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Evaluation of Selected Methods in the Control of Plant Parasitic Nematodes Infecting Carnation

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Abstract

Plant parasitic nematodes, whose control options are restricted, continue to cause enormous losses in crop production systems including carnations. This study was carried out with the aim of developing environmentally sound approaches for adoption in the management of phytonematodes affecting carnations. Various organic amendments were evaluated namely sugarcane bagasse, tea and assorted flower composts and molasses, in addition nematophagous fungus *Paecilomyces lilacinus* (PL plus®) and neem (Achook®). Meanwhile, a standard chemical nematicide, fenamiphos (Nemacur®), was used next to untreated plots were as control. The experiments were carried out under greenhouse conditions. Soil samples were collected before application of the amendments and at 90 and 180 days after treatment. Parasitic nematodes belonging to 16 genera were detected in plots where carnation had been produced under monoculture over several years. The most predominant of the nematodes detected were in the genera *Scutellonema, Helicotylenchus* and *Meloidogyne,* with 100, 82 and 100% frequencies of occurrence, respectively. Treatments effects caused a reduction in numbers of plant parasitic nematodes, with the exception of nematodes in the genera *Helicotylenchus, Criconema* and *Longidorus.* Galling due to root-knot nematode was reduced by between 53%, in plots treated with sugarcane bagasse, and 69% in those treated with neem. This study has established that application of organic substrates, neem and *P. lilacinus* reduced plant parasitic nematodes. The materials can be recommended for use in sustainable carnation production systems.

Keywords: Dianthus caryophyllus, Meloidogyne spp., neem, organic substrates and Paecilomyces lilacinus

1. Introduction

Carnation (*Dianthus caryophyllus* L.) is estimated to be grown on more than 500 ha mainly under greenhouse conditions in Kenya (HCDA, 2005). Production of the crop has been increasing gradually with expanding market demand. Despite the steady increase in carnation production, there are a number of limitations to the exploitation of the full potential of this industry. These include pests and diseases, excess pesticide usage, low quality produce and low yields (HCDA, 2000). Plant parasitic nematodes have been ranked among the principal constraints limiting carnation production in Kenya. The nematodes are known to damage roots individually but they also form complexes with other organisms which severely disrupts the ability of plants to take up water or nutrients from soil (Back et al., 2002; Masse et al., 2002). Yield loss on carnations due to nematodes alone is estimated at 10% - 20% on a worldwide scale (Phyllis, 1997).

A number of strategies have been developed for the management of plant parasitic nematodes. These strategies include chemical nematicides, fallowing, crop rotation, biological control, host resistance and organic soil amendments (Sikora & Fernandez, 2005). Application of chemical nematicides is the most widely used strategy, especially in intensive production systems involving high-value crops (Haydock et al., 2006). However, concerns about environmental health have raised the motivation to search for alternative methods that are less harmful to non-target species and to the environment (Pinkerton et al., 2000). An area that is fast gaining interest is the application of natural enemies coupled with application of organic amendments (Akhtar & Malik, 2000; El-Sherif et al., 2007). This approach optimizes the ecological synergies between biological components of the ecosystem, enhancing efficiency of soil processes in order to maintain soil fertility, productivity and crop protection. This strategy aims at minimizing the adverse effects on non-target organisms with the ultimate goal of enhancing biodiversity and ecosystem health (Pinkerton et al., 2000; Steinbeger et al., 2001). Information is required on options that can be adopted to reduce chemical use without compromising quality and crop productivity. The objective of this study was to determine the efficacy of locally available organic amendments, a commercial neem-based product and a nematophagous fungus in suppression of plant parasitic nematodes associated with carnations.

2. Materials and Methods

2.1 Experimental Site

The experiments were conducted under greenhouse conditions between January 2008 and March 2009 at James Finlays Company Farm, Kericho. Plots measuring 1 m x 4 m were laid out in randomized complete block design with six replications. Soil samples were collected prior to application of the treatments. Five soil sub-samples, each consisting of about 500 cm^3, were randomly collected to a depth of 30 cm from the three middle rows of each plot. The soil sub-samples were thoroughly mixed to form a composite sample which was placed in plastic bags and transported to the laboratory and stored at 10^oC. Soil sampling was repeated at 90 and 180 days after transplanting carnations into the plots.

