

BIBLIOGRAPHY

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ABSTRACT

The study was conducted at BIOCON laboratory and greenhouse of Benguet State University, La Trinidad Benguet from September 2010 to March 2011. Three levels of wax larva infected with approximately 100, 300 and 100 entomopathogenic nematodes (EPNs) were evaluated to determine their effect on root knot nematode, *Meloidogyne incognita* and on the growth and yield of carrot, cv. Tokita. Untreated plants and those treated with standard nematocide, Carbofuran (D'grand) were provided to serve as controls. The treatments were replicated five times and arranged randomly in the greenhouse using Completely Randomized Design (CRD).

Application of EPNs did not significantly affect the number of root knot nematodes in the roots. However, although not significantly different, the number of galls on the secondary root was generally higher in the control plants than those applied with EPNs.

Likewise, the growth parameters of carrots such as fresh top weight, fresh root weight, oven dry top weight, marketable and non-marketable root weight, were not significantly affected by EPN application.

TABLE OF CONTENTS

	Page
Bibliography.....	i
Abstract	i
Table of Contents	ii
INTRODUCTION	1
REVIEW OF LITERATURE.....	3
The Crop	3
The Importance of the Crop	3
The Causal Pathogen	3
Symptoms	4
Entomopathogenic Nematodes (EPNs)	4
Beneficial Effects of EPNs	5
Biology of Nematode/Bacterium Complex	6
MATERIALS AND METHODS	8
RESULTS AND DISCUSSION	12
Meteorological Condition	12
Number of Nematodes in Roots	12
Number of Galls on Secondary Root	13
Fresh Top Weight	14
Fresh Root Weight	14
Yield	15

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS	16
Summary	16
Conclusions	17
Recommendations	17
LITERATURE CITED	18
APPENDICES	20



INTRODUCTION

Carrot is one of the principal crops usually raised in the highlands and thus have gained great importance. Carrot is a good source of calcium and carotene. It is also a good source of protein, thiamine and ascorbic acid. It is commonly used as spice and can be eaten raw (Abuan, 2003).

In Benguet particularly, it is usually produced from October to May, or even the whole year round depending on the topography of the land, the environment and the availability of the factors of production.

In the Philippines especially in Benguet, root-knot nematode has already been found affecting the production of carrots. Depending on the variety and nematode population densities, the nematode could significantly affect the quality of the tap root making them commercially undesirable. According to Townshend (1962), many carrots are discarded in the fresh market and processing industry because of the tap root galling due to *M. hapla* infection. The more number of nematodes present/penetrating the roots the more damage is done on plants. But in the case of susceptible variety, even at low inoculum level, the quality and quantity of carrots could be significantly reduced.

Farmers however, often encounter problems with carrot plants, despite proper cultural practices and control of insects, weeds and other diseases. The possibility that their plants have been attacked by nematodes is great. These tiny organisms cause primary infection on plants by creating avenues or entry points for other pathogens.

Root knot nematodes, *Meloidogyne incognita* are obligate parasite capable of feeding inside the roots. The first of four molts occur in the egg and the nematodes hatch as second stage juveniles (J2s) which are the infective form. Typically, root knot



nematode development begins inside the egg. Adult female deposits eggs into a protective gelatinous matrix, near or just outside the root surface. A single female lays about 500 to 1500 eggs during her life, which last about two to three months. After the completion of embryogenesis, the first stage juvenile remains inside the egg until it molts into second stage – juvenile. After the second stage juvenile hatches from the egg, it moves freely in the soil in search of suitable host plant (Williamson and Gleason, 2003).

Crop production is the main source of income for most farmers in Benguet. Control of the damage caused by root knot nematodes in agricultural settings often requires the use of toxic pesticides. The use of entomopathogenic nematodes (EPNs) as an alternative to pesticides will be of great help to farmers to improve their income and to the country in general to increase food supply for the rapidly increasing population. Reduction of pesticide use will be contributory to ecosystem conservation and protection and ultimately to sustainable agriculture.

The study aims to evaluate the effect of different levels of EPNs on root knot nematode, *Meloidogyne incognita* and on the growth and yield of carrot.

The study was conducted at the BIOCON laboratory and greenhouse of Benguet State University, La Trinidad, Benguet from September 2010 to March 2011.



