
Survey of Nematode Destroying Fungi from Selected Vegetable Growing Areas in Kenya

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Abstract: Plant parasitic nematodes cause up-to 5% yield losses to a wide range of economic crops. In Kenya vegetables yield loss attributed to plant parasitic nematodes is estimated to 80%. Over the years, nematode control has heavily on the use of chemical nematicides which unfortunately leads to biological magnification and elimination of the beneficial microorganisms in the soil. This has triggered a growing interest in search of alternate management strategies. The objective of this study was, therefore, to document nematode destroying fungi in selected major vegetable growing areas in Kenya as a step towards developing self-sustaining system for management of plant parasitic nematodes. Soil samples were collected from five vegetable production zones in Kenya which were Kinare, Kabete, Athi-river, Machakos and Kibwezi and transported to the laboratory for extraction of the nematode destroying fungi. Soil sprinkle technique as described by Jaffee *et al.*, (1996) was used to isolate the fungi from the soil while identification was done using identification keys described by Delgado *et al.*, (2001). From the study a total of 171 fungi isolates were identified as nematode destroying fungi. The highest population was recorded at Kabete area recording 33.9% of the total record, followed by Machakos, Kibwezi, Athi-river and the least in Kin are with 24.6, 22.2, 11.7 and 7.6% of the total population in that order. *Arthrobotrys* was the most frequent genera with a mean occurrence of 7.3 followed by *Monacrosporium* with 6 and *Stylophage* with 5.2. *A.dactyloides* was significantly (P=0.002) affected by the agro-ecological zones with the highest occurrence being recorded in Kabete and the least in Athi-river. The highest diversity index and species richness of nematode destroying fungi was recorded in Kibwezi while the least was recorded in Athi-river. The genera *Arthrobotrys* had the highest number of trapped nematodes with a total population of 57, followed by *Monacrosporium* and least was *Stylopage* with 45 and 36 respectively, within a period of 104 hours. From the study, it is evident that agricultural practices affect the occurrence and diversity of nematode destroying fungi and *Arthrobotrys* can be developed as a bio-control agent for management of plant parasitic nematodes.

Keywords: *Arthrobotrys*, Biological Control, Plant Parasitic Nematodes

1. Introduction

Horticultural crops both for local consumption and export are important in Kenya. One-tenth of vegetables in Kenya are grown for export. They are recognized for their health and nutritional benefits; provide cash income and employment for close-to two million people in Kenya (Dobson, *et al.*, 2004). Production of vegetables in Kenya, especially for the expanding domestic market, is still limited by major pest and disease problems (Dobson, *et al.*, 2004). Plant parasitic nematodes have been identified as a major production constrains affecting vegetable production, reducing its yield quality and quantity (Nchore *et al.*, 2010). They are responsible to up to 80%, on vegetables production

(Kaskavalci, 2007).Vegetable production in Kenya is characterized by high chemical inputs for pest and soil fertility management (Mutsotso *et al.*, 2005). These practices have been associated with increases in soil born disease and decline in beneficial soil microorganisms (Wachira *et al.*, 2008). Specifically vegetable damage by Root-Knot nematodes in Kenya has been reported with infected plants being rendered unacceptable for international markets (Nchore *et al.*, 2010). The root knot nematodes increase wounding of the root system providing points of ingress for the pathogen. They may also modify the tissue becoming more suitable for bacteria colonization (Hayward, 1991). Globally it is estimated that US\$500 million is spent on root-knot nematode control (Keren-Zur *et al.*, 2000, Pinkerton *et*

al. 2000.) where range of strategies are used to control them. These include the use of nematicides, organic manure amendment and resistant cultivars. Overall, although nematicides are effective in managing root-knot nematodes and other plant parasitic nematodes, they are expensive and environmental pollutants when not applied at the right time, in the right way and in the right dosage increasing the cost of production and reducing the profit for the farmers (Republic of Kenya, Taita District Development Strategies 2002-2006). Their use is also curtailed by their threat to groundwater, soil biodiversity as well as long waiting periods between use, harvesting and marketing of crops (Bridge, 1996).

Alternatively, soil beneficial microorganisms could be used to reduce the effect of plant parasitic nematodes hence, reducing the application of chemical nematicides to the soil. The beneficial microorganisms are non-polluting and thus environmentally safe and acceptable. Usually they are species specific to target pest and therefore no chances affecting non target species unlike the chemicals which are broad spectra in action. 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 naturally capture, kill and digest nematodes in the soil (Rodrigues *et al.*, 2001, Nordbring-Hertz *et al.*, 2002). They comprise three main groups of fungi, the nematode trapping, the endoparasitic fungi and the egg- and cyst-parasitic fungi (Nordbring-Hertz *et al.*, 2002; Masoomah, *et al.*, 2004). After trapping the nematodes, they penetrate the cuticle and invade the entire body cavity and then digest them completely. This group of fungi has drawn much attention due to their potential for development as biological control agents of plant parasitic nematodes (Jansson and Persson, 2000; Sanyal, 2000; Masoomah, *et al.*, 2004). About 70% of fungi genera and 160 species are associated with nematodes but only a few of them can be used as biological control agents of nematodes (Elshafie *et al.* 2006). This study was therefore aimed at documenting the occurrence and diversity of nematode destroying fungi and testing their as bio-control against plant parasitic nematodes.

