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Natural Occurrence, Distribution and Isolation of Native Strains of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) in Vegetable Crops in Haryana, India

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ABSTRACT: Entomopathogenic nematodes (EPNs), Steinernematidae and Heterorhabditidae have a potential bio-control agents since these organisms have a wide range of geographic areas, soil types and are adapted to several hosts. They showed better performances over chemical and microbial insecticides. But for the better performances of EPNs, should be adapted to the local environmental conditions. The search for local EPN isolates though systematic survey is the first critical step in building an effective biological management program for insect pests. The populations of EPNs were isolated by *'Galleria* trap' method from the soils around the roots of various crops in Haryana (Hisar, Jind, Panipat, Sonipat, Karnal, Mewat, Palwal and Fatehabad districts). A total of 160 soil samples were collected from the rhizosphere of different crops and were processed for the detection of EPNs by insect bait method, 40 samples (25%) were found to be positive for EPNs. Based on samples collected from different vegetable crops, the frequency of EPNs observed were chilli (40.0%), cucurbitaceae (31.1%), capsicum (33.3%) and tomato (27.6%). Out of 40 samples found positive for EPNs collected from various vegetable crops, the frequency of occurrence of Steinernematid and Heterorhabditid nematodes were 80 and 20 percent, respectively. Identification of EPNs was done at generic level (*Heterorhabditis* and *Steinernema*) based upon the colour of host cadaver. *Galleria mellonella* larvae infected with EPN of the genus *Heterorhabditis* may impart reddish-orange colouring while those infected with *Steinernema,* imparts grey-brown colouring. So, the bio-efficacy of EPNs proved better of native strains which already compatible to the same environment.

Keywords: Distribution, Frequency, Isolation, *Heterorhabditidae*, *Steinernematidae*.

INTRODUCTION

Commercial production of entomopathogenic nematodes (EPNs) from the families Steinernematidae and Heterorhabditidae is used to manage insect pests biologically, especially for those that live in soil and cryptic environments. From various nations throughout the world, 21 species of *Heterorhabditis* and about 100 genuine species of *Steinernema* have been identified. But till now, only fifteen species of EPNs (12 for *Steinernema* and 3 for *Heterorhabditis* species) are recorded from different parts of India (Kumar *et al.*, 2022). Identification of naturally adapted species in a particular location is crucial for EPN to be a successful biological pesticide (Stock *et al*., 1999). Surveys showed that, with the exception of Antarctica, these nematodes are present throughout the earth (Campos-Herrera *et al.*, 2012). As a result of many surveys, numerous new species have been described, as well as numerous new isolates of previously described species. Entomopathogenic nematode research on *Steinernema carpocapsae, S. glaseri, S. feltiae*, and *Heterorhabditis bacteriophora* was started in India utilizing alien species and strains. However, the nematodes' weak capacity to adapt in a strange environment is what caused the results to be so uneven (Kaya *et al.*, 2006). Since then, surveys have been concentrated on identifying native species, leading to the recovery of a number of species and strains (Lalramliana and Yadav, 2010; Hussaini *et al.*, 2001; Kulkarni *et al.*, 2012; Bhat *et al*., 2017).

In the genera *Xenorhabdus* for steinernematids and *Photorhabdus* for heterorhabditids, these nematodes are linked to symbiotic bacteria. Once it has located an

appropriate host, the infectious juvenile stage of the nematode enters it through a natural entrance and moves into the hemocoel. Then the associated bacteria quickly grow in insect hemocoel and cause septicemia, which kills the host within 24 to 72 hours. The nematodes start the development process, then eat the bacterial cells and tissues of the host until there are no more food supplies left in the host carcass. Finally, they appear in the soil as a fresh batch of infectious juveniles (IJs) looking for fresh hosts (Hazir *et al.*, 2003). After two or three generations in the cadaver, the nematodes feed on the bacterial cells and host tissues before emerging as infectious juveniles into the soil environment. When they infect a new insect host, the infectious juveniles start the life cycle all over again (Kaya *et al.*, 2006).

