# DEPLOYMENT OF ORGANIC SUBSTRATES AND BIOCONTROL AGENTS FOR NEMATODE MANAGEMENT IN CARNATIONS



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## DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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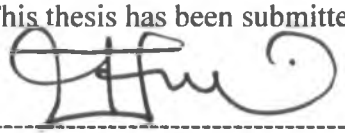
11/8/2009.

DATE

This thesis has been submitted with our approval as University supervisors.



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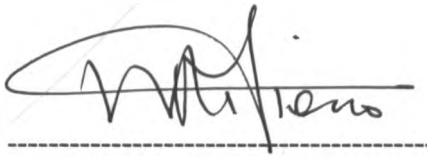

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**DEDICATION**

To my Family

## ACKNOWLEDGEMENT

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## ABSTRACT

Plant parasitic nematodes cause yield reductions in ornamental crops of up to 11%, however, intensive use of nematicides is being discouraged worldwide making environmental friendly management approaches a necessity. Thus a study was carried out with the aim of developing environmentally sound approaches in management of nematodes in carnations. Various organic substrates namely sugarcane bagasse, tea and flower composts, molasses, nematophagous fungus *Paecilomyces lilacinus* (PL plus®), neem (Achook®), and the standard chemical nematicide fenamiphos (Nemacur®) and a control were evaluated against plant parasitic nematodes and assessed their effect on non-target free-living nematodes and on overall nematode biodiversity populations associated with carnation plants. The experiments were carried out under greenhouse conditions using complete randomized design with six replications each. Soil samples were collected before application/planting (0 DAP), 90 DAP and 180 DAP. Nematode diversity was determined using the methods of Shannon-Wiener, abundance, richness, dominance, evenness and plant parasite indices of diversity. At the start of the experiments, there were no significant differences in populations of all nematodes. At 180 DAP out of the 16 genera of plant parasitic nematodes recovered only *Helicotylenchus, Criconema* and *Longidorus* populations had not been depressed by all treatments. Galling due to root-knot nematode was reduced by between 53 and 69% in treated plots compared to the control. The most predominant plant parasitic nematodes recovered at the end of the experiments were in the genera *Scutellonema, Helicotylenchus* and *Meloidogyne* with percentage densities of 14.7, 13.6 and 11.6 % respectively. Application of organic substrates namely bagasse, molasses, tea and flower composts resulted in increased abundance of free-living nematodes compared to the control. Among the 14 genera of the free-living nematodes recovered, bacterial feeders accounted for 73% while fungal feeders and predators accounted for 14% and 13% respectively. Members of the genus *Rhabditis* were predominant among the bacterial feeding nematodes representing 10% of the nematodes while *Mononchus* (10%) and

*Aphelenchoides* (14%) dominated predacious and fungivorous trophic groups respectively. A significant reduction in abundance of free-living nematodes was recorded in plots treated with fenamiphos and neem except in the genera *Cephalopus* and with the highest counts recorded in the control plot (796) followed by flower compost at 600 while the least was in plots treated with fenamiphos. In the genera richness, organic substrates and *Paecilomyces lilacinus* recorded the highest while fenamiphos plots had the least. Nematode diversity, according to Shannon-Weiner diversity index (H) at 180 DAP was 2.91, 2.98 and 2.95 in plots treated with bagasse, tea and flower composts respectively, compared to 1.8 in plots treated with fenamiphos. Dominance of nematodes was observed only in plots treated with fenamiphos and neem at 180 DAP. Evenness and Plant parasite indices were lowest before treatments were administered and increased with time reaching a peak at 180 days. This study has established that application of organic substrates and *Paecilomyces lilacinus* was suppressive to plant parasitic nematodes and also stimulated build-up of free-living nematodes. The products can be strongly recommended for use in sustainable carnation production systems.

# CHAPTER ONE

## 1.0 INTRODUCTION

The horticulture sub-sector has registered phenomenal growth of a record 15%-20% in the past decade to rank third in foreign exchange earnings in Kenya (HCDA 2005). It is currently the fastest growing agricultural sub-sector, with earnings of US$ 300 million (ILO, 2000). Fruits, vegetables and cut-flower production are the main aspects of horticultural production in Kenya (KFC, 2004). The cut flower industry dominates horticultural exports, earning US$ 500million in 2003 and provides direct employment to over 500, 000 people (HCDA, 2005). Roses make up 74% of Kenya's flower exports, followed by carnation which has a niche in some European countries.

Carnation (*Dianthus caryophyllus* L.) is presently estimated to be grown on more than 500 ha mainly under greenhouse conditions. Production of the crop has been increasing gradually with expanding market demand (HCDA, 2005). Kenya has become the European Union's biggest source of cut-flower and overtaken Israel as market leader (ILO, 2000). Despite the high growth rate recorded over the years, 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). Nematodes have been reported to be among the principal constraints limiting carnation production. Other soil pathogens known to present challenges include the fungal parasite *Fusarium oxysporum* f.sp. *dianthi* which causes severe damage in the areas where carnation is cultivated worldwide (Garibaldi and Gullino, 1987). These organisms are known to damage roots and reduce the ability of plants to take up water or nutrients from soil (Masse *et al.,* 2002). The problem is compounded in ornamentals because of foliar nematodes *Aphelenchus* spp., *Aphelenchoides* spp and *Ditylenchus* spp. which also cause qualitative losses. Yield loss due to

nematodes is estimated to be 10% - 20% worldwide (Phyllis, 1997), and about $24.5 million for the ornamentals in Kenya (HCDA, 2005).

A number of strategies have been developed for the management of root knot nematodes. These strategies include chemical nematicides, fallowing, cover copping, crop rotation, biological control, host resistance and organic soil amendments (Sikora *et al.,* 2005). Currently, control of root-knot nematodes is based mainly on the use of soil fumigants, and organophosphate and carbamate nematicides (Mutua *et al.,* 2007). Until recently, when concerns were raised about environmental health, due to chemicals, there is an added impetus to switch to biological methods from the deleterious environmental effects and non-sustainability of some of the more traditional methods in the management of nematodes (Pinkerton, *et al.,* 2000). But little is known about the effects of these management strategies on the diversity of native fauna in below-ground food webs. An area that is fast gaining interest in nematode management in these agricultural systems is what may be collectively termed 'novel biological' methods which include the use of soil amendments from a variety of sources (Hodda, 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 ecologically based pest management. This approach aims at minimizing the adverse effects on non-target species and the environment (Steinbeger *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. According to Sanchez-Moren and Navas (2007) free-living nematodes are a good indicator of soil health and soil recovery given their role in decomposition and regulation of bacterial and fungal microbes. It is therefore imperative to conserve biodiversity of these free-living nematodes (Pinkerton *et al.,* 2000).

More work is required to develop environmentally sound agricultural systems that minimize chemical usage while maintaining high production standards. It is therefore necessary to carry out selective suppression of plant-parasitic nematodes with no adverse effect on the beneficial free-living nematodes. Previous work has recommended that field studies should be conducted to obtain insights into the relationships between soil biodiversity and ecosystem functioning (Wall *et al.*, 2001; Robert and Louis, 2004). This study was therefore designed with the overall objective of developing an environmentally sound approach for management of nematodes in carnations.

The specific objectives were;

1. To evaluate efficacy of locally available organic substrates, a commercial neem-based product and a nematophagous fungus for suppression of plant-parasitic nematodes associated with carnations.
2. To determine the effects of various organic substrates, a nematophagous fungus and a commercial neem-based product on beneficial free-living nematodes.
3. To determine the effect of various organic substrates, a nematophagous fungus and a commercial neem-based product on the diversity of nematodes in carnations.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Origin and classification

Carnation (*Dianthus caryophyllus)* originated from the Mediterranean region. The name *Dianthus,* meaning lover's flower or divine flower, was designated to the genus by Theophrastus in the 4$^{th}$ century B.C. and was used by ancient Greeks while installing a queen thus was given a common name coronation flower and later carnation (Coats, 1968). Carnations belong to the widely cultivated genus *Dianthus*, a group of hardy and semi-hardy plants which also grow wild in southern Europe. Carnations are members of the family *Caryophyllaceae* with wild ancestors that are endemic to the Mediterranean region of Europe and China (Holdings, 1999).

### 2.2 Constraints to carnation production

Carnation production in Kenya is constrained by a number of factors. These include competition from other countries, poor infrastructure, pests and diseases and hence excess use of pesticides, lack of capital, lack of good quality propagation materials and appropriate growing technologies, inadequate research, adverse publicity and regulatory constraints (HCDA, 2005). Among these constraints, pests and diseases rank high with plant parasitic nematodes being of greatest importance accounting for 10-20% yield loss estimated annually (Phyllis, 1997).

Cut flower growing which has experienced phenomenal growth is highly dependent on external inputs mainly in the form of fertilizers and pesticides (Tenenbaum, 2002). The development of agroecological technologies and systems which emphasizes on conservation and regeneration of biodiversity, soil, water and other resources are urgently needed to meet the growing array of socioeconomic and environmental

challenges. Enhancing functional diversity in agroecosystems is a key ecological strategy to bringing sustainability to production (Altieri, 1999, Mutua *et al.,* 2007). Root-feeding nematodes are detrimental to plant growth. As few as one endo-parasitic nematode per plant may be enough to cause decreased productivity or death, while plants may tolerate several hundred ecto-parasitic nematodes per root system without reduction in yield (Santhosh *et al.,* 2005)

**2.3 Plant parasitic nematodes**

Nematodes that attack plants are worms, mostly microscopic in size, ranging from 0.25 mm to 3.0 mm long (Dunn and Crow, 2001). They are generally cylindrical in shape, tapering toward the head and tail. Females of a few species lose their worm shape as they mature, becoming greatly enlarged in diameter and assuming varying forms, such as pear, lemon, or kidney shapes (Agrios, 2005). In spite of their small size, nematodes are complex in organization. Plant parasitic nematodes possess all of the major organ systems of higher animals except respiratory and circulatory systems (Bird and Bird, 2001). The body is covered by a multi-layered cuticle which bears surface marks which are used when identifying nematode species. The cuticle of most nematodes is transparent, so that sufficient anatomical detail can be seen with the aid of a low-power binocular microscope (Dunn and Crow, 2001).

Plant parasitic nematodes have some form of oral stylet or spear, which is used somewhat like a hypodermic needle to puncture the host cell wall (Agrios, 2005). Many (probably all) plant nematodes inject enzymes into the host cell before feeding. These enzymes partially digest the cell contents before they are sucked into the gut. Most of the injury that nematodes cause to plants is related in some way to the feeding process (Wesemael *et al.,* 2006). Nematodes may feed on plant tissues from outside the plant (ectoparasitic) or inside the tissues (endoparasitic). If the adult female moves freely through the soil or

plant tissues, the species is said to be "migratory". Species in which the adult females become swollen and permanently immobile in one place in or on a root are termed "sedentary" (Tzortzakakis and Trudgill, 2005).

The development of disease in cultivated crops has long been known to depend on the complex interrelationship between host, pathogen and prevailing environmental conditions (Agrios, 2005). In the case of soil-borne pathogens, further opportunities exist for interactions with other microorganisms occupying the same ecological niche. The significant role of nematodes in the development of diseases caused by soil-borne pathogens has been demonstrated in many crops throughout the world (Back *et al.,* 2002). Plants infested with endoparasitic nematodes – *Meloidogyne* spp included - are usually subject to various nematode-induced modifications. Since most plant nematodes affect root functions, most symptoms associated with them are the result of inadequate water supply or mineral nutrition to the tops: chlorosis or other abnormal coloration of foliage, stunted top growth, failure to respond normally to fertilizers, small or sparse foliage, a tendency to wilt more readily than healthy plants, and slower recovery from wilting (Dunn and Crow, 2001).These can vary from localized forms of damage caused during invasion and feeding to overall systemic effects such as retarded plant growth. It is these changes which influence infections by soil-borne pathogens (Bird and Bird, 2001).

Cyst nematode *Heterodera daverti,* root knot nematode *Melodogyne incognita,* and *Paratylenchus dianthus* have previously been reported to be associated with field grown carnations (Del Sorbo *et al,* 2003)

**2.4 Trends in Nematode Control**

In refocusing crop protection from pest control to pest management, greater emphasis on ecological research and systems approach led to the term 'ecologically based pest management' (NRC, 1996).

