J.Biol.Control, 7 (1), 24-28, 1993

Biology of *Heterorhabditis indicus* Poinar, Karunakar & David, 1992

G.KARUNAKAR[1], H.DAVID and GEORGE O. POINAR Jr.[2]
Sugarcane Breeding Institute
Coimbatore 641 007

ABSTRACT

The life cycle of *Heterorhabditis indicus* Poinar, Karunakar & David, 1992 closely resembles that of other heterorhabditids. It has two cycles - a hermaphroditic cycle, one with an oviparous generation with a complete set of four juvenile stages and another amphimictic generation with four juvenile stages. These hermaphroditic and oviparous generations are completed in 11-12 days in *Galleria mellonella* (L.) (Gallerlidae:Lepidoptera) at 24°C.

KEYWORDS : *Heterorhabditis indicus*, biology, *Galleria mellonella*

A survey was conducted to find out the natural occurrence of entomophilic nematodes in soils in and around Coimbatore (Tamil Nadu state) in India during March-August 1989 by adopting the techniques described by Bedding and Akhurst (1975) using larvae of the sugarcane top borer, *Scirpophaga excerptalis* Walker (Pyralidae:Lepidoptera) and eggs of the white grub, *Holotrichia serrata* F. as trap materials. During this survey a new species of *Heterorhabditis* was isolated from a soil sample collected in Ramanathapuram village near Coimbatore and subsequently described by Poinar *et al.* (1992) as *Heterorhabditis indicus* Poinar, Karunakar & David 1992. Four species of *Heterorhabditis* have been described so far. These include *H. bacteriophora* (Poinar, 1975), *H.megidis* (Poinar *et al.*, 1987), *H. zealandica* Poinar, 1990 and *H. indicus* (Poinar *et al.*, 1992).

A detailed study was carried out on the biology of *H.indicus* at the Sugarcane Breeding Institute, Coimbatore.

MATERIALS AND METHODS

The population of *H.indicus* used for this study came from the original isolate which was subcultured on greater wax moth larvae, *Galleria mellonella* (L) and as per the basic *in vitro* production method outlined by Woodring and Kaya (1988). Observations on the life cycle of the nematode were made with infected 5th instar larvae of *G.mellonella* held at 24 ± 1°C. The infected hosts were obtained by placing 10 larvae in each of the Petri dishes infected with 200-400 infective juveniles per Petri dish. At 24 hours intervals 8 larvae were dissected and examined for the developmental stages of the nematode. The experiment was repeated twice and final observations were based on analysis of the combined results.

Observations on the fecundity, time requirement for moulting and duration of each stage were made by placing 10 IJ and first generation hermaphroditic forms on separate cavity slides containing insect haemolymph. Fresh haemolymph of wax moth larvae was added every 12 hours' interval with a hypodermic syringe. These slides were kept on moist cotton in a Petri dish and covered with another larger Petri dish to prevent evaporation.

RESULTS AND DISCUSSION

Observations on the life cycle of *H.indicus* in the host *G.mellonella* showed the following distinct stages.

1. Kallakurichi Co-op. Sugar Mills Ltd, Vadamathur Village, Kanji
2. University of California, Berkeley, California - 94720

Table 1. Survey for entomophilic nematodes

Location of survey	Period of survey	No.of surveys	Trap hosts used	No.of trap hosts/ survey	Details of entomophilic nematode infection
I. Sugarcane Breeding Institute					
A. Additional area - Field No.28	March-Aug. 1989	20	Larvae of sugarcane top borer, or fresh eggs of white grub	20 larvae or 20 eggs	No infection
- do -	March-Aug. 1989	20			
- do -	March-Aug. 1989	20			
B. Main farm - Field No.19	March-Aug. 1989	3			
21	March-Aug. 1989	3			
C. East Chithirai Chavadi (ECC) Farm	June-Aug. 1989	3	-do-	30 larvae or 30 eggs	No infection
D. Vedapatti (VPT) Farm	June-Aug. 1989	3			
II. Anaikatti hills	Aug. 1989	1	Larvae of sugarcane top borer, + fresh eggs of white grub	40 larvae + 40 eggs	No infection
III. Ramanathapuram village	Aug. 1989	1	Larvae of sugarcane top borer	20 larvae	Four larvae infected by *Heterorhabditis indicus*

L_2: Developmental stage which can develop into the third developmental stage or infective juveniles.

L_3: Free living infective third stage juvenile, characterised by heavily sclerotised dorsal tooth.

L_4: Fourth larval stage or pre-adult showing absence of Sclerotization.

Female: characterised by female sex organs and the presence of vulva.

L_1: First larval stage characterised by its small size.

Male L_4: Pre-adult male characterised by developing ventrally located testis.

Male: Characterised by developing male organs and the presence of spicules.

