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Diversity of nematode destroying fungi in Taita Taveta, Kenya

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ABSTRACT

The diversity of nematode destroying fungi in Taita Taveta, Wundanyi division, Coast Province, Kenya, was investigated between May 2006 and December 2007 aiming at harnessing their potential in the biological control of plant parasitic nematodes in the area. Given that the intensity of land cultivation is continually increasing in the study area, it is prudent to document the status of the nematode destroying fungi before the remaining forest habitats are ultimately disrupted. Soil samples were collected from forest, maize/bean, napier grass, shrub and vegetable fields, which represented the main land use types in the study area. The soil sprinkle technique method was used to isolate the nematode destroying fungi from the soil. The fungi were identified to species level. Eighty-five isolates, distributed in eight genera and 14 taxa were identified as nematode destroying fungi. The species identified were *Arthrobotrys dactyloides*, *Arthrobotrys oligospora*, *Arthrobotrys superba*, *Acrostalagamus obovatus*, *Dactyllela lobata*, *Harposporium aungulilae*, *Harposporium liltiputanum*, *Harposporium* spp, *Haptoglosa heterospora*, *Monacrosporium asterospernum*, *Monacrosporium cianopagum*, *Myzocytium*, spp, *Nematoctonus georgenious* and *Nematoctonus leptosporus*. Vegetable land use had the highest diversity of nematode destroying fungi. The results show that the study area is rich in nematode destroying fungi with *A. oligospora* being widespread and a possible candidate for biological control of plant parasitic nematodes.

Introduction

The Taita Hills are located in Southeastern Kenya, 25 km west of Voi town in the Taita Taveta district, at approximately 03° −20°S, 38 degrees −15°S. Due to their age (290–180 million yrs), isolated location and relatively stable conditions, they contain unique plants and animals with very high levels of endemism. The forests have been recognized as one of the 25 biodiversity 'hotspots' in the world (Rogo & Oguge 2000). Subsequent work ranked the area among the top-ten biodiversity hotspots in the world (Mittermeier *et al.* 2005). According to Pellikka *et al.* (2004), the area had 74 endemic vertebrates, 265 endemic invertebrates and 66 endemic trees. Some species, however, are critically endangered, e.g. the Taita Thrush, *Turdus helleri* (Bytebier 2001). Taita Hill forests hold a unique biodiversity with 13 taxa of plants and nine endemic animal species.

Although such inventories of aboveground biodiversity have been documented in this area, none of the studies focused on soil biodiversity despite its importance (Davet & Francis 2000; Moreira *et al.* 2006). Currently the forest area is under serious threat from fragmentation through agricultural activities leading to loss of biodiversity (Githiru & Lens 2007). Hence there is urgent need for documenting the belowground biodiversity.

Plant parasitic nematodes contribute greatly to loss of vegetable crops in Taita hills and the cost of production has

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increased due to the cost of chemical nematicides (Republic of Kenya, Taita Taveta District Development Plan 2002–2006). There is considerable concern about the use of chemical nematicides globally (Pinkerton *et al.* 2000), and some nematodes may have developed resistance to these chemicals (Kerry 2000; Larsen 2000). Thus, alternative nematode control strategies are being sought. About 70 % of fungal genera and 160 species are associated with nematodes but only a few of them are suitable for use in biological control of nematodes (Elshafie *et.al.* 2006). They continuously destroy nematodes in virtually all soils because of their constant interaction in the soil rhizosphere.

