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DIVERSITY AND DISTRIBUTION OF ENTOMOPATHOGENIC NEMATODES (EPNS) IN DIFFERENT AGRO ECOLOGICAL HABITATS OF TELANGANA STATE, INDIA

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KEYWORDS

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ABSTRACT

Different agro-climatic zones of seven districts in Telangana state were surveyed to study the diversity of native Entomopathogenic nematodes (EPNs) and characterize their distribution and habitat during 2013-2014. Among the 1226 soil samples collected randomly and subjected to baiting of EPNs with *Galleria mellonella* larvae, 17 soil samples (1.38%) tested positive for the presence of Steinernematid and Heterorhabditid nematodes. *Steinernema* spp. were found in 15 (88%) samples and only 2 (12%) samples confirmed to be *Heterorhabditis* spp. Highest samples (6) from Kareem nagar district were confirmed for the presence of EPNs, whereas samples from Nalagonda and Mahbubna gardistricts had no presence of EPNs. The *Steinernema* spp. was found both in field and horticultural crops, but, *Heterorhabditis* spp. was isolated only from field crops. Irrespective of species, all the isolates recovered were from irrigated cropping systems and none was found in rainfed cropping systems. The experimental results revealed that, EPNs prefer pest prone crop habitats for their survival, multiplication and could be potentially used for management of insect pests in organic farming and in integrated pest management practices.

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INTRODUCTION

Entomopathogenic nematodes (EPNs) from Heterorhabditidae (Poinar, 1976, Valadas*et al*., 2014) and Steinernematidae (Travassos, 1927) families are obligate insect parasites, which can infect and kill a broad range of insect hosts (Kaya andGaugler, 1993). Understanding their habitat, distribution and active soil zones will enable their use in insect pest management.

Entomopathogenic nematodes have a global distribution (Hominick, 2002). The only continent where they have not been found is Antarctica. Their distribution on a global scale is strongly influenced by climate and chance dispersal events, including those associated with human activities. Both biotic and abiotic factors such as soil texture, temperature, vegetation and availability of suitable hosts are amongst the factors determining the local distribution patterns. There is growing evidence of preferences of EPNs species for certain habitats. *Steinernema affine* is found largely in arable lands and grasslands, and virtually absent in non-cultivated fields(Hominick and Briscoe, 1990; Griffin *et al*., 1991; Stock *et al*., 1999; Kary*et al*., 2009; Pervez *et al*., 2014). Hence it is worth to understand the native species before embarking upon their exploitation. The survey for EPNs distribution in Kodaikanal Hills of South India (Razia and Sivaramakrishnan, 2014) has enabled to understand that EPNs prefer frequent cultivated crop zones where pests persists more compared to forest lands. The species of *Trichoderma*a fungal bio-control agent too had preferential survival in groundnut rhizosphere (Rashmi*et al*., 2015). The insect bio-control agent coccinellid beetles was also surveyed in different agro-climatic zones of Himachal Pradesh and found its species richness varied with agro-climatic conditions and was highest (22 species each) under sub-tropical and sub-temperate climate followed by 10 and 7 species under wet temperate and dry temperate conditions (Sharma *et al*., 2015). Thus, thecurrent research emphasizesto know the existence of native EPNs and their distribution for potential use in insect pest management of field crops and horticultural crops.

EPNs have been successfully used as bio-control agents of insect pests in many developed countries but their use in India is limited to academic studies due lack of information and understanding of their habitat and spread. With growing demand for organic products necessity has increased for potential bio-control agents against insect pests and EPNs fit well in acting against wide range of insect pests. However, their survey and documentation is very poor across the country including erstwhile Andhra Pradesh. The present investigation is undertaken in seven districts of Telangana state with an objective of identifying the EPNs preferential zone of survival, their distribution, species diversity and spatial variabilityin different agro climatic conditions.The study is expected to initiate further exploitation of EPNs in insect pest management suitable to the respective agro climatic conditions.

MATERIALS AND METHODS

Field Survey

For collection of soil samples, field survey was undertaken in different cropping

systems of Telangana state during 2013 to 2014. Total seven districts were surveyed (Fig. 2) and in each district 5 to 6 representative places with wide cropping diversity under both irrigated and rain-fed ecosystem were surveyed. The survey was planned such that to include representation of different agro climatic conditions of the region, cropping patterns, soil types, and ecosystems (Mutsuhiro*et al*., 1998). The distance between two sampling sites was minimum 5km and sampling sites consisted of both field and horticultural crops.

