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Survey of nematode-destroying fungi from selected vegetable-growing areas in Kenya

P.M. Wachira, J.N. Muindi and S.A. Okoth
University of Nairobi, P.O. Box 30197, Nairobi, Kenya
Email: pwachira@uonbi.ac.ke

ABSTRACT

Plant-parasitic nematodes cause severe damage to a wide range of economic crops, causing upto 5% yield losses globally. In Kenya, vegetables are affected, among other pests, by parasitic nematodes, causing upto 80% loss in yield. Nematode control is very difficult and relies heavily on use of chemical nematicides. Use of these chemical nematicides leads to biological magnification, and elimination of natural enemies of other pathogens, thus creating a need for greater application of pesticides, increased production costs, and development of insecticide-resistance. These factors have led to a growing interest in search for 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 a self-sustaining system for management of plant-parasitic nematodes. Soil samples were collected from five vegetable-production zones, viz., Kinare, Kabete, Athi-river, Machakos and Kibwezi, and transported to the laboratory for extraction of nematode-destroying fungi. The soil-sprinkle technique described by Jaffee *et al* (1996) was used for isolating the nematode-destroying fungi from soil, while, their identification was done using identification keys described by Soto Barrientos *et al* (2001). From this study, a total of 171 fungal isolates were identified as nematode-destroying. The highest population was recorded in Kabete, at 33.9% of the total, followed by Machakos, Kibwezi, Athi-river, with the least in Kinare, at 24.6, 22.2, 11.7 and 7.6% of the total population, in that order. *Arthrobotrys* was the most frequent genus, with 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 zone, with the highest occurrence recorded in Kabete, and the least in Athi-river. Kibwezi recorded highest diversity index, with a mean of 1.017, while, Athi-river recorded the least, with a mean of 0.333. Kibwezi had the highest species richness, recording a mean of 3.4, while, the least mean of 1.6 was recorded in Athi-river. Mean species richness of 2.2 was recorded for both Kabete and Machakos, and 1.8 for Kinare. From the three genera recorded, *Arthrobotrys* was more effective at trapping nematodes compared to *Monocrosporium* and *Stylopage*. The genus *Arthrobotrys* had the highest number of trapped nematodes, with a total population of 57, followed by *Monacrosporium,* the least being *Stylopage*, with 45 and 36, respectively, in a period of 104 hours. From the study, it is evident that agricultural practices affect occurrence and diversity of nematode-destroying fungi, and, *Arthrobotrys* can be used as a bio-control agent for managing plant-parasitic nematodes.

Key words: *Artabotrys*, biological control, plant-parasitic nematodes

INTRODUCTION

Horticultural crops, both for local consumption and export, are important in Kenya. One-tenth of the vegetables in Kenya are grown for export. They are recognized for their health and nutritional benefits, and provide cash income and employment for close to two million people in Kenya (Dobson *et al*, 2004). Production of vegetables in Kenya, especially for an expanding domestic market, is even now limited by major pests and diseases (Dobson *et al*, 2004). Plant-parasitic nematodes have been identified as a major production constraint, affecting vegetable production, resulting in reduced yield quality and quantity (Nchore *et al*, 2010). They are responsible for upto 80%, on vegetable production (Kaskavalci, 2007). Vegetable production in Kenya is characterized by high chemical input for pest and soil fertility management (Mutsotso *et al*, 2005). These practices have been associated with increase in soil-borne diseases and decline in beneficial soil micro-organisms (Wachira *et al*, 2008). Specifically, vegetable damage by root-knot nematode has been reported in Kenya, with infected plants rendered unacceptable to international markets (Nchore *et al*, 2010). The root knot nematode increases wounding of the root system, thus providing points of ingress of the pathogen. The nematode may also modify the tissue in a way that it becomes more amendable to bacterial colonization (Hayward, 1991). Globally, it is

