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Occurrence of entomopathogenic nematodes in Osun State, Southwestern Nigeria

M. Rufai[1†], A. A.Wahab[2], Q. O. Adeshina[1], J. C. Umehokoh[1] and O. O.Ologunro[1]

[1]*Parasitology Unit, Department of Zoology, Faculty of Basic and Applied Sciences, College of Science and Engineering, P.M.B. 4494, Osun State University, Osogbo, Nigeria*
[2]*Department of Microbiology, Osun State University, Osogbo, Nigeria*

[†]Corresponding email: akinlabi.rufai@uniosun.edu.ng

Abstract

In this study the occurrence of entomopathogenic nematodes (EPNs) was determined in three senatorial districts of Osun State in relation to soil texture, vegetation, moisture and pH. A total of 110 soil samples were randomly collected from various cultivated fields in different locations. The soil samples were baited twice using last instar larvae of the *Galleria mellonella* (greater wax moth) for presence of entomopathogenic nematodes. Four EPN isolates were recovered viz., *Heterorhabditis indica*, *Heterorhabditis bacteriophora*, *Steinernema karii* and *Steinernema wesieri*. Of a total of 110 soil samples examined, EPNs were found in 90.90% soil samples, they belonged to the genera *Steinernema* and *Heterorhabditis*. *Steinernema* spp. was found prevalent in both cultivated and undisturbed soils. Frequency of occurrence of EPNs in the sampled soils from the different senatorial zones was determined: soil samples from the Central zone recorded the lowest prevalence 87.50% while the soil samples from Western senatorial zone revealed 91.43% presence of the EPNs. The highest frequency of occurrence of EPNs-94.29% was recorded in the soil samples from Eastern senatorial zone. Nematode distribution and frequency was found to be related to soil pH (pH<4.5 to pH>7.2) and soil moisture. This is the first report of these nematodes from Osun State, Nigeria.

Key words: Entomopathogenic nematodes, EPNs, Occurrence, Soil pH, Moisture contents.

Entomopathogenic nematodes (EPNs) are found in diverse habitats all over the world. EPNs provide environmentally safe insect pest control and are used as biocontrol organisms. EPNs belonging to the families Steinernematidae and Heterorhabditidae have been proven as biopesticides against insect pathogens of agricultural crops (Denno *et al*., 2008). Globally 70 species of Steinernematidae and 20 species of Heterorhabditidae have been described so far (Orozco *et al*., 2014).

EPNs have been successfully used in agriculture due to their excellent qualities *viz*., wide host range, ability to quickly kill their host, easy mass production and being environmentally safe (Gaugler, 2007).

In Nigeria reports on the occurrence and distribution of entomopathogenic nematodes are scanty. The only survey of EPNs in Nigeria was the work of Akyazi *et al*., (2012) who identified nematode isolates of the genera *Steinernema* and *Heterorhabditis* based on morphological and molecular characterization in three of the thirty-six states of Nigeria.

Present study aims at investigating local strains of EPNs and their abundance in different parts of Osun State in relation to soil texture, vegetation, the impacts of moisture content and pH.

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Materials and Methods

Soil sample collection: During the survey of three senatorial zones of Osun State in Nigeria, altogether 110 soil samples were collected. Thirty five (35) soil samples of 1kg each were collected from each senatorial district. Each soil sample (1 kg) was collected randomly at a depth of 20cm from an area of 30 $m^2$with hand shovel and kept in a polyethylene bag. The samples were labeled with a water proof marker with the site information (site name, vegetation, soil type, date of collection); soil samples were stored in a cooler equipped with ice packs to maintain standard temperature range.

Screening and extraction of EPNs: The soil samples were baited twice, using last instar larvae of the *Galleria mellonella* (greater wax moth) for the extraction of entomopathogenic nematodes (Bedding & Akhurst, 1975).

Pathogenicity of EPN isolates in the sampled soil: In 500 ml plastic containers moistened soil samples with ten last-instar *Galleria mellonella* larvae were placed; containers were closed with a lid, turned upside down and kept at room temperature. After every two to three days the containers were checked for *G. mellonella* larvae. After seven days, dead insects were collected and placed in modified White traps (Kaya & Stock, 1997).Nematodes that emerged from the dead insects were collected, rinsed thoroughly with distilled water to remove debris. Each nematode isolate was cultured on fresh *G. mellonella* larvae to produce nematodes for identification and establishment of cultures.

Morphological identification of EPNs: For morphological characterization, nematodes were preserved in 4% formalin for identification based on morphological and morphometric features of infective juvenile (J_3) and second generation adults; for light microscope observations of at least 20 IJs, males and females were examined alive (Nguyen, 2007).

