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Survey for the presence of entomopathogenic nematodes (EPNs) in sugarcane growing regions of Telangana state

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Abstract
Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are lethal parasites of insects. They are widely distributed throughout the world and have a wide range of insect hosts. Introduction of exotic EPNs may induce exclusion of the local populations and/or species, thus eroding natural diversity. Keeping this in view soil survey was conducted in four districts of Telangana state for isolation of native EPN species. The results indicated that, out of 90 soil samples, 17 soil samples showed the infestation of *G. mellonella* with EPNs. The infected cadavers turned to brick red colour indicating the existence of *Heterorhabditis* sp. in the 17 soil samples collected from Sugarcane fields. The per cent occurrence of EPNs obtained from four districts of Telangana state revealed that, maximum occurrence of EPNs was observed in Kamareddy (33.33%), while, lowest prevalence of EPNs (5.55%) was recorded in Nizamabad district. Sangareddy district which recorded 23.33% EPNs occupied the second place, followed by Khammam with 16.67% soil samples tested positive for the presence of EPNs.

Keywords: Entomopathogenic nematodes, soil survey, *Heterorhabditis* sp., Telangana state

1. Introduction
Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate or sometimes facultative parasites of insects. EPNs belonging to genera, *Steinernema* and *Heterorhabditis* (Rhabditida: Steinernematidae and Heterorhabditidae) have become the subject of intensive research as they are lethal parasites of insects and have been used for inundative, augmentative or inocculative biological control of crop pests during the past two decades (Gaugler and Kaya, 1990; Bedding *et al.*, 1993; Parkman and Smart, 1996) [4, 3, 11]. EPNs are widely distributed throughout the world and have a wide range of insect hosts (Hominick, 2002) [5]. They are environmentally safe, widely acceptable, mass cultured in large quantities on artificial media and are easily applied with standard spraying equipments or through irrigation water. They fit nicely into integrated pest management (IPM) programmes because they are considered non-toxic to mammals, fishes, and birds and specific to their target pests. Introduction of EPNs as biological control agents in a particular site requires prior knowledge on their occurrence and proper identification of native species. Introduction of exotic EPNs may induce exclusion of the local populations and/or species, thus eroding natural diversity and be inefficient towards local insect pests as they may not be adapted to local environmental conditions (Miller and Barbercheck, 2001) [9]. Therefore, Isolation and identification of native entomopathogenic nematodes existing in the field is necessary before embarking upon their exploitation as biological control agents. Keeping in view of the diverse agro-climatic conditions in the country, isolation and molecular identification of native entomopathogenic nematodes in the field level are necessary for successful control of endemic pests in a particular location without causing any imbalance in the biodiversity of the locality.

2. Material and Methods
Insect parasitic nematodes can be recovered from naturally infested soils by baiting with host insects (Bedding and Akhrust, 1975) [2]. Hence, keeping in view soil samples were collected from Sugarcane growing regions of Telangana state and isolation of entomopathogenic nematodes from the soil was done using *Galleria mellonella,* the commonly used laboratory host for isolation and multiplication of EPNs.

2.1 Survey and sampling of EPNs: Field survey was carried out during July to December, 2016 (six months), in four districts of Telangana state *viz.,* Sangareddy, Kamareddy, Nizamabad and Khammam. A total of 90 soil samples were collected from four districts. The representative places from each district were selected based on the area covered under sugarcane cultivation. The information pertaining to the soil survey conducted in each district was presented in Table 1.

2.2 Collection of soil samples from field: For collection of soil samples, two representative places from Kamareddy, three representative places from Nizamabad and five representative places each from Sangareddy and Khammam districts, respectively were selected in sugarcane growing areas cultivated under irrigated ecosystem (Fig. 1a to 1e) and a total of six soil samples were collected randomly from each representative place of selected district. Thus, a total of 90 soil samples were collected from the four districts. The survey route was planned by taking into consideration of various parameters like, cropping systems, soil types and ecosystems. For sample collection the distance between two sampling sites was maintained at 5 km and above.