Collection of soil samples

During the survey, soil samples were collected randomly from different cultivated and non-cultivated areas. Each soil sample comprised of 5-20 random sub-samples at 10 m away from each other. Soil samples of each 500 gm were collected using hoes and shovelat a depth of 30 cm (ZdenekMracek*et al*., 1999). The number samples collected from each one acre varied between 10-15 for field crops and 8-15 for vegetable crops. While sampling, the shovel was thoroughly rinsed with water and air dried after every sampling to prevent contamination of the next sampling unit.

For each group of crops, different soil sampling pattern was followed. For horticultural plantation crops of annual and perennial nature, five random spots (Fig. 1a) were sampled for each tree. In case of field crops and vegetable crops zigzag pattern and hexagonal patterns (Fig. 1b i & ii) were followed. The soil samples collected were labeled, brought to laboratory under cold condition and stored in BOD incubator at 20^{0}C until used for extraction of EPNs (Kaya & Stock, 1997).

Extraction of Entomopathogenic nematodes

The field collected soil samples were first mixed properly. Around 200g of soil from each sample was transferred to cylindrical shape plastic containers (15x7cm) and moistened with distilled water up to field capacity. The EPNs were isolated using baiting method according to the modified methodology of Bedding and Akhurst (1975). Five last-instar *Galleria mellonella* L. larvae were placed in each plastic container of respective soil sample and covered with a perforated lid, turned upside down and incubated for 15 days at room temperature 23 ± 1 °C. Water was added to the samples to maintain the moisture during baiting. Two replications were maintained for each sample. *Galleria mellonella* larvae were checked once in every three days and dead larvae were replaced with fresh ones. Dead larvae were thoroughly rinsed in distilled water and placed individually in modified White traps (Kaya and Stock, 1997) for emergence of EPNs. The emerging nematodes collected from single dead larva were designated as an isolate. Each isolate was cultured on *G. mellonella* larvae to produce nematodes for identification and establishment of cultures. Based on the colour of the infected cadavers, culturing of bacteria on NBTA medium from haemolymph of infected cadavers and amphimixis/ hermaphroditism, the EPNs were identified (Akhurst, 1980; Wouts, 1981).

Bacterial symbiont isolation and identification

The symbiotic bacteria associated with EPN isolates was isolated following the procedure of Akhurst (1980) with modification in the sterilization process. Cleaned EPNs IJs approximately 100 were immersed in 85% sodium hypochloride solution for 8 min to digest the sheath of larvae and rinsed twice in sterile Rin gersolution and crushed in fresh sterile Ringer solution. The IJs are transferred to sterile nutrient broth. This suspension was streaked onto NBTA medium and incubated at 28ºC for 48h. Conventional phenotypic criteria appearance was used to verify generic identity (Xenorhabdus and Photorhabdus) of bacterial isolates (Boemare and Akhurst, 1988).

Data analysis

Percent frequency of occurrence (F) of entomopathogenic nematode in different locations as well as crops and soil types were calculated using the following formula:

$$\text{Frequency of occurrence (\%)} = \frac{\text{(EPN positive samples)}}{\text{(Total number of samples)}} \times 100$$

RESULTS AND DISCUSSION

The study assessed 1,226 soil samples during the survey for the presence of entomopathogenic nematodes (EPNs) collected from seven districts of Telangana state. The baiting technique yielded 17 EPN isolates out of all the samples tested which constitutes 1.38 per cent recovery of EPNs. The lower recovery of EPNs is a regular trend found by many workers, who surveyed EPNs earlier (Choo *et al*., 1995; Rosa *et al*., 2000; Grewal *et al*., 2001; Razia and Sivaramakrishnan, 2014). The EPNs recovery was found 4.72 percent (Ozer *et al*., 1995), 2% per cent (Hazir *et al*., 2003) and 12.1 per cent from Turkey. Zepeda-Jazo *et al*. (2014) recovered 12 Steinernematid isolates (85.7%) and two Heterorhabditid isolates (14.3%) in their study comprising 14 soil samples collected across the Mexico. In a similar study conducted within India, 33.3, 23.8, 23.8 and 19 per cent EPNs were recovered from cultivated fields of Gorakhpur, Deoria, Kushinagar and Maharajganj districts respectively. A ginger filed surveyed for occurrence of EPNs also showed higher presence of *Steinernema carpocapsae* (Rashid Pervez *et al*., 2014). However, 30, 30, 20, 20 and 20, 20, 20, 40 per cent EPNs were isolated from non-cultivated

Table 1: Occurrence of EPNs from different soil samples surveyed from seven districts of Telangana state.

