Efficacy of native isolates of Entomopathogenic Nematode against Spodoptera frugiperda

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***Abstract*—Entomopathogenic nematodes (EPN) are found to be very effective against Lepidopteran and Coleopteran insects. The EPN enters the insect larva through the natural openings and within a short period capable of killing the insect larva in 48 hrs due to septicemia. The effectiveness of EPN depends upon the virulence of the organism based on lethal concentration (LC) and lethal time (LT) of exposure. The fall armyworm (*Spodoptera frugiperda*) causes serious damage to fodder maize grown for livestock feed. A study was made to collect the native isolates of EPN *Heterorhabditis indica* (Hi) and *Steinernema glaseri* (Sg) in Tirunelveli and Kanyakumari districts. The EPN isolated from these districts were screened for virulence against third instar of *S. frugiperda.* The inoculum used were 1.25 x 10^9, 2.5 x 10^9 and 5 x 10^9 IJ/insect @ 10 larvae per treatment and three replicates maintained for each level. The mortality was recorded at every 12 hrs upto 72 hrs. The LC50 and LT50 were arrived by analyzing the data by probit analysis. The study revealed that among the native isolates of entomopathogenic nematodes, the isolate** JU-EPN-Hi-3 **from Nalloor village, Kanyakumari district, was the highly virulent against *S. frugiperda* (LC_{50} = 3.50 IJ/larva). The least virulent strain of EPN (LC_{50} = 5.54 IJ/larva) was** JU-EPN-Sg-1**, isolated from Vallavillai village, Kanyakumari district. Regarding lethal time 50 per cent mortality was obtained to the larva of *S. frugiperda* least duration in LT50 33.52 hrs. Whereas the highest duration (LT 50) of 44. 88 hrs was taken to kill the larva using the strain JU-EPN-Sg-1 from Kanyakumari (Vallavillai).**

***Index Terms*—Entomopathogenic nematodes, *Steinernema glaseri* and *Heterorhabditis indica, Spodoptera frugiperda,* Fodder maize, lethal concentration, lethal time.**

I. INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a highly destructive pest of maize and has become a serious threat in India since its recent invasion. Its polyphagous nature, rapid lifecycle and ability to develop resistance against insecticides complicate management strategies. Fodder maize, cultivated for silage production, is severely affected by this pest, leading to significant yield loss.

odder maize is a variety of maize (*Zea mays*) grown specifically for livestock feed, providing nutritious green fodder rich in carbohydrates, dry matter, and protein. It is an ideal non-legume fodder crop, especially for making silage (Sudip *et al.,* 2023). *S. frugiperda* causes foliar damage of maize resulting in lesser yield in plants on which it infects. The symptoms of *S. frugiperda* infestation involves irregular holes and window-like patches on leaves, shredded or ragged leaf edges, skeletonized foliage and whorl damage with signs of tunneling which are responsible for significant loss of fodder for animal stock.

Entomopathogenic nematodes (EPN) belonging to the families *Steinernematidae* and *Heterorhabditidae* are promising biological control agents due to their ability to cause rapid mortality in insect hosts. Infective juveniles (IJs) enter the host body through spiracles, mouth or anus and release symbiotic bacteria (*Xenorhabdus* or *Photorhabdus* spp.) into the hemocoel, causing septicemia. Virulence of EPN is influenced by nematode species, isolate, environmental factors and host susceptibility.EPN are very effective against Lepidopteran and Coleopteran insects and capable of killing the insect larva within 48 hrs (Caoili *et al*., 2018 and Garcia-del-Pino *et al*., 2013). The effectiveness of EPN depends upon the virulence of organism with respect to Lethal Concentration (LC) and Lethal Time (LT).

Native isolates often exhibit better adaptability to local environmental conditions than exotic strains. In this

context, the present study aimed to isolate native EPN from Tirunelveli and Kanyakumari districts and to evaluate their virulence against third-instar larvae of *S. frugiperda* under laboratory conditions.

