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Response of Free-Living Nematodes to Treatments Targeting Plant Parasitic Nematodes in Carnation

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Abstract: This study was carried out with the aim of evaluating the effect of ecologically sound approaches for nematode management on non-target organisms, free-living nematodes. The materials tested were sugarcane bagasse, molasses, tea and flower composts, neem (Achook), a biological agent (*Paecilomyces lilacinus*) and fenamiphos (Nemacur). The treatments were administered before planting carnation *var.* White Natila in flower beds that were naturally infested with nematodes. Application of bagasse, molasses, tea and flower composts resulted in increased abundance of free-living nematodes compared to the control where nothing was applied. Bacterial feeders, fungal feeders, and predators comprised 73, 14 and 13%, respectively of the free-living nematodes recovered. Members of the genus *Rhabditis* were the most abundant (10%) among the bacteriovores while *Mononchus* (10%) and *Aphelenchoides* (14%) dominated among the predators and fungivorous trophic groups, respectively. The highest numbers of free-living nematodes were recorded at 90 Days after Planting (DAP) in plots treated with bagasse and molasses but the numbers declined at 180 DAP. A steady increase in numbers of free-living nematodes was observed in plots treated with tea and flower composts up to 180 DAP. Significant reductions in abundance of free-living nematodes were recorded in plots treated with fenamiphos and neem. This study has established that application of organic substrates serve as a stimulus to processes leading to build-up of free-living nematodes. The organic substrates can strongly be recommended for use in sustainable carnation production systems.

Key words: Bacteriovores, fenamiphos, fungivores, neem, organic amendments

INTRODUCTION

The horticulture sub-sector has registered a phenomenal growth of 15-20% in the past decade to rank third as a foreign exchange earner in Kenya. It is currently the fastest growing agricultural sub-sector, with annual earnings of US$ 300 million (Waceke *et al.*, 2005). Fruits, vegetables and cut-flower production are the main sectors of horticultural production in Kenya. The cut flower industry dominates the horticultural exports, earning US$ 500 million in 2003 and provides direct employment to over 500,000 people (Manda and Sen, 2005).

Carnation (*Dianthus caryophyllus* L.) is grown on more than 500 ha mainly under greenhouse conditions. Production of the crop has been increasing steadily in response to the expanding market demand (Waceke *et al.*, 2001). Ultimately, Kenya has become the European Union's biggest source of the carnations, having overtaken Israel as market leader. Nematodes have been rated among the principal constraints to carnation production since they damage roots and reduce the ability of plants to take up water or nutrients from soil (Masse *et al.*, 2002). In ornamentals, the problem is compounded by foliar nematodes (*Aphelenchus* spp., *Aphelenchoides* spp. and *Ditylenchus* spp.) which cause qualitative losses. Losses due to nematodes in cut flower production are estimated to be 10-20% worldwide (Agudelo, *et al.*, 2006). Chemical nematicides have dominated in the management of the nematodes but environmental concerns have provided an added impetus to the search for alternative strategies (Wang, 2004). Biological control, combined with other feasible strategies is an area that is fast gaining popularity as a sustainable strategy in nematode management (Mostafa, 2001; Kiewnick and Sikora, 2004). This approach optimizes ecological synergies between biological components of the ecosystem, enhancing biological efficiency of soil processes in order to maintain soil fertility, productivity and crop protection in a new approach referred to as

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ecologically based pest management, which aims at minimizing adverse effects on non-target species and the environment (Steinberger *et al*., 2001). While plant parasitic nematodes have a negative impact on the plant, free living nematodes are known to play a role in nutrient cycling among other benefits. According to Sánchez-Moreno and Navas (2007), abundance and diversity of free-living nematodes is a good indicator of soil health, given their role in decomposition and regulation of bacterial and fungal microbes (Al-Sayed *et al*., 2007). It is, therefore, imperative to conserve biodiversity of these free-living nematodes. Whilst large data sets are available on the effect of organic substrates on plant parasitic nematodes, few investigations have examined their effects on the free-living nematode fauna (Ekschmitt *et al*., 2001). This study was undertaken with the aim of determining the effects of treatments targeting plant parasitic nematodes on free-living nematodes.

MATERIALS AND METHODS

The study was carried out between June 2006 and July 2007 at Kericho, Kenya. The experiments were conducted under greenhouse conditions in an area situated at an altitude of 1950 m a.s.l and receives mean annual rainfall of 2000 mm with mean annual day and night temperatures of 25 and 11°C, respectively. The soil type is sandy loam humic nitisols with an inherent pH of 4-4.5 but it is usually amended using agricultural lime to pH 6 to make it suitable for growing of carnations.

