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Interactions between soil additives and a variety of naturally occurring nematode-demolishing fungi in banana fields of Meru and Embu Counties, Kenya

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Abstract. *Wairimu WJ, Kimenjul JW, Muiru WM, Wachira PM. 2022. Interactions between soil additives and a variety of naturally occurring nematode-demolishing fungi in banana fields of Meru and Embu Counties, Kenya. Cell Biol Dev 6:* 82-93. Plant-parasitic nematodes pose a significant danger to banana production, as they reduce the productivity, quality, and lifetime of banana orchards. This study aimed to evaluate the diversity, quantity, and occurrence of nematode-demolishing fungi in banana production farms to use them to manage plant-parasitic nematodes. Also examined was the impact of organic and inorganic soil additives on nematode-demolishing fungi. The study region was divided into three agroecological zones: UM3 (Low), UM2 (Middle), and UM 1 (Upper). Ten farms were chosen randomly for a soil sample in each zone to determine the diversity and abundance of nematode-demolishing fungi. One farm was chosen randomly for soil additive treatments in each zone. The gathered soil samples were used to isolate and identify nematode-demolishing fungi at the species level. The diversity of nematode-demolishing fungi varied significantly between zones, with the highest variety and number of fungi found in the highest zone. *Arthrobotrys, Monacrosporium, Nematoctonus, Harposporium,* and *Paecilomyces* were the identified genera. *Arthrobotrys* was the most often isolated genus, with a frequency of 45%, followed by *Harposporium,* with a frequency of 18%. The remaining three genera each had a frequency of 9%. The *A. dactyloides, A. oligospora, A. robusta, A. longispora, A. superba, H. anguillulae, H. crassum, Meria coniospora, Monacrosporium cionopagum, N. leiosporus,* and *P. lilacinus* were among the species identified. The *A. oligospora* had captured and destroyed 98 plant parasite nematodes, whereas *M. cionopagum* and *Dactyllela phymatopaga* had eliminated 88 and 76 plant parasitic nematodes, respectively, within the same time frame. The amount of nematode-demolishing fungi was significantly different between the various soil additives, with chicken dung having the greatest number of 74 isolates, followed by cow manure, goat manure, the control treatment, and inorganic additive with 71, 69, 54, and 39 isolates, respectively. The amount of isolated nematode-demolishing fungi fluctuated throughout time, from 89 pre-treatment isolates to 122 after three months and 96 after six months. The variation of nematode-demolishing fungi over time was significantly different, demonstrating the impact of diverse soil additives on their existence. *Arthrobotrys* spp. is a suitable option for field efficacy studies since it was the most diversified, had the highest demolishing rate, and the organic additives facilitated its dispersion in banana plantations.

Keywords: Banana farm, Embu, fungi, Meru, nematode, soil additives

INTRODUCTION

Bananas (*Musa* spp.) are the most widely consumed fruit in the world, with consumers spending over £10 billion on bananas every year (Trade Fair 2013; Hapsari et al. 2017). About two percent of all international trade is in fresh bananas. Evidence of widespread dependence on fruit shows that only 15% are traded on the global market (FAO 2005), with the remaining 85% consumed inside individual countries. The banana is the fourth most valuable crop in the world (Muchui et al. 2013), after rice, wheat, and maize, to ensure food security in developing countries.

Bananas, both raw and cooked, are a nutritious choice because they include a variety of useful nutrients. For subsistence farmers, their attractive features include adaptability to intercropping, rapid growth, and a prolonged harvesting time (Macharia et al. 2010). Bananas are consumed not only by humans but are also utilized as animal fodder and material for roofing, flooring, and even matting. Furthermore, they provide a beneficial ground cover that shields the soil from wind and decreases the likelihood of erosion.

Bananas are widely traded and consumed in both underdeveloped and developed nations. Bananas play an important role in East African agriculture, both a staple crop and a source of revenue for subsistence farmers (Seshu et al. 1999). Almost 15 million tons of bananas are produced annually in the East African highlands (Ng'ang'a et al. 2011), making this food staple for over 20 million people. According to the data compiled by Ng'ang'a et al. (2011), small-scale farmers in Kenya are responsible for cultivating the vast bulk of Kenya's banana crop. Lacatan, Uganda Green, Apple, Gros Michel, Dwarf Cavendish, Giant Cavendish, Grand Nain, Williams, Valery, Muraru, Sukari, and Kiganda are some of the most popular types planted in Kenya.

Giant Cavendish, Apple (Sweet Banana), Valery, Uganda Green (for use in cooking), and Red Banana (used primarily in baking) are the most desirable export kinds (Mburugu 2013). Bananas are mostly grown in Kenya's Central, Eastern, Western, Nyanza, and Coast provinces (Ministry of Agriculture 2008; Kabunga et al. 2012; Mwombe et al. 2013; Karienye and Kamiri 2020; Kirimi et al. 2021; Wahome et al. 2021). Recently, coffee fields in the East and Central have given way to banana plantations (Muchui et al. 2013). As a result, growing bananas has been singled out as an industry that helps those in need. However, the banana product is in decline because of

knowledge gaps and constraints experienced by small-scale farmers and other players in the banana value chain, as indicated in a situational analysis of Imenti south districts conducted by Muchui et al. (2013).

Production of bananas in Kenya has dropped drastically during the past few decades. An increase in pests and illnesses, made worse by a lack of efficient control techniques, is mostly to blame for the decline, say Kahangi et al. (2002). Deteriorating soil fertility, poor crop management, inadequate sanitary planting material, poor marketing arrangement, post-harvest damage, genetic corrosion, and high costs are only some of the factors contributing to this decrease (Macharia et al. 2010).

Banana yields can drop by a factor of one hundred due to pests and illnesses, and the quality of the crop will suffer as a result (Viljoen 2010). Given their minuscule size, high rate of destruction, and the section of the plant they attack, root-lesion nematodes are a serious and economically significant pest of many cultivated crops worldwide (Trifonova and Karadjova 2009). Above-ground symptoms of nematode damage to roots include nutrient deficiency, incipient wilt, stunting, and poor yield; however, these symptoms are often misinterpreted as being caused by a lack of soil nutrition (Viljoen 2010), delaying the diagnosis and, as a result, management measures and increasing crop losses. In addition, crop damage caused by root-invading nematodes sometimes goes unnoticed by cultivators because of a lack of awareness of the symptoms created by parasitic nematodes, such as root galls, root lesions, and cysts. As a result, the banana crop is still threatened by nematodes in every place it is farmed (Mitreva et al. 2005).

This research aimed to examine the impact of organic and inorganic additives on the population and diversity of nematode-demolishing fungi in various agroecological zones and estimate the abundance and diversity of nematode-demolishing fungi in banana fields.

