International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 11, No. 1, p. 81-89, 2017

INNSPUB

RESEARCH PAPER OPEN ACCES

Influence of NPK fertilizer on tolerance to damage by root knot nematodes on coffee

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Article published on July 31, 2017

Key words: Galling index, Nematode damage, Nutrition, Shoot and root weight.

Abstract

Nematodes pose a serious threat to coffee production and applications of both organic and inorganic amendments have suppressive effects on nematode populations. The study was carried out to evaluate the effect of NPK fertilizer on nematode infection on coffee seedlings. A greenhouse experiment was conducted using coffee variety SL 28 grown in sterile soil media amended with NPK (2.5g/kg). Three levels of nematode inoculum namely 1000, 2000 and 5000 eggs and juveniles per seedling were evaluated for the effect on nematode damage with and without NPK application. The experiment was terminated ninety days after inoculation and the parameters assessed were galling index, egg mass indices, fresh shoot and root weight, length of internodes and plant height. Inoculation with the higher level of inoculum resulted, to more damage by the nematode especially without NPK. Plants inoculated with higher levels of inocuum had higher and significant differences ($P=0.05$) in galling indices and egg mass indices. Egg mass indices increased were significantly fewer in the roots of plants amended with NPK and the scores were 1.6, 2.7 and 4.1 compared to those of roots grown without NPK at 2.2, 3.1 and 4.6 for inoculation rates of 1000, 2000 and 5000 eggs/kg respectively. Differences in galling indices were observed among the different treatments with higher scores being registered for treatments without NPK. Proper and balanced plant nutrition is crucial for effective nematode management. Manipulation of plant nutrient can be employed with other strategies to manage root knot nematodes in coffee.

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Introduction

Plant parasitic nematodes are among the main coffee pests that pose the greatest threat to the coffee industry (Campos and Villain, 2005; Alban and Guerrero, 2013). Nematodes in the genus *Meloidogyne* are the most damaging in coffee however other nematodes in the genus *Pratylenchus* spp., *Helicotylenchus* spp., *Xiphinema* spp, *Radopholus* spp and *Rotylenchulus* spp have been associated with the crop (Campos *et al.,* 1990). Nematodes penetrate and feed on the roots of growing plants, drawing nutrients vital for plant growth and predisposing the roots to attack by other soil borne pathogens resulting in the development of disease complexes with fungi, bacteria and viruses (Campos and Villain, 2005; Khan, 2008; Orisajo and Fademi, 2013). In many situations, plant varieties resistant to fungi and bacterial diseases are rendered susceptible when parasitized by nematodes.

Less attention has been given to nematodes in coffee due to the hidden nature of damage they cause (Campos *et al.,* 1990; Khan, 2008). Under severe nematode infestations, seedlings under 5 years dry up and mature coffee suffers leaf drop, yields decline and even drying up (Whitehead, 1998; Campos and Villain, 2005).

Nutrient deficiencies resulting from root damage and impaired nutrient uptake due to nematode parasitism may be offset, in part, by proper nutrition, moisture and protection from adverse conditions (Wintgens, 2009). Adequate potassium levels may enable a number of plants to tolerate large populations of nematodes. High rates of chemical fertilizers, in particular, ammonium sources of nitrogen such as urea have been shown to suppress a number of nematodes including *Heterodera glycines* (Nyasani *et al.,* 2008; Wintgens, 2009). Soil nutrients are fundamental to plant growth in agriculture and studies have shown that fertilization of coffee with NPK and micro-elements strengthens plants with a response capacity to withstand effect of pests (Melakeberhan *et al.,* 1997; Coyne *et al.,* 2004, Wintgens, 2009).

High concentration of Ammonium ions (324mg/litre of Ammonium nitrate) suppresses or inhibits egg hatch and juvenile penetration into the roots; alters the degree of nematode attraction and nematode surface feeding behaviour due to changes in electrical potential created around the root tip area where juveniles penetrate (Berberchek and Duncan, 2004; Wintgens, 2008).

