Penetration and Emergence behavior of *Hexamermis vishwakarma* Dhiman (Nematoda:Mermithidae), a parasitoid of *Leptocoris augur*.

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Abstract

***Leptocoris augur** is a pest of Kusum plant, (**Schleichera oleosa**), which in turn is a host of Lac insect. This bug is a gregarious feeder and by its desapping habit loss the viability of seeds (Dhiman and Gulati, 1986). It is parasitized by a mermithid nematode, Hexamermis vishwakarma Dhiman, which naturally checks rapid built up of bug population.*

Emergence, of parasitic juveniles occurs through weak body point having thin arthrodial membranes such as tergal joints, pleuron membrane of abdomen, cervical membrane of neck, coxal joint with thorax, wing axiliaries and genitalia. Least preferred points include occular sclerite, antennal socket, scape and pedical joint of antenna, anal segments (post genital segments) and anal aperture (Figure 1). These points are selected randomly by trial-and-error pattern by the parasitic stage. Once parasitic development is completed, then this stage has to come out of the host body to enter into soil for moulting into adult to resume next generation.

***Key words:** Leptocoris augur, Hexamermis vishwakarma, Emergence behaviour, Parasitoid.*

1. Introduction

Leptocoris augur) is a pest of Kusam plant, (***Schleichera oleosa***), which in turn is a host of Lac insect. This bug is a gregarious feeder and by its desapping habit loss the viability of seeds (Dhiman and Gulati, 1986). It is parasitized by a mermithid nematode, *Hexamermis vishwakarma* Dhiman, which naturally checks rapid built up of bug population. Emergence, of parasitic juveniles occurs through weak body point having thin arthrodial membranes such as tergal joints, pleuron membrane of abdomen, cervical membrane of neck, coxal joint with thorax, wing axiliaries and genitalia. Least preferred points include occular sclerite, antennal socket, scape and pedicel joint of antenna, anal segments (post genital segments) and anal aperture. These points are selected randomly by trial-and-error pattern by the parasitic stage.

In India, the reports on endoparasitic nematodes appeared as early as 1927 about mermithids from *Anopheles* mosquitoes. Reports of mermithids on different hosts were continuously reported since then, ***H. vishwakarma*** **Dhiman** causing 88% mortality of *L.augur*,

Kusam bug in natural condition during rainy season, June to August and cent-percent mortality in Laboratory condition was reported.

2. Materials and Methods

Collection of parasitized bugs, parasitized nymphs and adults of host, *L. augur*, were collected along with fresh leaves and seeds of kusam plant by hand picking method from the field (Horticulture Experiment and Training Centre, Saharanpur) in polyethylene bags during rainy season, July to September. The specimens were brought to the laboratory at atmospheric temperature and humidity and restored in hurricane glass lamp chimneys.

Collected bugs were reared in lab, in hurricane glass lamp chimneys (24×36 cm) covered at top by fine muslin cloth. Fresh food supply was maintained daily and stale food was replaced. To maintain necessary relative humidity (70%), a cotton swab was placed in a water filled glass vial and temperature was maintained at 28°C. The emergence of parasitic nematode, *Hexamermis vishwakarma* was keenly observed and rearing petridishes were examined daily. This is the post-parasitic stage of emerged nema. Dial hygrometer and field thermometer were used for R.H. and temperature measurements.

Emerged post-parasitic nematodes, were collected with the aid of fine camel hair brush and were kept into the petridishes containing clean, sterilized coarse sand and then water was filled to submerge the nematodes in petridishes. Now each petridish was placed into another big petridish filled with water, to avoid escape of post-parasitic nematodes as well as to prevent the entry of ants, *etc.* (Double tray system).

3. Results

The mermithid nematode, *H. vishwakarma* passes through 3 stages prior to become adult, *i.e.,* pre-parasitic, parasitic and post-parasitic. Among these, only parasitic juvenile is the feeding stage which parasitizes the host bug, *L. augur* and rest stages are nonfeeding. Hence, following account pertain only to this stage under the penetration and feeding behaviour. It is being described here pointwise in a systematic way.