2. Materials and Methods

Soil samples were collected from five different vegetable growing areas in Kenya. These areas were Kinare, Kabete, Athi-river Machakos and Kibwezi in the order of altitude and temperature. Kinare was a high altitude area and the coldest among the zones and Kibwezi being the lowest and hottest. The vegetable gardens in each zone were mainly dominated by spinach, kales, tomatoes, cabbage and pepper among other vegetables. From each of the study areas five farms under intensive vegetable production were randomly selected for this study. From each of the farm, five different vegetable gardens were sampled. From each vegetable garden, five soil samples were collected and mixed in a bucket to make one composite sample. One kilogram of soil was then re-sampled from the composite sample in the bucket, put in plastic bags,

labeled and placed in a cool box. Soil sampling was done using a soil auger which was sterilized using ethanol after every sampling point to avoid cross contamination. All the samples were later transported to the laboratory for isolation of nematode destroying fungi.

Isolation of nematodes destroying fungi was done using the soil sprinkle technique as described by Jaffee *et al.*, 1996 where tap water agar (TWA) was prepared by dissolving 20 g agar in one liter of tap water. The medium was autoclaved and cooled before use after amending it with 0.1 g l⁻¹ of streptomycin sulfate under a laminar flow. One gram of soil sample was sprinkled on the medium in the petri dish and a suspension of *Meloidogyne* species of approximately 1000 nematodes was added into the petri dishes as baits (Christina *et al.*, 1999). The plates were then incubated at room temperature and observed daily from the third week up to sixth week under a dissecting microscope. The examination was focused on trapped nematodes, trapping organs and conidia of the nematodes destroying fungi (Wachira *et al.*, 2008).

Taxonomic classification of the nematode destroying fungi was done using the slide culture technique where slides were observed under a microscope while identification of the genus was done using identification keys described by Delgado *et al.*, (2001). After identification of nematode destroying fungi; pure cultures of the three most frequently isolated fungi isolates were made for efficacy experiment. A mycelia block (5 mm) was inoculated into PDA media in a petri dish and allowed to grow for five days before approximately 50 plant parasitic nematodes were added. The efficacy of the fungi isolates was monitored for a period of 3-6 weeks. Trapped nematodes were counted for five days, after 3 weeks of incubation. All the data in this study was analyzed through analysis of variance (Kindt & Coe 2005)

3. Results

171 fungi isolates were identified as nematode destroying fungi. They were grouped into three genera and five taxa. The three genera were *Arthrobotrys*, *Monacrosporium* and *Stylopage*. *Arthrobotrys* was the frequently encountered fungi genera. This genera was represented by, *A. oligospora*, *A. dactyloides* and *A. longispora*, the genera *Monacrosporium* was represented by *M. cionopagium* while genera *Stylopage* was represented by *Stylopage grandis*. *A. oligospora* had the highest frequency of occurrence followed by *A. dactyloides*, *M. cionopagium*, *S. grandis* and the least was *A. longispora* with occurrence frequencies of 46.20, 45.61, 5.85, 1.17 and 1.17% in that decreasing order (Fig 1).

The fungi isolates were recovered in all the vegetable production zones. Except for the *A. dactyloides*, all the isolates were not significantly ($P > 0.05$) affected by the agro-ecological zones. The highest occurrence of *A. dactyloides* was 40 being recorded in Kabete while the least was 4 being recorded in Athi-river. This species was also recorded in Machakos, Kibwezi and Kinare with records of 19, 10 and 5, respectively, in that decreasing order. Among all the isolates,

only *A. oligospora* and *A. dactyloides* occurred in all agro-ecological zones. *M. cionopagium* occurred in all zones except in Kinare while *S. grandis* was present in both

Kibwezi and Kinare with *A. longispora* being recorded in Kibwezi only (Table 1).