Low numbers of nematodes have been reported from optimal nutrient treatments compared with high numbers from nutrient deficient treatments, suggesting that nutrients may cause changes in soil chemistry that adversely affects nematode mobility and nematode mortality (Melakeberhan *et al.,* 1997). Similarly higher Pf/Pi (final/initial population) ratios have been recorded among rootstocks in nutrient deficient environments (Maredia *et al.,* 2003; Khan, 2008). In view of this, nematode management requires a multidisciplinary approach. An experiment was carried out to assess the effect of NPK on tolerance of coffee seedlings to damage by root knot nematodes (*Meloidogyne incognita*).

Materials and methods

Raising coffee seedlings

Coffee seedlings variety SL 28 were raised from selected quality cherries, processed into parchment coffee and sterilized in 10% (v/v) sodium hypochlorite (NaOCl) for 1.5 min, rinsed in sterile water for 3 minutes and sown in sterile sand in pots. Two hundred seeds were sown and after emergence, at soldier stage (unopened cotyledons) in 32 days period; they were then selected for growth uniformity. At 2 months of age or when first pair of true leaves appeared (butterfly stage), individual seedlings were used in the experiment and subjected to the various treatments.

Preparation of the potting mixture

Potting media comprising of loamy top soil and sand at a ratio of 1:1 was sterilized following a method described by Wintgens, (2009) and the nutrient status analyzed at the University of Nairobi, Chemistry laboratory. One kg pots measuring 8 cm in diameter and 20 cm deep were filled with the potting media and placed in the greenhouse.

The coffee seedlings were transplanted into the pots some of which of had been amended with NPK at the rate of 2.5g/kg of the soil and some pots which had not received the fertilizer depending on the treatments. Three levels of *Meloidogyne incognita* inoculum (1000, 2000 and 5000 eggs/kg of soil) and without inoculations were evaluated with and without NPK and the experiment treatment combinations are as shown in Table 1. The treatments were arranged in a completely randomized design.

Preparation of root knot nematode inoculum

Nematode egg inoculum was prepared in the laboratory following blender maceration and filtration technique as described by Hooper (2005).

The nematodes were later enumerated by examining three replicates of 2ml of the egg and Juvenile stage 2 suspension from a known volume (1000ml/litre) of nematode extract. Counting and recording was done in 7 randomly selected cells of the 49 cells/small squares on the grid in the counting slide and then averaged to obtain the mean (i.e. eggs/2ml). The concentration of the 1 litre extract was then determined by extrapolation from the known volume.

Inoculation of coffee seedlings

Meloidogyne incognita was inoculated into the coffee seedlings at two pair leaf stage by placing 10 ml aliquots containing a suspension of a mixture of 1000, 2000 and 5000 eggs and juveniles (respectively for the different treatments) into 2 or 3 pencil-size holes of 3-5 cm depth made around the seedling and soil pushed back after inoculation. The controls received equal amount (10 ml) of clean tap water only. The experimental design was a randomized complete block in a 4x2 factorial arrangement.

Assessment of galling, egg mass indices and nematode population

At the termination of the experiment after 90 days, data on galling and egg mass indices, nematode population, fresh root weights, leaf expansion and growth, plant height and length of internodes were taken. Other observations such as nutrient deficiency symptoms and signs of moisture stress were also made. Nematode population was determined in 200cm^3 soil samples assessed as the differences between the initial (Pi) and final (Pf) soil nematodes, fresh root weights were determined from the five seedlings of each treatment; leaf expansion and growth was determined by taking the length and width of the 3rd and 4th leaf pair from the tip.

Statistical analysis

Analysis of variance of each variable was carried out at 5% probability level using GENSTAT 7.2 programme and the means compared using the Least Significant Difference tests at 5% probability level.

Results

Evaluation of NPK fertilizer on plant growth parameters

NPK fertilizer application significantly increased all growth parameters (height, root and shoot weights, leaf size and internode).

Table 1. Treatment combinations with and without NPK at the different levels of inoculum.

Inoculum Density (eggs/kg soil)	NPK (Optimum)	NPK (Zero)
(control)	NPK_1	NPK_0
(1000)	NPK_1	NPK_0
(2000)	NPK_1	NPK_0
(5000)	NPK_1	NPK_0

Key: $NPK_1$with fertilizer application

NPK_0 without fertilizer application

The plant height and fresh shoot weights (FSW) of plants grown in soil amended with 2.5g NPK/kg soil were significantly ($P \le 0.05$) higher than those grown without NPK fertilizer at all levels of inoculum (Fig. 1). Plants treated with NPK_1 were taller and branching commenced earlier than in those grown without NPK_0 regardless of *M. incognita* inoculum densities (Fig. 2).