Parallel flower beds, measuring 100 cm in width and raised to a height of 25 cm, were made in a greenhouse, leaving a path of 50 cm between them. Experimental plots, measuring 100×400 cm, were marked out in the flower beds. The treatments tested were bagasse, tea and flower composts, molasses (a by-product of sugarcane processing), neem, *Paecilomyces lilacinus* (PL plus) and fenamiphos (Nemacur) while untreated plots were included as a control. Bagasse, tea and flower composts were sun dried to a constant weight. The composts and bagasse were applied at the rate of 300 t ha^{-1} as recommended for greenhouse usage (McSorley and Gallagher, 1995). Molasses was applied at the rate of 667 mL m^{-3} (Schenck, 2001) while neem was applied following the manufacturer's recommendation at 1.5 mL m^{-2} dissolved in 1000 L of water. *Paecilomyces lilacinus* (PL-plus) was applied at the rate of 2 kg ha^{-1} while fenamiphos was applied 1 week before planting at the rate of 30 g m^{-2}. Spray carnations cv. White Natila seedlings were transplanted into the plots. Treatments were arranged in a completely randomized design with 6 replications.

Characterization of nematodes in the research site: Five soil sub-samples were randomly collected to a depth 30 cm from the middle rows of each plot. The nematodes were extracted from 200 cm^3 soil using the procedure described by Hooper *et al.* (2005). The sub-samples were thoroughly mixed to form a composite sample before being placed in plastic sampling bags, transported to the laboratory and stored at 4°C. Soil sampling was done at planting, 90 and 180 days after planting. Nematodes from each sample were fixed using rapid Seinhorst technique and thereafter mounted on Cobb-type aluminium double cover glass slides that allow examination from either side (Siddiqi, 2000). Identification of the nematodes was based on morphological characteristics and pictorial keys using a high power microscope (Hunt *et al*., 2005). After identification, the nematodes were assigned to trophic groups as described by Yeates *et al.* (1993). Nematode abundance was transformed to Log (x+1) and different treatments were compared by two-way Analysis of Variance (ANOVA). Comparison of the means was done with the Least Significant Difference (LSD) test.

RESULTS

Effect of organic substrates and bio-control agents on non-parasitic nematodes in carnations: Application of bagasse, molasses, tea and flower composts as organic amendments led to significant ($p \leq 0.05$) increase in numbers of free-living nematodes in the soil (Table 1). A decline in nematode numbers was recorded in plots treated with fenamiphos and neem, compared to the control. The differences in mean numbers of free-living nematodes were not significant in plots that were treated with *P. lilacinus*, compared to the control.

Nematodes from 14 genera were recovered from carnation plots treated with organic substrates and the bio-control agent, *P. lilacinus* (Table 2). Among the free-living nematodes, bacterial feeders accounted for 73% while fungal feeders and predators accounted for 14 and

Table 1: Effect of different treatments on the free-living nematodes in carnation production systems

	Treatments							
	Fenamiphos	Neem	*P. lilacinus*	Control	Flower compost	Molasses	Tea compost	Bagasse
Nematodes/200 cm^3 soil	66c*	76c	220b	240b	270a	280a	290a	300a

*Mean followed by different letter(s) are significantly different along the row ($p \leq 0.05$)

Table 2: Occurrence and abundance of free living nematodes following application of the various organic substrates in carnations

Nematode genera	Trophic group	Density (%)	F-value Treatment	Treatment×Time interaction
Aphelenchoides	Fungivore	13.9	**	**
Mononchus	Predator	10.2	**	**
Rhabditis	Bacterivore	10.0	**	**
Cephalobus	Bacterivore	9.8	NS[A]	NS
Chromadora	Bacterivore	9.8	NS	**
Acrobeles	Bacterivore	8.7	**	**
Prodorylaimus	Bacterivore	8.1	**	**
Bunonema	Bacterivore	7.4	**	**
Aphelenchus	Bacterivore	4.8	**	**
Eucephalobus	Bacterivore	4.6	**	**
Heterocephalobus	Bacterivore	3.9	**	**
Labronema	Bacterivore	3.3	**	**
Plectus	Bacterivore	2.8	**	**
Nygolaimus	Predator	2.7	**	**

[A]NS: Not Significant, **Significant differences

Table 3: Effect of different treatments on free-living nematodes after varying durations from planting to 180 days after planting

Days after planting	Treatments Fenamiphos	Neem	Control	*P. lilacinus*	Flower compost	Tea compost	Bagasse	Molasses
0 DAP	30d*	25d	20d	65b	25d	80a	85a	45c
90 DAP	40c	90c	280b	320b	350b	350b	500a	520a
180 DAP	120e	100e	290c	200d	480a	460a	320b	250b