3.1 Penetration behaviour: – For the biological control of *L. augur*, pre–parasitic juvenile of *H. vishwakarma* first enters in the body cavity of the bug and then settles, in it. They are minute and measures 0.25 to 0.80 mm in length and 0.04 mm in width. Body is transparent white in colour and their internal structure clearly visible under the microscope mode of penetration is presented in pointwise as follows –

1. Pre–parasitic juvenile (infective stage) moves actively in search of a suitable terrestrial host (*L. augur*). The host feeds gregariously on the fallen seeds of kusum tree on the soil and became easy target (Figure- 1).
2. uefree living pre–parasitic juvenile is positively phototropic and come out in large number from the moist soil after hatching from the eggs.

3. In field, during rainy season (July to August) when plants are covered with a layer of moisture, these also move up on the plants upto certain length with water film performing undulating movements to infest host feeding on the foliage.

4. The infective juvenile penetrates the host with the help of a mouth stylet, through the weak sclerotized parts of the host body. The weak body parts through which penetration occurs include coxal joints of legs, under surface of wing pads near joint (in 3^{rd} to 4^{th} instar nymphs), (Figure- 1) genital region, pleuron, joints of abdominal sternite and tergal plates, cervical membrane and wing axillaries.

The stylets are used during penetration. These are hard, cuticular and pointed structures occurring in a pair, slightly protrude out through mouth. An aperture is created in the arthrodial membranes of joints through which preparasite penetrate inside the host body by performing undulating or wriggling movements. The entire body of the pre–parasite enters inside the host within 2 to 15 minutes, average being 5.4 minutes.

5. Most preferable penetration points have been observed as wing axilliaries, external genital part and cervical membrane.

6. Super-parasitism was observed as several pre-parasitic juveniles enter the same host. But these do not grow longer and generally more males are produced.

7. Second moulting occurs within the haemocoel of the host (*L. augur*) after penetration, and changes into the parasitic form.

8. The morphology and function of the esophagus of mermithids differ from that of all other nematodes and reflect the unusual habitat of this group of nematodes.

9. The digestive system consists of onchiostylet, pharyngeal tube, stichosome and intestine.

10. Each stylet is pointed out posteriorly it becomes stronger.

11. On the anterior portion of pharynx there are two penetration glands, two rows of stichocytes as accessory structures are present. Each stichocyte row contains glandular cells or stichocytes with prominent nuclei and nucleoli.

12. Intestine of pre–parasitic juvenile is jointed to the base of stichosome by several cells. This connection ruptures soon after the pre-parasitic stage enters the host's haemocoel and the intestine grows anteriorly and overlaps the stichosome. In the artificial culture, the connective ruptures as the pre–parasites start feeding.

13. The anterior half of intestine generally contains fluid while the posterior part has large cells filled with droplet and Glycogen particles (Trophosome).

14. The muscular system of pre-parasitic juvenile is well developed.

15. Tail is pointed, narrow and stout, move rapidly in water, working as a propelling organ.

16. Genital primordium represented by a group of cells is situated on the ventral body wall just posterior to the junction of the stichosome and intestine.

17. In laboratory, penetration lasts for 15 minutes to 4 hours and after that the pre-parasite settles in the body cavity. The initial growth is fast.

During the host searching, sensory organs of the pre-parasites play a vital role because sensory organs such as amphids and phasmids present in the anterior and posterior region of the body can detect the presence of host in nearby vicinity.

During their movement, the parasitoids come in contact with the host and when touched at the week points of the body such as wing auxillaries, pleural membrane, coxal joints, genital area (Figure 1.) etc., penetrates within the host body. It has been further observed that if pre-parasitic stages, then avoid penetration and move to search another host. After finding suitable penetration site on the host body, the infective larvae (pre-parasitc juvenile) penetrate through various weak parts, viz., coxal joints

Host bug, also, tries to avoid penetration by moving legs, antennae and fluttering wings but could not escape the penetration power of pre-parasitic stages.

