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Stimulation of Nematode-Destroying Fungi by Organic Amendments Applied in Management of Plant Parasitic Nematode

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Abstract: A screenhouse experiment was conducted to evaluate the effect of cow manure, chicken manure and their combinations on nematode destroying fungi, nematode community and growth of tomato (*Solanum lycopersicum* L.). The amendments were applied at the rate of 5% w/w in all the treatments. Isolation of nematode destroying fungi was done using the soil sprinkle technique. Nematodes were extracted from soil using the modified Baermann technique. Tomato growth was estimated through plant height and dry weight. Application of the organic amendments resulted in significant differences ($p \leq 0.05$) in occurrence of nematode destroying fungi amongst the treatments. The nematode destroying fungi occurred at frequencies of 50, 29.4, 17.6 and 2.9% in soil amended with chicken manure, cow/chicken combination, cow manures and the control, respectively. Eight species of nematode destroying fungi were identified in this study. The fungus *Arthrobotrys oligospora* (Fresenius) was most dominant fungus in all the treatments including control pots with an isolation frequency of 38.2%. Addition of organic amendments into the soil also resulted in an increase of bacterial and fungal feeding nematodes and reduction of plant parasitic nematodes. Specifically there was a 225, 96 and 62% increase in bacterial feeding nematodes and 391, 96 and 74% increase in fungal feeding nematodes in soil amended with chicken manure alone, combination of chicken and cow manure alone in that order. Numbers of plant-parasitic nematodes were 92% lower in soil treated with chicken manure compared to the control. Plant height and leaf widths were highest in plants treated with combination of cow and chicken manures. The plants mean dry weight were 6.6, 5.6, 2.0 and 1.5 in combination of chicken and cow manure, chicken manure alone, cow manure alone and control, respectively. This study has therefore, revealed that organic amendments stimulate the occurrence of nematode destroying fungi in the soil and also reduce plant parasitic nematodes. In addition, the combination of cow and chicken manure stimulates plant growth.

Key words: Biological control, *Arthrobotrys oligospora*, chicken manure, nematode community

INTRODUCTION

Although nematodes are generally regarded as silent enemies, losses of up to 80% have been associated with them in vegetable fields that are heavily infested (Siddiqi, 2000; Kaskavalci, 2007). For decades, the control of plant-parasitic nematodes has mainly depended on chemical nematicides (Akhtar and Malik, 2000). Although, nematicides are efficient and fast-acting, they are currently being reappraised with respect to the environmental hazards associated with them. In addition they are relatively unaffordable to many small-scale farmers. The persistent pressure on farmers to adopt strategies that do not pollute the environment has increased urgency in the search for alternative sustainable methods to regulate plant parasitic nematodes (Pinkerton *et al*., 2000; Mashela *et al*., 2008).

One of the alternative strategies for management of plant pararsitic nematodes is the application of organic amendments in the soil (Agyarko and Asante, 2005). Oka *et al.* (2000) pointed that organic amendments have consistently been shown to have beneficial effects on soil nutrients, soil physical conditions, soil biological activity and thereby improving the health of plants and reducing populations of plant parasitic nematodes. On the other hand, populations of free-living nematodes have also been shown to increase rapidly following the addition of organic substrates (Akhtar and Malik, 2000). Kimenju *et al*. (2004) reported that application of organic amendments stimulated the activity of natural antagonists of plant parasitic nematodes. However, the available reports do not mention the contribution of nematode destroying fungi in the reduction of plant parasitic nematodes yet they are known to destroy nematodes in the soil. *In vitro* experiments have shown that nematode destroying fungi increase in numbers or activity when organic substrates are incorporated into the soil (Gomes *et al*., 2000; Timm *et al*., 2001). In a related study,

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Jaffee (2006) reported that alfalfa (*Medicago sativa* L.) leaves enhanced the populations of *Dactylellina candidum* (Nees) but the study did not mention other nematode destroying fungi.

Nematode destroying fungi are natural enemies of plant parasitic nematodes (Nordbring-Hertz *et al*., 2002). Some of these fungi use adhesive conidia, branches, knobs and mycelia to parasitize nematodes. These devices are used to capture and destroy nematodes by means of an adhesive layer covering part or all of the device surfaces (Yang *et al*., 2007). Other fungi immobilize or kill nematodes by releasing toxins. This group of fungi has recently 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; Masoomeh *et al*., 2004).

