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# Effect of Land Use on Occurrence and Diversity of Nematode Destroying Fungi in Taita Taveta, Kenya

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**Abstract:** This study was undertaken with the objective of determining the occurrence of nematode destroying fungi in soil under different land use systems, with the ultimate goal of harnessing their potential in the control of plant parasitic nematodes. Soil samples were collected from an indigenous forest, maize/bean, napier grass, shrub and vegetable fields, which represented the main land use types in Taita Taveta district of Kenya. The fungal isolates obtained were grouped into seven genera the species identified were *Arthrobotrys oligospora*, *A. dactyloides*, *Monacrosporium cionopagum*, *A. superba*, *Harposporium anguillulae*, *Harposporium* sp., *Dactyllela lobata*, *Acrostalagums obovatus*, *Haptoglosa heterospora* and *Nematoctonous georgenious*. Occurrence of nematode destroying fungi was significantly (P: $3.81\times10^{-7}$) different among the land use systems in the study area. Out of the isolates that were positively identified, 33.7, 27.9, 20.9, 11.6 and 5.8% were from fields under vegetable, maize/bean, napier grass, shrub and forest, respectively. The diversity of nematode destroying fungi was highest in the maize/bean fields and lowest forest soil. Fungal isolates from vegetable gardens were most diverse but the least even while the forest land use was most even but least diverse. The total richness of nematode destroying fungi was 9, in vegetable and maize/bean fields while was 7, 6 and 3 in napier, shrub and forest habitats, respectively. This study has established that nematode destroying fungi are widely distributed and that land use has a significant effect on their diversity.

**Key words:** *Arthrobotrys oligospora*, evenness, vegetable field, natural forest

## INTRODUCTION

Nematode destroying fungi are a group of cosmopolitan microfungi that are natural enemies of plant parasitic nematodes (Birgit *et al*., 2002; Yang *et al*., 2007). They comprise fungi which parasitise nematode eggs and other life stages (Jansson and Persson, 2000). Although taxonomically diverse, this group of microorganisms is capable of destroying, by predation or parasitism, microscopic animals such as nematodes, rotifers and protozoans. Collectively, they have the unique ability to capture and infect nematodes in the soil and appear to be widespread in distribution (Birgit *et al*., 2002).

The actual mechanisms by which the fungi are attracted to the nematodes have not been fully understood. However, it is generally accepted that the cuticle is penetrated and the nematode is immobilized through infection bulbs, being finally digested by the trophic hyphae produced by the fungus (Bordallo *et al*., 2002). Some fungi use adhesive conidia, branches, knobs and mycelia to capture nematodes (Jaffe and Muldoo, 1995). In some cases, nematode destroying fungi produce toxins that immobilize or kill nematodes (Araújo *et al*., 1999). The group also includes endoparasitic species in such genera as *Harposporium*, *Nematoctonus* and *Meria* (Timm *et al*., 2001) which spend their entire vegetative lives within infected nematodes.

Nematophagous fungi have 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). Unfortunately, there exist multidimensional drawbacks to the realization of the full potential of the nematode destroying fungi. Unavailability of reliable methods to visualize the fungi and demonstrate their activity in their natural habitats is a major impediment. Consequently, activity of the fungi in the soil has been inferential through the reduction in numbers of nematodes or reduction of their damage to plants (Jaffee *et al*., 1998). Although fluorescence microscopy can be used to monitor the nematode destroying fungi in the soil, the sampling procedure available is inappropriate due to its destructive nature and heterogeneity of the soil (Jensen *et al*., 1998). Apart from disagreements on

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methods that can be used in monitoring organisms in the soil, the process is cumbersome (Persson *et al*., 2000). Some authors have recommended the soil dilution method and the most probable number as well as Polymerase Chain Reaction (PCR) for the estimation of the nematode destroying fungi population in the soil (Mauchline *et al*., 2002). Bioassay for conidia and parasitism assay or predatory index has also been recommended (Jaffee, 1999; Sanyal, 2000). Above all the gaps in knowledge the ecological factors that influence the occurrence and abundance of nematode destroying fungi are largely unclear.

