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Effects of Soil Chemical Characteristics on the Occurrence of Entomopathogenic Nematodes

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Authors' contributions

All the authors collaborated in carrying out this work. Authors JK, SAO designed the study and supervised field experiments while authors GA, SM, JM and GO supervised laboratory experiments. Author FK carried out the laboratory experiments, collected the data, performed data analysis and compiled the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study was conducted to determine the effect of selected soil chemical characteristics on the occurrence of entomopathogenic nematodes (EPNs) under different land uses in Embu and Taita districts in Kenya.
Study Design: The sampling points were systematically marked in a grid-mesh construction using GPS marking.
Place and Duration of Study: Soil sampling was done between January 2008 and May 2008 in Embu district in the highlands of Central and Taita-Taveta district in the Coastal

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highlands of Kenya.
Methodology: EPNs were baited from soil using *Galleria mellonella* larvae and infective juveniles identified using morphological- biometric characteristics. The nematode occurrence was evaluated through relative abundance and recovery frequency expressed as percentage from the soils.
Results: EPNs were detected in 43.3% of the samples with *Steinernema spp* being the dominant species. The occurrence of EPNs is affected by selected soil chemical properties, land use systems and heavy metals.
Conclusion: Soil fertility management practices and heavy metals influence the occurrence of EPNs and should be considered for their effective use as biological control agents.

Keywords: Steinernema; abundance; infective-juveniles; frequency; fertility.

1. INTRODUCTION

Entomopathogenic nematodes (EPNs) in the families of *Steinernematidae* and *Heterorhabditidae* are used to control insect pests primarily in the soil. These nematodes use symbiotic bacteria in the genera *Xenorhabdus* and *Photorhabdus* to kill and develop inside their hosts [1]. Once inside the host, infective juveniles penetrate the hemocoel, usually via natural openings, and release the symbiotic bacteria which kill the host usually within 24–48 h and provide essential nutrients for nematode development. The nematodes complete generally two to three generations within the host's cadaver and emerge as infective juveniles which forage for new hosts [2]. These soil nematodes have been found worldwide, except in Antarctica [3]. EPNs possess many desirable attributes such as broad host range, safety to non-target organisms and the environment, ease of mass-production and application, ability to search for pests, potential to recycle in the environment and compatibility with many agricultural chemicals [4]. EPNs are attractive alternatives to chemical insecticides and are ideally suited for integrated pest management (IPM) and organic systems particularly for soil inhabiting pests [5]. Even though EPNs are common, their efficacy has been reported to depend on soil characteristics, agricultural management practices as well as the competition with native EPNs [6,7]. Previous studies have found that agricultural management affects both soil quality variables and EPN distribution [8]. The use of pesticides, fresh manure, fungicides and chemical fertilizers has also been shown to have detrimental effects on the survival and efficacy of EPNs [7,9]. Environmental variables, soil characteristics and anthropogenic activities are also known to affect their diversity and occurrence [5]. The effects of agricultural management practices and soil pollutants have been extensively studied on other members of the nematode community [10]. However, the impact of soil physical and chemical characteristics on the occurrence on EPNs still remains unknown [11]. The aim of this study was to evaluate the influence of selected soil chemical characteristics on the occurrence of entomopathogenic nematodes in Taita and Embu districts, Kenya.

2. MATERIALS AND METHODS

2.1 Description of the Study Site

The study was conducted in Embu and Taita Taveta districts, Kenya (Fig. 1). The Embu area is located around Irangi forest in the northern part of Embu town which is in the Mount

Kenya region in the central part of the country. The site is located at longitudes 37º18' East and Latitudes 0ºS and 0º28'S and was selected because of its land use intensification gradient ranging from natural forests to intense utilization in the cultivated crop farm lands. The cultivated regions had altitude that varied between 1,200 and 2,000 metres above the mean sea level while the snowline is at 5,000 metres above the sea level. This Alfa-alpine belt occurs between 3,800 and 4,500 metres above the sea level and contains a more varied flora than the snow caped mountain tops. The lower alpine moorland occurs between 2,800 and 3,500 metres above the sea level and is characterized by high rainfall, thick humus layer and species richness. The Taita-Taveta District is situated in the South-Eastern Kenya, Coast Province lying along longitude 38º20´ and latitude 3º25 (Fig. 1). The area of covers 1000 km^2 and forms the northernmost part of the Eastern Arc Mountains which falls within the tropical moist highlands. The ecological regions in this area include mountain forest, with the highest point, at 1952 meters above the sea level. The indigenous and planted forests of Taita hills occupy the upper highland regions, mainly mountains, while agro forestry and shrubs occur on the foot slopes.