Fundamental goals of the new concept are three fold: one is to minimize adverse effects on non-target species and the environment, secondly is to develop an approach that is economically viable to the end user and thirdly to establish an approach that is sustainable (Steinbeger *et al.,* 2001). More recently alternative nematode management strategies have been explored since chemical nematicides, especially methylbromide, which have been the primary management tool for over fifty years are now either being removed from the market or are under review (UNEP, 2001). Such work is necessary to develop environmentally sound agricultural systems that minimize chemical use while maintaining high production standards (Robert and Louis, 2004). Fumigation, which gives excellent control of nematodes in soil and therefore a major option in most flower farms, is being discouraged by the European cut flower market through EUREPGAP control points and compliance criteria (EUREPGAP, 2003). The Kenya Flower Council, the industry's regulatory body, through their code of practice is restricting the use of 'extremely hazardous' and 'highly hazardous' products (WHO class 1a and 1b) where most nematicides (fenamiphos, carbofuran, cadusafos, aldicarb, and oxamyl) are rated (KFC, 2002).

### 2.4.1 Host resistance

The use of natural resistance for control can initially be highly effective. Early successes were made in crops diverse as soybean, cotton and potato. Plant host resistance and tolerance describes the effects of host genes that restrict or prevent nematode multiplication in a host species (Atkinson *et al.,* 2003). Resistance genes can be introgressed into crop plants from wild or economically less desirable relatives using traditional breeding methods and by genetic manipulation (McHenry *et al*, 2004). Genetically modified (GM) crops have been adopted at a dramatic rate over the past decade, despite divided opinion about the risks they may pose to consumer and the environment. Significant increases in crop yields due to engineered resistance to biotic stresses cannot be disputed (Thomason, 2003). The best known and most

successful of these is probably that of insect resistance conferred by the *Bacillus thuringiensis* gene on diverse insects and the *Mi* gene against *M. incognita (*Atkinson *et al.,* 2003*).* As a result, resistances are often highly specific and in current agricultural practice act as strong selectors for resistance-breaking races. These can take the form of related nematode species like the *Mi*-conferred resistance against *Meloidogyne incognita.*

Ideal plant resistance should be durable, providing an efficient protection against the target organism during prolonged and widespread use in an environment conducive to disease development (Castagnone-Sereno *et al.*, 2007). However, such a control strategy is frequently made inefficient due to the rapid emergence in pathogen populations of new variants that are able to overcome the plant resistance (Enjalbert *et al.,* 2005). In tomato, resistance to the major root-knot nematode species *Meloidogyne arenaria, M. incognita* and *M. javanica* is controlled by a single dominant gene named *Mi* and all the modern fresh-market and processing resistant tomato cultivars carry this gene. Although it is still highly efficient in most agronomical situations, the intensive use of the *Mi* gene about 60 years, along with the pathogenic variability of root-knot nematodes, raises concern about the durability of the resistance (Castagnone-Sereno *et al,* , 2007).

### 2.4.2 Soil amendments

Organic substrates and green manure are potential alternatives to the harmful chemical control means currently used against plant-parasitic nematode (Bar-Eyal *et al.,* 2006). The most effective amendments are those with narrow C:N ratios and high protein or amine-type N content. Organic soil amendments stimulate the activities of microorganisms that are antagonistic to plant-parasitic nematodes. The decomposition of organic matter results in accumulation of specific compounds in the soil that may be nematicidal. Amendments are mainly bio-products and wastes from industrial, agricultural, biological and other

activities. Control of plant-parasitic nematodes can be by improvements of soil structure and fertility, increasing the level of plant-resistance, release of nemato-toxic compounds, fungal and bacterial pathogens and other nematode antagonists. The mode of action of organic amendments leading to plant disease control and stimulation of microorganisms is complex and dependent on the nature of the amendments (Koenning *et al.,* 2003).

Sugarcane bagasse is a fibrous residue of cane stalks left over after the crushing and extraction of the juice from sugar cane and consists of approximately 50% cellulose and 25% each of hemicellulose and lignin. Chemically, bagasse contains about 50% α-cellulose, 30% pentosans, and 2.4% ash. Among many other uses of this agro – industrial by – product include pulp, paper production and products based on fermentation (Pandey *et al.,* 2000). When sugarcane bagasse was applied before planting tomatoes, there was a 22% reduction in root-galling, while 100-150 days after planting the reduction was 90% in the number of root-knot nematodes juveniles. (Sikora *et al.*, 2005). The actual product that does the control (active ingredient) is furfural, a by-product of pentosan hydrolysis. Bagasse was used in this experiment to test its efficacy against nematodes as well as potential effect on economic yield of carnations.

Cut flower and tea industry wastes from the post-harvest grading and field cultural practices represent large quantities of plant-derived organic wastes requiring environmentally safe disposal. Application of these wastes in the form of composts to agricultural sites may provide a practical means of disposal and may result in agronomic benefits (McSorley and Gallagher, 1995). Yard-waste compost incorporated into the soil in an experiment had little effect on nematodes densities although differences were observed among the various crops but immediate nematode response to compost incorporated into the soil was shown not to be the case within a short term of four months (Sikora *et al.,* 2005).

Observations, when powdered pine bark was applied at different rates as an amendment, indicated that populations of *Heterodera glycines* juveniles were not detected in significant numbers while populations of free-living nematodes declined and *Meloidogyne arenaria* juveniles increased with increasing rates of pine bark powder applied into the in soil. In contrast, *H. glycines* juveniles detected in the roots decreased in number at highest concentration of pine bark. Galling did not occur although *M. arenaria* juveniles were found in the roots. Increase in microbial populations (*Penicillium chrysogenum* and *Paecilomyces variotii*) with time is attributed to these changes (Kokalis-Burelle and Rodriguez-Kabana, 1994).

Composts applications have resulted in disease suppression (Hoitink and Grebus, 1994). The mechanisms put forth for this suppression have included changes in the physical and chemical properties of soil including nutrition and the production of nematotoxic substances released directly or by microbial breakdown, changes in the microbial ecology of soil that affect antagonistic organisms or the release of antagonistic microbial metabolites, and the induction of plant resistance or tolerance to nematodes as well as systemic acquired resistance (Akhtar and Malik, 2000).

Molasses – a brown viscous liquid from sugar manufacture - does not pose a threat to the environment since they are readily decomposed in the soil to carbon dioxide and harmless organic products (Schenk, 2001). Vawdrey and Stirling (1997) observed a reduction in severity of root galling in tomato upon application of molasses. Molasses applied to papaya field plots in achieved a degree of root-knot nematode control comparable with that of the chemical nematicide fenamiphos. Interestingly, the molasses were not suppressive to nematodes when applied to soil under sterile conditions. The ability of molasses to reduce high populations of reniform nematode *Rotylenchulus reniformis,* was observed in papaya plantation where

yields and crop quality remarkably improved (Schenck, 2001). In addition, numbers of cyst nematodes, *Heterodera schachtii* were significantly reduced. Little effect was recorded on yield of Chinese cabbage unlike in onions where the cyst numbers declined and the yield improved. Since there are a number of spiral nematodes associated with carnations, it would therefore be prudent to reveal the effect of molasses on spiral nematodes.

### 2.4.3 Biological agents

Biological management of nematodes principally concerns the exploitation of microbial agents. It involves the use of predators and parasites of pathogens that kill or damage their hosts. Although many organisms derive their nutrition from nematodes, the most studied natural enemies of nematodes are bacteria and fungi (Karssen and Moens, 2006). Biological control agents like *Paecilomyces lilacinus, Pochonia chlamydosporia*, *Trichoderma* spp., *Plectosphaerella cucumeria, Agrobacterium radiobacter*, *Bacillus subtilis*, and *Pseudomonas spp.* have demonstrated potential as control agents for nematodes (Atkins *et al.,* 2003). However an effective bionematicide has not yet emerged from this body of work. Biological control is more inconsistent, less effective and slower acting than control normally achieved with chemicals. Although improvements in performance might be expected from more research on individual agents, it seems likely that these limitations are inherent in most biological control agents and that their successful application will depend on integration with other control measures (Kerry, 2000). An ideal biocontrol agent should have a relatively broad spectrum of activity against plant-parasitic nematodes and ability to successfully colonize a variety of soil environments and cropping systems, with little or no effect on beneficial non-target organisms (Mutua, 2007). According to Somasekhar *et al* (2002), there is growing awareness that biological control carries risks as well as benefits. Therefore, there is need to carry out selective suppression of plant-parasitic nematodes with no adverse effect on free-living nematodes.

Root-feeding nematode numbers can be reduced by competition for root space. Vasicular abuscular mycorrhizal (VAM) fungi may prevent root-feeding nematodes from reaching the roots through a variety of mechanisms. According to Akhtar and Malik (2000), studies conducted to determine the protection of olive plants against parasitism of root-knot nematodes using abuscular mycorrhizal fungi, showed that AMF significantly improved growth of olive plants irrespective of cultivar, age and infection by root-knot nematodes *M. javanica* and *M.incognita*. Further, establishment of AMF in plant root systems is considered a biological means of protection against plant diseases caused by soil borne pathogens, through improvement of P absorption by plants as well as direct competition with the pathogen for infection sites and nutrients, among other mechanisms (Castillo *et al* 2006).

Nematode trapping fungi are common soil inhabitants which infect nematodes through different strategies. *Arthrobotrys* spp. are nematode trapping fungi which immobilize nematodes by using mycelial traps such as non-adhesive knobs and constricting rings (Viaene *et al*., 2006). *Arthrobotrys conoides* has a remarkable morphological adaptation as a non-obligate nematode trapping fungus and has been commercialised for the control of *Meloidogyne* spp. (Kiewnick *et al*., 2004). Application of the fungi into the soil has often resulted in inconsistent levels of nematode control, but improvements in product development indicate that more promising results can be obtained (Viaene *et al*., 2006).

The potential of *Pasteuria penetrans,* an obligate bacterium, has been investigated as a biocontrol agent mainly against root-knot nematodes (*Meloidogyne* spp) and significant reduction attained (Tzortzakakis and Serafim, 2003). *Pasteuria* species are gram-positive, dichotomously branched, endospore-forming bacteria with septate mycelium. The use of this bacterium to suppress plant-parasitic nematodes has been tested on many crops, mostly in greenhouse pots (Sikora *et al.,* 2005). *Pasteuria penetrans* suppressed *Meloidogyne*

spp. on bean, brinjal, chickpea, cucumber, eggplant, gape, hairy vetch, kiwi, mung, okra, peanut, pepper, rye, soybean, tobacco, tomato, and wheat (Tzortzakakis and Trudgill, 2005). The mode of action of *Pasteuria penetrans* is by reduced number of second stage juveniles (J2) penetrating roots, number of females in roots, female fecundity, number of J2 in soil, and number of eggs on roots (Weibelzahl-Fulton *et al.*, 1996). Movement and mobility of J2 were reduced and their ability to locate host roots was affected when J2 were encumbered with endospores (Tzortzakakis and Trudgill, 2005).

The nematophagous fungus *Pochonia chlamydosporia* Zare & Gams (syn. V*erticillium chlamydosporium* Goddard) (Zare *et al.*, 2001) is a widespread and naturally occurring facultative parasite of root-knot nematode eggs and has shown potential as a biocontrol agent against these nematodes. The fungus is able to colonize the rhizosphere of the host plant from where it can infect egg masses deposited on the root surface by mature females that reside inside the roots (Hirsch *et al.*, 2001). Furthermore, the biocontrol activity of this fungal species has been extensively studied in the past two decades. Its ecology is therefore better understood than most other nematode-destroying fungi (Kerry and Bourne, 2002).