This study was undertaken with the aim of determining the effects of chicken manure, cow manure and their combinations on occurrence of nematode-destroying fungi, nematode community in general and plant growth.

MATERIALS AND METHODS

Screenhouse experiments were carried out in the period between August 2007 and April 2008 at the University of Nairobi, Kenya. The amendments namely chicken manure, cow manure and their combinations were dried at 70°C until a constant weight was achieved. The amendments were then applied at the rate of 5% w/w (Kimenju *et al*., 2004) into soil that was naturally infested with nematodes and nematode-destroying fungi. The pots were irrigated and two-week old tomato seedlings (cv. Moneymaker) were transplanted into them. Un amended soil was used as a control. Treatments were arranged in a completely randomized design with five replications.

Isolation of nematode destroying fungi was done using the soil sprinkle technique as described by Jaffee *et al*. (1996). Tap water agar was prepared by dissolving 20 g of agar in 1 L of tap water. The medium was autoclaved and cooled to 45°C before amending it with 0.1 g L^{-1} of streptomycin sulfate to suppress bacterial growth. Approximately 1 g of soil from each sampling point was sprinkled onto the surface of water agar in petri dishes. Plant parasitic nematodes were added into the petri dishes as baits. The plates were incubated at room temperature and observed daily from the third week up to the 6th week under a microscope at low (40 x) magnification. The examination was focused on trapped nematodes, trapping organs and conidia of the nematode destroying fungi that grew from the soil. Nematodes were extracted from 200 cm^3 soil using the modified Baermann funnel technique as described by Hooper *et al*. (2005). The nematodes were identified to genus levels using the descriptions described by Bongers and Bongers (1998) and Mai and Mullin (1996) and then counted. Growth of tomato plants was monitored at the 4th and 7th weeks by assessing the plant height, leaf width- apical leaf of 3rd branch, internodal length (between 3rd and 4th branch) and the type of flower/flowering pattern. Shoot and root dry weights were taken at the end of the experiment after drying the samples at 70°C to constant mass.

All the data were tested for homogeneity and subjected to analysis of variance (Kindt and Coe, 2005). Where the overall F test was significant, means were compared using the Tukey Honest Significance test (HSD) at $p \leq 0.05$.

RESULTS

The differences in occurrence of nematode destroying fungi was significant ($p = 0.05$) among the treatments with means of 3.4 in chicken manure alone, 2.0 in combinations of cow and chicken manure, 1.2 in cow manure and 0.2 in control (Table 1).

The nematode destroying fungi occurred in frequencies of 50, 29.4, 17.6 and 2.9% in chicken manure, cow/chicken combination, cow manures and control, respectively (Fig. 1). Out of the nematode destroying fungi isolated, 71% were in the category of nematode trapping fungi while 29% of them were endo-parasitic. 50% of all the nematode destroying fungi were recorded in the soil treated with chicken manure. The mean richness of nematode destroying fungi was 3.4 in chicken manure and 0.2 in the control. Combinations of cow and chicken manure had a mean richness of 2.0 while cow manure had 1. Soil amended with chicken manure was the most diverse in terms of nematode destroying fungi with mean Shannon of 1.2. Combinations of cow and chicken was 0.64 diverse followed by cow manure alone with, 0.28 while the control had 0.

Of all the fungal isolates, *Arthrobotrys oligospora* (Fresenius) was most dominant and its occurrence was significantly different ($p \leq 0.05$) across the treatments with

Table 1: Effect of organic amendments on occurrence of nematode destroying fungi

Amendment	Mean occurrence of nematode destroying fungi
Control	0.2
Cow manure	1.2
Cow/Chicken manure	2.0
Chicken manure	3.4
p-value	3.604×10^{-05}