MATERIALS AND METHODS

Study site

Embu and Meru Counties, both in Kenya's Eastern province, were the focus of the research (Figure 1). Embu is found 120 kilometers northeast of Nairobi, at the height of 1,350 meters above sea level on the southern slopes of Mount Kenya (coordinates: 0.5333° S, 37.4500° E). At 1,462 meters above sea level and 275 kilometers from Nairobi, the town of Meru is a popular spot. The soils are rich, deep, and volcanic in origin, making them ideal for growing tea, coffee, bananas, and other crops. Study areas included the UM1 (upper midland high zone), UM2 (upper midland intermediate zone), and UM3 (lower upper midland zone). Since most bananas are grown in these areas, they made for good study locations. Land in the higher zone is used mostly for growing tea, intercropped with maize and banana; in the middle zone, tea and coffee are grown alongside banana plantations and horticultural fields; and in the lower zone, banana plantations predominate. Livestock was also kept in the upper and middle zones.

Assessing the diversity and abundance of nematode-demolishing fungi in banana fields

Experimental design

To contrast the effects of cultural practices and climate on the distribution and prevalence of nematode-demolishing fungi, the region was separated into three zones ranging from high altitude to low altitude at each study site. In Embu and Meru counties, 60 farms were chosen using a completely random design. Banana plantations were prioritized in the selection process of farms. Thirty samples of soil were gathered from each of the two sites. In June 2013, ten (10) samples were obtained randomly from each zone.

Soil sampling procedure

A random sampling technique was employed to collect five soil samples from each banana farm in Meru and Embu counties and three samples from each banana stool from the Embu trial locations. Soil cores were collected from a depth of 10-20 cm around the banana roots using core collection equipment (soil auger). The 500-gram composite sample was sealed in plastic and placed in a cool box for transit to the lab, where it was kept in a cold room at 10°C until the nematode-demolishing fungi could be isolated. To prevent contamination across sites, the auger was sterilized by dipping it in 70% ethanol between each sampling. Subsequently, the soil samples were utilized to extract nematode-demolishing fungi and plant parasitic nematodes.

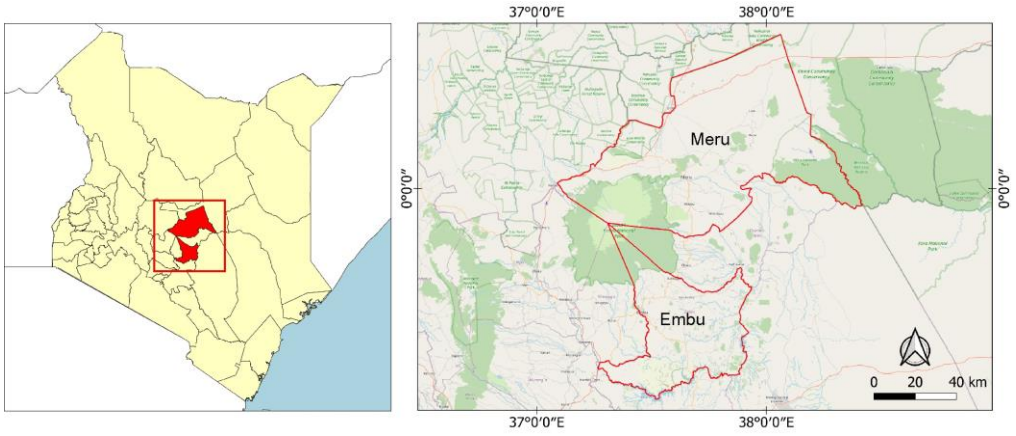

Figure 1. Maps of Embu and Meru, Kenya (Google Maps 2016)

Isolation of nematode-demolishing fungi

The 60 samples collected from Embu and Meru counties and the 45 samples collected from farms in Embu County that had been treated with organic and inorganic manure were used in an isolation process to look for nematode-demolishing fungi. Tap water agar was made by dissolving 20 grams of agar in 1 liter of regular tap water. The medium was autoclaved at 121 ° C for 15 minutes, cooled to 45 ° C, and then modified with 0.1 grams of streptomycin sulfate to prevent further bacterial growth. To isolate nematode-demolishing fungi, the soil was dusted on plates following the method described by Jaffe (1996). On the surface of the tap water agar plates, one gram of soil from each sample was evenly distributed, and this process was repeated three times for each soil sample. *Melodogyine* spp. J2 plant parasitic nematodes were used as bait, and plates were incubated at room temperature before being checked daily beginning in the third week and continuing through the sixth week using a dissecting microscope to see how much growth had occurred. Experiment nematodes were collected from soil and root samples following Kleynhans's method (1999). Prepared slides of the deceased nematodes were studied with a compound microscope. Fungal nematode demolishers in the study region were classified by genus and species based on their capturing organ and conidia form. Each farm's status regarding the presence or lack of fungi capable of demolishing nematodes was noted. The detected fungi were subcultured on potato dextrose agar to obtain pure cultures for the in-vitro nematode-demolishing fungi trapping ability trial. Prepared by dissolving 39.5 grams of potato dextrose agar into one liter of purified water. When the medium was modified with 0.1 g of streptomycin sulfate, it was autoclaved at 121°C for 15 minutes, cooled to 45°C, and then used to inhibit bacterial growth.

Identification of the nematode-demolishing fungi

Identification of the fungi to the species level was achieved by analyzing their morphology and trapping characteristics in relation to nematodes. Conidial shape, septation, conidiophore morphology, adhesive hyphae, adhesive traps, non-constricting rings, constricting rings, adhesive knobs, and inward invasions were the key features used. The fungi were observed on glass slides using a 1000x compound microscope. Nematodes, their trapping organs, and the conidia of the nematode-demolishing fungi were all photographed.

Determining the potential of the nematode-demolishing fungi under laboratory conditions

The 60 samples collected from Embu and Meru counties and the 45 samples collected from farms in Embu County that had been treated with organic and inorganic manure were used in an isolation process to look for nematode-demolishing fungi. Tap water agar was made by dissolving 20 grams of agar in 1 liter of regular tap water. The medium was autoclaved at 121°C for 15 minutes, cooled to 45°C, and then modified with 0.1 grams of streptomycin sulfate to prevent further bacterial growth. To isolate nematode-demolishing fungi, the soil was dusted on plates following the method described by Jaffe (1996). On the surface of the tap water agar plates, one gram of soil from each sample was evenly distributed, and this process was repeated three times for each soil sample. *Melodogyine* spp. J2 plant parasitic nematodes were used as bait, and plates were incubated at room temperature before being checked daily beginning in the third week and continuing through the sixth week using a dissecting microscope to see how much growth had occurred. Experiment nematodes were collected from soil and root samples following Kleynhans's method (1999). Prepared slides of the deceased nematodes were studied with a compound microscope. Fungal nematode attackers in the study region were classified by genus and species based on their capturing organ and conidia form. Each farm's status regarding the presence or lack of fungi capable of demolishing nematodes was noted. To test the fungi's ability to capture nematodes in vitro, each species was subcultured on potato dextrose agar to produce pure cultures. Prepared by dissolving 39.5 grams of potato dextrose agar into one liter of purified water. When the medium was modified with 0.1 g of streptomycin sulfate, it was autoclaved at 121°C for 15 minutes, cooled to 45°C, and then used to inhibit bacterial growth.