Fig. 1. The location of the Embu and Taita Taveta district, Kenya

2.2 Soil Sampling and Isolation of EPNs

The sampling points were systematically marked in a grid-mesh construction using GPS marking. Soil were collected from the 6 identified land use systems namely; coffee, maize, Napier, natural forest, planted forest, tea and vegetables from Embu and Taita districts, 20 points were randomly chosen to give a total of 120 and 100 samples from Embu and Taita sites respectively. Soil samples from the surface horizon (0-30 cm depth), most impacted by land use practices [12,13], were collected using soil auger between January-May 2008. The EPNs were isolated using the insect baiting technique [14]. Plastic boxes were filled with 500 cm^3 of each soil sample and ten last instar larvae of *Galleria mellonella* (Lepidoptera: Pyralidae) were placed on the surface of each soil box, boxes were covered with a lid and incubated at 24±1ºC and 55% relative humidity. Cadavers were recovered after 3 days, washed in tap water and individually placed on White traps to allow the emergence of infective juveniles (IJs). When EPNs were not recovered from soil, the bioassay was repeated twice using another 500 cm^3 soil samples and 10 fresh *G. mellonella* larvae to confirm the negative results [15]. Morphological identification of EPNs was done using criteria suggested by Hominick et al. [16]

2.3 Soil Chemical Characteristics

The soil properties analyzed were available plant nutrients (P, K, Na, Ca and Mg) using the Mehlich double Acid Method [17]. Total organic carbon and total nitrogen were determined using Calorimetric method [18] and Micro-Kjeldahl method [19] respectively. The micronutrients were analyzed by the EDTA method [20] while pH in 1:1 (w/v) soil by water suspension.

2.4 Data Analysis

The occurrence of Entomopathogenic nematodes and their relationship with soil chemical properties across land use systems in Embu and Taita regions were subjected analysis of variance (ANOVA) and Pearson correlation coefficients using SPSS version 16.

3. RESULTS

3.1 Distribution of EPNs and Soil Chemical and Physical Properties in Embu

Entomopathogenic nematodes were isolated from 42 (35%) and 52 (43.3%) of the samples from Embu and Taita sites respectively. Laboratory identification based on morphological analysis of IJs (infective juveniles) using standard light microscopy [21] revealed the presence of nematodes in the genus *Steinernema* in the soils from all the sites. In Embu district, the highest mean amount of EPNs of 15 (75%) was recovered in soils from coffee farm which contained elevated levels of Iron (Fe) while the least occurrence was observed in napier (Table 1).The soil pH of the soils ranged from 3.4 in natural forest to 4.2 in both maize and Napier. Acidity, which is an important measure of soil fertility status in terms of nutrient release, was found to be highest under tea and lowest in Napier. The lowest C: N (4.5) and highest (12.7) ratios were found in tea and coffee respectively. The contents of heavy metal levels (expressed as ppm) ranged between 0.2-89.4 ppm. The Mn (89.4 ppm) and Fe (21.9 ppm) content was highest in soils from natural forest and coffee respectively. The phosphorus (P) content was significantly higher (10.4 Mg/kg) in maize as compared to tea (3.4 Mg/kg). Significant differences ($P< 0.05$) were found in sodium (Na), magnesium (Mg) and potassium (K) content in soils from coffee, maize, Napier, natural forest, planted forest and tea. Soil from tea contained the highest amount of Calcium (20.9 Cmol/kg) with lowest in coffee [13 Cmol/kg] (Table 2). There was significant ($P < 0.05$) association between the occurrence of EPNs with iron while the other soil parameters did not show any relationship (Table 3).

