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# Mode of infestation and biocontrol potential of *Hexamermis viswakarma* Dhiman, a parasitoid of *Leptocoris augur* (Fabr), a pest of Kusum Plant, *Schleichera oleosa* lour

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**ABSTRACT**

*Hexamermis vishwakarma* Dhiman (Nematoda – Mermithidae) is a parasitoid of Kusum tree pest *Leptocoris augur* (Fabr). The pest severely causes damage to the seeds, bark and leaves of this tree. The parasitoid infests the bug population in large number during rainy season July to September at Saharanpur and Jharkhand forest area. Its mode of infestation and biocontrol potential is studied during present course of investigation. The parasitoid occurs in eggs, preparasite and post parasitic phases during its life cycle. The infective stage of parasite are preparasitic juveniles which are hatch out from the laid eggs after first rain of rainy season in first week of July. The preparasites are very active and emerges out in large number on soil and even reach on herbs under Kusum tree by undulating movement in search of host. On getting host ($I^{st}$ to $V^{th}$ instar nymphs as well as adults of *L. augur*). These move on host surface and search weak body points for entrance which are usually coxal joints, wing axillaries under surface of wing pods of nymphs, genital region and joints of abdominal tergites, sternites and pleurites. For penetration mouth stylet (penetration tooth) is used by preparsite and penetration lasts for about 15 to 35 minutes. Finally, these reach in the haemocoelomic cavity of host and begins to feed on host tissues such as haemocoelomic fluid, adipose tissue, body muscles, reproductive organs etc. and grow rapidly to become parasitic stage. Impact of parasitization on host is analysed which revealed that physical, anatomical and physiological changes are caused in host. The parasitized bug turns restless, its abdomen swells up resume biconvex shape, flight ability reduced feeding requirement of host greatly increased, copulation and oviposition stop, cuticle of abdomen turns transparent and coils of parasitic stage are externally visible. Depletion of haemocoelomic fluid, fat bodies and muscles starts and becomes distorted. Lipids and proteins content greatly decreases. Ecdysis in nymph stops and nymph prolong their duration till emergence of parasitic stage. After completing parasitic life within the host, it emerges out making a hole in weak body part of host and enters into moist soil to become adult after moulting. The host dies after emergence of parasitic stage within 10 to 15 minutes. Thus, cent percent mortality occurs due to parastitization which is good for biocontrol potential.

**KEYWORDS**

Infestation, Biocontrol, Preparasites, *Hexamermis viswakarma* Dhiman, *Leptocoris augur* (Fabr), *Schleichera oleosa* lour

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Mermithid nematodes are potential bio control agents of insect pests of agriculture forest and veterinary importance (Dhiman, 1994) *Hexarnermis vishwakarma* Dhiman (Nematode - Mermithidae) is an endoparasite of *Leptcoris augur* (Fabr.) (Heteroptera – coraidea - Rhopalidae) which in turn is a pest of Kusum *Schleichera oleosa* lour on which best quality of Lac is cultivated.

The pest extensively damage the seeds and bark of the host plant by gregarious feeding habit and damaged seeds loss viability (Dhiman, 1983, Dhiman and Gayyur, 1993, Dhiman and Gulati, 1986 and Shikha *et al*., 2021). The first record of Mermithids from insect can be found in fossil record where *Heydonius antique* and *H. matutinus* were collected from *Rhine Legmite* (Eocene) and Balticamber (Taylor, 1935). There are few scattered references to the entomophilic nematodes in the seventeenth and eighteenth century but last 30 years have certainly been the most productive period for the researchers on nematode including mermethids as described by a number of workers and papers in the literature *H. viswakarma* was recorded for the first time by (Dhiman, 1984) from *L. augur*. Thereafter some researcher are carried out on this time to time by (Dhiman and Singh, 1988, 1991, Dhiman and Gayyur, 1993, Dhiman and Kumar, 1996, Dhiman and Gujral, 2000, Tomar and Dhiman, 2017). In present paper an endeavour is made to describe its mode of infestation and biocontrol potential**.**