Sl. No.	Districts	No of samples collected	No of (+) ve samples	% of (+) vesamples
1	Adilabad	160	4	2.50
2	Kareemnagar	121	6	5.00
3	Medak	193	2	1.03
4	Rangareddy	200	3	1.50
5	Warangal	165	1	0.60
6	Nalagonda	111	-	-
7	Mehbubnagar	176	-	-

Table 2: Habitat diversity of EPNs isolated from seven districts of Telangana state

Sl. No.	Districts	EPNs Spp. isolated	Isolates	Soil type	Irrigated / rain-fed	Crop/Vegetation
1	Adilabad	*Steinernema* spp.	ASR- 1	Red	Irrigated	Brinjal
2		*Steinernema* spp.	ASB -1	Black	Irrigated	Paddy
3		*Steinernema* spp.	ASMB- 1	Medium black	Irrigated	Mango
4		*Steinernema* spp.	ASR-2	Red	Irrigated	Groundnut
5	Kareemnagar	*Steinernema* spp.	KSR-1	Red	Irrigated	Rose
6		*Steinernema* spp.	KSMR -1	Medium red	Irrigated	Grass
7		*Steinernema* spp.	KSMB -1	Medium black	Irrigated	Guava
8		*Steinernema* spp.	KSB -1	Black	Irrigated	Chilli
9		*Steinernema* spp.	KSR -2	Red	Irrigated	Maize
10		*Steinernema* spp.	KSR-3	Red	Irrigated	Castor
11	Medak	*Hetrorhabditid* spp.	MHB -1	Black	Irrigated	Cotton
12		*Steinernema* spp.	MSMB -1	Medium black	Irrigated	Banana
13	Rangareddy	*Steinernema* spp.	RSB -1	Black	Irrigated	Methi
14		*Hetrorhabditid* spp.	RHMB -1	Medium black	Irrigated	Sorghum
15		*Steinernema* spp.	RSB -1	Black	Irrigated	Chilli
16		*Steinernema* spp.	RSR -1	Red	Irrigated	Sugarcane
17	Warangal	*Steinernema* spp.	WSR -1	Red	Irrigated	Lime

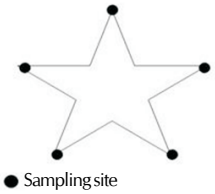

Figure 1a: Soil sampling pattern used for perennial horticultural fruit crops

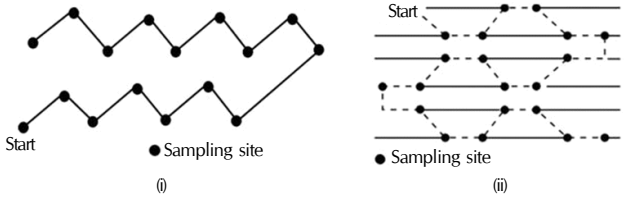

Figure 1b: Soil sampling pattern used for i) field crops and ii) vegetables

and garden/forest region fields of Gorakhpur, Deoria, Kushinagar and Maharajganj districts respectively which is contrasting, and rarely noticed and reported in any other studies. The active zone for EPNs survival is confirmed to be under the crop canopy of cropped areas where most of the insects are found (Singh *et al*., 2015). Maximum numbers of 6 isolates of EPNs were recovered from Karimnagar district representing 5 per cent of samples collected from the district (Fig. 2). Soil samples from two districts *viz*.,Nalagonda and Mahbubnagar did not yield any EPN isolates.There recovery of EPNs from the samples collected from different districts ranging between 0.60 to 5 per cent (Table 1).

The baited *Galleria mellonella*larvae displayed typical discolouration characteristics after incubation due to infection by EPNs. The discolouration is unique to the respective species of EPNs infection and symbiotic bacteria associated. Dark brick red to brown colour was noticed in two isolates, one each from Medak and Rangareddi districts which confirms the Hetrorhabditidspp. and corresponding symbiotic bacteria *Photorhabdus* (Fig. 3a & b). These nematodes showed a characteristic hermaphroditism in their first generation with slimy appearance of infected cadavers. The light yellow to creamy colour was shown by rest of the samples due to infection by *Steinernema* spp. and its association with symbiotic bacteria *Xenorhabdus* (Boemare and Akhurst, 1988). These nematodes were found amphimictic in all their generations and non-slimy in appearance on dead cadavers. Out of 17 isolates recovered (positive samples) 15 were found to be *Steinernema* spp. comprising 88% of the total recovery (positive samples). The remaining 12% were *Hetrorhabditidis*