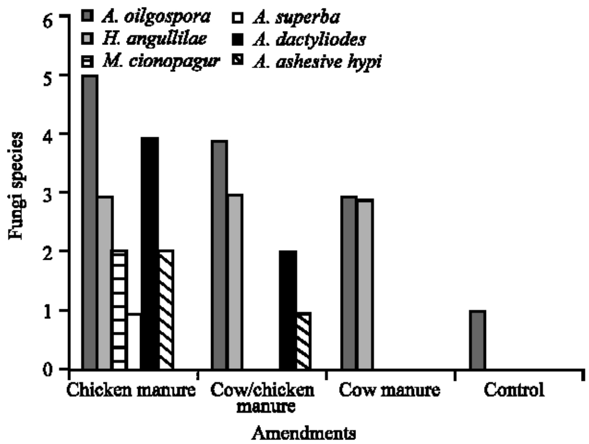

Fig. 1: Effect of organic amendments on occurrence of nematode destroying fungi

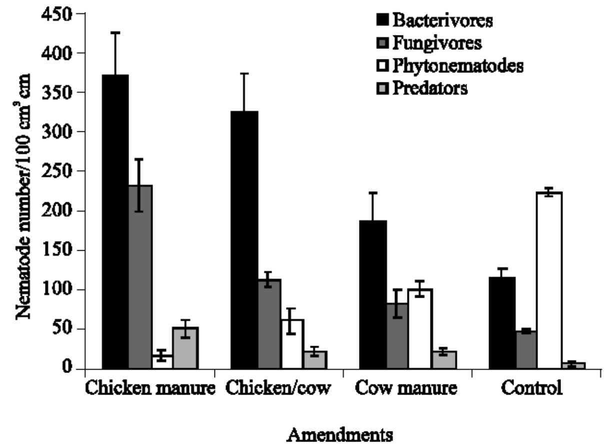

Fig. 2: Influence of organic amendments on nematode community structure

an isolation frequency of 38.2%. The other nematode destroying fungi had isolation frequencies of 26.5, 17.6, 8.8, 5.9 and 2.9% in *Harposporium aungulilae* (Lohde), *Arthrobotrys dactyloides* (Drechsler), *Monacrosporium cionopagum* (Drechsler), *Adhesive* hyphae and *A. superba* (Corda), respectively. *Arthrobotrys superba*, *H. aungulilae*, *M. cionopagum* and the Adhesive hyphae did not seem to respond to the treatments. The occurrence of *Athrobotrys dactyloides* was significantly different ($p = 6.837\times10^{-05}$) in all the treatments. Soils amended with chicken manure alone were characterized by presence of *A. oligospora*, *H. angullilae*, *A. superba*, *A. dactyloides*, *M. cionophagum* and nematode trapping structures such as adhesive hyphae. Soils amended with the combination of chicken and cow manure harbored populations of *A. oligospora*, *A. dactyloides*, *H. angullilae* and Adhesive hyphae. Nematode destroying fungi, *A. oligospora*, *H. angullilae* and *A. dactyloides* were isolated from soils amended with cow manure alone.

On nematode community, organic amendments resulted in a significant change in composition of the nematode community (Fig. 2). Application of the organic amendments caused an increase in numbers of bacterial and fungal feeding nematodes. There was an increase of 225, 96 and 62% in bacterial feeding nematodes in soils amended with chicken manure, combination of chicken and cow manure and cow manure, respectively. Similarly, application of chicken manure alone, combination of chicken and cow manure and cow manure alone led to a 391, 96 and 74%, respective increase in fungal feeding nematodes. In addition, application of the amendments suppressed the numbers of plant parasitic nematodes. Chicken manure led to 92% reduction in plant parasitic nematodes compared to 73 and 55% reduction after

Table 2: Effect of organic amendments on plant height, leaf width, number of branches, internode lengths and shoot widths

Treatment	Plant height	Stem diameter	Internode length	Leaf width
Control	32.1	2.46	6.17	3.99
Cow manure	31.5	2.68	3.67	3.52
Chicken manure	34.8	2.90	3.59	4.43
Cow/Chicken	46.8	2.76	4.47	5.03
p-value	0.03319	0.2778*	3.830×10^{-08}	1.822×10^{-08}

* Growth parameters not affected by the treatments

application of chicken and cow manure in combination and cow manure alone, respectively. The populations of predacious nematodes increased in response to application of the organic amendments. The highest number of predacious nematodes was recorded in soils amended with chicken manure.

Further results showed that organic amendments caused significant differences ($p\leq0.05$) in the plant height. The mean heights were 46.8, 34.8, 31.5 and 32.1 cm in combinations of cow and chicken manure, chicken manure alone, cow manure alone and control, respectively (Table 2). The number of branches had significantly increased in all the treatments in the 7th week than 4th week ($p = 2.2\times10^{-16}$). In the 7th week, the plants were significantly taller and with thicker shoot than in 4th week. Flower induction was earliest and more pronounced in tomato plants grown in soil amended with chicken manure, chicken/cow manure, cow manure and least in control pots. The combination of chicken and cow manure had the highest mean dry weight of 6.6 kg. Chicken manure alone recorded 5.6, cow manure alone 2.0 and 1.5 kg in control in descending order.