estimated that US $ 500 million is spent on root-knot nematode control strategy (Keren-Zur *et al,* 2000; Pinkerton *et al,* 2000), including use of nematicides, organic-manure amendment and use of resistant cultivars (Akhtar & Malik, 2000). Overall, though nematicides are effective in managing root-knot nematode and other plant-parasitic nematodes, they are expensive and become environmental pollutants when not applied at the right time, in the right manner and in the right dosage. This increases cost of production to the farmers, reducing their profit (Republic of Kenya, Taita District Development Strategies 2002-2006). Use of nematicides is also curtailed by their threat to groundwater, soil biodiversity, as well as long waiting-periods between use, harvesting and marketing of a crop (Bridge, 1996).

Alternatively, soil beneficial microorganisms can be used as an alternative, thereby helping reduce application of chemicals to the soil. This entails the use of natural enemies to control nematode pests. Beneficial micro-organisms are non-polluting and, thus, environmentally safe and acceptable. Usually, these are species-specific to the target pest, therefore with no chances of affecting non-target species (unlike chemicals, which are broad-spectrum in their action (Hein *et al,* 2007). Nematode-destroying fungi are one such group of beneficial microorganisms for use in control of plant-parasitic control of plant-parasitic nematodes. These micro-fungi are natural enemies of the nematodes. They naturally capture, kill and digest nematodes present in the soil (Rodrigues *et al,* 2001; Nordbring-Hertz *et al,* 2002). They comprise three main groups: the nematode trapping fungi, the endoparasitic fungi, and the egg-and cyst-parasitic fungi (Nordbring-Hertz *et al,* 2002; Masoomeh *et al,* 2004). After trapping the nematodes, the fungi penetrate their cuticle, invade their entire body-cavity and, then, digest them completely. This group of fungi has drawn much attention due to their potential as biological control agents for plant-parasitic nematodes (Jansson *et al,* 2000; Sanyal, 2000; Masoomeh *et al,* 2004). About 70% of the fungal genera and 160 species are associated with nematodes, but, only a few can be used as biological control agents for nematodes (Elshafie *et al,* 2006). This study was, therefore, aimed at documenting occurrence and diversity of nematode-destroying fungi and testing their efficacy on plant-parasitic nematodes, to harness their potential as bio-control agents against plant-parasitic nematodes.

MATERIAL AND METHODS

Soil samples were collected from five different vegetable-growing areas in Kenya, viz., Kinare, Kabete, Athi-river, Machakos and Kibwezi, in the order of altitude and temperature. Kinare was a high-altitude area and the coldest, with Kibwezi being the lowest and hottest. Vegetable gardens in each zone were dominated mainly by spinach, kale, tomato, cabbage and pepper, among other vegetables. From each of the study areas, five farms under intensive vegetable-production were selected randomly for this study. From each of the farms, five different vegetable gardens were sampled. From each vegetable garden in turn, five soil samples were collected and mixed together in a bucket to make a composite sample. One kilogram of soil was then re-sampled from the composite sample in the bucket, put into plastic bags, labelled and placed in a cool box. Soil sampling was done using a soil auger sterilized using ethanol after every sampling, to avoid cross-contamination. All the samples were later transported to the laboratory for isolating nematode-destroying fungi.

Isolation of nematodes-destroying fungi was done using the soil-sprinkle technique described by Jaffee *et al* (1996) where, tap water agar (TWA) was prepared by dissolving 20g agar in one litre of tap water. The medium was autoclaved and cooled before use after amending it with 0.1g per litre of streptomycin sulfate under a laminar air flow cabinet. One gram of soil sample was sprinkled on the medium in the Petri dish, and a suspension of *Meloidogyne* species consisting of approximately 1000 nematodes, was added to the Petri dishes as bait (Christina *et al,* 1999). The plates were then incubated at room temperature and observed daily from the third week, up to the sixth week, under a dissecting microscope. The examination was focussed on trapped nematodes, trapping organs and conidia of the nematode-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 Soto Barrientos *et al* (2001). After identification of nematode-destroying fungi, pure cultures of the three most-frequent fungal isolates were made for the experiment on efficacy. A 5mm mycelial block was inoculated into PDA in a Petri dish and allowed to grow for five days, before approximately 50 plant-parasitic nematodes were added. Efficacy of the fungal 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 by Analysis of Variance (Kindt & Coe, 2005)