II. MATERIALS AND METHODS

The present studies were conducted in the laboratory at the Department of Agricultural Entomology and Nematology, The Indian Agriculture College (TIAC), Radhapuram, Tirunelveli District, Tamil Nadu.

Survey, Occurrence and Distribution of Entomopathogenic Nematodes in Tamil Nadu

A total of 200 soil samples were collected from Kanyakumari and Tirunelveli districts to assess the presence and distribution of entomopathogenic nematodes (EPNs). Samples were collected from diverse cropping systems including banana, dragon fruit, coconut, cashew, rubber, teak, mango, sapota, jackfruit and star fruit plantations. At each location, two composite soil samples (0 - 10 cm depth) were collected, thoroughly mixed, and a subsample of 250 cc was retained. Data on soil type, geographic location, cropping system and recent rainfall were recorded. Samples were placed in labelled polythene bags and stored at 4°C until baiting for multiplication and extraction of EPN. EPN isolation was performed following the soil-baiting technique of Bedding and Akhurst (1975) using late instar larvae of *Corcyra cephalonica*. Infected larvae showing typical symptoms were transferred to clean Petri dishes for confirmation.

Physico-Chemical Analysis of Soil Samples

Soil types were confirmed using the existing soil maps available at the TIAC Soil Testing Laboratory, Radhapuram. Soil samples were analysed for major physico-chemical properties using standard procedures:

pH (1:2.5 soil-water suspension) - Percival (1993), Jackson (1965)

Electrical conductivity (EC) - Jackson (1965)

Available nitrogen - Subbiah and Asija (1956)

Available phosphorus - Watanabe and Olsen (1965)

Available potassium - Jackson (1965)

These parameters were used to understand ecological factors influencing EPN distribution.

Extraction of Nematodes

EPNs were extracted from foam culture medium four weeks after inoculation (Bedding, 1981). Foam chips were transferred to tissue paper placed over a 20-mesh aluminium screen. The setup was placed over a Petri dish containing distilled water such that water barely touched the foam. Emerging nematodes were collected every 24 hours for 72 hrs. The suspension was repeatedly washed to remove bacterial debris, and infective juveniles (IJ) were quantified.

Culture of *Corcyra cephalonica* (Pyralidae: Lepidoptera)

Broken cumbu grains (*Pennisetum glaucum* L.) were used as the rearing medium for *C. cephalonica*. Two kilograms of cleaned grains were dispensed into plastic trays and inoculated with 1 cc of freshly collected *C. cephalonica* eggs obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore. The trays were covered with muslin cloth and maintained at ambient room temperature (27 ± 2°C) and relative humidity **(**65–75%). Final instar larvae were harvested 25 days after inoculation for use in baiting and bioassays.

Mass Multiplication of EPNs

EPN isolates recovered from soil were mass-cultured using *C. cephalonica* larvae following Bedding and Akhurst (1975). Infected cadavers were transferred to White traps (White, 1927) for IJ emergence. The harvested IJ were surface sterilized with 0.25% sodium hypochlorite, washed thrice with sterile distilled water and stored at 13–15°C until further use.

Extraction of Infective Juveniles (IJ)

cadavers showing nematode infection were washed and individually placed on White's trap. IJs emerging from cadavers were harvested daily until emergence ceased. Collected juveniles were stored in tissue culture flasks and maintained at 20 ± 1°C in a BOD incubator with periodic aeration.

Sub-Culturing of EPN Isolates

Final instar larvae of *C. cephalonica* were used for routine sub-culturing. Ten larvae were exposed to 20 IJ per larva (200 IJ/ml suspension) on moistened Whatman No.1 filter paper in Petri dishes (Bedding, 1984). Dishes were sealed with cling film to maintain humidity. Cadavers were transferred to White traps 6

- 10 days post-infection, depending on EPN species (*S. glaseri* - 6 days; *H. indica* - 10 days).