Field trials, design, and application of treatments

To study the impact of organic and inorganic soil additives on the presence and diversity of nematode-demolishing fungi, researchers applied several treatments on soil in the Embu county region. In the experiment, Randomized Complete Block Design was utilized. The soil additive studies were conducted on a single farm in each zone with a monoculture banana crop and employed good agronomic techniques before the trials. Each treatment was applied three times on the farm, yielding a total of 45 samples for each sampling period and 135 samples for the three sampling periods. Species cumulative curves were utilized to validate the required sample size. Banana stools comprised four/three to six-month-old Israel cultivar banana plants per treatment application. The treatments employed were (i) cow manure, (ii) goat manure, and (iii) chicken manure that had been composted, cured, and dried at the recommended rate of 5% wet weight for six months (Wachira et al. 2009a), (iv) a compound fertilizer, and (v) a control banana stool where no treatment was administered. Cow manure, goat manure, and chicken manure were each applied at a rate of 40 kg per banana stool, along with compound fertilizer (17:17:17 100g/stool) and a control stool where no treatment was administered. The carbon-to-nitrogen ratio of organic additives for cow, goat, and chicken was 16:0.8, 26:2.3, and 20:0.6, respectively. The additives were mixed into the soil to a depth of 10 cm in each banana stool.

To allow for full decomposition and interaction with the rhizosphere, soil samples were taken (i) immediately before the application (pre-treatment) of the additives in the third week of July 2013, (ii) three months after the application date in the second week of September 2013, and (iii) six months after the application date in the second week of December 2013 to determine the presence and diversity of nematode-demolishing fungi.

Data analysis

Microsoft Excel 2013 was used to input data on the presence/absence of various nematode-demolishing fungi, and this information was then analyzed using tables and charts. Frequency of occurrence, Renyi profiles, the Shannon diversity index, and evenness were examined with R commander software to determine the diversity of nematode-demolishing fungi (Kindt and Coe 2005). The predatory fungal activity was calculated using Microsoft Excel 2013 by comparing the number of plant parasitic nematodes caught to the total number of plant parasitic nematodes. Soil additives' effects on the fungal population were compared by analyzing variance in the number of fungal isolates collected from each additive using the Genstat 15th version. The Least Significant Difference (LSD) test was used to compare the means at the 5% confidence interval.

RESULTS AND DISCUSSION

Diversity and abundance of nematode-demolishing fungi in banana fields

Characterization of nematode-demolishing fungi in Meru and Embu counties

This research determined 138 different fungal isolates to be nematode-demolishing agents. There were a total of 11 different species, divided into 6 different genera. *Arthrobotrys, Meria, Monacrosporium, Nematoctonus, Harposporium,* and *Paecilomyces* were the recognized genera. In terms of frequency, *Arthrobotrys* was the most often isolated species (45%), followed by *Harposporium* (18%) and the other three genera (9% each). There were many different types of *Arthrobotrys* found in the samples taken, including *A. oligospora, A. longispora, A. superba, A. dactyloides, A. robusta, Harposporium anguillulae, Harposporium crassum, Meria coniospora, Monacrosporium cionopagum, Nematoctonus leiosporus,* and *Paecilomyces lilacinus.* The species with the highest prevalence was *M. cionopagum* (26.6%), followed by *A. oligospora* (17.4%) and *A. robusta* (0.7%) (Table 1). The prevalence of *M. cionopagum* was highest in Embu (40.5%) and Meru (21%). The second most common species in both locations was *Arthrobotrys* spp., whereas the least common was *P. lilacinus*. The agroecological zone had a significant impact on only two species: *A. longispora* and *H. crassum*, with P values of 0.014 and 0.059, respectively ($P<0.05$) (Table 1).

There was no noticeable difference in the distribution of nematode-demolishing fungi, which was discovered in all three agroecosystems. The highest concentration of nematode-demolishing fungi (53 isolates) was found in the uppermost zone (UM1). The highest incidence (49 occurrences) was found in the intermediate zone, while the lowest incidence (36 occurrences) was found in the lowest altitude (Figure 2).

Diverse nematode-demolishing fungi were found in only one of the agroecological regions, while the other two had many more. The mean Shannon diversity of nematode-demolishing fungi was 0.511 in the low zone, 0.758 in the middle zone, and 0.799 in the upper zone (see Figure 3). The mean Shannon value across all samples was 0.689.

Figure 4 shows that the upper zone had the most diversity richness, followed by the middle and low zones. The *M. coniospora, A. robusta, N. leiosporus, H. anguillulae,* and *H. crassum* were detected in Meru but not in Embu (Table 2).

The total species cumulative curve for this study showed that the 60 soil samples used were sufficient for estimating nematode-demolishing fungi (Figure 5).

Table 1. Frequency of occurrence of nematode-demolishing fungi in Embu and Meru Counties, Kenya

Isolate	Rank	No. of isolates	%	Cumulative frequency	P- value (P=0.05)
M. cionopagum	1	37	26.8	1.6	0.6233
A. oligospora	2	24	17.4	44.2	0.8199
A. longispora	3	21	15.2	59.4	0.01396
A. superba	4	19	13.8	73.2	0.5964
A. dactyloides	5	15	10.9	84.1	0.3564
H. anguillulae	6	7	5.1	89.1	0.8572
H. crissum	7	5	3.6	92.8	0.0590
N. leiosporus	8	4	2.9	95.7	0.3547
P. lilacinus	9	3	2.2	97.8	0.3613
M. coniospora	10	2	1.4	99.3	0.1305
A. robusta	11	1	0.7	100	0.3742

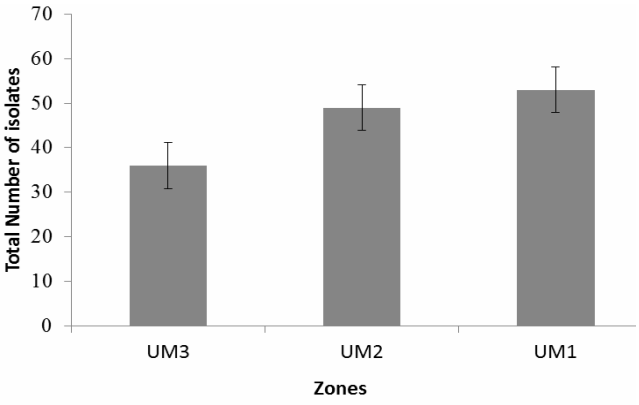

Figure 2. Frequency of nematode-demolishing fungi in Embu and Meru Regions, Kenya