Table 1. Mean occurrence of entomopathogenic nematodes in different agroecosystems in Embu and Taita districts, Kenya

Land use	Occurrence (%)	
	Embu	Taita
Coffee farm	75	60
Maize farm	50	50
Natural forest	35	35
Planted forest	50	45
Napier	20	-
Tea farm	30	-
Vegetable farm	-	30
Mean	43.3	40

3.2 Distribution of EPNs and Soil Chemical and Physical Properties in Taita

The highest mean occurrence of EPNs was observed in coffee (60%) while the least number was recorded in vegetables (Table 1). Planted forest had the highest level of acidity, Carbon(C), Manganese (Mn), sodium (Na) and Iron (Fe) but the lowest level of calcium (Ca), Na and Mn was found in Coffee. Coffee and vegetables had the highest C: N (9.0) ratios while natural and planted forests recorded the least ratios at 5.2 and 5.6 respectively. The C, Ca and Mg levels in all the land use systems were higher compared to the N levels (Table 4). Among the available plant nutrients, the levels of P, Ca and Na were found to be high in Napier with significant differences ($p<0.05$) in all the land use systems. Further significant variation was noted in the levels of Fe compared to Zn with the planted forest recording the highest level of Fe. There was no significant variation in the levels of K and Mg. There was significant ($P < 0.05$) negative association between the occurrence of EPNs with all soil parameters except pH, Ca, Na, K and Zn with Mg showing a positive correlation (Table 5).

Table 2. Land use influence on the soil chemical characteristics in Embu district, Kenya

Land use	Soil Properties											
	pH	acidity	C	N	Ca	Mg	Na	K	P	Mn	Zn	Fe
Coffee	3.8	2.1	3.8	0.3	13	1.8	0.4	0.2	8.4	39.1	0.5	21.9
Maize	4.2	1.1	3.3	0.4	16	2.4	0.5	0.4	10.4	40.9	0.7	3.9
Napier	4.2	0.8	3.5	0.3	11.4	2.5	0.3	0.3	9.2	33.3	0.7	4.9
Natural forest	3.4	2.3	5.6	0.6	21	3.3	0.4	0.3	5.9	89.4	0.5	0.9
Planted forest	3.9	2.1	6.4	0.8	13.1	1.9	0.2	0.2	5.1	58.9	0.2	3.6
Tea	3.5	2.9	4.5	0	20.9	1.9	0.2	0.1	3.4	52.1	0.4	0.6
P value	0.001*	0.001*	0.001*	0.001*	0.03*	0.001*	0.001*	0.002*	0.001*	0.001*	0.001*	0.001*

** Significant at P< 0.05*

Table 3. Association of soil chemical characteristics with the occurrence of entomopathogenic nematodes in Embu district, Kenya

Variables	pH	Acid	C	N	Ca	Mg	Na	K	P	Mn	Zn	Fe
Occurrence	0.206	0.092	-0.003	0.129	-0.336	-0.377	-0.003	0.159	0.268	-0.137	0.035	0.796*
P Value	0.207	0.358	0.495	0.304	0.086	0.061	0.495	0.264	0.141	0.294	0.445	0
PH		-0.664*	-0.150	0.218	-0.613*	0.133	-0.045	0.346	0.628*	-0.249	0.357	0.226
P Value		0.001	0.276	0.192	0.003	0.299	0.430	0.079	0.003	0.159	0.073	0.174
Acidity			0.607*	-0.034	0.640*	-0.224	0.037	-0.246	-0.888*	0.559*	-0.620*	-0.053
P Value			0.004	0.446	0.002	0.186	0.441	0.162	0	0.008	0.003	0.418
Carbon				0.572*	0.225	0.010	0.081	0.168	-0.712*	0.703*	-0.732*	-0.300
P Value				0.007	0.184	0.484	0.375	0.252	0	0.001	0	0.113
Nitrogen					-0.212	0.298	0.133	0.167	-0.031	0.475*	-0.392*	-0.186
P Value					0.199	0.115	0.300	0.253	0.452	0.023	0.054	0.230
Calcium						0.231	0.262	-0.261	-0.642*	0.592*	-0.129	-0.442*
P Value						0.097	0.146	0.148	0.002	0.005	0.305	0.033
Magnesium							0.402*	0.028	0.136	0.566*	0.167	-0.397*
P Value							0.049	0.456	0.295	0.007	0.254	0.051
Sodium								0.590*	-0.052	0.356	0.124	-0.151
P Value								0.005	0.418	0.073	0.312	0.275
Potassium									0.181	-0.016	0.135	0.034
P Value									0.236	0.476	0.296	0.446
Phosphorus										-0.600*	0.712*	0.402*
P Value										0.004	0	0.049
Manganese											-0.513*	-0.429*
P Value											0.015	0.038
Zinc												0.316*
P Value												0.101

