STUDIES ON THE LIFE CYCLE OF HETERRORHABDITIS INDICA – AN ENTOMOPATHOGENIC NEMATODE

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Abstract - Entomopathogenic nematodes are soil born parasitoid which offers pathogenicity to various soil dwelling plant pathogens. EPN's generally gets mutually associated with bacteria which is responsible for pesticidal actions. The two most studied species of EPN's are Steinernematidae and Heterorhabditidae families which has mutualistic association with bacteria of genus Xenorhabdus and Photorhabdus. Present study focus on the life cycle study of Heterrorhabditis spp. where H. indica is taken as reference organism to represent the entire Heterorhabditidae family. It was observed that 1st generation hermaphroditic females were observed after 3 days of infection in wax moth larvae and simultaneously lays egg on 4th day followed by hatching on 5th day. Second generation males and females can be differentiated after 7th day of infection and the phenomenon Endotokia matricida for infective juvenile (IJ) development were observed on 8th day in hermaphroditic female and in between 10th and 12th day in amphimictic females.

Key word: amphimictic females, eggs, infective juvenile, hermaphroditic females, wax moth.

I.INTRODUCTION

Entomopathogenic nematodes (EPN) are safe bio control agents [1] that are mainly used to manage soil borne insect pests [2]. The nematodes of the two families Steinernematidae Chitwood and Chitwood [3] and Heterorhabditidae Poinar [4] of the phylum Nematoda, are small parasitic round worms that infect and kills the insects. These nematodes are found to be adaptable microorganisms because they can able to overcome the acquire immunity developed against them in their corresponding insect pest (Gaugler and Kaya 1990) [5]. Both entomopathogenic nematode families are monogeneric in nature. Riddle et al. (1997) proved that EPNs are closely related to *C. elegans*, which is used as model organism for studying animal development and genetics[6].

Kaya (1993) discussed that within *Steinemematidae, Steinernema* is the accepted genus and in Heterorhabditidae it is *Heterorhabditis*. They had also described that free-living, non-feeding infective juveniles of these nematodes possess attributes of both insect parasitoids or predators and microbial pathogens. Like parasitoids/predators, they have chemoreceptors and are motile; like pathogens, they are highly virulent, kills their hosts rapidly, can be cultured easily *in vitro*, have a high reproductive potential, and have a numerical but no functional response[7]. *Steinernema* and *Heterorhabditis* have a mutual symbiotic association with the bacteria of genera Xenorhabdus and Photorhabdus, respectively, and the nematode–bacteria complexes are used in the biocontrol agents against insects [5].

The third stage of the nematode called dauer juvenile (DJ) occurs freely in the soil and its role is to seek out and infect an insect larva. *Heterorhabditis* enters host by abrading the intersegmental membranes of the insect using a dorsal tooth. Once the DJ reaches haemocoel of insect, it releases cells of a symbiont bacterium that the nematode carries in its intestine. The haemolymph of the insect provides rich medium for the bacterial cells where they begins to grow, release toxins and exo-enzymes and kill

the insect. The insect dies rapidly, usually within 24-48 h. Nematode reproduction continues over two to three generations until the nutrient status of the cadaver deteriorates where upon adult development is suppressed and DJ accumulate. These non-feeding infective stages emerge into the soil where they may survive for several months in the absence of a suitable host [4]. In *Heterorhabditis* spp., the DJ after reaching the insect haemoceol tend to fourth juvenile stage (J4), within 48-72 hrs followed by pupal stage which lays egg and mature to give first generation hermaphrodite females called J1 (within 8 to 10 days). Further but these hermaphroditic females give rise to a second generation (J2) amphiteric males and females and to self-fertile hermaphrodite females and DJ [8]-[9].