The modified Baermann funnel technique (Hooper et al., 2005) was used to extract nematodes from 200 cm^3 of soil. The soil was spread on a double layer of milk filters supported by a sieve then placed in a shallow dish before adding water to a level where it just touched the soil. After 24 hours, the sieve was carefully removed and the nematodes suspension concentrated by passing through a series of four 45µm-aperture sieves and the nematodes collected from each of the sieves. Aliquots of 1 ml of a well-agitated nematode suspension was pipetted into a counting slide and observed under a light microscope. Counting was repeated for three aliquots and the mean nematode count recorded. Nematodes were then placed in vials and stored at 4^oC awaiting fixation. Nematodes were killed by subjecting them to a temperature of 55 – 60^oC for two minutes and then fixing them in 3% of formalin (Hooper, 2005). Twenty five nematodes from each suspension were selected for identification to the genus level based on morphological features (Hunt et al., 2005).

2.2 Organic Amendment for Plant Parasitic Nematodes Control

The materials tested were sugarcane bagasse which is a fibrous residue of cane stalks left over after the crushing and extraction of the juice. Tea and flower composts are decomposed green materials derived from leaves and shoot trimmings from grading sheds. Molasses (a by-product of sugarcane processing) is thick syrup derived from the processing of the sugarcane into sugar. Neem (Achook®) is a commercial botanical pesticide extracted from the neem tree and contains azadirachtin (0.15%). *Paecilomyces lilacinus* (PL plus®) is a nematophagous fungi known to parasitizes nematode eggs and is formulated in powder form containing 4×10^9 spores/gram of strain 251. Fenamiphos (Nemacur 5Gr®) is a non-volatile systemic nematicide that has been registered for use on a wide range of crops in Kenya. Plots that were not subjected to any treatment were considered as control.

Sugarcane bagasse, tea and flower composts were sun-dried until a constant mass was achieved in about 7 days. The composts and bagasse were incorporated into the soil, just before planting, using a hand hoe at the rate of 300 tons/ha as recommended for greenhouse usage (McSorley & Gallagher, 1995). Molasses was applied at the rate of 667 ml/m^2 (Schenck, 2001) while neem was applied following the manufacturer's recommendation at 1.5 ml/m,^{2} dissolved in one litre of water. *Paecilomyces lilacinus* was applied at the rate of 2 kg/ha in planting holes while fenamiphos was broadcasted and mixed into the soil, one week before planting, at the rate of 30 g/m^2. All the materials were applied once. One month old rooted spray carnation cultivar White Natila cuttings were transplanted into the plots at a spacing of 12.5 x 25 cm to a achieve a plant density of 32 plants/m^2. Treatments were arranged in a randomized complete block design replicated six times.

Root galling and eggmass indices were quantified using a scale of 1-9 where: 1 = 0 galls/eggs masses, 2 = 1-5, 3 = 6-10, 4 = 11-20, 5 = 21-30, 6 = 31-50, 7 = 51-70, 8 = 71-100, and 9 = 100 galls/egg masses (Sharma et al., 1994). All the data collected was subjected to analysis of variance and where applicable means were compared using LSD at $P\leq0.05$.

3. Results

Plant parasitic nematodes belonging to sixteen genera were identified in plots where carnation had been grown as a monocrop for over ten years. The genera identified were *Scutellonema, Helicotylenchus, Meloidogyne, Pratylenchus, Tylenchus, Hemicyclophora, Tylenchorhynchus, Rotylenchus, Tylenchulus, Criconema, Trichodorus, Hoplolaimus, Hemicriconemoides, Longidorus, Xiphinema* and *Paratylenchus* spp. (Table 1). The most predominant nematodes were in the genera *Scutellonema, Helicotylenchus* and *Meloidogyne* spp., with 100, 81 and 100 % frequencies of occurrence, respectively. The mean numbers of nematodes belonging to the genus *Scutellonema* was highest followed by those in the genus *Meloidogyne*.