Nematode destroying fungi are regarded as cosmopolitan microfungi that are natural enemies of plant parasitic nematodes (Nordbring-Hertz *et al.* 2002). Some of these fungi use adhesive conidia, branches, knobs and mycelia to parasitize nematodes. These devices are used to capture nematodes by means of an adhesive layer covering part or all of the device surface (Yang *et al.* 2007). According to Luo *et al.* (2004), some of the fungi immobilize or kill nematodes by releasing toxins. Fungi in the genera *Harposporium*, *Nematoctonus* and *Meria* are known to parasitize nematodes (Jansson *et al.* 1985). This group of fungi has drawn much attention because of their potential as biological control agents of plant and animal parasitic nematodes (Araújo *et al.* 1999; Jansson & Persson 2000; Sanyal 2000; Masoomeh *et al.* 2004; Yan *et al.* 2005). They are also sometimes used as indicators of environmental pollution (Ming *et al.* 2006). The objective of this study was to determine the diversity and frequency of occurrence of nematode destroying fungi in the Taita district, Kenya.

Material and methods

The study was conducted in Taita district, Werugha location of Wundanyi division located at 03° 23′S 380 18′E, along a land use intensity gradient spanning from the valley bottoms of Werugha and the Ngangao forest, of the Taita hills (Beentje, 1988). The valleys are rich in vegetable cultivations which is the main economic activity of the Taita community (Pellikka *et al.* 2004). The study area was divided into the five main land use types: natural forest, shrub, vegetable, napier grass and maize/bean intercrop. The natural forest consisted of a broad diversity of indigenous trees including *Strombosia scheffleri* (*Olacaceae*), *Dicralonepis usambarica* (*Thymeleaceae*), *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*), *Sporobolus pyramidalis* (*Gramineae*) and *Ficus thoningii* (*Moraceae*). The vegetable gardens were mainly dominated by cabbage (*Brassica oleraceae*), spinach (*Chenopodium spinacia*), tomato (*Solanum lycopersicum*), kale (*B. oleraceae var. acephala*) and cucumber (*Cucumis sativus*). Different vegetable crops are grown separately under rotation cycles. Maize is commonly intercropped with beans, forming the main food crops in the study area. Napier grass fields (*Pennisetum purpureum*) are widely used as fodder for the dairy animals under restricted grazing systems.

Eight soil samples were randomly taken from each of the five land uses, for isolation of nematode destroying fungi. From each sampling point, five sub-samples were taken three meters from the centre. These were mixed homogeneously to constitute a composite sample from which 500 g soil was taken, placed in a plastic bag, sealed and then transported to the laboratory and stored at 10 °C before isolation of the nematode destroying fungi. The soil auger was sterilized in ethanol between sampling points.

Isolation of the fungi was done using the soil sprinkle technique as described by Jaffee (1996). Approximately 1 g of soil from each sampling point was sprinkled onto the surface. Tap water agar (20 g of agar (Biotec, UK) in L^{-1} tap water amended with 0.1 g L^{-1} streptomycin sulfate after autoclaving to suppress bacterial growth). A suspension of 1000 larvae of *Meloidogyne incognita* was added to the Petri dish as bait. The plates were incubated at room temperature, approximately 28 °C ± 1 and observed daily from the third week for 5–6 weeks, under a Carl Zeis ×40 dissecting microscope, for trapping organs, conidia and trapped nematodes.

After the sixth week, all the fungal colonies that had emerged were sub-cultured on potato dextrose agar (Fluka, India) for pure cultures and multiplication. To confirm the status of the fungi, observations were made daily. Records were made after the third day for trapped nematodes, trapping organs and conidia. Photographic records were made. Identification of the fungi was done using the key of Cooke & Godfrey (1964). Frequency of occurrence, evenness, Renyi profiles and the Shannon diversity index were determined (Kindt & Coe 2005).

Results

Eighty-five isolates of nematode destroying fungi were identified and grouped into 13 taxa and eight genera (Table 1). *Arthrobotrys* spp. represented by *Arthrobotrys dactyloides*, *Arthrobotrys oligospora*, and *Arthrobotrys superba*, were the most frequently isolated with a cumulative frequency of 61 %. *Harposporium* spp. (10.6 %) was represented by *Harposporium aungullilae, Harposporium liltiputanum* and *Harposporium* sp. The rest of the genera had cumulative frequency of less than 10.