After finding suitable penetration site on the host body, the infective larvae (pre-parasitic juvenile) penetrate the host through various weak body parts, viz. coxal joints of legs, under surface of wing pads near joints (in III-V instar nymphs), genital region, pleuron, between the abdominal sternal and tergal plates, cervical membrane and wing auxiliaries (Table 1.), with the help oof a mouth stylet and settle in the body cavity. The initial growth rate is very fast. After two days, the larval length has doubled and after 10 days it has increased more than 20-fold. 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 (Table 2.). Penetration (in laboratory condition) lasts for 15 minutes to 2 hours. The infective larvae (pre-parasitic juveniles) penetrate the host with help of a mouth stylet and settle in the body cavity. After reaching in the body cavity of the host bug, these feed on the haemocoelomic fluid, adipose tissue, body muscles etc. Now this stage of pre-parasitic juvenile is term as “parasitic juvenile”. Once parasitic development is completed, then this stage has to come out of the host body to enter into soil for moulting into adult to resume next generation. This emergence of parasitic stage from host body is being described as follows in points.

1. Emergence, of parasitic juveniles occurs through weak body point having thin arthrodial membranes such as tergal joints, pleuron membrane of abdomen, cervical membrane of neck, coxal joint with thorax, wing axiliaries and genitalia. Least preferred points include occular sclerite, antennal socket, scape and pedical joint of antenna, anal segments (post genital segments) and anal aperture. These points are selected randomly by trial-and-error pattern by the parasitic stage (Figure- 1 and Plates- 1 A, B & C).

2. The parasitic stage uses its lance like tooth to perforate the arthrodial membrane.
3. After perforation, it brings it body out slowly by wriggling or peristalsis movement. First, it protrudes out cephalic end then pulls entire body.
4. The time taken for emergence varies according to sex, length of parasitic juvenile and emergence point. Generally, it lasts for 2 to 8 minutes. Average being 5 minutes.
5. The parasitic juvenile emerges out as post–parasitic stage. If emergence occurs on soil, it enters into soil upto the depth of 10 to 30cm for moulting into adult. If emergence occurs on host plant of bug, then it falls down on soil by performing peristalsis movement.
6. It was further observed that, if the emerging parasitic stage emerged out its 50% body outside and nema is disturbed then the emerging nema goes back inside the host. If, however more than 50% part of the nema has been emerged out, then on disturbance, it quickly emerges out.

7. Time taken from penetration and emergence is calculated 18 to 22 days with an average of 20–22 days. After emergence, host bug dies due to loss of haemolymph and other vital body organs. However, if parasitic stage emerges through leg, or antenna, it takes more time and sometimes dies during emergence (Figure 2. and Plate 1 A, B, & C).
8. The post-parasitic stage (non feeding stage) moults into adult, mate, then oviposits in soil. The life cycle is completed when the embryonated eggs hatch to release the infective pre-parasites (Figure 5.).

   There are four moults in the life cycle of *H. vishwakarma,* the first in the egg, the second during parasitic development, and the final two moults in the post-parasitic juvenile (Figure 5).