Frequency of occurrence of EPNs: For assessing entomopathogenic nematode population absolute and relative frequency of entomopathogenic nematode species in the samples were calculated (Hominick, 2002).

Possible relationship between soil moisture and soil pH, and the occurrence of EPNs: The moisture content (%) was calculated from the sample weight before and after drying. The soil pH was determined by weighing out about 10g of the each soil sample into the container with 50ml of distilled water. The pH value of water above the soil in the container was then measured.

Results

Out of 110 soil samples examined for EPNs in the three senatorial districts of Osun State, 100 soil samples representing 90.90% were positive for one or more species of EPNs (Table 1). Soil from the Eastern senatorial district and vegetables had higher number of positive samples for EPNs as compared to the soils from the Western senatorial district under the cassava field. Soils from the Central senatorial district and land under cassava had the least number of positive samples for nematodes (Table 1).

Table 1. Soil samples collected from different locations in three senatorial districts of Osun State, Nigeria.

Zone	Sampling code	Total samples	+ve samples	Location coordinate
Osun East	OSE	35	33	N07.5165°E004.5286°
Osun West	OSW	35	32	N07.65536°E004.20505° Elevation; 827
Osun Central	OSC	40	35	N07.75793°E004.60625° Elevation; 1050
Total		110	100	

The soil was taken from different types of ecosystems and categorized into three groups: sand, sandy loamy and clay. Clay provided the lowest percentage of samples (78%) with EPNs, while EPNs were found in 100% of the sandy loamy soil samples.

Occurrence of entomopathogenic nematodes in relation to textural class and vegetation in the sampled soils from East, West and Central senatorial zones was determined (Table 2). The dominant species in all the soils from East, West and Central senatorial districts was *Steinernema* spp. The soil from the vegetable field had the highest number of EPNs as compared to the soil from other agro-ecosystems.

The absolute frequency of occurrence of EPNs was lowest (87.50%) in the soil from Central zone, followed by in the Western senatorial zone soil (91.43%). EPNs were present in 94.29% of the soil samples from Eastern Senatorial zone soil (Table 3).

Four isolates of EPNs namely; *Steinernema karii*, *Steinernema wesieri*, *Heterorhabditis indica* and *Heterorhabditis bacteriophora* were discovered from the sampled areas (Fig. 1). Table 4 shows that the total EPNs count per $30m^2$ of sample location was 666 EPNs in Osun Central senatorial zone soil; of which 325 (44.89%) were recorded for *Steinernema karii*, 159 (21.96%) for *Steinernema wesieri*; 95 (13.12%) for *Heterorhabditis bacteriophora* and 87 (12.02%) for *Heterorhabditis indica*. A total of 261 EPNs were counted in Osun West senatorial zone; of which 75 (26.88%) were recorded for *Steinernema karii*, 66 (23.66%) for *Steinernema wesieri*; 92 (32.98%) for *Heterorhabditis bacteriophora* and 28 (10.04%) for *Heterorhabditis indica*. Some 486 EPNs were counted in Osun East senatorial zone; of which 115 (16.69%) were *Steinernema karii*, 103 (14.95%) *Steinernema wesieri*; 145 (21.05%) *Heterorhabditis bacteriophora* and 123 (17.85%) were *Heterorhabditis indica*. *Steinernema karii* had higher occurrence of 325 (44.89%) and 75 (26.88%) in Osun Central and Osun West, respectively. The relative frequency of EPN species varied in the three senatorial districts.

Table 2. Occurrence of EPNs in relation to textural class and vegetation in the three senatorial zones of Osun state, Nigeria.

Textural class	Vegetation	Osun Central		Osun West		Osun East	
		No. of samples	+ve samples	No. of samples	+ve samples	No. of samples	+ve samples
Sandy	Vegetable	9	8	10	8	11	10
Sandy loam	Cassava	9	9	10	10	10	9
Sandy	Cassava	11	8	8	8	8	7
Clay		11	10	7	7	6	6
		40	35	35	33	35	32

Table 3. Absolute frequency of EPNs in the sampled areas.

Locations	No. of +ve samples	No. of sample collected	AF*(%)
Osun East	33	35	94.29
Osun West	32	35	91.43
Osun Central	35	40	87.50
Total	100	110	90.90

*Absolute frequency = No. of samples containing entomopathogenic nematode species /Total number of samples collected x 100

Table 4. Relative frequency of EPNs in the sampled areas.

