Tropical and

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Agroecosystems

INFLUENCE OF LAND USE AND SOIL MANAGEMENT PRACTICES ON THE OCCURRENCE OF NEMATODE DESTROYING FUNGI IN TAITA TAVETA, KENYA

[INFLUENCIA DE LAS PRÁCTICAS DE USO Y MANEJO DE SUELO SOBRE LA OCURRENCIA DE HONGOS NEMATOFAGOS EN TAITA TAVETA, KENYA]

Peter M. Wachira[1*] Sheila Okoth[1], John Kimenju[1] and Richard K. Mibey[2]

[1] *University of Nairobi P.O. Box 30197 00100 Nairobi, Kenya*
E-mails: wachirapm@yahoo.com, pwachira@uonbi.ac.ke, dorisokoth@yahoo.com,
wkimenju@yahoo.com
[2] *Moi University P.O. Box 3900 - 30100 Eldoret, Kenya; E-mail: rmibey@mu.ac.ke*
*Corresponding author

SUMMARY

Due to the increased concerns about the effect of agro-chemicals on soil health and soil biodiversity, use of biological methods has become most acceptable alternative methods for farmers to control soil pathogens during crop production. A study was therefore undertaken to determine the occurrence of nematode destroying fungi in Taita Taveta with the aim of isolating and characterizing them for biological control of plant parasitic nematodes. Twenty eight fungal isolates, distributed in three genera, were identified as nematode destroying fungi from all the positive soil samples. Out of the isolates that were identified, 71, 25 and 4 % were in the genera *Arthrobotrys, Monacrosporium* and *Nematoctonus* respectively. *Arthrobotrys oligospora* had an occurrence frequency of 42.9% which was the highest followed by *A. dactyloides, M.cionopagum, Monacrosporium* sp and *Nematoctonus* sp with frequencies of 28.6, 17.9 and 7.1and 3.6% respectively. The occurrence of nematode destroying fungi was affected by land use and organic inputs (P ≤ 0.05) while it was not affected by crop rotation (P ≥ 0.05). Napier land use was more diverse than the other land uses with a mean shannon diversity index of 0.717 followed by horticulture (index 0.497). Maize /bean, coffee/beans, fallow and shrub land uses had a mean shannon index of 0. The same trend was observed on richness where napier had a mean richness of 2.2, horticulture 1.8, maize bean 1 while shrub, fallow and coffee/ beans all had mean richness of 0.2. *A.oligospora* was the most frequently isolated fungi (42.9 %) and showed high potential in biocontrol of plant-parasitic nematodes and was recommended for further studies and development as a biological control agent.

Key words: Plant parasitic nematodes; land use; biological control.

RESUMEN

Debido a la preocupación creciente sobre el efecto de los agroquímicos en la salud del suelo y su biodiversidad, el empleo de métodos de control biológicos para el control de patógenos del suelo es ahora una alternativa aceptable para los agricultores. El estudio se realizó para determinar la presencia de hongos nematofagos en Taita Taveta con el objetivo de aislarlos y caracterizar su potencial para el control biológico de nematodos parásitos. Se obtuvieron 28 aislados fungales, distribuidos en 3 generos, los cuales fueron identificados como nematofagos. De los cultivos identificados, 71, 25 y 4% fueron de los generos *Arthrobotrys, Monacrosporium y Nematoctonus* respectivamente. *Arthrobotrys oligospora* tuvo la mayor incidencia con 42.9%, seguido de *A. dactyloides*, *M. cionopagum, Monacrosporium* sp y *Nematoctonus* sp (28.6, 17.9, 7.1 y 3.6% respectivamente). La presencia de los hongos nematofagos fue influenciada por el uso del suelo y los productos orgánicos (P<0.05) pero no por la rotación de cultivo (P>0.05). El suelo empleado para Napier fue más diverso con un media de diversidad de shannon de 0.717, seguido por el suelo hortícola (index 0.497). El suelo empleado para maíz/fríjol, café/fríjol, y las tierras en descanso y con herbaceas tuvieron un índice de 0. La misma tendencia fue observa para la riqueza, donde el suelo de Napier tuvo una riqueza de 2.2, hortícola (1.8), maíz/fríjol (1) y herbacea, en descanso, y café/fríjol (0.2). *A. oligospora* fue el aislado más frequente (42.9%) y mostro el mayor potencial para el control biológico de nematodos parasitos de las plantas. Se recomiendan estudios para evaluar su empleo como agente de control biológico.

