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IMPACT OF LAND USE ON THE DISTRIBUTION AND DIVERSITY OF ENTOMOPATHOGENIC NEMATODES IN EMBU AND TAITA DISTRICTS, KENYA

[IMPACTO DEL USO DEL SUELO SOBRE LA DISTRIBUCIÓN Y DIVERSIDAD DE NEMATODOS ENTOMOPATOGÉNICOS EN LOS DISTRITOS DE EMBU Y TAITA, KENIA]

J. Fanuel Kawaka[1], John W. Kimenju[3], George Ayodo[1], Shelmith W. Mwaniki[2], John O. Muoma[1], Sheila A. Okoth[3*] and George O. Orinda[1]

[1]Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya. Emails: fkjairo@hotmail.com; gayodo@hotmail.com; jmuoma@gmail.com; rudevsol@yahoo.com.
[2]National Agricultural Research Laboratories, Kenya Agricultural Research Institute, P.O. Box 14733-00800, Nairobi, Kenya. Emails : smwaniki@yahoo.com
[3]Department of Plant Science and Crop Protection, University Of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya. Emails: wkimenju@yahoo.com, dorisokoth@yahoo.com.
**Corresponding author*

SUMMARY

Natural entomopathogenic nematodes (EPNs) are considered as potential biological control agents against soil-borne insect pests. This study was conducted to determine the impact of land use on the distribution, occurrence and diversity of entomopathogenic nematode community. Isolation of EPNs was done using the baiting technique and application of morphological identification methods revealed presence of the genus *Steinernema*. Land use intensification negatively affected the occurrence and recovery frequency in soils of Embu and Taita districts. The occurrence of EPNs was high in soils from coffee than maize and beans which had more nematodes than planted forest and napier grass followed by natural forest and tea respectively. PCR-RFLP of the internal transcribed spacer region on the ribosomal(r) DNA of the EPN isolates and digestion of the products by *Alu I* enzyme showed molecular variations among the isolates. The study has demonstrated that the frequency of occurrence and species variation of EPNs is different in various land uses.

Key words: Entomopathogenic nematodes; land use intensification; occurrence; diversity; PCR RFLP technique.

INTRODUCTION

Entomopathogenic nematodes are widely distributed in soils throughout the world (Adams *et al*., 2006; Hominick, 2002). The nematodes are considered good alternative to insect pest control due to their high reproductive potential coupled with mutualistic existence with vertebrates and plants in soils(Burnell and Stock, 2000; Gaugler, 2002). Application of non-native Entomopathogenic nematodes as biocontrol agents is used worldwide but their efficacy, reproductive potential, virulence and survival may be influenced by environmental factors. Introduction of nematode strains in new ecosystems may have negative effects on non-target organisms and partially or completely displace endemic EPNs (Lynch and Thomas, 2000).

In order to increase the efficacy of biological control products and reduce the environmental risks, knowledge on the impact of land use on the effectiveness of EPNs strains. Several studies have been conducted to determine the habitat preferences and distribution of EPNs in temperate areas of Europe: Austria (Hozzank *et al.,* 2003), Belgium (Midituri *et al.,* 1997). However, there is limited information available about EPNs in Africa (Waturu *et al.,* 1997). The current study was therefore undertaken to establish the effects of land use and agro-ecosystem management on the distribution and diversity of entomopathogenic nematodes.

METHODOLOGY

The study was conducted in two benchmark sites namely Embu district in the highlands of Central and Taita-Taveta district in the Coastal highlands of Kenya. Embu site covers Irangi forest area and the adjacent farmlands. It is also recognized as a mega-biodiversity site and the adjacent farmlands with a *Humic Nitisoils* (Kimenju *et al*., 2005). Taita site covers Ngangao forest area and the adjacent farmlands in Wundanyi division. This is one of the 25 globally recognized biodiversity hotspots with a *Humic Cambisols* (Kimenju *et al*., 2005). Soil samples were

taken from pre-determined sampling points under natural forests, planted forest, napier, coffee, tea, vegetable and maize and beans. Soil samples were augered from a depth of 5-30cm from three points making a triangular grid in each sampling point (Nyasani *et al*., 2008). A total of 20 sub-soils were taken and placed in polythene sheet where were mixed and then a sample was taken and placed in a polythene bag before transporting to the laboratory.

Nematode isolation: EPNs were isolated using the insect baiting technique and soil samples were incubated at room temperature for 24 hrs (Bedding and Akhurst, 1975). Ten last instars larvae of *Galleria mellonella* (Lepidoptera: Pyralidae) were placed on the surface of each soil sample, plastic boxes measuring 8 x 8 x 2 cm^3 were covered with a lid, and incubated at 24 ±1°C and 55% relative humidity. Cadavers were recovered after three days, washed in tap water then placed on White traps to allow the emergence of infective juveniles (IJs) (Raquel Campos-Herrera *et al.,* 2007). All analyses were based on the relative occurrence and abundance of EPNs. Analysis of variance (ANOVA) was carried out on the data sets.