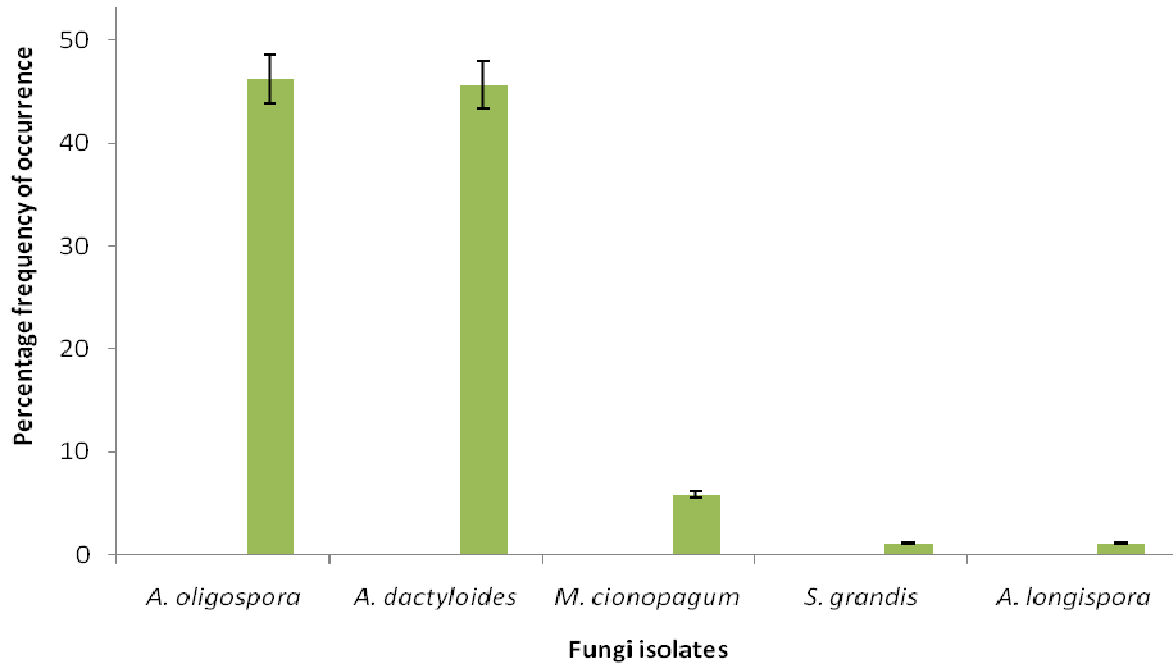


Figure 1. Percentage occurrence of nematode destroying fungi in vegetable production areas in Kenya.

Table 1. Occurrence of nematode destroying fungi in major agro-ecological zones in Kenya.

Zone	<i>A. dactyloides</i>	<i>A. oligospora</i>	<i>A. longispora</i>	<i>M. cionopagium</i>	<i>S. grandis</i>	Total
Kabete	20	17	0	1	0	58
Machakos	19	22	0	1	0	42
Kinare	5	7	0	0	1	13
Kibwezi	10	20	2	5	1	38
Athi-river	4	13	0	3	0	20
P.value	0.002	0.395	0.062	0.165	0.062	

The highest number of nematode destroying fungi was recorded in Kabete (Table 1), followed by Machakos, Kiwezi, Athi-river and finally Kinare with total mean abundance of 11.6, 7.6, 7.4, 4.0, and 2.6 in that decreasing order (Table 2). Kibwezi recorded the highest diversity index with a mean of 0.930, then Machakos with 0.637 while Kabete recorded the least diversity index mean of 0.411. Mean richness and

abundance varied between the vegetable production zones. The highest mean species richness was recorded in Kibwezi while the least was recorded at Athi-river. All the agro ecological zones differed significantly ($P = 9.587 \times 10^{-4}$) in terms of species abundance. Kabete had the highest species abundance with a mean of 11.6 and least was Kinare with a species mean abundance of 2.6 (Table 2)

Table 2. Mean shannon, species richness and abundance of nematode destroying fungi in different vegetable growing areas in Kenya.

Zone	N	Mean Shannon	Mean richness	Mean abundance
Kibwezi	5	0.930	3.4	7.6
Machakos	5	0.637	2.2	7.4
Athi-river	5	0.483	1.6	4.0
Kinare	5	0.482	1.8	2.6
Kabete	5	0.411	2.2	11.6
P value				9.587×10^{-4}

Detection of nematode destroying fungi increased with increase in number of the soil samples taken. It was evident that all possible isolates of nematode destroying fungi were recorded in this study from the samples collected. Collecting

and processing additional samples would not significantly increase the number of isolates (Fig 2).

There was a significant ($P=0.003$) difference on efficacy between the three most frequent nematode destroying fungi

species. *Arthrobotrys oligospora* was the most efficient nematode destroying fungi with a mean of 7.3, followed by

Monacrosporium and the least was *Stylopaga* with mean records of 5.9 and 5.1 respectively.