The main objective of this work is to isolate native EPN from the district of Haryana, India, a biodiversity hotspot location, in addition to analyzing the occurrence and their potential use as a biological control agent moving forward.

MATERIALS AND METHODS

District surveyed: Hisar, Jind, Panipat, Sonipat, Karnal, Mewat, Palwal and Fatehabad districts of Haryana were surveyed for EPNs (Fig. 1).

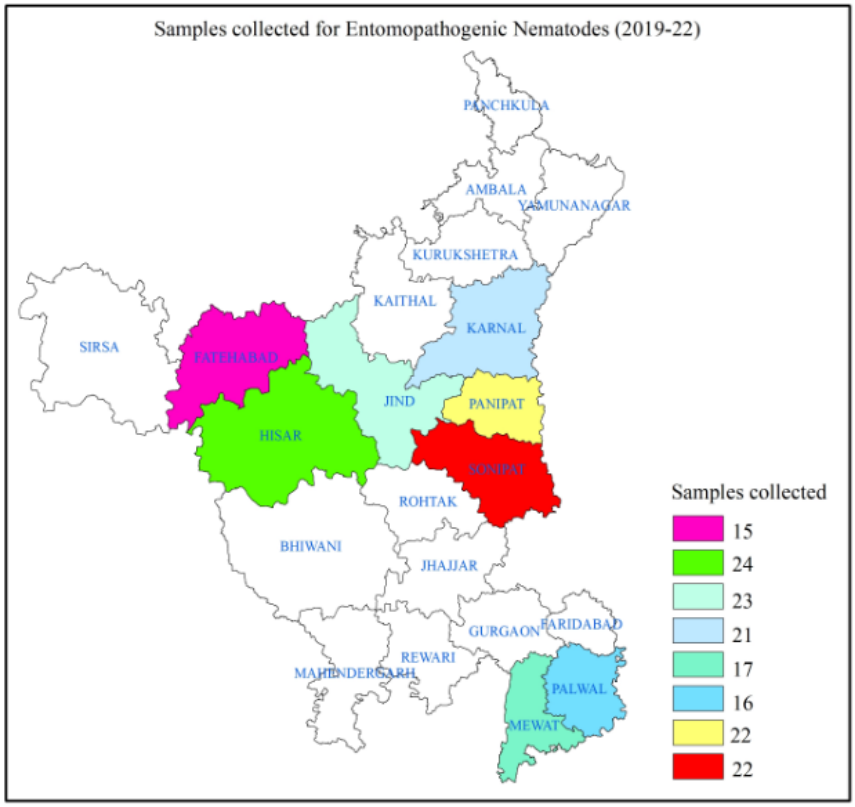

Fig. 1. Map showing the districts of Haryana from where the soil samples of EPNs were collected.

Soil sampling: From 2019 to 2022, soil samples were taken at random from a diversity of vegetable-growing regions in the districts of Haryana, India. A total of 160 samples, spanning around 8 districts, were taken from different sampling sites (Plate 1). Using a hand shovel, 5 subsamples from each site were obtained from a 20–25 cm depth and combined to obtain roughly 1 kg of composite samples (Orozco *et al.*, 2014). The appropriate elevations and geographic coordinates were noted. For further processing, all soil samples were collected in polyethylene bags to avoid dehydration, proper tagged, sealed, and placed in boxes before being transported to the laboratory, Department of Nematology, College of Agriculture, CCS Haryana Agricultural University, Hisar.

Plate 1. Taking soil samples from potato field.

Culture of insects (*Galleria mellonella*): Entomopathogenic nematodes were isolated on greater wax moth, *G. mellonella* (Woodring and Kaya 1988). In a lab conditions, the larger wax moth culture was kept alive on an artificial diet. 100 g of wheat flour, 200 g of corn flour, 100 g of wheat bran, 100 g of milk powder, 50 g of yeast extract powder, 175 ml of honey, and 175 ml of glycerin were used to make an artificial diet for *Galleria* cultivation.