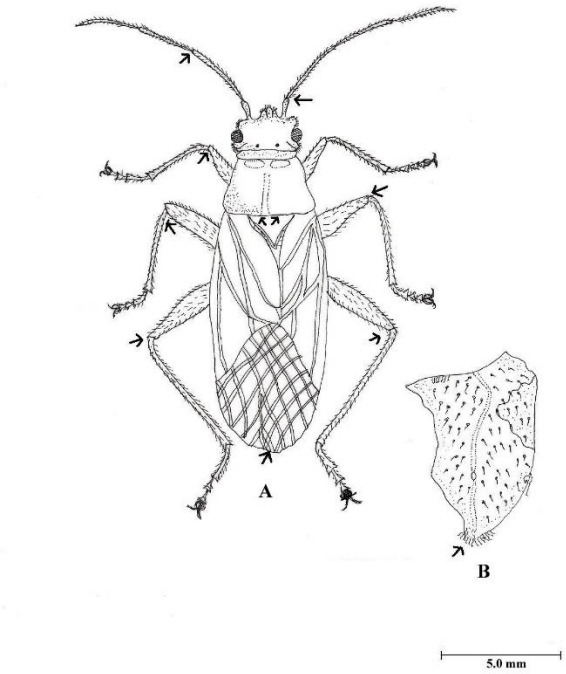

Figure 1. A Penetration points for the entry of pre-parasitic juvenile

B Genital part of *L. augu*

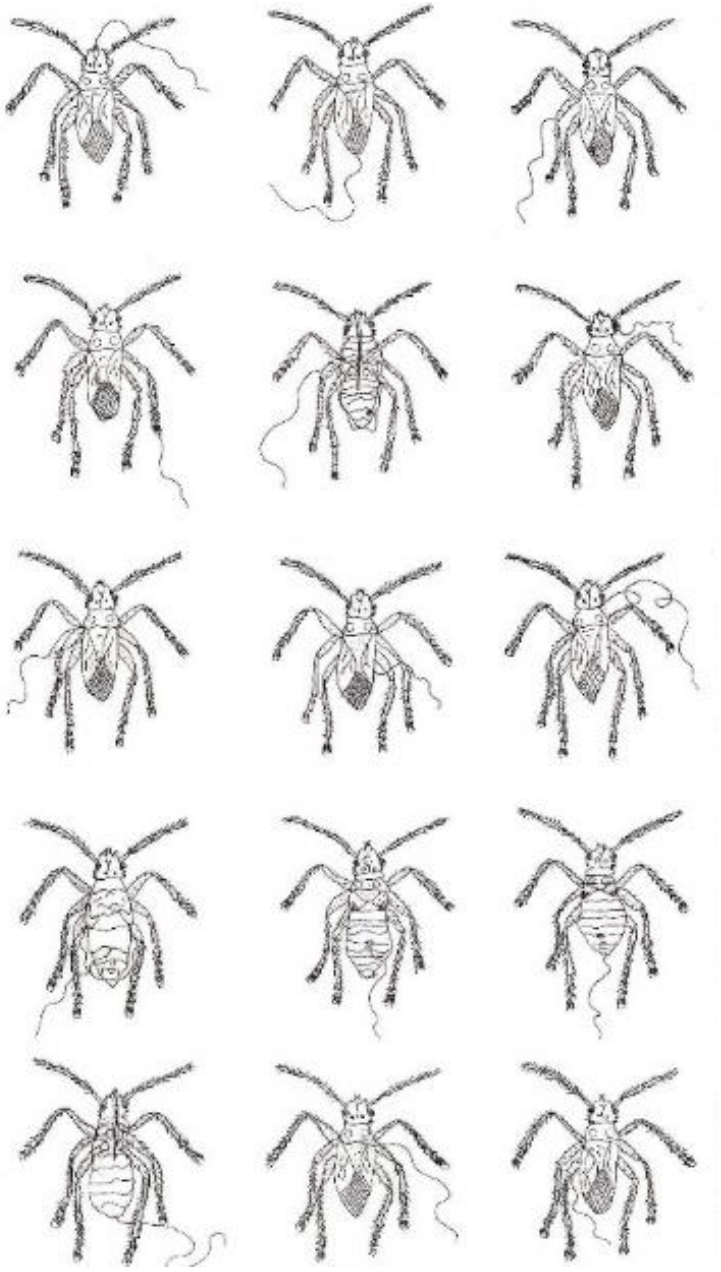

Figure 2. Points of emergence on the body of *L. augur* for the emergence of parasitic juvenile stage.