RESULTS AND DISCUSSION

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

Fungal isolates were recovered from all the vegetable-production zones. Excepting *A. dactyloides,* all the isolates were not significantly (*P> 0.05*) affected by the agro-ecological zone. Frequency of occurrence of *A. dactyloides* was significantly (*P=0.002*) affected by the agro-ecological zone. The highest occurrence of *A. dactyloides* was recorded in Kabete, while the least was recorded in Athi-river, with a total record of 40 and 4, respectively. The species was also recorded in Machakos, Kibwezi and Kinare, with occurrence of 19, 10 and 5, respectively, in decreasing order. Among the isolates, only *A. oligospora* and *A. dactyloides* were found to occur in all the agro-ecological zones. *M. cionopagium* occurred in all the zones except Kinare, while, *S. grandis* was present in both Kibwezi and Kinare. *A. longispora* was recorded in Kibwezi only (Table 1).

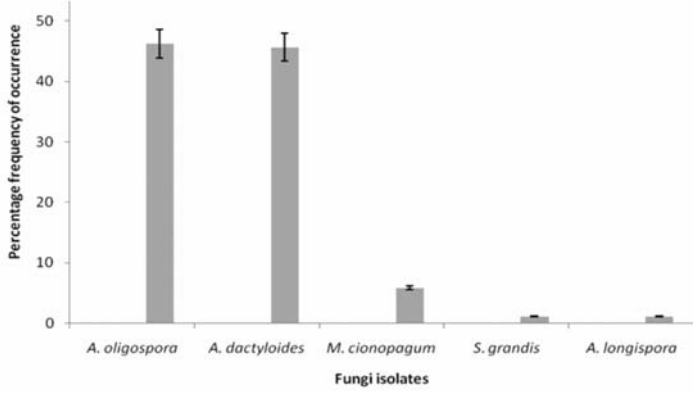

Fig. 1. Percentage occurrence of nematode-destroying fungi in some vegetable-producing areas of Kenya

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

Zone	*A. dactyloides*	*A. oligospora*	*A. longispora*	*M. cionopagium*	*S. grandis*	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, 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. Kibwezi recorded the highest diversity index, with a mean of 0.930, followed by Machakos with 0.637, while Kabete recorded the least diversity index mean of 0.411. Mean richness and abundance varied between vegetable-production zones. The highest mean species richness was recorded in Kibwezi, while the least was recorded in 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, the least was seen in Kinare with species mean abundance of 2.6 (Table 2).

More nematode-destroying fungi were detected with increase in the number of the soil samples under study. It is evident that all the possible isolates of nematode-destroying fungi were recorded in this study, from the samples collected. Collecting and processing additional samples may not have significantly increased the number of isolates (Fig. 2).

There was a significant (*P=0.003*) difference on efficacy between the three most-frequent nematode-destroying fungal species. *Arthrobotryrs oligospora* was the most efficient nematode-destroying fungus, with a mean of 7.3, followed by *Monacrosporium;* the least was *Stylopage,* with mean records of 5.9 and 5.1, respectively.