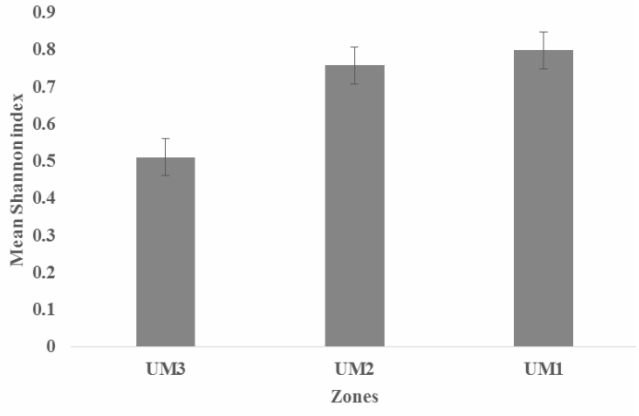

Figure 3. Mean Shannon indexes of the nematode-demolishing fungi in the study area

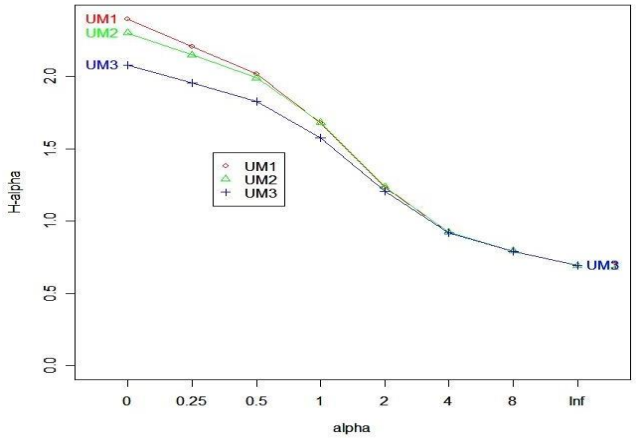

Figure 4. Renyi diversity profile for nematode-demolishing fungi in the study area

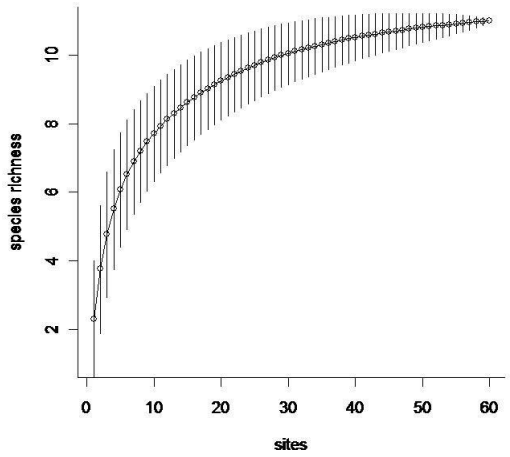

Figure 5. Frequency cumulative curve for the species of nematode-demolishing fungi

Table 2. Comparison of the nematode-demolishing fungi species present in banana production sites in Embu and Meru Counties, Kenya

Isolate	Embu in %	Meru in %
M. cionopagum	12	14.5
A. oligospora	5.8	11.6
A. longispora	3.6	10.7
A. superba	2.9	10.7
A. dactyloides	4.3	7.2
H. anguillulae	-	5.1
H. crassum	-	3.6
N. leiosporus	-	2.9
P. lilacinus	1.4	1.4
M. coniospora	-	0.7
A. robusta	-	0.7
Total	30	69.1

Occurrence of nematode-demolishing fungi in Embu

The Embu research site yielded 42 nematode-demolishing fungal isolates. The fungi in the Embu research site belonged to *Arthrobotrys*, *Monacrosporium*, and *Paecilomyces*. Seven *Arthrobotrys* were discovered: *oligospora, longispora, superba, dactyloides, robusta, cionopagum,* and *lilacinus*. The three agroecological zones in the Embu study site did not differ significantly in terms of the prevalence of the fungi that demolish nematodes. The number of isolates was largest in the upper zone (15), then in the middle zone (14), and lowest in the lower zone (13). It was found that nematode-demolishing fungi were less likely to be isolated at lower altitudes (Figure 6). The presence or absence of agroecological zones has no effect on their dispersal.

Occurrence of nematode-demolishing fungi in Meru

Ninety-six fungal isolates were found to be nematode-demolishing agents in the Meru investigation. Nematode-demolishing fungi were found in six different genera and eleven different species. There were 40 isolates in the high zone, 35 in the middle zone, and only 21 in the low zone. The results showed that ecological zones significantly (P=0.04) influenced the occurrence of nematode-demolishing fungi, with the frequency of occurrence decreasing with altitude from upper, middle, to low zones (Figure 7).

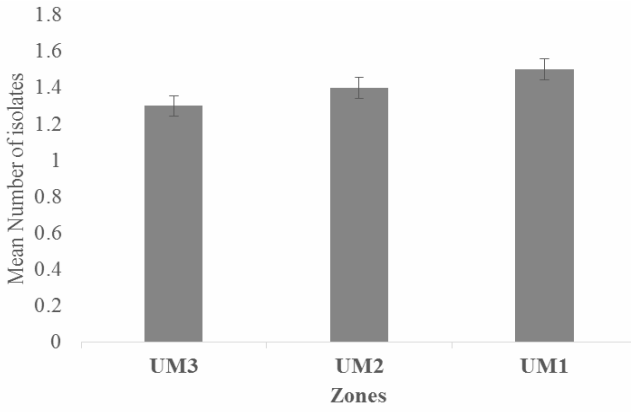

Figure 6. Occurrence of nematode-demolishing fungi in banana orchards in Embu County, Kenya

Determining the potential of the nematode-demolishing fungi under laboratory conditions

The growth habits, conidial morphology, and nematode-trapping structures of the isolated fungi were characterized (Figure 8). Characteristics of *Arthrobotrys* include a conidium made up of two cells, one at each end, and a mycelium that grows primarily outside of the nematode hosts. Conidia of *A. oligospora* had a small distal cell and a huge proximal cell, and the conidiophore was erect and tree-like in appearance (Figures 8A and 8B). The conidium of *A. dactyloides* was long and slender, and the distal cell was nearly the same size as the proximal cell. The conidia were born in a terminal cluster on the conidiophore and formed constricting rings. Although the conidia of *A. superba, A. robusta,* and *A. longispora* all possessed a single septum and clustered together in three dimensions (Figure 8C), the three species' conidium were significantly different in size and shape. The conidia of the genus Harposporium were carried on phialides and typically were lodged outside of the dead nematode's body, contrasting with the mycelium's internal location. Arcuate conidia can be seen in *H. anguillulae* (Figure 8E) and *H. crassum*, with the latter having smaller conidia. In contrast to *N. leiosporus*, whose conidium does not create chlamydospores, Nematoctonus was identified based on its

hyphae, which lacked adhesive cells but developed adhesive knobs on the conidium. Mycelium that became tangled around nematode eggs allowed us to identify the fungi as belonging to the species Paecilomyces, which is distinguished by its cylindrical conidia that hang in chains from phialides. The worm became tangled in the sticky mycelium of *M. cionopagum*, which formed a trap, and a single apical conidium was carried on an erect conidiophore (Figure 8D). The endoparasitic fungi *M. coniospora* was distinguished by its nearly conical conidium, which formed a knoblike structure at the apical end. The *Dactyllela phymatopaga* may be identified by the nematodes it caught on its distinctive sticky knobs (Figure 8F).