Palabras clave: Nematodos parásitos; uso del suelo; control biológico.

INTRODUCTION

Nematodes are microscopic multicellular roundworms that inhabit marine, freshwater and terrestrial environments. Some are beneficial soil microorganisms that play an important role in essential soil processes while others cause plant diseases (Dufour *et al.*, 2003). The plant parasitic nematodes infect the root tissues of the plant causing root galls that lead to reduced water and mineral uptake in the plant root system. They have been reported to cause up to 50% and 60% yield loss in both maize and common beans respectively in heavily infested fields in Kenya. They are also associated with huge crop loss in tomato for smallholder growers in Kenya (Kimenju *et al.*, 1998, Oruko and Ndungu, 2001). In Taita Taveta, which is the study site, horticulture is the main source of livelihood accounting for 95% of household income (Spoerry, 2006). However, Kimenju *et al.*, (2005), reported high populations of plant parasitic nematodes in horticulture farms in this area, while Spoerry (2006) reported that nematodes are the major soil pests identified by the farmers that cause low vegetable production. These soil pest problems together with the poor degraded soils found in the area has led to heavy use of agro chemicals in the farms. The negative effects of this land intensification on soil health has however been recorded over the years in the study area (Pellikka *et al.*, 2004) and include decrease in useful organisms in the soil and soil erosion (Sirvio *et al.*, 2004). In agreement with these reports, the District Development Strategies 2002-2006 for Taita, crop production has been identified as an area of focus while high chemical costs, soil degradation, inadequate technical know how and low soil fertility are identified as factors contributing to low farm production.

Soil beneficial microorganisms could be used to reduce the effect of some soil pests and therefore reducing the application of chemicals to the soil. Nematode destroying fungi are such beneficial microorganism that can be used to control plant parasitic nematodes. They are micro fungi that are natural enemies of nematodes. They capture, kill and digest nematodes (Rodrigues *et al.*, 2001, Nordbring-Hertz *et al.*, 2002). They comprise three main groups of fungi, the nematode trapping and the endoparasitic fungi that attack vermiform living nematodes by using specialized structures, and the egg-and cyst-parasitic fungi that attack these stages with their hyphal tips (Masoomeh, *et al.*, 2004, Nordbring-Hertz *et al.*, 2002). After trapping there is the penetration of the nematode cuticle, invasion and then digestion. This group of fungi has drawn much attention due to their potential as biological control agents of nematodes that parasitize plants or animals (Jansson and Persson, 2000, Sanyal, 2000, Masoomeh, *et al.*, 2004).

The objective of this study therefore was to investigate how the land use types and farm inputs in Taita Taveta affect the occurrence of nematode destroying fungi with the aim of developing them as biological control agents of plant parasitic nematodes.

MATERIAL AND METHODS

Study site and selection of sampling points

The Taita Hills are located in Southeastern Kenya, 25km west of Voi town in the Taita-Taveta District, at approximately 03 degrees -20ºS, 38 degrees -15ºS. It covers an area of 16,965. The forests have been recognized as one of the 25 biodiversity 'hotspots' in the world (Rogo and Oguge, 2000, Mittermeier *et al.*, 2005). Taita Hill forests hold a unique biodiversity with 13 taxa of plants and nine endemic animal species (Bytebier, 2001). Currently the forest area is under serious threat from fragmentation through agricultural activities leading to loss of biodiversity (Githiru and Lens, 2007). Hence there is urgent need for documenting the belowground biodiversity.

