Entomology

Development of *Hexamermis vishwakarma* Dhiman (Nematoda: Mermithidae), a parasitoid of *Leptocoris augur* (Fabr.)

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Abstract

Leptocoris augur, a well-known pest of the "Kusum" plant *Schleichera oleosa* Lour, is endoparasited by *Hexamermis vishwakarma* Dhiman. Using a two-tray technique, this worm has been successfully raised in a lab to learn about its biology. The five stages of this parasitoid's life cycle are eggs, pre-parasitic juvenile, parasitic juvenile, post-parasitic juvenile, and adult. Within 20 to 72 hours of the male and female post-parasitic nematodes mounting into adults, copulation takes place, lasting approximately 30 minutes, according to the biology of *H. vishwakarma*. After 10 to 25 days, ovipositions start on average 14 days after copulation. They produce 40 to 60 eggs per day for approximately a week, following which a few days must pass. Approximately 1200 to 1600 (on average, 1400) eggs are deposited during the course of 40 to 50 days. Internal fertilization occurs in the oviduct, which is the upper portion of the uterus. The incubation period, which lasts from July to September during the wet season, averages 16.5 days but can range from 14 to 20 days. The process of "spontaneous" egg hatching takes roughly 13 minutes on average, with an average of 1.5 minutes. The pre-parasitic juvenile enters the surrounding water after hatching. Its length ranges from 0.25 to 0.80 mm (average of 0.70 mm); they can live for three to five days without the host or culture media. Within 15 to 4 hours, the pre-parasitic juvenile enters the host bug and begins consuming nutrients from the adipose tissue, muscles, and haemo-coelomic fluid. As it grows in size, it is referred to as a "parasitic juvenile."

The size of parasitic- Juvenile juvenile parasites range in length from 12.0 to 194.0 mm and breadth from 1.25 to 2.25 mm. With an average of 20.5 days, the duration from penetration to emergency is estimated to be between 18 and 22 days. Parasitic juveniles are now known as "post-parasitic juveniles" once they emerge. While female nematodes are 52.0 to 194.0 mm in length and 2.00 to 2.25 mm in width, males are smaller, measuring 12.0 to 49.0 mm in length and 1.25 to 1.40 mm in width. Finally, within 17 to 30 days (on average, 24 days), post-parasitic juveniles moult in two adults. Adults resemble post-parasitic nematodes, with the exception that their vulva, spicules, and other features are completely formed. Furthermore, depending on changes in temperature and relative humidity as well as the condition of post-parasitic nematodes, the lifespan of male adults of *Hexamermis vishwakarma* ranges from 30 to 40 days, while that of female adults varies from 40 to 50 days.

Keywords: *Hexamermis vishwakarma* Dhiman, *Leptocoris augur*, Mermithid, Biology, Biocontrol agent

1. Introduction

Entomophilic nemetodes are extremely adaptable and effective against a wide variety of insect pests. They have the potential for biocontrol and are environmentally beneficial. *Hexamermis vishwakarma* an endoparasite of *Leptocoris augur*, a renowned pest of the "Kusum" plant, Lour, is a mermithid nematode. It feeds gregariously on the falling seeds of Kusum plants (Dhiman and Gulati 1986) [1], and Dhiman observed that it is an endoparasite of *L. augur* Fabr. (Dhiman 1984) [2]. Though there are few study articles on other mermithids, research on entomophilic nematodes is still in its infancy. However, there is limited material accessible on Hexamermis sp. by Williams *et al.* (2001) [3], Luckmann and Poinar (2003) [4], and Arijit and Satpathi (2005) [5]. On *H. vishwakarma*, good work has been done (Dhiman and Kumar, 1996a) [6]. We don't yet know the biology of this possible biocontrol agent. As a result, the current research describes the biological events in detail, equipping the economic entomologist to raise this parasitoid in large quantities in a lab.