A. oligospora had the highest frequency of occurrence, followed by *A. dactyloides*, *Monacrosporium cionopagum*, *A. superba* and *H. aungullilae*. They had an occurrence frequency of 32.9, 18.8, 9.4, 9.4 and 8.2 %, respectively. *Acrostalagums obavatus*, *Nematoctonus georgenious*, *Haptoglosa heterospora* and *Dactyllela lobata* all had an occurrence frequency of 3.5 % each having been recorded only three times. All the rest, *Nematoctonus leptosporus*, *Harposporium* sp., *Myzocytium* sp. and *H. liltiputanum* were recorded only once and had an occurrence frequency of 1.2 %. Eight of the fifteen species recovered were endoparasitic while the rest were trapping fungi, and different nematode destroying structures were recorded (Table 1).

The frequency of occurrence of nematode destroying fungi was significantly different among the land uses evaluated (p-value: 2.112×10^{-07}). The frequencies were 30.6, 27.1, 5.9, 10.6 and 25.9 % in vegetables, maize/bean, forest, shrub and napier land uses, respectively (Fig 1).

Table 1 – Frequency of occurrence of nematode destroying fungi in different land use systems in Taita Taveta district, Kenya

Species	No. of isolates	Percentage of isolation	Cumulative percentage	Nematode destroying mechanism
A. oligospora	28	32.9	32.9	Three dimensional adhesive network, non constricting rings
A. dactyloides	16	18.8	51.8	Constricting rings
M. cionopagum	8	9.4	61.2	Adhesive mycelia columnar
A. superba	8	9.4	70.6	Three dimensional adhesive network
Harposporium aungulilae	7	8.2	78.8	Endoparasitic: ingested conidia
Acrostalagums obavatus	3	3.5	82.4	Endoparasitic
N. georgenious	3	3.5	85.9	Endoparasitic
Haptoglosa heterospora	3	3.5	89.4	Endoparasitic producing elongate thalli in the nematode
Dactyllela lobata	3	3.5	92.9	Adhesive network
N. leptosporus	1	1.2	94.1	Endoparasitic, adhesive knobs on conidium
Harposporium sp	1	1.2	95.3	Endoparasitic; adhesive conidia
Myzocytium sp	1	1.2	96.5	Endoparasitic
Harposporium liltiputanum	1	1.2	97.6	Endoparasitic; adhesive conidia
Unidentified sp. 1	1	1.2	98.8	Endoparasitic
Unidentified sp. 2	1	1.2	100.0	Endoparasitic
Total	85	100.0		

The total number of nematode destroying species in all the land uses was 15 while overall richness and Shannon indices were 2.125 and 0.731, respectively. Richness and diversity varied significantly between different land uses (Table 2). Napier and vegetable had the highest number of species (10) while the forest had least (4). Vegetables had the largest richness and Shannon indices, with forest having the lowest.

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, shrub and forest respectively (Fig 2a). The evenness profiles shows that the forest was the most even followed by maize/bean, shrub napier and vegetable in that order (Fig 2b).

Discussion

The nematode destroying fungi comprise more than 200 species of taxonomically diverse fungi that all share the ability to infect and kill living nematodes. The study is of interest because of their potential use as biological control agents against both plant and animal nematodes (Larsen 2000, Yang *et al.* 2007). From the study, it is evident that all the sampled land uses differed in terms of occurrence of nematode destroying fungi, consistent with previous reports indicating that nematode destroying fungi were present in all habitats but at different densities and diversities (Nordbring-Hertz *et al.* 2002). However, contrary to expectation that beneficial microorganisms decrease with increased intensity in land use (Vandermeer *et al.* 1998), the diversity and richness of the nematode destroying fungi was higher in intensively cultivated lands, like the vegetable gardens, compared to the forest ecosystem. According to Wang *et al.* (2003), some agricultural inputs stimulate build-up of nematode trapping fungi hence the observed diversity, evenness and richeness with increased land use intensity compared to land uses which are materially unchanged by human activity (forest and shrub land). Also agricultural practices can exert positive or negative impacts on other microorganisms in the soil (Sanchez 1997; Akhtar & Malik 2000). Adhesive and ingestive conidia were found, but with a much lower frequency than other mechanisms. All the