Nematode-destroying fungi were isolated from all the selected vegetable-production zones. These occurred at different frequencies and diversity. The study demonstrated

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}

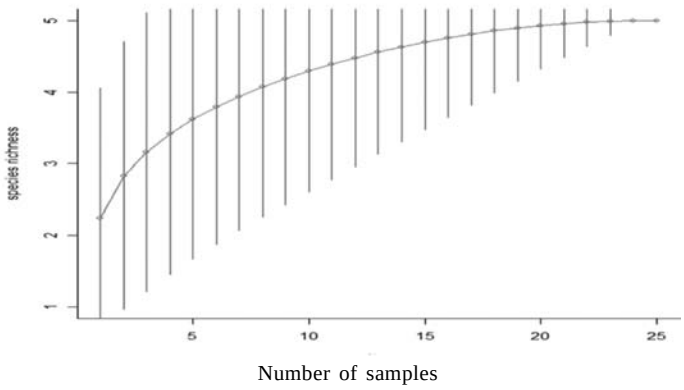

Fig. 2. A total-species cumulative curve for nematode-destroying fungi in some vegetable production zones of Kenya

the occurrence of diverse nematode-destroying fungi in nature, especially, in vegetable-production zones. These findings agree with previous reports on nematode-destroying fungi that indicate that these are wide-spread in all habitats, but, at 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 area under study. Other studies on nematode-destroying fungi report the same observation. It has never been clear why this is the most frequently encountered fungus. Wachira *et al* (2008) had suggested that farming practices like weeding could be the cause of such high occurrence. It has also been suggested that it is due to the ability of the fungus to exist both as a saprophyte and a plant-parasitic nematode feeder (Sobita and Anamika, 2011). Due to this high occurrence, this fungus has attracted several other interesting studies (Niu and Zhang, 2011).

It was expected that the highest divergence of fungi would be isolated from areas under low temperature (Kinare). However, this was not the case. Kinare had the least variety of fungi. This was attributed to the high use of chemical fertilizers and pesticides, since, all the vegetables were aimed for the market. In a study on long-term effects of manures and fertilizers on soil productivity and quality, it was reported that chemically fertilized soils had lower content of organic matter and fewer 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 farm at University of Nairobi to which animal manure had been frequently applied. This may explain the high number of nematode-destroying fungi here, since, these are associated with increase in beneficial micro-organisms in the soil (Wachira and Okoth, 2009). In our study, a high fungal population was found in areas where manure had been applied, and low fungal population in areas where chemical fertilizer was applied.

Temperature is an important factor in regulating microbial activity and shaping soil microbial communities. It determines moisture level in the soil, which is key to fungal spore germination and growth. High temperatures lead to low soil-moisture, which leads to low fungal-spore germination. A study by Haugen and Smith (1992) reported that at high temperatures, there was low germination of fungal spores, leading to low fungal population, while, under low temperatures there was high fungal germination, leading to a high fungal population. This was found to be in reverse in our study. Although Machakos and Kibwezi experience high temperatures, a high population of nematode-destroying fungi was seen here by us. This could be attributed to the irrigation activities undertaken at the farm which ensured moist conditions throughout the growth season. This soil enhanced moisture, coupled with high temperature, improved fungal spore germination (as, fungal spores germinate better under moist and warm conditions).

Efficacy test showed that the genus *Arthrobotrys* was most effective in trapping plant-parasitic nematodes. Previous studies on fungi of this genus have consistently showed that it is able to trap 90% of all the nematodes in Petri dishes and liquid cultures in 16-40 hours (Rajeswari and Sivakumar, 1999). Therefore, due to its high occurrence, this fungus can be used in management of plant-parasitic nematodes, and its potential for this ability should be investigated. The investigation should focus on finding suitable carrier/s for the fungus and its mode of application. This would reduce over-reliance on chemical nematicides and help develop a self-regulating system in the soil for control of soil borne pests.

CONCLUSION

Additional evidence has been provided by this study that nematode-destroying fungi naturally occurr in agricultural habitats. It is also evident that agricultural activities targeting high crop-production, like, application of chemical fertilizers and pesticides, directly affect soil biodiversity adversely. Results from this study can be used in further research for establishing the potential of nematode-destroying fungi in regulation of plant-parasitic nematode population.

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