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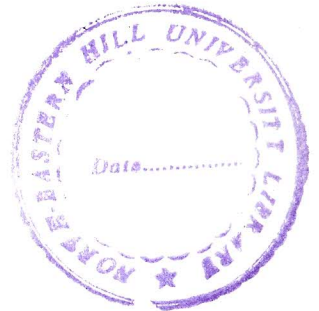
**FAUNAL COMPOSITION AND DISTRIBUTION OF  
ENTOMOPATHOGENIC NEMATODES AND THEIR BIOEFFICACY  
AGAINST MAJOR INSECT PESTS IN RI-BHOI DISTRICT OF  
MEGHALAYA**

**ABSTRACT**

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## ABSTRACT

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Entomopathogenic nematodes (*Heterorhabditis* spp. and *Steinernema* spp.) are promising biological control agents for a variety of soil-dwelling insect pests. The present work deals with a study on ascertaining the faunal composition, distribution, ecological characterization and bioefficacy of entomopathogenic nematodes in the Ri-bhoi District of Meghalaya. The objectives of study were:

1. To ascertain the faunal composition and distribution of EPNs in Ri-bhoi District of Meghalaya.
2. To study the seasonal prevalence of EPNs.
3. To characterize the EPN species with respect to ecological parameters.
4. To test the bioefficacy of locally isolated EPN species against major insect pest in the area.

To study the occurrence and distribution of EPNs in the area, soil samples from different habitats (Dry land, Wet land, Jhum land and Forest land) were collected and baited by *Galleria* traps. Entomopathogenic nematodes were recorded from 89 samples (5.37%) out of 1656 samples collected from various habitats. Out of 89 positive samples, the frequency of occurrence of *Steinernema* spp. was recorded to be more (73.03%) than *Heterorhabditis* sp. (26.97%). All the EPN positive

samples were from the forest soils. *Steinernema* spp. were mostly isolated from sandy loam soils while *Heterorhabditis* sp., was isolated from red loamy soil.

Based on their morphometric measurements, and light/scanning electron microscopic studies the isolated nematodes were identified as, *Heterorhabditis indica*, *Steinernema thermophilum* and *Steinernema glaseri*. It is for the first time that their occurrence is reported herein from north-east region of India, Meghalaya in particular. A brief description is provided for all the species recorded.

For the study of seasonal population fluctuations of EPNs, two nematode positive sites, one for *Heterorhabditis* sp. and another for *Steinernema* sp. were selected in the study area. The seasonal prevalence was studied using indirect method and the presence of EPNs was evaluated using *Galleria* traps. *Heterorhabditis* sp. was detected in the soil samples throughout the study period, causing moderate to high mortality of the host insect. The prevalence of *Heterorhabditis* and *Steinernema* species was found to be positively correlated with soil temperature, soil moisture and rainfall. The prevalence of *Heterorhabditis* was recorded to be considerably high during March to November. The prevalence of *Steinernema* sp. started increasing from March onwards and reached a peak in May; thereafter it maintained almost a uniform trend till November. The prevalence declined abruptly to a very low level in the month of December.

With respect to ecological characterization of EPN species, effects of following parameters were taken into consideration: 1). temperatures on infectivity/production of IJs 2.) relative humidity on production of IJs 3). storage temperatures, population densities and duration on survival and pathogenicity of IJs 4). Time period and soil depth on attachment of IJs to a mobile host 5.) soil moisture levels on establishment of IJs in the host 6). IJs population densities on their infectivity/production 7). different pesticides on survival/pathogenicity of IJs.

Effect of temperatures on infectivity and reproduction of EPNs was determined by numbers of IJs established per *G. mellonella* and the numbers of IJs produced per insect host at different temperatures (10 – 35°C). The optimum temperature (high establishment and production of IJs) of EPNs were recorded to be, *H. indica* from 20–30°C, *S. thermophilum* from 25–35°C and *S. glaseri* from 15-35°C.

Effect of relative humidities on reproduction of IJs was determined by the first day of emergence of IJs and the number of IJs produced by the insect host exposed to 55 – 100% R.H. The optimum R.H range for all three species was found to be 80 – 100%.