2.3 Sampling: Soil samples were collected from fields having adequate moisture (40-60%) content. For soil sampling, random method of soil sampling was done, *i.e.* by following zig-zag method (Fig. 2) and a minimum of 8-10 random sub-samples were collected from each sampling site. Preferably 8 sub-samples were collected from a moist root zone (Plate. 1) and 2 sub-samples near the irrigation channel at a depth of 15-25cm. Thus, a total quantity of 2kg of soil was collected from each field. Precaution was taken to maintain a minimum of 10m distance between two consecutive sub-samples. Collection of soil samples from 2m Peripheral area was avoided. The collected soil samples were mixed thoroughly and stored in a sampling polythene bags. Each bag was labelled with: Date, location, soil type and stage of crop.

2.4 Selection of representative soil sample: Quadrant method of selection was followed for collection of representative soil sample. The collected soil sample was spread over a polythene sheet and cleaned from plant debris, pebbles and stones. Then the soil was divided into four equal samples and two opposite samples were selected randomly and the other two samples were discarded. Again this selected soil was divided into four equal samples and two samples were selected and two samples were discarded, same procedure was followed until the representative sample of 250-300 grams was obtained (Plate. 2). Selected soil sample was baited with factitious host Greater wax moth larvae, *Galleria mellonella* for isolation of EPNs from soil.

2.5 Isolation of EPNs by baiting technique with *G. mellonella*: For isolation of entomopathogenic nematodes from the soil, the baiting technique proposed by Bedding and Akhurst, (1975) [2] was followed. The representative soil sample was transferred to plastic containers (12 X 12cm) having perforated lid and 8-10 fourth instar larvae of Greater wax moth (*G. mellonella*) were released into each container. These containers were placed in cool and well ventilated place and kept undisturbed under laboratory conditions for 4-5 days. Care was taken to avoid compression of soil in the plastic container and optimum space was provided for easy movement of larvae into the soil and to facilitate infection with EPN (Plate. 3).

2.6 Infection of *G. mellonella* with EPNs: The mortality of *G. mellonella* due to EPNs infection was recorded starting from 24 hrs up to 96 hrs (1-4 days). The mortality of larvae due to EPN was identified based on the colour of dead cadavers. If infected with *Heterorhabditis*; cadavers usually turn to brown to dark brick red colour. The infected cadavers do not emit any bad odour and the body does not putrify. The skin of the cadavers does not rupture on pressure and will be free of any mycelial growth on the body. The EPN infected dead cadavers exhibiting the above symptoms were used for extraction of EPNs.

3. Results and Discussion

The primary goal of the present investigation was to identify the native species of EPNs with superior strains to make use of them for the effective management of root grubs in Sugarcane growing regions of Telangana state. A total of 90 soil samples representing the different soil type and irrigation systems were collected from four districts and were subjected to soil baiting technique with *Galleria mellonella,* the commonly used laboratory host for isolation of EPNs from the soil.

The results indicated that (Table 2) out of 90 soil samples, 17 soil samples showed the infestation of *G. mellonella* with EPNs. The infected cadavers turned to brick red colour (plate 4) indicating the existence of *Heterorhabditis* sp. in the 17 soil samples collected from Sugarcane fields.

Among the four districts surveyed for the presence of EPNs in Telangana state, in Sangareddy, out of 30 soil samples collected from three mandals *viz*., Sangareddy, Sadasivapeth and Zaheerabad and five villages, *viz*., Vykuntapuram, Girmapur, Pasthapur, Chota Hyderabad and Nadikudi, seven soil samples showed the presence of EPNs. In Kamareddy, 12 soil samples were collected from two villages, *viz*., Adloor and Chinna-mallareddy, of Kamareddy mandal in which four samples tested positive for *Heterorhabditis* sp. While in Nizamabad district soil samples were collected from three villages, viz., Rampurgadda, Rudrur and Varni, of two mandals, viz., Gandhari and Bodhan, from which out of 18 soil samples one sample showed the presence of EPNs. In Khammam district 30 soil samples were collected from five villages, *viz*., Mallepalle, Ammagudem, Rajeswarapuram, Konaigudem and Motapuram, of two mandals, viz., Nelakondapalle and Kusumanchi, where EPNs presence was detected in five soil samples.