Trapping ability of the isolated nematode-demolishing fungi

The average plant parasitic nematodes trapped and destroyed by the three selected nematode-demolishing fungi were 262 (72%) out of approximately 360 juveniles of *Meloidygyne spp*. After 96 hours, *A. oligospora* trapped and destroyed 98 plant parasitic nematodes, while *M. cionopagum* destroyed 88 plant parasitic nematodes. The least was *D. phymatopaga*, with 76 plant parasitic nematodes destroyed in the same period (Table 3). No trapping was recorded from all isolates between 0-24 hours. *A. oligospora* had the highest trapping ability at 82%, followed by *M. cionopagum* at 73%, and the least was *D. phymatopaga* at 63%. A paired test showed that the time taken by *A. oligospora* to trap nematodes was significantly different from that of *D. phymatopaga* (P value= 0.047) but did not differ significantly from the time taken by *M. cionopagum (P value 0.378).* The time taken by M. cionopagum to trap nematodes was not significantly different from that of *D. phymatopaga* (P value= 0.075).

The effect of organic and inorganic additives on the population and diversity of nematode-demolishing fungi

The highest number of nematode-demolishing fungi were isolated from the soils amended with chicken manure, with 74 isolates, followed by soils amended with cow manure 71, goat manure 69, and control plot 54, while the least was from the soils amended with fertilizer, with 39 isolates (Table 4). A significant difference was observed in the nematode-demolishing fungi population on different soil additives. Application of cow, goat, and chicken manure did not differ significantly in their interaction with the nematode-demolishing fungi in the banana plots, as indicated by the means. The mean for control banana plots where no additive was applied was significantly compared with the plots where a synthetic additive (fertilizer) was applied (Table 4).

In the soils amended with chicken manure, the population of nematode-demolishing fungi increased from 24 isolates to 31 isolates in the first 3 months. It decreased to 19 isolates below the initial numbers isolated in the sixth month. In soils amended with cow manure and goat manure, the populations increased after three months from 19 to 30 and were maintained above the initial isolation after 6 months at 22 and 20 isolates, respectively. Soils with no additive increased the population of nematode-demolishing fungi from 14 isolates to 20 isolates after 3 months, and the numbers did not change after six months. Soils amended with fertilizer decreased the nematode-demolishing fungi population in the first three months from 13 to 11 isolates; the population, however, increased after six months to reach 15 isolates (Table 4).

Seventy-four nematode-demolishing fungi were isolated from soils added with chicken manure, 71 from soils amended with cow dung, 69 from soils modified with goat manure, and 54 from the control plot. Only 39 nematode-demolishing fungi were identified from soils improved with fertilizer (Table 4). The number of fungi that feed on nematodes varied significantly across treatments. The results showed no discernible difference between the effects of applying cow, goat, or chicken manure to the banana plots and the fungi that kill nematodes. However, there was a considerable difference between the mean of amended and unamended banana plots (Table 4).

Soil nematode-demolishing fungi increased from 24 to 31 isolates in the first three months after being introduced to the soil treated with chicken manure before declining to 19 isolates by the sixth month. After three months, populations in soils altered with cow manure and goat manure rose from 19 to 30, and after six months, they remained at 22 and 20 isolates, respectively. After 3 months, the number of nematode-demolishing fungi in soils that had not been amended had increased from 14 to 20. However, this rise had stalled by 6 months. Table 4 shows that after six months of fertilizer replenishment, the number of nematode-demolishing fungi in soils rose from 11 to 15.

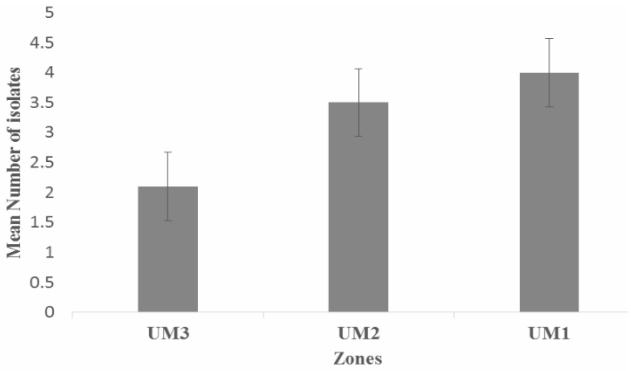

Figure 7. Occurrence of nematode-demolishing fungi in banana orchards in Meru County, Kenya

Table 3. The trapping ability of the nematode-demolishing fungi

Isolate/hours	0	6	12	24	30	36	42	48	54	60	66	72	78	84	90	96	Total
A. o	0	0	0	0	9	11	8	11	11	11	11	13	7	3	2	1	98
M. c	0	0	0	0	4	4	3	10	11	12	15	14	8	4	2	1	88
D. p	0	0	0	0	2	3	7	8	10	11	12	12	6	3	1	1	76

Note: *A.o= Arthrobotrys oligospora, M.c= Monacrosporium cionopagum, D. p= Dactyllela phymatopaga.* Figures in the table represent the mean numbers of plant parasitic nematodes trapped in three experiments