**Correlation is significant at the 0.05 level.*

Table 4. Land use influence on the soil chemical characteristics in Taita Taveta district, Kenya

Land use	Soil Properties											
	pH	**acidity**	**C**	**N**	**Ca**	**Mg**	**Na**	**K**	**P**	**Mn**	**Zn**	**Fe**
Coffee farm	4.8	0.4	1.8	0.2	2.1	2.3	0.2	0.3	14.4	0.3	3.8	41.3
Vegetables	4.9	0.3	1.8	0.2	2.3	2.4	0.3	0.6	53.4	0.8	4.1	49.8
Natural forest	4	1.1	2.7	0.5	2.6	1.2	0.3	0.3	27	0.7	3.6	76.7
Maize farm	4.6	0.3	1.7	0.2	2.6	2.2	0.2	0.4	12.5	0.7	4.5	31.1
Planted forest	3.5	2	2.3	0.4	2.4	1.2	0.3	0.2	58	0.8	4.6	89.4
P value	0.001*	0.001*	0.001*	0.001*	0.02*	0.2	0.002*	0.3	0.02*	0.001*	0.001*	0.001

** Significant at P< 0.05*

Table 5. Association of soil chemical characteristics with the occurrence of entomopathogenic nematodes in Taita district, Kenya

Variables	pH	Acid	C	N	Ca	Mg	Na	K	P	Mn	Zn	Fe
Occurrence	0.365	-0.459	0.500*	-0.483	-0.029	0.412*	-0.161	-0.046	-0.439*	0.663*	0.116	0.618*
P Value	0.068	0.028	0.017	0.021	0.455	0.045	0.262	0.429	0.034	0.001	0.324	0.003
PH		-0.922*	-0.550	-0.504*	0.126	0.639*	-0.062	0.564*	-0.251	-0.380	0.131	-0.878*
P Value		0	0.009	0.017	0.310	0.002	0.403	0.007	0.157	0.060	0.302	0
Acidity			0.563*	0.473*	-0.119	-0.666*	0.111	-0.558*	0.340	0.450*	-0.121	0.938*
P Value			0.008	0.024	0.320	0.001	0.331	0.008	0.084	0.031	0.316	0
Carbon				0.920*	0.127	-.0444*	0.310	-0.495*	0.173	0.302	-0.178	0.753*
P Value				0	0.308	0.033	0.105	0.018	0.246	0.111	0.239	0
Nitrogen					0.185	-0.331	0.291	-0.378	0.252	0.355	-0.058	0.671*
P Value					0.232	0.090	0.121	0.061	0.156	0.074	0.410	0.001
Calcium						0.603*	0.051	0.500*	0.340	-0.027	0.836*	-0.107
P Value						0.004	0.420*	0.017	0.084	0.457	0	0.336
Magnesium							-0.042	0.739*	0.247	-0.430*	0.763*	-0.659*
P Value							0.434	0	0.161	0.037	0	0.001
Sodium								0.058	0.360*	0.318	0.066	0.226
P Value								0.410	0.071	0.099	0.398	0.183
Potassium									0.427*	-0.135	0.608*	-0.506*
P Value									0.039	0.297	0.004	0.016
Phosphorus										0.394*	0.587*	0.427*
P Value										0.053	0.005	0.039
Manganese											-0.029	0.486*
P Value											0.454	0.021
Zinc												-0.179
P Value												0.239

