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Natural Occurrence of Entomopathogenic Nematodes Associated with Ginger (*Zingiber officinale* Rosc.) Ecosystem in India

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ABSTRACT: Random survey for naturally occurring entomopathogenic nematodes (EPNs) from ginger (*Zingiber officinale* Rosc.) ecosystem were undertaken from different agro-climatic regions in India. Two hundred and two soil samples were collected from different locations of districts Kozhikode, Wayanad, Kottayam, Idukki (Kerala), Kodagu (Karnataka), Coimbatore (Tamil Nadu), Kolasib (Mizoram), Faizabad (Uttar Pradesh) and Barapani (Meghalaya) for determination of EPN population. Out of 202 soil samples baited out, eight samples were found to be positive to EPNs. Among these strains, three EPNs were from Kozhikode district, three from Idukki district and one each from Wayanad and Faizabad districts. Out of the eight EPNs isolated, three species belong to genus *Steinernema*; one to *Heterorhabditis* and four to *Oscheius*. These EPNs have been identified on the basis of morphometric and morphological characterization. Four hundred and twenty four pseudostems of ginger infested with shoot borer (*Conogethes punctiferalis*) larvae were collected from the different localities of Kozhikode, Wayanad and Kodagu districts and 112 larvae were found dead. Among dead larvae, only one EPN, namely *Oscheius* sp. was recorded from the shoot borer larvae. Our survey revealed that, *Oscheius* spp. and *Steinernema* spp. widely occur and *S. ramanai* and *O. gingeri* reported as news species from the rhizosphere of ginger. These EPNs have great potential for biological control of insect pests of ginger.

Key words: Entomopathogenic nematodes, Survey, Natural occurrence, Ginger, Shoot borer

Ginger (*Zingiber officinale* Rosc.) is a perennial herb belonging to the family Zingiberaceae and rhizome or rootstalk used as spice and medicine. From 1975 onwards till the 80s, India was the major producer of ginger with a share of 30-35% of world production. However, in the later part of 90s, ginger production fall down. Ginger production in India is sustained losses due to several reasons. Among them, one of the major constraints is insect pests. Among the insect pests, shoot borer (*Conogethes punctiferalis* Guen.) is the most serious, which causes significant yield losses (Devasahayam *et al.*, 2012). The only effective method to manage this pest is the use of insecticides resulted pesticide residues in the produce affecting human health and also causing other ecological hazards. There has been a renewed interest in developing environment-friendly pest management schedules in agriculture.

Entomopathogenic nematodes (EPNs) have got little attention by researchers though they have a great potential in reducing pest population and with little manipulation their role can be enhanced (Ali *et al.*, 2005). To improve upon their biocontrol potential is to isolate new strains or to detect species which can tolerate local climatic condition and in this way several biocontrol successes have been achieved in many parts of the world (Lorio *et al.*, 2005).

EPNs have been reported to occur in tropical, subtropical and temperate countries (Gaugler & Kaya, 1990) except Antarctica (Griffin *et al.*, 1990). In addition, many surveys have revealed natural occurrence of EPNs associated with different ecosystem in India such as, in Meghalalaya (Lalramlina & Yadav, 2010), in Andman and Nicobar islands (Prasad *et al.*, 2001), Gujarat (Vyas, 2003), Kerala (Banu *et al.*, 2004; 2005), New Delhi (Ganguly & Singh, 2000), Tamil Nadu (Josephrajkumar & Sivakumar, 1997) and Uttar Pradesh (Pervez and Ali, 2007). However no such information is available about the EPNs associated with ginger crops.

Hence, random survey for naturally occurring EPNs from ginger ecosystem was undertaken from different agro-climatic regions in India and evaluated pathogenicity of isolated EPNs against shoot borer larva (SBL), *Conogethes punctiferalis* and greater wax moth larva (GWML), *Galleria mellonella*.