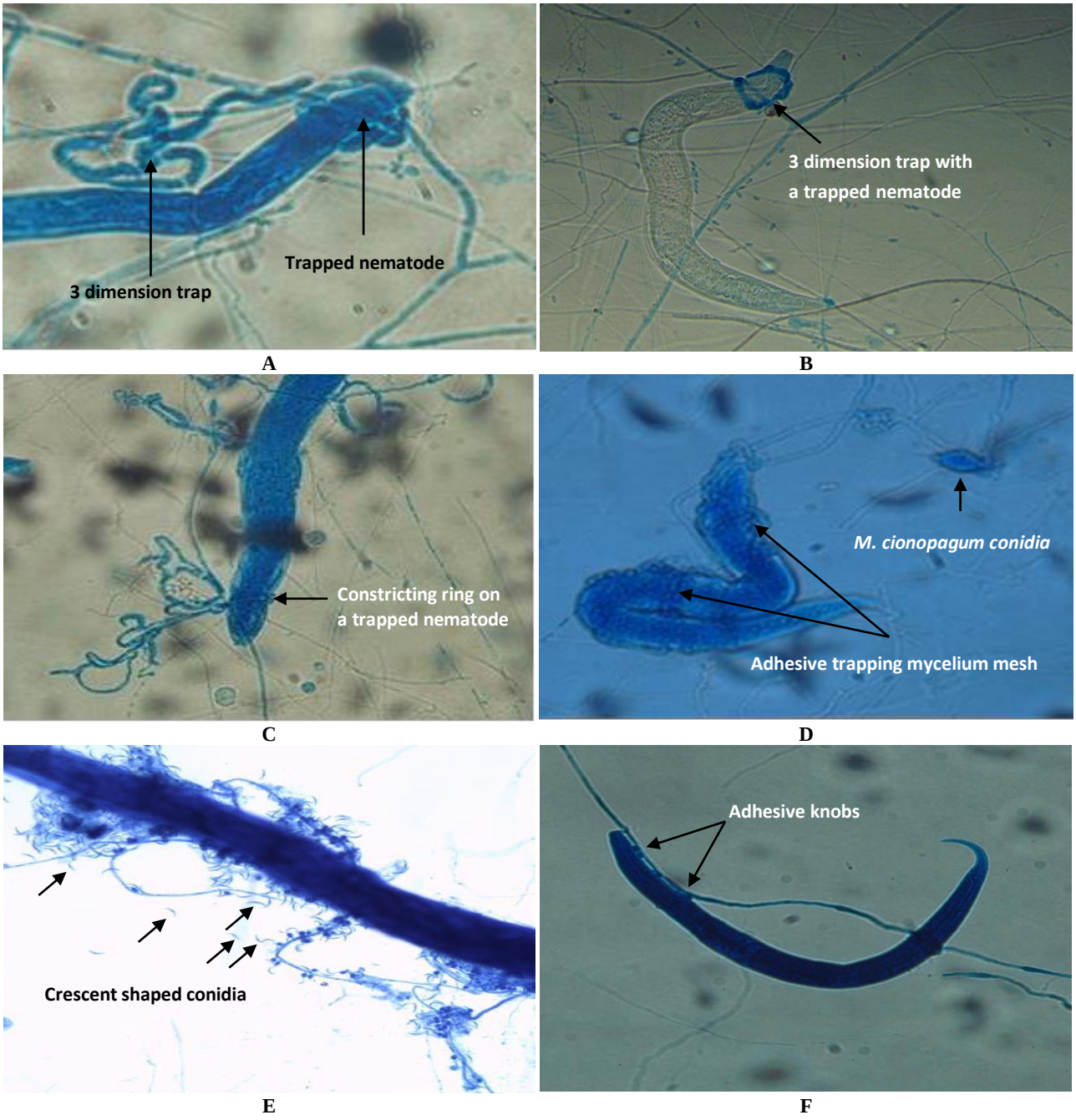

Figure 8.A. *A.oligospora* three dimension trap with the fungal (Magnification X1000). B. *A. oligospora* three dimension trap (Magnification X1000). C. Constricting rings of *A. longispora* (Magnification 1000). D. Adhesive mycelium and conidia of *M. cionopagum* (Magnification X1000). E. Arcuate conidia of *H. anguillulae* emanating from a digested nematode (Magnification X1000). F. Sticky knobs of *Dactyllela phymatopaga* (Magnification X1000)

Individual isolates were affected by the soil additives. *M. cionopagum* had the highest population in all treatments, followed by *A. longispora*, while *H. anguillulae* and *D. phymatopaga* had the lowest populations in all the treatments. Only *A. dactyloides* distribution was significantly affected by the different additives (P= 0.003) (Table 5).

Soil additives had an impact on individual isolates. The most abundant species across treatments were *M. cionopagum* and *A. longispora*, while *H. anguillulae* and *D. phymatopaga* were consistently the least numerous. The distribution of *A. dactyloides* was the only factor that changed noticeably due to the various adjustments (P= 0.003) (Table 5). The total number of nematode-demolishing fungi varied from month to month. When nematode-demolishing fungi were isolated before and after treatments, there was a notable difference between the two populations; however, by the sixth month, there was no longer a discernible difference between the two (Table 5).

Following the implementation of changes, the number of retrieved isolates grew from 89 to 122, then decreased to 96 by the sixth month (Table 6, Figure 9).

Table 4. The population of nematode-demolishing fungi (NDF) in banana plots treated with different additives

Additives	Months after application			Total isolates	NDF means
	Pre-treatment	3	6		
Chicken	24	31	19	74	2.8 a
Cow	19	30	22	71	2.6 ab
Goat	19	30	20	69	2.6 ab
Control	14	20	20	54	2.0 bc
Fertilizer	13	11	15	39	1.4 c

Note: Means with the same letter along the columns are not significantly different. LSD = 0.7

Table 5. The abundance of nematode-demolishing fungi in different banana plots treated with organic soil additives

Fungal isolates	Additives						
	Chicken	Cow	Goat	Control	Fert.	Total	P value
M. cionopagum	22	18	22	19	15	96	0.182
A. longispora	16	17	17	13	10	73	0.24
A. oligospora	16	14	19	13	9	71	0.08
A. dactyloides	12	10	5	5	1	33	0.003
A. superba	7	9	4	4	3	27	0.215
H. anguillulae	2	0	2	0	0	4	0.188
D.phymatopaga	1	1	0	0	1	3	0.735
Total	74	71	69	54	39	307	

Note: Systematic presentation of the species names in full (*Monacrosporium cionopagum, Arthrobotrys longispora, Arthrobotrys oligospora, Arthrobotrys dactyloides, Arthrobotrys superba, Harposporium anguillulae, Dactyllela phymatopaga.*

Table 6. Monthly variation of the nematode-demolishing fungi in soils amended with organic soil additives

Months	No. of isolates	Means
Pre-treatment	89	1.977b
3	122	2.7111a
6	96	2.1333b

Note: Means with the same letter along the columns are not significantly different. LSD = 0.518

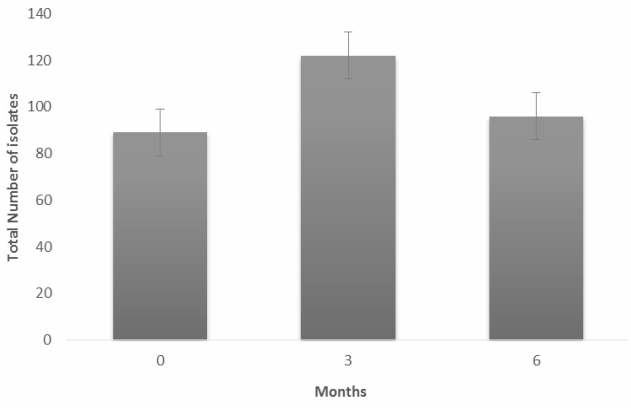

Figure 9. Nematode-demolishing fungi in amended soil over time

Discussion

This study has confirmed that nematode-demolishing fungi are widespread, and their diversity differs from one agroecological zone. Furthermore, they were present in all banana production farms in the two study sites; this concurs with previous studies as reviewed by Swe et al. (2011) that reported the presence of nematode-demolishing fungi in a wide range of environments.

The fungi isolated used various mechanisms to capture and destroy plant parasitic nematodes: these included; constricting rings, non-constricting rings, adhesive nets, adhesive knobs, and ingested spores. Different genera and species isolated were; *M. cionopagum* and *Arthrobotrys* spp. from all three regions were the highest in occurrence. It reflects past research work, as recorded by Birgit et al. (2002), on the occurrence of nematode-demolishing fungi. Wachira et al. (2014) also recorded that *Arthrobotrys* and *Monacrosporium* species were the most diverse in the three zones in the Embu region in banana farms.

