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# Isolation of Native Strains of Entomopathogenic Nematodes against Fall armyworm, *Spodoptera frugiperda* (Smith.) in Maize

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## ABSTRACT

*Entomopathogenic Nematodes (EPNs) found in a variety of soil types, geographical regions, and hosts, which belong to the genera Steinernematidae and Heterorhabditidae, have the potential to act as biocontrol agents. In comparison to chemical and microbial pesticides, they performed better. A total of 87 soil samples were taken from regions where maize was grown in Tuticorin district, Tamil Nadu, India and they were examined for the presence of EPN in 2022–2023. By using the Corcyra baiting approach, a total of 9 samples (10.33%) showed EPN EPN-positive sites. A total of 8 Steinernema sp. (13.33%) and 1 Heterorhabditis sp. (5.00%) were isolated from that population. EPN is identified at a generic level using the cadaver's colour. Heterorhabditis displays brick red, while Steinernema exhibits creamy white. At a dose of 100–600 IJs/larva for the third and fifth instar, the isolated efficient native EPN strain (Kayathar strain) demonstrated mortality of 95.00–100.00% and 94.50–99.80%. According to the study, EPN shown showed considerable potent against Spodoptera frugiperda. So, EPNs may be used as a promising bio-control agent to battle pests of the maize crop.*

***Keywords:*** *Native strains, Entomopathogenic nematodes (EPNs), Pathogenicity, Spodoptera frugiperda, Maize, Corcyra, Steinernema sp., Heterorhabditis sp.*

## Introduction

Entomopathogenic nematodes (EPNs) are obligate parasites that belong to the family Steinernematidae and Heterorhabditidae and thus the Genera were *Steinerema* and *Heterorhabditis*, respectively [1]. About 100 *Steinernema* species and 21 *Heterorhabditis* species have been discovered worldwide; Of these, 12 species of *Steinernema* and 3 species of *Heterorhabditis* exist in India [2]. The free-living infective juveniles (IJs), which are members of the genera *Xenorhabdus* and *Photorhabdus*, have the ability tocan kill the host insect by carrying symbiotic species-specific bacteria in their intestines. Nematode-bacteria complexes are used to kill insect pests most frequently within 48-72 h post-infection, and the host itself serves as the site of subsequent reproduction. EPNs are well amenable to being used as a bio-control agent due to their wide host range and large-scale *in vitro* and *in vivo* cultureeability [3]. In recent decades, insects have developed resistance due to the indiscriminate use of insecticides [4]. Therefore, the major objective of this study was to isolate native EPN strains from the maize maize-grown tracts of the Tuticorin district, Tamil Nadu, India and to test the pathogenicity of native EPN strains against an exotic pest, fall armyworm, *S. frugiperda* in maize.

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## Materials and Methods

### *Isolation of entomopathogenic nematode*

**Site selection:** Soil samples were collected from the maize-growing regions of the Tuticorin district as well as from the fields of Agricultural College and Research Insitute, Killikulam campus during 2022-2023 in order to isolate native EPN strains.


***Fig. 1. Map showing the blocks of Tuticorin, Tamil Nadu, India***

***Soil Sampling***

By adopting methods described by [5], hand shovels were used to collect 200-250 g of samples from each sample site at a depth of 10 to 15 cm near the rhizosphere region and vegetation [6]. Samples were packaged in polythene bags, stored at the optimal room temperature to conserve moisture, and labelled with the location, kind of soil and crop ecosystem. Using 70% ethanol, hand shovels were sterilized prior to sampling another site.

***Culturing of host insect, Corcyra cephalonica Staint.***

EPN was isolated from soil using the Rice moth, *C. cephalonica* as a bait insect. Additionally, employed for mass rearing of EPN as well [7]. *C. cephalonica* was mass reared on half-broken pearl millet in the biocontrol laboratory at Agricultural College and Research Institute, Killikulam in accordance with standard protocol by [8].

***Soil baiting technique***

Ten final instar larvae of *C. cephalonica* were employed as bait along with 250 g of soil from each sampling site was placed in a plastic container under laboratory conditions and sprayed a small quantity of water to retain moisture; holes are made in the lids to allow aeration. Containers were then incubated in a dark environment at a temperature of 25±2 °C. Dead cadavers were extracted from the soil after an incubation period of 6–7 days [7]. Cadavers were transferred to White traps after being completely rinsed with sterile distilled water.

