International Journal of Agriculture, Environment and Biotechnology
Citation: *IJAEB*: **14**(03): 393-397, September 2021
DOI: 10.30954/0974-1712.03.2021.16

ASSOCIATION FOR AGRICULTURE ENVIRONMENT AND BIOTECHNOLOGY

ENTOMOLOGY

Occurrence and Distribution of Entomopathogenic Nematodes in Horticultural Crops Soils of Solan and Sirmaur District of Himachal Pradesh, India

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Paper No. 925 **Received:** 02-07-2021 **Revised:** 09-08-2021 **Accepted:** 09-09-2021

ABSTRACT

Two years the random roving survey was conducted for occurrence and crop-wise distribution pattern of Entomopathogenic Nematode (EPN) in Solan and Sirmour districts of Himachal Pradesh under the Nematology Laboratory, Department of Entomology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, -173230, Himachal Pradesh, India, Sirmour during 2019-2020. Total 225 soil samples were collected from all Solan and Sirmaur districts, of which 68 samples were found EPN positive. The percent occurrence of Steinernema and Heterorhabditis was 27.03 % and 27.45 %, respectively. The maximum frequency of occurrence (56 %) was recorded from Rajgarh and the minimum (12 %) from Halonipul, Sirmaur district. In the crop-wise distribution pattern, the maximum frequency was observed in fruit crops (80 %) which was followed by plantation crops (35 %), ornamental crops (25 %).

HIGHLIGHTS

- Distribution of Entomopathogenic Nematodes in mid-hills of Himalyas.
- Morphological identification in the laboratory.
- Study of Frequency of occurrence of EPN from two districts of Himachal Pradesh. India.
- Entomopathogenic nematode distribution pattern in crop-wise.

Keywords: EPN, Survey, Steinernema, Heterorhbditis, Frequency, Horticulture Crops

Entomopathogenic nematodes (EPN) from Heterorhabditidae (Poinar 1976) and Steinernematidae (Travassos 1927) families are obligate insect parasites that can infect and kill a broad range of insect hosts (Kaya and Gaugler 1993). These nematodes are symbiotically associated with entomopathogenic bacteria *Photorhabdus* (Boemare *et al*. 1993) and *Xenorhabdus* (Thomas and Poinar 1979). These nematodes are currently marketed globally for the biological control of insect pests (Grewal *et al*. 1998).

Entomopathogenic nematodes (EPNs) belong to the genus Steinernema, and Heterorhabditis can control a diverse of insect pests due to their efficiency within a short period, compatibility with pesticides, and active host-seeking ability (Dowds and Peters 2002). They partially owe their pathogenicity to their symbiotic relationship with bacteria of the genus *Xenorhabdus* and *Photorhabdus* (Bedding *et al*. 1983; Forst *et al*. 1997; da Silva *et al*. 2000). Although there are some constraints for successful application of EPNs in the field, like environmental extremes or the resistance of certain pests to EPN penetration (Lewis *et al*. 2006; Georgis *et al*. 2006; Toepfer *et al*. 2010), they can be as effective as chemical

How to cite this article: Kasi, I.K., Singh, M., Waiba, K.M. and Monika. 2021. Occurrence and Distribution of Entomopathogenic Nematodes in Horticultural Crops Soils of Solan and Sirmaur District of Himachal Pradesh, India. *IJAEB*, **14**(03): 393-397.

Source of Support: None; **Conflict of Interest:** None

insecticides under certain conditions (Laznik and Trdan 2017). Also, EPNs can be applied together with other agro-chemicals, time-saving, providing cost-effective and long-lasting pest control (Ozdemir *et al*. 2020a). For this purpose, many studies have been conducted with local EPN species and isolates, testing their pathogenicity on important pests and their compatibility with registered pesticides to design more sustainable pest control programs (Baimey *et al*. 2015; Ferreira *et al*. 2016; Hazir *et al*. 2018; Kwizera and Susurluk 2017; Laznik and Trdan 2014, 2017; Ogretmen *et al.* 2020; Ozdemir and Evlice 2020; Ozdemir *et al*. 2020b).

EPN belongs to the family; Heterorhabditidae and Steinernematidae are represented by the genera, Heterorhabditis Poinar and Steinernema Travassos and Neosteinernema Nguyen & Smart, respectively. The state of Assam is hot with humid rich in a wide range of flora and fauna, and has a vast diversity of both beneficial and harmful insect pests. There has been no systematic survey for EPNs carried out in Himachal Pradesh till today. The current survey was the first conducted in Himachal Pradesh and specially focused on the Sirmaur district of Himachal Pradesh. The primary objectives of this study were to survey entomopathogenic nematodes from the families Heterorhabditidae and Steinernematidae in the Solan and Sirmaur districts of Himachal Pradesh to find out the distribution pattern of EPN, their ecosystem, and habitat.