Various agricultural practices and systems significantly impact the soil biota, their occurrence, and diversity (Scott et al. 2010). For example, cleared forest land for cultivation negatively disturbs the soil environment and decreases the number and species of beneficial soil organisms. High zones had the highest occurrence and diversity of nematode-demolishing fungi, which decreased with altitude. The variations in nematode-demolishing fungi populations can be attributed to the farming and cultural practices practiced in the study sites. High altitudes were characterized by mixed cropping and animal farming. Animal farming may have resulted in the use of animal manure in the farms hence the higher soil organic matter content in the high zone (Kaskavalci 2007), which positively influences the establishment and diversity of nematode-demolishing fungi (Wachira et al. 2011). In addition, Intercropping in the high zone offered alternative hosts to the nematodes, hence higher numbers of plant parasitic nematodes and a subsequent high number of the antagonists.

This research confirms the ubiquitous prevalence of nematode-demolishing fungi and shows that these fungi vary between agroecological zones. Furthermore, it is consistent with previous research evaluated by Swe et al. (2011), which indicated the prevalence of nematode-demolishing fungi in various environments. It was found in all banana production farms in the two study sites.

The separated fungi used strategies to trap and eliminate plant parasitic nematodes, including constricting rings, non-constricting rings, adhesive nets, adhesive knobs, and ingested spores. Common to all three areas, *M. cionopagum* and *Arthrobotrys* spp. were the most frequently found isolated genera and species. It is consistent with the findings of previous studies, as Birgit et al. (2002) documented on the prevalence of nematode-demolishing fungi. Furthermore, Wachira et al. (2014) found that across all three zones in the Embu region's banana fields, Arthrobotrys and Monacrosporium species exhibited the highest levels of diversity.

The diversity and abundance of soil biota are strongly influenced by farming methods (Scott et al. 2010). When

forests are cut down to make way for farms, the soil is thrown out of balance, and the number and variety of beneficial organisms in the soil decline. The abundance and variety of nematode-demolishing fungi peaked at higher altitudes and gradually declined. It has been hypothesized that differences in farming and cultural methods at the research sites are responsible for the observed variability in nematode-demolishing fungi populations. A variety of crops and livestock were raised at high elevations. Soil organic matter content is higher in the high zone, possibly due to the application of animal manure in farming (Kaskavalci 2007), which has a beneficial effect on the establishment and diversity of nematode-demolishing fungi (Wachira et al. 2011). Plant parasitic nematodes proliferated because of the increased availability of alternate hosts provided by intercropping in the high zone.

The low zone had fewer nematode-demolishing fungi, characterized by banana monoculture and high irrigation practices. Moisture in soil is known to affect fungal spore dispersal (Dieterich and Sommer 2009). The running water and soaked soils may have harbored the growth of fungal spores, resulting in the few nematode-demolishing fungi in this zone. There was also minimal land disturbance in low altitudes as tillage was reduced in the banana plantations (Wachira et al. 2009a). Cultivation increases the chance of dispersing fungal spores to other parts of the farm.

The three nematode-demolishing fungi used for the trapping ability test were positive. They managed to trap plant parasitic nematodes within the first thirty hours and exhausted the available nematodes in 96 hours. The high trapping ability of the nematode-demolishing fungi reveals their potential for field trial tests to confirm their viability for commercial production in managing vermiform plant parasitic nematodes. Commercial products of nematode-demolishing fungi of the species *Paecilomyces* that parasitizes nematode eggs have been released in the market.

Some nematode-demolishing fungi, such as *A. oligospora*, have been shown to boost plant growth and improve the nutritional value of fruits, all while being kind to the environment and lowering farmers' agricultural production costs (Singh et al. 2013). The nematode-demolishing fungus *A. oligospora*, as documented by Bíró-Stingli and István (2011), significantly decreased the population of female *Meloidogyne* larvae in a pepper field by 35%. The use of microorganisms in the control of agricultural pests and disease pathogens is becoming increasingly common. One example is *Beauveria bassiana* formulations, which are becoming popular for controlling insect pests.

The study confirmed a significant difference between the various soil additives tested; the highest population of nematode-demolishing fungi was isolated from the soils treated with organic additives. Conversely, soils added with inorganic fertilizers had the lowest nematode-demolishing fungal population. Mugwe et al. (2009) noted that fertilizer strongly influenced the soil microbial community structure and function.

Various farm practices, such as monoculture, tillage, soil pollution, and pesticide use, have undesirable impacts on many soil organisms (Scott et al. 2010; Xue and Zhang 2011). Levels of soil organic matter are decreasing due to intensive land use and excessive use of high external chemical inputs for crop production. Disturbance of soil physicochemical and biological processes leads to soil and water pollution, which results in the destruction and reduced effectiveness of useful soil microorganisms leading to increased numbers of pathogens and parasitic organisms.

Organic additives are known to increase organic carbon and nitrogen in the soil, as recorded by Bouajila and Sanaa (2011). Swe et al. (2011) also noted that animal manure is high in nitrogen, phosphorous, and potassium which the nematode-demolishing fungi utilize saprophytically as they establish themselves. An example is *Arthrobtrys* spp., known to thrive in the soil as a saprophyte and as a predator; this reflects earlier findings as documented by Jaffe (2004), Farell et al. (2006), Wachira et al. (2009b) and Xue and Zhang (2011) who noted that the *A. oligospora* obtains its carbon and energy from organic matter as a saprophyte and from trapping nematodes as a parasite which makes it adaptable to the big range of habitations. It explains the abundance and diversity of *Arthrobotrys* spp. in all agroecological zones and across all additives (Connell et al. 2006).

Since there were fewer nematode-demolishing fungi in the low zone, typified by its high rates of irrigation and banana monoculture, nematodes thrived there. Soil moisture is known to play a role in spreading fungi spores (Dieterich and Sommer 2009). The low concentration of nematode-demolishing fungi in this area may be because rushing water and saturated soils did not provide ideal conditions for germinating fungal spores. Tillage was reduced in banana plantations, causing less land disturbance at low elevations (Wachira et al. 2009a). The spread of fungi spores to unaffected areas of the farm is made more likely by cultivation.

Three nematode-demolishing fungi were tested for their ability to trap nematodes; all three passed, successfully entrapping plant parasitic nematodes within the first 30 hours and demolishing them all in 96 hours. For the control of vermiform plant parasitic nematodes, field trial testing is needed to confirm the viability of the nematode-demolishing fungi for commercial production due to their high trapping ability. Fungi of the genus *Paecilomyces*, which feed on nematode eggs, are now available commercially.

When correctly introduced into a farm, nematode-demolishing fungi eliminate the need for repeated sprays, which helps the environment and the farmers' bottom-line needs. According to the research of Singh et al. (2013), *A. oligospora* both stimulate plant development and improves the dietary value of harvested fruits. The nematode-capturing fungi were documented by Bíró-Stingli and István (2011). In a pepper field, oligospora cut the population of female Meloidogyne larvae by 35 %. Pests and disease-causing organisms in crops can be controlled with the help of certain microorganisms nowadays. Beauveria bassiana formulations, increasingly used to control insect pests, are gaining favor.