REVIEW OF LITERATURE

The Crop

Carrot (*Daucus carota* L.) a biennial plant grown for its edible fleshy root is usually orange in color, aromatic and sweet. A member of the carrot family (Umbelloferae), carrot is believed to have originated from the Middle East. It is now found worldwide, either as a cultivated plant or a weedy wild plant with long, dry roots.

Carrot thrives best on a deep loose loamy soil. The long smooth, slender carrot desired for fresh marketing can be successfully grown only on deep well drained, high soils. Experiments in Virginia and New York indicated maximum yields at around pH 6.5 and extremely low yields at pH 5.2 or below. Carrot requires an abundant and well distributed water supply (Abuan, 2003).

Importance of the Crop

Carrot is rich in carotene, a precursor of Vitamin A, and contains appreciable quantities of thiamine and riboflavin. Carrot is now gaining in importance because of its high nutritive value, as stated by quantities of thiamine and riboflavin. It is also used in many food preparations. It can be a principal ingredient of a dish – like carrot salad. It gives attractive color and appearance to pickles. It is excellent for garnishing and is very rich in vitamins.

The Causal Pathogen

Root knot nematodes are the most important plant parasitic nematodes in the Philippines. It represents a relatively small, but economically important group of obligate



plant pathogens (Agrios, 1997). It is the most common and widely distributed all over the country attacking several vegetable crops, field crops, fruit crops and ornamentals.

Meloidogyne spp. is considered to be sedentary endoparasites. They invade the roots and the development of the vermiform second stage juveniles (J2) depends upon the modification of the phenotype and function of specific root cells to form specialized cells that become the permanent source of nutrients for the parasites.

Symptoms

Damage caused by root-knot nematode is best determined by examining the roots for the presence of galls. Nematode galls should not be confused with the root nodules of leguminous plants. Nematode galls are characterized by swelling of the roots whereas root nodules are merely attached to the roots.

Above ground symptoms of plants attacked by root – knot nematodes are stunting and yellowing of leaves. In some vegetables, the plants may also show wilting during warmer part of the day as if the plants lack water and eventually die. Plants with fleshy roots are likely to be more severely damaged by root – knot nematodes than plants with grass – type roots.

In carrots, symptoms of *Meloidogyne* spp. infection include galling, large proliferation of secondary roots and tap root malformation such as severe forking and stunting (Vrain, 1982 as cited by Belair, 1992).

Entomopathogenic Nematodes (EPNs)

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are obligate parasites of a wide range of insects (Fallion *et al.*, 2002). The infective stage, known as the infective juvenile (IJ), carries a symbiotic bacterium



that is released following infection of the insect host. Steinernematids are associated with *Xenorhodus* spp., and Heterorhabditids are associated *Photorhabdus* spp. Once infection has occurred, the bacteria release anti-microbial agents that help prevent colonization of the insect cadaver by contaminating fungi and bacteria, and act as a food source for developing EPNs (Kaya and Gaugler, 1993). The bacteria also produce stillbene and indole metabolites that are nematicidal to a range of nematode species, including some plant parasites.

Beneficial Effects of EPNs

Entomopathogenic nematodes have been used successfully to control a number of compost and soil insects (Gouge and Hague, 1995). These same nematodes have shown some potential as antagonists to plant parasitic nematodes (PPNs). Application of EPNs to soil reduced a number of important PPN species including *Meloidogyne* spp., *Belonolaimus* spp., *Tylenchorhyncus* spp., and Criconematidae (Grewal *et al.*, 1997). EPNs tested in laboratory (Bird and Bird, 1986) and greenhouse studies and applied to tomato plants inoculated with *Meloidogyne* spp., reduced nematode penetration and egg production. Perry *et.al.* (1998) reported a reduction of *Globoderarostochiensis* penetration in potato tubers treated with *S. carpocapsae* in greenhouse and outdoor trials.

A number of interactive effects may be involved in suppression of PPNs and EPNs. Bird and Bird (1986) proposed the spatial competition at the mutually attractive root tip may affect root - knot nematode penetration. Ishibashi and Kondo (1986) suggested increased number of predators from the application of additional nematode biomass.



Biology of Nematode/Bacterium Complex

Steinernatids and Heterorhabditids are obligate pathogens in nature. Only the non-feeding third stage infective juvenile (IJ) or dauer juvenile is capable of surviving outside the insect host. The IJ carries cells of its bacterial symbiont in its intestinal tract. After locating suitable host, the IJ invades it through natural openings (mouth, spiracles, and anus) or thin areas of the host's cuticle and penetrate into the host hemocoel. The IJ releases its symbiont, and the bacterium and nematode cooperate to overcome the host's immune response (Chen *et al.*, 2004).

