Tropical and Subtropical Agroecosystems

USE OF NEMATODE DESTROYING FUNGI AS INDICATORS OF LAND DISTURBANCE IN TAITA TAVETA, KENYA

[USO DE HONGOS NEMATOFAGOS COMO INDICADORES DE PERTUBACIÓN DEL SUELO EN TAITA TAVETA, KENIA]

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SUMMARY

This study was undertaken to determine whether nematode destroying fungi can be used as indicators of soil disturbances. 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 and identified. The species identified were, *Acrostalagums obovatus, Arthrobotrys dactyloides*, *Arthrobotrys oligospora*, *Arthrobotrys superba, Dactyllela lobata*, *Haptoglosa heterospora, Harposporium anguillulae, Harposporium.sp, Monacrosporium cionopagum* and *Nematoctonous georgenious.* Occurrence of nematode destroying fungi was significantly ($P = 3.81 \times 10^{-7}$) 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. Soil disturbance accounted for the highest occurrence of nematode destroying fungi (60.77 %) while moisture, accounted for 23.35%. Fungal isolates from vegetable gardens were most diverse while soils from the forest were most even with least diversity. The total richness of nematode destroying fungi was nine, seven, six, and three in maize bean, napier, shrub and forest habitats respectively. This study has established that nematode destroying fungi increases with increased land disturbance.

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 parasitize 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* (Tim *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). In their natural ecosystems, nematode destroying fungi are in constant interaction with the nematode communities and about 70% of fungi genera and 160 species are associated with nematodes (Elshafie *et al.,* 2006). Although such interactions have been thought to reduce the population of plant parasitic nematode in the fields, no such results have been achieved from the field experiments. Jaffee and Strong, (2005), Jaffee *et al.,* (2007) found no direct involvement of nematode destroying fungi in nematode suppression in Bodega Marine Reserve. Such conclusions have only been made in laboratory experiments (Elshafie *et al.,* 2006). 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).

Nematodes are used as indicators in the ecosystems because of their permeable cuticle which allows them to respond to pollutants and correspond with the restorative capacity of soil ecosystems. They contribute to nitrogen mineralization indirectly by grazing on decomposer microbes, excreting ammonium and immobilizing nitrogen in live biomass (Dufour *et al.,* 2003). The nematode community structure in the soil is affected by many factors and among them soil fertility practices. Despite the existence of gaps in knowledge on ecological factors that influence occurrence and abundance of nematode destroying fungi, (Wachira *et al.,* 2008), soil restorative factors that affect nematode population, like organic amendments, (Kimenju *et al*, 2008) also affect the population of nematode destroying fungi in the soil (Gomes *et al.,* 2000; Timm *et al.,* 2001).

The objective of this study was therefore to determine how the diversity and occurrence of nematode destroying fungi in the soil can be used as indicator of soil disturbance.

MATERIAL AND METHODS

This study was conducted in Taita Taveta, Wundanyi division which is located at approximately 03 degrees -20'S, 38 degrees -15'S. 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 trees. Natural shrub consisted of mainly *Croton megalocarpus* and *Lantana camara.* The vegetable gardens had different types of vegetable crops 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. *Pennisetum purpureum* commonly called napier grass is used as fodder to dairy animals under zero- 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 samples were taken. One sub- sample was taken from the center and four sub-samples at a distance of 3 meters from the centre. An auger was used to take soil cores from the 0-20 cm soil depth.

Five sub-samples were mixed homogeneously to constitute a composite sample from which 500g soil was taken, placed in a plastic bag and then in to a cool box. The auger was sterilized by dipping it in 70 % ethanol between sampling points to avoid cross contamination. These soil samples were transported to the laboratory where they were kept in a cold room at about 10^0C before isolation of the nematode destroying fungi.

