



EFFECT OF SOIL FERTILITY MANAGEMENT PRACTICES ON NEMATODE DESTROYING FUNGI IN TAITA, KENYA

[EFECTO DE PRÁCTICAS DE MANEJO DE LA FERTILIDAD DEL SUELO SOBRE HONGOS NEMATÓFAGOS EN TAITA, KENIA]

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SUMMARY

The study aimed at identifying soil fertility practices that promoted nematode destroying fungi in the soil and the treatments comprised of Mavuno fertilizer, Triple super-phosphate and calcium ammonium nitrate (TSP+CAN), cow manure and a control where no amendments were applied. This experiment was replicated in ten farms for three planting seasons.

There were significant difference ($P= 1.705 \times 10^{-06}$) in occurrence of the nematode destroying fungi between soil fertility treatments. The highest mean occurrence of nematode destroying fungi was 1.6 which was recorded in soils amended with cow manure and the least was in soils from the control plots. A mean of 0.78 was recorded in soils from both TSP+CAN and Mavuno fertilizers. Plots amended with cow manure gave the highest diversity of nematodes followed by the control, then TSP+CAN and least in Mavuno with shannon indices of 0.34, 0.15, 0.13 and 0.11 respectively. Sixty percent of all the isolated nematode destroying fungi genera were from plots treated with cow manure and only twenty percent were from plots amended with the inorganic fertilizer.

Key words: Nematode destroying fungi; *Arthrobotrys oligospora*; organic amendments; plant parasitic nematodes.

INTRODUCTION

Taita district is a horticulture production zone where farmers experience high losses caused by the plant-parasitic nematodes (Mutsotso *et al.*, 2005). This has led to increased cost of production through purchase of nematicides (Taita District Development Strategies 2002-2006). A survey conducted in the area revealed that farmers used inorganic fertilizers, pesticides and non-conventional methods to control nematodes and increase soil fertility (Mutsotso *et al.*, 2005). Increasingly environmental concern on the use of nematicides have been reported (Pinkerton *et al.*,

2000; Kerry, 2000; Larsen, 2000) which has prompted the search for alternative methods which are cost effective and environmental friendly. About 70% of fungi genera are natural enemies of plant parasitic nematodes and they have drawn much attention because of their potential as biological control agents of nematodes that are parasitic on plants and animals (Jansson and Persson, 2000; Sanyal, 2000; Birgit *et al.*, 2002, Masoomah, *et al.*, 2003; Yan *et al.*, 2005).

Although the use of inorganic fertilizers and chemical pesticides have led to considerable increases in overall food production worldwide, they have disregard the potential benefits of soil biological activities in maintaining soil health. Furthermore, overuse of these chemicals led to soil and environmental degradation (i.e., depletion or loss of soil fertility and its physical and biological components, contamination of surface and ground water) and declines in productivity in certain areas of the world (Vandermeer *et al.* 1998). In addition, the vast majority of the world's farmers particularly in the developing countries have limited access to, external inputs necessary to apply the principles and practices of high external input agriculture (Vandermeer *et al.*, 1998). Therefore, soil organic matter was thought to be a key factor in plant production, since it represents the main source of mineral nitrogen assimilable by crops (Chotte *et al.*, 1998). There is evidence that soil biotic communities are associated with the aboveground vegetation (Lavelle, 2000). The high occurrence of plant parasitic nematodes in sin intensively cultivated soils of Taita Taveta could be associated with land management practices which alter soil conditions and this justifies the need to study the belowground biodiversity of these oils found at the study site (Ramakrishnan *et al.*, 2005; Saha, 2009). The aim of the study was to identify the soil fertility management which favored buildup of nematode destroying fungi in the soil. The aim was to explore potential of NDF for use as biological control agents of plant parasitic nematodes.

MATERIALS AND METHODS

The farms were located in both Werugha and Wumingu locations of Wundanyi division, Taita district. They were divided into four plots measuring 3 x 3 meters and separated by one meter path around each plot. In one of the plots, nine kg of cow manure were broadcasted all over the plot (10 tons per ha), on the next, 0.8 kg per plot of triple super phosphate (TSP) and 0.5 kg per plot of calcium ammonium nitrate (CAN) and 0.9 kg per plot Mavuno fertilizer (blend of fertilizers containing 11 nutrients) to a third plot and these were spread uniformly over the plots. A control plot where not amendments were applied was maintained. This experiment was replicated ten times.