In a bowl, the aforementioned components were well mixed. Then, glycerin and honey were put in a separate container and well combined with the flour mixture. Healthy *G. mellonella* larvae were removed, placed into the rearing jars' feed, and allowed to complete their life cycle and flourish (Plate 2). These containers were kept in an incubator adjusted at 25±1ºC. Later, *G. mellonella* pupae were kept separate from the feed and kept in plastic containers until they emerged as adults. The adults were taken out of the glass jar each day and placed on a piece of white card stock with folded paper strips to serve as egg-laying sites. The jars were once again incubated at 25±1ºC in a BOD incubator. The adults of *G. mellonella* were kept on cotton that had been dipped in a honey solution. Following egg laying, papers containing egg mass were kept in artificial diets until the larvae emerged. The larvae were then given adequate amounts of the food. Larvae of a consistent age were used for mass production.

Plate 2. *Galleria mellonella* culturing in artificial diet.

Insect-baiting and nematode culture: According to Bedding and Akhurst's original report (1975), insect-baiting was undertaken. Each glass bottles containing soil samples received ten *G. mellonella* larvae in their last instar, which were subsequently inserted and incubated at 25±2°C. Regular inspections were conducted on the samples to assess for successful insect infection and soil moisture. Until infective juveniles (IJs) emerged, all collected cadavers were cleaned with distilled water and placed in a White trap (White, 1927). Next, IJs were collected, cleaned, and kept in storage at 16±2°C. Re-inoculation of *G. mellonella* larvae was performed three times in order to get pure culture of the nematodes retrieved from the soil (Hoy *et al.*, 2008).

RESULTS AND DISCUSSION

The main objective of the current investigation was to identify native EPN species with excellent strains so that they may be used for successful bio-management of various insect pests in different parts of the state of Haryana. Eight districts were intended to obtain a total of 160 soil samples that represented various soil types and irrigation systems. These samples were then processed to the soil baiting technique with *G. mellonella*, a widely preferred host for isolating EPNs from soil. Out of 160 soil samples, 40 were found to have *G. mellonella* infestations with EPNs, according to the data. Out of 40 positive samples for EPNs, the eight soil samples taken from vegetable fields contained *Heterorhabditis* spp. which were identified based on the colour of the afflicted corpses, that turned brick red. Remaining 25 soil samples with positive EPNs results were determined to include *Steinernema* spp. based on the cadaver *Galleria*'s grayish-white colour (Plate 3).

According to data on the prevalence of EPNs acquired from eight districts in the state of Haryana (Table 1), the highest frequency of EPNs was found in Panipat (40.9%) followed by Hisar (37.5%) and Sonipat district (36.4%), while Fatehabad district had the lowest incidence of EPNs (20%). Mewat and Palwal districts were found to have no EPN prevalence. Following Jind, whose soil samples tested positive for EPNs in 30.4 percent of cases, was Karnal district, which had a 19.0 percent EPN presence. Six (Hisar, Jind, Panipat, Sonipat, Karnal, and Fatehabad) of the eight districts in Haryana state that were surveyed for the presence of EPNs were found to be positive for *Steinernema* spp., while five (Hisar, Jind, Panipat, Sonipat, and Fatehabad) were found to be positive for *Heterorhabditis* spp.

Out of 160 soil samples, 45 came from cucurbitaceae crops, including 29 tomato, 23 brinjal, 18 okra, 11 potato, 7 carrot, 5 capsicum, 5 chilli, 5 onion, 4 cruciferous, 4 coriander and 3 garlic crop.

Based on samples collected from different vegetable crops, the frequency of occurrence of EPNs were observed maximum in chilli (40.0%) followed by capsicum (33.3%) cucurbitaceae (31.1%), tomato (27.6%), brinjal (26.1%), cruciferous (25.0%), onion (20.0%), potato (18.2%), okra (16.7%) and carrot (14.3). Coriander and garlic crops were found with none EPNs frequency. Out of 40 samples found positive for EPNs collected from various vegetable crops, the frequency of occurrence of Steinernematid and Heterorhabditid nematodes were 80 and 20 percent, respectively (Table 2).