The per cent occurrence of EPNs obtained from four districts of Telangana state (Table 2 and Fig 3) revealed that, maximum occurrence of EPNs was observed in Kamareddy (33.33%), while, lowest prevalence of EPNs (5.55%) was recorded in Nizamabad district. Sangareddy district which recorded 23.33% EPNs occupied the second place, followed by Khammam with 16.67% soil samples tested positive for the presence of EPNs.

The above results are in accordance with the findings obtained by Uribe-lorio *et al.* (2005) [14]. They reported that, 20.50% of the total soil samples were tested positive for the presence of EPNs. Whereas, Barbosa-Negrisoli *et al*. (2010) [1] and Myers *et al*. (2015) [10], recorded the presence of entomopathogenic nematodes in, 15.7% and 21% of the soil samples, respectively. The present findings also support the results of Hussaini *et al.* (2000) [6]. They reported the wide distribution of EPN species in Andra Pradesh. Sunanda *et al,*

(2016) [3] reported the distribution and occurrence of EPNs in 1.38% of total soil samples collected from Telangana state. Josephrajkumar and Sivakumar (1997) [7], Lalramliana and Yadav (2010) [8], Singh *et al*, (2015) [12], have also reported the occurrence and distribution of EPNs from various parts of the country.

Table 1: Survey carried out in sugarcane growing districts of Telangana state for isolation of Entomopathogenic nematodes

Sl no.	Date of soil survey	GPS information		Sample location			Growth stage of crop	Soil type	Type of irrigation
		Latitude	Longitude	District	Mandal	Village/Town			
1.	09/07/2016	17.630086	78.099496	Sangareddy	Sangareddy	Vykuntapuram	Grand growth stage	Black, clay	Drip
2.	09/07/2016	17.789829	78.568312			Girmapur	Grand growth stage	Black, clay	Drip
3.	14/07/2016	17.807993	80.896407		Sadasivapeth	Nadikudi	Seedling stage	Red, sandy	Furrow
4.	28/07/2016	17.672189	77.540484		Zaheerabad	Pasthapur	Formative stage	Black, clay	Drip
5.	28/07/2016	17.704484	77.629011			Chota hyderabad	Formative stage	Red, sandy	Furrow
6.	24/08/2016	18.385402	78.314397	Kamareddy	Kamareddy	Adloor	Grand growth stage	Black, clay	Furrow
7.	24/08/2016	18.286520	78.326502			Chinnamallareddy	Grand growth stage	Black, clay	Furrow
8.	03/09/2016	18.371829	78.075890	Nizamabad	Gandhari	Rampurgadda	Seedling stage	Black, clay	Furrow
9.	17/10/2016	18.575139	77.875678		Bodhan	Rudrur	Grand growth stage	Black, clay	Furrow
10.	17/10/2016	18.541617	77.894217			Varni	Grand growth stage	Black, clay	Furrow
11.	13/11/2016	17.169223	79.973439	Khammam	Nelakonadapalle	Mallepalle	Formative stage	Red, loamy	Furrow
12.	13/11/2016	17.136504	80.010521			Ammagudem	Formative stage	Red, loamy	Furrow
13.	13/11/2016	17.136955	80.003299			Rajeswarapuram	Formative stage	Black, clay	Furrow
14.	03/12/2016	17.119521	80.036408			Konaigudem	Grand growth stage	Black, clay	Furrow
15.	03/12/2016	17.159360	79.975992		Kusumanchi	Motapuram	Grand growth stage	Black, clay	Furrow