A similar trend was observed in the repeat experiment as shown in Table 3. The diversity and richness of nematode destroying fungi was higher in soil amended with chicken manure alone, combination of chicken and

Table 3: Effect of organic amendments on plant height, flower production, diversity and richness of nematode destroying fungi and plant parasitic nematodes

Treatment	Plant height	Flower production	Dry weight	Mean Shannon index of fungi	Mean richness of fungi	Mean PPN
Control	30.3	0.1	1.36	0.4564	1.6	222.0
Cow manure	42.6	0.5	2.25	0.7049	2.0	141.5
Chicken and cow	53.3	1.0	3.37	0.9247	2.7	84.0
Chicken manure	57.3	1.0	4.23	1.0450	3.0	58.5
p-value	6.189×10^{-07}	7.906×10^{-08}	1.33×10^{-05}	0.02466	0.03596	3.039×10^{-09}

PPN: Plant Parasitic Nematodes

cow manure and cow manure alone as compared to the control. Plant parasitic nematode numbers were significantly lower ($p = 3.039\times10^{-09}$) in soils amended with chicken manure alone and all the other amendments compared to the control.

Plant growth parameters were significantly higher in soils with organic amendments compared to the control. The plant height and mean dry weight were higher in chicken manure treatments, followed by a combination of cow and chicken manure, cow manure and least in the control. Amendments with chicken manure also recorded more flowers than cow manure alone and control.

In summary, the results from this study indicate that the organic amendments stimulated the occurrence of nematode destroying fungi, changed the nematode community by reducing the plant parasitic nematodes. In addition, amendments enhanced plant growth vigor. Specifically, chicken manure alone enhances the diversity and richness of nematode destroying fungi and reduction of plant parasitic nematodes. The combination of chicken and cow manure was the best in stimulation of plant growth.

DISCUSSION

This study has revealed that organic amendments and especially chicken manure stimulated build-up of nematode destroying fungi, *Arthrobotrys oligospora*, *Harposporium angullilae*, *A. superba*, *A. dactyloides*, *Monacrosporium cionopagum* and related nematode-destroying structures in the soil. The organic amendment supplies the needed food sources to the nematode trapping fungi hence their enhancement. This is supported by the findings of Timm *et al.* (2001), who suggested that the increase in nematode-trapping fungi after addition of organic amendment is due to available carbon and energy from the organic amendment and nitrogen from consumed nematodes. In a related study, Jaffee (2006), also showed that organic amendments enhanced build-up of nematode-trapping fungi *Dactylellina candidum* (Nees) though no other fungi were mentioned and are thought to be influenced differently depending on their feeding mechanism (parasitic of saprophytic).

The fungi *Arthrobotrys oligospora* was the most enhanced in this study by the organic amendments and especially by the chicken manure. Probably compounds containing ammonia also enhance the population of nematode destroying fungi. From this study, chicken manure and then combination of chicken and cow manures stimulated the buildup of nematode destroying fungi as well as reducing the population of plant parasitic nematodes. The biological control efficacy of ammonia like the one found in chicken manure, has been shown to be equivalent to that of 1,3-D, chloropicrin, metam-sodium, cadusafos, or metam-sodium (Yücel *et al*., 2002; Koenning *et al*., 2003). In a related study (Jaffee, 2004) absent in the literature, reported that *Arthrobotyrs oligospora* was enhanced by large quantities of alfalfa amendments. Alfalfa leaves supplied nitrogen in the soil which in turn increased the population of *A. oligospora* in the soil. Viaene *et al.* (2006) reported that *A. oligospora* which immobilizes nematodes by using mycelial traps such as non-adhesive knobs and constricting, rings, could be used as a biological control agent of plant parasitic nematode. Other species of *Arthrobotrys* have been used as biological control of plant parasitic nematodes with recommendable success (Kiewnick *et al*., 2004). What is not clear is whether it is the nematode destroying fungi that reduces the population of plant parasitic nematodes in the soil. Although, strong indications of nematode trapping fungi suppressing nematodes have been demonstrated in the laboratory using Petri dishes (Elshafie *et al*., 2006), it is till not clear with field and green house experiments (Jaffee and Strong, 2005; Jaffee *et al*., 2007).