The mutualistic bacterium propagates and produces substances that kill the host and protect the cadaver from colonization by other microorganisms. The nematode initiates its development, feeding on bacterial cells and host tissues that have been metabolized by the bacterium and has 1-3 generations, depending on host size. As the food resources in the host cadaver in search of a new host (Chen *et al.*, 2004).

A major difference between Steinernatids and Heterorhabditids is that the species in the former group are amphimictic, whilst species in the latter group are hermaphrodites in the first generation but amphimictic in the following generations. Therefore, Steinernatids require at least two IJs, a male and a female, to invade the host to produce progeny, and Heterorhabditids need only one IJ to penetrate into the host as the resulting hermaphroditic adult is self-fertile.

Each nematode species is specifically associated with one bacterial symbiotic species; however, the symbiotic species maybe associated with more than one nematode species. This specificity operates at 2 levels. First, the best nematode reproduction occurs on their natural symbiont even though, in some cases, the nematode develops on other



bacterial species. Second, the natural bacterial symbionts are retained better than other bacterial species. The nematode is dependent upon the mutualistic bacterium for quickly killing its insect host; creating a suitable environment for its development, producing antibiotics that suppress competing microorganisms and transforming the host tissues into a food source. The bacterium requires the nematode for protection from external environment, penetration into the host's hemocoel and inhibition of the host's antibacterial proteins (Chen *et al.*, 2004).



MATERIALS AND METHODS

The study was conducted at the BIOCON Laboratory and greenhouse of Benguet State University, La Trinidad, Benguet from September 2010 to March 2011.

Source of Inoculum (*M. incognita*)

The root-knot nematode *Meloidogyne incognita* was used in the experiment. These inocula have been maintained in a susceptible variety of garden pea grown in pots under greenhouse condition in order to have a ready source of inoculum.

Plant Material

Carrot seeds cv. Tokita was directly sown in 7x7x14 cm diameter plastic pots containing 3 kg heat sterilized soil. Thinning was done six weeks from the time of seed sowing, maintaining 1 plant per pot. The plants were inoculated a week after thinning.

Preparation of Inocula

Eggs of root-knot nematode were collected from egg masses by dissolution of the gelatinous matrix. The infected roots were washed thoroughly and cut into small sizes. The cut roots were shaken for four minutes in stoppered flask containing 5.25% liquid bleached (Clorox) diluted with four volumes of water making 1% NaOCl solution. The liquid was poured over rested sieves and collected eggs were washed thoroughly with tap water to remove the bleach. Suspensions were poured over the blue sieve lined with tissue paper. Tap water was added reaching the bottom of the sieve. This allowed the eggs of the root knot nematode to hatch. After 48 hours, J2s were collected in a beaker.



Source of Entomopathogenic Nematodes

Steinernema sp. All strain IJs were produced in the last instar wax moth *Achroia grisella* (Figure 1). EPN infected *A. grisella* cadavers (hereafter termed as cadavers) were obtained by infecting each last instar *A. grisella* larva with approximately 100 *Steinernema* sp. IJs.

Lesser wax moth infected with EPNs were focused under dissecting microscope (Figure 2).



Figure 1. Lesser wax moth (*Achroia grisella*) used for the mass propagation of EPNs