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 grams of agar in one liter of tap water. The medium was autoclaved and cooled to 45^0C before amending it with 0.1 g/L of streptomycin sulfate to suppress bacterial growth. Approximately one gram 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 (40 x) magnification, from the third week up to the sixth 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). The Principal Component Analysis was conducted to estimate the factors that influence the occurrence of nematode destroying fungi in the area. Multivariate analysis using ADE4 software was done on the temporal association of nematode-trapping fungi and soil characteristics (Thioulouse *et al.,* 1997).

RESULTS

All the sampled land use types were significantly different in terms of occurrence of nematode destroying fungi (NDF) (p-value=3.81x 10^{-07}). NDF were present in all the land use types but at varying frequencies and abundance and their frequency was 33.7% and 5.8% in vegetable and forest ecosystems, respectively. The vegetable and maize/bean land uses harbored all the species recorded in this study, apart from *Acrostalagums obovatus* and *Monacrosporium cionopagum.* The forest land use had the lowest abundance of NDFs and those present belonged to the genera, *Arthrobotrys, Monacrosporim* and *Harposporium*. The proportions NDFs isolated from maize/bean, shrub land and napier grass plots were

27.9, 11.6 and 20.9 % of the total fungal occurrence, respectively (Table 1).

Differences in evenness were significant (P-value: 3.8 x 10^{-07}) among the five land use systems. Evenness of species of NDF was highest in the forest and lowest in the vegetable gardens. Species richness ranged from three to nine being highest in soils collected from the intensively cultivated areas which comprised maize beans intercrops and in the vegetable fields (Table 1).

Detection of nematode destroying fungal species increased with increase in number of the soil samples taken (Fig 2). However, the curve indicates that all possible species in the area were recovered in 37 samples collected from all land-uses, which implied that additional samples were not likely to increase chances of isolating 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% and the main species were *A.oligospora*, *A.dactyloides* and *A. superba* (Table 3). 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 soil samples collected each from vegetable and maize/beans land use.

Table 1.Effect of land use on Frequency of isolation, richness and diversity of NDFs in soils from Taita Taveta district, Kenya

Land use	n	Frequency of isolation %	Mean evenness	Mean richness	Mean Shannon
Forest	8	5.8	0.375	0.625	0.17.
Maize/bean	8	27.9	1.000	3.000	1.07
Napier	8	20.9	1.000	2.250	0.76
Shrub	8	11.6	0.625	1.250	0.36
Vegetables	8	33.7	1.000	3.625	1.26
P-value		3.81x 10^{-07}	7.14 x 10^{-9}	3.81x 10^{-07}	1.062 x 10^{-06}