The objective of this study was therefore to determine how land use influences the diversity and occurrence of nematode destroying fungi in the soil.

## MATERIALS AND METHODS

Survey of nematode destroying fungi was conducted in Taita Taveta, Wundanyi division from January to May 2007. The five land use types selected were natural forest, shrub, vegetable, napier grass and maize/bean intercrop. The natural forest consisted of a broad diversity of indigenous tress which included; *Strombosia scheffleri* (Olacaceae), *Dicralonepis usambarica* (Thymelaceae), *Graibia zimmermanii* (Papilionaceae), *Oxyanthus speciosa* (Rubiaceae), *Dracaena deremensis* (Dracaenaceae), *Rauvolvia mannii* (Apocynaceae), *Rytiygynia schumanii* (Rubiaceae) and *Chassalia discolor* (*Rubiaceae*). Natural shrub consisted of mainly *Croton megalocarpus* (Euphorbiaceae), *Lantana camara* (Verbenaceae), *Sporoblus pyramidalis* (Gramineae) and *Ficus thoningii* (Moraceae). The vegetable gardens were mainly dominated by cabbage (*Brassica oleraceae*), spinach (*Chenopodium spinacia*), tomato (*Solanum lycopersicum*), kale (*Brassica oleraceae* var. *acephala*) and cucumber (*Cucumis sativus*), grown separately in randomly selected rotation systems. Maize intercropped with beans was selected because it was the main food production system in the study area. Napier grass fields (*Pennisetum purpureum*) are also widely distributed in the area and serve to supply fodder to the dairy animals under restricted grazing systems.

Eight soil samples were taken from each of the five land uses. In total, 40 main sampling points were randomly identified from which five sub sampling were taken. One sub- sample was taken from the center and four sub-samples at a distance of 3 m from the centre (Fig. 1). An auger was used to take soil cores from the 0-20 cm soil depth.

The five sub-samples were mixed homogeneously to constitute a composite sample from which 500 g soil was taken, placed in a plastic bag and then placed in a cool box. The auger was sterilized by dipping it in ethanol between sampling points to avoid cross contamination. The soil samples were transported to the laboratory where they were kept in a cold room at about 10°C before isolation of the nematode destroying fungi.

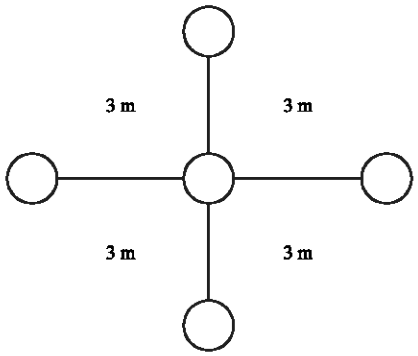


Fig. 1: Schematic representation of the five soil sampling points which comprised one main sampling point

Isolation of the fungi was done using the soil sprinkle technique as described by Jaffee *et al*. (1996). Tap water agar was prepared by dissolving 20 g of agar in one liter of tap water. The medium was autoclaved and cooled to 45°C before amending it with 0.1 g $L^{-1}$ of streptomycin sulfate to suppress bacterial growth. Approximately 1 g of soil from each sampling point was sprinkled onto the surface of water agar in Petri dishes. Plant parasitic nematodes were added into the Petri dish as baits. The plates were incubated at room temperature and observed daily under a microscope at low (40x) magnification, from the third week up to the 6th week. The examination was focused on trapped nematodes, trapping organs and conidia of the nematode destroying fungi that grew from the soil.

After the sixth week, all the fungal colonies that had emerged were sub-cultured on potato dextrose agar to obtain pure cultures. To verify the status of the fungal isolates as predators of nematodes, observations were made on a daily basis, after the third day, for trapped nematodes, trapping organs and conidia. Photographs of trapped nematodes, trapping organs and conidia were taken for use in identification of the nematode destroying fungi. Data were analyzed by calculating the frequency of occurrence, evenness, Renyi profiles and the Shannon diversity index (Kindt and Coe, 2005).