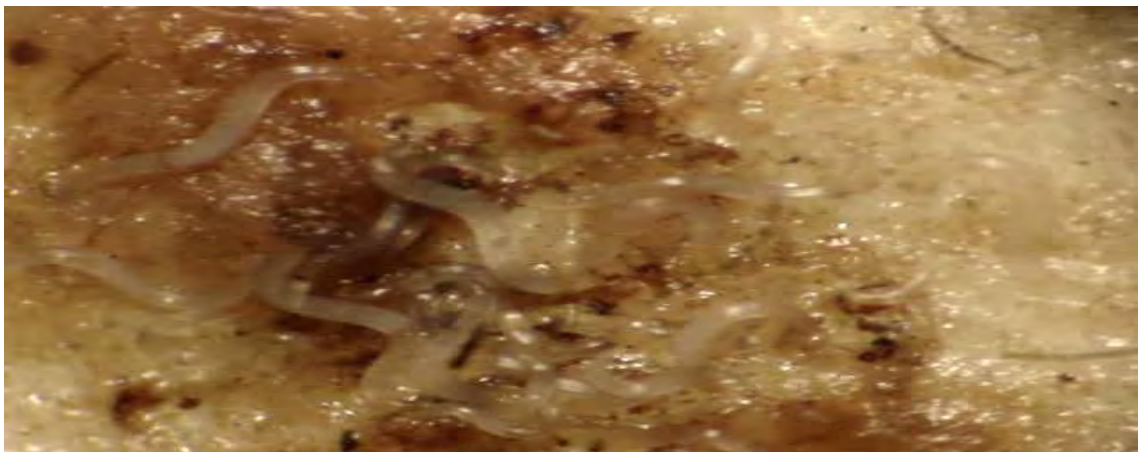


Figure 2. Close up view of EPNs inside *Achroia grisella*

Nematode Inoculation

After seedling emergence and when the plants were already well established, all treatments were inoculated with 1000 second stage juveniles per plant. Four holes were made in the soil near the plant zone. Using a pipette, the nematode suspension was distributed in each hole and covered again with soil.

Entomopathogenic nematodes were applied immediately after nematode inoculation.

The treatments were:

T₁ – 0 wax moth cadaver

T₂ – 1 wax moth cadaver (approximately 100 EPNs)

T₃ – 3 wax moth cadavers (approximately 300 EPNs)

T₄ – 5 wax moth cadavers (approximately 500 EPNs)

T₅ – D'grand- (.4g)

The different treatments were replicated 6 times. The plants were arranged in the greenhouse using CRD (Figure 3).



Figure 3. Experimental lay-out

All the cultural management practices for commercial carrot production such as watering, fertilization control of insect pest and diseases except nematodes were employed to all treatments to insure the good growth and yield of the plants.

Data Gathered

1. Number of nematodes in soil (200cc). About 200cc of soil was processed using the modified- tray method.
2. Number of nematodes in roots (2g). Two grams of roots was stained by using acid-fuchsin and the number of nematodes was counted using a dissecting microscope.
3. Number of galls. The individual galls in the whole root system was counted.
4. Fresh top weight. The vegetative part of the plants was cut at about 6cm from the soil then weighed.
5. Fresh root weight (g). The roots were washed thoroughly with running water and weighed separately.
6. Oven dry top weight (g). The upper part of the plant was oven dried at 72⁰ C for 48 hours.
7. Yield (kg). The marketable and non-marketable roots were weighed separately.

Data Analysis

The data were analyzed statistically using analysis of variance (ANOVA) and treatment means were separated using Duncan's Multiple Range Test (DMRT).



RESULTS AND DISCUSSION

Meteorological Condition

Table 1 shows the average temperature and relative humidity during the conduct of the study. Apparently, the above conditions were not favorable for the development and reproduction of root-knot nematodes. According to Hine (1965) the duration of the life cycle depends on the temperature; when it is maintained at a low level the nematode numbers increase slowly. All eggs do not hatch simultaneously; some of them hatch only several months after egg laying resisting cold and dryness. Luc *et al.* (2004) reported that one cycle was completed in 19 days at 30⁰C versus 43 days at 21.8⁰C.

Number of Nematodes in Roots

The number of nematodes observed from the 2g root of the different treatments did not differ significantly. However, higher number of nematodes was recorded from the roots obtained from the control plants than those applied with different levels of EPN (Table 2). Surprisingly, no nematode was recovered from the soil which indicates that perhaps some of the nematodes died and were not able to infect the plants.

Table 1. Average temperature and relative humidity during the conduct of the study

MONTHS	TEMPERATURE			RH
	MAXIMUM	OPTIMUM	MINIMUM	(%)
November	24.80	15.20	20.00	86.00
December	25.10	13.60	19.35	92.00
January	24.30	13.00	18.65	85.30



Number of Galls on Secondary Roots

Table 2 shows the effect of the different treatments on the number of galls in the secondary roots of carrot. Although not significantly different, the inoculated plants without EPNs had the highest number of galls compared to those plants treated with EPNs. Apparently, application of three infected cadavers wax moth (approximately 300 EPNs) was better than those applied with the highest number of wax moth cadavers (approximately 500 EPNs). On the other hand, the standard nematicide, Carbofuran, (D' grand) showed the lowest number of galls. Perez and Lewis (2004) reported that *M. hapla* inside the roots and egg recovery from seedlings treated with EPNs was significantly less than those in the control. The low rate of *Steirnerema glaseri* suppressed *M. incognita* penetration into tomato roots and the high rate of *S. glaseri* reduced egg production.