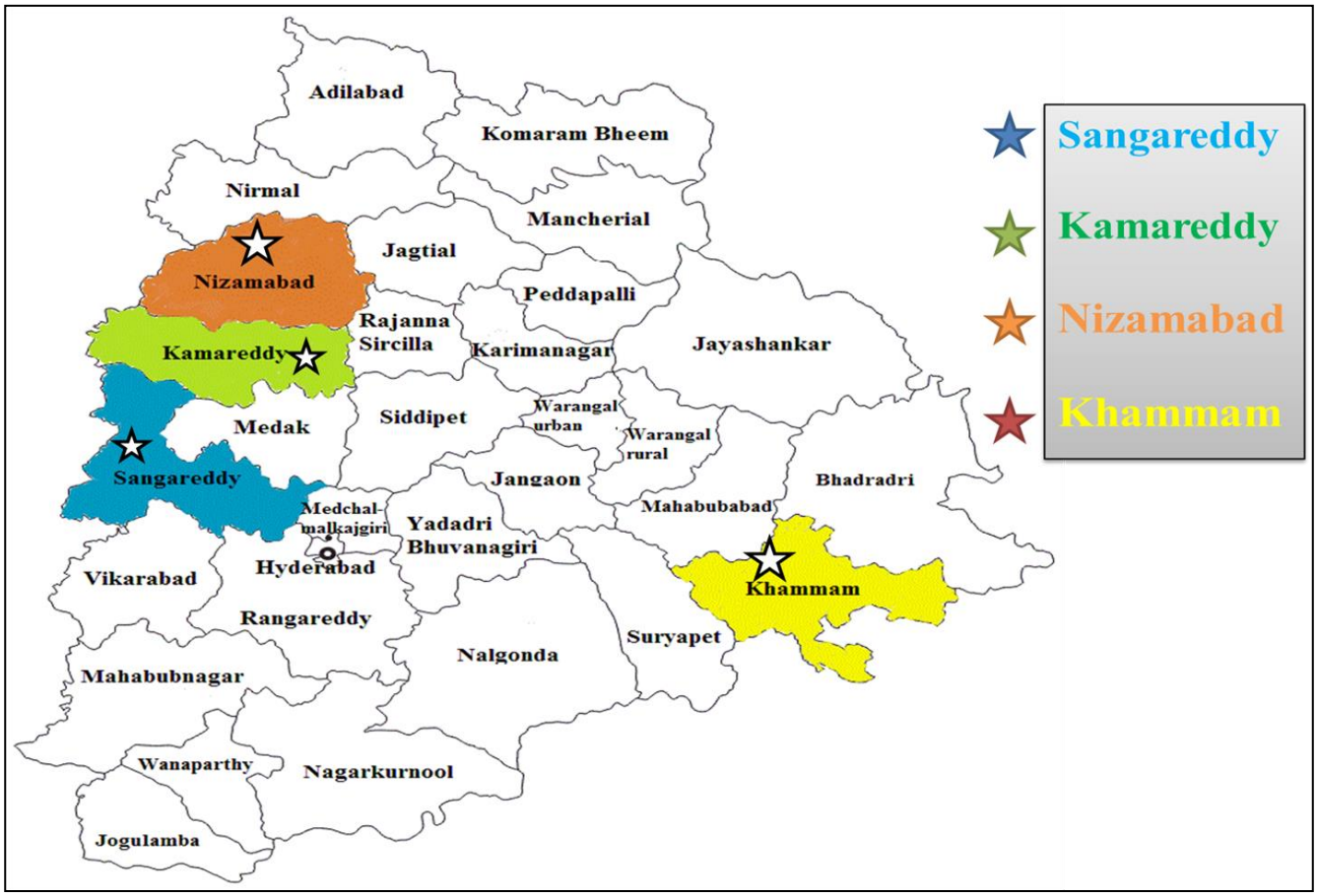

Fig 1a: Survey carried out in Sugarcane growing districts of Telangana state for entomopathogenic nematode