2. Materials and Methods

Parasitized insects were collected from the HRI and Training Center Saharanpur fields. This worm was successfully raised in a lab using a two-tray technique in order to learn about its biology. The technique outlined by Dhiman and Uzma (1993) [7] was used to continue rearing *H. vishwakarma*. In a lab, copulation and oviposition were examined using a binocular microscope.

3. Observations and Discussion

Eggs, pre-parasitic juvenile, parasitic juvenile, post-parasitic juvenile, and adult are the five phases of *Hexamermis vishwakarma's* life cycle (Plate-8). The adult stage, which is free-living and non-feeding, occurs in most soils between 8.0 and 10.0 cm below the surface, depending on the soil's moisture content and temperature.

3.1 Copulation

Copulation and oviposition take place in adults in coiling stage in soil. For copulation high humidity (80-100%) is required.

Adult male bears one pair of spicules (copulatory organ) (Plate–6 and Plate-7). While female bears vulva like other nematodes. Male bears sensory organs (amphids) at cephalic end which help in locating the female in moist soil (Plate-4). On feeling the presence of female in nearly vicinity the male moves towards it and finally get success in it. On finding

female, the excited male moves its body attaching with female. This process last for few minutes to hours. Generally, 5 to 15 minutes are taken. By this act male locates vulva of the female and tries its protruded spicules (one or both) to insect in the vulva of female. On getting success, vulva is opened spicules are inserted into vagina and spermatic fluid is injected in it by force. Copulation stage last four half an hour generally retracts its spicules. The male, now, separates off retracts its spicules and moves away of female without showing any affection. After making the same female by water proofing ink, further possibility of copulation was observed. it has been seen that the female mated several times (Plate-1d). During copulation spicules are used to spread open the female gonopore (vulva)

3.2 Fertilization

During population tailless sperm amoeboid are regulated into the vagina of female. The sperms by performing amoeboid movement or wrangling movement travel to over duct or lower part of uterus. Where they fertilize eggs. The fertilized eggs are collected into uterus before we position in large number. Thus, fertilization is internal (Plate-1e).

3.3 Oviposition

During oviposition mature eggs move towards the vulva of female by the Paristaltic movement of uterine wall. Humidity is very essential for oviposition. *20* ± 2^0C temperature and 80-100% R.H. is required for opposition. In field of oviposition frequently occur during rainy months, from July to September. A pre-oviposition period of 10-26 days with an average of 17 days is required prior to egg laying. Eggs are laid at a rate of 40 to 60 per day, for about a week and then a gap of few days is necessary oviposition lasts for 40-50 days during which about 1100-1600 eggs with an average of 1360 eggs are deposited. Unfertilized females also lays egg which develop parthenogenetically. In laboratory rearing, eggs were also seen releasing from the body wall by rapturations. At many places, body wall ruptures and eggs are set free in soil or in petridish in lab.

3.4 Egg structure

Newly laid eggs of *H. vishwakarma* are almost special and dull milky white. Their diameter varies from 0.033 to 0.040 mm with an average of 0.039 mm. Eggs are dull transparent and free of the gelatinous coverings. The egg chorion of newly laid egg is slightly soft which gradually changes into the hard covering or coat which is rough externally having many blunt protuberances (Plate-1).

3.5 Incubation period

It varies according to temperature and humidity and hence, rainy, winter and summer seasons effect incubation period greatly. During rainy season, July to September, minimum incubation period of 14 days and maximum of 20 days is required. Egg chorion being hard and cuticular protects it from unfavourable condition.

3.6 Hatching

Hatching process last for about 1 to 3 minutes with an average of 1.5 minutes. It does not occur in winter and summer seasons. During hatching the Larva (pre- parasitic Juvenile) also moults (Plate-2).

3.7 Migration

Pre-parasite migrates to soil surface on host plant. Pre- parasite hatch in large number (up to few hundreds). Their length varies from 0. 25 to 0.80 mm with average length of 0.07 mm and width 0. 40 mm.