The aforementioned findings are consistent with those made by Uribe-Lorio *et al.* (2005). According to their findings, 20.50 percent of all soil samples tested positive for the presence of EPNs. In contrast, Barbosa-Negrisoli *et al.* (2010) and Myers *et al.* (2015) found

entomopathogenic nematodes in 15.7 percent and 21 percent, respectively, of the soil samples. The conclusions of Hussaini *et al.* (2000) are likewise supported by the current findings. They noted that Andhra Pradesh has a diverse range of EPN species. The distribution and incidence of EPNs were reported by Sunanda *et al.* (2016) in 1.38 percent of all soil samples taken from the state of Telangana. Lalramliana and Yadav (2010), Singh *et al.* (2015), and Josephrajkumar and Sivakumar (1997) have also reported the prevalence and distribution of EPNs from various sources. Gowda *et al.*, 2020 also recovered 3 soil samples containing EPNs out of 130 samples during a survey of Uttar Pradesh, India from 2016-2017 with frequency of 2.3 percent. Out of 200 soil samples collected from 40 soil sites in Thialand and found the prevalence of EPNs was 8.0 percent (Ardpairin *et al.*, 2020).

Out of 313 soil samples collected from different districts of Haryana during 2018-2021, 99 samples (31.6%) were found to be positive for the EPNs. Maximum frequencies of occurrence of EPNs was found in ber orchards (Steinernematid 65.6% and Heterorhabditid 34.4%), followed by sugarcane, wheat and cluster been, as only 71.1, 37.5, 35.7 and 35.3 percent, respectively (Kumar *et al.*, 2021).

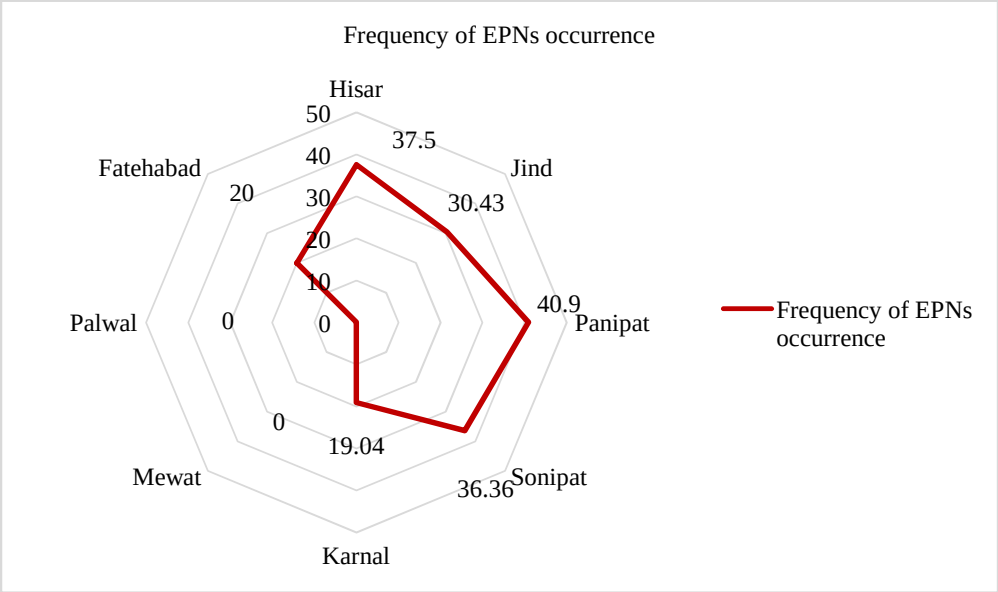

Fig. 2. Occurrence of EPNs in various districts of Haryana, India.

Table 1: District wise distribution and frequency of EPNs in Haryana, India.