In *Heterorhabditis* spp., most of the times first generation of offspring emerge as IJs. These IJs were developed *in utero* of the parental hermaphrodite in a process known as *endotokia matricida*. *Endotokia matricida* occurs as a result of self-fertilization; where fertilized eggs hatch into juveniles of Stage 1 (J1) within the hermaphroditic nematode [10]. In field, these entomopathogenic nematodes are mobile and persistent in soil; furthermore, they are highly effective as biocontrol agents and often render better results than those obtained by means of chemical compounds used also as control [11]. At present, 35 species in *Steinernema* and 10 species in *Heterorhabditis* have been identified, but only few of them are commercially available as biocontrol agents [12].

Current data suggests that two species of *Heterorhabditis* namely *H. indica* and *H. bacteriophora*, have a global distribution. Of which, *H .indica* occurs widely in the tropics and subtropics, having been isolated in southern India and is used for the biological control of the white grubs *Holotrichia serrata* and *Leucopholis lepidophora* in sugarcane plantations in India [13]. Since detailed studies on life cycle of *H. indica* were not available, studies were done to examine the life cycle this species which can be interrelated with the entire *Heterorhabditis* spp.

II.MATERIALS AND METHOD

A. Source of Organism

Heterrorhabditis indica was procured in the form of commercially available biopesticide Multiplex Soldier, Bangalore. *Galleria mellonella* (wax moth) was procured from National Bureau of Agriculturally Important Insects (NBAII), Bangalore and Sugarcane Breeding Institute, Coimbatore. The procured wax moth were provided with the artificial diet (Wheat flour 350 g, corn flour 200 g, milk powder 130 g, baking yeast powder 70 g, honey 100 ml, and glycerin 150 ml) until the larvae reaches desired instar stage.

B. Infection of wax moth with multiplex soldier

Two methods of infection of wax moth with nematodes were carried out. In first method, 30g of bio pesticide powder was dissolved in 100ml of distilled water and maintained at 150 rpm for 30 minutes in rotary shaker to activate it. Later, 10 wax moth larvae were placed on moist Whatmann filter paper no.1 in a petridish and exposed to a concentration of 100 infective juveniles (IJs) per wax moth larvae. The entire setup was sealed with parafilm and maintained at room temperature until the mortality of larvae occurs.

In the second method of infection, 30g of sterile sand was taken in plastic box and mixed with 5g of bio pesticide powder along with 50ml of distilled water to maintain moisture in the mixture. 10 wax moth larvae were placed in each plastic box, covered and kept in room temperature until the death of the larvae occurs. This method is more efficient since Ijs by nature occurs in soil and the number of nematodes infecting each larva would be more when compared with water suspension of bio pesticide.

C. Studies on the life cycle of the nematode

To study the life cycle of *H. indica,* the host wax moth was infected with nematode according to the method described earlier and the plates were stored in well aerated clean environment. After the death of larvae, two larva's were taken on every 24 hours cleaned with ringer's solution (NaCl – 9g, $CaCl_2$ – 0.37g, KCl – 0.42g, $NaHCO_3$ – 0.2g, Dis.H_2O –

1000ml) followed by distilled and were subject to dissection with sterile needle and blade in petriplate containing ringer's solution. The emerged nematode larvae were carefully examined under the light microscope with different resolution to study the detailed morphology and life cycle of nematode. This was repeated until the complete life cycle was studied.

III. RESULTS AND DISCUSSION

A. Infection of wax moth with multiplex soldier

In first method of infection of wax moth with *H.indica*, 10 larvae's of wax moth were found to be death after 48 hours of infection and the red pigmentation were developed after 96 hours of infection. In the second infection method all the 10 larvae's were found to be death after 24 hours followed by the red pigmentation after 72 hours. Since, in second method nematode was mixed up with soil as like in the natural environment (nematodes usually occur in soil) the rate of infection was better than the first method. The pigmented cadavers were taken and washed with distilled water thrice and in ringer solution once and was used for further experiments.

