Seasonal prevalence of *Hexamermis vishwakarma* Dhiman (Nematoda: Mermithidae) parasitism in *Leptocoris augur* Fabr. (Heteroptera: Rhopalidae) infesting *Schleichera oleosa* in relation to environmental factors and its biocontrol potential

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Abstract

Hexamermis vishwakarma Dhiman, an entomophilic nematode, is thought to be a possible bio-control agent for *Leptocoris augur*, a pest of *Schleichera oleosa*. The nematodes develop inside the host insect through a number of nymphal and adult stages of *L. augur* prior to emerging from the host as fully developed, non-feeding postparasites. Host mortality results from the nematodes leaving the bug.

The objective of this study was to identify the nematodes that parasitize *L. augur* and to chart (record) the level of parasitism in the population of *L. augur* in response to environmental factors like temperature and rainfall. The possibility for *H. vishwakarma* to exert biological control over the *L. augur* population through the occurrence of epizootics is discussed.The study was carried out in the HRI and Training Centre, which is situated in the Indian district of Saharanpur, from January to December and (fortnightly from May to October). By comparing the number of *H. vishwakarma* Dhiman parasitized nymphs and adults bugs with the current rainfall and temperature at biweekly intervals, it was possible to determine the effect of abiotic conditions on the degree of parasitism in *L. augur*.

In *S. oleosa*, there was a highly substantial positive association between the amount of parasitism and rainfall. From July to August, when there was the most rainfall in 2006, post-parasitic juvenile caused mortality in *L. augur* infesting *S. oleosa* was (81 and 88%, respectively), and the parasitism decreased as the amount of rainfall decreased. In the lab, the parasitization rate ranged from 80% to 100%. The percentage of parasitization in the bug population in the field was 10–88%.

The majority of the bug population (80 to 90%) died in the cage within a month, which was a promising result for *H. vishwakarma* Dhiman. As a result, *H. vishwakarma* can be successfully used in India as an *L. augur* biocontrol agent. The inverse relationship between rainfall and *H. vishwakarma* Dhiman (mermithids) infection will be useful in planning when to apply pesticides in IPM plans to control this pest.

Keywords: *Leptocoris augur,* parasitization percentage, *Hexamermis vishwakarma* Dhiman, abiotic factors, temperature, rainfall, biocontrol potential

1. Introduction

Hexamermis vishwakarma Dhiman, an entomophilic nematode, is thought to be a possible bio-control agent for *Leptocoris augur*, a pest of *Schleichera oleosa*. Despite the nematode spending a portion of its life cycle in the soil or infecting an insect consuming the seeds and bark of the kusam tree, the infectious nematode is still there. the nematode's juvenile or pre-parasites come into contact with the host insect (Kumkum, 2021) [1]. The nematodes develop inside the host insect through a number of nymphal and adult stages of *L. augur* prior to emerging from the host as fully developed, non-feeding postparasites. Host mortality results from the nematodes leaving the bug. Because the nematodes have left the bug, host mortality occurs. Upon entering the soil, the postparasites transform into adults, breed, and lay eggs.

Although *H. vishwakarma* Dhiman has a number of beneficial properties as a biocontrol agent, such as high lethality, host specificity, simplicity of use, and possibility for long-term recycling in the environment are all advantages. (Dhiman and Kumkum, 2005) [2], the challenges in traditional taxonomy of the juvenile stage (Poinar, 1979) [3] and the absence of guidelines for mass cultivating Hexamermis sp. for distribution in the field (Kery and Hominick, 2002) [4].

The parasitism of *L. augur* by a *Hexamermis vishwakarma* Dhiman (Mermithidae: Nematoda) is described in this paper, and its potential for biocontrol in an integrated pest management (IPM) programme is discussed.

The goal (objective) of this study was to identify the nematodes that parasitize *L. augur* and to chart the level of parasitism in the population of *L. augur* in response to environmental factors like temperature and rainfall. The possibility for *H. vishwakarma* Dhiman to exert biological control over the *L. augur* population through the occurrence of epizootics is discussed. However, compared to field research, the parasitization percentage was greater (80 to 100%) in laboratory studies for the biological control of *L. augur*. This is due to the fact that abiotic variables, like as temperature and relative humidity, differ from laboratory conditions in the environment.

2. Materials and methods

2.1 Study area

The study was carried out in the Saharanpur area of India's HRI and Training Centre from January to December. During the studied period, the location's annual average rainfall, number of wet days, and maximum and lowest temperatures were 2006, 31.7 °C and 14.2 °C, respectively.