3.8 Penetration

Pre- parasitic Juvenile actively move for three to five days prior to penetration in the host bug. Penetration (in laboratory condition) lasts for 15 minutes to 4 hours. After entering the host bug's body cavity, they grow larger and form numerous coils while feeding on adipose tissue, hemocoelomic fluid, and body muscles, among other things. At this point, the juvenile is said to as "parasitic." (Plate-8).

3.9 Development of Parasitic juvenile

The size of parasitic Juvenile varies from ***12***.0 to ***194***.0 mm in length and 1.80 mm in width. The parasitic Juvenile emerges out as post parasitic stage from the different parts of the host body after completion of its parasitic life. The emergence points are external genitalia, tergal joints, pleuron membrane of abdomen, cervical membrane of neck, coxal joints and thorax, wing auxiliaris and abdominal sternite joints (Plate-3). For complete emergence 2 to 8 minutes with an average of 5.1 minutes are required. The time taken from penetration of Pre parasitic nematode into the host to emergence of parasitic nematode is calculated 18 days to 22 days with an average of 20.5 days (Fig. 1).

3.10 Emergence

(Plate-1a, b) After emergence parasitic juveniles now termed as “post parasitic juveniles”. Migration of post parasitic Juvenile occurs into soil to parasitic moults into adult. Male measures 12.0 to 49.0 mm in length and 1.25 to 1.40 mm in width, with an average length of 25.0 mm and width 1.30 mm while female nematodes extend 52.0 to194.0 mm in length and 2.o to 2.25 mm in width, average being 114.0 mm in length and 2.14 mm in width. Spicules of male nematode and vulva and vagina and other structure are found clearly visible in development and developing stage. After emerging out the post found clearly visible in developing stages after emerging out, the post parasitic juveniles reach in soil under 3 to 10 depths in field and remain in coils for moulting into adult. Similar to the post parasitic juveniles’ adults do not require any feeding material (Plate-4,5,6,7).

Doucet, *et.al.*, (1998) [8] mentioned that in *Agamermis decaudata* parasitizing *Castinia dedalus* (host) in Argentina male was larger than female.

At emergence, a hole is created by the Juvenile form in the host body and if more than one nematode emerges out from the host body then the number of holes increasing accordingly. Through, one or rarely two nematodes emerge out (Paine and Mullens, 1996) [9], also observed that a single nematode. Biological work by mermithid nematodes has been done by various workers, viz., Simmons, and Edman (1978) [10]. Poinar, and Otieno (1974) [11] mentioned the four moults in the Mermithidae. The haemocoelomic fluid start seeping out through the holes and the insect become quite sluggish. It dies within 2 to 25 minutes with an average of 15 minutes after the emergence of the nemas. Juvenile forms have also been found in the body cavity of the dead host when dissections were

carried out under binocular microscope. Thus 100% mortality of the host is caused.

Generally, emergence occurs on soil surface but in few cases, emergence has been seen on the leaves of nearby host a shelter plant or goes for oviposition, such emerged parasitic stages if not fall down on the ground, dries up.

In this way *Hexamermis vishwakarma* completes its life cycle (Fig-1 and 2b) in five stages, eggs, pre- parasitic stage, parasitic stage, post parasitic stage and adult (Dhiman and Kumkum, 2005) [12].

