Research Paper :

Distribution of entomopathogenic nematodes in natural habitats

L.S. VANITHA, MANJU KUMARI, J. JAYAPPA AND S.C. CHANDRASHEKAR

International Journal of Plant Protection (October, 2010), Vol. 3 No. 2 : 179-181

See end of the article for authors' affiliations

Correspondence to :
L.S. VANITHA
Department of Plant Pathology, University of Agricultural Sciences, G.K.V.K., BENGALURU (KARNATAKA) INDIA

SUMMARY

A total of 13 different habitats were evaluated for the presence of rhabditid entomopathogenic nematodes in University of Agricultural Sciences, GKVK, Bengaluru, Karnataka. The EPNs were collected by using *Galleria mellonella* baited traps at 13 locations representing different cropping systems. In each location, 50 traps were placed and these traps were harvested at 4th, 7th and 12th days after placing them. The data on infestation of *G. mellonella* larvae in each trap were recorded and data was converted to per cent infestation of total larvae sampled. The total per cent mortality of *Galleria mellonella* larvae to EPNs was recorded and it varied from (0-20%). The recovered isolates were identified as a *Steinernema* spp.

Key words : *Galleria mellonella*, Entomopathogenic nematodes, *Steinernema* spp., *Xenorhabdus, Photorhabdus*

Accepted :
April, 2010

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are obligate parasites of mainly soil-inhabiting insects and have great potential as biological control agents of many insect pests and these are in association with symbiotic bacteria, *Xenorhabdus* and *Photorhabdus* spp. which have insecticidal and antimicrobial properties. EPNs were found in a variety of soil habitats, and the various species and isolates exhibit considerable variation in terms of host range, reproduction, infectivity and conditions for survival (*i.e.* temperature, soil moisture etc. (Kung *et al*., 1991). Surveys for EPNs were conducted in temperate, subtropical and tropical regions to further advance the use of EPNs as biological control agents in South Africa (Ehlers, 1996). Exploration of entomopathogenic nematodes from native habitats is needed to know their diversity and distribution for their utilization as a biocontrol agent.

MATERIALS AND METHODS

Isolation of entomopathogenic nematodes from soil:

Isolation of EPNs was done using *Galleria mellonella,* a susceptible host by baiting method (Bedding and Akhurst, 1975). Small plastic vials of 50 ml capacity with wire mesh on both sides with last instar larvae (8th instar) of greater wax moth, *Galleria mellonella* (Fig.1) were placed in the pits at a depth of 15 cm dug in different locations, later these were covered with soil. The traps were harvested at 4th, 7th and 12th days to know the per cent infectivity of *Galleria mellonella*

Fig. 1 : ***Galleria mellonella*** **baited trap**

larvae to EPNs. Water containing infective juveniles in the White's trap (White, 1927, Fig. 2) were poured in 100 ml beaker filled with distilled water. Nematodes were left to settle for about 30 minutes at the bottom of the dish, the washing process was repeated three times until water became clear (Fig. 3).

In vivo mass multiplication of nematodes:

Galleria larvae which were infected with EPNs were kept on White's trap and collected nematode culture suspensions were sterilized with 0.1% hyamine to get rid of all contaminants

Fig. 2 : White's trap for collection of entomopathogenic nematodes

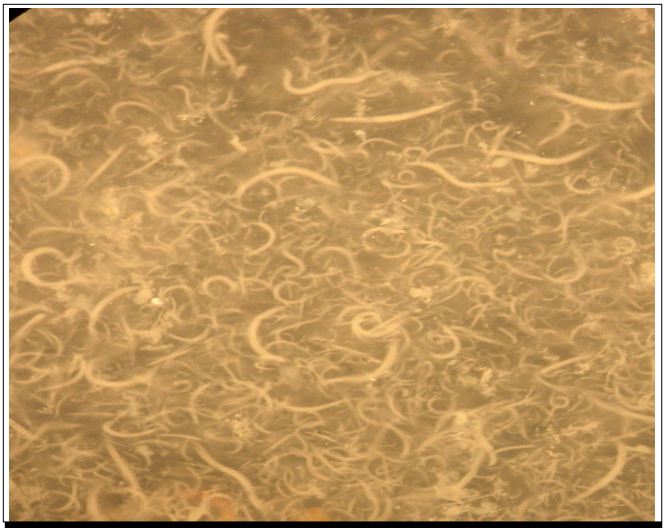

Fig. 3 : Entomopathogenic nematodes culture

Details of locations sampled for EPNs in University of Agricultural Sciences, GKVK, Bengaluru, Karnataka		
Location 1	Mango orchard	Mango
Location 2	Play ground	-
Location 3	Tree arboretum	Forestry tree species
Location 4	Botanical garden	Cashew, forestry tree species
Location 5	Gauva plot	Guava
Location 6	Jatropa field	Jatropa
Location 7	Dryland	Chilli, maize etc.
Location 8	Crop Physiology Research plot	Groundnut
Location 9	Medicinal and Aromatic garden	Ornamental and medicinal plant
Location 10	Horticulture nursery	Tomato, pumkin etc.
Location 11	Genetics and Plant Breeding plot	Rice
Location 12	Pulse crop	Cowpea, field bean etc.
Location 13	Agronomy plot	Sunflower

associated with infective juveniles and also to kill non-infective juveniles. A small suspension of nematode culture was added to a clean Petri plate using moist filter paper. Later, few last instar *G. mellonella* larvae were placed and incubated at room temperature. After two days, the larvae were observed for mortality. They were surface sterilized and again kept on White's trap.