DNA extraction and PCR amplifications: Total genomic DNA was extracted from ten entomopathogenic nematodes each (mainly juveniles and adults) isolated from natural forest, planted forest, napier, tea, coffee, vegetables and maize and beans land uses using the DNeasy Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions(Susurluk *et al*.,2003). DNA quantification and concentration was estimated using direct comparison with known DNA standards in per cent agarose minigel containing ethidium bromide (0.5µg/ml) buffered in 1X TBE(89Mm boric acid and 0.5M EDTA) in horizontal electrophoresis apparatus. A 5µl DNA was mixed with loading dye (0.25% bromophenol blue and 40% w/v sucrose), and 2µl of each of the series of standard DNA solutions were each mixed with 0.4µl of the gel loading dye and loaded into individual wells of the agarose gel. Electrophoresis was carried out at 100 volts for 1 hour until the bromophenol blue had migrated approximately 1-2cm. The intensity of the fluorescence of the unknown DNA was compared and estimated with that of the known standards. PCR amplifications of the extracted DNA were carried out for each strain using PuReTaq Ready-To-Go PCR beads in a standard 25 µl reaction volume. The reaction was set up by adding 0.25µl of the 18 S forward, 0.25µl of 26 S reverse (Vrain *et al., 1992*), 18.9µl of double distilled water and 5µl of the purified DNA. The amplifications were conducted as follows; 1 cycle at 94 °C for 2 minutes followed by 40 cycles at 94 °C for 30 seconds, 45 °C for 60 seconds, and 72 °C for 90 seconds. The last step was 72 °C for 5 minutes as suggested by Hominick *et al*., (1997). Agarose gel electrophoresis (180 V for 35 min) was carried out at the end of PCR amplifications using standard protocols (Reid *et al.,* 1998). *Alu I* enzyme obtained from Amersham Biosciences, Freiburg, Germany was used for the digestion of the PCR products of the ITS region. The amplified rDNA products and restriction patterns were separated by electrophoresis in a two per cent agarose gel containing ethidium bromide (0.2%) in 0.5xTBE (54.0 g Tris base + 27.5 g boric acid + 20 ml 0.5 M EDTA) at 100 volt for 1.5 h

RESULTS

Morphological identification revealed that the dominant species in the soils were *Steinernema spp*. In Embu, soils collected from the coffee and maize/beans intercropping plots contained the highest number of samples with nematodes compared to the soils from tea farms and napier grass. Samples from planted forest had higher EPNs than natural forests. Similarly, in Taita, the highest number of EPNs was recorded in soils from maize/bean, planted forest and the napier grass where significant differences (*P=0.001*) between the land uses in abundance was recorded.

According to the RFLP profiles generated, all the isolates generated 3 fragments except those from coffee farm with two fragments. The fragment sizes ranged between 190bp and 330bp with the largest generated by isolates from tea while the smallest fragment was from planted forest (table 3). In Taita, the largest fragment size of 490bp was generated by all the isolates while the least fragment of 150bp was generated by isolates from napier grass and coffee soils.

Table 1: Occurrence and abundance of *Steinernema* spp in different agroecosystems in Embu district, Kenya

Land use	Occurrence (%)	Abundance	Mean	95% Confidence interval for mean	
				Lower bound	Upper bound
Natural forest	35.0	2175	293.14	54.41	531.88
Planted forest	50.0	2121	566.10	367.55	764.65
Napier	20.0	6450	471.50	101.06	841.94
Tea	30.0	2155	1885.80	-42.82	3814.42
Coffee	75.0	385500	481.50	177.74	785.26
Maize/beans	50.0	8400	705.00	312.80	1097.20
Mean	43.3	67783.5			

Table 2: Occurrence and abundance of *Steinernema* spp in different agroecosystems in Taita district, Kenya

Land use	Occurrence (%)	Abundance*	Mean	95% Confidence interval for mean	
				Lower bound	Upper bound
Natural forest	35.0	15547	544.43	245.78	843.08
Planted forest	45.0	9400	1159.11	775.25	1542.97
Grassland	40.0	12400	615.25	390.85	839.65
Vegetables	30.0	13200	728.00	548.62	907.38
Maize/beans	50.0	8160	1890.50	812.75	978.00
Mean	40.0	11741.4			
LSD	5.22				

* P=0.001, highly significant

Table 3: Estimated fragment sizes (bp) of ITS region of the rDNA of 10 *Steinernema* isolates from Embu and Taita districts, Kenya