## Material and Method

Studies are carried out mainly in field area of 'HRI and Training center', Saharanpur where host plant *S. oleosa* and pest are in good number. The parasite *H. viswakarma* infest the host bug during rainy season July to September, hence, investigations are mainly made during these months. In laboratary rearing of *H. viswkarma* was done by using the method of double tray system as described by (Dhiman and Gayyur, 1993) for rearing the parasitic nematodes. Culture was maintained in the laboratory rearing culture medium was prepared as suggested by (Cantwell, 1974) 170 gms of dried whole egg solid was taken and reconstituting the solids in 500 ml of distilled water in a blender operated at low speed to prevent foaming. Now 50-100 ml. of this medium was placed in a 250ml. flask and steam sterilized small amounts were also taken in test tubes. Surface sterilized nematodes with 0.05% sodium hypochloride for 20 minutes was added especially to the rearing medium held at 24$^{o}$C nematodes may be held in this manner for up to one year without transfer. The media contained all stages of nematode at one time. For parasitization, a hurricane glass chimney with moist soil taken along with 50 bugs of different stages (II$^{nd}$ to V$^{th}$ instar nymphs and adults). In it preparsitic names were sprayed using hand sprayer (about 250 preparasities in 250 ml in saline solution (0.1 N). The chimney was covered at top by fine muslin cloth. Penetration behaviour of preparsitic juveniles was keenly observed using 20X hand lens. After 3-4 hrs, these bugs were taken out and reared separately on moist crushed seeds of Kusum plant. Now the effect of parasitism on the bug and its behaviour was clearly observed. Time to time several dissections of parasitized bugs was also made under binocular or microscope to ascertain the effect of parasitism on the anatomy of the bug. Time period from the entrance of preparasite into the host till emergence was also recorded. Penetration and movement of preparasites into the host till emergences was also observed in field during rainy season.

## Results and Discussion

### Mode of Infestation or Penetration Behavior of Preparasites

Infective stage of *H. viswakarma* is preparasitic stage or juvenile. On first shower of rain in the soil preparasite hatch out in large number and come on soil surface move very rapidly in surrounding environment and even on herbs under the Kusum plant in search of host. The length of preparasite measures from 0.24 to 0.75 mm with an average of 0.65 mm and the average width is 0.04mm. These nemas remain active for 3 to 4 days and if not find host become die. However, on encounter of host preparasite move on host surface and search weak body parts for entrance such as throdial membrane of body joints, wing axillaries, under surface of wing pad joints, genital region, cervical membrane, antennal base joints with head, thoracic and abdominal tergal, sternal and pleuron joints. For penetration a pointed penetration tooth or mouth stylet is present on the mouth. Entrance through these points lasts for about 10 to 25 minutes by performing undulating or wriggling movement After penetration on reaching haemocol of host bug, these begins feeding by absorbing heamocoelomic fluid. Adipose tissue, body muscle, reproductive organs etc. and grow rapidly in size and makes many complicated coils. Now the stage is called 'parasitic stage' which may grow up to 12 mm to 194.0 mm in length and 1.26 to 2.27 mm in width equal to the size of adult worm.

**Bio-Control Potential**

For seeing biocontrol potential of *H. viswakarma*, impact of parasitism on host was observed and then parasitization capacity was recorded.

**Impact of Parasitism on Host**

It was observed under following headings.

**Behavioural Change**

Early parasitized bug becomes irritated actively move for feeding but as development of parasitic stage proceed inside, it become lethargic, no copulation and oviposition occurs. Color of the host bug turns light. Flight ability is lost and at last when parasitic stage emerged out from it through weak body points. It dies within 15 to 30 minutes.

**Morphological Change**

The abdomen of parasitized bug become swollen and attain biconvex shape, movement of antennae, legs and wings fluttering ability reduced. Feeding requirement of host bug greatly increased and it devotes more time for feeding on the seeds and leaves of the host plant. Body cuticle becomes thin and transparent, so that coils of nematodes externally visible through it. External genetalia of parasitized host found distorted. A bluish spot develops preferably on left side on the postero-ventral part of abdomen.

**Fig 1. Plate 1: Showing Coils of *H. vishvakarma* inside host bug**

**Anatomical Changes**

First there is quantitative loss in the haemocoelomic fluid of the host as it absorbed by the body wall of the parasite up to some extent. Secondly fat bodies of the host which lie around the gut, reproductive organs and other body parts are consumed. Thereafter, thoracic and abdominal muscles are first dissolved by parasitic enzymes and then absorbed by body wall of the parasite. Super parasitism which is a general tendency also results in the destruction of the posterior part of alimentary canal, testis and ovaries are greatly reduced and genital ducts are distorted at many points and reduced.