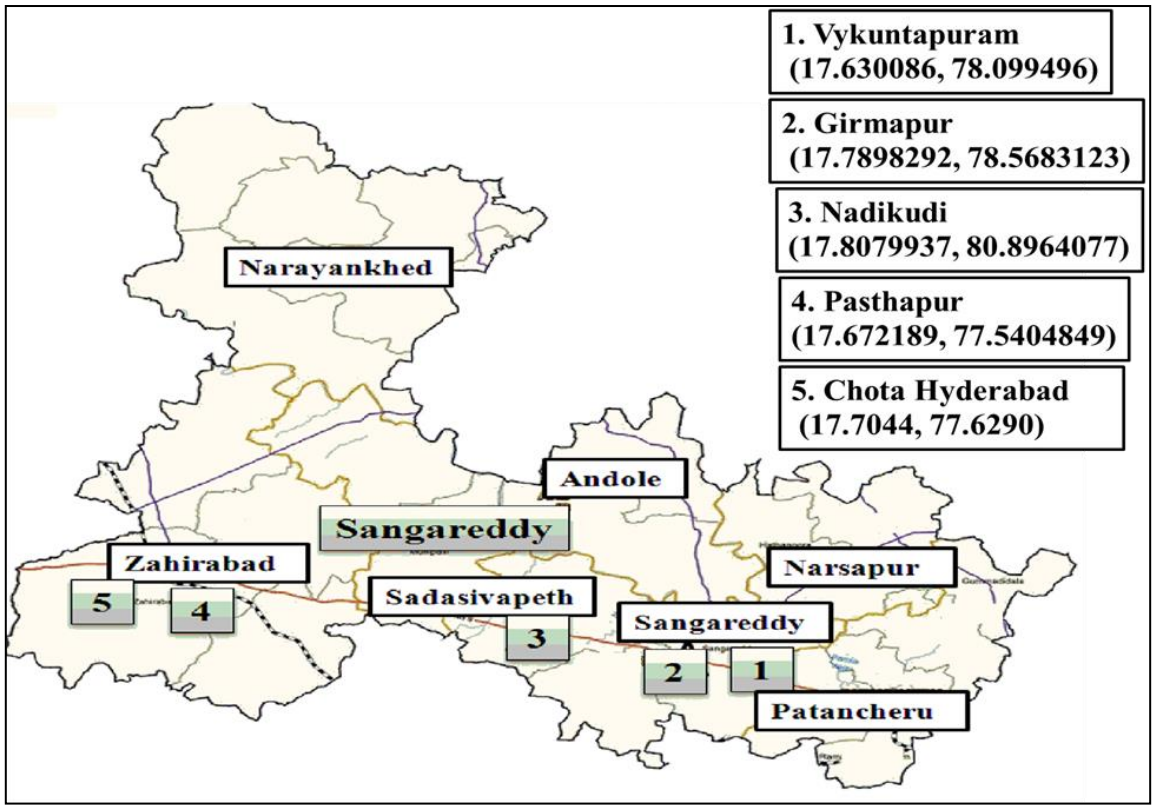

Fig 1b: Survey carried out in Sugarcane growing regions of Sangareddy district for entomopathogenic nematodes