## RESULTS

All the sampled land uses were significantly different in terms of occurrence of nematode destroying nematodes

(p-value: $3.81\times10^{-07}$). Nematode destroying fungi were present in all the land use types but at varying frequencies and abundance. The frequency of isolating nematode destroying fungi was 33.7 and 5.8% in vegetable and forest ecosystems, respectively. The vegetable ecosystem harbored all the species recorded during this study, apart from *Acrostalagums obovatus* which was absent. With the exception of *Monacrosporium cionopagum*, all the other nine species were recovered from the maize/bean fields. The forest land use had the least counts of nematode destroying fungi, which were in the genera, *Arthrobotrys*, *Monacrosporim* and *Harposporium*. The proportions of nematode destroying fungi isolated from maize/bean, shrub land and napier grass plots were 27.9, 11.6 and 20.9%, respectively (Table 1).

Differences in evenness were significant (p-value: $3.8\times10^{-07}$) among the five land use systems tested. Evenness of species of nematode destroying fungi was highest in the forest and lowest in the vegetable gardens. The total species richness ranged from three to nine being highest in the intensively cultivated ecosystems under maize intercropped with beans and in the vegetable fields. The total richness of the nematode destroying fungi was equal in maize/bean and vegetable fields but the evenness was slightly higher in the former than the latter (Table 1).

The diversity profiles of nematode destroying fungi in the five land uses shows that maize/bean and the vegetable fields exhibited the highest diversity, followed by napier grass fields. The diversity was lowest in the forest ecosystem (Fig. 2a).

The evenness profiles show two distinct categories in the study area (Fig. 2b). The evenness profile in the forest was distinct and above those of the other land uses. Evenness in the maize/bean field was almost equal to that in the shrub land.

Detection of nematode destroying fungal species increased with increase in number in number of the soil samples taken (Fig. 3). However, the curve indicates that all possible species in the area were recovered in 37 samples, meaning that processing of additional samples would yield no new species.

Eighty six isolates of nematode destroying fungi were identified and grouped into ten taxa and seven genera. Fungi in the genus *Arthrobotrys* were the most frequently isolated, with a cumulative frequency of 64% (Table 2). Species in the genus were *A. oligospora*, *A. dactyloides* and *A. superba*. The genus was represented in all the land use systems. It was followed by the genus *Harposporium* which was represented by *H. aungullilae* and *Harposporium* sp. Members of the genus *Nematoctonus* were least frequent (2.3%), being isolated only in two samples in this study.

Table 1: Effect of land use on frequency of isolation, richness and diversity of nematode destroying fungi in Taita Taveta district, Kenya

| Land use | n | Frequency of isolation (%) | Mean evenness | Mean richness | Mean Shannon |
|---|---|---|---|---|---|
| Forest | 8 | 5.80 | 0.375 | 0.625 | 0.17 |
| Maize/bean | 8 | 27.90 | 1.000 | 3.000 | 1.07 |
| Napier | 8 | 20.90 | 1.000 | 2.250 | 0.76 |
| Shrub | 8 | 11.60 | 0.625 | 1.250 | 0.36 |
| Vegetables | 8 | 33.70 | 1.000 | 3.625 | 1.26 |
| p-value | | $3.81\times10^{-07}$ | $1.39\times10^{-07}$ | $3.81\times10^{-07}$ | $1.062\times10^{-06}$ |