B. Studies on the life cycle of the nematode on *in vivo* condition

The life cycle of nematode *H. indica* consists of four important stages which include hermaphroditic females, eggs, amphimictic females and males. Each stage of them is described below.

i). Hermaphroditic females

Hermaphroditic females which came from IJ's after development inside the wax moth were observed on 3rd day after infection. They are reported to be largest of all other stages which vary from 2.7 ± 1 mm [13]. Anal region of this stage was appeared to be swollen and the reproductive part vulva was placed above the anus. *Endotokia matricida* were observed after 8th day of infection in first generation hermaphroditic females. Fig1 shows the adult hermaphrodite stage and Fig 2, 3, 4 shows the mouth, vulva and anus of first generation *H.indica.*

Fig 1. Hermophroditic female

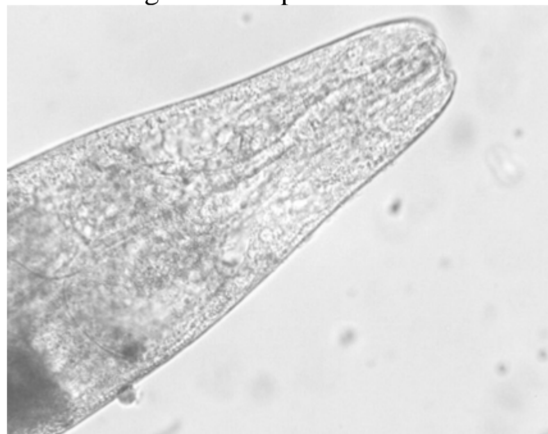

Fig 2. Mouth of 1st generation *H. indica*

In Steinrernema reproduction is observed to be amphimictic where the corresponding DJ matures to become either a male or a female and no hermaphroditic females as in *Heterrorhabditis* spp. [9] and hence life cycle becomes even simpler and shorter duration. Approximately to complete one life cycle it takes 10-12 days in *Heterrorhabditis* spp. but in case of Steinrernema spp. life cycle can be completed within 8 – 12 days because of the absence of Hermaphroditic females in the first generation.

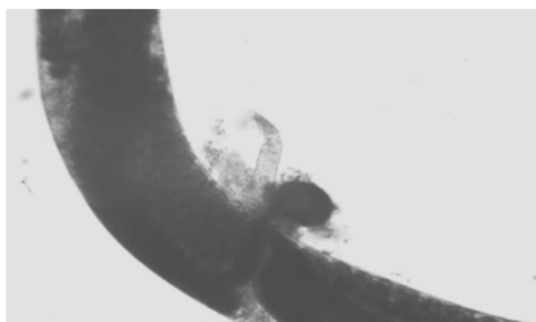

Fig 3. Vulva of female *H. indica*

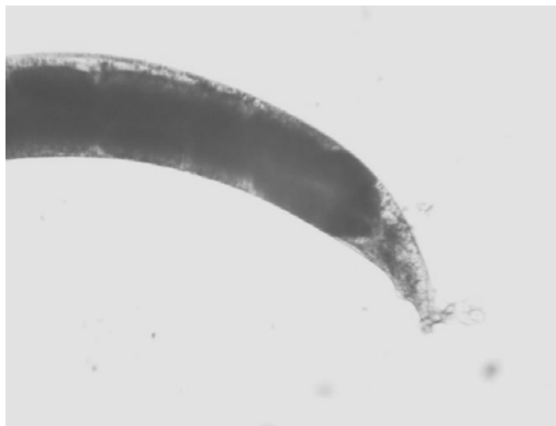

Fig 4. Anus of *H. indica*

ii). Eggs

Eggs of *H.indica* starts developing after 4th day of infection on wax moth and the eggs were found to be present in the entire body region of *H.indica* covered by thick cuticle. Eggs start hatching after the fifth day of infection and develop into amphimictic females and males. Fig 5 & 6 shows the clear image of eggs and oozing of eggs from 1st generation *H.indica.* Wang and Bedding (1996) studied the dynamics of population development of *H. bacteriophora* and *S. carpocapsae* in larvae of G. mellonella and described that *Endotokia matricida* was observed from first generation of Hermaphroditic females as in *Heterrorhabditis* spp. but only in third generation in case of Steinernema family[10].