The study was conducted along the valley bottoms of Werugha and the Ngangao forest. The valleys are rich in vegetable cultivations. The soils are composed of a high-humic A-horizon overlaying a pinkish acid sandy loam. Two rain seasons are experienced; (i) Long rain season- March to May, (ii) Short rains - November to December. The crops planted are maize, beans, sweet potatoes, cassava, arrowroots, bananas, fruit trees and horticulture crops like tomato, kale, cabbage and lettuce, that are limited to valley bottoms. Horticulture is the main income generating activity in the area and supplies the coast province of Kenya with vegetables.

The study area was stratified into six strata based on the land uses identified. Sixty (60) points, 200m apart were randomly chosen using GPS mappings. These points fell within the six land use systems; Coffee farms (*Coffea arabica*), Maize based farming (Zea *mays*), Fallow land mainly *Lantana camara* (Verbenaceae), Napier farms (*Pennisetum purpureum*) and Horticulture mainly cabbage and kales (*Brassica oleraceae*) and tomatoes *(Solanum lycopersicum)*. The sixty points were distributed unequally in the land uses. In order to have equal representation, equal numbers of replicates (5) were picked randomly from each land use. Therefore from those sixty points, thirty (30) sampling points were selected with each land use type being represented by five (5) sampling points.

Soil Sampling and laboratory analysis

At each sampling point, two vertically crossing lines and two concentric circles of radius 3 and 6m were drawn. An auger (7cm diameter) was used to take four

cores of soil from the 0-20 cm depth in the small circle and eight in the outer circle (Fig. 1). The 12 sub-samples were homogeneously mixed to constitute a composite sample from which 500g soil was taken, placed in a plastic bag, and double sealed and then kept under shade. The soil auger was sterilized with ethanol between sampling points to avoid cross contamination. The soil samples were transported to the laboratory where they were kept at room temperature before isolation of nematophagous fungi. Information on soil fertility management at the sampling points was obtained using questionnaire, observation and interviews. The following attribute data was collected: land use, organic inputs used, inorganic input (fertilizer) and crop rotation.
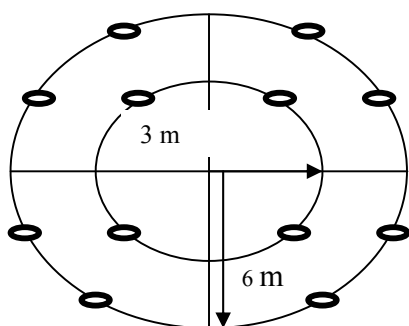

Figure 1: Schematic representation of the 12 soil sampling points

Isolation of nematode destroying fungi was done using the soil sprinkle technique (Jaffee *et al.,* 1996) on tap water agar amended with 0.1 g/l of streptomycin sulfate to reduce bacterial growth. Approximately 1 g of soil sample from each of the sampling point was sprinkled on the surface of the water agar media in a Petri dish. A suspension of 500 -1000 juveniles of *Meloidogyne incognita* was added as bait and the plates kept at room temperature. All samples were replicated five times. The inoculated and incubated plates were examined daily for 6 weeks under a dissecting Leica, microscope at x 40 magnification. Identification slides were prepared by staining the isolates with lacto-phenol blue on a microscope slide and observed under Olympus compound microscope at a magnification of x 40. Photographs of the slides were taken for identification of the fungus. The various genera were differentiated from each other

through the conidia, trapping structure and conidiophores characteristics. Identification of the fungi was done using the key described by Cooke and Godfrey (1964).

In-vitro study

Obtained pure culture of each fungi species was later inoculated in another tap water agar without the streptomycin but with nematode suspension to determine their predatory activity on the nematodes.

Statistical Analysis

Generalized linear models were fitted to test the effect of land use on the occurrence of nematode destroying fungi since the data were found to be over dispersed. These effects were analysed through ANOVA while their diversity was explored using Richness and the Shannon diversity index from R Statistical software Version 2.1.1 (Kindt and Coe, 2005)

RESULTS

Land use and soil fertility management practices

There are six major land use types in the study area; maize beans system that produces food for consumption, coffee bushes which are sometimes intercropped with beans or with maize, napier, shrub land, fallow and horticulture Soil fertility practices varied with land use. Cow manure was recorded in horticulture, maize bean and napier only. It was recorded in 50 % of all the collected samples. Fertilizers are used in vegetable and maize farms while pesticides that target soil pests are applied only on vegetable farms. Chicken manure was reported in only two sampling points from horticulture. Crop rotation is practiced only in horticulture production and food crop farms (maize and beans) (Table 1). Thirty eight percent of the total fungal isolates were isolated from samples with cow manure only. Soil samples from farms with chicken manure had at least two different fungal isolates recovered and they formed ten percent of all the recovered isolates.