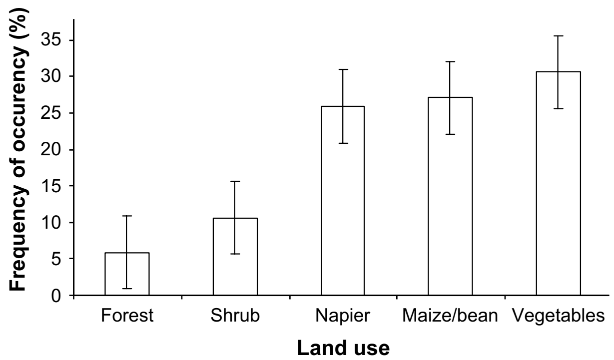

Fig 1 – Frequency of occurrence of nematode destroying fungi under different land use types in Taita Taveta, Kenya.

Table 2 – Effect of land use on richness and diversity of nematode destroying fungi in Taita Taveta district, Kenya

LUT	*n*	Mean richness	Mean Shannon
Forest	8	0.625	0.173
Maize beans	8	2.875	1.048
Napier	8	2.750	0.968
Shrub	8	1.125	0.311
Vegetables	8	3.250	1.156
P-value		2.112×10^{-7}	6.345×10^{-8}

LUT: land use type; *n*: number of samples.