On the other hand, since the nematodes were not able to reproduce due to unfavorable conditions for growth and development, no egg mass was observed in the roots.

Table 2. Effect of entomopathogenic nematode (EPNs) on the number of galls and nematodes on the roots

TREATMENTS	NUMBER OF GALLS	NUMBER OF NEMATODES IN ROOTS
0 wax moth cadavers	8.00 ^a	5.17 ^a
1 wax moth cadavers	3.17 ^a	1.67 ^a
3 wax moth cadavers	2.83 ^a	1.67 ^a
5 wax moth cadavers	3.00 ^a	1.33 ^a
D'grand (0.4 g)	0.50 ^a	0.50 ^a

Means followed by similar letters are not significantly different at 5% level using DMRT.



Fresh Top Weight

Table 3 shows the effect of entomopathogenic nematodes (EPNs) on the fresh top weight of carrot inoculated with root knot nematode. Evidently, no significant differences were noted among the treatments. Nevertheless, application of three wax moth cadavers resulted in the highest fresh top weight (31.83 g) while the lowest (2.33 g) was obtained from the plants applied with the standard nematicide, D'grand. The same trend was observed in the oven dry top weight.

Fresh Root Weight

Similar to the fresh top weight, application of EPNs did not significantly affect the fresh root weight of inoculated carrots (Table 3). Numerically however, plants inoculated with root-knot nematode and applied with one (1) wax moth cadaver gave the highest fresh root weight (89.83 g). Unexpectedly, the lowest fresh root weight was recorded from inoculated carrots applied with five (5) wax moth cadavers (61.17 g).

Table 3. Effect of entomopathogenic nematodes (EPNs) on the growth parameters of carrot (g)

TREATMENTS	FRESH TOP WEIGHT	FRESH ROOT WEIGHT	OVEN DRY TOP WEIGHT	MARKETABLE ROOT (g)	NON MARKETABLE ROOT
0 wax moth cadavers	30.00 ^a	80.83 ^a	5.85 ^a	78.17 ^a	1.17 ^a
1 wax moth cadavers	28.67 ^a	89.83 ^a	4.997 ^a	61.17 ^a	28.67 ^a
3 wax moth cadavers	31.83 ^a	87.67 ^a	6.25 ^a	59.00 ^a	28.67 ^a
5 wax moth cadavers	24.5 ^a	61.17 ^a	4.28 ^a	32.82 ^a	28.33 ^a
D'grand (.4 g)	22.33 ^a	72.67 ^a	3.96 ^a	59.00 ^a	13.67 ^a

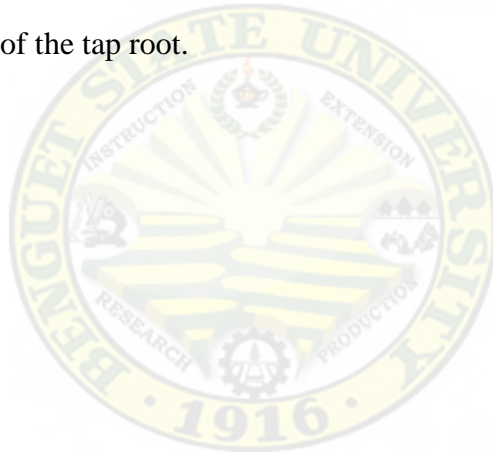
Means followed by similar letters are not significantly different at 5% level using DMRT.



Yield

The effect of EPN on the marketable yield was not significant (Table 3). Unexpectedly; higher yield was obtained in the untreated compared to the treated plants. However, lower yield on the treated ones was not attributed to nematode infection but rather to improper management of the crop that could have affected the growth. Due to very dense planting, thinning of the plants may have disturbed the main root resulting to forking of the tap root.

The effect of entomopathogenic nematodes (EPNs) on the non-marketable yield was also not significant (Table 3). Symptoms on non-marketable root include rotting, forking and cracking of the tap root.



SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

A greenhouse experiment was conducted at the BIOCON laboratory and greenhouse of Benguet State University, La Trinidad, Benguet from September 2010 to March 2011 to determine the effect of entomopathogenic nematodes (EPNs) on root-knot nematode, (*Meloidogyne incognita*) and on growth and yield of carrot.