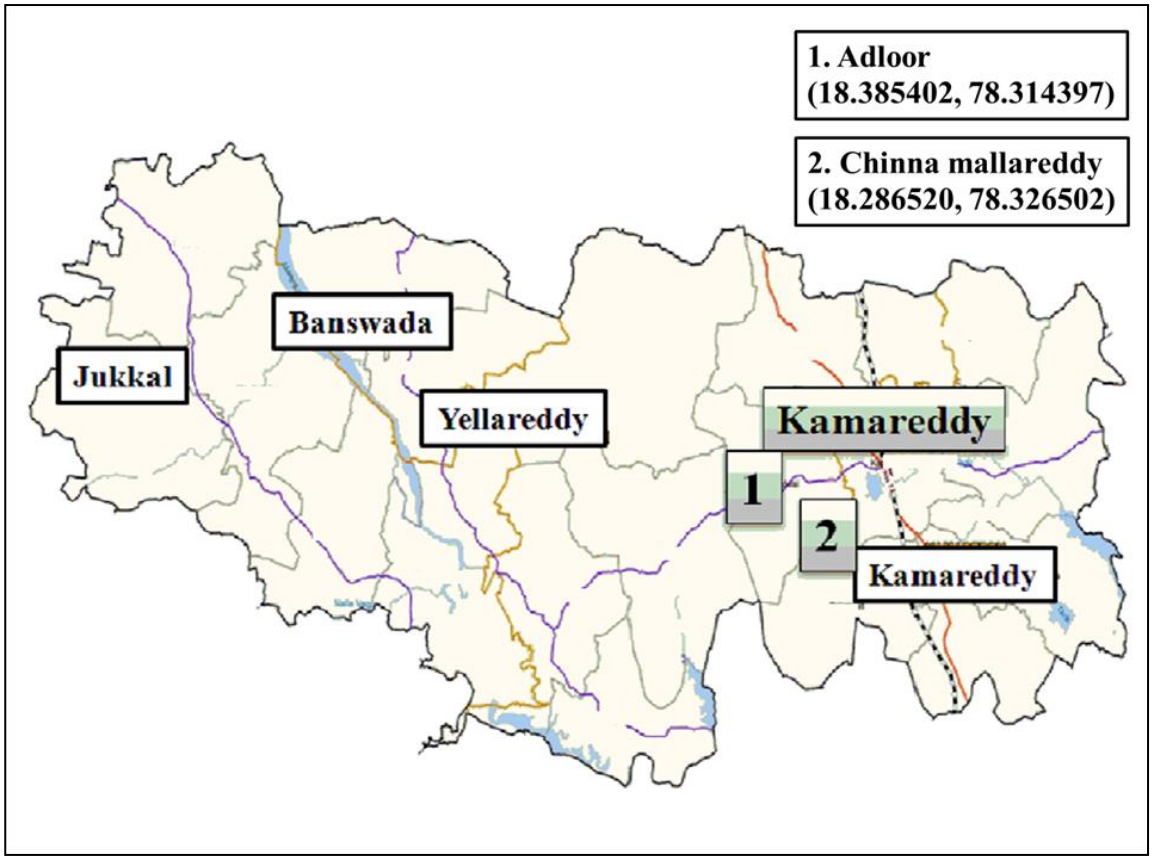

Fig 1c: Survey carried out in Sugarcane growing regions of Kamareddy district for entomopathogenic nematodes