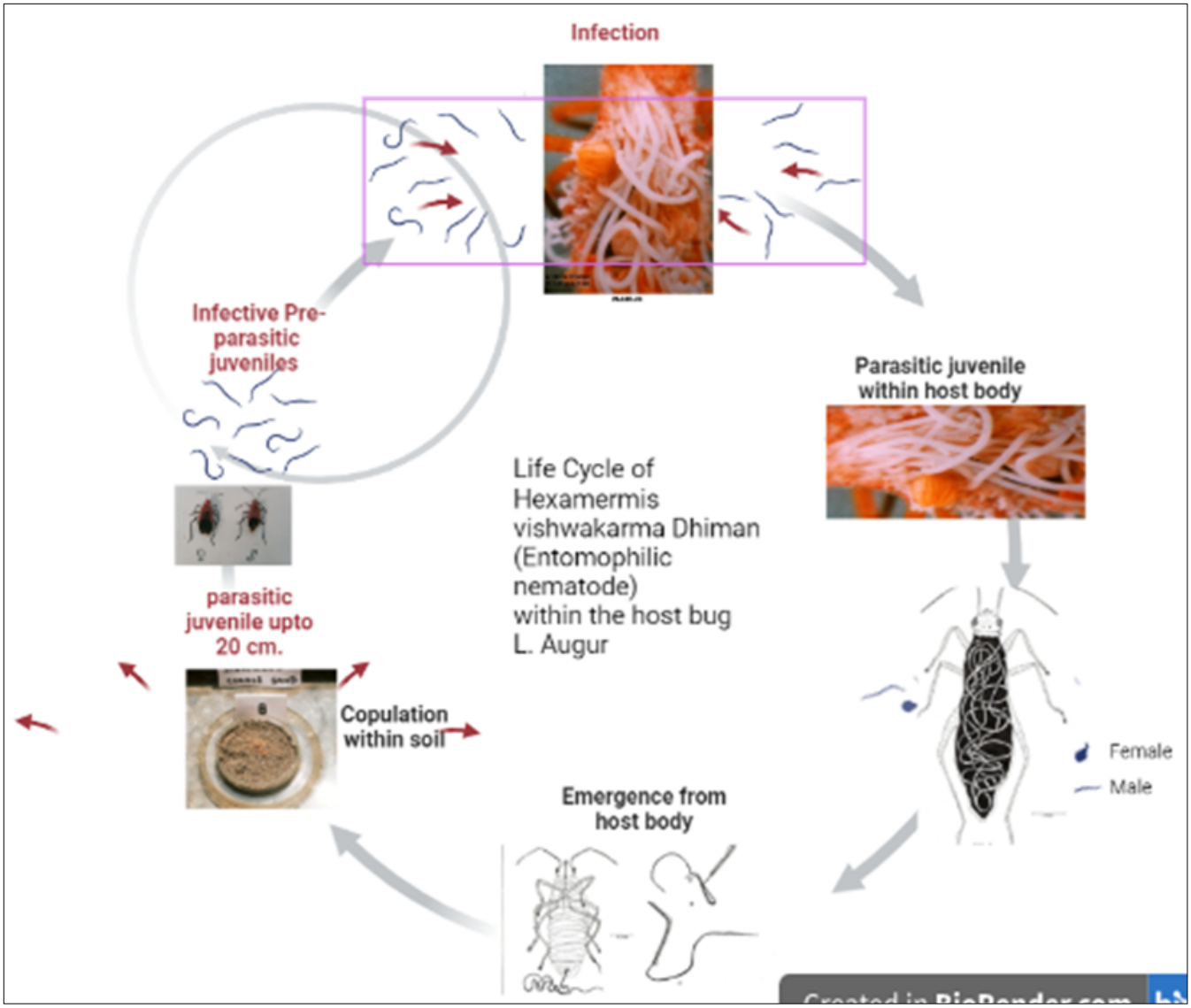
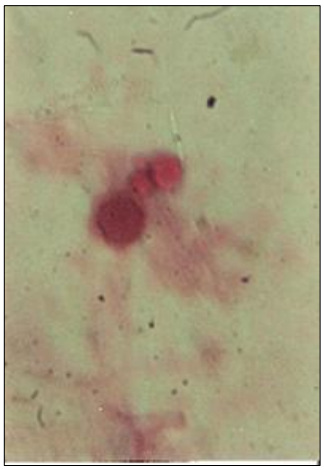
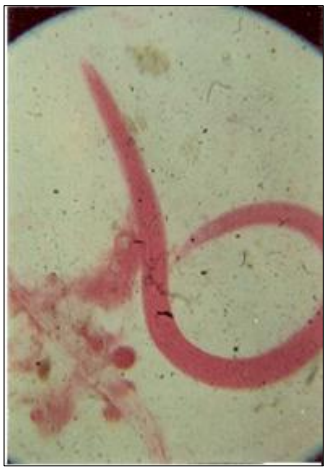


