15	P > 0.05 (NS)[1]
	vs *H. indicus*	2.37	P < 0.05 (S)
Tail length	HA vs HSL 6	7.65	P < 0.05 (S)
	vs HSL 10	4.28	P < 0.05 (S)
	vs HSL 105	21.16	P < 0.05 (S)
	vs *H. indicus*	1.80	P > 0.05 (NS)[1]
	HD vs HSL 6	6.22	P < 0.05 (S)
	vs HSL 10	2.41	P < 0.05 (S)
	vs HSL 105	21.45	P < 0.05 (S)
	vs *H. indicus*	4.44	P < 0.05 (S)
	HT vs HSL 6	12.83	P < 0.05 (S)
	vs HSL 10	8.19	P < 0.05 (S)
	vs HSL 105	31.38	P < 0.05 (S)
	vs *H. indicus*	0.15	P < 0.05 (S)
Body width	HA vs HSL 6	14.17	P < 0.05 (S)
	vs HSL 10	11.08	P < 0.05 (S)
	vs HSL 105	8.72	P < 0.05 (S)
	vs *H. indicus*	8.72	P < 0.05 (S)
	HD vs HSL 6	2.22	P < 0.05 (S)
	vs HSL 10	3.14	P < 0.05 (S)
	vs HSL 105	0.37	P > 0.05 (NS)[1]
	vs *H. indicus*	0.37	P > 0.05 (NS)[1]
	HT vs HSL 6	6.26	P < 0.05 (S)
	vs HSL 10		P < 0.05 (S)
	vs HSL 105	4.27	P < 0.05 (S)
	vs *H. indicus*	4.27	P < 0.05 (S)

All the *Heterorhabditis* (HSL 6, HSL 10 & HSL 105) populations previously detected from South-west coast of Sri Lanka did not display similarity in their total body lengths to that of HA population. However, it is peculiar that the total body length of them have close similarity to that of HT population. Total body length of *H. indicus* showed similarity only to HD population. Tail length of HA, HD and HT did not show similarity to that of any of the three HSL isolates. However, a close relationship could be seen between the tail length of both HA and HT to that of *H. indicus*. HD did not exhibit such a relationship. Further, body width of HA and HT have similarity to that of all HSL isolates and HD has similarity only to HSL 105 isolate. Only HT population showed similarity to body widths of *H. indicus* .

3.3 Distribution of EPN along the Southern coast:

Majority of EPN were recovered from the region of 30- 40 m from the tidal zone (0 m) to the interior. Number of soil samples that were positive for EPN in relation to the distance from the sea is shown in **Table 5.**

Table 5: Number of soil samples that were positive for EPN in relation to the distance from the sea

Site	Distance from the tidal zone (m)			
	0m	**10m**	**20m**	**30-40m**
Matara	00	01	04	02
Dondra	02	02	06	06
Weligama	00	01	02	05
Hungama	01	00	01	00
Tangalle	00	00	01	04
Ahangama	00	00	01	02
Dickwella	00	00	00	02
Total	**03**	**04**	**15**	**21**

However, the rate of recovery declines towards the tidal zone as well as towards the dry zone in this survey. PH 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 in all the sampling occations. Hence, their survivability remains still a question at the coastal line.

4. Conclusions drawn and recommendations, if any, for implementation

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 are dominated.

All the *Steinernema* populations that found in this survey were new to exiting pool of EPN. According to the IIP identification the undescribed *Steinernema* isolate detected from Matara and Hungama have close similarity to that of SSL 82 isolate which was recovered earlier (Amarasinghe, *et al*, 1994). This *Steinernema* isolate showed a characteristic behaviour of sudden coiling after a few shakes in water. However, other undescribed *Steinernema* isolate detected from Weligama 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 most probably belonged to the *H. indicus*. However only taxonomical studies may lead to misidentification. Therefore, it is necessary to perform DNA based study for further confirmation.