Application of 1, 3 and 5 wax moth cadavers infected with EPNs in carrot cv. Tokita inoculated with 1000 juveniles of *Meloidogyne incognita* did not significantly affect the number of nematodes in the roots. The absence of nematodes in the soil and the presence of very few nematodes in the roots indicate that some of the nematodes died and were not able to infect the plants. This was attributed to unfavorable weather condition that could have affected the growth and development of root-knot nematodes.

Although not significantly different, the number of galls in the secondary root was generally higher in the untreated control than those applied with entomopathogenic nematodes (EPNs). Because of very low temperature, the nematode development and reproduction was delayed, thus no egg masses were produced.

Likewise no significant differences were also noted on the growth parameters of carrot: fresh top weight, fresh root weight, oven dry top weight, marketable and non-marketable yield.



Conclusions

1. Results of the experiment shows that application of different levels of wax moth cadavers infected with EPNs did not significantly affect the number of root knot nematodes that penetrated the roots including the number of galls. Since the temperature was very low, the growth and development of both the root knot nematode and EPNs were delayed. Thus, no egg mass was also detected.

2. Because of the above reason, the growth and yield parameters of carrot were also not significantly affected by EPN application.

Recommendations

Since the experiment was greatly affected by unfavorable temperature additional studies are necessary to fully evaluate the efficacy of entomopathogenic nematodes against root-knot nematode in carrot. It is recommended that studies should be done during summer when the temperature is conducive for the growth and development of both RKN and EPNs in order to have more conclusive results.



LITERATURE CITED

- ABUAN, M. M. 2003. Response of two carrot (*Daucus carota* L.) cultivars to varying levels of root-knot nematode. BS Thesis. (Benguet State University. La Trinidad, Benguet. P. 12.
- AGRIOS, G. N. 1997. Plant Pathology. 4th Edition. San Diego, California. Pp. 565-567.
- BIRD, A. F., and J. BIRD. 1986. Observations on the use of entomopathogenic nematodes as means of biocontrol of root-knot nematodes. International Journal of Parasitology 16:511-516.
- BELAIR, G. 1992. Effect of Cropping Sequences on Population Densities of *Meloidogyne hapla* and Carrot Yield in Organic Soil. Journal of Nematology. 24 (3): 450-456.
- CHEN, Z., S. CHEN and D.W. DICKSON. 2004. Nematology-Advances and Perspective, CABI Publishing. Pp.1098-1099.
- FALLION, D. J. KAYA, R. GAUGLER and B. S. SIPES. 2002. Effect of Entomopathogenic Nematodes on *Meloidogyne incognita* on Tomato and Soybean. Journal of Nematology 34(3):239.
- GREWAL, P. S., W. R. MARTIN, R. W. MILLER and E. E. LEWIS. 1997. Suppression of plant-parasitic nematode populations in turf grass by entomopathogenic nematodes. Nematology 1:735-743.
- GOUGE, D. H. and N. HAGUE. 1995. Glasshouse control of fungus gnats, *Bradysia paupera* on fuchsias by *Steinernafeltiae*. Fundamental and Applied Nematology 18:77-80.
- HINE, R. B., O. V. HOLTZMAN, and R. D. RAABE. 1965. Disease of Carrot in Hawaii. Hawaii Agric. Exp. Stn. Bull.136, Univ. of Hawaii. P. 26.
- ISHIBASHI, N., and E. KONDO. 1986. *Steinernafeltiae* (DD-136) and *S. glaseri*: Persistence in soil and bark compost and their influence on native nematodes. Journal of Nematology 18:310-316.
- KAYA, H. K., and R. GAUGLER. 1993. Entomopathogenic nematodes. Annual Review of entomology 38:181-206.
- LUC, M., RICHARD.S., J. BRIDGE. 2005. Plant parasitic Nematodes in Subtropical and Tropical Agriculture. CABI Publishing P. 331.
- PEREZ, E. E., and E. E. LEWIS. 2004. Entomology Department, Virginia Tech, Blacksburg, VA 24061, USA Biological Control. Vol 30. Pp .336-341



- PERRY. R. N., HOMONICK. W. M., BEANE. J., BRISCOSE. B., 1998. Effect of the entomopathogenic nematodes, *Steinernafeltiae* and *S. carpocapsae*, on the potato cyst nematode, *Globoderarostochiensis*, in pot trials. *Bocontrol Science and Technology* 8:175-180.
- TOWNSHEND, J. L. AND T. R. DAVIDSON. 1962. Some Weed Hosts of the Northern Root- knot Nematode *Meloidogyne hapla* (Chiwood,1948) Ontario, Canada. *Journal of Botany*. 17:154-66.
- WILLIAMSON, V. M. and C. A. GLEASON. 2003. Plant-nematode Interactions. *CurrentOpinion in Plant Biology*. 6:327-333.