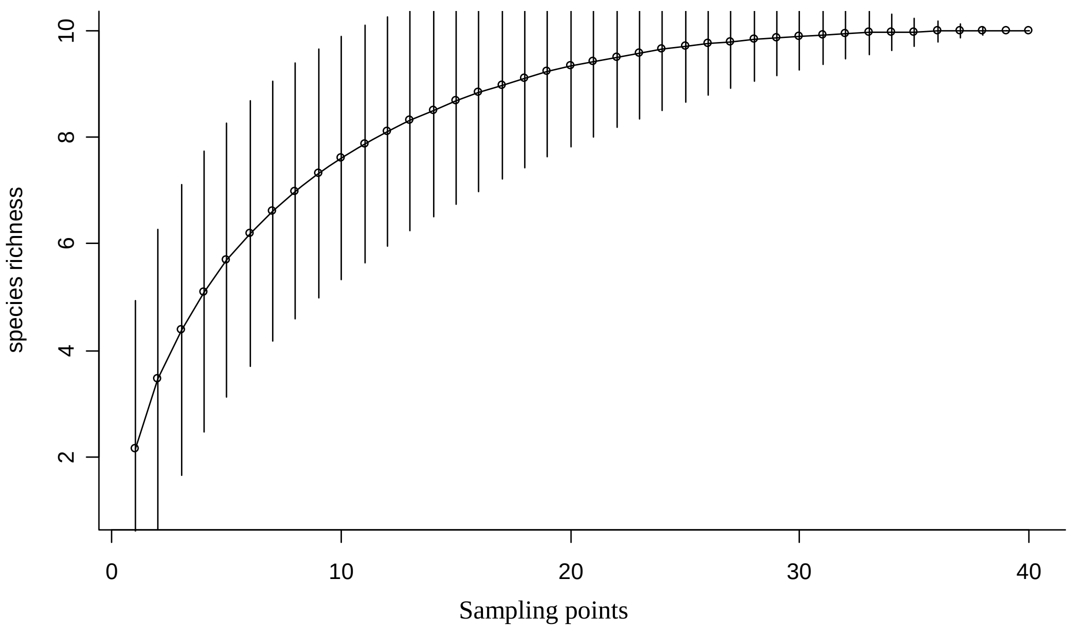

Figure 2. Species accumulation curve of nematode destroying fungi isolated from Taita Taveta district in Kenya.

Table 3. 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*
Harposporium.sps	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* (Table 3). 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 respectively. *Harposporium anguillulae*, *Dactyllela lobata*, *Acrostalagums obovatus* and *Nematoctonous georgenious* were not sensitive to the land- use disturbances.

A principal component analysis (PCA) on the data, showed that two main factors explaining 84.12% (Fig. 5a). The main factor, explained 60.77 % while the second factor explained 23.35%.The first factor separated maize/beans and vegetables on one hand and shrub, napier and forest on the other. The second factor separated forest and vegetables on one hand and napier, shrub and maize/bean on the other. Factor one separated intensively cultivated land uses from the least cultivated land use systems while factor two sepaterd the landuse with the highest canopy covers from the open land uses.

The nematode destroying fungi species loaded more towards the horticulture and the maize/bean land uses. *Monacrosporium cionopagum* and *Acrostalgmus obovatus* loaded strongly to Vegetable and maize/bean respectively. 60% of all the species were associated with vegetable land use while 40 % loaded with maize (5b).

Nematode destroying fungi are partly affected by the soil chemical properties as the two main factors accounting for 50%. Factor 1 separates phosphorus (P) on one side and nitrogen (N), carbon (C), Cat ions Exchangeable Capacity (CEC) and potassium (K) on the other hand. pH and Calcium are not significant in the survival of fungi. The second factor C, N and Ca on one side and P, K and pH and CEC on the other side (Fig. 5c)

Some species (*A. oligospora, A. dactyloides, A. supreba, Dactyllel lobata, Haptogrosa heterospora, Acrostalagums obovatus*) depend on soil pH, K and CEC for survival. *M. cionopagum, Harposporium aungulilae* and *Harposporium georgenous* prefers soil with high N, C, and Ca while P deficient soils seem to deter the growth of nematode destroying fungi (Fig. 5c)

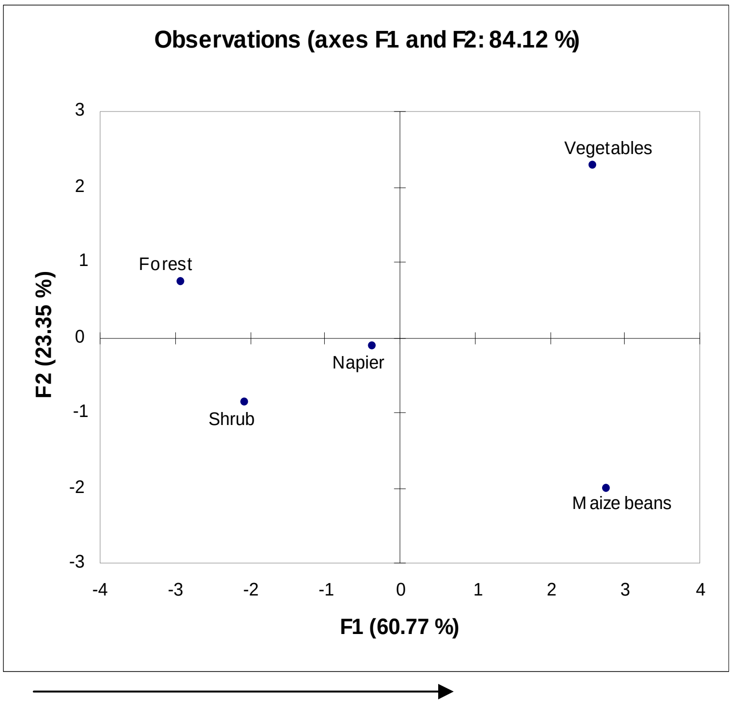

Figure5a. Increasing agricultural intensification: increase soil disturbance, additional of organic and inorganic fertilizer