(A)

(B)

(C)

Plate 1 A Parasitic nematode emerging out through head region B & C Post-parasitic stage of H. vishwakarma after emergence

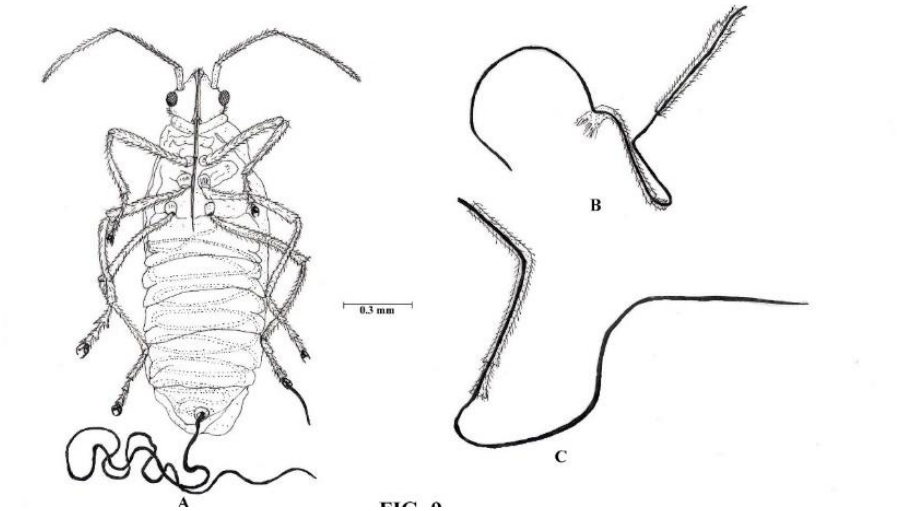

Figure 3. A- An emergence of parasitic stage through genital aperture.
B, C- Emergence through legs.

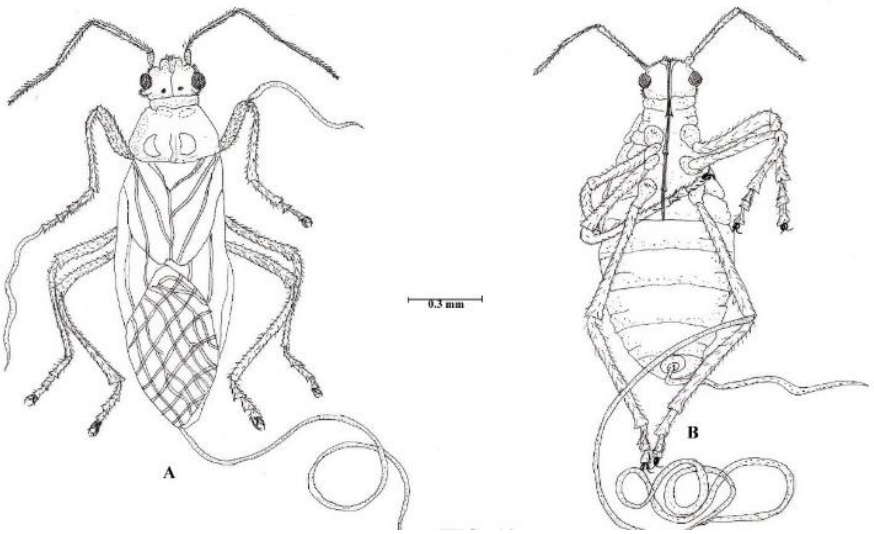

Figure 4. A & B. Emergence of parasitic stage through genitalia and leg (Femarotibial joint)