Sr. No.	Districts surveyed	GPS Information	No. of samples collected	EPNs positive samples	Frequency (%)	EPNs identified
1	Hisar	N 29.151-29.190 E 75.720-75.744	24	9	37.5	*Steinernema* spp. *Heterorhabditis* spp.
2	Jind	N 29.169-29.442 E 75.705-76.413	23	7	30.4	*Steinernema* spp. *Heterorhabditis* spp.
3	Sonipat	N 28.870- 29.943 E 76.943-77.042	22	9	40.9	*Steinernema* spp. *Heterorhabditis* spp.
4	Panipat	N 29.387-29.443 E 76.968-76.985	22	8	36.4	*Steinernema* spp. *Heterorhabditis* spp.
5	Karnal	N 29.692-29.678 E 76.984-76.992	21	4	19.0	*Steinernema* spp.
6	Mewat	N 28.124-28.310 E 76.953-76.952	17	0	0	Nil
7	Palwal	N 28.120-28.139 E 77.268-77.337	16	0	0	Nil
8	Fatehabad	N 29.511-29.555 E 75.450-75.455	15	3	20.0	*Steinernema* spp. *Heterorhabditis* spp.

Plate 3. *Galleria mellonella* larvae infected with (A) *Steinernema* spp. (B) *Heterorhabditis* spp.

Table 2: Frequency of occurrence of EPNs associated with vegetable crops in Haryana, India.

Sr. No.	Crops surveyed	No. of sample collected	No. of samples positive for EPNs					
			EPNs		*Steinernema* spp.		*Heterohabditis* spp.	
			No.	%	No.	%	No.	%
1.	Cucurbitaceae	45	14	31.1	12	80	2	14.28
2.	Tomato	29	8	27.6	6	75	2	25
3.	Brinjal	23	6	26.1	5	83.33	1	16.66
4.	Okra	18	3	16.7	2	66.7	1	33.3
5.	Potato	11	2	18.2	1	50	1	50
6.	Carrot	7	1	14.3	1	100	0	0
7.	Capsicum	6	2	33.3	2	100	0	0
8.	Chilli	5	2	40.0	2	100	0	0
9.	Onion	5	1	20.0	0	0	1	100
10.	Cruciferous	4	1	25.0	1	100	0	0
11.	Coriander	4	0	0	0	0	0	0
12.	Garlic	3	0	0	0	0	0	0
Total		160	40	25.0	32	80	8	20

CONCLUSION

The safest biocontrol agents, and a potential alternative to insecticides, are entomopathogenic nematodes. EPNs can be utilised on a number of crops because of their broad host range. However, only 40 of the 160 soil samples taken during the survey from various vegetable crops in Haryana detected entomopathogenic nematodes. The survival of *Steinernema* species and *Heterorhabditis* species emphasises the significance of carrying out more thorough surveys in Haryana.

FUTURE SCOPE

By evaluating the potential of these organisms, we need to do more survey for EPNs in native locations. They are more virulent than many other biocontrol agents, so need to mass multiply and use at large scale for management of insect-pests as alternate to insecticides.

Conflict of interest. Nil

REFERENCES

Ardpairin, J., Muangpat, P., Sonpom, S., Dumidae, A., Subkrasae, C., Tandhavanant, S., Thanwisai, A. and Vitta, A. (2020). A survey of entomopathogenic nematodes and their symbiotic bacteria in agricultural areas of northern Thailand. *Journal of Helminthology, 94*.

Barbosa-Negrisoli, Carla, R. C., Mauro, S., Garcial, Claudia, D. and Aldomario, S. (2010). Survey of Entomopathogenic Nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) in Rio Grande do Sul State, Brazil. *Nematologia Brasileira Piracicaba, 34*(4): 189-197.

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

Bhat, A. H., Istkhar Chaubey, A. K., Půža, V. and San-Blas, E. (2017). First report and comparative study of *Steinernemasurkhetense* (Rhabditida: Steinernematidae) and its symbiont bacteria from subcontinental India. *Journal of Nematology,49*: 92–102.

Campos-Herrera, R., Barbercheck, M., Hoy, C. W. and Stock, S. P. (2012). Entomopathogenic nematodes as a model system for advancing the frontiers of ecology. *Journal of Nematology, 44:* 162-176.

Gowda, M. T., Patil, J., Vijayakumar R., Halder, J., Veereshkumar, Divekar, P. A., Rai, A.B. & Singh, J. (2020). Isolation, identification and biocontrol potential of entomopathogenic nematodes occurring in Purvanchal and Bundelkhand regions of Uttar Pradesh,

India. *Egyptian Journal of Biological Pest Control,30*: 95.