Virulence Assay of EPN Isolates

Virulence of native EPN isolates was assessed following Dunphy and Webster (1986). Third instar *S. frugiperda* larvae were exposed to graded concentrations of 0, 5, 10, 20, 40, 80 and 100 IJ per larva. Each treatment consisted of 10 insects per replicate and four replicates. Mortality was recorded every 12 hrs up to 72 hrs. Lethal concentration (LC_{50}) and lethal time (LT_{50}) values were computed using Probit Analysis (Finney, 1962). The most virulent isolates were selected for further pathogenicity studies.

III. RESULTS

Virulence against *S. frugiperda*

Median lethal concentration

Among all the isolates of entomopathogenic nematodes, *H. indica* isolate JU-EPN-Hi-3 Kanyakumari (Nalloor) was highly virulent against *S. frugiperda* (LC50 - 3.50 IJ/larva). It was followed by JU-EPN-Sg-4 (4.28) Kanyakumari (Mulankuzhi), JU-EPN-Hi-1 (4.33) Kanyakumari (Kanchapuram), JU-EPN-Hi-6 (4.70) Tirunelveli (Neduvalli) and JU-EPN-Sg-3 (4.70) Kanyakumari (Karavillagam). JU-EPN-St-1 Kanyakumari (Vallavillai) was less virulent with LC50 of 5.54 IJ/larva (Table 1) (Fig: 1)

Fig : 1. LC_{50} and LT_{50} of Entomopathogenic Nematode isolates against *Spodoptera frugiperda*

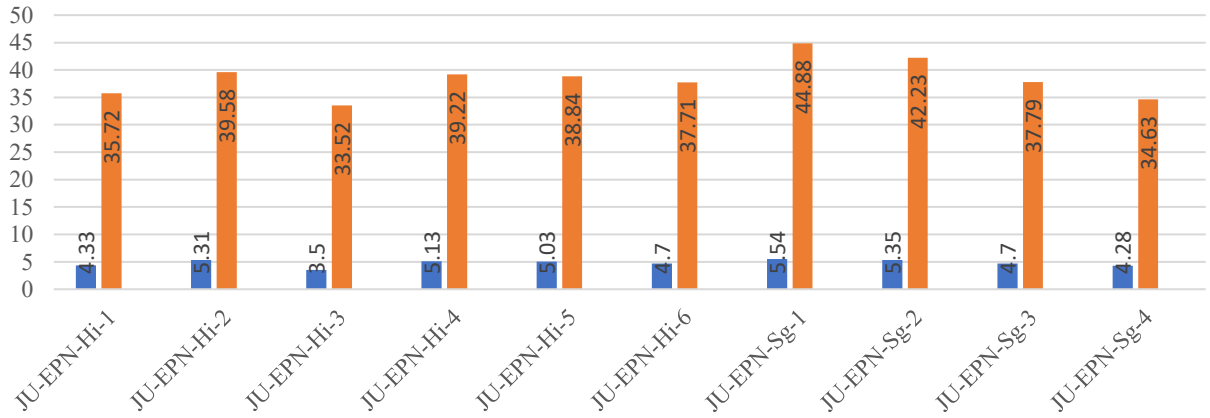

Table 1: Median lethal concentration (LC50) of EPN isolates to *S. frugiperda*

EPN isolates	$\chi 2$ (n-2)	b	+SE	LC50	Fiducial limits	
					Lower	Upper
JU-EPN-Hi-1	0.36	3.63	0.75	4.33	2.70	5.37
JU-EPN-Hi-2	0.12	4.67	0.96	5.31	4.23	6.17
JU-EPN-Hi-3	0.73	3.08	0.71	3.50	1.54	4.74
JU-EPN-Hi-4	1.02	3.69	0.66	5.13	3.74	6.19
JU-EPN-Hi-5	0.18	4.42	0.79	5.03	3.80	5.91