Organic inputs (cow manure and chicken manure) significantly affected the occurrence of nematode destroying fungi in the study area (P ≤ 0.05). Inorganic inputs (chemical fertilizers and pesticides) did not show any effect on the occurrence of nematode destroying fungi (P ≥ 0.05).

Table 1: Land use types and farm inputs

| Land use | Farm No. | Organic inputs | | Inorganic inputs | | Cropping system |
		Cow manure	Chicken manure	Fertilizers	Pesticide	Crop rotation
Horticulture	1, 2 & 5	Applied	Not applied	Applied	Applied	Practiced
	3 & 4	Applied	Applied	Applied	Applied	Practiced
Maize/bean	1	Applied	Not applied	Not applied	Not applied	Practiced
	2	Applied	Not applied	Applied	Not applied	Practiced
	3	Applied	Not applied	Not applied	Not applied	Not practiced
	4 & 5	Applied	Not applied	Not applied	Not applied	Practiced
Napier	All 5 farms	Applied	Not applied	Not applied	Not applied	Not practiced
Coffee/bean	All 5 farms	Not applied	Not applied	Not applied	Not applied	Not practiced
Shrub land	All 5 farms	Not applied	Not applied	Not applied	Not applied	Not practiced
Fallow	All 5 farms	Not applied	Not applied	Not applied	Not applied	Not practiced

Effect of Land use on nematode destroying fungi

Land use significantly affected the occurrence of nematode destroying fungi (P $\leq$ 0.05). The land use explained 63.73% of the observed absence or presence of nematode destroying fungi in the study area (P $\leq$ 0.05). All the sampled land uses were positive of nematode destroying fungi with some land use having one genus while others had more than one genera. These isolates were obtained from sixty percent of the plated soil samples while forty percent of the samples either had contaminants or did not have nematode destroying fungi. All the isolates of nematode trapping fungi were twenty eight in number and after identification they were grouped into three genera, *Arthrobotrys, Monacrosporium* and *Nematoctonus*. The genus *Arthrobotrys* was reported in all the land uses except in fallow. The genus *Monacrosporium* was reported in four land uses; vegetables maize, napier, and fallow while *Nematoctonus* occurred only in maize bean (Table 2). *A. oligospora, A.dactyloides, M.cionapoagum, Monacrosporium* sp and

Nematoctononus sp occurred in frequencies of 42.9, 28.6, 17.9, 7.1 and 3.6% respectively. Napier land use was the richest and most diverse land use followed by horticulture, maize bean, coffee, fallow and shrub land in that order (Table. 3). The species cumulative curve indicates that if the number of samples was increased, there is a possibility of isolating other nematode destroying fungi from the study site (Fig. 2).

Table 2. Effect of land use, inorganic inputs, organic inputs and crop rotation on occurrence of Nematode destroying fungi.

Source of variation	% Deviance explained	P- value
Land use	63.73	0.0003152
Inorganic inputs	13.47	0.1298
Organic inputs	59.32	2.123×10^{-05}
Crop rotation	9.5	0.1005

Table 3: Nematode destroying fungi in different land use types.