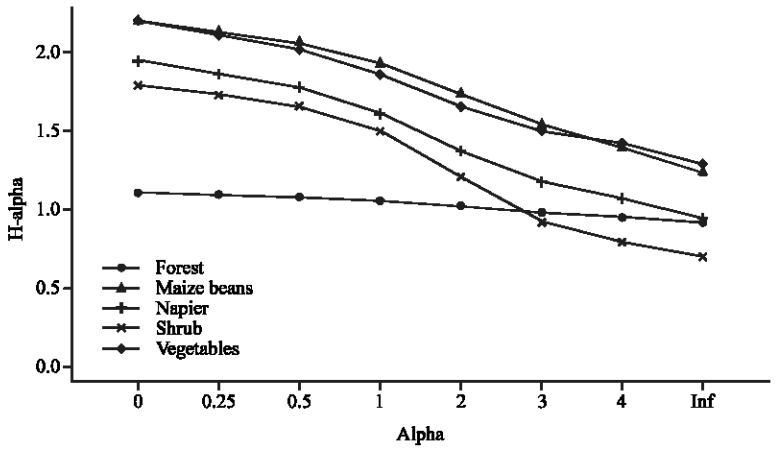


Fig. 2a: Diversity of nematode destroying fungal species under varying land use systems in Taita Taveta district, Kenya

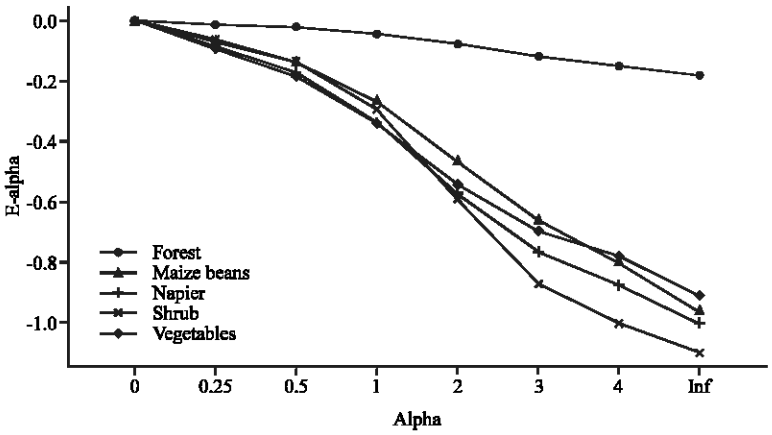


Fig. 2b: Evenness of nematode destroying fungal species isolated from soil under different land use systems in Taita Taveta district, Kenya

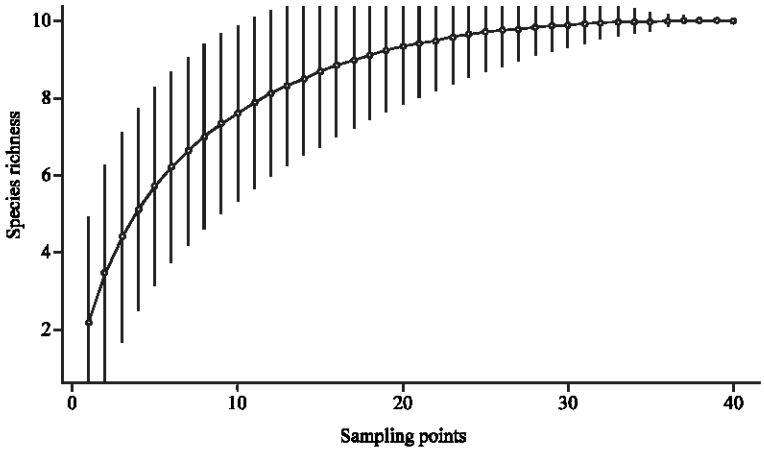


Fig. 3: Species accumulation curve of nematode destroying fungi isolated from Taita Taveta district in Kenya