2.2 Seasonal incidence of *Hexamermis vishwakarma*

Live nymphs and adults were collected near the host plant *S. oleosa* (Plate-1. a, b) at intervals of two weeks in order to evaluate the seasonal occurrence of the *H. vishwakarma* Dhiman population. Nymphs and adults were then carefully separated from the adult in the lab. The nymphs were moved to trays (25 cm in diameter) covered with fine muslin cloth at a rate of 15 per tray, where they were maintained on Kusam seed and fresh leaves in line with their host plants under ambient natural circumstances (29 ± 2 °C, 70% RH). Every day, until the bugs or nymphs died because of the exit of nematodes, or until the bugs or nymphs' diet changed because of the exit of nematodes, or until the nymphs moulted, the number of bugs examined depending on the pest's field indicendence, this number varied from 13 to 180 every month. A hurricane glass lamp chimney measuring 9.0 cm in diameter and 20.0 cm in length was taken and placed in a Petri dish filled with clean, moist, sterilised sand to measure the parasitization percentage in the lab. Crushed leaves and paralysed insects were placed in the chimney, which was then covered with a thin muslin cloth. Water was added as needed to maintain the proper wetness. Every day, fresh food was provided, and old food was replaced. The bugs were raised until an *H. vishwakarma* Dhiman post-parasitic juvenile emerged from the host bugs. Post-parasitic juveniles were raised using the technique described by Petersen and Willis (1972) [5]. Pre-parasite to bug ratio was maintained at roughly 20 to 1.

Large wooden wire gauge cages measuring 1.5 x I.0 x 0.5 metres were made for outdoor observation. Each cage included a wooden base, fine wire mesh on all four sides, and a partially openable top. Wire gauze was also used to cover the top and removable lid. The cage's bottom lacked any supporting framework. To offer a natural atmosphere, these cages were kept in the field area under the kusam tree from July to September.

The following formula was used to compute the parasitization rate: –

$$\frac{\text{Total no. of dead bugs collected from the cage}}{\text{Total no. of healthy bugs released in the cage}} \times 100$$

2.3 Influence of temperature and rainfall on the parasitism of *Hexamermis vishwakarma*

By comparing the number of *H. vishwakarma* Dhiman parasitized nymphs and adults with the current rainfall and temperature at biweekly intervals, it was possible to determine the effect of abiotic conditions on the degree of parasitism in *L. augur*. The meteorology department of HRI and Training Centre in Saharanpur provided the weather information for the research period.

2.4 Statistical analysis

The one-way analysis of variance (ANOVA) and LSD multiple range test were used to investigate the association using Excel software and SPSS20.0, respectively. At the level of P< 0.05, significance between components was taken into consideration.

3. Results

3.1 Evaluation of parasitization percentage in field after release

Utilising data from experimental cages, the parasitization % was computed. To offer a natural atmosphere, wooden cages were kept in the field area under the kusam tree from July to September. To make it simple for everyone to obseve the parasitic juveniles emerge, the bottom of each cage was set on a thick, white polyethene sheet. A precise number of host populations, including male and female adults and nymphs in the second to fifth instars, were let release in each cage along with some crushed kusam seeds for bugs. Each cage also contained a large cotton swab to maintain the required R.H. The *H. vishwakarma Dhiman* parasitized bugs that were discovered dead were removed from the appropriate cages.

The percentage of parasitization in the field (in its natural setting) ranged from 80 to 90%. At least 50% and no more than 90%.

3.2 *Hexamermis vishwakarma* Parasitism in *L. augur.*

The parasitization rate of the pest (*L. augur*) was clearly higher from July to August in 2006, according to data on parasitization % under field conditions. length executing wavy motions to infest host while feeding on the leaves. *L. augur* nymphs and adults had pale orange colouring, were slow, and resembled other nymphs and adults in terms of size and morphology.

There were one to five post-parasitic nematodes that might be produced by a single insect. The anal entrance or the legs of the cuticle of bugs or nymphs are the two places where nematodes can leave the host bug (Figure: 3-h, i). Nevertheless, in situations of superinfections, the post-parasitic juvenile invariably burst from the anal orifice of the larva and nematode escape.

Young postparasitic juveniles (Figure: 3. j) were creamy or light white when they first emerged. The post-parasitic juvenile's length, which ranged from 12.0 to 25 cm in length and 1.25 to 2.25 mm in diametre, was inversely correlated with the degree to which the worms parasitized the host. The post-parasitic nematodes' front head tip was blunt, and tail-like appendages were observed at the back (Figure: 2. g). The host bug went dark, flattened down as soon as the parasites emerged, lost all of its haemocoelomic contents, and eventually died.

3.3 Parasitization percentage in laboratory

The cages were then sprayed with freshly hatched pre-parasitic suspension in the 1.0% saline solution. With the use of a little hand sprayer, these nemas were sprayed (Figure: 1. c). Stale food was replaced with fresh food after two days. The proportion of the bug population that was parasitized was carefully monitored. The parasitization of *H. vishwakarma* Dhiman was confirmed by dissecting the bugs in 1.0% saline water after 5 days of pre-parasite exposure.

After the parasitic stage emerged in the second set of studies, mortality of the insects was noted. After 25 days, the remaining bugs were removed and dissected.