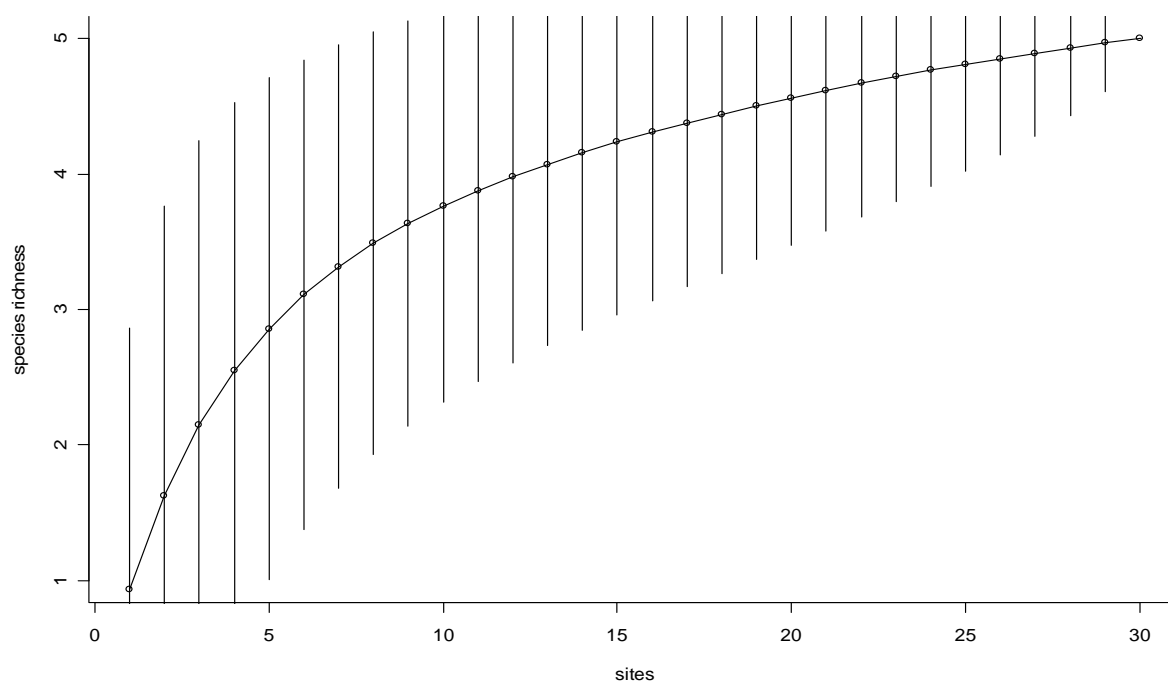| Land use	*A. oligospora*	*A. dactyloides*	*M. cionopagum*	*Nematoctonus*	*Monacrosporium. sp*
Horticulture	4	3	2	0	0
Shrub land	0	1	0	0	0
Coffee/bean	1	0	0	0	0
Maize/ bean	3	0	1	1	0
Napier grass	4	4	1	0	2
Fallow	0	0	1	0	0

Figure 2. Nematode destroying fungi species accumulation curve

Table 3. Effect of land use on richness and diversity of nematode destroying fungi .

Land use	Samples	Mean richness	Mean shannon
Coffee/Bean	5	0.2	0.000
Fallow	5	0.2	0.000
Maize/Bean	5	1	0.000
Napier	5	2.2	0.717
Shrub	5	0.2	0.000
Horticulture	5	1.8	0.497