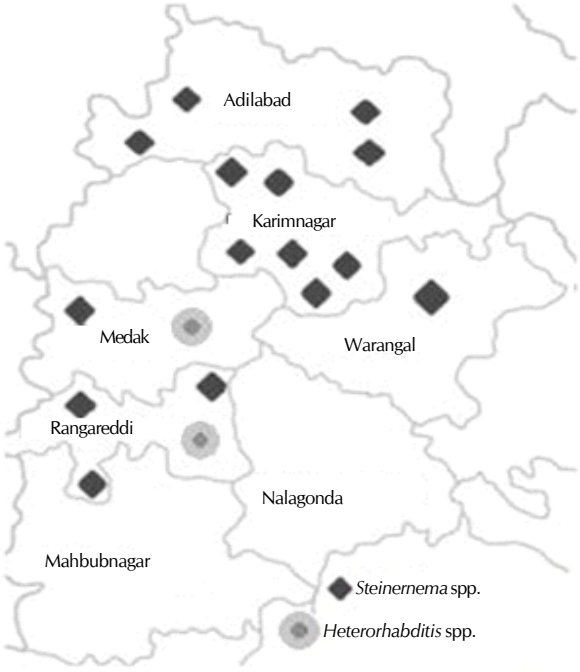

Figure 2: Map showing the surveyed area and recovery of EPN species from different districts of Telangana state

spp. (Table 2).

All the isolated EPNs were from irrigated conditions and none found from rain-fed conditions. It clearly indicates that moisture favours the occurrence, survival and multiplication of EPNs

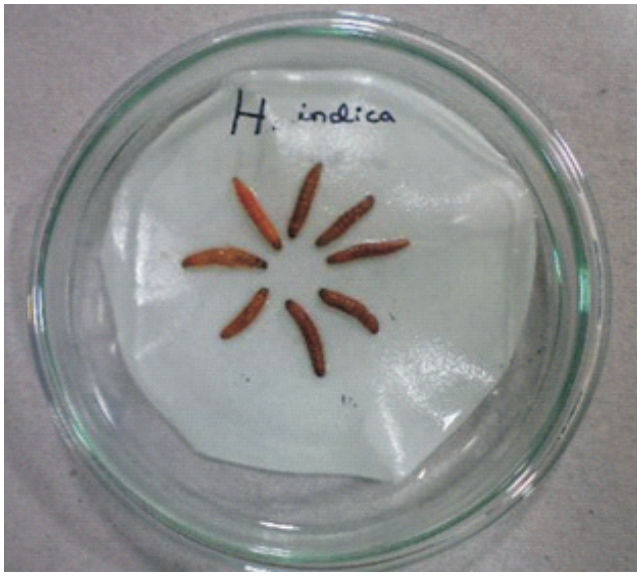

Figure 3(a): *Heterorhabditid* spp. with dark brick red colour infected cadavers of *Galleria mellonella* larvae

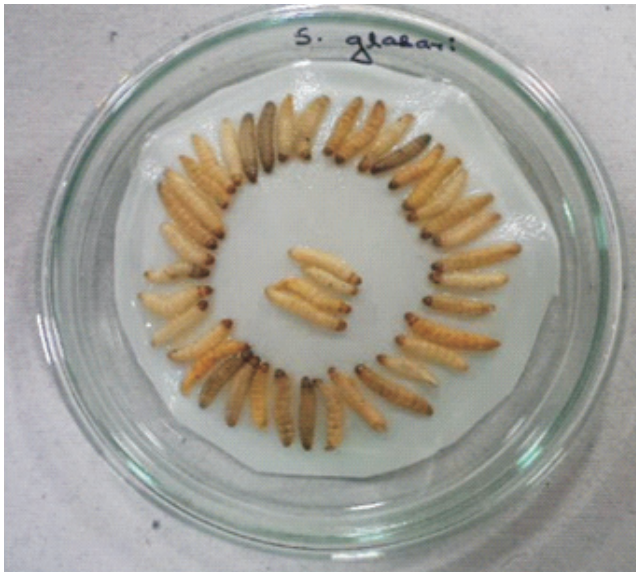

Figure 3(b): *Steinernema* spp. with light yellow colour infected cadavers of *Galleria mellonella* larvae