In the laboratory, the high parasitization percentage benefits from the high humidity (80–86%) and moderate temperature (29–31ºC). In the laboratory, the range of parasitization was 80.5 to 100%, with an average of 87.3. The easy accessibility of hosts in the lab's constrained environment to the pre-parasites was identified as the primary cause of the greater percentage of parasitization.

The juvenile infective nematodes enter the host using a mouth stylet and weak sclerotized areas of the host body after the pre-parasitic nematodes use their undulating movements to locate a suitable penetration site on the bug. Examples of weak body parts include the cervical membrane, coxal joints in the legs, the area between the abdominal sternal and tergal plates, under the surface of the wing pads near a joint (in III to IV instar nymph), the genital region, and the pleuron. Stylets are then used to enter the host bug's body and settle in the haemolcoelomic cavity. These are known as parasitic juveniles and gain growth at the expense of host tissue (Figure: 2. e).

It is estimated that it takes 18 to 22 days, on average 20 to 22, from penetration to emergence. The host bug dies after emergence as a result of losing haemolymph and other essential body organs. The parasite stage pierces the arthrodial membrane with its lance-shaped tooth (Figure: 2. f). After the parasite stage, the parasitic juvenile appears. For the purpose of growing into an adult, it burrows into the soil up to a depth of 10 to 30 cm. When the embryonated eggs hatch to release the contagious pre-parasites, the life cycle is complete. The mermithid nematode *H. vishwakarma* Dhiman goes through four moults egg, pre-parasitic, parasitic, and post-parasitic before becoming an adult. Only the parasitic juvenile stage among these feeds on the host beetle; the *L. augur* and remainder stages do not. In 72 days, the life cycle is finished.

Large anatomical alterations, including biochemical and physiological changes, are brought on by parasitic juvenile inside the hemocoel of the bug (*L. augur*). When pre-parasitic juveniles initially enter the host body (Figure: 1: c, d), the bug feels slightly irritate and moves around a lot. However, as the parasitic stage develops inside the host, the bug appears lethargic and eventually becomes rather slow before emerging. The parasitized bug's abdomen develops a biconvex form and swells up significantly. The parasitized bug developed an incapacity to fly due to the significant interference with the movement of the antennae, legs, and wings. On the posteroventral portion of the bug's abdomen, a bluish patch forms, preferably on the left side. The coils of the parasitic stage can be seen externally under a binocular microscope or even with the unaided eye when the host's abdomen's body cuticle thins and becomes transparent. The parasitized bug prefers alone and avoids social situations. The host bug's appetite grows, and it spends more time eating Kusam seeds and suckling sap from neighbouring host plants' leaves. A general trend known as multi-parasitism promotes the continuous delivery of nutrients by greatly lengthening feeding intervals.

In the middle and late phases of the parasitized bug, the host bug's ability to reproduce is decreased, and eventually, no reproduction takes place. Male bugs have also been noted to have deformed external genitalia. In nymphal instars, parasitization prevents ecdysis and prolongs the nymphal stage until the nematode emerges, killing it. Changes were also brought on by parasitism. First quantitative haemolymph loss occurs. The ovum's development in the ovary in females is significantly impacted. The size of the testes is significantly reduced in parasitized male bugs, and these bugs are 100% sterile.

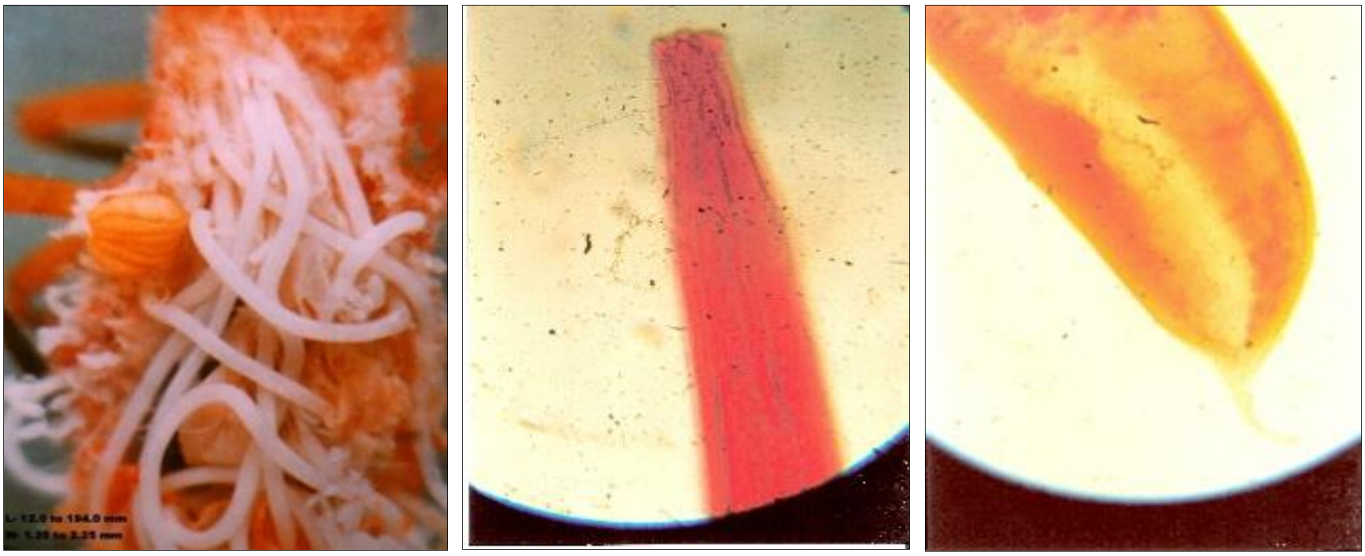
In the lab, the parasitization rate ranged from 80 to 100% (Figure:1. d), while in field cages, the bug mortality rate was found to be between 80 and 90%. The parasitization rate in a population of bugs was found to be between 10 and 88%.