Characteristics of isolates

Arthrobotrys oligospora formed adhesive nets, non constricting rings and three dimensional structures which caught nematodes. The Conidia were indented at the septa producing two distinct cells. The species was also differentiated by the upright conidiophores in which conidia were in groups of more than ten (Plate 1a and b). *A. dactyloides* developed an-upright and un-branched conidiophores. The conidia were ellipsoid and slightly curved and almost equal two cells. It produced three cell constricting rings (Plate 2). The *Monacrosporium* species mycelium developed adhesive columnar branches, which looked like ladder or a mesh where all the nematodes were held. The conidia had two to many cells. The conidiophores were colourless, erect, bearing single terminal conidia (Plate 3 a and b). The genera *Nematoctonous* produced mycelia net work originating from the destroyed nematode. Its conidia germinated after being released from the destroyed nematode (Plate 4). The nematode was attacked by germinated adhesive hourglass shaped knob conidia. The mycelium showed a clamp connection.
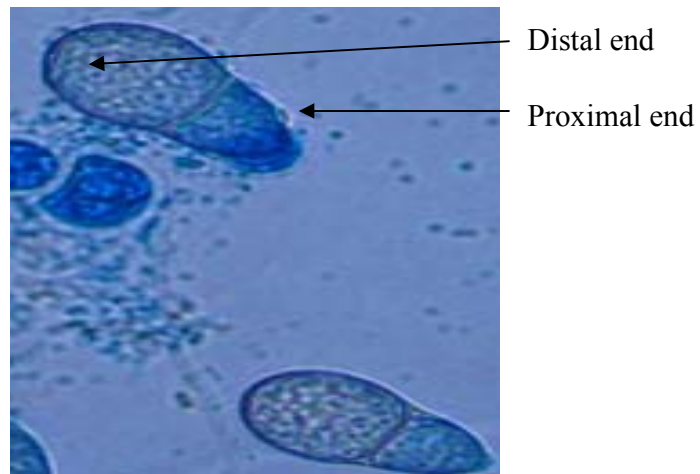
Plate 1a. *Arthrobotrys oligospora*: Conidia with two distinct cells, the distal cell; is almost twice as big as the proximal cell.
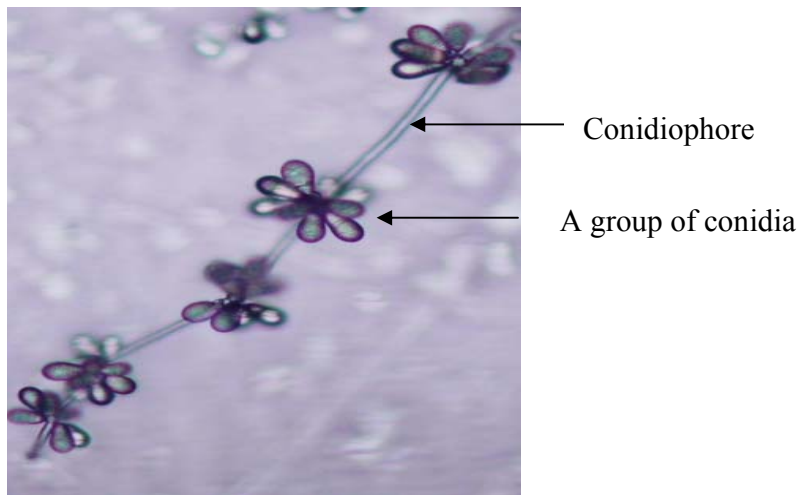

Plate 1b. A Conidiophore of *A.oligospora* with groups of conidia. The conidia occur in series of more than ten in each conidiophore.
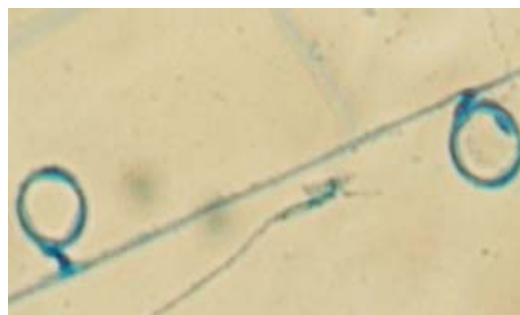

Plate 2. Constricting rings of *Arthrobotrys dactyloides*; the ring is made up of three cells which expand towards the centre and squeeze the nematode when touched from the inner side.

Plate 3a. *Conidia* of *Monacrosporium cionopagum:* The conidia has two to several cells. The conidia are strongly spindle shaped.
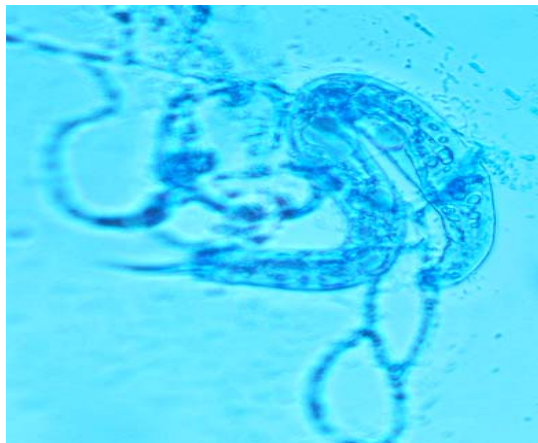