Fig 1: Life cycle of of *Hexamermis vishwakarma* Dhiman in host (*L. augur*) bug and in soil

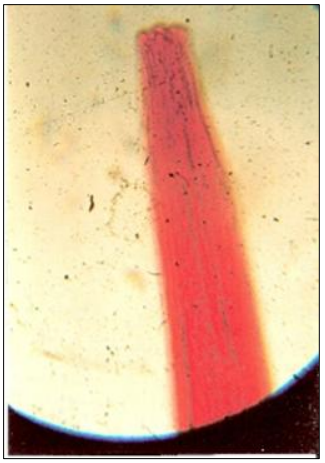


(1) Egg structure



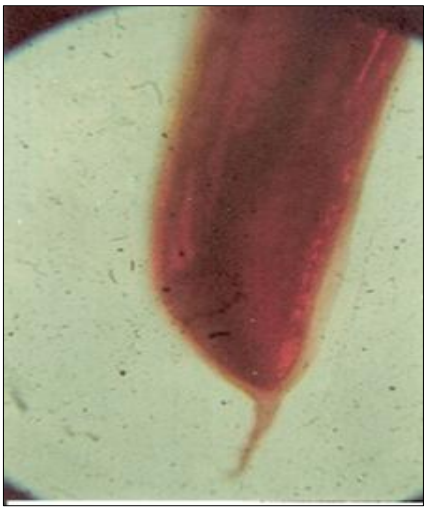
(2) Pre-parasitic stage

(3) Parasitic juvenile in Host Body

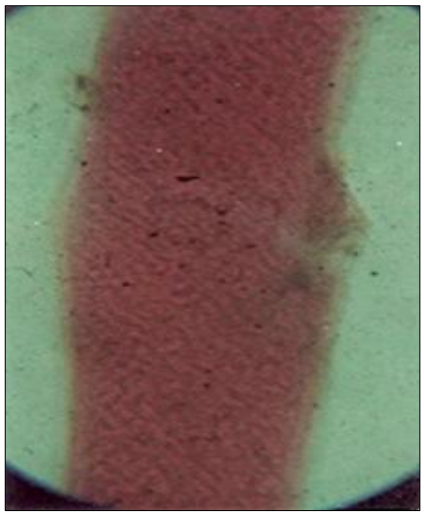


(4) Cephalic end

(5) Mid body part



(6) Caudal end of Male part



(7) Vulva of female

(8) Prarasitic juvenile

(9) Emergence of Parasitic stage through anus of *L. augur*

(10) Emergence of Parasitic stage thgrough axilliary part of *L. augur*

(11) Host Bug *Leptocoris augur*

(12) Copulation of Parasitic juvenile of male and female (13) Fertilization of Parasitic juvenile of male and female occurs in soil