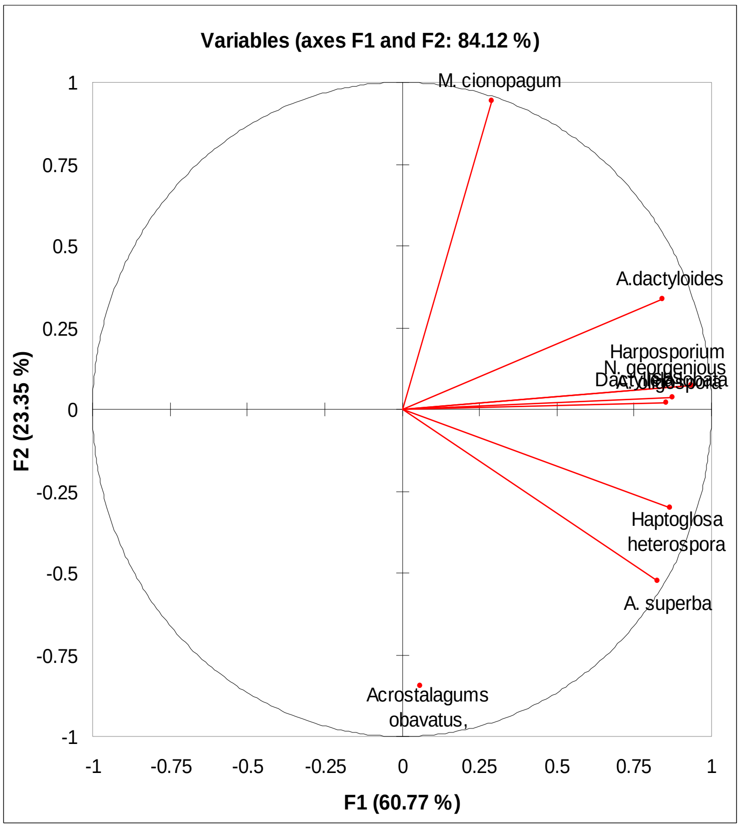

Figure. 5b. Effect of land use in distribution of nematode destroying fungi.