(a)

(b)

(c) (d)

Fig 1: (a) *Leptocoris augur*-infested *S. oleosa* bark; (b) Gregarious feeding on the seeds of *Schleichera oleosa;* (c) Parasitization in *Leptocoris augur;* laboratory condition*; (d) Pre–parasitic juvenile of H. vishwakarma* Dhiman

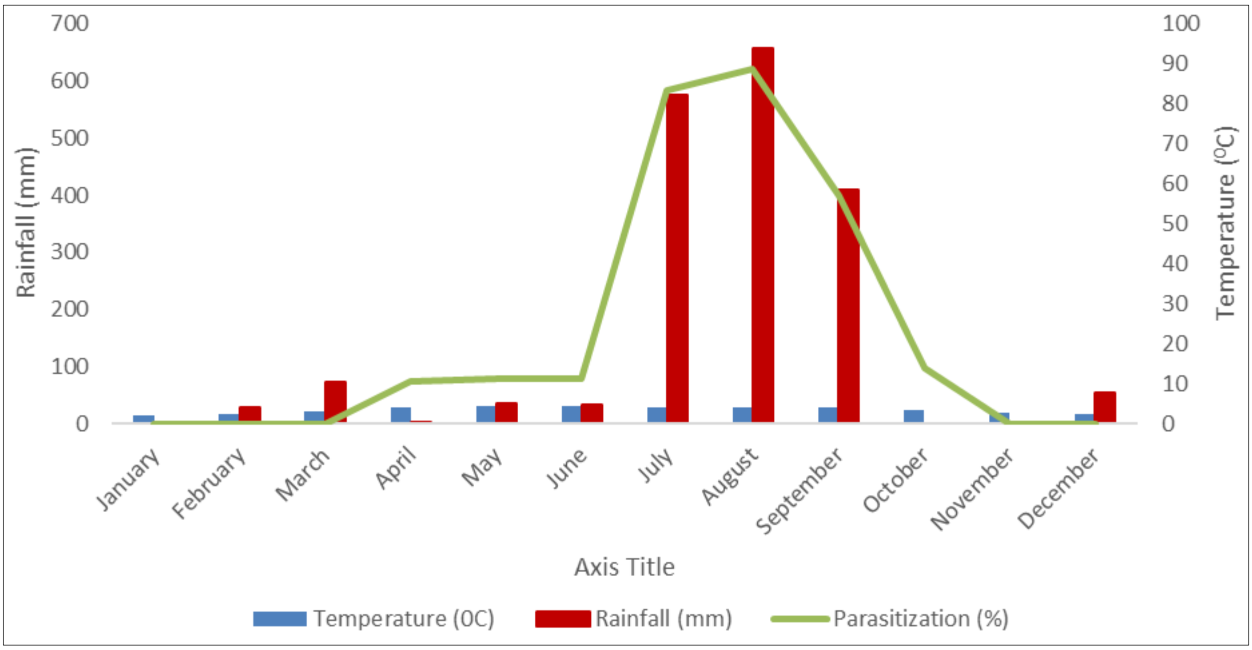


(e) (f) (g)

Fig 2: (e) Parasitic juvenile of *H. vishwakarma* Dhiman inside the haemocoel of L., (f) Juvenile juvenile post-parasitic head (Scale bar: 96.8 mm); Bugs, (g) Juvenile with papillae in the tail area (scale bar: 98.5 m)

(h) (i) (j)