***White trap method***

The white trap contains a 90 mm petri dish filled to a depth of 1 cm with 0.01 per cent formalin solution. Inverted petri dishes of 50 mm diameter were placed within 90 mm petri dish. Whatman No. 1 filter paper was placed on top of the dish. Dead cadavers were arranged over filter paper. To ensure that the filter paper came into contact with the formalin water. Petri plates were stored in a dark environment at a temperature of 28 °C. IJs start to emerge from the cadavers and gather in the formalin solution [9].

***Harvesting and Enumeration of EPNs***

Harvested EPNs were added to a 250 ml Erlenmeyer flask, which was then rinsed thrice with distilled water and allowed to settle, supernatants were removed. The process was repeated until it attain clear EPN suspension. For counting, 1 ml of EPN suspension was spread over a nematode counting chamber (8 x 8 x 1.5 cm) and examined using a stereozoom microscope (40 x)

.

***Mass culturing of Fall armyworm, Spodoptera frugiperda Smith.***

From the corn fields, egg masses and larvae were collected and they were reared separately in plastic containers measuring 30 mm in diameter by 40 mm in height, fed with an artificial diet based on the TNAU artificial diet composition. Pupa was placed inside a 30 x 30 x 30 cm oviposition chamber with adult feeding solution as provided by TNAU and young Nerium plant branches were used as a substrate for egg laying. First and second instar larvae are reared with maize leaves and shifted to a semi-synthetic diet.

Based on preliminary bioassays conducted in the laboratory, effective strain is carried over to the pathogenicity study.

***Pathogenicity of effective EPN strain against S. frugiperda***

For pathogenicity assays of FAW, EPN suspensions of different doses (50, 100, 200, 250, 400, and 600 IJs/larva) against third and fifth instar larvae. To avoid cannibalism, one larvae per petri-plate (90 mm), was fed with maize leaf. To conserve moisture, plates were sealed with parafilm tape and kept in a polythene cover under a dark room temperature of 28 °C. Larval mortality was examined 48, 72, and 96 hours of the inoculation period.

***Statistical analysis***

The "Probit analysis in Excel" application was used to compute the $LC_{50}$ [10].

## Results and Discussion

***Isolation and Identification of native entomopathogenic nematodes***

A total of 87 soil samples were collected from maize-growing areas of Tuticorin district, Tamil Nadu, India. Out of 87 samples, nine samples were EPN positive. From the soils, EPN were isolated by the soil baiting method using *C. cephalonica*. One of nine samples was determined to have *Heterorhabditis* from Kayathar, which was distinguished by the brick red colour of the *Corcyra* cadaver. Based on the cadaver's creamy white and greyish colouring, the remaining 8 samples were discovered to have *Steinernema*.

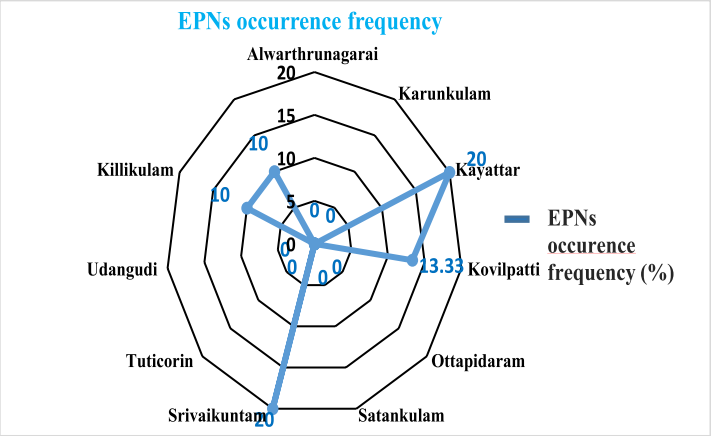


***Fig. 2. Occurrence of EPN in Tuticorin, Tamil Nadu, India.***

***Plate. 1. Insect baiting method using Rice moth, Corcyra cephalonica***

***Plate. 2. White trap method to collect Ijs***

[11] reported that soil surveys showed an EPN recovery rate of 6.00-35.00%. With a frequency of 10.34%, retrieved 9 EPN positive samples out of 87 samples from the Tuticorin district's maize-growing tracts. In the Northern regions of Tamil Nadu, a rate of 16.30% was observed [12]. Recovery rates in the Karur district were 0.25 per cent [13] while,, Andhra Pradesh were was 1.20 per cent [14]. The method of detection and sample intensity used in the various surveys showed differences in the recovery rate of EPN.