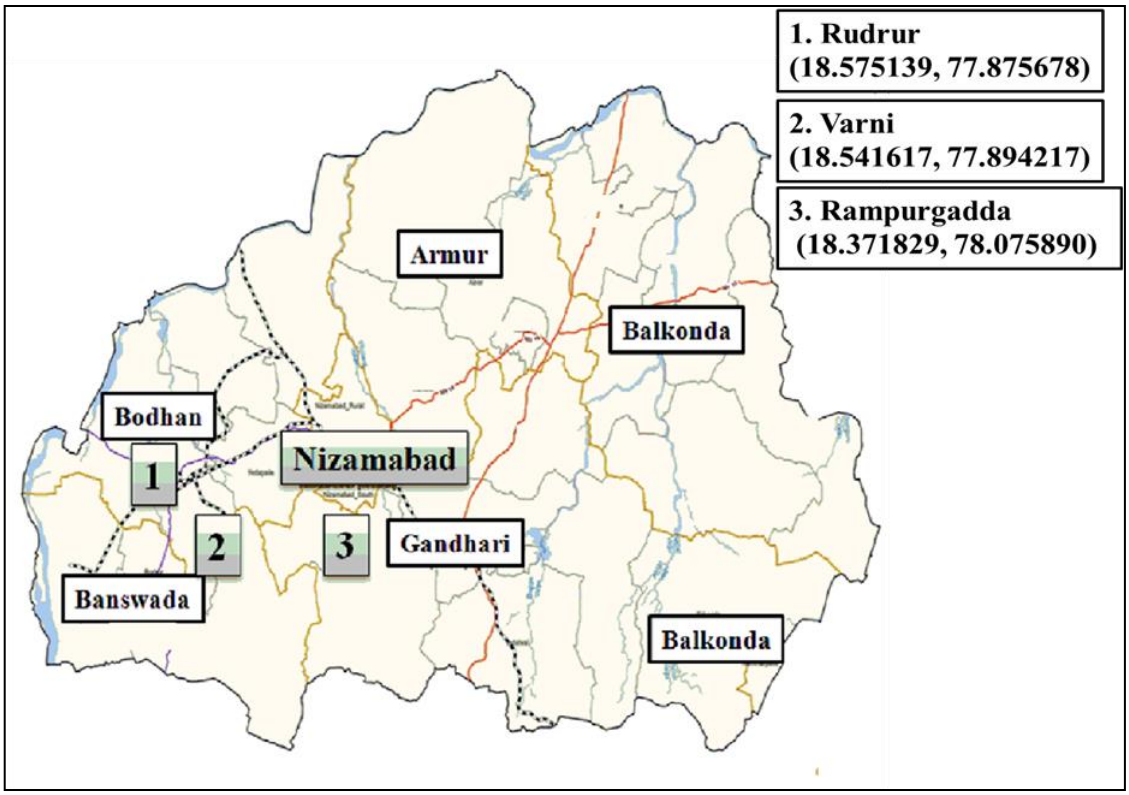

Fig 1d: Survey carried out in Sugarcane growing regions of Nizamabad district for entomopathogenic nematodes

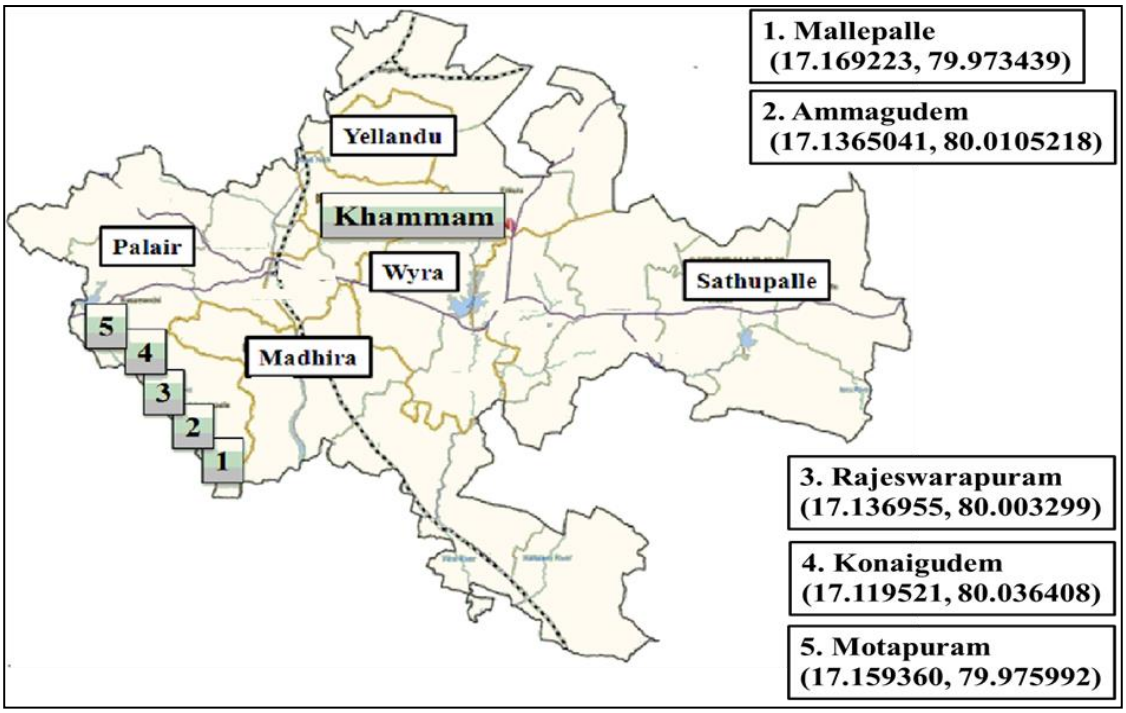

Fig 1e: Survey carried out in Sugarcane growing regions of Khammam district for entomopathogenic nematode