To study the effect of storage temperature, population densities and duration, IJs of EPNs were exposed to four different temperatures (5, 10, 25 and 30°C) and three population densities (100, 500 and 1000 IJs/ml) for a period of 120 days and their survival and pathogenicity were determined at different time intervals. Study revealed that 10°C, 100

IJs/ml but not more than 60 days was the optimum condition for *H. indica* and *S. glaseri*, whereas 25°C, 100 IJs/ml but not more than 60 days was the best condition for *S. thermophilum*.

Foraging behavior of isolated EPNs was studied by two methods 1). attachment of IJs to a mobile host 2). capability of IJs to infect host at different soil depths. The attachment *H. indica* IJs to a mobile host was higher at all observation time (1, 5 and 10 minutes) as compared to *S. thermophilum* and *S. glaseri*. However, at different soil depths *S. glaseri* appeared as the most effective species as it showed the highest establishment of IJs in the host at the deepest soil depth of 10 cms.

To study the effect of soil moisture on infectivity of IJs, establishment of IJs in the insect host was observed at different soil moisture levels. It emerged that IJs of *S. thermophilum* could establish at 4% soil moisture level. At 5% moisture level IJs establishment was observed for all EPNs. Numbers of IJs established/host increased along the soil moistures reaching their peak at 16 – 18% soil moisture level and declined from 20% onwards, except for *S. glaseri* where the establishment rate increased till the highest soil moisture level studied.

The effect of IJs population densities on their infectivity and production were studied by exposing *G. mellonella* larvae to different concentrations of IJs. The infected dead larvae were observed for the establishment, first day of emergence and production of IJs of EPNs. It appeared that the number of IJs established per insect host significantly

increases at higher concentrations for all the EPNs. The first day of IJs emergence from the host cadavers was also affected by the population densities. Earlier emergences were observed at higher densities but no IJs were produced at establishment of IJs higher than  $551.5 \pm 41.92$ ,  $428.4 \pm 74.73$  and  $321.4 \pm 22.96$  IJs per host for *H. indica*, *S. thermophilum* and *S. glaseri*, respectively. The highest numbers of IJs were produced at a concentration of 200 IJs/larva for both *H. indica* and *S. thermophilum*.

In order to ascertain the effect of different pesticides on survival and pathogenicity, IJs of EPNs were exposed to two (lower and higher) recommended field dose of carbaryl, nimbecidine, endosulfan, quinolphos, fenvalerate, mancozeb and carbofuran for a period of 72 hrs. It was observed that IJs of *H. indica* was found compatible with mancozeb and nimbecidine at both lower and higher concentrations. IJs of *S. thermophilum* were found compatible with carbaryl and nimbecidine at lower and higher concentrations, and to mancozeb at lower concentration, whereas IJs of *S. glaseri* were found compatible with nimbecidine, endosulfan, quinolphos, fenvalerate and mancozeb.

Bioefficacy of the isolated EPNs were tested against four major insect pests of the study area, namely larvae of colocassia corm borer, *Haplosonyx chalybaeus*, larvae of cabbage butterfly, *Pieris brassicae*, larvae and pupae of brinjal fruit and shoot borer, *Leucinodes orbonalis* and larvae and pupae of mustard saw fly, *Athalia lugens proxima*. Bioefficacy

was determined by percent insect larval/pupal mortality, and the total production of IJs per host to ensure their recycling potential.

The bio-efficacy of nematode species against *H. chalybaeus* larvae was studied by petridish assay. The larvae were exposed to 25, 50, 75, 100 and 200 concentrations of IJs for 120 hrs and host mortality was monitored at every 24 hrs intervals. 100% of larval mortality was observed for all EPNs at a concentration of 200 IJs/larva within 48 hours after inoculation (HAI) in case of *S. glaseri* and 72 HAI in case of both *H. indica* and *S. thermophilum*. Progeny production increased along with the IJs concentrations in EPN species. The maximum number of IJs produced was observed in case of *H. indica* ( $168.9 \pm 2.67 \times 10^3$  IJs/larva at 200 IJs/larva) whereas the least infective juveniles yield was observed in case of *S. glaseri* ( $18.9 \pm 0.57 \times 10^3$  IJs/larva at 100 IJs/larva).