MATERIALS AND METHODS

Location and Area

The district Solan and Sirmaur of Himachal Pradesh is hill zones of the Himalayas. The district comes under mid-hills. In the case of the vegetation structure of Solan, Sirmaur is concerned, the district comprises of broad types of horticulture land to forest areas. Localities where sampling for EPNs was conducted covering in the district (Table 1).

Methods of collection

A random traveling survey collected total 225 soil samples from all the two districts of Himachal Pradesh from June 2019 to November 2020, covering all cropped areas and horticulture crop habitats (Apple, Peach, Apricot, Plum, Persimmon… etc) (Fig. 1).

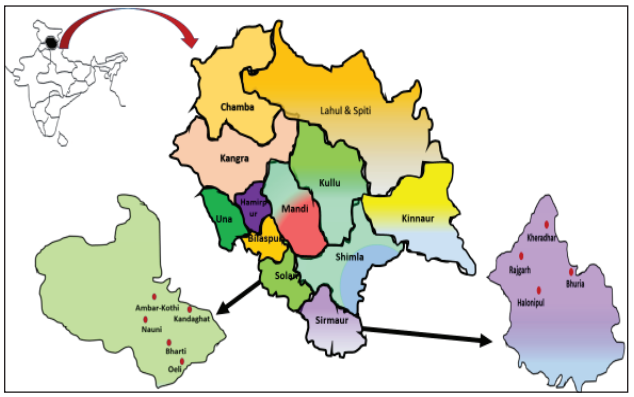

Fig. 1: Solan and Sirmaur district of Himachal Pradesh showing selected locations for survey work. (Source: QGIS)

Relatively more excellent, shady, and moist sites were chosen for soil sample collection. About 1000 g of soil was collected from each malty sampling site at a 10-15 cm depth from the soil surface. The EPNs were isolated from the soil samples by insect baiting technique using late instar larvae of *G. mellonella* (Bedding and Akhrust 1975). The initial culture was obtained from the Nematology Laboratory, Department of Entomology, Dr. YSP

Table 1: Localities where sampling for EPNs was conducted covering in the district of Solan and Sirmaur, Himachal Pradesh, India

Districts	Localities	Latitude	Longitude	Altitude (m)
Solan	Nauni	30° 51′33 N	77° 10′ 30 E	1179
	Ambar-kothi	30° 51′ 07 N	77° 13′ 26 E	1300
	Oeli	30° 51′.02N	77° 14′ 27 E	1350
	Bharti	30° 59′ 45 N	77° 01′ 08 E	1150
	Kanda ghat	30° 57′ 23 N	77° 06′ 49 E	1480
Sirmaur	Rajgarh	30° 53′ 15 N	77° 16′ 07 E	1682
	Kheradhar	30° 08′ 24 N	77° 15′ 05 E	2032
	Bhuira	30° 49′ 38 N	77° 20′ 07 E	1650
	Halonipul	30° 51′ 03 N	77° 18′ 44 E	1349

University of Horticulture and Forestry, Himachal Pradesh. After thoroughly mixing the soil sample a representative volume of soil (ca 250 g) will be transferred to a plastic jar (ca 500 ml) (8 cm height and 6 cm diameter) containing 5 -7 full-grown larvae of *Galleria larvae*. The jars were incubated at 25 ± 1 °C temperature for 7 days. Every alternate day samples were observed for EPN infection. The larval cadavers, if any, were collected from the soil sample, washed with distilled water, and placed on White's trap (White, 1927) to observe the emergence of IJs. The collected nematode isolates were identified as EPNs after confirming their pathogenicity against the larvae of *G. mellonella*.

Identification

To identify EPN species, the isolated infective juveniles (IJs) were killed by gentle heating at 60°C in water and fixed in TAF (7 ml formalin + 2 ml triethanolamine + 91 ml distilled water). After keeping the nematodes in fixative for 5-7 days, they were transferred to a cavity block with ethanol, water, and glycerine in the ratio of 15: 5: 1 and kept in desiccators for 15 days. After that the IJs were mounted in glycerol on a glass slide. EPN species were identified with the help of published literature (Adam *et al*. 2006; Adams and Nguyen 2002; Hominick *et al*. 1997). Measurements were taken using a Leica IM50 microscope fitted with a light distortion difference. Some of the varieties have been identified and verified by comparisons with equipment from the Nematology Laboratory, in Dr. YSP UHF, Himachal Pradesh. India and the identified species were arranged in a systematic order.