**Reproductive Ability**

As testis and ovaries are greatly reduced, there is no spermatogenesis and ovulation and eventually no egg formation. Parasitized bugs are unable for copulation Cent percent sterility occurs in the either sex. Fecundity reduced to zero. No sexual dimorphic changes are seen in parasitized bugs.

**Changes in Physiology of Host**

Haemocoelomic fluid diminish feeding requirement increases as parasitized bug devotes more time on feeding. Ecdysis or moulting in parasitized nymphs cases. As, there is no considerable damage to the nervous system and respiratory system, the bug survive till the emergence of parasitic stage from the host body and the host dies after sometime of emergence.

**Parasitization Capacity**

Parasitization capacity was observed both in field as well as laboratory.

**Percent Parasitization in Field**

It is difficult to calculate the exact percentage of parasitization in field due to multiplication of host and parasitic population both. For this, field surveys were conducted monthly during the year 2020 to 2022. Bugs were collected by hand picking method randomly from 5 square meter area and brought alive in laboratory in polythene bags and then examined for parasitization by doing dissections.

Percent of parasitization was calculated using the formula.

**No of Parasitized Bugs / Total No of Bugs Collected × 100**

It has been observed in the field that parasitization only occurs during rainy month after year from June to September or early October.

Maximum 35% parasitization is recorded during August and minimum 1 percent during late June. Preparasites after hatching from eggs requires high humidity or water for its undulating movement in search of host. This condition is only available during rainy months in this locality.

**Table 1. Percent of Parasitization of *L. augur* by *Hexamermis viswakarma* in field**

| S. N. | Date of Collection of Bugs | No. of Bugs Collection | No of Bugs Found Parasitized | Parasitization Percent |
|---|---|---|---|---|
| 1 | 24 July 2020 | 200 | 40 | 20 |
| 2 | 26 Aug 2020 | 150 | 45 | 30 |
| 3 | 5 Sept. 2020 | 120 | 30 | 25 |
| 4 | 20 June 2021 | 100 | 01 | 01 |
| 5 | 27 July 2021 | 75 | 15 | 20 |
| 6 | 30 Aug 2021 | 125 | 25 | 20 |
| 7 | 15 July 2022 | 70 | 20 | 28.5 |
| 8 | 29 Aug 2022 | 100 | 35 | 35 |
| 9 | 2 Oct 2022 | 150 | 20 | 13.3 |

**Table 2. Parasitization percentage of *L. augur* by *H. viswakarma* in lab**

| S. N. | Observation Period | No of Bugs Released in the Cage | No of Bugs Found Parasitized | Percent of Parasitization |
|---|---|---|---|---|
| 1 | 2 July to 27 July 2020 | 75 | 72 | 96 |
| 2 | 6 Aug to 28 Aug 2020 | 90 | 81 | 90 |
| 3 | 6 Sep to 24 Sep 2020 | 125 | 120 | 96 |
| 4 | 5 July to 27 July 2020 | 100 | 98 | 98 |
| 5 | 2 Aug to 24 Aug 2021 | 105 | 105 | 100 |
| 6 | 5 Sep to 25 Sep 2021 | 94 | 90 | 95.7 |
| 7 | 4 July to 24 July 2022 | 80 | 75 | 74 |
| 8 | 5 Aug to 23 Aug. 2022 | 95 | 90 | 95 |
| 9 | 4 Sep. to 25 Sep. 2022 | 120 | 112 | 93 |

## Parasitization in Laboratory

For this big wooden wire gauze cages were taken (1.5 × 1 × 1.5 meter). In it healthy bugs were reared on the crushed Kusum seeds. Wet cotton swab was placed in it for maintaining necessary R. H. In this cage laboratory reared preparasites were sprayed on the bugs and parasitization was observed after a week period. Fixed number of bugs was used in each experiment and parasitized bugs number was calculated. Approximately 5 nemas per host were used a hand sprayer was and nemas were sprayed using water media. The data of table 2 reveals that parasitization in lab varied from 95-100 percent.

The reason for this high percent is due the easy availability of host to the preparasitic nemas in limited space of the wooden wire gauze cage. The parasitized bugs are always dies, hence, parasitization may be considered as mortality percent. Looking to the high mortality percent, *H. viswakarma* has very good potential to be used as biocontrol agent.

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