Fig 2a: Different stages of *Hexamermis vishwakarma* Dhiman

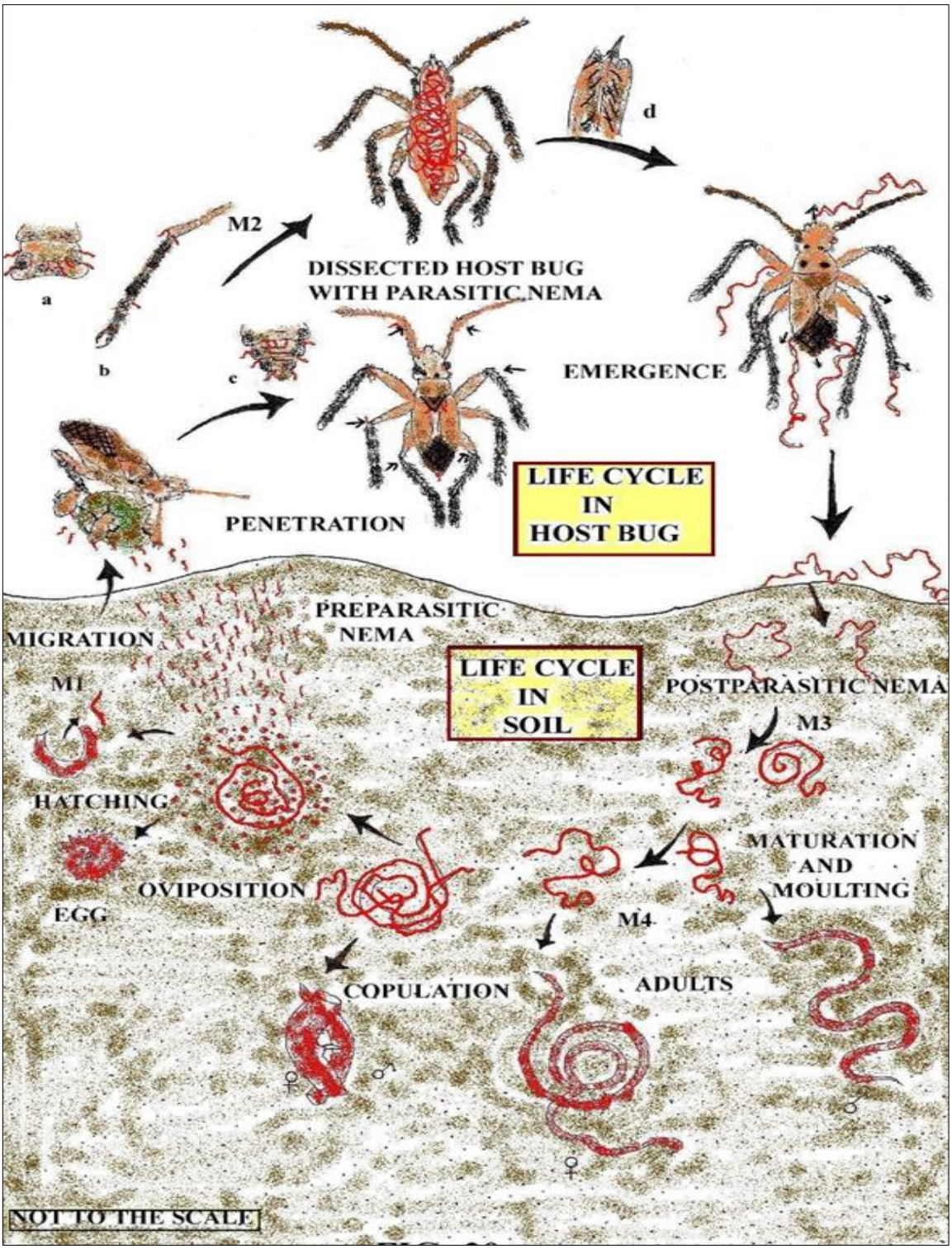


Fig 2b: Diagrammatic representation of Life cycle of of *Hexamermis vishwakarma* Dhiman in host (*L. augur*) bug and in soil

3. Conclusion

Thus, the entomophilic mermithids offer a promising potential for insect control and these nematodes have been used in different parts of worlds as biological insecticides. However, it seems that the economic benefits of mermithids in controlling insect pests have not yet been completely recognized in India. Experiment and field trials to control the *Leptocoris augur* (Fabr.) by using *H. vishwakarma* has yielded significance success. Hence, its use as a bicontrol agent is suggested.

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