*Paecilomyces lilacinus* is a ubiquitious soil hyphomycete which parasitizes eggs of root-knot nematodes thus regulating populations of the nematodes in field soil (Schenk, 2004). Its effectiveness was comparable to several chemical nematicides tested. More recently, yields of tomato indicated that *Paecilomyces lilacinus* strain 251 was as effective as Vapam soil fumigant (Schenck, 2004). It is however worth noting that *Paecilomyces* species have been reported to cause allergic reactions, eye and skin infections in humans and animals except for strain 251 that poses no health risk to humans (USEPA, 2003). As the environmental awareness of the hazards of toxic products remains the subject of restrictive use, the Pest Control Products Board (PCPB) is encouraging efficacy trials of alternatives which do not fall under the category of 'extremely hazardous' or 'highly hazardous' products (PCPB, 2004). *Paecilomyces lilacinus* is

thus under scrutiny in this experiment to test if it can be a viable option to manage parasitic nematodes in carnations compared to the standard products in the market.

### 2.4.4 Neem (Achook®)

Neem is a botanical pesticide extracted from neem tree. 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, phenos, formaldehyde and fatty acids are released during decomposition of neem-based products Javed *et al.,* (2007). Pot tests conducted against key nematodes parasitic on banana nematodes indicated a reduction in populations of *Pratylenchus goodeyi* by 60%, *Meloidogyne* spp by 35% but less than 5% reduction in *Radopholus similis, Helicotylenchus multicinctus,* and *Hoplolaimus* spp. Damage to nematodes on banana roots in plants treated with this botanical nematicide was significantly less compared to those treated with carbofuran and the negative control. Field tests, however, showed that *Pratylenchus goodeyi* and *Meloidogyne* spp. appeared to be highly sensitive to neem treatments Pesticidal properties of azadirachtin, the active ingredient in neem, have been clearly documented (Akhtar and Malik, 2000; Agyarko and Asante, 2005). The commercial use of neem based products, as a crop protection agent in Kenya is fairly young. This is evident in the temporary registration accorded to neem (Achook®), an emulsifiable concentrate containing azadirachtin 0.15% w/w (1500 ppm) by Pest Control Products Board (PCPB). The efficacy of this product labelled to be ecologically friendly and to control *Meloidogyne* spp, *Radopholus similis, Pratylenchus* among others has had mixed results in field where flowers are grown and therefore needs to be investigated.

### 2.4.5 Chemical control

Nematicides are chemical compounds that directly kill nematodes, whereas the terms 'nematistat' or nematistatic' are sometimes used for compounds that do not kill nematodes directly, but are effective in paralysing the nematodes for a variable period of time during which they deplete their lipid reserves to such an extent that they are unable to cause plant injury (Haydock et al., 2005). Nematicides have been effectively in use since the late 19$^{th}$ century when the fumigant carbon disulphide was introduced. Development of further fumigants took place in the first-half of the 20$^{th}$ century with the introduction of chloropicrin, 1,3-dicholropropene 91,3-D), methyl bromide, 1,3-dichloropropene and 1,2-dichloropropene mixture (DD). Organophosphates, such as fenamiphos, ethoprophos, carbamates carbofuran, aldicarb, and oxamyl were developed later. Methylbromide has since been revoked for usage under the Montreal Protocol for the reduction of gases contributing to global warming (Strurz and Kimpiniski, 1999).

Fenamiphos (Nemacur®) is a powerful non-volatile systemic nematicide that kills ectoparasitic, semi-parasitic and free living nematodes in ornamentals, horticultural and field crops. It is applied as a pre-plant broadcast treatment in granular formulation (Tenenbaum, 2002). The environmental fate of this product shows that it is of moderate persistence in the soil environment, with a reported soil half-life of about 50 days (Wauchope, *et al*, 1992). The compound appears to have no effect on the activity of soil bacteria (USPHS, 1995). It has been shown that it disappears quickly from water in acidic and alkaline water, but it is stable in neutral water when held in the dark. The compound, when in the presence of artificial light, disappears very rapidly. In a neutral solution, half of the initial amount of the compound degraded within 4 hours (Extoxnet, 1996). In plants, the compound is absorbed through the roots and translocated to the leaves. It is broken down within the plant. The products of its breakdown are relatively persistent and can also inhibit cholinesterase (Smith, 1993). Its World Health Organisation toxicology rating is class 1b and

therefore on the restricted products list of Kenya Flower Council (KFC, 2002).Due to its preferred use in the flower industry to manage nematodes, it is used in this study as the standard product in the market.

**2.5 Diversity of plant parasitic nematodes**

Soil biodiversity is usually defined as the variety and variability of living organisms and the ecosystems in which they occur. The variety of life in the soil encompasses not only plants and animals but also the invertebrates and micro-organisms that are interdependent on one another and the higher plants they support (Ettema and Yeates, 2003). Ideally, biodiversity should be sufficient so that soil sustainability is achieved (Mulder *et al.,* 2003). The natural soil environment, which is the traditional media for carnation growing in Kenya, harbours a multitude of microorganisms. As many as $10^6$–$10^8$ bacterial cells, $10^6$–$10^7$ actinomycete cells, $5 \times 10^4$–$10^6$ fungal colony-forming units (CFU), $10^5$–$10^6$ protozoa and $10^4$–$5 \times 10^5$ algae are estimated to be present in a gram of field soil taken from the surface (Bunning and Jimenez, 2003). A single acre of soil is estimated to harbour as many as $3 \times 10^9$ nematodes (Decaremer and Hunt, 2005). Although many of these organisms are saprophytic, having little, if any effect on cultivated crops, the moist soil environment is favourable for the activities of plant-parasitic nematodes (PPN) and for the growth and multiplication of pathogenic fungi (Back *et al.*, 2002).

Nematodes are widely distributed in the soil, and their communities are made up of diverse species that, according to their feeding habits, can be classified into five major groups: plant parasites, bacterial and fungal feeders, predators and omnivores (Yeates, 1999). Nematode communities can be classified on the basis of colonizer – persister (c-p) of 1 to 5 (Bongers, 1990). Here nematodes with a c-p value equal to one are short lived, and have high fecundity; whereas those with c-p values of five are long lived, have large bodies, low fecundity and the greatest sensitivity to disturbance. Depending therefore on the prevailing condition in the soil in terms of disturbance or amendments, succession on nematode communities has been

reported by Ferris and Matute (2003) where during decomposition of organic matter with a mixture of C:N ratios populations of enrichment-opportunist bacterivorous nematodes increased rapidly in response to low C:N materials. Fungivorous nematodes increased gradually as higher C:N ratio residues became more abundant. Generally, nematode succession, which is not limited to the trophic group level, but also occurs among taxa (genera or species) within a feeding group, follows the pattern cp1→ cp2→ cp3 to 5 Nematodes are crucial in transfer of energy and matter through the soil food web because of their central and diverse trophic positions (Dawson *et al.,* 2000), and thus can be used as bioindicators. Other functions and ecosystem services carried out by nematodes include maintenance of soil structure, parasitizing soil pests and other microbes, soil detoxification and nutrient cycling (Yeates *et al*, 2003).

According to Sanchez-Moren 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. It is, therefore, imperative to conserve biodiversity of these free-living nematodes (Pinkerton *et al.,* 2000). 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).

Practices such as monoculture have been shown to change soil biophysical, chemical and hydrological conditions adversely affecting biodiversity (Mulder *et al.*, 2003). Further, Yeates and Bongers, (1999) reported that diversity and maturity indices were higher in mixed species grass swards than under monoculture. Nematode diversity was shown to be lowest in ecosystems experiencing long-term human

interference suggesting that changes in nematode community may be a reflection of changes in soil and ecological processes (Yeates, 1999).

Soil disturbance leads to changes in microbial species and their composition (Bloemers *et al.,* 1997), and as agriculture is intensified disturbance in soil increases (Yeates *et al.,* 1999). In the long run soil disturbance disrupts normal soil functions causing pests and diseases like parasitic nematodes populations to build-up (Giller, *et al.,* 1997) consequently resulting in yield and quality reduction of the affected crop. The use of organic substrates offers an alternative of restoring or maintaining soil diversity by influencing overall composition of the nematode fauna (Yeates and Bongers, 1999) and therefore improved quality and crop yields.

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; Otipa *et al.,* 2006). 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, 2000). 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 (Widmer and Abawi, 2002; Kimenju *et al.,* 2004).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study site

The study was carried out between June 2006 and July 2007 at Kericho, Kenya. The experiments were conducted under a 0.5 ha greenhouse alongside a commercial crop in an area situated at an altitude of 1950m above sea level that receives mean annual rainfall of 2000 mm. The greenhouse records mean annual day and night temperatures of $25^0$ C and $11^0$ 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 carnations.

### 3.2 Land preparation

Land preparation included uprooting of the old crop, removal of the debris, ploughing to fine tilt and then watering of beds to field capacity. Parallel beds of 100cm width were raised to a height of 25cm, with a pathway in-between of 50cm (Plate1). On the beds, experimental plots of 400cm long were separated by guard rows measuring 50cm while taking care of the edge effects.

Plate 1. Experimental site showing the parallel raised beds.

### 3.3 Description of test materials used and method of application

The treatments tested were:

Sugarcane bagasse which is a fibrous residue of cane stalks left over after the crushing and extraction of the juice from sugar cane and consists of approximately 50% cellulose and 25% each of hemicellulose and lignin (Plate 3). Chemically, bagasse contains about 50% α-cellulose, 30% pentosans, and 2.4% ash.

Tea and flower composts are decomposed green materials derived from Tea and flower leaves that have been swept out of the grading shades and allowed to naturally rot as shown in plate 2.

Molasses (a by-product of sugarcane processing) is a thick by-product from the processing of the sugar cane into sugar.

Neem (Achook®) is a commercial botanical pesticide extracted from neem tree and contains azadirachtin (0.15%) active ingredients.

*Paecilomyces lilacinus* (PL plus®) is a commercial product available in the market containing $4\times10^9$ spores/gram.

Fenamiphos (Nemacur 5Gr®) is labeled a powerful non-volatile systemic nematicide that kills ectoparasitic, semi-parasitic and free living nematodes in ornamentals, horticultural and field crops.

Control used here is where the plot is not treated at all.

Plate 2. A heap of flower clippings in the process of decomposing to become compost

Plate 3. A photo showing sugarcane bagasse blocks

Sugarcane bagasse, tea and flower composts were sun dried to a constant weight. The composts and bagasse were worked in to the soil using a hand hoe at the rate of 300 tons/ha as recommended for greenhouse usage (McSorley and Gallagher, 1995) just before planting. Using a watering can, molasses was applied at the rate of 667 ml/$m^3$ (Schenck, 2001), neem was applied following the manufacturer's recommendation at 1.5 ml/$m^2$ dissolved in one litre of water and *Paecilomyces lilacinus* at the rate of 2 kg/ha. Fenamiphos was broadcasted and worked in to the soil one week before planting at the rate of 30 g/$m^2$. All applications were done only once. One month old rooted spray carnations cultivar white Natila cuttings were transplanted into the plots at plant density of 32 plants per $m^2$ - where pre made net-support

wires and drip lines for fertigation had been laid (Plate 4). Treatments were arranged in a completely randomized design with six replications. (Appendix 7.3a and b).

Plate 4. Carnation rooted cuttings just transplanted onto the beds.

### 3.4 Estimation and characterization of parasitic nematodes inoculum in the soil

#### 3.4.1 Soil Sample collection

200 $cm^3$ soil samples were collected after the land was prepared for planting prior to the application of treatments. Five soil sub-samples were randomly collected to a depth of 30 cm from the three middle rows of each plot. The sub-samples were thoroughly mixed to form a composite sample for each treatment before being placed in plastic sampling bags, transported to the laboratory and stored at 4 $^0$C. Soil sampling was also done at 90 days and 180 days after planting.

#### 3.4.2. Nematode extraction

The modified Baermann funnel technique (Hooper) 2005, was used to extract nematodes from 200$cm^3$ soil. The soil was spread on a double layer of milk filters supported by a sieve then placed in a shallow dish and water added 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 juveniles collected from each of the sieves. Aliquots of one ml of a well-agitated nematode suspension was pipetted into a counting slide and observed under a light microscope. Counting was repeated for 3 aliquots and the mean recorded. Nematodes were then placed in vials and stored at 4 $^0$C awaiting fixation. Nematodes were heat killed by subjecting them to temperatures of 55 – 60$^0$C for about one minute and fixed in 3% of formalin (Hooper, 2005). This way, nematodes internal organs were left intact for ease of identification. A suspension of the nematodes was homogenised before taking 2ml of solution into a counting chamber. One hundred nematodes from the slides in each sample were selected at random for identification to genus level under a light microscope at a magnification of ×400.