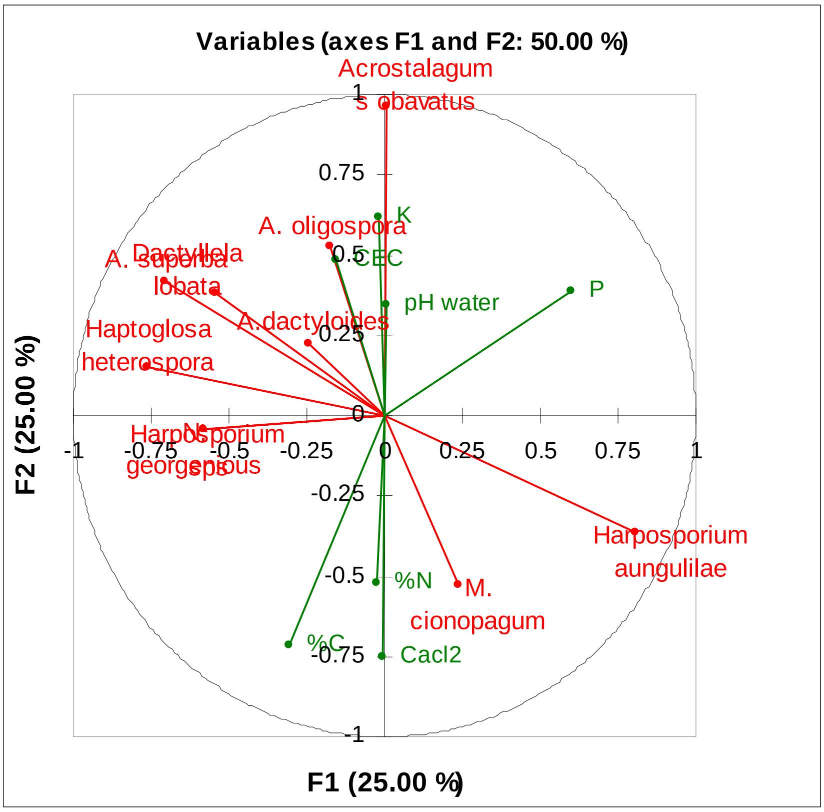

Figure 5c. Canonical correlation analysis between the fungi species and the soil chemical parameters.

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 intensification resulted in increased occurrence and diversity of nematode destroying 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 in the management of plant parasitic nematodes. 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 less disturbed land use systems such as the forest and shrub land, few isolates of NDFs were recovered.

A number of explanations could account for the higher frequency of occurrence of NDFs in the habitats that are subject to regular disturbance compared to the stable which include of organic and inorganic soil enhancement materials. The form of these inputs crop had varied effects on the soil ecosystems for instance animal manures and composits increased populations of beneficial microorganism. It has been demonstrated that composted cow manure reduced aphid's population in tomato plants hence reducing the effects of those pests in tomatoes compared to the synthetic fertilizers thus making the soil healthy (Yardım and Edwards, 2003). The most common organic fertilizer applied by the farmers in Taita on their farm is livestock manure. Synthetic fertilizers have constantly been associated with increased pathogenic microorganisms and decreased beneficial microorganism (Bulluck *et al.*, 2002). There is some evidence that synthetic fertilizers reduce plant resistance to insect pests, tend to enhance insect pest populations and can increase the need for insecticide applications (Yardım and Edwards 2003). Soil amendments were reported to have an impact on nematode community structure and diversity. Populations of bacterivorous nematodes mainly in the Rhabditidae and Cephalobidae, and fungivorous nematodes were greater after planting in soils amended with swine manure, composted cotton-gin trash, or rye-vetch, than in soils amended with synthetic fertilizer (Bulluck *et al.*, 2002). According to Wang *et al.,* 2003, some of the agricultural stimulate build-up of nematode trapping fungi while Dackman *et al.,* (1987) found greater numbers of nematode- trapping fungi in two plots treated with manure compared to inorganic fertilizer.

It is 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 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 were less disturbed meaning that certain species of NDFs were established and suppressed.

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. Crop management practices (such as addition of organic amendments) have varying effects on indigenous microorganisms in the soil (Akhtar and Malik, 2000). This may account for the higher evenness of NDFs in the forest, which is a more stable ecosystem, when compared to the vegetable gardens which are subjected to the management practices adopted by farmers in a given area.

Arthrobotrys oligospora was the most abundant species of NDF in the study area as it was found in the land use types with occurrence frequency of 33.7%. These findings were 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 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. Apart from introduction of particular species from the genus, agricultural practices that stimulate build-up of the fungi need to be identified for use by small scale farmers.

Factors affecting the occurrence of NDFs are agricultural intensification through soil disturbance and addition of agrochemicals and crop diversity. Soils from vegetable and maize /bean supported higher populations of NDFs compared to other LUTs due to their varied plant types which are host to many nematodes which are n constant interaction with NDFs. Disturbances of soil through activities like digging and weeding have an effect of dispersing the fungal fragments resulting into high chances of detecting NDFs in the soil. Another factor which influenced NDFs was the amount of moisture in the soil which was high in natural forest and vegetable plots which were irrigated and mulched.

CONCLUSION

Nematode destroying fungi were more frequently isolated from the intensively cultivated land under annual and vegetable crop production. Fungi survived in highly disturbed ecosystems and therefore have potential of being good indicators of soil disturbances.

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