The plots were then planted with maize, Hybrid (H513) at a spacing of 90 x 30 cm, two seeds per hole and beans (Mwezi moja) at spacing of 30 cm in alternate rows. Soil sample was collected from each corner of the plot, then at half length of each side and at the middle of the plot. This gave a total of nine sub samples. The soils were collected at two levels, 0 – 10 cm and 10 – 20 cm. The two composites samples from the nine sub samples of soil collected from 0 – 10 cm and from 10 – 20 cm were used to estimate nematode destroying fungi during three consecutive seasons. The soils were collected after maturity of maize (during harvest period of maize). The soils were transported to the laboratory and kept in a cold room at about 10°C before isolation of the nematode destroying fungi.

Isolation of the fungi was done using the soil sprinkle technique 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°C 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. A pure culture of plant parasitic nematodes (*Meloidogyne* spp.) was added into the Petri dish as baits. The plates were incubated at room temperature and observed daily under a microscope at low magnification, from the third week up to the 6th week. The examinations were 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 (PDA) to obtain pure cultures. Identification was carried as according to the key described by Cook and Godfrey, 1964.

Data analysis

Occurrence and diversity of nematode destroying fungi was compared using Frequency of occurrence,

evenness, Renyi profiles and the Shannon diversity index (Kindt & Coe 2005). Principal Component Analysis and Multivariate analysis using ADE4 software were done on the temporal association of nematode-trapping fungi and soil fertility treatments (Thioulouse *et al.*, 1997).

RESULTS

Soil fertility management practices caused significant ($P= 1.705 \times 10^{-06}$) difference on the occurrence of nematode destroying fungi (NDF) across the treatments. The highest occurrence of NDF was in plots treated with cow manure. Although the farmer practice (TSP + CAN) and Mavuno fertilizer were significantly different, TSP + CAN had a higher occurrence of NDF than Mavuno, while the control had the lowest (Fig. 1).

Plots treated with cow manure had the highest diversity of the fungi followed by control plots and Mavuno the lowest (Table 1).

Table 1. Effect of soil fertility treatment on richness and diversity of nematode destroying fungi

Treatment	Mean richness	Mean Shannon
Control	0.72	0.145
TSP + CAN	0.77	0.134
Cow manure	1.5	0.338
Mavuno	0.78	0.106

Although there were no significant differences occurrence of NDF in the two soil depths, (0-10 and 10 – 20), the 0 – 10 centimeters level had higher mean compared to level 10 – 20 centimeters with 1.02 and 0.9 being recorded in level 0-10 and 10-20 respectively. There was no significant difference on seasonal variation of NDF. Some NDF were not affected by the interventions (Table 2).

The nematode trapping fungi were more sensitive to soil fertility improvement interventions. Except for *Acrostalamus ganoides* all the endo parasitic nematode destroying fungi were not affected by the fertility management. Addition of cow manure in the soil increased the chances of isolating NDF and their diversity in the soils are presented in Fig.2.

Addition of cow manure in the soil accounted for 61.98 of the diversity of nematode destroying fungi in the soil (Fig.3). It was observed that 60 % of the isolated genera were present in soils amended with cow manure while the remaining 40 % was due to inorganic fertilizer and control in equal proportions which accounted for 28.73% of the NDFs. *Monacrosporium cionopagum* and *Arthrobotrys*

longispora species were more responsive to application of inorganic fertilizers while *Arthrobotrys superba* and *Nematoctonus georgenous* were prevalent where no chemical fertilizer had been applied. Mavuno inorganic fertilizer supported proliferation of NDFs compared to the TSP+CAN (Fig. 3).

A total of 218 isolates were identified with 58 % of the isolates belonging to the genus *Arthrobotrys* with *A.*

oligospora being the most frequently isolated species with 44% occurrence. Other species isolated were, *A. longispora*, *A. oligospora*, *A. dactyloides* and *A. superba*. Distribution of other genera in the soils was as presented in Figure 4.