EPN species	Osun Central	Osun West	Osun East
Steinernema karii	325 (44.89%)	75 (26.88%)	115 (16.69%)
Steinernema wesieri	159 (21.96%)	66 (23.66%)	103 (14.95%)
Heterorhabditis bacteriophora	95 (13.12%)	92 (32.98%)	145 (21.05%)
Heterorhabditis indica	87(12.02%)	28 (10.04%)	123 (17.85%)
Total	666	261	486

Relative frequency = Frequency of entomopathogenic nematode species /Sum of frequencies of all species x100

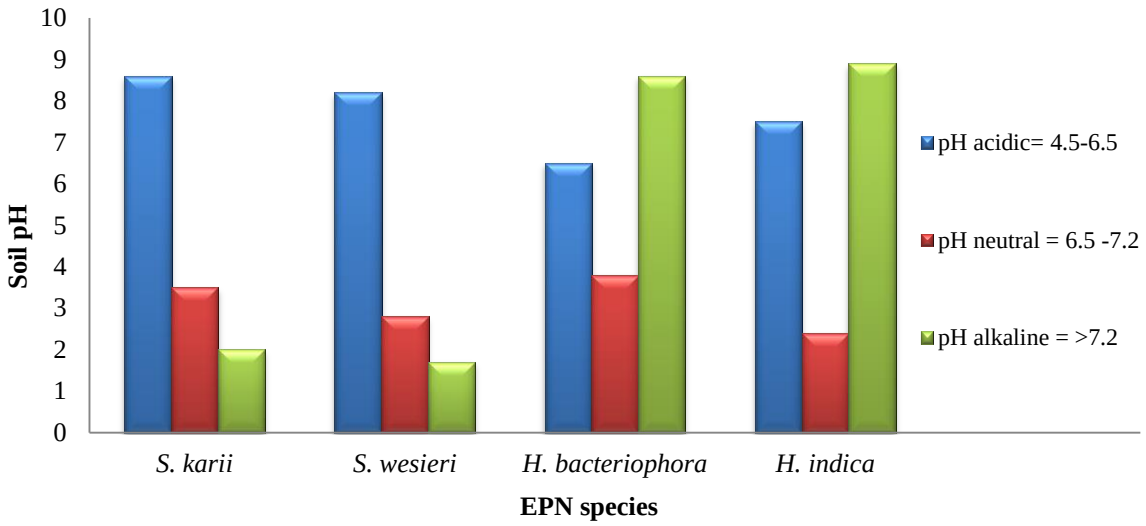

Fig. 1. Relationship of soil pH and occurrence of EPN in the sampled soil.

The research confirms a significant impact of pH and soil moisture content, on the occurrence of EPNs discovered in this study. EPNs were found in soils of varying pH levels, although individual species seemed to be thriving at a certain degree of acidity (pH<4.5). *Steinernema* spp. were dominant in acidic soil while *Heterorhabditis* spp. were found dominant in both acidic and alkaline soils (Fig. 1).

The highest EPN count 43 was found in soil with moisture content (**W_1-W_1 / W_2-W_1 x 100)** i.e., 26.6000% and pH 6.51 followed by 38 EPN count in soil with moisture content 26.9461 and pH 6.56. The EPN count was drastically low at the moisture content 36.2000% and pH 6.53. The least EPN 02 count was recorded in soil with moisture content 4.0000% and pH 7.88 (Table 5).

Determination of pathogenicity of EPNs in the sampled soil showed all the soil samples (containing EPN isolates) used for the baiting caused mortality to the *Galleria mellonella* larvae (Table 6). The EPN species caused up to 96.0, 93.3, and 92.0% mortality, respectively in the three soil samples used, within 72 h. The exposure time of the individual EPN isolates tested had significant variable ($p<0.05$) effects on mortality of the *Galleria mellonella* larvae. The number of larvae mortality increased with increased exposure time to all the EPNs.

Table 5. EPN counts in relation to moisture content and pH in Eastern senatorial zones of Osun State, Nigeria.

Soilcode	% Moisture = W_1-W_1 / W_2-W_1 x 100	pH	Nematode count
OSE001	1.1952	7.83	04
OSE002	4.0000	6.88	02
OSE003	4.1916	7.85	06
OSE006	3.6000	7.80	08
OSE007	6.4000	7.76	05
OSE009	3.8000	8.74	03
OSE010	8.6000	7.20	19
OSE011	2.2000	6.73	04
OSE016	18.000	6.62	10
OSE018	7.1856	6.62	11
OSE026	26.6000	6.51	43
OSE027	36.2000	6.53	21
OSE029	26.9461	6.56	38
OSE030	30.8000	6.54	15
OSE031	26.9460	6.55	35
OSE033	23.6000	6.52	20

Table 6. Lethal time fifty (LT_{50}) levels of the EPNs on 3rd instar larvae of *Galleria mellonella* larvae at 36, 48 and 72 hour exposure time and number of IJs.