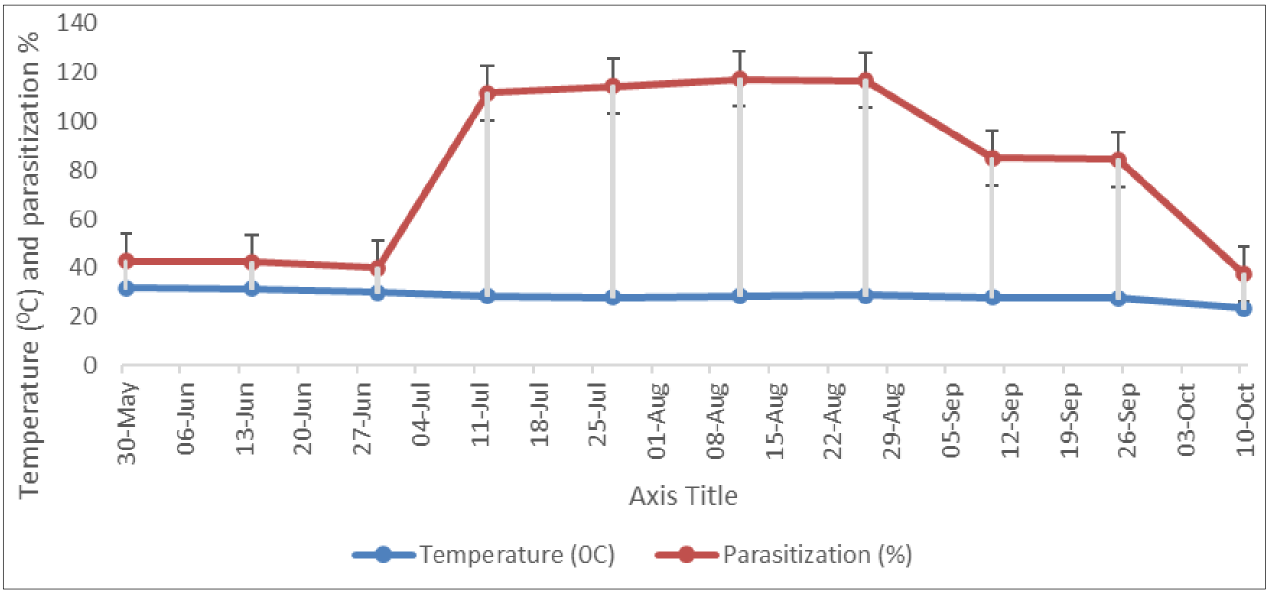
Fig 3: (h) A mermithid nematode's exit, through the legs of *L. augur* nymph; (i) Young post-parasitic parasites can emerge through the mouth aperture.*;* (j) Parasitic juvenile (12.0 to 25. cm length and 1.25 to 2.25 mm width)



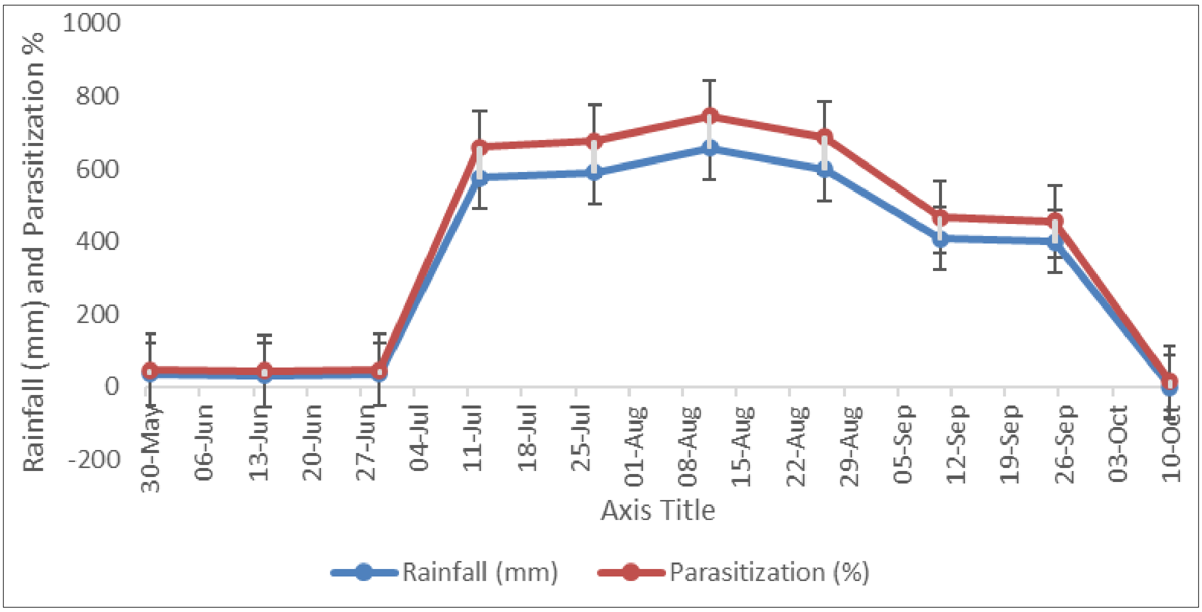
Graph 1: Seasonal prevalence of *H.vishwakarma* Dhiman parasitism in *L. augur* infesting *S.oleosa* in relation to temperature and rainfall