Table 2: Frequency of occurrence of nematodes destroying fungi in different land use systems in Taita Taveta district, Kenya

| Species | Rank | Occurrence | % | Cumm. freq. | p-value |
|---|---|---|---|---|---|
| *Arthrobotrys oligospora* | 1 | 29 | 33.7 | 33.7 | 0.006872 |
| *Arthrobotrys dactyloides* | 2 | 17 | 19.8 | 53.5 | 0.001228 |
| *Monacrosporium cionopagum* | 3 | 11 | 12.8 | 63.3 | 0.01092 |
| *Arthrobotrys superba* | 4 | 9 | 10.5 | 76.7 | 0.03096 |
| *Harposporium anguillulae* | 5 | 5 | 5.8 | 82.6 | 1.00* |
| *Harposporium* sp. | 6 | 4 | 4.7 | 87.2 | 0.005619 |
| *Dactyllela lobata* | 7 | 3 | 3.5 | 90.7 | 0.3382* |
| *Acrostalagums obovatus* | 8 | 3 | 3.5 | 94.2 | 0.3382* |
| *Haptoglosa heterospora* | 9 | 3 | 3.5 | 97.7 | 0.018 |
| *Nematoctonous georgenious* | 10 | 2 | 2.3 | 100.0 | 0.1028* |

*The species occurrence is not significantly affected by the land use types

*Arthrobotrys oligospora* had the highest frequency of occurrence, followed by *A. dactyloides*, *Monacrosporium cionopagum*, *A. superba* and *Harposporium aungullilae* on the species rank curve (Table 2). Land use had a significant effect on occurrence of nematode destroying fungi. Occurrence of *A. oligospora* had a p-value of 0.006872 while *A. dactyloides*, *M. cionapagum* and *A. superba* had p-values of 0.001228, 0.01092 and 0.03096, respectively. Some rare isolates also reflected the effect of land use on their occurrence, *Harposprium* sp. and *Haptoglosa heterospora* with a p-value of 0.005619 and 0.018,


Fig. 4a: Probability of isolating *Arthrobotrys oligospora* in soil under different land use systems in Taita Taveta district in Kenya

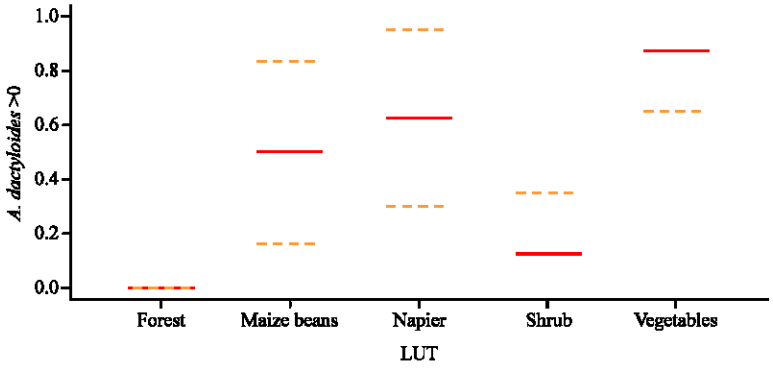


Fig. 4b: Probability of isolating *Arthrobotrys dactyloides* in soil under different land use systems in Taita Taveta district in Kenya

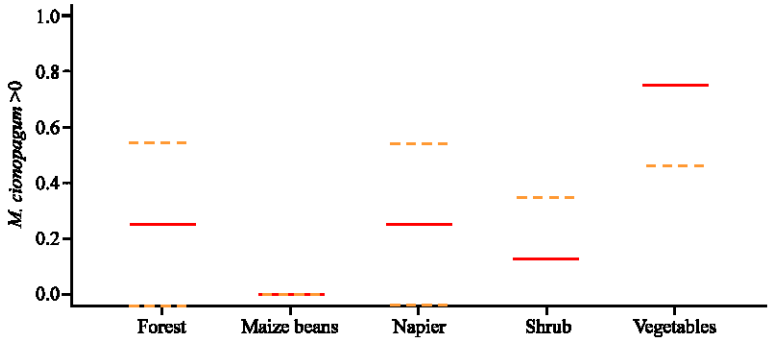


Fig. 4c: Probability of isolating *Monacrosporium cionopagum* in soil under different land use systems in Taita Taveta district in Kenya

respectively. *Harposporium anguillulae*, *Dactyllela lobata*, *Acrostalagums obovatus* and *Nematoctonous georgenious* were not affected by the land use.