irrespective of cropping patterns. Both field crops and horticultural crops fields had EPN distribution. However, among the two isolates, *Hetrorhabditid* spp. was abundant in black and medium black soils under field crops habitat. *Steinernema* spp. was found both in red and black soils of field crops and horticultural crops habitat. The fruit crops, vegetables and cereals had more number of EPN recovery followed by oilseeds, cotton, sugarcane and flower crops (Fig. 5). The dominant nematode species observed from all the soils was *Steinernema* spp. This indicates that *Steinernema* spp. is probably more versatile and sustains adverse environmental conditions compared to *Heterorhabditis* ssp. in Telangana state. Our findings are in concurrent with Stock *et al*. (2004) who also found *Steinernema* spp. from both orchanrds and grass lands and *Heterorhabditis* spp. only from field crops. This implies that *Steinernema* spp. is more persistent and has a high recycling ability. Mracek *et al*. (1999) observed higher frequency of EPNs (> 50%) in areas with fruit trees, forests and pastures. However, our results are in contrary to these observations. But irrigation significantly influenced the survival of EPNs. The latest observations by Singh *et al*. (2015) also showed high percent of EPNs recovery from cultivated garden soils which received frequent irrigation.

The density of EPNs is attributed to the level of moisture which determines the infectivity against the insect pests. The maximum isolation of EPNs in the present study from vegetable and fruit crops is an indication of more scope for prediction and isolation of EPNs under these crop habitats (Fig. 4). Pervez *et al*. (2014) in their study noted the presence of *S. carpocapsae* from ginger rhizosphere. Thus our results confirmed the ubiquitous distribution of these nematodes in natural ecosystems favoured by crops and vegetation coverage. Abundance of native EPNs found in the surveyed area may be also due to prevailing ecosystems where human intervention is substantial like in perennial orchards and also recorded by Gaugler (2002). The districts surveyed have fertile loam to

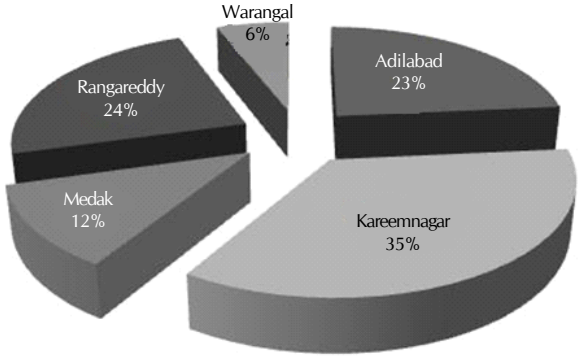

Figure 4: Per cent of EPNs recovery in different districts of Telangana state

sandy loam soil with vegetation cover. These conditions with adequate rain prevailing in Telangana supported the survival and prevalence of host insects and high occurrence of EPNs especially in fruit crops due to shade and higher moisture under canopy.

The results support the hypothesis of natural epizootics of entomopathogenic nematodes connected with insect outbreaks similar to those of other entomopathogens such as viruses and bacteria. Mracek and Webster (1993), and Zepeda-Jazo *et al*. (2014) reported high occurrence of Steinernematids in habitats damaged by insects. Similarly the current study showed that the occurrence of EPNs tends to be dependent on the presence of insect hosts, which are usually found more in vegetable cropping habitats. Entomopathogenic nematodes were recorded during the whole year which is also reported by Hominick and Briscoe (1990). There was no evidence for seasonality in occurrence and pathogenicity of EPNs (Divya and Sankar, 2009). The surveys concluded that all seven districts of Telangana are rich in EPN diversity for occurrence of Steinernematids and Heterorhabditids in fruit crops,

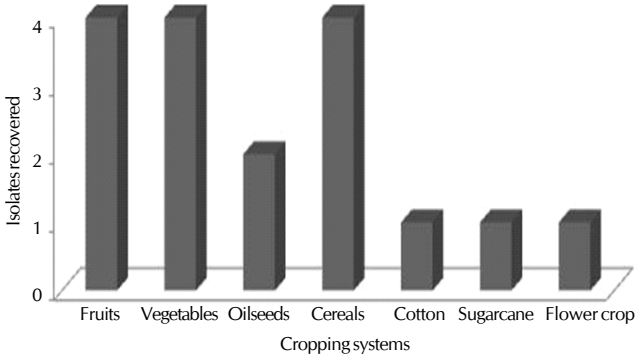

Figure 5: EPN isolates recovered from different cropping systems in Telangana state

vegetables and field crops.

The study uncovered the abundance diversity of EPNs in Telangana and right places for their collection. Even though use of EPNs at field level in India is yet to develop, but understanding the native species helps in formulating the strategies for their deployment in organic pest management. Therefore, there is a greater interest in finding EPN populations with traits suitable to local conditions. As native species multiply thrive and perform well. The abundance of *Steinernematid* and *Heterorhabditid* species of EPNs in Telangana state gives further scope for their use in biological pest management and ensures food and security.

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