**Correlation is significant at the 0.05 level.*

4. DISCUSSION

Soil is an ecosystem with very complex relationships between EPNs and abiotic factors. Soil contains various components that are likely to influence the natural occurrence and population of EPNs. In natural ecosystems, soil type might have a greater influence on heterorhabditids than on steinernematids [15]. The isolation of *Steinernema* as the dominant species from these regions confirms previous studies that showed *Steinernema* spp to be more frequent than *Heterorhabditis* spp [15,22,23]. In field studies, organic manure used as fertilizer has either increased or decreased EPN establishment or recycling [24]. The application of organic manure has been reported to result in increased densities of native *S. feltiae*, whereas NPK fertilizer suppressed nematode densities [25]. Stuart et al.[26] concluded that inorganic fertilizers are likely to be compatible with EPNs in tank mixes and should not reduce the effectiveness of EPNs applied for short-term control as biological insecticides, but might interfere with the use of EPNs as inoculative agents for long-term control of insect pests. The soil pH alone does have a strong effect on the EPN activity however at pH values above 10, the nematode activity declines rapidly [27]. In our study, the soil pH ranged from 3.5 to 4.9, this is considered favorable for the survival of EPNs.

The low occurrence of EPNs in the vegetable farms may be due to a very high level of soil management intensity as well as frequent application of pesticides. The high level of management in the vegetable is aimed at controlling insect pests which would otherwise serve as hosts to EPNs. The use of insecticides has also been reported to have direct toxic effects on nematodes [28]. Fungicides and herbicides are poorly tolerated by EPNs and have toxic effects on their survival and virulence [29]. The highest values of C and N in natural and planted forests could be due to poor transformation and mineralization of organic matter that favour occurrence of EPNs. Napier, tea and vegetable farms are associated with increased microbial activity resulting from soil management practices such as frequent tilling enhance mineralization of the organic matter and this could reduce the occurrence the nematodes. Tea farms in Embu had the lowest P and K while in Taita maize and planted forest had the lowest P and K respectively, which could be related to the fertility management practices such as the application of fertilizers. The negative correlation of EPNs with acidity, C, N, P and Mn observed in Taita district could suggest that soils with increased levels of these elements may lead to reduced occurrence of EPNs. However previous laboratory studies have shown that Mn slightly stimulated the reproduction of *S. feltiae* [30]. Positive correlation between Magnesium and EPNs confirms previous laboratory studies on *Heterorhabditis bacteriophora* [31], that showed both manganese and magnesium to neutralize negative effect of lead on *S. feltiae* mortality [30]. In the two sites, Sodium (Na) did not have any impact on the nematode population but has been reported to kill developing nematodes leaving behind infective juveniles [32]. Amounts of metal ions such as Iron (Fe^{2+}) have been reported to have a weak vitalizing effect on the infectivity of the nematodes with respect to wax moth caterpillars, *Galleria mellonella* [33], however Barrios et al. [34] showed that metals such as Cr, Fe, Mo, Ni, Va, Cd, Zn and Li negatively affect virulence and infectivity of EPNs. EPN survival and activity has showed that high levels some heavy metals negatively affect virulence and infectivity of EPNs [10,30]. Field soil samples from the Taita showed that some soil chemical properties as such as acidity, C, N, Mg, P, Mn and Fe had a negative effect on the EPN occurrence. The negative correlations in Taita could be due to factors such as altitude, soil texture and rainfall pattern. Therefore continued exposure to these soil properties may lead to drastic reduction of natural EPN population.

5. CONCLUSION

The results of this study suggest that many factors interact to determine the occurrence of EPNs. Soil parameters such as heavy metals, plant nutrients and land use systems may help predict the natural occurrence of EPNs in varying land uses. The study shows that soil fertility management practices should be taken into account for effective use of EPNs as effective biological control agents.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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