Nematodes were determined up to genus and family level according to Bongers (1988). Permanent mounts in formaldehyde on mass slides contained about 150 individuals that were examined under a light microscope at a magnification of ×400–600, following the keys described by Bongers (1988). Taxa identified were further assigned to functional groups according to their life strategies (Bongers, 1990) and then allocated into five trophic groups (bacterial and fungal feeders, plant parasites, omnivores, and predators) based on the descriptions by Yeates *et al.* (1993).

### 3.4.3 Root galling index

Galling was quantified using a scale of 1-9 where:

1 = 0 galls/eggs masses;

2 = 1-5 galls/egg masses;

3 = 6-10 galls/egg masses;

4 = 11-20 galls/egg masses;

5 = 21-30 galls/egg masses;

6 = 31-50 galls/egg masses;

7 = 51-70 galls/egg masses;

8 = 71-100 galls/egg masses;

9 = 100 galls/egg masses (Sharma *et al*, 1994).

### 3.4.4 Damage index

Damage index of the roots was expressed as a percentage of infected root converted using the scale of 1 – 10 described by Zeck (1971).

0 – healthy root system, no infection

1 – Very few galls only detected upon close examination

2 – small galls easy to detect

3 – numerous small galls

4 – numerous small galls and few big galls

5 – 25% of the root system severely galled and not functioning

6 – 50% of root system severely galled and not functioning.

7 – 75% system severely galled and not functioning

8 – no healthy root but plant still green

9 – rotting, completely galled root system and plant dying

10 – plant and root dead

### 3.4.5 Shoot dry weight

Nematode effects on plant growth were assessed using shoot dry weight. Shoots were pulled out of the ground, the roots cut off at the crown and plants weighed before being placed in an oven at $80^0$ C. Weights were recorded daily for four days until constant weight was achieved. Constant weight was achieved on the third day.

### 3.5 Estimation and characterization of non - parasitic nematode inoculum in the soil

#### 3.5.1 Soil Sample collection

Refer to section 3.4.1

#### 3.5.2. Nematode extraction

Refer to section 3.4.2

### 3.6 Effect of various organic substrates, nematophagous fungi and neem on diversity of nematodes in carnations rhizospere.

#### 3.6.1 Soil Sample collection

Refer to section 3.4.1

#### 3.6.2. Nematode extraction

Refer to section 3.4.2

#### 3.6.3 Nematode diversity indices

General indices to nematodes as given by Norton and Niblack (1991) and Bernard (1992) were used.

#### 3.6.3.1 Species richness

$$SR = \frac{S-1}{\log_e N}$$

where S = number of taxa identified
P = the proportion of individuals in the $i^{th}$ taxon
N = the number of individuals identified

#### 3.6.3.2 Shannon-Wiener diversity index

$$H' = \Sigma\, pi \log_e pi$$

### 3.6.3.3 Maturity index

Index for measuring soil disturbance level:

- Maturity Index (MI): For soil nematodes, except plant parasitic nematodes.

$$MI = \sum_{i=1} c\text{-}p\ i\ .\ pi$$

where n=the number of individuals identified, *c-p* is the *c-p* value (Table 3.1) and pi is the proportion of individuals in the $i^{th}$ taxon.

**3.6.3.4 Plant parasitic Index (PPI):** Index for measuring soil disturbance level, it is only for plant parasitic nematodes. It is calculated like maturity index but care has to be taken to pick the right *c-p* values (Table 3.1).

Table 3.1 Nematode families with the *c-p* values which are used in calculating the maturity index (MI) and plant parasite index.

| **Family** | ***c-p* value** |
|---|---|
| Alaimidae | 4 |
| Aphelenchidae | 2 |
| Aphelenchoididae | 2 |
| Anguinidae | 2[a] |
| Aporcelaimidae | 5 |
| Bastianiidae | 3 |
| Belondiridae | 5 |
| Bunonematidae | 1 |
| Cephalobidae | 2 |
| Chromadoridae | 3 |
| Criconematidae | 3[a] |
| Diphtherohporidae | 3 |
| Diplogagasteridae | 1 |
| Dolichodoridae | 3[a] |
| Hemicycliophoridae | 3[a] |
| Hoplolaimidae | 3[a] |
| Leptonchidae | 4 |
| Longidoridae | 5[a] |
| Monhysteridae | 2 |
| Monochidae | 4 |
| Nordiidae | 4 |
| Panagrolaimidae | 1 |
| Paratylenchidae | 2[a] |
| Plectidae | 2 |
| Pratylenchidae | 3[a] |
| Prismatolaimidae | 3 |
| Qudsianematidae | 4 |
| Rhabditidae | 1 |
| Teratocephalidae | 3 |
| Thornenematidae | 5 |
| Tobrilidae | 3 |
| Trichodoridae | 4[a] |
| Tripylidae | 3 |
| Tylenchidae | 2[a] |

Adopted from Yeates and Bongers (1999) and includes changes proposed by Bongers *et al.* (1995). [a] are values for families which should be included in the PPI rather than MI.

### 3.7 Carnation yield assessment

Marketable stems harvested were recorded as yield. The marketable stems were identified by the market quality parameters which included strong, long stems with an average of four or more bloom counts. Data were subjected to analysis of variance and means separated using LSD at $P \leq 0.05$ (Steel and Torrie, 1981). The SAS Release 8.1 for Windows (2000) was used in the analysis.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Plant parasitic nematode population in the research field.

Plant parasitic nematodes belonging to sixteen genera were isolated in the soil rhizospheres of carnations. These were *Scutellonema, Helicotylenchus, Meloidogyne, Pratylenchus, Tylenchus, Hemicyclophora, Tylenchorhynchus, Rotylenchus, Tylenchus, Criconema, Trichodorus, Hoplolaimus, Hemicriconemoides, Longidorus, Xiphinema* and *Paratylenchus* (Table 4.1). The most predominant nematodes were in the genera *Scutellonema, Helicotylenchus* and *Meloidogyne* with percentage densities of 14.7, 13.6 and 11.6 % respectively. Nematodes in the genera *Criconema, Helicotylenchus, Longidorus, Hemicyclophora* and *Xiphinema* were not influenced by application of the soil with the various organic substrates. The organic substrates when applied over time had significant effect on all nematodes except those in the genera *Helicotylenchus*, *Criconema* and *Longidorus.*

Table 4.1 Occurrence and abundance of plant parasitic nematodes and their reaction to various treatments in carnations.

| Nematode genera | % Density (in 200 $cm^3$ soil) | F- value | |
|---|---|---|---|
| | | All Treatments | Treatment×Time interaction |
| *Scutellonema* | 14.7 | ** | ** |
| *Helicotylenchus* | 13.6 | Ns | Ns |
| *Meloidogyne* | 11.6 | ** | ** |
| *Pratylenchus* | 10.5 | ** | ** |
| *Tylenchus* | 7.8 | ** | ** |
| *Hemicyclophora* | 7.4 | Ns | ** |
| *Tylenchorynchus* | 6.1 | ** | ** |
| *Rotylenchus* | 5.5 | ** | ** |
| *Tylenchulus* | 5.3 | ** | ** |
| *Criconema* | 3.4 | Ns | Ns |
| *Trichodorus* | 3.0 | ** | ** |
| *Hoplolaimus* | 2.9 | ** | * |
| *Hemicriconemoides* | 2.3 | ** | ** |
| *Longidorus* | 2.2 | Ns | Ns |
| *Xiphinema* | 2.1 | Ns | ** |
| *Paratylenchus* | 1.6 | * | ** |

*Significant at P≤0.05; ** Significant at P≤0.01; Ns=not significant; ×-interaction

Differences in numbers of plant parasitic nematodes were significant among the treatments applied (Figure 4.1). Fenamiphos and neem led to the highest reductions of 72% and 71% in nematode populations, respectively compared to the control. Inspite of their significant reduction, differences in the means of nematode populations in plots treated with tea compost, molasses and *P. lilacinus* were not significant. bagasse recorded the least nematode reduction (31%).

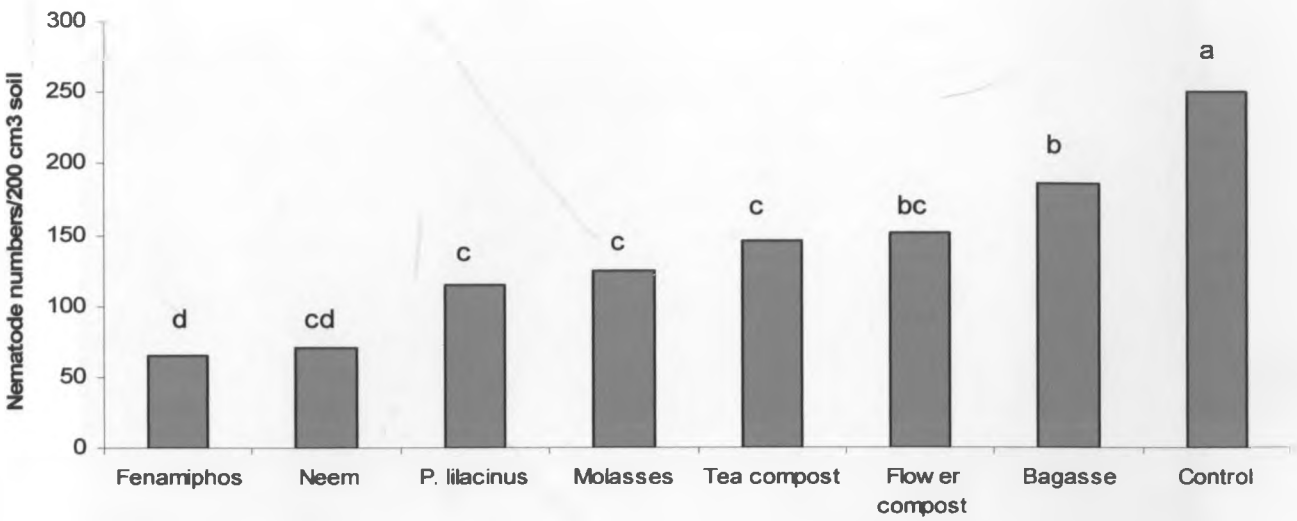


Figure 4.1 A general representation of the effects of seven control agents on plant parasitic nematodes in carnations. Bars headed by different letters are significantly different ($P \leq 0.05$).

Plant parasitic nematodes from different genera had varying responses to the various treatments (Figure 4.2). Fenamiphos and neem led to a steady decline in populations of PPNs (Plant Parasitic Nematodes). At 90 DAP, an increase in numbers of PPNs was observed before declining on plots treated with organic substrates. But flower compost led to a gradual increase up to 90 DAP before stabilizing at 180 DAP. There was an exponential increase in PPNs in the control where no treatment was applied as shown on figure 4.3. This was shown by patches of poorly growing plants (Plate 6).

Plate 5. Carnation crop showing the characteristic patches of poorly growing plants due to the effect of nematode infestation.