The prevalence of EPN in maize fields of the Tuticorin district ranged widely from 10.00 to 20.00 per cent, with Killikulam (10.00%) having the lowest prevalence and Kayathar and Srivaikuntam having the most (20%), followed by Kovilpatti (13.33%) having the moderate. The discrepancy could be a result of the EPN's patchy distribution, which is caused by temporal and spatial fluctuation and also relies on the species [15].

***Table 1: Distribution and frequency of EPN in Tuticorin, Tamil Nadu, India***

| SI. No. | Blocks/ Areas | | No. of samples collected | EPNs positive samples | Frequency (%) | Identified EPNs Species |
|---|---|---|---|---|---|---|
| 1. | Alwarthirunagarai | | 4 | 0 | 0 | Nil |
| 2. | Karunkulam | | 5 | 0 | 0 | Nil |
| 3. | Kayattar | | 20 | 4 | 20.00 | *Steinernema* sp. *Heterorhabditis* sp. |
| 4. | Kovilpatti | | 15 | 2 | 13.33 | *Steinernema* sp. |
| 5. | Ottapidaram | | 5 | 0 | 0 | Nil |
| 6. | Satankulam | | 5 | 0 | 0 | Nil |
| 7. | Srivaikuntam | | 5 | 1 | 20.00 | *Steinernema* sp. |
| 8. | Tuticorin | | 3 | 0 | 0 | Nil |
| 9. | Udangudi | | 5 | 0 | 0 | Nil |
| 10. | Killikulam | Blackgram | 10 | 1 | 10.00 | *Steinernema* sp. |
| | | Orchard | 10 | 1 | 10.00 | *Steinernema* sp. |
| TOTAL | | | 87 | 9 | 10.34 | |

Additionally, only Steinernematids are reported in soil samples taken from several locations in Tamil Nadu [16]. Steinernematids alone were also reported by [10]. Furthermore, *Steinernema* (2.20 %) was most prevalent than *Heterorhabditis* (0.30 %) [13]. *Steinernema* (74.00 %) was more predominant in the cotton ecosystem of Tamil Nadu [17].

Furthermore, samples that tested positive for EPN ranged from sandy loam (lower) to clayey (higher) which supports EPN's survival and mobility [18] [19].

***Preliminary study***

Based on the preliminary bio-efficacy study, *Steinernema* sp. (Kayathar strain) was found more effective than others. So this strain was further tested against S. *frugiperda* at different concentrations of EPN.

***Pathogenicity of Steinernema sp. (Kayathar strain) against S. frugiperda***

Based on the computed $LC_{50}$ values, *Steinernema* sp. required 33.13 IJs/larva and 37.09 IJs/larva at 96 hours after infection, and 37.80 IJs/larva and 54.33 IJs/larva at 72 hours after infection for third and fifth instar larva of *S. frugiperda,* respectively. According to [20] *Heterorhabditis indica* consumed 20.26 and 62.07 IJs per third and fifth instar larva at 72 hours. According to [21], S. *riobrave* 500 IJs/larva were ineffective against FAW. The present findings, are in line with those of [22], who found that the isolated strain of S. *carpocapsae* RW14-GR3a-2 from Rwanda was the most virulent. Additionally, S. *riobrave* MEX-15 was the most virulent strain.