It has been demonstrated in this study that application of organic soil amendments resulted in changes in nematode community structure by increasing the abundance of free-living nematode populations and suppressing plant parasitic nematodes. Application of soil amendments is becoming a conventional practice that helps in the control of nematodes and other soil-borne diseases. Comprehensive studies like those of Koenning *et al.* (2003) have revealed the nematicidal potential of organic products used as soil amendments. When incorporated into the soil, organic substrates undergo biologically mediated decomposition to release

NH_4^+, formaldehyde, phenols and volatile fatty acids, among other compounds (Wang *et al*., 2004). The involvement of soil micro-organisms in nematode control in amended soils has been confirmed by the fact that soil irradiation disrupts the nematicidal effect of these amendments (Kaskavalci, 2007). It has been established that application of organic substrates leads to build-up of micro-organisms which serve as are food substrates for free-living nematodes hence their build-up. Populations of free-living nematodes such as bacteriovores, fungivores and predacious have been shown to rapidly increase following the addition of organic amendments (Akhtar and Malik, 2000; Jaffee, 2002). In addition, free-living nematodes may accelerate the decomposition of soil organic matter and increase mineralization of nitrogen and phosphorous thus triggering a chain reaction that favours their build-up (Widmer and Abawi, 2000; Kimenju *et al*., 2004). Yücel *et al.* (2002) categorical that organic amendments that have high nitrogen content and release ammonia upon decomposition are more effective in nematode suppression.

In this study plants grown on soil amended with organic substrates grew and differentiated faster reaching flowering stage earlier than the control. Unlike the nematode destroying fungi and the nematode communities which were enhanced by the chicken manure, the growth of the plants was more enhanced by the combination of chicken and cow manures. The increase in growth is attributed to the release of macro - and micronutrients, plant growth regulators and stimulation of beneficial micro flora such as the mycorrhizae fungi (Kaskavalci, 2007). In the current study, the plants grown on organic amendments were taller and heavier compared to the control. Since dry weight is used to estimate productivity (Opik *et al*., 2005) productivity would be expected to be higher in plants treated with organic amendments. Though the inorganic fertilizers were not tested in the current study, the findings are in agreement with Widmer and Abawi (2000), who reported that plants grown in plots receiving organic manures were always larger than those receiving inorganic fertilizers. In a separate study, weights of tomato plants grown in the ammonia-treated soils were about six fold greater than the control. Amendments therefore represent important resource for the improvement of soil fertility because decomposed materials ultimately serve as sources of nutrients for plants and thus improve crop yields.

The increase in crop vigour may partly be attributed to reduced plant-parasitic nematode populations, but nutrients availability cannot be ignored. In a earlier study, decrease in populations of parasitic nematodes has been associated with increased in crop yield (Zolda, 2006). In turn, the decline in plant parasitic nematodes in this study could probably be attributed to the high number of nematode destroying fungi. The association between nematode-trapping fungi, organic matter, plant growth and nematodes community is complex. It would be difficult to conclude that the increase of nematode destroying fungi would lead to automatically reduction of plant parasitic nematodes hence health plants. Akhtar and Malik (2000) suggested that free-living nematodes reproduce rapidly when presented with organic substrate and play an important role in recycling of plant nutrients making them available to plants. Such organic substrates again have been seen to support high numbers of nematode destroying fungi and reduced populations of plant parasitic nematodes. Therefore, low numbers of plant parasitic nematodes coupled with high numbers of nematode destroying fungi and high nutrient levels in the soil has a positive effect to plant growth. It would be impossible to attribute the performance of the crop to either of them. In order to access the contribution of nematode destroying fungi, there is need to address the quantification and efficacy methods (Jaffee, 2006). More study should be devoted to the correlation between populations of nematode destroying fungi, plant parasitic nematodes and the actual plant yield.

CONCLUSION

This study has demonstrated the potential of organic amendments in stimulation of nematode destroying fungi for the management of plant-parasitic nematodes. The occurrence and diversity of nematode destroying fungi was associated with the decreased number of plant parasitic nematodes. Amendments cause improved plant growth and changes the nematode community structure particularly leading to decreased plant parasitic nematodes. However, such alternative nematode management strategies are unlikely to be as effective and fast-acting as nematicides. Although nematicides would reduce the plant parasitic nematodes, other environmental and soil fertility issues arise. Therefore, sustainable management of plant-parasitic nematodes from addition of organic amendments to the soil overrides all other considerations.

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