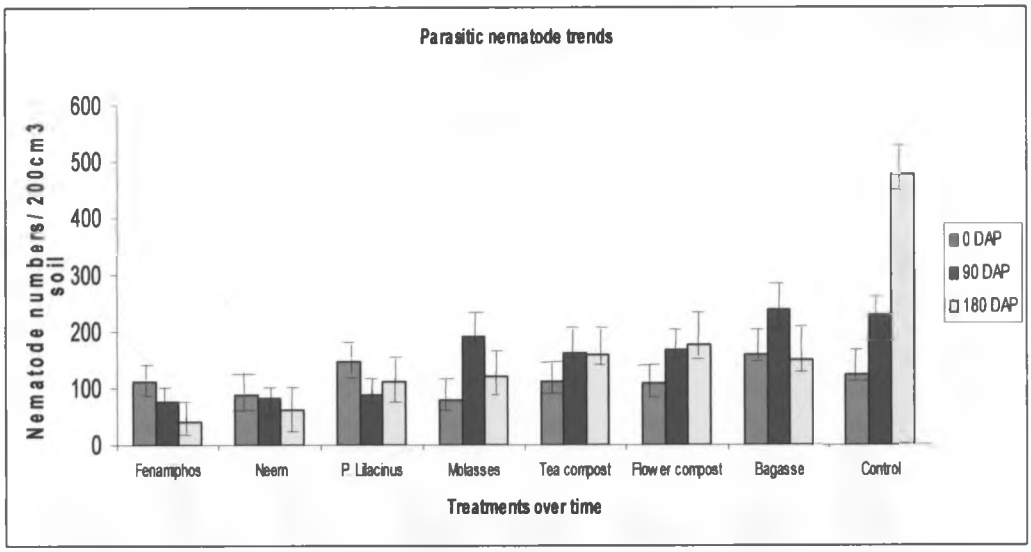


Figure 4.2 Effect of treatments and time on plant parasitic nematode populations in carnation

Three nematode genera, *Scutellonema*, *Pratylenchus* and *Meloidogyne* mainly showed variable responses to the treatments (Fig. 4.3a, b and c). Application of organic substrates - bagasse, molasses, and flower compost led to an increase of the nematode numbers in the three genera at 90 DAP followed by a decline at 180 DAP. Compared to the control, application of fenamiphos, neem and *P.lilacinus* led to a continuous decrease of the numbers of the three genera after 90 and 180 DAP.

Application of various treatments had variable effects on the numbers of the plant parasitic nematode genera (Table 4.2). Bagasse, tea compost and molasses did not influence the numbers of *Hemicriconemodes, Pratylenchus, Paratylenchus, Trichodorus* and *Tylenchus.* Nematodes in the genera *Hoplolaimus* and *Scutellonema* were suppressed by all treatments applied as shown in Table 4.2. With the exception of fenamiphos and neem, the rest of the treatments did not influence *Trichodorus* populations. Nematodes in the genera *Hemicyclophora, Paratylenchus, Trichodorus* and *Tylenchus* did not show any response due to the application of *P. lilacinus*.



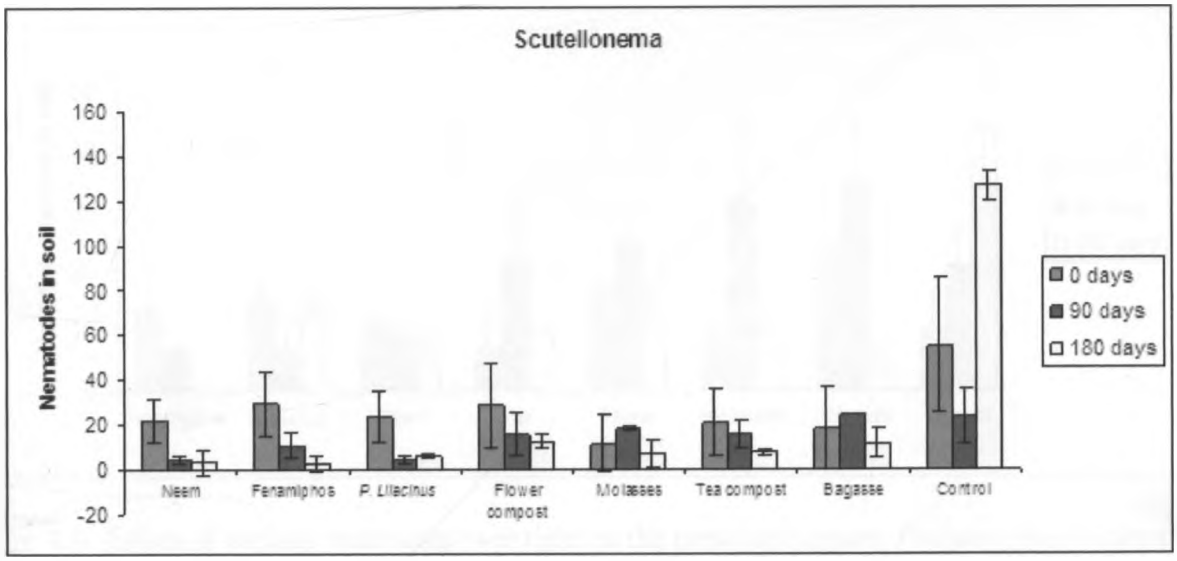


Figure 4.3a Effect of treatments over time on the genera *Scutellonema* in carnations

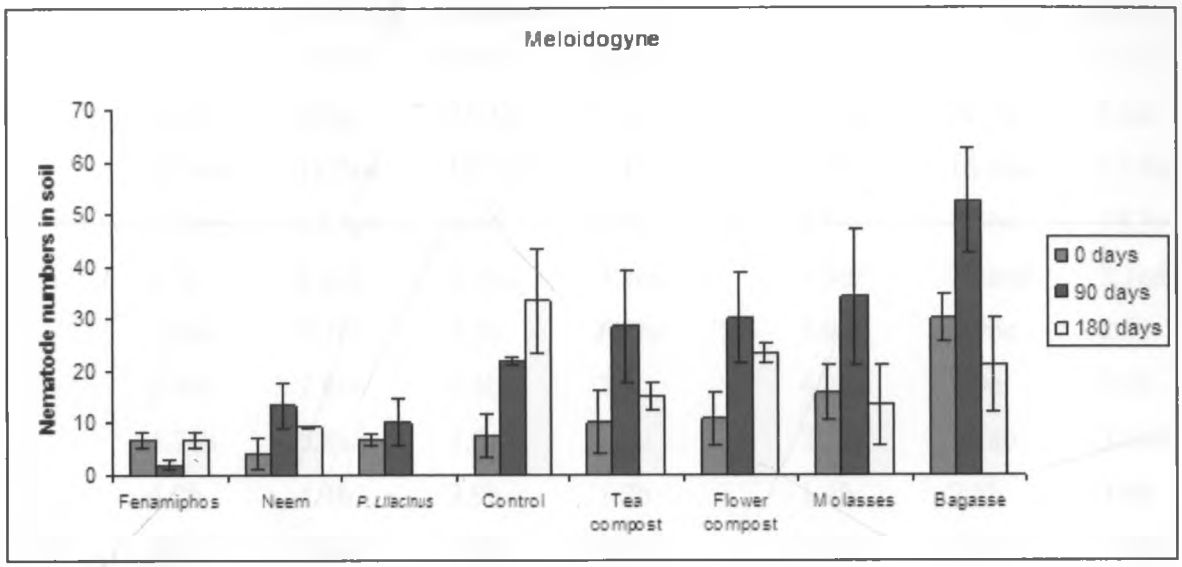


Figure 4.3b Effect of treatments over time on the genera *Meloidogyne* in carnations.

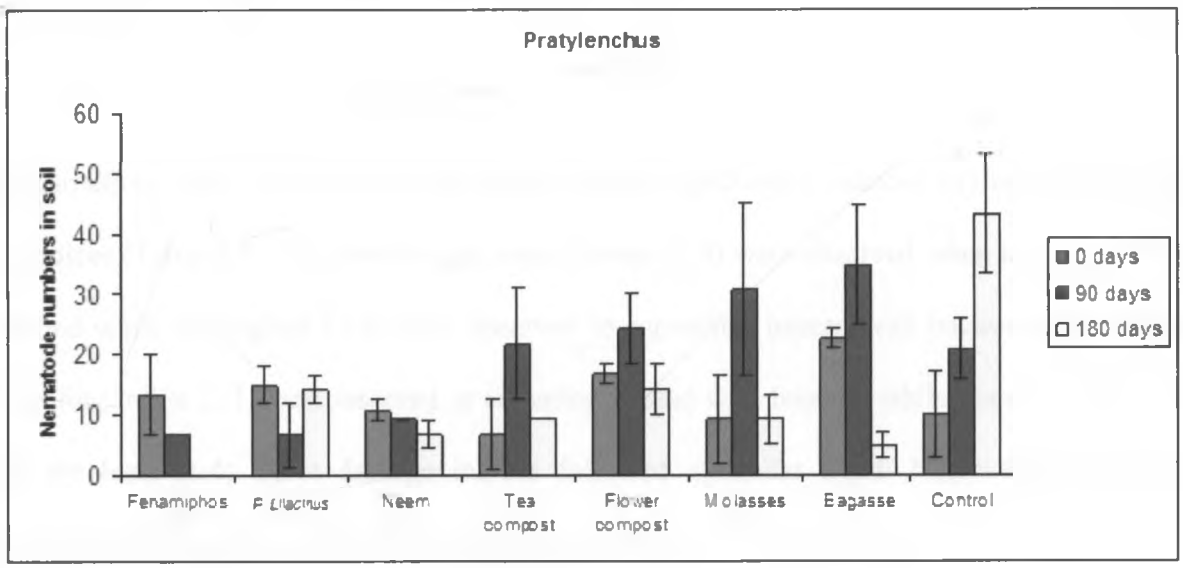


Figure 4.3c Effect of various treatments over time on the nematode genera *Pratylenchus* in carnations.

Table 4.2 Effect of different treatments on plant parasitic nematodes in carnations.

| matode genera | Mean number of nematodes in 200 $cm^3$ soil | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bagasse | Tea compost | Flower compost | Fenamiphos | Neem | Molasses | *P lilacinus* | Control |
| *utellonema* | 18.1b | 14.7b | 18.9b | 14.2b | 9.7b | 12.2b | 11.1b | 68.3a |
| *loidogyne* | 20.8b | 15bc | 21.4b | 4.4d | 8.9cd | 21.1b | 5.6d | 34.4a |
| *atylenchus* | 20.8ab | 12.5cd | 18.3abc | 6.7d | 8.9d | 16.4bc | 11.9cd | 23.6a |
| *lenchus* | 13.9ab | 13.3ab | 6.4c | 5.6c | 6.1c | 7.8bc | 18.3a | 17.1a |
| *lenchorynchus* | 9.7b | 8.6bc | 8.9bc | 3.3cd | 1.9d | 7.2bcd | 3.1cd | 26.9a |
| *ylenchus* | 7.8bc | 8.1b | 8.3b | 6.9bc | 3.9bc | 6.9bc | 2.5c | 18.3a |
| *lenchulus* | 5.8bc | 7.8bc | 6.4bc | 3.9c | 4.2bc | 3.9c | 9.2b | 18.7a |
| *ichodorus* | 4.7ab | 5.8a | 5.0ab | 1.1c | 3.1bc | 5.3ab | 3.6abc | 5.6ab |
| *plolaimus* | 4.2b | 4.4b | 3.9b | 1.7b | 1.4b | 2.8b | 4.4b | 10.0a |
| *micriconemoides* | 3.9a | 3.3ab | 5.0a | 0.8b | 1.1b | 4.7a | 3.1ab | 4.4a |
| *ratylenchus* | 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 ($P \leq 0.05$).

Treating carnations with various nematode control agents significantly reduced eggmass, galling, and root damage indices (Table 4.3). The lowest eggmasses indices (1.4) were observed when neem and *P. lilacinus* were applied while the highest (3.9) were observed in carnations treated with bagasse and molasses. The highest galling index (2.1) was observed in carnation treated with bagasse while plants treated with neem recorded the least (1.4). Root damage indices followed a similar trend. Neem recorded the highest reduction galling index (69%) followed by fenamiphos (67%).

Table 4.3 Effect of different treatments on eggmass, root damage and galling indices in carnations.

| Treatment | Indices | | | |
|---|---|---|---|---|
| | EMI | Root damage | Galling index (GI) | % reduction in GI |
| Bagasse | 3.9b | 1.8b | 2.1b | 53% |
| Tea compost | 3.1c | 1.6c | 1.7c | 62% |
| Flower compost | 2.9c | 1.2d | 1.8c | 60% |
| Fenamiphos | 1.7d | 0.8e | 1.5d | 67% |
| Neem | 1.4d | 0.7e | 1.4d | 69% |
| Molasses | 3.9b | 1.7b | 1.9c | 58% |
| *P. lilacinus* | 1.4d | 1.8b | 1.9c | 58% |
| Control | 6.2a | 3.5a | 4.5a | - |
| CV(%) | 20.8 | 12.1 | 13.9 | - |

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

**4.2 Effect of various organic substrates, nematophagous fungus and neem on non-parasitic nematodes in carnations**

Non – parasitic nematodes from 14 genera were recovered from carnation plots treated with organic substrates and the nematophagous fungus *P. lilacinus* (Table 4.4). Among these nematodes, bacterial feeders accounted for 73% while fungal feeders and predators accounted for 14% and 13%, respectively. The bacterivorous nematodes were members of the genera *Acrobeles, Rhabditis, Cephalobus, Prodorylaimus, Bunonema, Eucephalobus, Heterocephalobus, Plectus,* and *Chromadora.* The predators

were members of the genera *Mononchus*, *Labronema* and *Nygolaimus* 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*.