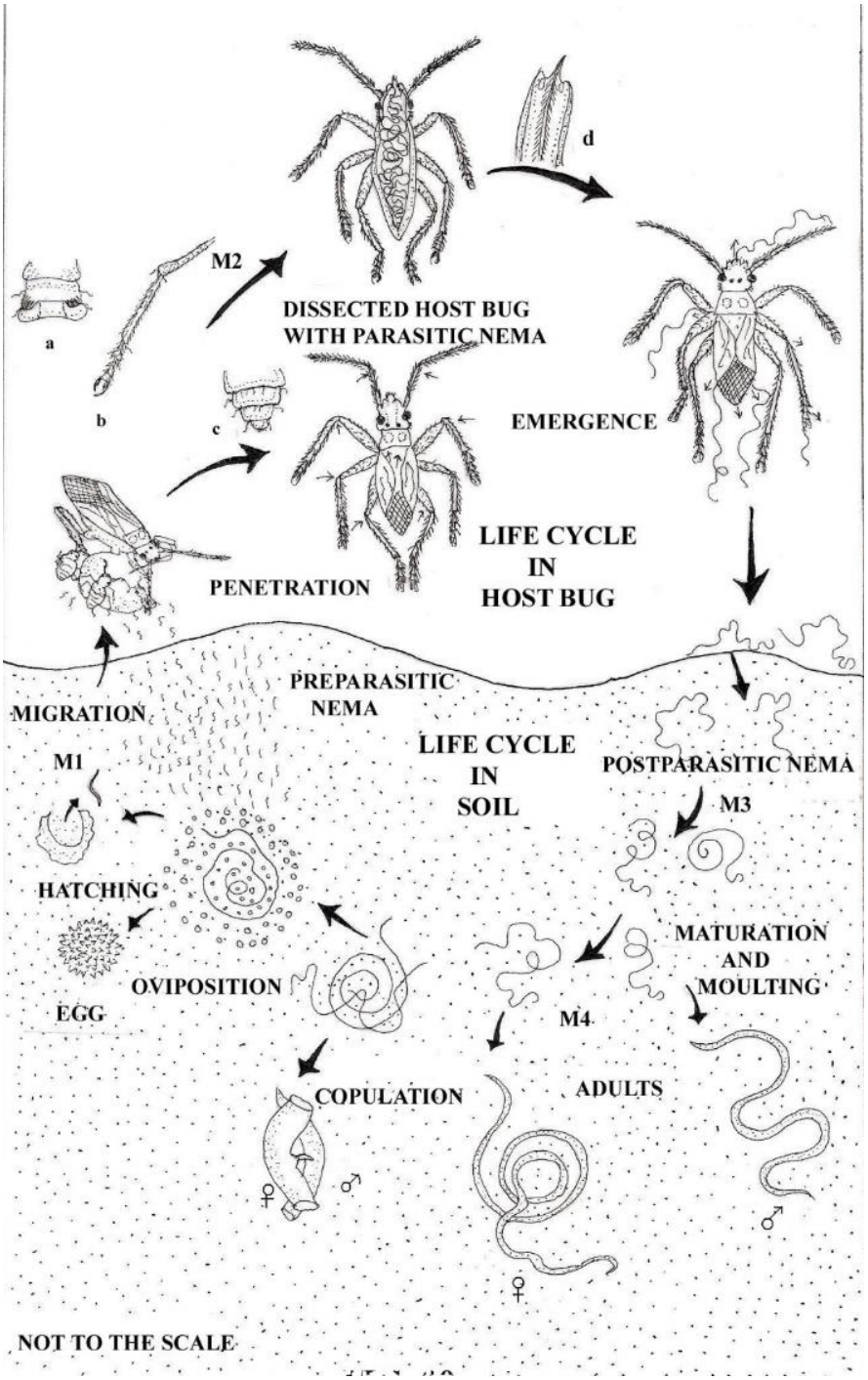

Figure 5. Life cycle of *Hexamermis vishwakarma* Dhiman in Bug (Complete in 72 days)

Abbreviation – M1- First moulting, M2- Second Moulting, M3- Third Moulting, M4-Fourth Moulting

Table 1. Penetration points of host bug *L.augur* through which pre-parasitic *Hexamermis vishwakarma* emerges from body of bug.

No. of observations	Penetration points	Stages of the host nymph					Adult	
		I	II	III	IV	V	Male	Female
1	Cervical membrane	-	-	++	+++	+++	+	++
2	Wing auxiliaris	-	-	+	++	++	++	+++
3	Pleuron	-	-				+	++
4	Between abdominal Sternal and tergal plates	-	-	-	+	+	+	++
5	Coxal joints of legs	-	-	+	-	++	+	+
6	Under surface of wing pads near joints	-	-	+++	+++	+++	++	++
7	Genital region	-	-	+++	+++	++	+++	+++

Abbreviation

+ = Indicates intensity of penetration

- = Nil

\+ = Less

++ = Medium

+++ = More

Table 2. Emergence points of host bug *L.augur* through which post-parasitic *Hexamermis vishwakarma* emerges from body of bug.