Hazir, S., Kaya, H. K., Stock, S. P. and Keskin, N. (2003). Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. *Turkish Journal of Biology*, *27*: 181-202.

Hoy, C. W., Grewal, P. S., Lawrence, J. L., Jagdale, G. and Acosta, N. (2008). Canonical correspondence analysis demonstrates unique soil conditions for entomopathogenic nematode species compared with other free-living nematode species. *Biological Control*, *46*: 371-379.

Hussaini, S. S., Ansari, M. A., Ahmad, W. and Subbotin, S. A. (2001). Identification of some Indian populations of *Steinernema* species (Nematoda) by RFLP analysis of ITS region of rDNA. *International Journal of Nematology,11*: 73-76.

Hussaini, S. S., Singh, S. P., Parthasarathy, R. and Shakeela, V. (2000). Storage effects on activity of native *Steinernema* and *Heterorhabditis* spp. *Indian Journal of Nematology*, *30:* 231-232.

Josephrajkumar, A. and Sivakumar, C. V. (1997). A survey for entomopathogenic nematodes in Kanyakumari district, Tamil Nadu, India. *Indian Journal of Entomology*, *59*: 45-50.

Kaya, H. K. and Stock, S. P. (1997). Techniques in insect nematology. In: Lacey LA, editor. Manual of techniques in insect pathology. San Diego: Academic Press; pp. 281–324.

Kaya, H. K., Aguillera, M. M., Alumai, A., Choo, H. Y., de la Torre, M., Fodor, A., Ganguly, S., Hazir, S., Lakatos, T., Pye, A., Wilson, M., Yamanaka, S., Yang, H. and Ehlers, R. U. (2006). Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. *Biological Control,38*: 134-155.

Kulkarni, N., Rizvi, A. N., Kumar, V., Paunikar, S. and Mishra, V. K. (2012). Morphological and molecular characterization of *Steinernemadharanaii* sp. n.: a new entomopathogenic nematode from India. *Indian Journal of Tropical Biodiversity*, *20*: 107-116.

Kumar, D., Kumari, P., Kamboj, R., Kumar, A., Banakar, P. and Kumar, V. (2022). Entomopathogenic nematodes as potential and effective biocontrol agents against cutworms, *Agrotis* spp.: present and future scenario. *Egyptian Journal of Biological Pest Control*, *32*: 42.

Kumar, V., Kumar, A., Banakar, P. and Mann, S. S. (2021). Natural occurrence and distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in Haryana. *Haryana agric. Univ. J. Res.*, *49*: 13-17.

Lalramliana and Yadav, A. K. (2010). Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Meghalaya, NE India. *Science Vision,10*(3): 89-100.

Myers, R. Y., Sipes, B. S., Matsumoto, T. K., Mello, C. L. and Mello, J. S. (2015). Occurrence and distribution of Heterorhabditid populations in the Hawaiian Islands. *Nematropica,45*: 198-207.

Orozco, R. A., Lee, M. M. and Stock, S. P. (2014). Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). *Journal of Visualized Experiments, 89*. https://doi.org/10.3791/52083.

Singh, S. P., Yadav, A., Vardhan, S. and Tripathi, C. P. M. (2015). Diversity analysis of entomopathogenic nematodes against *Helicoverpa armigera* (Hübner) from Tarai region of IGP, India. *Current Life Sciences*, *1*(1): 15-23.

Stock, S. P., Pryor, B. M. and Kaya, H. K. (1999). Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California. *Biodiversity Conservation*, *8*: 535–549.

Sunanda, B. S., Jeyakumar, P. and Vijayalakshmi, K. (2016). Diversity and distribution of entomopathogenic nematodes (EPNs) in different agro ecological habitats of Telangana State, India. *The Ecoscan.*, *9*: 67-71.

Uribe-Lorío, L., Marielos, M. and Patricia, S. S. (2005). First record of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Costa Rica. *Journal of Invertebrate Pathology*, *88*: 226-231.

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

Woodring, J. L. and Kaya, H. K. (1988). Steinernematid and heterorhabditid nematodes. A handbook of techniques. *Southern Cooperative Series Bulletin* 331.

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