Table 1. Plant parasitic nematodes associated with intensive carnation production under greenhouse conditions

Nematode genera	% Density (in 200 cm^3 soil)	Mean numbers	Frequency of occurrence (%)
Scutellonema	14.7	22	100
Helicotylenchus	13.6	13	81
Meloidogyne	11.6	15	100
Pratylenchus	10.5	13	64
Tylenchus	7.8	11	72
Hemicyclophora	7.4	6	50
Tylenchorynchus	6.1	11	61
Rotylenchus	5.5	10	48
Tylenchulus	5.3	10	42
Criconema	3.4	6	39
Trichodorus	3.0	7	41
Hoplolaimus	2.9	6	30
Hemicriconemoides	2.3	6	24
Longidorus	2.2	7	22
Xiphinema	2.1	7	20
Paratylenchus	1.6	7	18

Differences in numbers of all the plant parasitic nematodes were significant among the treatments applied (Figure 1). 90 days after treatment, fenamiphos and neem led to the highest reductions of 72% and 71% in nematode populations, respectively. Sugarcane bagasse was the least effective among the materials tested, causing a 31% reduction in nematode numbers compared to the control. Fenamiphos was the superior treatment, but it was not significantly different from neem. Among the organic materials, molasses was more effective than bagasse.

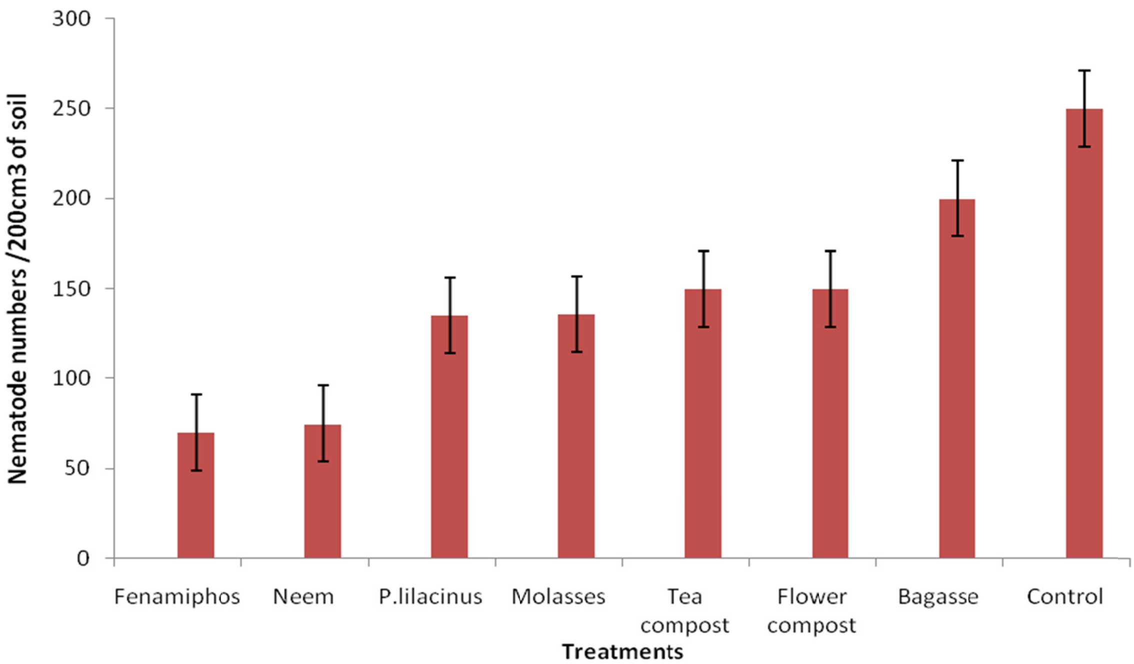

Figure 1. Effects of seven control agents on plant parasitic nematodes in carnations

A decline in nematode numbers was recorded in plots treated with fenamiphos and neem from planting up to 180 days after planting. The nematode numbers declined 90 days after treatment with *P. lilacinus* but an increase was recorded at 180 days. Numbers of plant parasitic nematodes was about two times more in the control compared to sugarcane bagasse, which was the least effective among the treatments at the end of the experiment.

Application of the different treatments had variable effects on plant parasitic nematodes from different genera (Table 2). The effects of sugarcane bagasse, tea compost and molasses were not significantly different on the number of nematodes in the genera *Hemicriconemoides, Pratylenchus, Paratylenchus, Trichodorus* and *Tylenchus*. Nematodes in the genera *Meloidogyne*, *Tylenchorhynchus*, *Rotylenchus*, *Tylenchus* and *Hoplolaimus* were suppressed by all the materials tested as shown in table 2. With the exception of fenamiphos and neem, the rest of the treatments did not cause significant effects on nematodes in the genus *Trichodorus*. The effect of *P. lilacinus* was not significant to nematodes in the genera *Hemicyclophora, Paratylenchus, Trichodorus* and *Tylenchus*.