JU-EPN-Hi-6	0.26	4.08	0.91	4.70	3.31	5.64
JU-EPN-Sg-1	0.83	2.69	0.50	5.54	3.91	6.91
JU-EPN-Sg-2	0.26	2.76	0.53	5.35	3.73	6.66
JU-EPN-Sg-3	0.62	2.63	0.50	4.70	3.00	6.02
JU-EPN-Sg-4	2.02	1.66	029	4.28	2.21	6.21

Median lethal time

JU-EPN-Hi-3 Kanyakumari (Kanchapuram) caused 50 per cent mortality of *S. frugiperda* larvae in a minimum time of 33.52 hrs. It was followed by JU-EPN-Sg-4 Kanyakumari (Mulankuzhi) with LT50 as 34.63 h/larva. Maximum time was taken by JU-EPN-Sg-1 Kanyakumari (Vallavillai) which recorded LT50 as 44.88 h/larva (Table 2).

Table 2: Median lethal time (LT50) of EPN isolates to *S. frugiperda*

EPN isolates	χ2 (n-2)	b	+SE	LT50	Fiducial limits	
					Lower	Upper
JU-EPN-Hi-1	8.00	4.70	6.47	35.72	27.56	43.57
JU-EPN-Hi-2	4.73	6.01	0.62	39.58	36.22	42.90
JU-EPN-Hi-3	2.82	3.09	0.39	33.52	29.55	39.40
JU-EPN-Hi-4	7.67	2.60	0.29	39.22	27.75	56.74
JU-EPN-Hi-5	1.41	4.51	0.82	38.84	34.90	42.86
JU-EPN-Hi-6	1.83	2.78	0.29	37.71	32.46	43.68
JU-EPN-Sg-1	7.00	4.01	0.44	44. 88	35.41	56.00
JU-EPN-Sg-2	1.75	3.70	0.40	42. 23	38.15	48.26
JU-EPN-Sg-3	6.85	5.14	0.52	37.79	30.57	44.90
JU-EPN-Sg-4	11.87	4.01	0.48	34.63	23.81	43.89

IV. DISCUSSION

The present study revealed significant variation in the virulence of native isolates of *H. indica* and *S. glaseri* against third instar larvae of *S. frugiperda*. Among the isolates, JU-EPN-Hi-3 (*H. indica*) from Nalloor was the most virulent, recording the lowest LC_{50} (3.50 IJ/larva) and LT_{50} (33.52 hrs). The high pathogenicity of *H. indica* may be attributed to its rapid penetration ability and the fast multiplication of its symbiotic *Photorhabdus* bacteria, which is consistent with earlier reports on its efficacy against noctuid pests.

In contrast, JU-EPN-Sg-1 (*S. glaseri*) exhibited higher LC_{50} and LT_{50} values, indicating lower virulence. This aligns with earlier findings that *S. glaseri* typically show slower infection rates against foliage-feeding lepidopterans.

Differences in virulence among isolates collected from various agro-ecosystems suggest that soil conditions, microclimate and host availability influence EPN

performance. The superior efficacy of isolates from Kanyakumari indicates better ecological adaptation.

Overall, the results highlight JU-EPN-Hi-3 as the most promising native isolate for use against *S. frugiperda*. Its high infectivity and rapid killing ability support its potential for incorporation into integrated pest management programmes. Further studies on field evaluation and formulation are necessary to enhance its practical application.

V. CONCLUSION

The present study demonstrated significant variation in the virulence of native entomopathogenic nematode isolates against *S. frugiperda*. Among the isolates evaluated *H. indica* JU-EPN-Hi-3 from Nalloor exhibited the highest pathogenicity, expressed through the lowest LC_{50} and LT_{50} values, indicating superior infectivity and rapid mortality induction. In contrast, *S. glaseri* JU-EPN-Sg-1 showed comparatively lower virulence.

The findings highlight the potential of locally adapted *H. indica* isolates, particularly JU-EPN-Hi-3, for the development of effective biological control strategies against *S. frugiperda*. These isolates can be considered promising candidates for further mass production, formulation, and field-level evaluation to support their integration into sustainable pest management programmes.

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