The probability of isolating an *Arthrobotrys oligospora* from the vegetable, maize/bean and napier grass fields was above 0.8 while it was 0.2 in the forest soil (Fig. 4a). The fungus was present in all the target land uses. *Arthrobobrys dactyloides* was most frequent in vegetable gardens, followed by napier grass fields, but very low in the shrub land and completely absent in the forest (Fig. 4b). Chances of isolating *Monacrosporium cionopagum* were 0.8 in vegetable gardens but below 0.3 in all the other land uses and absent in the maize/bean fields (Fig. 4c).

## DISCUSSION

This study has demonstrated that nematode destroying fungi are wide spread in occurrence in the target habitats which were indigenous forest, shrub land, napier grass, maize/bean and vegetable fields. The fungi that were isolated exhibited several mechanisms of capturing and destroying plant parasitic nematodes which included constricting rings, adhesive nets and non-constricting rings. The study has also revealed that increased land use intensity resulted in increased occurrence and diversity of nematode trapping fungi. These findings are consistent with previous reports indicating that nematode destroying fungi were present in all habitats but at different densities and diversities (Birgit *et al*., 2002). Widespread occurrence and abundance of the fungi is thought to be an indicator of great potential that can be exploited to the benefit of crop production. Contrary to expectation that beneficial microorganism decrease with increased intensity in land use (Vandermeer *et al*., 1998), the diversity of the nematode destroying fungi was higher in the vegetable gardens compared to the forest ecosystem. In the more intact and stable land uses (forest and shrub land), only a few isolates of nematodes destroying fungi were recovered at lower frequencies.

A number of explanations can be used to account for the higher frequency of occurrence of nematode destroying fungi in the habitats that are subject to regular disturbance compared to the stable ecosystems like shrub land and indigenous forest. Addition of farm inputs in the form of organic and inorganic compounds has an effect on indigenous microorganisms in the soil. According to Wang *et al*. (2003), some of the agricultural inputs stimulate build-up of nematode trapping fungi. It's also possible that fungal tissues are fragmented and scattered in the course of farm operations, thus increasing their frequency of isolation. Intensive cultivation is characterized by increased movement of soil which may result in increased spread of the microorganisms in the field. Soil disturbance, coupled with frequent changes in crop cover, subjects the soil biota to stress making it difficult for a particular species to establish itself in the soil to out-compete the others. In contrast, soils under forest and shrub are less disturbed meaning that certain species of nematode destroying fungi are able to establish and suppress other species that are poorly suited to compete effectively.

Evenness of the nematode destroying fungi was lower in the highly disturbed habitats like in vegetable gardens. According to Sanchez (1997), agricultural practices can have positive or negative impacts on microorganisms in the soil. Intensive cultivation is usually accompanied by application of inorganic fertilizers and pesticides. Apart from the negative effects from synthetic inputs, human activities may also impose selective pressure on the naturally-present microorganisms. Crop management practices (e.g., addition of organic amendments) are known to have varying effects on indigenous microorganisms in the soil (Akhtar and Malik, 2000). This may account for the higher evenness of nematode destroying fungi in the forest, which is a more stable ecosystem, when compared to the vegetable gardens which are subject to the management practices adopted by farmers in a given area.

*Arthrobotrys oligospora* was the most abundant species of nematode destroying fungi in the study area. It was isolated from all the land uses with an overall occurrence frequency of 33.7%. This finding was consistent with results from a similar study conducted in South Africa (Durand *et al*., 2005; Farrell *et al*., 2006). The genus *Arthrobotrys* was the most frequently represented in all the habitats that were the subject of this study. It's 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. Apart from introduction of particular species from the genus, agricultural practices that stimulate build-up of the fungi could be identified and recommended for adoption by farmers.

## CONCLUSION

Additional evidence has been provided from this study that nematode destroying fungi are naturally occurring and widespread in agricultural and forest habitats. The fungi were more frequently isolated from the intensively cultivated land under annual and vegetable crop production. This unique observation sets the justification for continued work to establish the potential of nematode destroying fungi in regulation of plant parasitic nematodes.

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