*Means followed by different letter(s) are significantly different along the rows

Table 4: Effect of different treatments, applied to control parasitic nematodes, on free living nematodes in the soil

Nematode genera	Nematode numbers/200 cm^3 soil under various treatments Bagasse	Tea compost	Flower compost	Fenamiphos	Neem	Molasses	*P. lilacinus*	Control
Aphelenchoides	47.2a*	36.4b	34.4bc	10.6d	9.7d	40.8ab	35.3b	25.3c
Mononchus	34.2a	27.5bc	27.5bc	6.7e	10.8de	31.9ab	13.6d	23.1c
Rhabditis	30.8a	30.0ab	26.9abc	5.3e	13.3d	23.1bc	20.1cd	22.8bc
Acrobeles	25.6a	25.8a	22.8ab	6.1d	12.8c	22.5ab	15.8c	18.3c
Prodorylaimus	29.2a	21.4ab	16.7bc	8.3c	11.7c	27.8a	14.2bc	10.61c
Bunonema	20.0abc	22.8a	21.7ab	5.3e	11.4de	19.7abc	12.8cde	13.9bcd
Aphelenchus	15.6a	15.6a	13.1ab	1.1c	1.4c	9.7b	16.1a	10.3b
Eucephalobus	12.8ab	10.6bc	10.6bc	3.6d	8.1cd	16.7a	7.2cd	10.0bc
Heterocephalobus	10.8bc	19.4a	14.1ab	0.0e	1.4e	8.3cd	4.4de	9.2bcd
Labronema	11.4a	12.2a	10.0a	0.0c	0.8c	10.8a	3.9bc	7.8ab
Plectus	6.4abc	5.3bcd	9.7ab	0.6d	2.5cd	9.4ab	2.8cd	11.7a
Nygolaimus	5.6cd	8.9abc	11.4a	1.1e	0.8e	6.7c	2.2de	10.6ab

*Means within the same column followed by different letter(s) are significantly different ($p \leq 0.05$)

13%, respectively. The bacterivorous nematodes were members of the genera *Acrobeles*, *Rhabditis*, *Cephalobus*, *Prodorylaimus*, *Bunonema*, *Eucephalobus*, *Heterocephalobus*, *Plectus*, *Nygolaimus* and *Chromadora*. The predators were members of the genera *Mononchus* and *Labronema* while the fungivores were assigned to the genera *Aphelenchoides* and *Aphelenchus*. Members of the genus *Rhabditis* were predominant among the bacterial feeding nematodes, representing 10% of the nematodes while *Mononchus* (10%) and *Aphelenchoides* (14%) dominated the predacious and fungivorous trophic groups, respectively. The treatments had a significant effect on the free-living nematodes, with the exception of those in the genera *Cephalobus* and *Chromadora*.

The treatments had variable effects on numbers of free-living nematodes over time, from application during planting throughout to 180 days after planting (Table 3). With the exception of fenamiphos and neem, all the other treatments led to a sharp increase in numbers of free-living nematodes within the first 90 DAP. The nematode numbers continued to increase in plots treated with tea and flower composts, fenamiphos as well as in the control up to 180 days.

Free-living nematodes from different genera had varying responses to the various treatments aimed at controlling plant-parasitic nematodes (Table 4). The organic amendments namely bagasse, molasses, tea and flower composts induced an increase in numbers of nematodes from most of the genera of free-living nematodes. Bagasse led to an increase in all the nematodes, except *Nygolaimus* and *Eucephalobus* spp. While most organic substrates had a positive effect on the populations of bacterivorous nematodes, *P. lilacinus* (biocontrol agent) greatly increased the populations of fungivorous nematodes in the genera *Aphelenchus* spp. and *Aphelenchoides* spp. Plots treated with fenamiphos and neem had significantly lower numbers of free living nematodes compared to the other treatments.

DISCUSSION

This study has demonstrated that amending soils with organic substrates as well as with biological agents contribute to a change in nematode community structure by increasing the abundance of free-living nematode populations. Populations of free-living nematodes have been shown to increase rapidly following the addition of organic substrates (Akhtar and Malik, 2000; Agyarko and Asante, 2005). The mode of action of organic substrates leading to stimulation of free-living nematodes is complex and dependent on the nature of the substrate (El-Sherif *et al*., 2007). These mechanisms stem from the decomposition process that leads to changes in the physical and chemical properties of the soil. According to Sanchez and Navas (2007), the nematode community structure is strongly impacted by changes in soil systems since nematodes are highly dependent on soil properties. When incorporated into the soil, organic substrates undergo a series of processes that release NH_4^+, formaldehyde, phenols and volatile fatty acids, among other compounds (Wang *et al*., 2004). The compounds may act individually or collectively to stimulate build-up of beneficial microbes including free-living nematodes (Desaeger and Rao, 2002). According to Akhtar and Malik (2000), there could be a correlation between increase in NH_4^+ and an increase in numbers of free-living nematodes following addition of organic substrates. 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 increase of the nematodes (Kimenju *et al*., 2004).