Weather-related factors' effects on mermithid

The amount of parasitism and rainfall in *S. oleosa* were shown to be highly correlated (correlation values of 0.93 and 0.11, respectively; p values of 0.011, 0.000, and 0.05, respectively). Infested *S. oleosa* by *L. augur* saw post-parasitic juvenile-induced mortality rates of (81 and 88%, respectively) from July to August, which coincided with the year's peak rainfall (Graph 2). Parasitism decreased as rainfall decreased. The amount of *H. vishwakarma* incidence in the population of *L. augur* bugs infesting *S. oleosa* exhibited a statistically high degree of negative connection (correlation value -0.82 and 0.11), (p=0.00, 0.99, respectively) with the maximum temperature (r=-0.14, 0.000, respectively) (Graph 3). There was less mermithid parasitism in *L. augur* infesting *S. oleosa* throughout the months with higher temperatures during the season. Graph 1 also displays the seasonal frequency of *H. vishwakarma* parasitism in *L. augur* infesting *S. oleosa* in relation to temperature and precipitation.



Graph 2: Demonstrating a relationship between the Temperature and the parasitization rate of *H. vishwakarma* Dhiman in *L. augur*.



Graph 3: Showing correlation between parasitization percentage of *H. vishwakarma* Dhiman in *L. augur* in relation to Rainfall

Discussion

L. augur's prevalence on *S. oleosa* was first noted in January and persisted through December. Mermithid parasitism of L. augur populations in *S. oleosa* (rainy season) started in July and August, respectively, and persisted for the duration of the pest's stay around these trees.

The number of parasites that a nematode created inside of its host had an impact on the length of the parasite, with longer nematodes emerging from simple parasitism and shorter nematodes emerging from heavily parasitized bugs.

Similar observations were made about *Hexamermis sp.* parasitizing *Cydia hemidoxa* infesting black pepper, which were accounted for by the nematodes' restricted access to host-bound food sources for development (Devasahayam and Koya, 1994) [6].

According to Sanad *et al.*, (2017) [7], super parasitism and nutrition availability also have a big impact on mermithid nematode population regulation and sex selection.

Mermithid entrance into the host tissue has been described in three different ways. Thirdly, via a middle-ground insect host, as has been noted for ants, wasps, and spiders (Kaiser, 1991; Poinar, 1985; Yoshida, 2014) [8, 9, 10].

the potential for mature mermithids to deposit eggs on the ground. Alternately, as in the instance of the tobacco budworm *Helicoverpa armigera* infested by Ovamermis sinensis (Q. Li *et al.*, 2009) [11], to get to the insects' borehole, infected juveniles in the soil can ascend the pseudostem a short distance. Terrestrial mermithids, according to Nickle (1981) [12], can infect host insects directly from the earth or following a brief ascent up a plant stem.

Mortality rates from severe mermithid epizootics can surpass 50% and frequently exceed 90% (Bhatnagar *et al.*, 1985; Micieli *et al.*, 2012) [13, 14]. In our studies, *Laptocoris augur* populations experienced mortality rates from mermithid epizootics of 80 and 88%, respectively.

This discovery is consistent with past research on nematodes of the genera *Ovamermis* and *Hexamermis*, which prey on a variety of significant lepidopteran pests. For instance, the average parasitism of *Mythimna* seperata by *O. sinensis* ranged from 40 to 90%, whereas that of Agrotis ipsilon by *Hexamermis Agrotis* ranged from 44 to 67.6% (Chen *et al.*, 1991; Li and Xiong, 2005) [15, 16]. Investigations reveal that *L. augur's* mermithid parasitism dropped as a percentage after October. Since October, there has been a decrease in the number of bugs and the percentage of bugs that *H. vishwakarma* parasitizes. Our research revealed that temperature and rainfall had a significant impact on the degree of *H. vishwakarma* parasitism of *L. augur.*

Temperature and rainfall have been found to have a significant impact n the mermithid infection of host grasshoppers and locusts (Baker and Capinera, 1997; Prabhakar *et al.*, 2010) [17, 18]. The abiotic elements mentioned above, particularly rainfall, have an impact on the parasitization rate year after year (Presswell *et al.*, 2015) [19].

H. vishwakarma Dhiman nematodes were discovered to exist in areas receiving 800 to 1200 mm of yearly rainfall host (Helden, 2008; Poinar, 1981) [20, 21] and generally need a moist environment to infect their (Kaiser, 1991) [22]. Between 576 and 657 mm of rain fell in the study region for the Kusam tree (*S. oleosa*) throughout the months of July and August.

The weather and soil were continually moist at this time, which allowed the infectious juvenile (pre-parasitic) to creep up on *L. augur* nymphs and adults and infect them, resulting in significant levels of parasitism.