MATERIALS AND METHODS

Soil samples collection site: Random survey and soil samples were collected from ginger rhizosphere from different locations of Kozhikode, Wyanad, Kottayam, Idukki (Kerala), Kodagu (Karnataka), Coimbatore (Tamil Nadu), Guwahati (Assam), Faizabad (Uttar Pradesh), Kolasib (Mizoram) and Barapani (Meghalaya) districts. Within collection site, about 1 kg of soil sample was collected at a depth of 10-20 cm using a hand trowel, each sample containing a composite from five random subsamples. The hand trowel was sterilized with 70% ethanol before leaving the sampling site. Samples were placed in polyethylene bags to minimize dehydration, tag a label providing all necessary information and transported in to the laboratory. At each sampling site, temperature was taken and notation about physiographic regions, altitude, longitude, latitude data taken with the help of GCMS and annual average rainfall data collected from state Krishi Bhavan and KVKs.

Isolation of EPNs from soil: EPNs isolated from the soil using the insect baiting technique (Bedding & Akhurst, 1975). About 250 g composite soil was placed in a plastic container and five fourth instar GWML were used to bait out the EPNs, if any. The soil sample was checked every day up to 7-10 days or pupation of the GWML. If any GWML was found dead, to be placed in modified white trap for 2 weeks at room temperature for emergence of EPN. In case of the negative results, the isolation process in the soil was repeated two times for the confirmation of the result. Emerged IJs collected from White traps was used to infect fresh GWML to produce nematodes for establishment of cultures.

Maintenance of EPNs and insect cultures: All isolated EPNs were cultured as per the procedure described by Kaya and Stock (1997). The IJs were surface sterilised in 0.1% Hyamine solution and stored in distilled water in tissue culture flasks for identification. However, only fresh nematode culture was used in the bioassay.

GWML reared on artificial diet as per the procedure described by David and Kurup (1988) and SBL were collected from ginger fields of IISR Experimental Farm, Kozhikode. The larvae were sorted out and those of same size were used for the bioassay study.

Soil characterization: Soil that was used for baiting for nematodes was maintained at room temperature. Soil samples that were positive to EPNs were analyzed soil type and pH. The pH was measured from a 1:2.5 soil/m Q-water suspension. The properties of the soil contents were evaluated by Bouyoucos method (MAPA, 1975; 2005).

Detection of EPNs from SBL: Infected pseudostems of ginger with SBL were collected from different localities to detect the EPNs from naturally infected SBL. Pseudostems were placed in polyethylene bags and tag a label providing all necessary information. The shoot borer larvae isolated from the pseudostem of the ginger and dead SBL were placed in modified white trap for 15 days at room temperature for emergence of EPN.

Identification of EPN: Morphological and morphometric studies were carried out with 10 infective juveniles and 10 first generation males (Stock *et al.*, 2000). Nematodes were fixed and processed to dehydration following the method described by Steinhorst (1966). According to their morphological characteristics, EPN was placed into similar species-groups using taxonomic criteria suggested by Stock and Kaya (1996).

Entomopathogenicity: Pathogenicity of EPNs against SBL and GWML was tested in petri- plates. For this, ten larva of tested insect was kept in petri plate and 100 IJs of each tested species of EPNs were inoculated and their mortality was recorded after 72 h. The experiment was conducted at room temperature and replicated ten times along with control. The mortality was calculated according to following formula and mean value worked out.

Mortality (%) = D x 100 / N

Where:

D- Number of dead larvae; **N** – Total number of larvae

Statistical analysis: All data were subjected to analysis of variance (ANOVA) and means compared according to Duncan´s multiple range test. Before analysis, data of the nematodes were square root-transformed and those of percentages of insect mortalities were arcsine transformed. All means were transformed back to the original units for presentation.

RESULTS AND DISCUSSION

Soil samples collected: Two hundred and two soil samples were collected from ginger rhizosphere from different locations of Kozhikode, Wyanad, Kottayam, Idukki districts of Kerala, Kodagu district of Karnataka, Coimbatore district of Tamil Nadu, Kolasib district of Mizoram, Faizabad district of Uttar Pradesh and Barapani district of Meghalaya (Table 1).

Isolation of EPNs from soil: EPNs were recovered from 8 out of the 202 soil samples (3.96 %). Among these eight positive samples, steinernematidae (37.5%), Rhabditidae (62.5%) and heterorhabditidae (12.5%) isolates were recovered from the four regions. Out of the isolated EPNs, three each EPNs were found from Kozhikode and Idduki districts and one each from Wyanad and Faizabad districts (Table 1).