APPENDICES

Appendix Table 1. Effect of EPNs, on the number of nematodes in roots (2g) of carrots cv. Tokita inoculated with root knot nematode, *M. incognita*

TREATMENT No. of wax moth cadaver	REPLICATION			TOTAL	MEAN
	I	II	III		
T1-0	14.5	1.00	0.00	15.50	5.17
T2-1	0.50	1.00	3.50	5.00	1.67
T3-3	2.00	1.00	2.00	5.00	1.67
T4- 5	0.50	1.50	2.00	4.00	1.33
T5-D'grand	1.00	0.00	0.50	1.50	0.50

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F-VALUE	TABULAR	
					0.05	0.01
Replication	2	21.333	10.67	0.7232	3.84	7.01
Treatment	4	38.767	9.692	0.6602 ^{ns}		
Error	8	117.433	14.679			
Total	14	177.433				

^{ns} – Not significant

Coefficient of variation = 185.26%



Appendix Table 2. Effect of EPNs, on the number of nematodes in roots (2g) of carrots cv. Tokita inoculated with root knot nematode, *M. incognita*

TREATMENTS No. of wax moth cadaver	REPLICATION			TOTAL	MEAN
	I	II	III		
T1-0	3.87	1.22	0.71	5.80	1.93
T2-1	1.00	1.22	2.00	4.22	1.41
T3-3	1.58	1.22	1.58	4.38	1.46
T4- 5	1.00	1.41	1.58	3.99	3.00
T5-D'grand	1.22	0.71	1.00	2.93	0.997

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F-VALUE	TABULAR	
					0.05	0.01
Replication	2	1.41	0.43	0.58	3.84	7.01
Treatment	4	0.85	0.35	0.4838 ^{ns}		
Error	8	5.85	0.73			
Total	14	8.113				

^{ns} – Not significant

Coefficient of variation = 60.17%



Appendix Table 3. Effect of EPNs, on the number of galls of carrots cv. Tokita inoculated with root knot nematode, *M. incognita*

TREATMENTS No. of wax moth cadaver	REPLICATION			TOTAL	MEAN
	I	II	II		
T1-0	4.58	2.00	0.71	7.29	2.43
T2-1	1.22	1.73	2.55	5.50	1.83
T3-3	2.12	1.58	1.73	5.43	1.81
T4- 5	1.22	2.24	2.12	5.36	1.79
T5-D'grand		0.71	1.00	2.93	0.98

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F-VALUE	TABULAR	
					0.05	0.01
Replication	2	0.512	0.256	0.218	3.84	7.01
Treatment	4	3.212	0.803	0.685 ^{ns}		
Error	8	9.376	1.172			
Total	14	13.090				

^{ns} – Not significant

Coefficient of variation = 61.26%



Appendix Table 4. Effect of EPNs, on the fresh top weight of carrots cv. Tokita inoculated with root knot nematode, *M. incognita*

TREATMENTS No. of wax moth cadaver	REPLICATION			TOTAL	MEAN
	I	II	III		
T1-0	25.50	31.50	33.00	90.00	30.00
T2-1	28.00	38.00	20.00	86.00	28.67
T3-3	27.00	40.50	28.00	95.50	31.83
T4- 5	26.50	20.00	27.00	73.50	24.50
T5-D'grand	21.50	23.50	22.00	67.00	22.33

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F- VALUE	TABULAR	
					0.05	0.01
Replication	2	78.633	39.312	1.2034	3.84	7.01
Treatment	4	186.233	46.558	1.4251 ^{ns}		
Error	8	261.367	32.671			
Total	14	526.233				

^{ns} – Not significant

Coefficient of variation = 21.25%



Appendix Table 5. Effect of EPNs, on the fresh root weight of carrots cv. Tokita inoculated with root knot nematode, *M. incognita*