Because nematode eggs and adults, which both occur outside the host, are vulnerable to desiccation (Brown and Platform, 1977; Galloway and Brust, 1977) [23, 24], high humidity and mild temperatures generally have a beneficial impact on worm survival (Arthurs *et. al.*, 2004) [25]. The relationship between temperature and nematode parasitism has been well documented in many nematode parasites (Poinar, 1975) [26].

The study site's high moisture content and moderate temperature may have created the optimum environment for high levels of *H. vishwakarma* parasitism in *L. augur.*

Mermithids from the genera *Ovamermis*, *Agamermis*, *Octomyomermis*, and *Hexamermis* are effective biocontrol agents for a number of economically significant pests.

Similar reports on species against brown hoppers and white bugs are also known (Choo and Kaya, 1990) [27]. *Hexamermis sp.* and *Mermis sp.* against orthopterans, in addition to

Octomyomermis sp., Romanomermis sp., and Heleidomermis sp. (Platzer *et al.*, 2005) [28] *Hexamermis sp.* and *Mermis sp.* have been reported to be effective against orthopterans, dermopterans, coleopterans, dipterans, hymenopterans, and lepidopterans (Poinar, 2001) [29].

The present study's high mortality of *L. augur* caused by *H. vishwakarma* suggests that these organisms have considerable biocontrol potential and can be important players in the pest's natural control.

As seen in the current study, the time of year with the most rain (July to August) may be ideal for *H. vishwakarma* nematode parasitization. The early field populations of the pest are reduced as a result of the high level of parasitization by mermithid nematodes during these months, which would be more receptive to management than other methods.

Therefore, entomophilic nematodes have been utilised as biological insecticides and have a promising future for the management of insect pests.

By raising mermithids in vivo, like with vector insects, it is possible to overcome the challenges of mass mermithid rearing (Santamarina and Perez, 1997) [30].

When establishing IPM techniques for the environmentally friendly management of *L. augur* on *S. oleosa*, *Hexamermis vishwakarma* can play a significant role as a biocontrol agent.

Future recommendations

By boosting this natural enemy as a potent biological control agent against *L. augur*, our ongoing study aims to characterise the worm's morphology down to the species level and develop mass-rearing techniques for the nematode for field releases and efficient pest management.

Significance statement

The results from *H. vishwakarma* Dhiman were encouraging, and within a month, 80–90% of the population of bugs in the cage died. As a result, *H. vishwakarma* Dhiman can be successfully used in India as an *L. augur* biocontrol agent.

Conclusions

Finally, it should be noted that *Hexamermis vishwakarma* Dhiman, a major insect pest of *S. oleosa* and the commercially significant kusam tree, was discovered in *L. augur*.

There is an urgent need to try mermithids for the control of agricultural and forest pests and other nuisance insects in India, and to achieve this entomologist have to play a vital role. Dhiman and Kumkum (2006), conducted an experimental and field trial to control *Leptocoris augur* by using *Hexamermis vishwakarma* Dhiman.