Location	Land use	Isolated designate	Fragment size
	Natural forest	NF-1	200, 290 and 310
	Planted forest	PF-2	190, 240 and 300
	Napier grass	NP-3	210, 280 and 300
	Tea	TF-4	190, 220 and 330
	Coffee	CF-5	210 and 330
Taita district	Natural forest	NF-7	150, 200 and 490
	Planted forest	PF-8	190, 210 and 490
	Napier grass	GL-9	150, 190, 250 and 490
	Vegetables	VG-10	150, 190, 250 and 490
	Maize and bean intercrop MB-11		190, 210, 370 and 490

DISCUSSION

Results obtained from the soils from Embu and Taita districts showed that the entomopathogenic nematodes were distributed. The observation confirmed previous studies that have shown *Steinernema spp* to be more frequent than *Heterorhabditis spp* (Hominick, 2002). Disturbance of the natural habitats through felling of indigenous trees, followed by establishment of single species plantations have been noted to cause a decline of nematode abundance and species richness (Bloemers *et al.,* 1997). Moderate occurrence of EPNs in natural forest soils could be due to the presence of high proportion of clay, which has poor aeration and retains a lot of water thus affecting their mobility and survival. Most EPN strains used in biological control have been isolated from sandy soils (Mra´cˇek *et al*., 2005).

The presence of high organic matter in forest soils which helps the proliferation of other microbes which may be antagonistic to EPNs (Nyasani *et al.,* 2008). However, exceptions have been reported on the effect of clay soil content increasing the population, efficacy and survival of EPNs (Shapiro *et al.,* 2000). Other biotic and abiotic soil factors have also been reported to influence distribution and occurrence of EPNs (Bednarek, 1998). Natural forests, planted forests and napier grass have large insect diversity that is hardly controlled by natural enemies than agricultural areas (Kimenju *et al.,* 2005).

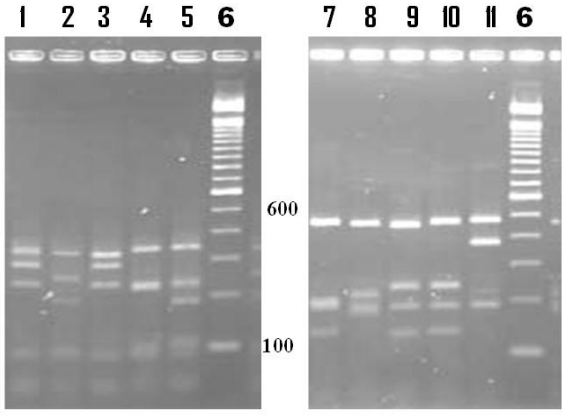

Figure 1: Banding patterns of the PCR-amplified products of the ITS region of *Steinernema* isolates from Embu (natural forest-1, planted forest-2, napier-3, coffee -4 and tea -5) and Taita (natural forest-7, planted forest-8, napier-9, vegetables-10 and maize and beans -11) digested by *Alu I* restriction enzyme. Lanes "6" correspond to the 100bp DNA marker

The presence of single vegetation in napier grass over many seasons is usually characterized with fewer pests and this could be responsible for reduced ability to sustain the EPNs (Mwaniki *et al*., 2008). The high occurrence of EPNs in coffee farms could be attributed to minimum soil disturbance in terms of tillage and low use of inorganic fertilizers and pesticides (Campos-Herrera *et al.,* 2008). The dense canopy in coffee farms provides little fluctuations of temperature and moisture, which favour EPN survival (Nyasani *et al*., 2008). In vegetable and maize/bean intercrops, the occurrence of EPNs is affected by frequent cultivation which exposes them to desiccation and direct lethal rays from the sun (Shapiro *et al*., 1999). Besides, the use of pesticides, fresh manure, fungicides and chemical fertilizers may have detrimental effects on the survival and efficacy of EPNs (Lawrence et al., 2006). These intensive agricultural practices are unsuitable for the insects and therefore may interfere with host-parasite relationship (Caroli *et al.,* 1996). In the intercrops, different crops are associated with different pests which could be used by nematodes for survival (Mwaniki *et al*., 2008). Generation of similar fragments in the isolates from Taita Taveta and Embu districts may suggest that there are species adapted to the same level of soil disturbance and land use. The unique bands generated by all the isolates revealed molecular variations within the genus *Steinernema*.

CONCLUSIONS

The study has demonstrated that various land uses affect the distribution, occurrence and diversity of EPNs. However, further surveys should focus on different agro ecological zones and seasons to ascertain these findings. There is also need to use more restriction enzymes to confirm the molecular variations observed during the study. .

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