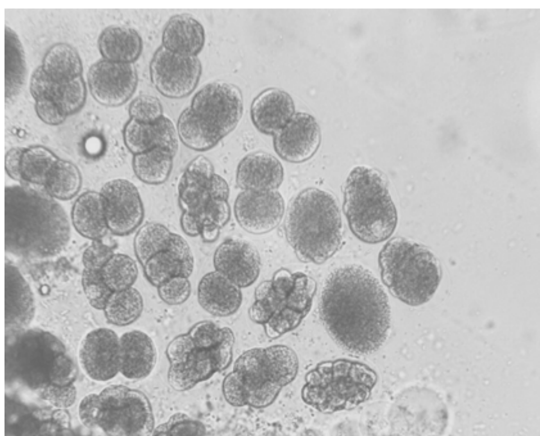

Fig 5. Eggs of *H. indica*

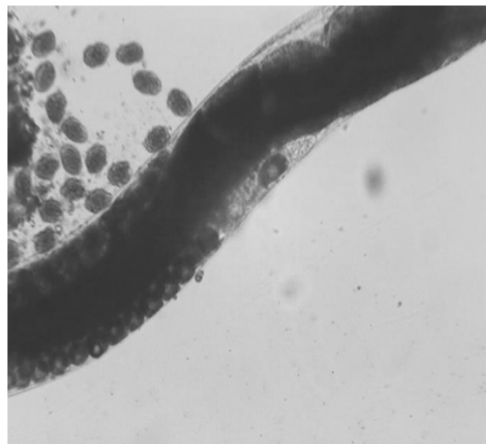

Fig 6. Oozing of Eggs from *H. indica*

iii). Amphimictic females and males

Second generation amphimictic females and males were observed after 5th day of infection of wax moth. The life span of male varies from 48 t0 72 hours which is short lived when compare to the second generation females which carries offspring's for the next generation. After hatching the size of both males and females were found to be similar but after 24 hours of hatching the size of female gets drastically increased. Pioner et al. (1992), reported that size of males 721±64 µm where the females are 1.6 ± 0.12 mm which is approximately the double the size of males[13]. Females can be easily observed with the naked eye but males can be observed clearly with the help of microscope only.

Fig 7. Hermaphroditic female, 2nd gen Male + female

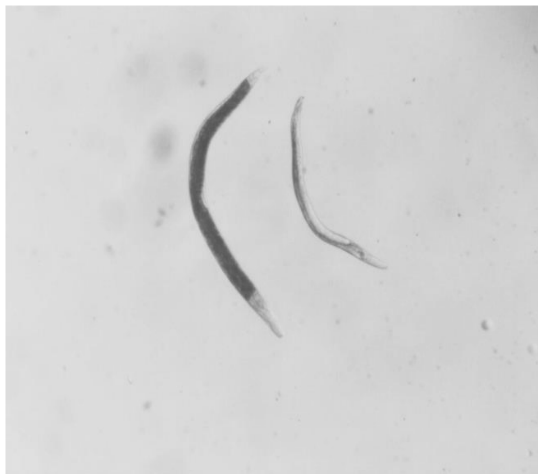

Fig 8. 2nd Gen Female and Male

Endotokia matricida were observed between 10th to 12th days of infection in second generation hamphimictic females. Fig 7 & Fig 8 shows all the three 1st generation female and 2nd generation male and female. Fig 9 & 10 shows *Endotokia matricida* of Hermaphroditic female.