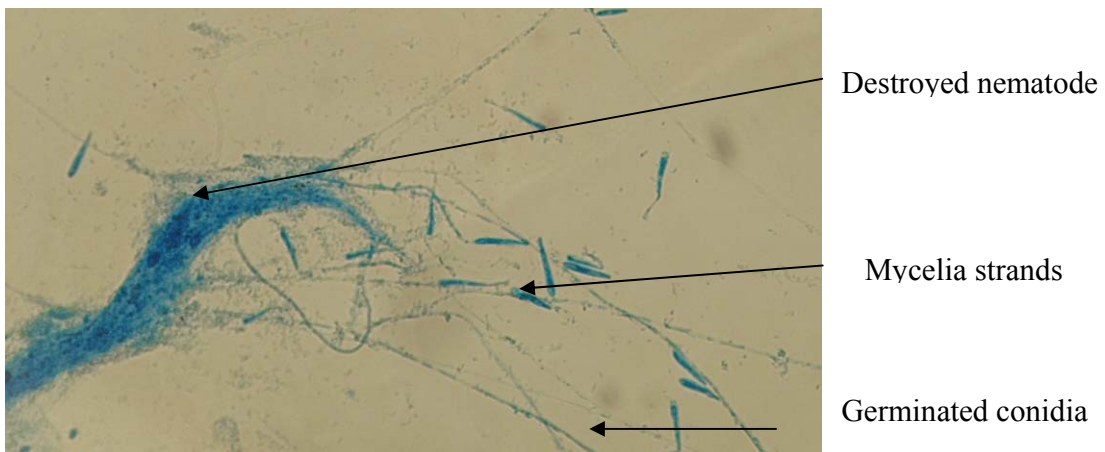Plate 3b. Adhesive column of *Monacrosporium cionopagum* with a trapped nematode.

Plate 4. *Nematoctonus leiosporus*

Destroyed nematode

Mycelia strands

Germinated conidia

In vitro studies of the isolates

A.oligospora formed adhesive nets, non constricting rings and three dimensional structures which caught nematodes and consumed them within twelve hours. The number of traps increased with increased number of nematodes reaching the highest pick on the eighth day (400 traps), that also resulted in the increase in the number of trapped nematodes. After the ninth day, the number of the traps went down as the conidiophores and conidia increased (Fig. 3). In the thirteenth day, the number of traps, trapped nematodes as well as the number of un-captured nematodes had declined. Time for the destruction of nematode by the genera *Nematoctonous* was between twenty-four and thirty six hours after incubation. Since the fungus is an endo parasitic, the actual time when the adhesion of the conidia to the nematode and infection occurred could not be recorded. In *Monacrosporium* sps the adhesion occurred on the ladder like mycelium. The nematodes were fully consumed between twelve and fifteen hours. In all of them, the newly hatched juveniles were easily trapped and killed faster than the big nematodes.
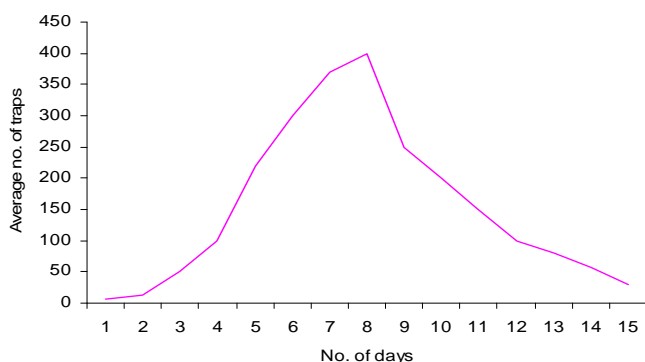