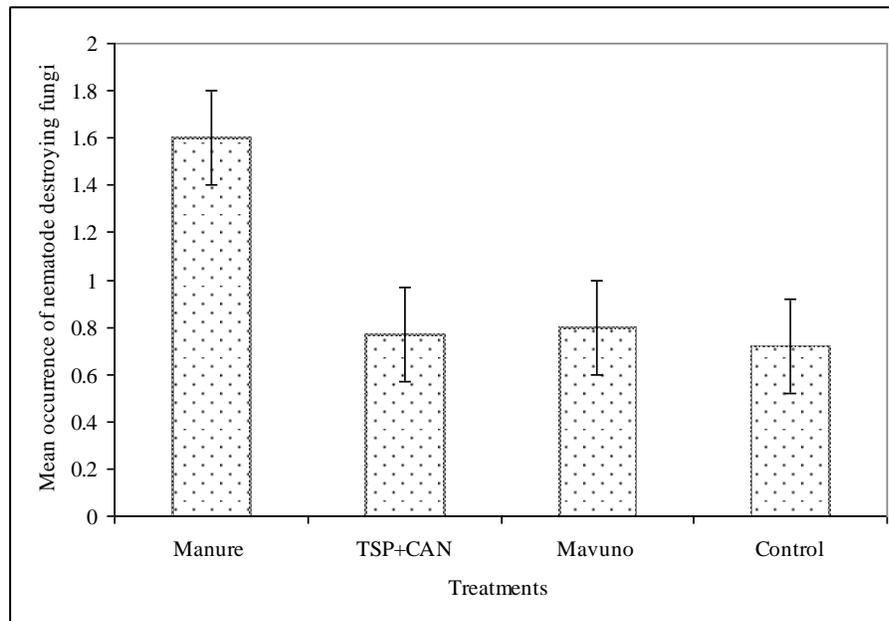


Figure 1. Effect of soil fertility amendments on mean occurrence of nematode destroying fungi.

Table 2. Effect of soil fertility amendments on occurrence of nematode destroying fungi species.

Treatment	<i>A l</i>	<i>A o</i>	<i>A d</i>	<i>A g</i>	<i>A s</i>	<i>H a</i>	<i>M c</i>	<i>M a</i>	<i>N g</i>
Control	0.02	0.3	0	0.05	0.12	0	0.1	0.03	0.12
TSP+CAN	0.07	0.3	0.02	0.02	0.3	0.02	0.15	0.02	0.12
Manure	0.01	0.6	0.1	0.1	0.13	0.18	0.13	0.07	0.13
Mavuno	0.1	0.4	0	0	0.02	0.02	0.15	0.05	0.02
P <0.05	0.05	0.005	0.002	0.013	0.03	4.819 x 10 ⁻⁶	0.8	0.56	0.12

A l - *Arthrobotrys longispora*

A o - *Arthrobotrys oligospora*

A d - *Arthrobotrys dactyloides*

A g - *Acrostalgmus goniodes*

A s - *Arthrobotrys superba*

H a - *Harposporium aungullilae*

M c - *Meria coniospora*

M a - *Meristacrum asterospermum*

N g - *Nematoctonus georgenous*

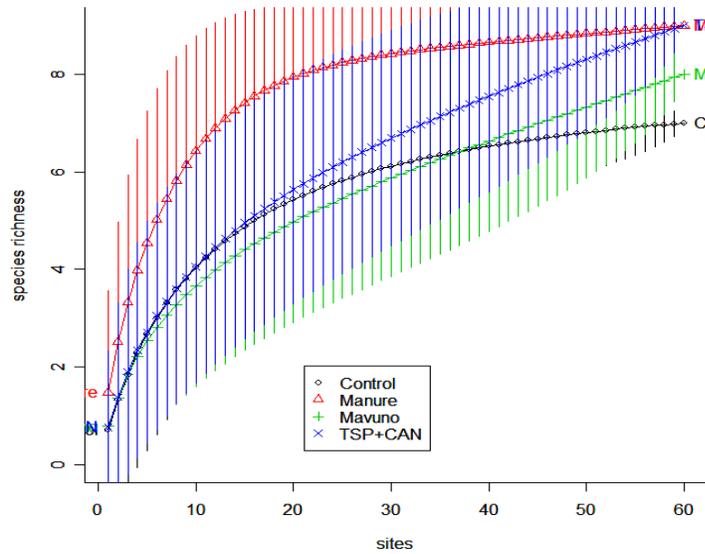


Figure 2. Nematode destroying fungi species cumulative curve as affected by soil fertility management practices.