TREATMENTS No. of wax moth cadaver	REPLICATION			TOTAL	MEAN
	I	II	III		
T1-0	86.50	68.50	87.50	242.50	80.83
T2-1	105.00	115.00	49.50	269.50	98.86
T3-3	72.50	90.00	71.00	263.00	87.67
T4- 5	72.50	57.00	54.00	183.50	61.17
T5-D'grand	79.00	63.50	75.50	218.00	72.67

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F- VALUE	TABULAR	
					0.05	0.01
Replication	2	1156.633	578.317	1.94	3.84	7.01
Treatment	4	1657.100	414.275	1.39 ^{ns}		
Error	8	2380.200	297.525			
Total	14	5193.933				

^{ns} – Not significant

Coefficient of variation = 21.99%



Appendix Table 6. Effect of EPNs, on the oven dry top weight of carrots cv. Tokita inoculated with root knot nematode, *M. incognita*

TREATMENTS No. of wax moth cadaver	REPLICATION			TOTAL	MEAN
	I	II	III		
T1-0	9.20	2.58	5.78	17.56	5.76
T2-1	5.14	6.22	3.63	14.99	4.997
T3-3	7.03	5.82	5.89	8.74	6.25
T4- 5	4.58	3.31	4.94	12.83	4.28
T5-D'grand	4.16	4.29	3.44	11.89	3.96

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F- VALUE	TABULAR	
					0.05	0.01
Replication	2	7.049	1.524	1.3384	3.84	7.01
Treatment	4	11.573	2.893	1.098 ^{ns}		
Error	8	21.065	2.633			
Total	14	39.686				

^{ns} – Not significant

Coefficient of variation = 32.02%



Appendix Table 7. Effect of EPNs, on marketable root weight of carrots cv. Tokita inoculated with root knot nematode, *M. incognita*

TREATMENTS No. of wax moth cadaver	REPLICATION			TOTAL	MEAN
	I	II	III		
T1-0	86.50	65.00	83.00	234.50	78.17
T2-1	105.50	61.00	17.50	183.50	61.17
T3-3	102.00	42.00	33.00	177.00	59.00
T4- 5	41.50	20.50	36.50	98.50	32.83
T5-D'grand	79.00	22.50	75.50	177.00	59.00

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F- VALUE	TABULAR	
					0.05	0.01
Replication	2	4719.433	2359.717	402574	3.84	7.01
Treatment	4	3156.233	789.058	1.4236 ^{ns}		
Error	8	4434.067	554.258			
Total	14	12309.733				

^{ns} – not significant

Coefficient of variation = 40.57%



Appendix Table 8. Effect of EPNs, on non- marketable root weight of carrots cv. Tokita inoculated with root knot nematode, *M. incognita*

TREATMENTS No. of wax moth cadaver	REPLICATION			TOTAL	MEAN
	I	II	III		
T1-0	0	3.50	0	3.50	1.17
T2-1	0	54.00	32.00	86.00	28.67
T3-3	0	48.00	38.00	86.00	28.67
T4- 5	19.50	19.50	34.50	85.00	28.33
T5-D'grand	0	41.00	0	41.00	13.67

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F- VALUE	TABULAR	
					0.05	0.01
Replication	2	1827.30	913.65	3.3497	3.84	7.01
Treatment	4	1843.27	460.82	1.6895 ^{ns}		
Error	8	2182.03	272.754			
Total	14	5852.75				

^{ns} – Not significant

Coefficient of variation =57.83%



Appendix Table 9. Effect of EPNs, on non- marketable root weight of carrots cv. Tokita inoculated with root knot nematode, *M. incognita*

TREATMENTS No. of wax moth cadaver	REPLICATION			TOTAL	MEAN
	I	II	III		
T1-0	0.71	2.00	0.71	3.42	1.14
T2-1	0.71	7.38	5.70	13.79	4.596
T3-3	0.71	6.96	6.20	13.87	4.623
T4- 5	5.76	4.47	5.92	16.00	5.333
T5-D'grand	0.71	6.44	0.71	7.86	2.62

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F- VALUE	TABULAR	
					0.05	0.01
Replication	2	35.602	17.801	3.9675	3.84	7.01
Treatment	4	36.112	9.028	2.0122 ^{ns}		
Error	8	35.893	4.4487			
Total	14	107.607				

^{ns} – Not significant

Coefficient of variation = 57.83%