From this study, the diversity of free-living nematodes recovered from the carnation production system was lower compared to previous studies in other production systems (Yeates *et al*., 1999; Zolda, 2006). This can be attributed to the fact that cut-flower production is characterized by usage of enormous amounts of agrochemicals, mainly in the form of fertilizers and pesticides (Tenenbaum, 2002). Loss of biodiversity has indeed been attributed to the adverse effects that are associated with agrochemicals, especially the use of nematicides (Yeates *et al*., 1999). Fumigation of soil to control soilborne pathogens and nematodes is recognized as one of the most serious threats to the beneficial organisms such as free-living nematodes (Pinkerton *et al*., 2000). Bacterial feeders dominated the trophic groups isolated in this study and this finding is consistent with reports from other workers (Wasilewska, 1997; Ekschmitt *et al*., 2001). According to Zolda (2006), the elevated numbers of bacterial feeding nematodes can be attributed to increased food resources for the microorganisms in the soil. Indeed, bacterivorous nematodes responded quickly to increased food supply (Yeates *et al*., 1999). In addition, decomposition pathways in agricultural systems are mostly driven by bacteria which serve as a stimulus to increased numbers of bacterial feeding nematodes (McSorley and Frederick, 2000). Rapid changes in numbers of bacterivorous nematodes can be anticipated, given their short generation time of 3-4 days (Ruess, 2003).

The dominance of fungal feeding genera (*Aphelenchoides* spp. and *Aphelenchus* spp.) in soils amended with *P. lilacinus* (PL Plus) is noteworthy because fungivorous genera are normally found at lower densities than bacteriovores and predators (Knudsen and Bae, 2001). *Paecilomyces lilacinus* is an ubiquitious soil hyphomycete which parasitizes eggs of root-knot nematodes thus regulating populations of the nematodes in field soil (Schenck, 2004). Numerous species of fungivores have been found in soil with the most common genera being *Aphelenchus* and *Aphelenchoides* (Yeates *et al*., 1999). A fungal-dominated decomposition pathway is however likely, considering that species of one of the most prevalent genus in this study, namely *Acrobeles* as well as plant parasitic *Filenchus* and *Tylenchus* may also feed on fungi (Zolda, 2006). The relative increase in fungal biomass occasioned by application of *P. lilacinus*, could have resulted in the relative increase of fungal feeding nematodes. Kiewnick and Sikora (1989) reported that numbers of the mycophagous nematodes, *Aphelenchoides* sp. and *Aphelenchus avenae*, increased several-fold within a few days after adding flax to roots that had been precolonized by *Rhizoctonia solani*. Moreover, abundance of bacterivorous nematodes has been shown to reduce bacterial biomass, occasioning a relative increase of fungal biomass (Soylu *et al*., 2005). Bae *et al*. (2001) observed many nematodes associated with *Trichoderma* hyphae presumably feeding and numerous nematode eggs adhering to hyphae. This led to the conclusion that populations of fungivorous nematodes may increase rapidly following addition of fungi as biocontrol agents.

The study revealed that neem and fenamiphos caused a reduction in numbers of free-living nematodes. Pesticidal properties of azadirachtin, the active ingredient in neem, have been clearly documented (Akhtar and

Malik, 2000; Agyarko and Asante, 2005). According to Agyarko and Asante (2005), neem based products reduced egg hatch and the mobility of nematode juveniles. Apart from azadirachtin, several other compounds namely salannin, nimbidin, thionemone ammonia, phenol, formaldehyde and fatty acids are released during decomposition of neem-based products (Yasmin *et al*., 2003). It is possible that these compounds are individually or collectively detrimental to free-living nematodes, thus accounting for the decline in their numbers in treated plots. In conclusion, organic substrates are increasingly gaining popularity as components of integrated pest management, developed with the goal of reducing chemical usage in the control of plant parasitic nematodes. The fact that addition of the organic substrates results in build-up of beneficial organisms such as free-living nematodes is an added advantage.

CONCLUSION

An increase in numbers of free-living nematodes was recorded in all plots where different organic substrates were applied with exception of neem. A decline in numbers of the nematodes was observed in plots treated with chemical nematicides (fenamiphos). It is recommended, the organic amendments should be incorporated in carnation production given the range of other benefits which include disease suppression, increased soil water holding capacity, improvement of soil fertility and structure.

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