Soil code	No. of IJs	No. of *G. mellonella*	Mortality (%) of *G. mellonella*	Mortality (%) at LT_{50} (h)
OSE026	43	7	7 (96.0)	28.1
OSE027	21	7	4 (93.0)	21.5
OSE029	38	7	6 (92.0)	20.3

The larvae of *Galleria mellonella* were found susceptible to the test at 36hr, 48hr and 72hr exposure time of EPN. The degree of susceptibility of *Galleria mellonella* larvae to EPNs infection varied according to the dose of their infective juvenile as well as the exposure period. The time taken for half the number of (LT_{50}) *G. mellonella* to die on exposure to the EPNs was significantly variable (Table 6). OSE026 recorded the highest LT_{50} (28.1 h) as compared to OSE029 which recorded the lowest LT_{50} (20.3 h). OSE026 had intermediate LT_{50} value of 21.5 h.

Discussion

The present study demonstrated the occurrence of local strains of EPNs in various soil samples across the three senatorial districts of Osun State, Nigeria. Overall, sandy loam soil samples had the largest percentage of samples with nematodes, but the greatest species diversity was found in sandy soil. Some nematode species were present in only one type of soil. For example, *H. bacteriophora* was present only in sandy soil. In all soil types, the presence of *Steinernema* spp. were recorded, though the species was most prevalent in sandy soils. This is in line with other researchers in North-west Poland who identified an association of *S. silvaticum* with sandy soils and the presence of *S. feltiae* in all of the soil types studied (Mracek *et al*., 2005). The obtained results confirm the fact that entomopathogenic nematodes are present in different types of soils.

The effect of various soil moistures and pH on the prevalence of entomopathogenic nematode was examined in the laboratory. Fluctuation in soil moisture generally seemed to have affected the

population and species of EPNs in the soil samples. A slight reduction in the moisture content caused a disproportionate decrease in the nematode population. At 3-10% moisture content, there were few EPNs; while there were practically no EPNs found in the soil with moisture content of less than 3%. Greatest number of EPNs was observed in sandy loam soil with a moisture content of 26.6000% and 26.9461%. Significantly higher ($p<0.05$) populations of EPNs were observed associated with higher moisture contents with highest and lowest values of 4.0000% and 1.1952% obtained. This increase was population-dependent and indicates that moisture may have promoted water distribution efficiency in the soil which could aid nematode survival and reproduction leading to increase in population and occurrence of different species. In this study, very high soil water contents tended to cause reduction in the EPNs count, this is in agreement with the work of Simons & Poinar (1973) who discovered that in water-saturated soil, nematode populations were reduced to about 20% after 20 weeks and there were indications that considerable reduction had occurred already after 12 weeks. This confirms the general opinion that water saturated soil is not favorable for most nematodes due to chemical properties of the soil solution as a result of microbiological activity which could overtime lead to reduction in the soil pH through soil acidification as a result of leaching from high amounts of rainfall.

It was observed that numbers of EPNs varied at different pH levels. Nematode counts were greatest at both pH 6.51 and 6.56. The least EPN count was found in soil with pH 6.88. The soil pH however did not seem to directly influence the species diversity, all the species recorded in this study were present in slightly acidic soil ($pH<6.53$) and neutral soil ($pH>7.2$). This agrees with the work of Hara *et al*., (1991) who established the presence of EPN species in a wide range of pH soil levels (from 4.6 to 8).

Determination of the pathogenicity of the EPNs in the sampled soil showed that larvae of *G. mellonella* died on exposure to the EPN isolates in the soil samples. Pathogenicity was also found in this study to be moisture dependent; this is in agreement with studies conducted by Alekseev *et al*., (2006) who recorded that the activity of infective juveniles of *S. carpocapsae* in the soil upper layer (1 cm depth) was strongly affected by the soil type, when the soil moisture was low, the number of nematodes found in the upper layer was correspondingly low. Puza & Mracek (2007) suggested that low water content in the soil may slow the migration of invasive larvae from dead insects to the environment and influence nematode density in the soil.

Conclusion

Results in this study have shown that EPNs were present and spatially distributed in Osun State. The study established the occurrence of *Steinernema* spp. and *Heterorhabditis* spp. in vegetables, cassava and maize growing farmland areas. The research has also shown that nematodes are specific to the environment and the soil type; and its physicochemical properties influence the abundance and presence of particular species of nematodes in the environment. This implies that their abundance could be a ready source of local strain EPNs for their mass production for bio-control measure against crop insect pests and diseases vectors. These local isolates may prove more effective in bio-control of important agriculture insect pests than exotic EPN isolate as insect pests of economically important crops in this region may be more susceptible to these native EPNs.

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