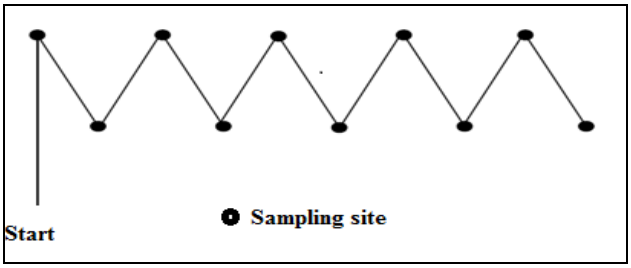

Fig 2: Zig-zag method of soil sampling

Plate 1: Collection of soil samples near the root zone in Sugarcane crop

Plate 2: Representative soil sample (250-300 grams) selected for isolation of EPNs bybaiting technique with *G. mellonella*

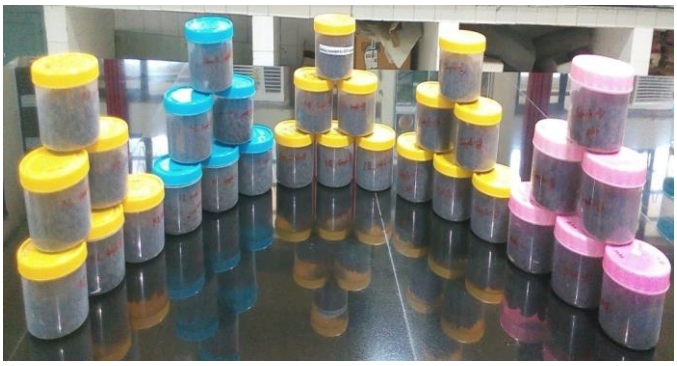

Plate 3: Baiting technique with *Galleria mellonella* for isolation of EPN's from the soil samples

Table 2: Survey carried out in Sugarcane growing districts of Telangana state for presence of entomopathogenic nematodes

Sl no.	Sample location			Total no. of soil samples collected	No. of samples tested positive for EPNs	Total no. of soil samples collected from each district	No. of Samples tested positive for EPNs	Per cent occurrence of EPNs in each district	EPN species base on visual observations
	District	Mandal	Village/Town						
1.	Sangareddy	Sangareddy	Vykuntapuram	6	1	30	7	23.33	*Heterorhabditis* spp
2.			Girmapur	6	2				
3.		Sadasivapeth	Nadikudi	6	2				
4.		Zaheerabad	Pasthapur	6	2				
5.			Chota Hyderabad	6	0				
6.	Kamareddy	Kamareddy	Adloor	6	1	12	4	33.33	*Heterorhabditis* spp
7.			Chinnamallareddy	6	3				
8.	Nizamabad	Gandhari	Rampurgadda	6	1	18	1	5.55	*Heterorhabditis* spp
9.		Bodhan	Rudrur	6	0				
10.			Varni	6	0				
11.	Khammam	Nelakonadapalle	Mallepalle	6	2	30	5	16.67	*Heterorhabditis* spp
12.			Ammagudem	6	2				
13.			Rajeswarapuram	6	0				
14.			Konaigudem	6	1				
15.		Kusumanchi	Motapuram	6	0				

Plate 4: Brick red colour dead cadavers of *G. mellonella* showing infection by *Heterorhabdtitis* sp.

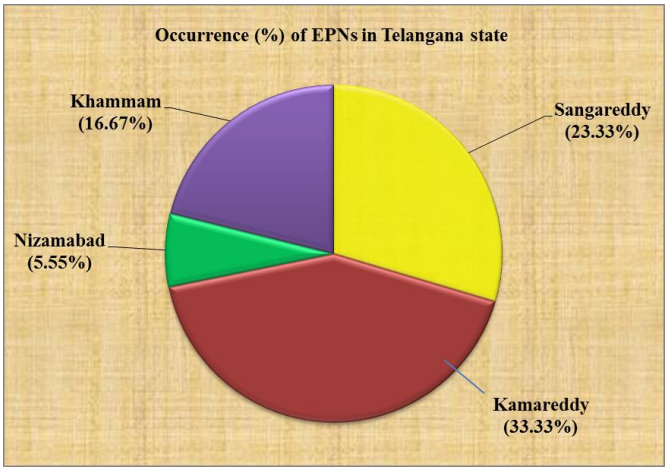

Fig 3: Occurrence of entomopathogenic nematodes in the Sugarcane growing districts of Telangana state

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