Figure 3. Traps formation in *A. oligospora.*

DISCUSSION

The nematodes destroying fungi occurred in all land uses in the study area, though their diversity varied, in which shrub, fallow and coffee/beans was low, while napier recording highest occurrence followed by maize/beans and vegetable farms. Land use and organic inputs were found to be significant factors affecting the occurrence of the nematode destroying fungi in Taita. Land uses that received cow manure and chicken manure (organic inputs) favored the presence of nematode destroying fungi. These were the horticulture, napier and the maize beans land uses. Though the inorganic inputs also significantly affected the occurrence of nematode destroying fungi, their

occurrence was greater with the availability of organic inputs (cow manure and chicken manure). This finding agrees with those of Dackman *et al,* (1992) who reported that organic matter may enhance nematode trapping fungi. Jaffee, (2004) showed that organic amendments enhanced build-up of resident nematode-trapping fungi in the soil. Higher soil organic matter content protects plants against nematodes by increasing soil water-holding capacity and enhancing the activity of naturally occurring biological organisms that compete with nematodes in the soil (Kaskavalci, 2007). The horticulture, napier and the maize/ bean land uses receives more attention in terms of inputs since they are the main source of income (horticulture and napier) and food (maize/bean) (Mutsotso *et al.,* 2005, Sylvie, 2006).

Of all the five fungal species identified in this exercise, *Arthrobotrys oligospora* was selected as the best candidate for bio control of nematodes since it had the highest occurrence frequency of 42.9% which was statistically significant compared to the other (P≤ 0.05). The fungus is also able to destroy the nematodes in a very short time, twelve hours was recorded here compared to twenty four hours for the *Monacrosporium cionopagum* and *Nematoctonus*. It is not clear why *Arthrobotrys oligospora* is the abundant isolate in this study. One possible explanation in this study would be the presence of inorganic and organic inputs in the soil applied by the farmers. Farrell *et al.* (2006) observed that *A. oligospora* was very abundant in Bodega Marine Reserve and attributed it to the organic matter of the soil which was estimated to be 6.5%. Apart from presence of organic matter, the fungi also obtain its carbon and energy from two sources, from organic matter (saprophyte) and also from trapping nematodes (parasite) making it adaptable to wide range of habitats. Due to its occurrence and widespread nature, it has attracted a lot of research (Duponnois *et al.,* 2001, Santos *et al.,* 2001, Bordallo *et al.,* 2001, Farrell *et al.,* 2006).

In 1961, Shepherd, in his study made a similar observation about the abundance of *A. oligospora*. It is possible that members of the genus were the best adapted to the biotic and abiotic conditions prevailing in the study area. This finding is of practical value to the search and utilization of biological agents for the control of plant parasitic nematodes. Whenever it occurred, it was easily identified through the shape of the conidia, trapping organs and also conidial arrangement in the conidiophores. Its interaction with nematodes was easily visible. When a nematode was captured and consumed, the fungus produced a lot of conidia attached to the conidiophores. This gives an advantage to the fungus as a bio control candidate as the abundant conidia are a mode of dispersal. The study also observed that young nematodes were caught

immediately after hatching. The nematodes were captured and destroyed by the nematode destroying fungi in the laboratory through non-constricting rings, adhesive hyphae, and adhesive mycelia columnar and constricting rings. This observation is ecologically important because the fungus would be able to kill the juvenile 2 (J2) stage of the nematodes which is the most destructive stage of the Root Knot Nematodes (RKN) that attacks the plant roots. If not controlled, the J2 attack the plant root, penetrating to the root tissue causing the root knots. This stage is characterized with small nematodes (in size) which when caught by the adhesive mycelia of the fungus are not able to escape. This observation was in agreement with the study conducted by Jasson *et al.,* (2000) who demonstrated that the nematode size determines the possibility of its capture with the big nematodes escaping the ring traps formed by the *A.dactyloides*. Although the nematode destroying fungi destroyed the nematodes in the laboratory using Petri dishes also as reported by Elshafie *et al.,* (2006), similar reports have not been obtained from field and green house experiments (Jaffee and Strong, 2005, Jaffee *et al.,* 2007).

CONCLUSION

This is the first account of a focused study on nematode destroying fungi in Kenya. The study has confirmed that nematode destroying fungi occur in the study area and their distributions are influenced by land use and land management system. This unique observation sets the justification for continued work to establish the potential of nematode destroying fungi in regulation of plant parasitic nematodes. Agricultural practices that stimulate build-up of nematode destroying fungi could be identified and recommended for adoption by farmers.

ACKNOWLEDGMENT

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