The life cycle of *H.indicus* is schematically represented in Fig.1 as it took place in *G. mellonella*. The life cycle showed that this nematode has a heterogonic cycle with a hermaphroditic oviparous (short generation and

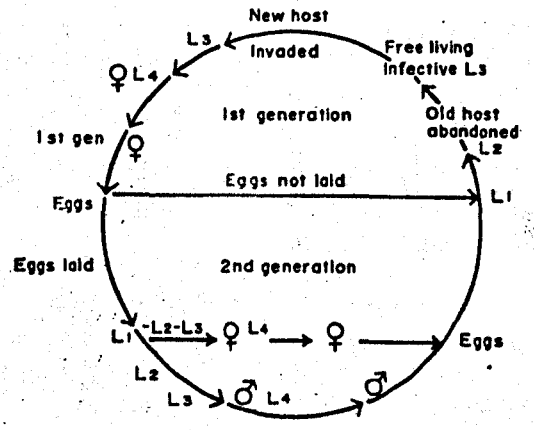

Fig.1 Schematic representation of life cycle of newly isolated *Heterorhabditis indicus* P.K. and D. in *G.mellonella*

Table 2. Life cycle of *Heterorhabditis indicus* in *Galleria mellonella* at 24°C

Days after death of host	Stage of nematodes
1 - 2	Third and fourth stage juveniles
3 - 4	Pre-adult and mature, pre-adult male and females (Second generation)
5 - 6	Giant females, pre-adult male and females (Second generation)
7	Giant females with juveniles, mature females and males. Early stage juveniles
8 - 9	Large number of females and dead males with eggs and juveniles
10	Infective juveniles start emerging from host

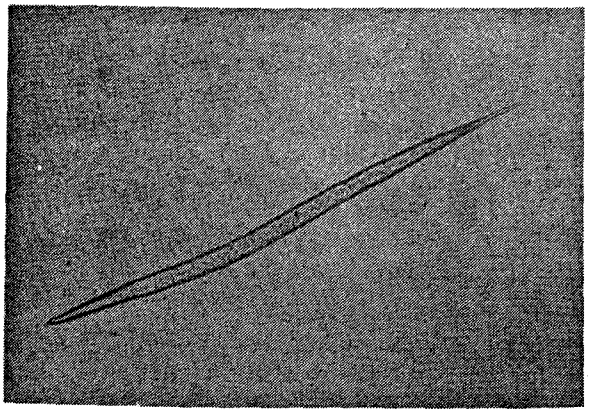

Plate 1. *H.indicus* - rosette formation

an amphimictic ovoviviparous (long) generation. Live IJ of this nematode frequently formed rosette aggregations in culture suspension (Plate 1). As many as 107 nematodes were fastened by their tails in such an aggregation and these individuals remained infective.

Penetration of the host took place through the spiracles, mouth and anus and the host larvae contained IJ throughout its body length within two hours after inoculation. The host larvae turned reddish to brick red in colour 36-48 hours after infection and the colour intensified progressively. The L_3

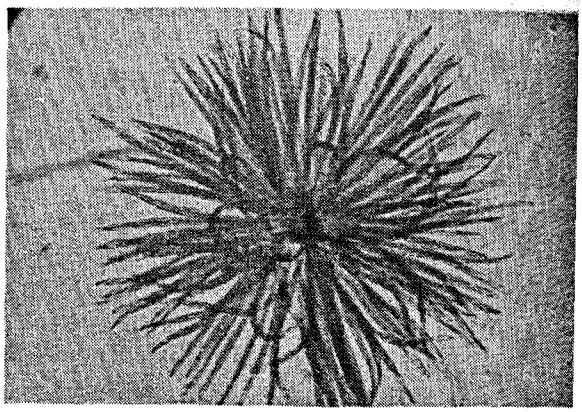

Plate 2. L3 stage of *H.indicus*

(Plate 2) moulted soon after penetration. These juveniles developed in the haemolymph, increased slightly in length, doubled in width and usually moulted in 17-30 hours. The L_4 increased in width and doubled in length. The first generation hermaphroditic forms were observed in the host about four days after penetration, as the L_4 moulted in 24-36 hours. It reached 5.7 mm in length and laid a maximum of 1215 eggs in its life span of three days. Within two hours of egg laying, embryonic development was observed and hatching of L_1 took place in 6.45 to 8.30 hours. The egg laying pattern was found to be erratic and it did not occur at regular intervals.

More number of eggs were laid when the population density in the host was low. Each egg measured 57 μm in length and 27 μ m in width (Plate 3). As the population density increased, eggs remained inside the female body, where they developed (*endotikia*