*Table 2. LC 50 values of the Steinernema sp. (Kayathar strain) against S. frugiperda*

| Instars | Exposure time | Regression equation | Chi square values (χ2) | LC 50 (IJs/ml) | Fiducial Limits | |
|---|---|---|---|---|---|---|
| | | | | | LL | UL |
| Third Instar | 48h | y = 2.12 + 1.88x | 1.21 | 41.39 | 18.11 | 94.58 |
| | 72h | y = 2.10+2.01x | 1.27 | 37.80 | 16.73 | 85.42 |
| | 96h | y = 2.55+1.90x | 1.17 | 33.13 | 13.38 | 82.03 |
| Fifth Instar | 48h | y = 1.80+1.74x | 4.38 | 72.17 | 38.56 | 135.08 |
| | 72h | y = 2.50+1.51x | 4.06 | 54.33 | 24.27 | 121.64 |
| | 96h | y = 1.91+2.25x | 2.38 | 37.09 | 16.66 | 82.56 |

Additionally, third instar mortality was 95.00% to 100.00% at 96 hours after infection due to 100 - 600 IJs per larva. For the fifth instar, 96-hour infections with 250, 400, and 600 IJs/larvae resulted in mortalities of 94.50% to 99.80%. The data suggest that FAW caterpillar mortality rises, as anticipated, with an increase in EPN concentration applied, but declines with increasing instar.

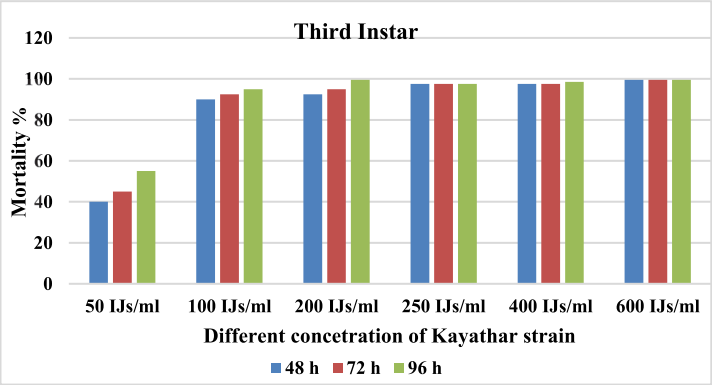


*Fig 3. Mortality per cent of Steinernema sp. against third instar of S. frugiperda*

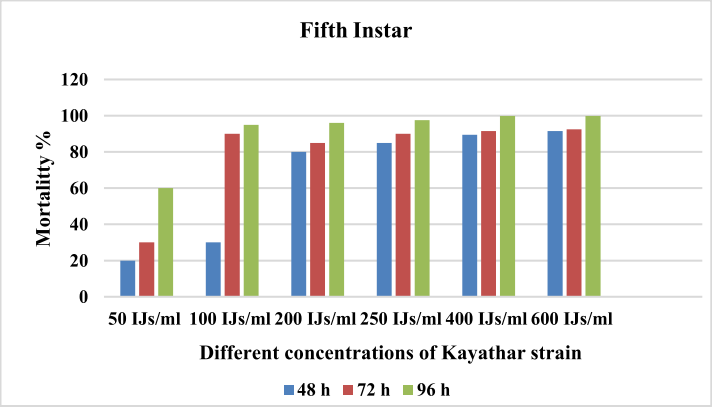


*Fig 4. Mortality per cent of Steinernema sp. against the fifth instar of S. frugiperda*

## Conclusion

The prevalence and geographic distribution of EPN in the Tuticorin district's maize-growing regions are highlighted in this study. Furthermore, this study shows that soils collected from maize fields are fairly rich in widespread EPN dispersion. However, the Steinerematids and Heterorhabditis discovered during the investigation may both have the ability to control insect pests in the maize ecosystem. Entomopathogenic nematodes are the most secure biocontrol agents and a potential substitutes for pesticides. Because of their wide host range, EPNs can be used on a variety of crops. The isolated *Steinernema* sp. (Kayathar strain), was extremely pathogenic to *S. frugiperda*. EPN as is one of the promising bio-control agents in the maize environment, further research is needed to determine their host range, lethal effects, pathogenicity, seasonal abundance, and biotic and abiotic variables. Implying that the EPN could be an effective biocontrol agent for the control of pests that impact maize crops.

### Future scope of the study

The findings of the present study will undoubtedly contribute to decrease decreasing the indiscriminate use of insecticides in the maize crop ecosystem. Additionally, EPN is more virulent than many other bio-control agents, also as an alternative to insecticides.

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### Conflict of interest

The authors declare that they have no conflict of interest in the publication.

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