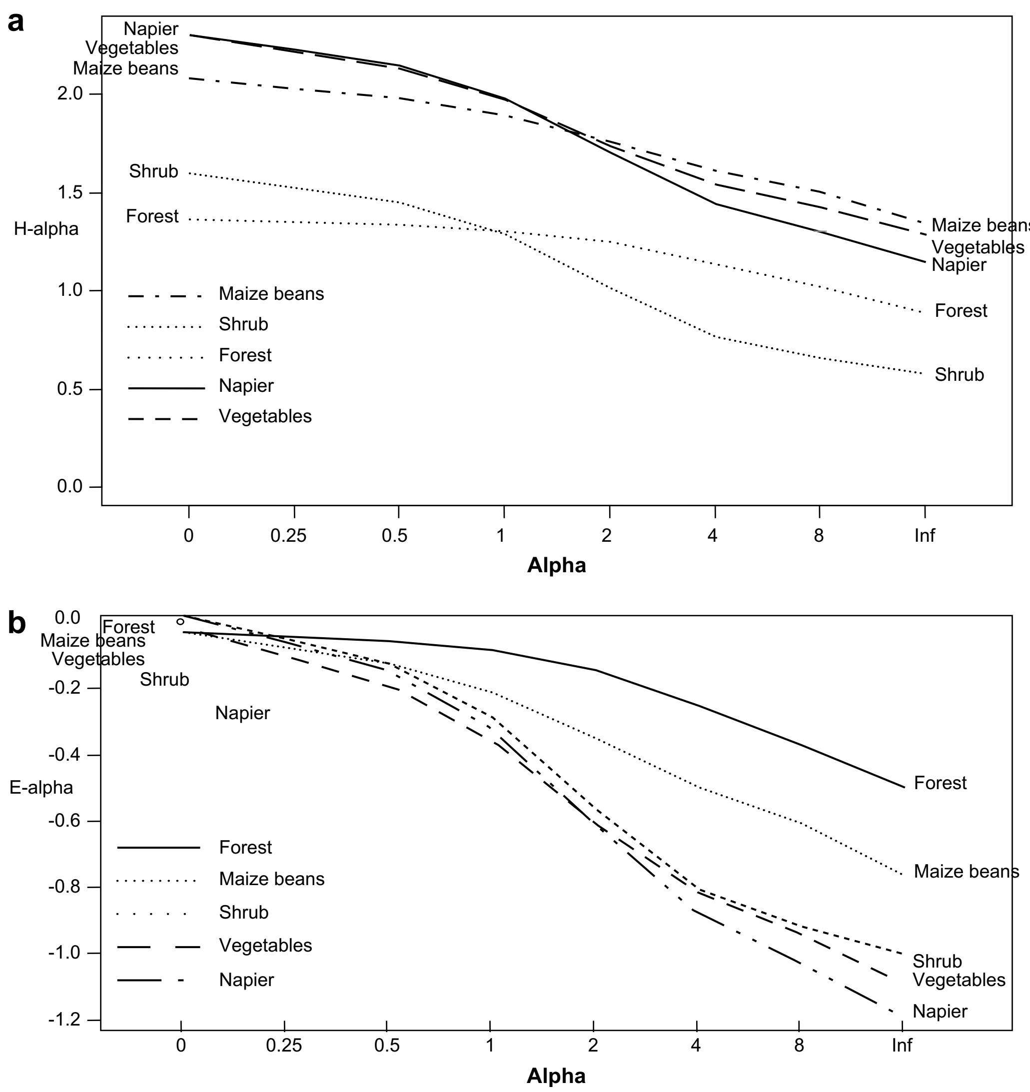

Fig 2 – (a) Renyi diversity profiles; (b) Renyi evenness profiles.

structures identified are consistent with the reports of other authors (Masoomeh *et al.* 2004; Farrell *et al.* 2006; Yang *et al.* 2007 and Jinkui *et al.* 2007), who identified three main groups of nematode destroying fungi – (1) the nematode trapping and (2) the endoparasitic fungi that attack vermiform living nematodes by using specialized structures, and (3) the egg- and cyst-parasitic fungi that attack these stages with their hyphal tips.

A. oligospora was the most commonly isolated nematode trapping fungus from this study (28 %). It was not clear why this was the dominant species. Similar observations were made by Jaffee and Strong (2005) and Jaffee *et al.* (1996). A possible reason might be the presence of organic matter and the utilization of two carbon sources, from organic matter (saprotroph) and also from trapping nematodes (parasite), making it adaptable to wide range of habitats. The species has been reported to be cosmopolitan in nature (Durand *et al.* 2005; Farrell *et al.* 2006). Its *in vitro* activity of nematode destruction was fascinating, being able to destroy the nematodes within 12 h. It has been reported to have trapped 90 % of all the nematodes (in Petri dish and in liquid culture) in 16–40 h (Rajeswari & Sivakumar 1999). This fungus would be recommended for further study with the aim of developing it as a biological control agent. Such a study should be geared towards growth parameters of the fungus, since biological, chemical and physical factors of the soil are known to inhibit fungal growth by fungistatic compounds (Xu *et al.* 2004; Yang *et al.* 2007), and this is made even more complicated by crop rotations. The ability of this fungus in biological control could be improved through genetic engineering and then packaged as a biological control agent (Yang *et al.* 2007).

There is no evidence so far to support the suggestion that the high numbers of nematode destroying fungi observed in the field result in suppression of plant parasitic nematodes, as our work did not look at the population changes of both the nematodes and the nematode destroying fungi. Strong indications of nematode trapping fungi suppressing nematodes have been obtained in the laboratory (Elshafie *et al.* 2006). Jaffee & Strong (2005) and Jaffee *et al.* (2007) found no direct involvement of nematode destroying fungi in nematode suppression in Bodega Marine Reserve. More studies on biological interactions are recommended in this area. Studies should focus on improving the fungal quantification method (Jaffee *et al.* 1996).

Conclusion

The study area is rich in diversity of nematode destroying fungi which are naturally occurring and widespread in agricultural and forest habitats. The results from this study could be used to formulate alternative nematode destroying practices which could be environmentally friendly and affordable by the farmers. Land use practices that promote high populations of the nematode destroying fungi would also be recommended for the farmers to boost occurrence of nematode trapping fungi in the soil.

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