We found that the prevalence of EPNs in soils is low compared with other surveys. The low recovery rate of EPNs in this survey could be attributed to the use of GWML as the bait insect because some EPN species that were more host specific. In using GWML, we isolated only those species that have a relatively broad host range or only infect lepidopteran larvae. The selected site may reflect variation in the availability of suitable host species and it is difficult to find EPNs in the soil in the absence of susceptible hosts (Mracek and Webster, 1993). In the future, the incorporation of coleopteran and dipteran bait insects may increase our knowledge on the diversity and distribution of these important natural control agents of insects.

Table 1. Soil sample analysis for detection of entomopathogenic nematodes.

Locality (state/ district)	No. of samples collected	Positive samples	EPN baited out
Kerala			
Kozhikode	40	03	*Heterorhabditis* sp. (IISR-EPN 01) *Steinernema* sp. (IISR-EPN 02) *O. gingeri* (IISR-EPN 07)
Wyanad	23	01	*Oscheius* sp. (IISR-EPN 05)
Idukki	42	03	*S. ramanai* (IISR- EPN 03) *Oscheius* sp. (IISR-EPN 04) *S. carpocapsae* (IISR-EPN 06)
Kottayam	14	-	-
Karnataka			
Kodagu	22	-	
Mizoram			
Kolasib	12	-	-
Tamiln Nadu			
Coimbatore	16	-	-
Meghalaya			
Barapani	17	-	-
Uttar Pradesh			
Faizabad	16	01	*Oscheius* sp. (IISR-EPN 08)
Total	**202**	**08**	

Rosa *et al.* (2000) have summarized the rate of recovery of EPNs from various soil surveys conducted throughout the world. Most surveys showed their recovery rate from soils between 6 - 35%. In the present study, the Steinernematids were found to be significantly predominating than heterorhabditids. Many other workers have also reported dominance of steinernematids recovery over heterorhabditids. In contrast, the dominance of

Table 2. Shoot borer larvae collected for the detection of EPNs

Location	No. of larvae collected	No. of dead larvae	No. of larvae infested with EPNs
1. Kozhikode			
IISR Experimental Farm, Peruvannamuzhi	201	77	*Oscheius* sp. (IISR-EPN 09)
Thamarassary	55	-	-
2. Wyanand			
Vythiri	76	12	-
Mananthavadi	8	8	-
Sulthan Bathery	14	3	-
3. Kodagu			
Gonnikopal	19	-	-
Madikeri	51	12	-
Total	**424**	**112**	**01**

heterorhabditids over steinernematids has been found in rather few surveys. For example, Rosa *et al.* (2000) in a study in nine islands of the Azorean archipelago noticed that *Heterorhabditis* spp. were present in 30 sites from six islands, whereas *Steinernema* spp. were found only in 16 sites from three islands.

Further, our findings were comparatively average to studies in India, where Raj Kumar *et al.* (2001) showed that out of 105 soil samples collected from Rajasthan, only 5 (4.76%) were found to be positive for steinernematids and heterorhabditids. Subsequently, Parihar *et al.* (2002) undertook another survey in Rajasthan and reported the presence of EPN in 8 samples out of 477 samples (1.68%) studied. They further mentioned that out of 8 positive samples, 5 (62.5%) were positive with *Heterorhabditis* spp. and the other 3 (37.5%) constituted *Steinernema* spp. Josephrajkumar and Sivakumar (1997) in their study in Tamil Nadu reported the prevalence of Steinernematids (94.44%) and heterorhabditids (5.55%). In contrast to this, Singh *et al.* (1992) reported a very low prevalence (1.82%) of *Steinernema* sp. at ICRISAT centre, Hyderabad.

Diversity among the species of *Oscheius* could not be compared due to lack of information but it may be assumed that diversity vary considerably with habitat, area and the number of individual (Pervez and Ali, 2007).