The results of this survey supports to that of first extensive study carried out in the South - est coast of Sri Lanka regarding the Genera of EPN found (Amarasinghe *et al*, 1994). In this study majority of EPN were recovered from the region of 30 – 40m from the tidal zone to the interior while the first study indicated that there was no obvious relationship

between the presence of these nematodes and the distance from the sea. This contrast may attributed to the site specificity and the season of sampling.

During the study same individual subsites of the major sites have produced both positive and negative results of EPN. This survey has been proved that EPN inhabit in the dry zone of Southern Sri Lanka. 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).

New species and strains of EPN are always being found from the various parts of the world and they exhibit different virulencies against a particular host. Therefore, it is essential to identify our new EPN using molecular biological techniques.

5. (a) Citation of periodicals reporting work done under this contract, giving author, title, journal, volume and page number.

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6. An explanation of any significant departure from the level of activity forseen by the contract

Although the duration of the project is one year it had to be extended beyond that period. The only reason for this delay was atrributed to the difficulties involved in the identification of isolated EPN at IIP, UK in the final part of the research.

D-10 Prevalence and distribution of entomopathogenic nematodes in Dondra coasal belt of Sri Lanka

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Soil samples from 4 sites comprising 16 subsites representing ecologically diverse habitats along the Dondra coastal belt of Sri Lanka were examined to study the prevalence and distribution of entomopathogenic nematodes (EPN) belonging to the Families of Heterorhabditidae and Steinernematidae which have a great potential in controlling number of economically important insect pests, using the *Galleria* baiting technique.

The 4 subsites sampled at 30m from the tidal zone (0m) to the interior were positive for EPN and both *Steinernema* spp. Travassos and *Heterorhabditis* spp. Poinar isolates which are new species to the existing pool of EPN were found. These soils were silt loam soils inhabiting vegetation such as *Ipomea, Cocos nucifera, Pandanus, Calotropis* and common grass. However, no insect species were recovered at the time of sampling.

The results of this study showed that these nematodes could survive in moderately alkaline soils (pH 7.6-8.5) and could tolerate 30°C temperature.

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OCCURRENCE OF ENTOMOPATHOGENIC NEMATODES IN SIX SITES ALONG THE SOUTHERN COASTAL BELT OF SRI LANKA

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The occurrence of entomopathogenic nematodes (EPN) of the families Heterorhabditidae and Steinernematidae was studies over a period of twelve months in six sites representing wet zone (Matara, Dondra and Weligama), intermediate zone (Tangalle) and dry zone (Hungama and Hambanthota) along the Southern coast of Sri Lanka.

Four sub-sites with ecologically diverse habitats were selected at each site. Soil samples were drawn at 0, 10, 20 and 30 - 40 m from the tidal zone (0 m) to the interior in each sub-site along a line transact. Altogether 384 soil samples were assessed on four occasions for EPN using Galleria baiting technique (Bedding and Akhurst, 1975). Nematodes were identified at International Institute of Parasitology, UK, using molecular biological techniques.

38 soil samples drawn from five sites excluding Hambanthota were positive (Dondra 16 / 64 , Matara 07 / 64 , Weligama 08 / 64 , Tangalle 05 / 64, Hungama 02 / 64 ,) for EPN. Dondra yielded *Heterorhabditis indicus* while Matara yielded an undescribed *Steinernema* isolate in addition to *H. indicus*. Another undescribe *Steinernema* isolate which is unique to Sri Lanka was recovered from Weligama. *Heterorhabditis* and *Steinernema* isolates of Tangalle and Hungama are yet to be identified.

Most of the isolates were recovered from the region of 30 - 40 m from the tidal zone to the interior in which vegetation like *Ipomea, Pandanus, Mimosa, Calotropis, Cocus nucifera* and common grass were present. Rate of recovery declines towards the tidal zone as well as towards the dry zone. Positive sites contained moderately alkaline sandy soil. Soil temperature was around 300 C at the time of sampling and insects were not associated with soil samples and vegetation.

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