Treating carnation plots with the various materials resulted in significant reduction in eggmass production and galling induced by root-knot nematodes (Table 3). The lowest eggmass indices (1.5) were observed in plots treated with neem or *P. lilacinus* while the highest (3.9) was recorded in plots treated with bagasse and molasses, excluding the control. Among the plots under treatment, the highest galling index (2.1) was observed in carnation treated with bagasse while plots treated with neem recorded the least (1.4). The highest reduction in galling index (69%) was recorded in plots treated with neem ,followed by the one treated with fenamiphos.

Table 2. Effect of different treatments on plant parasitic nematodes from different genera associated with carnations

Nematode genera	Numbers of nematodes in 200 cm^3 soil							
	Bagasse	Tea compost	Flower compost	Fenamiphos	Neem	Molasses	*P lilacinus*	Control
Scutellonema	18.1b	14.7b	18.9b	9.7b	14.2b	12.2b	61.1a	68.3a
Meloidogyne	20.8b	15bc	21.4b	4.4d	8.9cd	21.1b	20..6b	34.4a
Pratylenchus	20.8ab	12.5cd	11.9cd	6.7d	8.9d	16.4bc	18.3abc	23.6a
Tylenchus	13.9ab	13.3ab	6.4c	5.6c	6.1c	7.8bc	18.3a	17.1a
Tylenchorynchus	9.7b	8.6bc	8.9bc	1.9d	3.3cd	7.2bcd	3.1cd	26.9a
Rotylenchus	7.8bc	8.1b	8.3b	2.9c	6.9bc	6.9bc	8.5b	18.3a
Tylenchulus	5.8bc	7.8bc	6.4bc	3.9c	4.2bc	3.9c	9.2b	18.7a
Trichodorus	4.7ab	5.8a	5.0ab	1.1c	3.1bc	5.3ab	3.6abc	5.6ab
Hoplolaimus	4.2b	4.4b	3.9b	1.7b	1.4b	2.8b	4.4b	10.0a
Hemicriconemoides	3.9a	3.3ab	5.0a	0.8b	1.1b	4.7a	3.1ab	4.4a
Paratylenchus	2.2abc	2.5abc	2.8abc	0.8c	0.8c	1.7bc	3.3ab	4.2a

Data are means of 18 samples. Means followed by the same letter(s) along rows are not significantly different ($P \leq 0.05$).

Table 3. Effect of different treatments on eggmass (EMI) and galling (GI) caused by root-knot nematodes in carnations

Treatments	EMI	Galling index (GI)	% Reduction in GI
Bagasse	3.9b	2.1b	53%
Tea compost	3.1c	1.7c	62%
Flower compost	2.9c	1.8c	60%
Fenamiphos	1.7d	1.5d	67%
Neem	1.5d	1.4d	69%
Molasses	3.9b	1.9c	58%
P. lilacinus	1.5d	1.9c	58%
Control	6.2a	4.5a	-

Data are means of 18 samples. Means followed by the same letter(s) within columns are not significantly different ($P \leq 0.05$).

4. Discussion

Out of the sixteen plant parasitic nematodes associated with carnations, members of the genera *Scutellonema*, *Meloidogyne* and *Helicotylenchus* were the most frequently encountered. This finding is consistent with previous reports on distribution of plant parasitic associated with different cropping systems in Kenya (Kimenju et al., 1998; Kandji et al., 2003). Among the nematodes that were widespread in carnation plots, *Meloidogyne* spp. present the greatest threat, given their damage potential as endo-parasites, compared to the rest which are ectoparasites. Apart from the direct effects that root-knot nematodes have on plants, they form synergistic complexes with other disease causing organisms leading to severe destruction and reduction in yield (Back et al., 2002; Masse et al., 2002).