Soil characterization: Among the positive samples, maximum EPNs were recovered from laterite soil (50%), followed by alluvial (25%), whereas minimum occurrence of EPNs in sandy loam and clay soils (12.5%) (Table 3). An important indicator determining whether EPNs occur in the environment is the soil type. Soil texture influences nematode survival and mobility. Generally, higher clay content results in lower nematode survival. This is due to decreased pore size and reduced oxygen availability (Molyneux & Bedding, 1984; Kung *et al.*, 1990). Nematodes are generally more mobile in laterite and sandy soil and these factors greatly contribute to the distribution of nematodes in particular habitat (Barbercheck & Kaya, 1991; Georgis & Poinar, 1993).

There were no obvious trends was found for the occurrence of EPNs when soil temperature at the time of sampling (21- 36 ^{0}C), rainfall (681-6917 mm) and altitude (512- 1146 m) (Table 3). Similar effect was recorded for altitude, temperature and rainfall (Constant *et al.*, 1998; Garcýa del Pino and Palomo 1996; Mracek *et al.*, 2005). For pH, we isolated EPNs from acidic (pH 4.4) to slightly alkaline (pH 8.1) soils. This agrees with other studies where the pH of EPN positive soil samples varied from 4.6 to 8 (Mracek and Becvar, 2000; Hara *et al.* 1991; Griffin *et al.* 1991).

EPNs abundance, distribution and habitat preference are related to host-parasite relationships, environmental conditions and soil characteristics (Barbercheck, 1992). Consequently, the EPNs occurrence in soil samples can be variable in different surveys, ranging the recovery frequency from 0.7 to 70.1% (Bruck, 2004).

Detection of EPNs from the SBL: Four hundred and twenty four SBL isolated from infected pseudostem of the ginger from different localities of Kozhikode and Wyanad districts of Kerala and Kodagu districts of Karnataka. Out of 424 larvae, 112 natural dead larvae were found. Among these dead larvae, only one EPN was found from the districts Kozhikode (Table 2). This EPN are not identified up to species level due to they not be reproduced successfully in the laboratory.

Table 3. Environmental and soil characteristics of the positive samples for EPNs species.

EPNs	Physiographic region	Location/ district	Altitude (m)	Latitude, longitude	Temperature (°C)	Rainfall (mm)	Soil properties	
							pH	Texture
Heterorhabditis sp. (IISR-EPN 01)	Foot hills	IISR Experimental Farm, P'muzhi, Kozhikode	535	77°01'E 9°33'N	29	4120	5.3	Laterite
Steinernema sp. (IISR-EPN 02)	Foot hills	IISR Experimental Farm, P'muzhi, Kozhikode	610	77°13'E 9°69'N	34	4120	5.7	Laterite
S. ramanai (IISR-EPN 03)	Hills	Adimali, Idukki	1146	76°57'E 10°01'N	28	3830	7.8	Alluvial
Oscheius sp. (IISR-EPN 04)	Hills	Peerumedu, Idukki	1124	77°01'E 9°33'N	21	6917	7.4	Laterite
Oscheius sp. (IISR-EPN 05)	Hills	Ambalavayal, Wynand	918	76°12'E 11°36'N	32	3062	4.4	Clay
S. carpocapsae (IISR-EPN 06)	Plain	Kumarganj, Faizabad	107	81°50'E 26°32'N	36	681	7.8	Sandy loam
O. gingeri (IISR-EPN 07)	Foot hills	IISR Experimental Farm, P'muzhi, Kozhikode	512	77°81'E 9°73'N	35	4907	4.9	Laterite
Oscheius sp. (IISR-EPN 08)	Hills	Udumbanchola, Idukki	1067	77°10'E 9°53'N	26	3830	8.1	Laterite

Identification of EPNs: Total nine isolates of EPN isolated, Among them, eight from soil and one from SBL. Out of these isolates, three belonged to genus *Steinernema*; one to *Heterorhabditis* and five to *Oscheius*. Among them, two new species *viz., S. ramanai* (IISR-EPN 03). (Pervez *et al.*, 2011) and *O. gingeri* (IISR-EPN 07) (Pervez *et al.*, 2013) and one known species, *S. carpocapsae* (IISR-EPN 06) were identified on the basis of morphometric and morphological characterization. Although another 6 isolates could not be identified to species level. The description of these species along with their biological characterization is undergoing. Similar values were recorded from other Mediterranean regions.