Table 4.4 Occurrence and abundance of non parasitic nematodes and their reaction to various treatments in carnations

| Nematode genera | % Density | F-value | |
|---|---|---|---|
| | | All Treatments | Treatment×Time interaction |
| *Aphelenchoides* | 13.9 | ** | ** |
| *Mononchus* | 10.2 | ** | ** |
| *Rhabditis* | 10.0 | ** | ** |
| *Cephalobus* | 9.8 | Ns | Ns |
| *Chromadora* | 9.8 | Ns | ** |
| *Acrobeles* | 8.7 | ** | ** |
| *Prodorylaimus* | 8.1 | ** | ** |
| *Bunonema* | 7.4 | ** | ** |
| *Aphelenchus* | 4.8 | ** | ** |
| *Ucephalobus* | 4.6 | ** | ** |
| *Euphalobus* | 3.9 | ** | ** |
| *Labronema* | 3.3 | ** | ** |
| *Plenchtus* | 2.8 | ** | ** |
| *Nygolaimus* | 2.7 | ** | ** |

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 compared to control plot (Fig 4.4). 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.

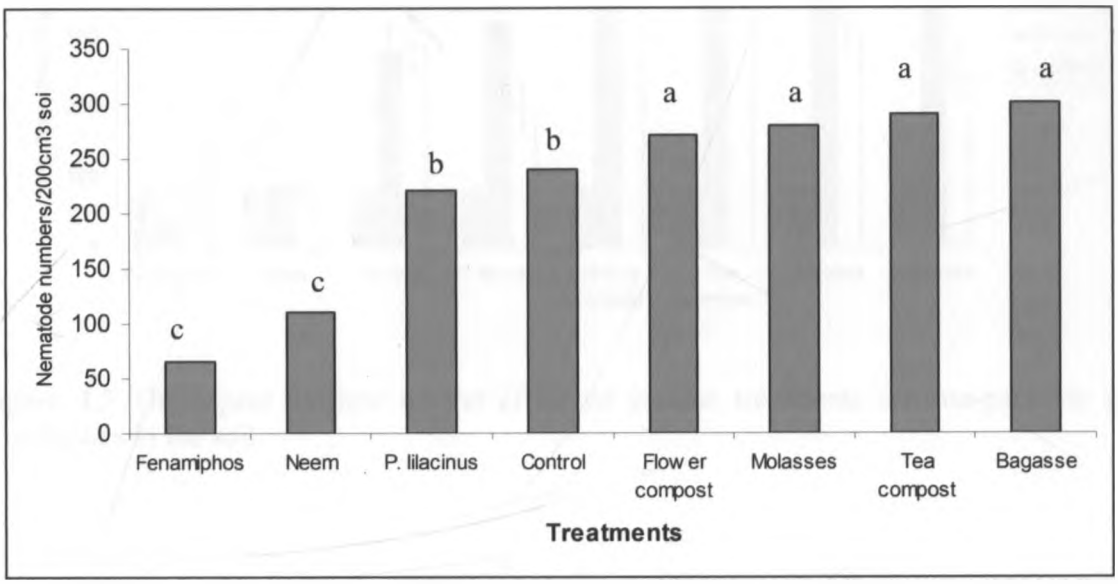


Figure 4.4 General trend on the effect of different treatments on the free-living nematodes in carnations at 180 DAP. Bars headed by different letters are significantly different ($P \leq 0.05$).

The treatments had variable effects on numbers of free-living nematodes over time, from application during planting throughout to 180 days after planting (Fig. 4.5). 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.

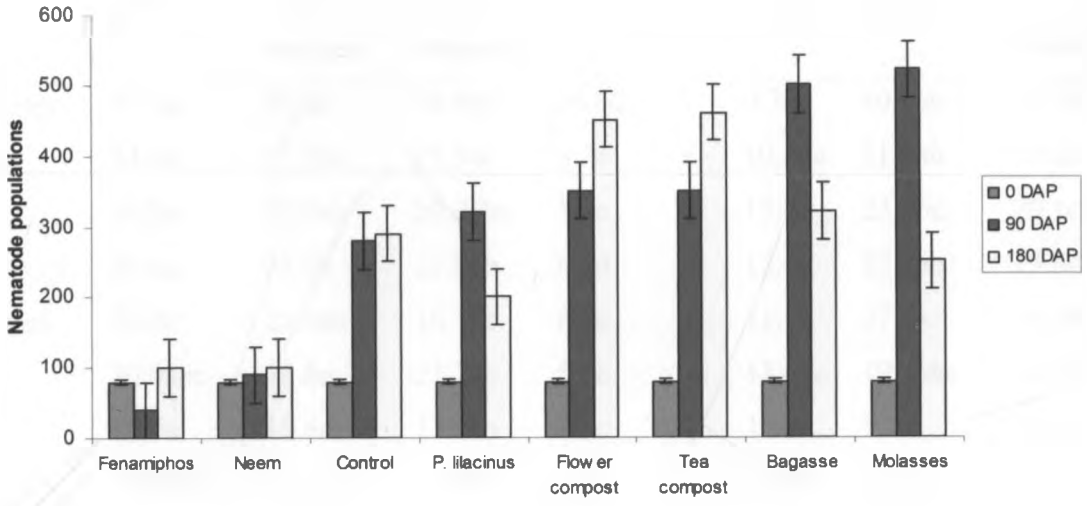


Figure 4.5 The aspect of time on the effect of various treatments on non-parasitic nematodes populations in the soil.

Free living nematodes from different genera had varying responses to the various treatments aimed at controlling plant parasitic nematodes (Table 4.5). 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* (nematophagous fungi) 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.

Table 4.5 Effect of different treatments on the non-parasitic free living nematodes in the experimental plots..

| Nematode genera | Mean number of nematodes in 200 $cm^3$ soil | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 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 |
| *Ucephalobus* | 12.8ab | 10.6bc | 10.6bc | 3.6d | 8.1cd | 16.7a | 7.2cd | 10.0bc |
| *Euphalobus* | 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 |
| *Plenchtus* | 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 |

Data are means of 48 samples. Means followed by the same letter(s) along rows are not significantly different ($P \leq 0.05$).

**4.3 Effect of various organic substrates, a nematophagous fungus and a commercial neem-based product on the diversity of nematodes**

Nematodes belonging to thirty genera of were recovered from soils planted with carnations subjected to different treatments. Nematodes isolated belong to four trophic groups namely plant feeders (PF), bacteriovorous (BF), fungivorous (FF) and predators (PR). The plant feeders were predominant in all the treatments followed by bacteriovores. Fungivores and predators were the least in all the treatments (Table 4.6)

Table 4.6 Overall mean numbers of nematode communities in carnation rhizospheres.

| Nematode genera | Trophic Group | *Mean numbers | Nematode genera | Trophic group | *Mean numbers |
|---|---|---|---|---|---|
| *Scutellonema* | PF | 22 | *Aphelenchus* | FF | 31 |
| *Helicotylenchus* | PF | 13 | *Aphelenchoides* | FF | 35 |
| *Meloidogyne* | PF | 15 | *Mononchus* | PR | 21 |
| *Pratylenchus* | PF | 13 | *Rhabditis* | BF | 25 |
| *Tylenchus* | PF | 11 | *Cephalobus* | BF | 14 |
| *Hemicyclophora* | PF | 6 | *Chromadora* | BF | 25 |
| *Tylenchorynchus* | PF | 11 | *Acrobeles* | BF | 13 |
| *Rotylenchus* | PF | 10 | *Prodorylaimus* | BF | 0 |
| *Tylenchulus* | PF | 10 | *Bunonema* | BF | 14 |
| *Criconema* | PF | 6 | *Ucephalobus* | BF | 30 |
| *Trichodorus* | PF | 7 | *Euphalobus* | BF | 25 |
| *Hoplolaimus* | PF | 6 | *Labronema* | PR | 21 |
| *Hemicriconemoides* | PF | 6 | *Plectus* | BF | 18 |
| *Longidorus* | PF | 7 | *Nygolaimus* | PR | 18 |
| *Xiphinema* | PF | 7 | | | |
| *Paratylenchus* | PF | 7 | | | |

*Mean number of nematodes in 200 $cm^3$ soil 180 days after application of treatments. PF-herbivores, BF-bacteriovores, FF-fungivores and PR-predatory.

There was a general increase in both parasitic and non-parasitic nematodes. However, non-parasitic nematodes were more dominant increasing to a peak at 180 days DAP. The numbers of plant parasitic nematodes showed slight but low increases up to 180 DAP.

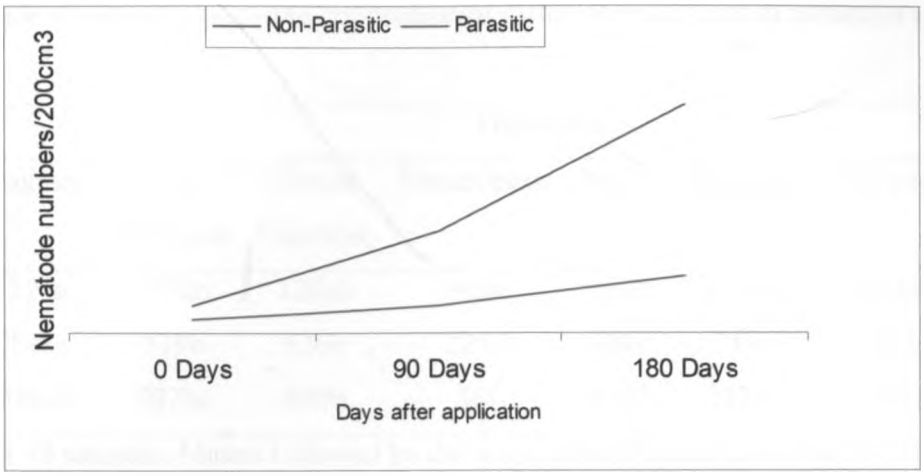


Figure 4.6 Response of plant parasitic and non-parasitic groups of nematodes to a nematophagous fungus and organic substrates.

Nematode diversity (parasitic and non parasitic) in carnations assessed by abundance was variable following application of different treatments (Table 4.7). Among the treatments, the highest nematode abundance was found in plots treated with bagasse (748) followed by those treated with molasses (710) after 90 DAP. Nematode abundance was least in plots treated with fenamiphos (221) followed by neem (344). At 180 days, however, the highest population was in control plots (797) followed by flower compost (600) while the least was in plots treated with fenamiphos at 56 followed by neem and 114 nematodes per $200cm^3$.

Table 4.7 Nematode abundance following application of different treatments in carnation experimental plots.

| Time | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bagasse | Tea Compost | Flower Compost | Fenamiphos | Neem | Molasses | *P.lilacinus* | Control |
| Pre-treatment | 224a | 177ab | 126ab | 145ab | 102b | 121ab | 193ab | 140ab |
| 90 days | 748a | 519b | 526b | 221d | 344c | 710a | 426bc | 513b |
| 180 days | 480cd | 597bc | 600b | 56f | 114f | 397de | 307e | 797a |

Data are means of 48 samples. Means followed by the same letter(s) along rows are not significantly different ($P \leq 0.05$).

Significant ($P \leq 0.05$) differences in nematode genera were observed in plots which were given different treatments (Table 4.8). After 90 days, soils treated with bagasse, tea and flower composts as well as molasses had higher genera richness than those treated with fenamiphos, neem and *P. lilacinus.* At 180 DAP, with the exception of fenamiphos and neem, the organic substrates and *P. lilacinus* recorded the highest genera richness.