This study has demonstrated that amending soils with organic substrates as well as incorporating a biological control agent (*P. lilacinus*) and neem resulted in significant reduction of numbers of plant parasitic nematodes associated with carnation. The organic substrates tested namely bagasse, tea and flower composts and molasses were suppressive to all genera of plant parasitic nematodes in carnation plots. In addition, the amendments reduced the reproductive potential of root knot nematodes as measured using eggmass densities. These findings are in agreement with previous reports by Akhtar and Malik (2000), Agyarko and Asante (2005) who found that incorporating organic amendments into the soil, have a an effect on soil organisms.

Organic substrates offer an exciting alternative or supplement to other strategies, with the ultimate goal of reducing chemical usage in general and nematicides in particular (Akhtar & Malik, 2000). The mode of action stems from the decomposition process that leads to changes in the physical and chemical properties of the soil. According to Sanchez-Moreno and Navas (2007), the nematode community is strongly influenced 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 (Walker, 2004; Wang et al., 2004). The compounds may act individually or collectively to stimulate build-up of beneficial microbes that are antagonistic to plant parasitic nematodes (Kerry, 2000; Akhtar & Malik, 2000; Viaene et al., 2006). In addition to the direct effects on nematodes, organic amendments also increase the water holding capacity of the soil, improve the soil structure and release nutrients into the soil (Walker, 2004). These attributes are known to increase plant's ability to overcome the negative effects of nematode infestation (Mcsorley & Gallagher, 1995).

Application of organic substrates led to an initial increase in numbers of some plant parasitic nematodes but there was an ultimate reduction in the numbers. The delay in response of nematodes to application of organic substrates may be explained by the fact that a substantial amount of time is required for the breakdown of the substrates and subsequent release of the active ingredients into the soil (Widmer & Abawi, 2002; Kimenju et al., 2004).

In this study, the efficacy of *P. lilacinus* was clearly demonstrated as a promising strategy in carnation production for the control of root-knot nematodes. *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). While, Khalil et al. (2012) found that *P. lilacinus* was the most effective bioagent between others against *M. incognita* on tomato under greenhouse conditions for galls, egg masses and nematode population in soil by 66.67, 75.97 and 85.22%, respectively. Also, Kiewnick and Sikora (2006) recorded that the fungal biocontrol agent, *P. lilacinus* strain 251 (PL251) was potential to control the root-knot nematode *Meloidogyne incognita* on tomato. The action of *P. lilacinus* against plant parasitic nematodes was interpreted in multitude investigations. Khan et al. (2006) and Khan et al. (2004) recorded the directed penetration of fungal hypha to the female cuticle of *M. javanica* by transmission electron microscopy. While, Park et al. (2004) reported that *P. lilacinus* could be produce leucino toxin and other nematicidal compounds. In the laboratory test this fungus infested eggs of *M. incognita* and destroys the embryos within 5 days because of simple penetration of the egg cuticle by individual hypha aided by mechanical and/or enzymatic activities, in addition to killing juveniles and females of *M. incognita* and *Globodera pallida* (Jatala, 1986). The pesticidal properties of azadirachtin, the active ingredient in neem, have been clearly documented (Akhtar & Malik, 2000; Agyarko & Asante, 2005). According to Agyarko and Asante (2005), neem based products reduced egg hatching and the mobility of nematode juveniles. Several other compounds namely salannin, nimbidin, thionemone ammonia, phenols, formaldehyde and fatty acids are released during decomposition of neem-based products (Javed et al., 2007) which antagonises the nematodes reproduction. in the same trend both Khalil et al. (2012) and Saad et al. (2012) found that Azadirachtin (Achook 0.15%) effective against *Meloidogyne* sp. on tomato plants. It was suggested that neem may be effect as repellent compound on plant parasitic nematodes (Khalil, 2013).

Carnation growing is mainly done under monoculture in intensive production systems. According to Giller et al. (1997) monoculture is one of the practices that deny the soil the long-term benefits of functional and taxonomic diversity. The practice results in biophysical, chemical and hydrological changes that interfere with the biological equilibria in the soil (Freckman & Ettema, 1993). Application of non-chemical strategies and especially organic substrates may help to maintain the soil's productive potential.

5. Conclusion and Recommendations

From the study, it is evident that organic amendments can play a role in managing plant parasitic nematodes in high value crops. Further work is required to assess to the potential of combining different methods for plant parasitic nematodes.

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