Entomopathogenicity: Results indicated that all the isolated EPNs were pathogenic to SBL and GWML but percentage mortality was varied. All the isolates caused cent per cent mortality to GWML except, *Oscheius* sp. (IISR-EPN 08) which caused only 92 % mortality after 72 h. Among the test EPNs, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *S. carpocapsae* (IISR-EPN 06) and *Oscheius* sp. (IISR-EPN 07 and 09) was found promising, it brought about cent per cent mortality to SBL, followed by *S. ramanai* (IISR-EPN 03) and *Oscheius* spp. (IISR-EPN 05) caused only 92 per cent, whereas minimum mortality (83 %) caused by *Oscheius* spp. (IISR-EPN 04 and 08) (Fig. 1).

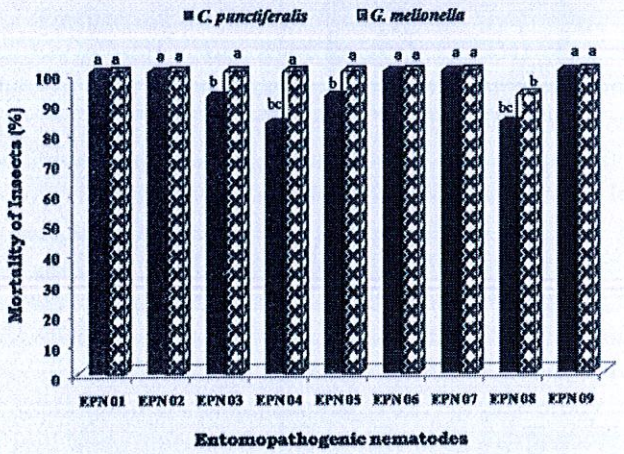

Fig. 1. Mortality of *C. punctiferalis* and *G. mellonella* caused by different entomopathogenic nematodes. EPN 01- *Heterorhabditis* sp. (IISR- EPN 01); EPN 02 - *Steinernema* sp. (IISR-EPN 02); EPN 03 - *S. ramanai* (IISR – EPN 03); EPN 04 - *Oscheius* sp. (IISR-EPN 04); EPN 05 - *Oscheius* sp. (IISR-EPN 05); EPN 06 - *S. carpocapsae* (IISR-EPN 06); EPN 07 – *O. gingeri* (IISR-EPN 07), EPN 08 - *Oscheius* sp. (IISR-EPN 08) and EPN 09 - *Oscheius* sp. (IISR-EPN 09)

In vitro screening of EPNs infectivity can be an important component of developing a biological control programme for a particular pest (Ricci *et al.*, 1996). In past, one of the basic reasons for failure of EPNs for biological control of insect pests is because of the wrong choice of nematode species or strain (Kaya and Gaugler, 1993) and results can vary greatly among strains of the same species (Pervez *et al.*, 2012; 2014). The variation mortality percentage within Steinernematidae, Heterorhabditidae and Rhabditida group indicated that, neither group was superior to the other. Differences among EPN species/strain in their efficacy against insect pests reported by earlier workers (Gaugler and Kaya, 1990; Pervez *et al.*, 2012; 2014).

Isolated EPNs showed promise as biological control agents against SBL, *C. punctiferalis*, hairy caterpillar, *Euproctis* sp. and leaf betel, *Lema* sp. in the laboratory (Pervez *et al.*, 2012; 2014). Therefore, evaluation of promising EPNs against SBL under field conditions is undergoing.

Our survey revealed that, *Oscheius* spp. and *Steinernema* spp. occur widely. These indigenous strains will be suitable for managing the insect pests of ginger. The results of this survey extend the knowledge on EPNs from ginger ecosystem. In conclusion, the present study constitutes the first report of EPNs associated with ginger rhizosphere. The information generated from present study may open the prospects for using EPNs in the biological control programs against insect pests in the area because indigenous EPNs are adapted to the local environmental conditions and are natural regulators of insect populations.

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