No. of observations	Emergence points	Stages of the host nymph					Adult	
		I	II	III	IV	V	Male	Female
1	Cervical membrane	-	-					
2	Wing auxiliaris	-	-	+	++	++	++	++
3	Pleuron	-	-	+	++	+	+	+
4	Between abdominal	-	-	+	++	+	+++	+++

	Sternal and tergal plates							
5	Coxal joints of legs	-	-	+	+	-	++	++
6	Under surface of wing pads near joints	-	-	+++	+++	+++	++	++
7	Genital region	-	-	+++	+++	+++	+++	+++

Abbreviation

+ = Indicates intensity of emergence

- = Nil

\+ = Less

++ = Medium

+++ = More

4. Discussion

The parasitization effects of mermithid on their hosts were studied by Rempel, (1940), Lesage & Harrison, (1980) & Rhamhalingham, (1987).

In India, the genus ***Hexamermis*** has been reported parasitizing various insects' hosts, *viz.*, *Agrotis influsa* (Welch 1963), *Vanessa atalanta*, *Polygonia comma*, *P. interrogationis* and *Isia Isabella* Puttler & Thewke (1971), ***Leptocoris augur***, (Dhiman, 1984), *Chilo infuscatellus*, *Sciropophaga excerptalis* and *Saccharipha*g*us indicus* (Srivastava 1964*), Atritibialis spodoptera sp*. (Bhatnagar, *et. al*., 1985), *Cydia leucostema* Subbiah (1986 &1989).

The infective stage of *H. vishwakarma* (pre-parasites) penetrate in the host with the help of mouth stylet and settle in the body cavity; the initial growth rate is fast. After four days, the larval length has doubled and after 11 days it has increased 12-fold. The full length of up to 30.0 cm is reached within one month. In super-parasitism length of parasitic stage not reach to maximum level and more males are produced (Paine & Mullens, 1994). During development of the parasitic stage, the characteristic digitate appendages of the post parasitic larva are formed. It is which generally assumed that this appendage is the result of extensive enlargement of the body in which the tail tip does not take part.

During the emergence of *H. vishwakarma*, the host bug, *L. augur* remains inactive and almost stationary. Emergence occurs through weak body parts using stylets and 2–8 minutes are required with an average of 5.1 minutes for emergence of parasitic nematodes (Plate 1 A, B & C). Death of host bug usually follows quickly after the emergence, due to loss of essential body fluids. About 3-week period is required for parasitic life with an average of 20.5 days. It enters into soil using pointed narrow cephalic end up to the depth of 8-10 cm in soil and then coils and moults for the last time to become adult in soil. It was further observed that if emerging stage emerged out its half or less than half body and nema or host is disturbed, then,

the emerging nema goes back inside the host. However, more than half emerged nema on disturbance emerged out quickly. This behavior is not observed by any other worker of this field.

In case of *H. vishwakarma*, normally the size of female was found larger than the male. But Doucet, *et. al.,* (1998) mentioned that in *Agamermis decaudata* parasitizing *Castnia dedalus* (host) in Argentina, male was larger than female. In case of *L. augur* maximum 5 and minimum 1 nema were emerged from single host body. Penetration of pre-parasitic makes the bug slightly irritated but as development of nematode proceeds inside the host, the bug becomes lethargic and the abdomen swells up greatly and attains biconvex shape. All the parasitized bugs are incapable of taking flight.

5. Conclusion

Thus, the entomophilic mermithids offer a promising potential for insect control and these nematodes have been used in different parts of world as biological insecticides. But, in India, the economic value of mermithids in insect pest control does not appear to have been fully appreciated yet. Experimental and field trials to control the *Leptocoris augur* (Fabr.) by using *H. vishwakarma* has yielded significant success to and its use as a biocontrol agent is suggested.

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