Table 4.8 Genera richness of nematodes following application of different treatments in carnation experimental plots.

| Time | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bagasse | Tea Compost | Flower Compost | Fenamiphos | Neem | Molasses | *P.lilacinus* | Control |
| Pre-treatment | 7.8ab | 7.1ab | 6.5b | 7.8ab | 6.4b | 7.5ab | 9.8a | 6.4b |
| 90 days | 20.4a | 20.3a | 20.0a | 16.8bc | 16.8bc | 19.4ab | 14.8c | 21.8a |
| 180 days | 24.5a | 25.7a | 24.5a | 6.08d | 12.0c | 21.7b | 23.3a | 25.7a |

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

The Shannon-Wiener diversity index (H) varied significantly ($P \leq 0.05$) following application of different treatments on carnations (Table 4.9). There were significant increases in Shannon diversity after 90 days,

through to 180 days except in fenamiphos where a decline was noted. After 180 days, the highest diversity was observed in the plots treated with bagasse, flower compost, molasses and *P. lilacinus*. The least diversity after 180 days was 1.8 observed under fenamiphos and this was followed by that of neem at 2.3.

Table 4.9 Mean diversity indices (H) for nematode genera in carnations treated with various management agents.

| Time | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bagasse | Tea Compost | Flower Compost | Fenamiphos | Neem | Molasses | *P.lilacinus* | Control |
| Pre-treatment | 1.79ab | 1.75ab | 1.54ab | 1.81ab | 1.62ab | 1.78ab | 2.10a | 1.41ab |
| 90 days | 2.78ab | 2.75ab | 2.40b | 2.62ab | 2.68ab | 2.69ab | 2.48b | 2.95a |
| 180 days | 2.91a | 2.95a | 2.98a | 1.78c | 2.30b | 2.89a | 2.80a | 3.00a |

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

There was significant ($P \leq 0.05$) dominance in nematode communities in soils treated with different agents (Table 4.10). Dominance was observed prior to application of treatments but was absent in all treatments at 90 DAP. At 180 DAP, only carnation plots treated with fenamiphos and neem showed dominance.

Table 4.10 Dominance of nematodes following application of different treatments in carnation rhizospheres.

| Time | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bagasse | Tea Compost | Flower Compost | Fenamiphos | Neem | Molasses | *P.lilacinus* | Control |
| Pretreatment | 0.20b | 0.22a | 0.25b | 0.20b | 0.25b | 0.19b | 0.15c | 0.34a |
| 90 days | 0.07a | 0.07a | 0.07a | 0.10a | 0.07a | 0.08a | 0.11a | 0.06a |
| 180 days | 0.07b | 0.06b | 0.06b | 0.18a | 0.12ab | 0.06b | 0.09b | 0.06b |

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

Significant variations ($P \leq 0.05$) in evenness were observed in carnation rhizospheres after applying different treatments (Figure 4.7). Evenness was lowest before treatments were administered and increased with time

reaching a peak at 180 days. Plant parasite index in carnations treated with various treatments also showed the same trends (Fig. 4.8).

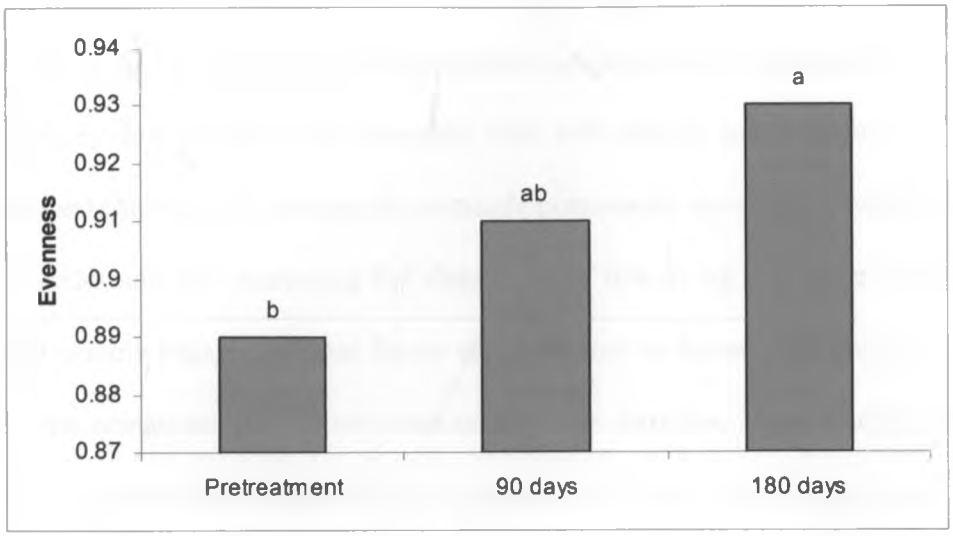


Figure 4.7 Effect of different nematode treatments on evenness. Bars headed by different letters are significantly different ($P \leq 0.05$).

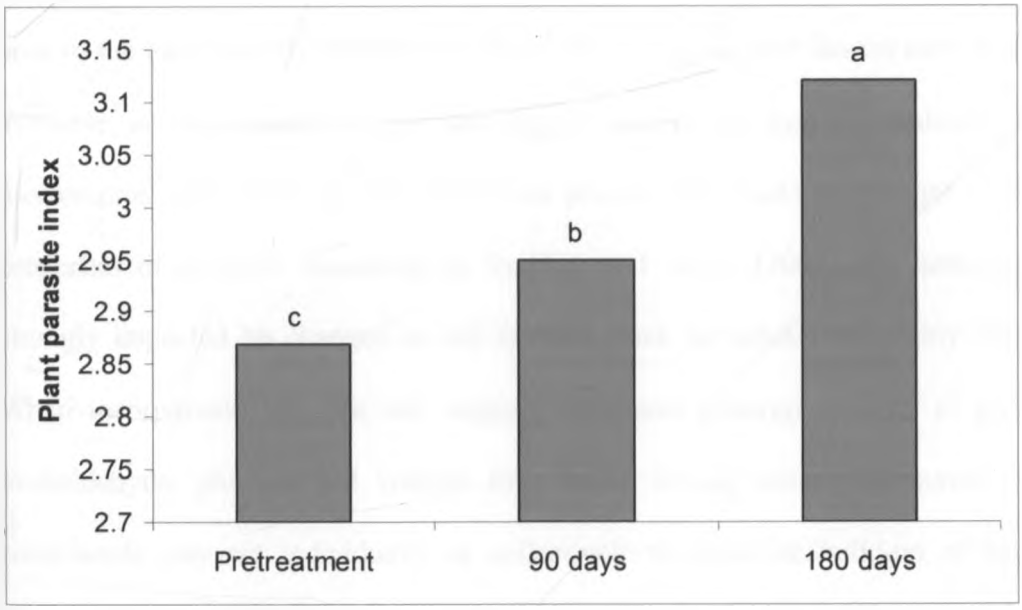


Figure 4.8 Effect of different nematode treatments on plant parasitic index. Columns headed by different letters are significantly different ($P \leq 0.05$).

# CHAPTER FIVE

## 5.0 DISCUSSION

### 5.1 Effect of different organic substrates a commercial neem based product and a nematophagous fungus in the management of plant parasitic nematodes in carnations

This study demonstrated that amending soils with organic substrates as well as with a biological agent (*P. lilacinus*) contribute to changes in nematode community structure by reducing populations of plant parasitic nematodes and also increasing the abundance of free-living nematode populations. The organic substrates tested namely bagasse, tea and flower composts and molasses were found to be suppressive to several plant parasitic nematodes (PPNs) and reduced eggmass densities. These findings are in agreement with previous reports by Akhtar and Malik (2000); Agyarko and Asante (2005); Otipa *et al.*, 2006).

Organic substrates offer an alternative or supplementary control measure following pressure on flower producers to reduce chemical usage in general and nematicides in particular because of the danger they pose to the environment (Akhtar and Malik 2000). The organic wastes used in the present study are easily available in large quantities and decompose without leaving any residues like the chemicals. 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 that are antagonistic to plant parasitic nematodes and as well elevate free-living nematodes numbers (Desaeger and Rao, 2002). The mode of action of organic substrates leading to stimulation of free-living nematodes is

complex and dependent on the nature of the substrate (Sikora *et al.,* 2005; Otipa *et al.*, 2006). 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 (Widmer and Abawi, 2002; Kimenju *et al.,* 2004).

Out of the sixteen plant parasitic nematodes isolated, the frequently encountered nematode in carnations as revealed in this study belong to the genus *Scutellonema* followed by *Helicotylenchus* and is similar to the findings by Kandji *et al.,* (2003). This genus nematode, except the later, was suppressed by all the treatments showing that all these can control nematode populations and can be considered to replace chemical nematicides. Application of organic substrates led to initial increases in *Meloidogyne*, however, molasses and bagasse soon reduced these levels. This temporary delay in control of these pest may be explained by the time required by the organic substrates to breakdown before they can release nematicidal compounds to the environment (Widmer and Abawi, 2002; Kimenju *et al.,* 2004).

There was an increase in the beneficial nematodes *Mononchus* and *Nygolaimus* following the application of organic substrates. It has been demonstrated previously that nematode antagonistic microbes multiply rapidly due to addition of organic matter which in turn provide control of plant parasitic nematodes (Mc Sorley and Frederick, 2000). It has been shown that dorylaimid predators (e.g *Nygolaimus*) are the most efficient and highly potent as biological control agents (Yeates *et al.,* 1999). Dorylaimid, and diplogasterid predators can be easily maintained by addition of organic matter to soil because they are polyphagous and remain abundant in soil without prey nematodes (Ruess, 2003). Predatory nematodes like *Mononchus*,

which was predominant in these findings, are comparatively large and therefore favoured by coarse soils of high organic matter content (Akhtar and Malik, 2000).

Bacterial feeders dominated the trophic groups isolated in this study and this finding is consistent with reports from other workers (Wasilewska, 1995; Ekschimitt *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 (Mc Sorley and Frederick, 2000). Rapid changes in numbers of bacterivorous nematodes can be anticipated, given their short generation time of 3-4 days (Ruess, 2003).

In this study, it was clear that *P. lilacinus* was best option to manage *Meloidogyne* spp. *Paecilomyces lilacinus* is an ubiquitious soil hyphomycete which parasitizes eggs of root-knot nematodes thus regulating populations of the nematodes in field soil (Schenk, 2004). The dominance of fungal feeding genera (*Aphelenchoides* spp. and *Aphelenchus* spp) in soils amended with *P. lilacinus* is noteworthy because fungivorous genera are normally found at lower densities than bacteriovores and predators (Bae and Knudnus, 2001). 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 as 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 fungivorous nematodes. Hoffman and S'jacob (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 (Yeates *et al*, 1993). Bae and Knudsen (2001) observed many nematodes associated with *Trichoderma* hyphae presumably feeding on 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 effectiveness of *Paecilomyces lilacinus* in this study is of great importance in view of the withdrawal of some nematicides from the market because of health and environmental hazards associated with their production and use (Stirling, 1991).

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, phenos, formaldehyde and fatty acids are released during decomposition of neem-based products (Javed et al., 2007). 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. This finding is worth noting since neem is labeled to be ecologically friendly (probably a marketing gimmick). Here it's not the case.

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. As the benefits of the use of amendments lead to improved

soil organic matter content and improved water holding capacity, it is possible that these agronomic benefits overcame the expected negative effects of nematode presence (Mcsorley and Gallagher, 1995). Flower growers as well have established 'optimal' nutritional requirements that provide the desired quality of flowers regardless of the existing impediments except that in this case it will be elevated compared to a nematode free media. However, it should be noted that when damaging nematodes are controlled, then the quality of flowers is improved because the lesions are reduced and water uptake improved

**5.2 Effect of various organic substrates, a nematophagous fungus and a commercial neem-based product on the diversity of nematodes**

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).

Nematode diversity in carnations was increased by using organic amendments such as bagasse, composts and molasses. The study goes further to reveal that *Paecilomyces lilacinus* - a biological agent - was also able to raise the level of nematode diversity. Both the organic amendments and *Paecilomyces lilacinus* were able to raise the level of nematode diversity in soil to the levels under control conditions. The increase

of nematode diversity under organic conditions is a finding that is consistent with that of Freckman and Ettema (1993).