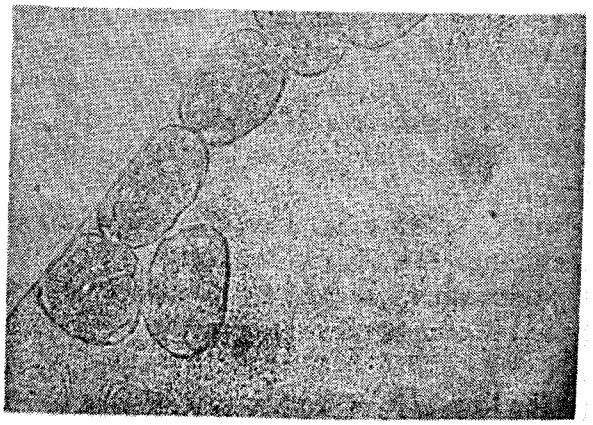

Plate 3. Egg stage of *H.indicus*

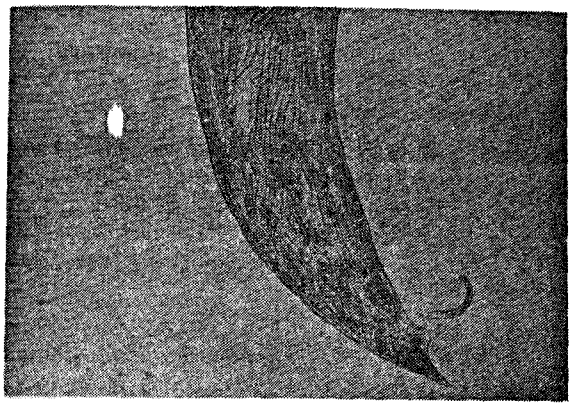

Plate 4. *Endotikia matricida*

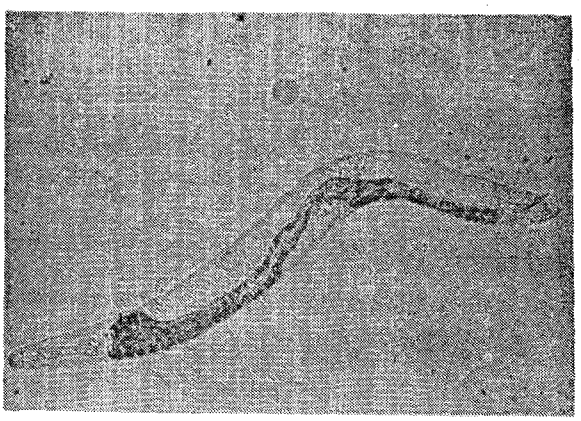

Plate 5. Male of *H.indicus*

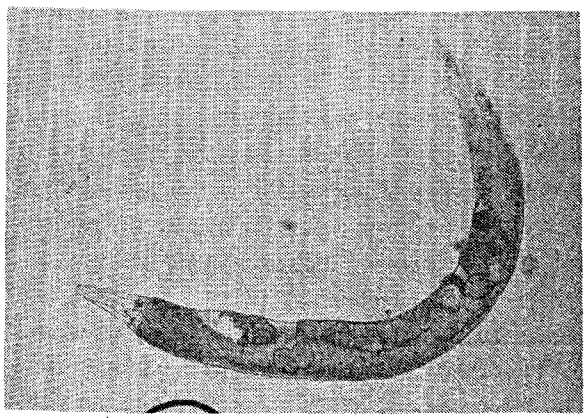

Plate 6.Female of *H.indicus*

matricida) (Plate 4). Whether the eggs were laid or retained within the body, they developed rapidly into males (Plate 5) or females (Plate 6) of the second generation. When the quantity of food decreased in the host larvae, L_2 from laid eggs developed into L_3 and stopped further development and the first generation females ceased egg-laying. L_1 from unlaid eggs developed inside the body of the female generaly upto L_3. In rare instances males and females were observed developing inside these hermaphroditic females.

Irrespective of stages, moulting took place in 2-3 hours. Males were observed about a week after penetration into the host. About 11 to 12 days after infection of the host, the IJ came out of the infected larvae.

The life cycle of *H. indicus* has much in common with the other species of *Heterorhabditis* as seen in the description and biology of *H. bacteriophora* (Poinar, 1975) by Khan *et al.* (1976) and Wouts, (1979). The infectives of *H. zealandica* enters through the mouth, spiracles and anus within 2 hours of exposure as the infectives are present in mouth, anterior portion of the digestive system, head and hind part of the body cavity of the host (Wouts, 1979) similar to *H. indicus*. Within 36-48 hours of exposure to infectives, the host larvae die and the dead larvae turn brick red in colour. Khan *et al.* (1976) reported that *H. bacteriophora* - infected larvae turned deep red, while it was yellow or pale orange in the case of *H. zealandica* (Wouts, 1979).

Like other heterorhabditids, this species also has two distinct generations - an oviparious (short) generation with amphimictic females and males as reported by Li *et al.* (1986), Doucet and Poinar (1985) and Poinar (1990). Since the first generation is hermaphroditic, a single invading juvenile is sufficient for multiplication. Their establishment in the host is more or less ensured, whereas at least two IJ (a male and female) must enter in the case of *Steinernema.*

ACKNOWLEDGEMENT

Grateful thanks are due to Dr.K.Mohan Naidu Director, and Dr.K.C.Alexander, Head, Division of Crop Protection, Sugarcane Breeding Institute, Coimbatore for giving all the necessary facilities for this study.

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