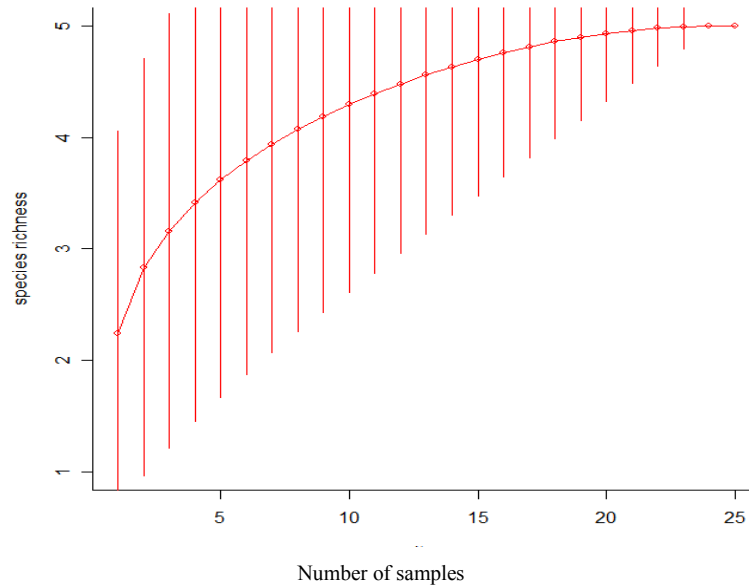


Figure 2. A total species cumulative curve for nematode destroying fungi in the vegetable production zones in Kenya.

4. Discussion

Nematode destroying fungi were isolated from all the targeted vegetable production zones. They occurred in different frequencies and diversity. The study has demonstrated the diverse occurrence of nematode destroying fungi in nature and especially in vegetable production zones. These findings agree with previous reports on nematode destroying fungi that they are widespread in all habitat but with different densities and diversities (Birgit *et al.*, 2002, Wachira *et al.*, 2008).

Arthrobotrys oligospora was the most abundant species of nematode destroying fungi in the study area. Other studies on nematode destroying fungi have made the same observation. It has never been clear why this is the most frequently encountered fungi. Wachira *et al.*, (2008) had suggested that farming practices like weeding could be the cause of this high occurrence. It has also been suggested that it is due to its ability to exist both as a saprophyte and a plant parasitic nematode feeder (Sobita and Anamika, 2011). Due to this high occurrence, this fungus is attracting a lot of other interesting studies (Niu and Zhang, 2011).

It was expected that the highest number of fungi would be isolated from areas with low temperature (Kinare). However this was not the case. Kinare had the least number of fungi. This was attributed to the high use of chemical fertilizers and pesticides since all the vegetables were all aimed for market. In a study on long-term effects of manures and fertilizers on soil productivity and quality, it was reported that fertilized soils had lower contents of organic matter and numbers of microfauna than manured soils (Edmeades, 2003). The highest number of nematode destroying fungi was recovered from Kabete. Soils in this area had been collected from the University of Nairobi farm where animal manure is

frequently applied. This may explain the high number of nematode destroying fungi since they have been associated with increase of beneficial microorganisms in the soil (Wachira and Okoth, 2009). From this study, there was high fungal population in areas where manure was applied and low fungal population being recorded in areas where chemical fertilizer was applied.

Temperature is an important factor regulating microbial activity and shaping the soil microbial community. It determines moisture levels in the soil which is key to fungal germination and growth. High temperatures lead to low soil moistures which is attributed to low fungal spore germination. A study by Haugen and Smith (1992) reported that at high temperatures there is low germination of fungi spores leading to low fungi populations and while in low temperatures there is high fungal germination leading to high fungal population. This was reported in reverse in our study. Although Machakos and Kibwezi had high temperatures, high population of nematode destroying fungi was reported. This could be attributed to irrigation activities which ensured moist conditions throughout the growth season. This soil moisture, coupled with high temperature enhanced the fungal germination since fungal spore germinates better in moist and warm conditions.

The efficacy test showed that genus *Arthrobotrys* was the most effective in trapping plant parasitic nematodes. Previous studies on fungi belonging to these genera have consistently showed that it was able to trap 90% of all the nematodes in petri dish and in liquid culture in 16-40 hours (Rajeswari and Sivakumar, 1999). Therefore, due to its' high occurrence, this fungi can be developed for the management of plant parasitic nematode and its potential should be investigated. The investigation should concentrate on suitable carriers for the fungi and also mode of application. This would reduce over

reliance on chemical nematicides and also develop a self regulation system in the soil that controls the soil born pests.

5. Conclusion

Additional evidence has been provided from this study that nematode destroying fungi are naturally occurring in agricultural habitats. It is also evident that agricultural activities targeting high crop production, like application of chemical fertilizers and pesticides are directly affecting the soil biodiversity. The results of this study can be used for further research to establish the potential of nematode destroying fungi in regulation of plant parasitic nematodes.

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