Bioefficacy against larvae of *P. brassicae* was also studied using petridish assay with 10, 25, 50, 75 and 100 IJs concentration for a period of 120 hrs. The insect mortality was monitored at every 24 hrs intervals. Positive correlation was observed between the concentrations and the time of insect larval mortality. 100% of insect larval mortality was observed for all EPNs at a concentration of 200 IJs/larva within 48 HAI in case of *S. glaseri* and 72 HAI in case of both *H. indica* and *S. thermophilum*. The highest number of infective juveniles produced by *P. brassicae* was observed in case of *H. indica* ( $33.8 \pm 2.46 \times 10^3$  IJs/larva at 100 IJs/larva).

In order to evaluate the bio-efficacy of nematode species against larvae and pupae of *L. orbonalis*, two assays were carried out *i.e.*, petridish assay for larvae and soil column assay for pupae. The larvae and pupae were also found to be susceptible to EPN species when exposed to different concentrations (10, 25, 50, 75 and 100 IJs/larva and 25, 50, 75, 100 and 200 IJs/pupa). In case of larvae, at the lowest concentration studied (10 IJs/larva), 100% mortality was observed only in case of *H. indica* within 120 HAI. At higher concentrations (100 IJs/larva) 100% mortality was observed for all the species within 48 HAI. The production of IJs by *L. orbonalis* showed the same trend as with other insect pests, where the maximum production of IJs was observed in case of *H. indica* ( $96.7 \pm 2.11 \times 10^3$  IJs/larva at 100 IJs/larva).

Pupae of *L. orbonalis* were less susceptible to EPNs. At the highest concentration studied (200 IJs/larva), 100% mortality was observed within 96 HAI in case of *H. indica* ( $LC_{50} = 31.0$ ), whereas *S. glaseri* caused only  $53.4 \pm 6.6\%$  mortality even within 120 HAI ( $LC_{50} = 159.8$ ). In case of *S. thermophilum*, an early mortality ( $20.00 \pm 11.6\%$ ) was observed at 24 HAI which reached to 100% within 96 HAI ( $LC_{50} = 50.6$ ). The pupa produced comparably less IJs than larva. Among the three species the production was observed highest in *H. indica*, yielding  $12.2 \pm 0.78 \times 10^3$  IJs/pupa at 200 IJs/pupa.

Bioefficacy against larvae and pupae of *A. proxima* were also studied by two assays (petridish assay for larvae and soil column assay for pupae)

at 10, 25, 50, 75 and 100 IJs/larva and 25, 50, 75, 100 and 200 IJs/pupa concentrations. In case of larvae, at the highest concentration (100 IJs/larva), 100% mortality was observed at 48 HAI for *H. indica* ( $LC_{50} = 30.6$ ), whereas it was observed within 72 HAI ( $LC_{50} = 5.6$ ) in *S. glaseri*. In case of *S. thermophilum*, mortality was first observed at 24 HAI which reached to 100% within 48 HAI ( $LC_{50} = 37.3$ ). Of all the insect pest studied, the production of EPN IJs was comparably less in *A. proxima* larvae. The highest IJs produced were found to be only  $29.3 \pm 1.39 \times 10^3$  IJs/larva at 75 IJs/larva in case of *H. indica*.

The pupae were found less susceptible to EPNs as compared to larvae. No species could cause 100% pupal mortality. However, at the highest concentration of IJs (200 IJs/pupa),  $93.4 \pm 6.60\%$  mortality was observed within 96 HAI in case of *H. indica* ( $LC_{50} = 81.7$ ;  $LT_{50} = 56.1$ ) and *S. glaseri* caused only  $46.6 \pm 6.60\%$  mortality even within 120 HAI ( $LC_{50} = 197.3$ ;  $LT_{50} = 112.3$ ).

In conclusion, the present study shows that the EPN fauna in the Ri-Bhoi District of Meghalaya is comprised of three nematode species, i. e., *H. indica*, *S. thermophilum* and *S. glaseri*. On the basis of bioefficacy assays the study proves that all the EPN species have considerable potentials to be used as biocontrol agents against the tested insect pests.

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