The study identified four feeding groups of nematodes in carnations, a range that is within that generally recognised by many workers (Yeates *et al.,* 1999). These were dominated by herbivores in which plant parasites fall. When allowed to function without any treatment, the herbivores led to extensive root damage on carnations thus justifying the introduction of management agents. Incorporation of various eco-friendly by-products reduced these damaging parasites while increasing the population of the harmless or even beneficial nematodes.

Organic substrates composts, bagasse, and molasses, were able to increase nematode abundance and genera richness in line with previously reported studies (Freckman and Ettema, 1993; Yeates, 1999). It is interesting to note that although nematode abundance increased sharply after addition of bagasse and molasses, it soon dropped to levels below those of flower compost. It is possible that these materials could have favoured multiplication of particular species or genera of nematodes which then started competing among themselves for resources or space. Yeates (1999) pointed out that host specific herbivorous nematodes are strongly correlated with plant host. Differences in the nature of these organic materials may help explain the observed variances in nematode abundance. Indeed, the nature and amount of organic material strongly affect nematode population and usually influence the overall composition of the nematode fauna (Yeates and Bongers, 1999).

Soil disturbance leads to changes in species and species composition (Bloemers *et al.,* 1997), and as agriculture is intensified, disturbance increases (Yeates *et al.,* 1999). In this study, overall nematode

evenness was observed to increase in the soil overtime meaning that soil disturbance became less. In the long run soil disturbance interferes with ecosystem functions which may lead to an increase in pests and diseases like parasitic nematodes (Giller *et al.,* 1997). However, the fact that soil disturbance has been found to decrease in this experiment means that the negative effects of interfering with the ecosystem functions are forestalled. This also helps to counter the effects of the observed increased plant parasite index with time.

One of the aims of this study was to try out different agricultural by-products with the aim of maintaining nematode diversity where it is high or restoring where it is low. Following soil disturbance during cultivation nematodes were disrupted and few genera recorded. However, restoration of nematode diversity occurred relatively fast under organic substrates and nematophagous fungus *Paecilomyces lilacinus*. Neem raised diversity slightly whereas fenamiphos suppressed it. Thus organic substrates and *Paecilomyces lilacinus* may be recommended for improving soil biodiversity to avoid impairing the ecosystem function.

This study has shown the herbivores to be the dominant genera followed by bacteriovores. A study on integrated management (lower agrochemical and tillage) reported consistently higher nematode biomass with the greatest increase being that of herbivores although predators were also always more under the system, but the dominant group was bacteriovores (Bowman and Zwart, 1994). Hansson *et al.* (1990) also reported the dominance of bacteriovores in arable cropping areas. Therefore, the studies agree with the present findings.

Carnation growing is a monoculture practice which denies soil the long term benefits of functional and taxonomic diversity among the soil biota (Giller *et al.,* 1997). Monoculture has been mentioned as one of

the management practices that can change soil biophysical, chemical and hydrological conditions so that abundance of members of different trophic levels changes (Freckman and Ettema, 1993; Yeates and Bongers, 1999). Therefore it is important to ensure that the change in soil occasioned by cultivating carnation as a monoculture crop is not detrimental, it is clear that incorporating organic substrates and nematophagous fungi in the management of plant parasitic nematodes in carnation is the way forward. The benefits of organic substrates and *Paecilomyces lilacinus* revealed by this study go alongside reduced injury to the plants and possibly increased longevity.

**5.3 Conclusion and recommendations**

Four trophic groups; herbivores, bacteriovores, fungivores and predators were identified in carnations and hence their use as indicators of the ecological status of soil is validated.

Organic substrates restored nematode diversity while at the same time reducing root damage in carnations. The restored nematode diversity points to the wellbeing of soil thus ensuring sustainability hence these substrates are recommended over agents that reduce diversity such as chemical nematicides.

The fungus *Paecilomyces lilacinus* also led to increased nematode diversity while keeping damage on crops low. Increased diversity means more nematodes with less dominance of those likely to cause damage to crops. Thus organic substrates and *Paecilomyces lilacinus* are useful in overcoming the negative effects of monoculture in carnation growing.

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 therefore recommended that organic substrates

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.

## CHAPTER SIX

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## CHAPTER SEVEN

## 7.0 APPENDICES

**Appendix 7.1** Galling index scale as described by Sharma *et al.*, 1994.

| Number of galls | Scale |
|---|---|
| 0 | 1 |
| 1 – 5 | 2 |
| 6 – 10 | 3 |
| 11 – 20 | 4 |
| 21 – 30 | 5 |
| 31 – 50 | 6 |
| 51 – 70 | 7 |
| 71 – 100 | 8 |
| >100 | 9 |

**Appendix 7.2** Mean square values from analysis of variance for the effect of various treatments on plant parasitic nematodes

| **Nematode genera (variable)** | **Source** | | |
|---|---|---|---|
| | **Treatment** | **Treatment×Time** | **Error** |
| *Scutellonema* | 6792.8 | 2676.7 | 472.6 |
| *Helicotylenchus* | 704.7 | 352.5 | 441.9 |
| *Meloidogyne* | 1828.9 | 455.7 | 191.5 |
| *Pratylenchus* | 626.2 | 657.1 | 99.6 |
| *Tylenchus* | 487.0 | 461.7 | 107.4 |
| *Hemicyclophora* | 75.7 | 238.6 | 63.9 |
| *Tylenchorynchus* | 1137.7 | 410.3 | 78.7 |
| *Rotylenchus* | 401.5 | 247.7 | 67.4 |
| *Tylenchulus* | 433.9 | 223.4 | 57.9 |
| *Criconema* | 61.9 | 51.8 | 30.2 |
| *Trichodorus* | 45.6 | 46.5 | 14.8 |
| *Hoplolaimus* | 128.9 | 52.7 | 26.1 |
| *Hemicriconemoides* | 45.0 | 36.4 | 15.3 |
| *Longidorus* | 29.8 | 23.7 | 16.2 |
| *Xiphinema* | 26.2 | 37.2 | 15.4 |
| *Paratylenchus* | 24.5 | 24.5 | 10.6 |

Degrees of freedom for Treatment, Treatment×Time and Error are 7, 14 and 120 respectively

**Appendix 7.3** Mean square values from analysis of variance for the effect of various treatments on nonparasitic nematodes

| Nematode genera (variable) | Source | | |
|---|---|---|---|
| | Treatment | Treatment×Time | Error |
| *Aphelenchoides* | 3378.7 | 1797.3 | 235.8 |
| *Mononchus* | 242.1 | 21.7 | 14.7 |
| *Rhabditis* | 1348.4 | 539.0 | 128.2 |
| *Cephalobus* | 637.6 | 492.0 | 384.3 |
| *Chromadora* | 1400.3 | 1625.8 | 819.4 |
| *Acrobeles* | 850.8 | 626.1 | 100.1 |
| *Prodorylaimus* | 1118.4 | 716.9 | 177.5 |
| *Bunonema* | 665.8 | 269.0 | 149.7 |
| *Aphelenchus* | 670.5 | 670.5 | 54.3 |
| *Ucephalobus* | 270.1 | 270.1 | 46.9 |
| *Euphalobus* | 763.9 | 763.9 | 67.1 |
| *Labronema* | 430.5 | 430.5 | 82.5 |
| *Plenchtus* | 284.8 | 284.8 | 63.75 |
| *Nygolaimus* | 317.8 | 317.8 | 42.3 |

Degrees of freedom for Treatment, Treatment×Time and Error are 7, 14 and 120 respectively

**Appendix 7.4** Analysis of variance for diversity following application of different treatments on carnations

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Period | 2 | 29.3771 | 14.6885 | 108.54 | <.001 |
| Treatment | 7 | 3.1582 | 0.4512 | 3.33 | 0.003 |
| Period. Treatment | 14 | 7.8988 | 0.5642 | 4.17 | <.001 |
| Residual | 120 | 16.2389 | 0.1353 | | |
| Total | 143 | 56.6731 | | | |

**Appendix 7.5** Analysis of variance for abundance following application of different treatments on carnations

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Period | 2 | 3166277. | 1583138. | 145.64 | <.001 |
| Treatment | 7 | 2196803. | 313829. | 28.87 | <.001 |
| Period.Treatment | 14 | 1853435. | 132388. | 12.18 | <.001 |
| Residual | 120 | 1304418. | 10870. | | |
| Total | 143 | 8520934. | | | |

**Appendix 7.6** Analysis of variance for dominance following application of different treatments on carnations

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Period | 2 | 0.630434 | 0.315217 | 57.96 | <.001 |
| Treatment | 7 | 0.053075 | 0.007582 | 1.39 | 0.214 |
| Period.Treatment | 14 | 0.185289 | 0.013235 | 2.43 | 0.005 |
| Residual | 120 | 0.652663 | 0.005439 | | |
| Total | 143 | 1.521460 | | | |

**Appendix 7.7** Analysis of variance for evenness following application of different treatments on carnations

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Period | 2 | 0.052254 | 0.026127 | 2.68 | 0.072 |
| Treatment | 7 | 0.086664 | 0.012381 | 1.27 | 0.270 |
| Period.Treatment | 14 | 0.131114 | 0.009365 | 0.96 | 0.496 |
| Residual | 120 | 1.167780 | 0.009731 | | |
| Total | 143 | 1.437812 | | | |

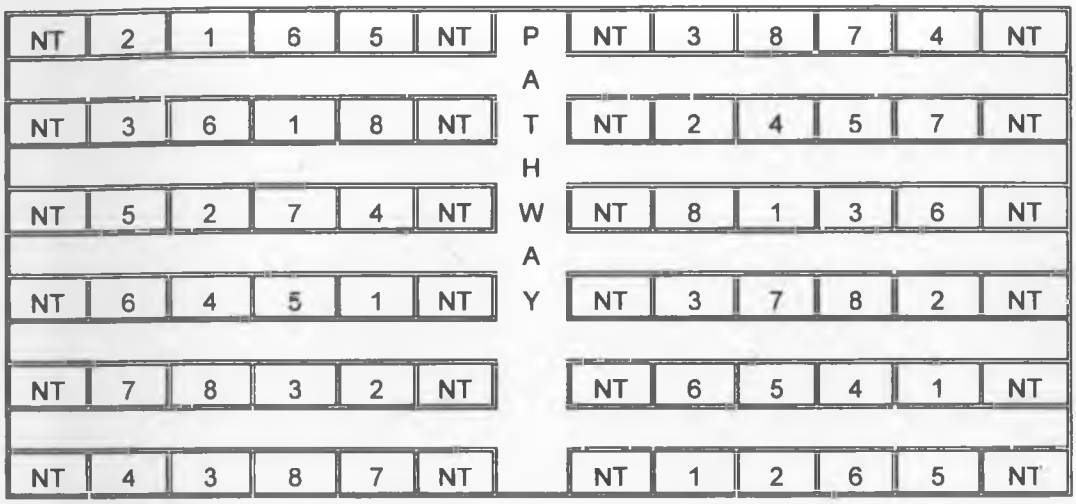
**Appendix 7.8** Analysis of variance for plant parasite index following application of different treatments on carnations

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Period | 2 | 1.55006 | 0.77503 | 24.15 | <.001 |
| Treatment | 7 | 0.38101 | 0.05443 | 1.70 | 0.116 |
| Period.Treatment | 14 | 0.69544 | 0.04967 | 1.55 | 0.104 |
| Residual | 120 | 3.85067 | 0.03209 | | |
| Total | 143 | 6.47718 | | | |

**Appendix 7.9** Analysis of variance for richness following application of different treatments on carnations

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Period | 2 | 4825.463 | 2412.731 | 272.71 | <.001 |
| Treatment | 7 | 1081.510 | 154.501 | 17.46 | <.001 |
| Period.Treatment | 14 | 1465.679 | 104.691 | 11.83 | <.001 |
| Residual | 120 | 1061.650 | 8.847 | | |
| Total | 143 | 8434.302 | | | |

**Appendix 7.10a** Experimental layout showing the completely randomised design used




**Key**

| Area | Sizes | Total |
|---|---|---|
| NT (no treatment) | 2m | 24 |
| Treatment plots | 4m | 48 |
| Pathway | 2m | 1 |
| Guard rows | 0.5m | 60 |

**Appendix 7.10b** A picture of the layout showing the raised beds arranged as a completely randomised design used