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References

1. Kumkum. *Un nématode entomophile, hexamermis sp., un agent de biocontrôle: Hexamermis vishwakarma dhiman, nématode mermithique de la punaise du kusum, de schleichera oleosa)*. Editions Notre Savoir Press. (French Edition) (1st ed.), 2021. Retrieved from https://a.co/d/d35LUTK.
2. Dhiman SC, Kumkum. Biocontrol potential of *Hexamermis vishwakarma* Dhiman in controling *Leptocoris augur* Fabr, a pest of Kusam plant. Jr. of App. Zoo. Res. Ass. 2005;16(1):34-35. http://www.cabdirect.org/abstracts/20053221393.html
3. Poinar GO. Nematodes for biological control of insects. CRC Press, Boca Raton, FL, 1979.
4. Kerry BR, Hominick WM. *Biological control in D.L. Lee (Ed)., The biology of nematodes*., London, England: Taylor and Francis, 2002, 483-509.
5. Petersen JJ, Willis OR. Procedures for mass rearing of a mermithid parasite of mosquitoes. Mosquito News. 1972;32:226-230.
6. Devasahayam S, Abdulla and Koya KM. Seasonal incidence of *Hexamermis sp.* (Dor., Mermithidae) parasitizing larva of top shoot borer *Cydia hemidoxa* Meyr. (Lep. : Tortricidae) on black pepper. National Research Centre for Species. 1994;117(1):31-34.
7. Sanad M, Sun JS, Shaseldean MSM, Wang Y, Gauglar R. Super parasites and population regulation of the mosquito paracetic mirmitha nematodes *Romanomermis iyengari* and *Strelkovimermis spiculatus*. Journal of Nematology. 2017;49:316-320.
8. Kaiser H. *Terrestrial and semiterrestrial Mermithidae In: Nickle WR. (Ed.) Manual of agricultural nematology.* Marcel Dekker, New York, 1991, 899-965.
9. Poinar GO Jr. Mermithid (Nematoda) parasites of spider and harvestmen. Journal of Arachnology. 1985;13:121-128.
10. Yoshida M. *Behave oral analysis of insect parasitic nematodes.* In T. Mizukubo and K. F. Kyoto (Eds), Nematological experimentation. Japan: Kyoto University in (Japanese), 2014, 120-122.
11. Li Q, Sun Y, Wang G, Liu X. Effects of the mermthid nematode on the haemocytes of its host *Helicoverpa armigera*. Journal of Insect Physiology. 2009;55:47-50.
12. Nickle WR. Mermithid parasites of agriculture pest insects. Journal of Nematology. 1981;13:262-266.
13. Bhatnagar VC, Pawar CS, Jadhav DR, Davies JC. Mermithid nematodes as parasites of *Heliothis spp.* And other crop pests in Andhra Pradesh, *India. Proc. Indian.* Acad. Sci. Anim. Sci. 1985;94:509-515.
14. Micieli MV, Risso P, Achinelly MF, De Los Milagros Villar M, Muttis E. Population dynamics between the mermithid, *Strelkovimermis spiculatus* (Nematoda: Mermithidae) and the flood water mosquito *Ochlerotalus albifosciatus* (Diptera: Culicidae) overtime. Biological Control. 2012;61:55-63.
15. Chen G, Jian H, Ren HF. A 15 year study on the natural control of Mythimna seperata (Lep: Noctuidae) by *Ovomemis sinensis* (Nematoda: Mermithidae). Chinese Journal of Biological Control. 1991;7:145-150.
16. Li FC, Xiong LX. Biological characteristic of *Hexamermis agrotis*. Chinese bulletin of Entomology. 2005;42:704-707.
17. Baker GL, Capinera JL. Nematodes and nematomorphs as control agents of grasshoppers and locusts. Memories of the Entomological Society of Canada. 1997;171:157-211.

18. Prabhakar M, Prasad YG, Venkateswarlu B. New record of *Hexamermis dactylocercus* Poinar jr. and Linares (Nematoda: Mermithidae) parasitizing red hairy caterpillar, *Amsacta albistriga* (Walker) (Lepidoptera: Arctiidae) from India Journal of Biological Science. 2010;24:285 -287.
19. Presswell B, Evans S, Poulin R, Jorge F. Morphological and molecular characterization of *Mermis nigrescens* Dujardin (Nematoda: Mermithidae) parasitizing the introduced European earwig (Dermaptera: Forficulidae) in New Zealand. Journal of Helminthology. 2015;89:267-276.
20. Helden AJ. First extent records of mermithid nematode parasitism of *Auchenorrhyncha* in Europe. Journal of Invertebrate Pathology. 2008;99:351-353.
21. Poinar GO JR. *Mermithid nematodes of blackflies, In: Blackfiles: The future of Biological Methods in Integrated Control (Laired, M.Ed.).* Academic, London, 1981, 159-170.
22. Kaiser H. Terrestrial and Semiterrestrial Mermithidae. In W.R. Nickle (Ed,). Manual of Agricultural Nematology New York, NY: Marcel Dekker, 1991, 899-965.
23. Brown BJ, Platzer EG. The effect of temperature on the infectivity of *Romanomermis culicivorax.* Journal of Nematology. 1977;9:166-172.
24. Galloway TD, Brust RA. Effects of temperature and photoperiod on the infection of two mosquito species by the mermithid, *Romanomermis culicivorax*. Journal of Nematology. 1977;9:218-221.
25. Arthurs S, Heinz KM, Prasifka JR. An analysis of using entomopathogenic nematodes against above-ground pests. Bulletin of Entomological Research. 2004;94:297-306.
26. Poinar GO JR. *Entomogenous nematodes. A Manual and host list of Insect Nematode Associations*. E.J. Brill, Leiden, 1975.
27. Choo HY, Kaya HK. Parasitism of brown planthopper and white backed planthopper *by Agamermis unka* in Korea. Journal of Nematology. 1990;22:513-517. [PMC free article] [PubMed] [Google Scholar].
28. Platzer EG, Mullens BA, Shaseldean MM. *Mermithid nematodes. In P. Grewal, R. Ehiers and D. Shapiro Ilan (Eds)., Nematodes as Biocontrol Agents.* Wallingford, England: CABI publishing, 2005, 411-418.
29. Poinar GO. *Heydenius brownie sp.* n. (Nematoda: Mermithidae) parasitizing a planthopper (Homoptera: achilidae) in Baltc amber. Nemtol. 2001;3(8):757.
30. Santamarina, Mijares A, Perez, Pacheco R. Reduction of mosquito larval densities in natural sites after introduction of *Romanomermis culicivorax* (Nematoda: Mermithidae) in Cuba. J Med Entomol. 1997;34:1-4.