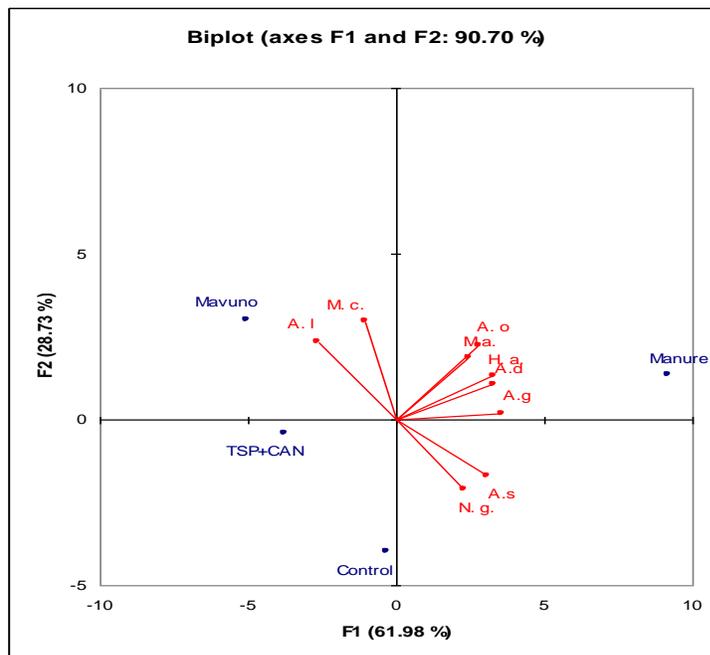


Figure 3. Principle Component Analysis on nematode destroying fungi and the soil fertility management practices where:

- A l* - *Arthrobotrys longispora*
- A o* - *Arthrobotrys oligospora*
- A d* - *Arthrobotrys dactyloides*
- A g* - *Acrostalgnus goniodes*
- A s* - *Arthrobotrys superba*
- H a* - *Harposporium aungullilae*
- M c* - *Meria coniospora*
- M a* - *Meristacrum asterospermum*
- N g* - *Nematoctonus geogenus*

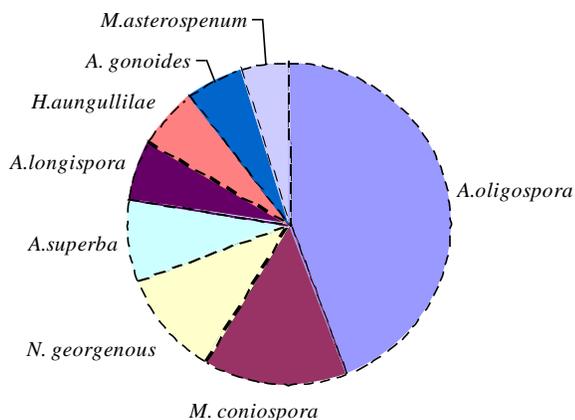


Figure 4. Proportion of the identified NDF species in Taita Taveta soils.

DISCUSSIONS

Nematode destroying fungi occurred in all farms however, their diversity varied with depth of soil, soil fertility management regime and the season. Gray and Bailey (1985) did not find any significant differences in the vertical distribution of the nematophagous fungi at 0-35 cm depths. However, they reported that the majority of the fungi were found in the upper organic level (0- 10). This could be explained by the fact that this is the zone with high organic matter contents which favor active microbial community with greater biomass of organisms including the nematodes which are their source of nutrition. *A. oligospora*, a ring former was frequent in this layer than in the second layer. This fungi was also reported to have been enhanced through addition of organic amendments in agricultural soil (Jaffee, 2004). The presence of nematodes and plants roots on the surface soil may have also caused this observation. Nematodes are usually attracted to the nematode destroying fungi and studies on cell biology on the interactions between roots cells and nematophagous fungi have indicated that the fungi have endophytic behavior in root cells (Lopez-Llorca *et al.*, 2002). In the lower horizons, the nematode destroying fungi survive as saprophytes enabling them to compete effectively with the other organisms for available organic matter, moisture and soil nutrients.

The number of nematode destroying fungi increased each season in the plots treated with cow manure which could be explained by the fact that there was a buildup of the organic materials in the soil coupled by residual effect. In other works, highly decomposed manure had a high preference of both the nematodes and nematode destroying fungi (Mahoney and Strongman 1994).

Addition of organic or inorganic amendments to the soil cause differences in occurrence, richness, diversity and evenness of nematode destroying fungi (Wang *et al.*, 2003). These different additions to the soil exert positive or negative impacts on the microorganisms in the soil (Sanchez 1997; Akhtar and Malik 2000). Additions of organic amendments in the soil have also been shown to stimulate the resident nematode destroying fungi (Wachira *et al.*, 2009). In the context of agrarian practices in organic agriculture, use of organic amendments is considered a way to restore biodiversity in the edaphic environment (Garcia *et al.*, 2004). Jaffee *et al.*, 1998, demonstrated that organically managed plots had slightly higher number of nematode destroying fungi than the convectional plots which were treated with inorganic fertilizers, which was also confirmed in this study.

CONCLUSION

It is evident that integrated soil fertility management practices have an impact on nematode destroying fungi. Use of animal manures could restore and maintain effective populations of natural plant parasitic nematode regulatory processes in the soil.

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