An abundance of nematode-demolishing fungi was isolated from soils treated with organic additions, confirming a significant difference between the soils treated with the different additives examined. The fungal population that feeds on nematodes was lowest in soils supplemented with inorganic fertilizers. The fertilizer regime's composition and activity of soil microbes are profoundly affected by Mugwe et al. (2009).

Many soil organisms are negatively impacted by common farming techniques such as monoculture, tillage, soil contamination, and pesticide use (Scott et al. 2010; Xue and Zhang 2011). Intense land usage and the overuse of high external chemical inputs have resulted in declining soil organic matter levels. When the soil's physicochemical and biological processes are disrupted, soil and water pollution leads to the death or impairment of beneficial soil microorganisms and the proliferation of diseases and parasites.

According to the research of Bouajila and Sanaa (2011), organic additions raise the levels of organic carbon and nitrogen in the soil. Animal dung is rich in nitrogen, phosphorus, and potassium, which the nematode-demolishing fungi use saprophytically throughout their establishment (Swe et al. 2011). Soil-dwelling Arthrobtrys spp., for instance, are both saprophytes and predators, before corroborating observations published by Jaffe (2004), Farell et al. (2006), Wachira et al. (2009b) and Xue and Zhang (2011), who all mentioned that the *A. oligospora* can thrive in various environments thanks to its ability to derive its carbon and energy needs from a combination of saprophytic (carbon from organic matter) and parasitic (energy from nematodes) sources. It explains why Arthrobotrys spp. can be found in every agroecological zone and every type of additive (Connell et al. 2006).

Farms amended with inorganic fertilizer decreased the nematode-demolishing fungal population after three months, and the population increased after six months. It can be attributed to the deterioration of soil microbe diversity and health by the chemicals in the inorganic fertilizers (Kar et al. 2007). The population, however, increased steadily but remained below the initial population after six months; this shows the recovery of the soils from the effects of the inorganic fertilizer. Using inorganic sources of phosphorus and nitrogen may enhance soil organic carbon. Still, long-term use of synthetic fertilizers is not advised as it negatively impacts soil structure and decreases soil macro aggregates. Using fertilizers pollutes the environment and degrades the soil (Lazcano et al. 2012).

Soils amended with chicken manure had the highest number of nematode-demolishing fungal isolates, which concurs with the findings of similar research work as Wachira et al. (2009b) and Iqbal et al. (2012). It can be attributed to the chicken manure composition. Chicken manure is slightly basic with a pH of 8.4, has a high electrical conductivity compared to other organic manures, and contains higher levels of nitrogen, phosphorus, and potassium than cow and goat manure (Karanja et al. 2007) which the fungi thrive on as it establishes itself for predatory activities. Analysis of different animal manure reveals that goat and chicken manure have more potential to provide nutrients, mainly phosphorous and nitrogen, than other manure sources. The highest amount of potassium was also delivered by the chicken manure (Noling 2012).

Previous work on *Bacillus subtilis* by Miriam et al. (2011) shows that a combination of *B. subtilis* and cow manure led to a reduction of 54% in the number of plant parasitic nematodes compared to the untreated control. In addition, it has been indicated that poultry litter reduced *Rotylenchulus reinformis* by 55 % (Ravichandra 2014) in a field experiment. It was attributed to the reduction of the number of eggs in the roots.

It is evident that soil additive management practices impact nematode-demolishing fungi (Romy and Robert 2014); the availability of various nutrients which the fungi feeds on as they establish their colonies in the soil to attack the nematodes. Therefore, using locally available animal manures could restore the regulatory processes of nematode-demolishing fungi and maintain effective populations of plant parasitic nematodes in the soil. In addition, the release of substances with nematicidal effect and organic acid during additives decomposition has been attributed to the reduction of the nematode population (Kimenju 2004; Georgis et al. 2006).

Soils contain huge amounts of various living organisms. These life forms play essential ecosystem services such as filtering water, parasitizing pests and pathogens, removing pollutants, and providing plant nutrients. Therefore, applying animal manure to enhance the establishment and conservation of beneficial soil organisms is one of the most convenient ways farmers can conserve nematode-demolishing fungi to reduce the number of plant parasitic nematodes in their farms to maintain general soil health (Renčo (2013).

After three months, the number of nematode-demolishing fungi decreased on farms that had been altered with inorganic fertilizer, and after six months, the population grew. This decline can be related to the inorganic fertilizers' detrimental effect on soil microbial variety and health (Kar et al. 2007). Despite being lower than the initial population, the steady increase in numbers suggests that the soils have recovered from the effects of the inorganic fertilizer. It's possible that using inorganic sources of phosphorus and nitrogen could increase soil organic carbon, but this wouldn't be a good idea as a prolonged application of synthetic fertilizers tends to reduce soil macro aggregates, which has a detrimental effect on soil structure. In addition, fertilizers impair soil quality and contribute to environmental pollution (Lazcano et al. 2012).

This finding is in agreement with those of related studies by Iqbal et al. (2009a) and Wachira et al. (2009b), which found that the greatest number of nematode-destructive fungal isolates were found in soils modified with chicken manure. Chicken manure's chemical makeup explains why this is the case. Due to its slightly basic pH of 8.4 and high electrical conductivity compared to other organic manures, as well as its higher levels of nitrogen,

phosphorus, and potassium than cow and goat manure, the fungi flourish in chicken manure as it sets up shop for predatory activities (Karanja et al. 2007). Compared to other types of manure, goat and chicken are the most promising in providing essential nutrients like phosphorus and nitrogen. Chicken manure also provided the highest levels of potassium (Noling 2012). Miriam et al. (2011) found that when *B. subtilis* was combined with cow manure, the quantity of plant parasitic nematodes dropped by 54 percent compared to the untreated control. Furthermore, according to data from a field study, the amount of *R. reinformis* was cut in half by using poultry litter (Ravichandra 2014). A decrease in the total number of eggs in the roots was blamed for this phenomenon.

Soil additive management strategies affect nematode-demolishing fungi (Romy and Robert 2014) because they affect the nutrients used by the fungi as their colonies spread across the soil in search of nematodes to devour. Keeping functional populations of plant parasitic nematodes in the soil requires balancing beneficial and harmful organisms. Using locally available animal manures could reestablish regulatory processes of nematode-demolishing fungi. In addition, the decomposition of additives causes the release of chemicals having a nematicidal impact, such as organic acid, which helps keep nematode populations in check (Kimenju 2004; Georgis et al. 2006).

Numerous creatures call soil home, and these organisms perform crucial ecosystem functions like filtering water, controlling pests and pathogens, detoxifying soil, and nourishing plants. To preserve nematode-demolishing fungi to decrease the number of plant parasitic nematodes on farms and maintain general soil health, one of the most practical methods farmers can employ is the application of animal manure to enhance the establishment and conservation of beneficial soil organisms (Renčo 2013).

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