Statistical Analysis

Microsoft Excel has been used to analyze morphometric variability in men and IJs. Data collected were analyzed using PASW 18 no the percent frequency of occurrence (F) of entomopathogenic nematode in different districts as well as crops were calculated using the following formula:

$$\frac{(EPN\ Positive\ samples)}{(Total\ number\ of\ samples)} \times 100$$

RESULTS

EPN were recovered from 68 samples out of 225 soil samples collected from two districts (Solan and Sirmaur) of Himachal Pradesh, and the frequency was recorded highest in Rajgarh 56 %, followed by 40 % in Kheradhar Sirmaur district, 36 % in Ambarkothi, and 32% in Kanda ghatSolan district respectively. The frequency of occurrence in the region ranges from 56-12 percent. Among 68 positive samples, 44 samples (27.03%) contained Steinernematid, and 24 contained Heterorhabditis. The highest recovery of EPN from Rajgarh of Sirmaur district is 56 % is an indication of the suitable climate for the survival and multiplications of EPNs that prevail in the area (Table 2).

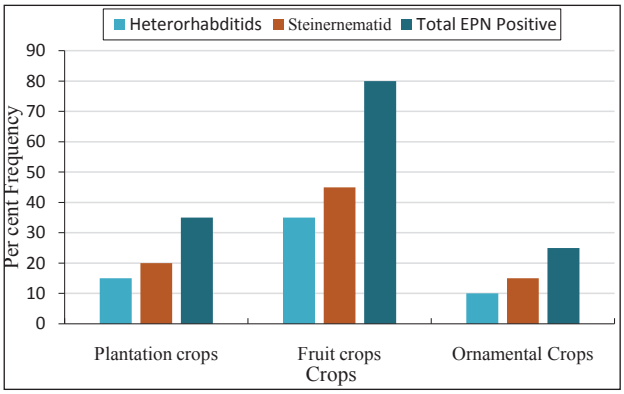

Fig. 2: Frequency of occurrence of EPN in different crops in Solan and Sirmaur district, Himachal Pradesh, India

The highest crop-wise distribution of EPN was recorded in fruit crops (80 %) followed by plantation crops (36 %) and ornamental crops (25 %) (Table 3 and Fig. 2).

DISCUSSION

The abundance of native EPNs was observed in the surveyed area (27 samples out of 120) may be due to prevailing ecosystems where human impact is substantial, like perennial orchards. This result conforms with Shahina *et al*. (1998). He reported that the abundance of native EPNs is high in an ecosystem with substantial human impact. Significant variations in the occurrence of EPNs can also be exacerbated by the combination of insecticides (Marcek and Becvar 2000). The region has fertile, compacted sandy loam with a good vegetative cover. In addition to the normal rainfall in Himachal Pradesh, these conditions should have

Table 2: Frequency of occurrence of EPN from two districts of Himachal Pradesh, India

Districts	Name of the location	Altitude (m)	Total no of sample collected	No of sample with EPN	Frequency of occurrence (%)
Solan	Nauni	1179	25	5	25
	Ambarkothi	1300	25	9	36
	Oeli	1350	25	7	28
	Bharti	1150	25	5	20
	Kanda ghat	1480	25	8	32
Sirmaur	Rajgarh	1682	25	14	56
	Kheradhar	2032	25	10	40
	Bhuira	1650	25	7	28
	Halonipul	1349	25	3	12

Table 3: Crop wise distribution pattern of EPN in the district of Solan and Sirmaur, Himachal Pradesh, India

Crops	Heterorhabdities		Steinernematid		Frequency of EPN Positive (%)
	Number/20 sample	Frequency (%)	Number/20 sample	Frequency (%)	
Plantation crops	3	15	4	20	35
Fruit crops	7	35	9	45	80
Ornamental Crops	2	10	3	15	25

supported the survival and proliferation of host pests and ultimately the high emergence of EPNs, especially in fruit plants due to the shade and high humidity under a large tree bed.

CONCLUSION

The surveys have shown that all five blocks of districts are rich in EPN diversity and Steinernematids and Heterorhabditids in fruit crops, plantation crops, ornamental crops, and field crops of the district indicates their potential role in the natural control of soil-inhabiting insect pests of major crops. The study has also necessitated further study on taxonomic and molecular characterization and host ranges of these EPN species to explore and ascertain their possible potential scope to use as a biological control agent.

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