Identification:

Isolated EPNs were relaxed in hot water, fixed in F.A. 4:10 permanent mounts were prepared by processing through lactophenol and finally mounted on glycerin. Nematodes were identified using taxonomic keys available in their respective groups.

Identification factors for genus *Steinernema*

-Excretory pore located posterior to swollen part of metacarpous

- Female without double valve epiptygma

-Spicule about 58µm long

-Tail less than 58µm long

-Infective juveniles have two cephalic horns

RESULTS AND DISCUSSION

Among thirteen locations, ten locations were positive for EPNs (Table 1). The results of the experiment are presented as a total percentage mortality of *Galleria mellonella* larvae to EPNs as follows, Agonomy plot(10%), Botanical Garden(0%), Crop Physiology(2%),

Table 1 : Per cent infection of *Galleria mellonella* larvae by entomopathogenic nematodes in soil, across different locations within GKVK campus

Locations	Total no. of traps	4th day		7th day		12^{th} day		Total no. sampled	Total no. infected	Total per cent infection
		No. of traps sampled	No. infected	No. of traps sampled	No. infected	No. of traps sampled	No. infected			
1. Mango orchard	50	10	1	20	1	20	3	50	5	10
2. Play ground	50	10	0	20	5	20	0	50	5	10
3. Tree arboretum	50	10	0	20	1	20	4	50	5	10
4. Botanical garden	50	10	0	20	0	20	0	50	0	0
5. Guava plot	50	10	2	20	1	20	1	50	4	8
6. Jatropa	50	10	0	20	1	20	0	50	1	2
7. Dryland	50	10	2	20	0	20	1	50	3	6
8. Crop physiology	50	10	0	20	1	20	0	50	1	2
9. Medicinal and Aromatic garden	50	10	4	20	6	20	0	50	10	20
10. Horticulture nursery	50	10	0	20	0	20	0	50	0	0
11.GPBexperimental plot	50	10	1	20	0	20	0	50	1	2
12. Pulses crops	50	10	0	20	0	20	0	50	0	0
13. Agronomy plot	50	10	2	20	2	20	1	50	5	10

Dryland (6%), Gauva plot (8%), Genetics and Plant Breeding Reseach plot(2%), Horticulture nursery(0%), Jatropa (2%), Mango orchard (10%), Medicinal and Aromatic garden (20%), Play Ground(10%), Pulse crop(0%) and Tree arboratum (10%) (Table 1).

The nematodes were found distributed in all habitats. The difference in per cent mortality of *G. mellonella* larvae by EPNs, across different locations, may be due to difference in the number of soil borne insects for EPNs, survival and reproduction or due to influence of farm parasites, environmental factors such as soil moisture, soil type, physical and chemical parameters across the locations and distribution of EPNs mainly varied with the seasons and many other unknown factors. Similar experiment was carried out by (Akhurst and Brooks, 1984) who reported that the recovery of EPNs was more in orchards, pasture and crop land compared to wood land.

Soil supports infinite species of fauna and flora to lead their live both in mutualistic and antagonistic relation. However, finite amount of resources in the soil limit the population to certain threshold level. The population of soil dwelling coleopteran insects are mainly controlled by EPNs and their associated symbiotic bacteria. Prevalance of EPNs in the region in Bengaluru, Karnataka and other parts of the india and in other countries indicates, that EPNs could be one of the best choices as a biocontrol agents against different insect pests. There is need to explore and use these nematodes in different crops.

Authors' affiliations:
MANJU KUMARI, Department of Biotechnology, Bangalore University, BENGALURU (KARNATAKA) INDIA
J. JAYAPPA, Raita Samparka Kendra, Challakere, CHITRADURGA (KARNATAKA) INDIA
S.C. CHANDRASHEKAR, Department of Plant Pathology, University of Agricultural Sciences, G.K.V.K., BENGALURU (KARNATAKA) INDIA

REFERENCES

Akhurst, R.J. and Brooks, W.M. (1984). The distribution of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) in North Carolina. *J. Invert. Pathol*, **44**: 140-145.

Bedding, R.A. and Akhurst, R.J. (1975). A simple technique for the detection of insect parasitic nematodes in soil. *Nematologica*, **21**:109–110.

Ehlers, R.U. (1996). Current and future use of nematodes in biocontrol: Practice and commercial aspects in regard to regulatory policies. *Biocontrol Sci. Technol.*, **6**: 303–316.

Kung, S.P., Gaugler, R. and Kaya, H.K (1991). Effect of soil temperature, moisture and relative humidity on entomopathogenic nematode persistence. *J. Invertbr. Pathol.*, **51**: 242-249.

White, G.F. (1927). A method for obtaining infective nematode larvae from soils. *Sci.*, **66**: 302-303.

****