Fig 9. *Endotokia matricida* of Hermaphrodite Female – Initation Stage

Fig 10. *Endotokia matricida* of Hermaphrodite Female – End Stage

IV. CONCLUSION

The detailed life cycle studies of H. indica were done. From the study, hermaphroditic females developed from activated IJ's were seen on 3rd after the infection of bio pesticide powder in wax moth. Eggs start developing on 4th day of infection onwards and start hatching by 5th day. It was also observed that not all the eggs were laid into the host few were retained inside the mother which later undergoes intrauterine development by the process of *Endotokia matricida. Endotokia matricida* was observed in both hermaphroditic and amphimictic females. Second generation amphimitic males and females were observed after 5th day of infection and male has very short life span which dies immediately after mating within 48 to 72 hours. *Endotokia matricida* were observed after 8th day of infection in first generation hermaphroditic females and were observed between 10th to 12th days of infection in second generation amphimictic females. As a result of this EM, IJ's develop which is then detached from the insect cadaver and searches for new host. On an average complete 3 life cycles can occur in wax moth larvae after which nutrient gets depleted and the nematode seeks new host in the form of IJ's.

REFERENCES

[1] R.U. Ehlers, and H.M.T. Hokkanen, "Insect biocontrol with non-endemic entomopathogenic nematodes (Steinrnema and Heterorhabditis spp.): conclusions and recommendations of a combined OECD and COST workshop on scientific and regulatory policy issues," Biocontrol. Science and Technology, vol. 5, pp. 184-291, 1996.

[2] R.U. Ehlers, and A. Peters, "Entomopathogenic nematodes in biological control: feasibility, perspectives and possible risks," Cambridge University Press, Cambridge, pp. 119-136, 1996.

[3] B.G. Chitwood, and M.B. Chitwood, "An Introduction to Nematology," Monumental Printing Co., Baltimore, Maryland, pp. 72-89, 1937.

[4] G.O. Poinar, "Description and biology of a new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* n. gen., n. sp. (Rhabditida:

Heterorhabditidae n. fam.)", Nematologica, vol. 21, pp. 463-470, 1976.

[5] R. Gaugler, and H.K. Kaya, "Entomopathogenic Nematodes in Biological Control," Soil ecology, CRC Press, Boca Raton, Florida, pp 93-115, 1990.

[6] D.L. Riddle, T. Blumenthal, B.J. Meyer, and J.R. Priess, "Introduction to *C. elegans,"* C. elegans II, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 1–22, 1997.

[7] H.K. Kaya, "Entomogenous and entomopathogenic nematodes in biological control," Plant Parasitic Nematodes in Temperate Agriculture, CAB International,Wallingford, United Kingdom, pp. 565-591, 1993.

[8] O. Strauch, S. Stoessel, and R.U. Ehlers, "Culture conditions de. ne automictic or amphimictic reproduction in entomopathogenic rhabditid nematodes of the genus *Heterorhabditis,"* Fundamental and Applied Nematology, vol. 17, pp. 575-582, 1994.

[9] I. Dix, A.M. Burnell, C.T. Griffin, S.A. Joyce, M.J. Nugent, and M.J. Downes, "The identification of biological species in the genus *Heterorhabditis* (Nematoda: Heterorhabditidae) by cross-breeding second generation amphimictic adults," Parasitology, vol. 104, pp. 509-518, 1992.

[10] J. Wang, and R.A. Bedding, "Population development of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in the larvae of *Galleria mellonella,"* Fundamental and Applied Nematology, vol. 19, pp. 363-367, 1996.

[11] G.O. Poinar, "Biology and taxonomy of Steinernematidae and Heterorhabditidae in Entomopathogenic Nematodes in Biological Control," CRC Press, Boca Raton, Florida, pp. 23-61, 1990.

[12] S.P Stock, and D.J. Hunt, "Nematode morphology and systematic," Nematodes as Biocontrol *Agents,* CABI Publishing, Wallingford, United Kingdom, pp 477-508, 2004.

[13] G.O. Poinar, G.K. Karunakar, and H. David, "*Heterorhabditis indicus* n. sp. (Rhabditida: Nematoda) from India: Separation of *Heterorhabditis* spp. by infective juveniles," Fundamental and Applied Nematology, vol. 15, pp. 467-472, 1992.