This was consistent with fresh weights of shoot and root weights of plants amended with NPK and inoculated at 1000 eggs/plant which recorded higher weights of 4.1g compared to those grown without NPK fertilizer whose weights were 1.8g and 0.7 g respectively (Table 2).

Table 2. Fresh shoot weight, fresh root weight and internode length of coffee seedlings with and without NPK and inoculated with different inoculum levels of *Meloidogyne incognita*.

Treatment	Fresh shoot weight (g)	Fresh root weight (g)	Internode (cm)
NPK_1+0 (control)	5.2a	5.2a	2.4a
NPK_1+1000	4.1b	4.1b	2.2a
NPK_1+2000	3.3ab	3.3b	2.3a
NPK_1+5000	0.8cd	0.8d	1.2b
NPK_0+0 (Control)	2.5bc	1.1c	1.8ab
NPK_0+1000	1.8bc	0.7d	1.3b
NPK_0+2000	1.3cd	0.5d	1.0b
NPK_0+5000	0.6cd	0.4d	0.4c
L.S.D.(P=0.05)	0.877	0.2084	0.4689

All data are means of 10 replicates. Means followed by the same letter within a column are not significantly (P=0.05) different.

It was also observed that plants in the control (treatment which had neither NPK nor nematode inoculum) recorded higher fresh weights of shoots (2.5g) and roots (1.1g) than those of inoculated plants at all levels grown without NPK. Similarly, plants without fertilizer at the highest inoculum of 5000 eggs/kg soil were severely damaged and stunted with glaring microelement deficiency symptoms (Fig. 2).

Fresh shoot weight of plants grown without NPK and inoculated with 1000 eggs/plant were significantly smaller (1.8g) than those amended with NPK (4.1g) at the same inoculum density (Table 2). At the highest density of nematodes (5000 eggs/plant) FRW of plants (0.8g) treated with NPK was not significantly (P=0.05) different from that of plants grown without NPK (0.7g) at lowest inoculum intensity of 1000eggs/plant. Similar observations were recorded for internode length where 5000 eggs/plant with NPK application was 1.2cm long plants without NPK application and inoculated with 1000 eggs/plant recorded 1.3cm internode length (Table 2). In a repeat experiment, the plants grown in soil added with NPK and non inoculated recorded the greatest height of 33.3cm, while the least height was recorded in the plants grown without fertilizer but inoculated with the highest level of inoculum and this had plant height of 16.5cm (Table 3).

Table 3. Egg mass index, fresh weight of roots and shoots: root ratios of coffee plants amended with NPK and inoculated with varying inoculum densities of root knot nematodes (RKNs).

Treatment	Egg mass index	Fresh shoot weight	Fresh root weight	S:R ratio	Height (cm)
NPK_0+No eggs (control)	1f	7.2d	5.3d	1.4e	25.5c
NPK_0+1000 eggs	2.2d	5.7e	4.2e	1.4e	22.7d
NPK_0+2000 eggs	3.1c	5.2e	3.3f	1.6d	20.1e
NPK_0+5000 eggs	4.6a	2.1f	1.1g	1.9c	16.5f
NPK_1+0 eggs	1f	20.6a	9.7a	2.1b	33.3a
NPK_1+1000 eggs	1.6e	12.6b	7.6b	1.7d	29.8b
NPK_1+2000 eggs	2.7c	11.5c	6.1c	1.9c	28.6b
NPK_1+5000 eggs	4.1b	7.9d	3.2f	2.5a	24.6c
L>S.D (P=0.05)	0.3683	0.949	0.2403	0.18	2.007

All data are means of 10 replicates. Means followed by the same letter within a column are not significantly (P=0.05) different.

Assessment of damage and levels of RKN

Above ground symptoms observed in the artificially infested coffee seedlings were dieback, stunting, yellowing of leaves and reduction in leave sizes. Plants treated with high inoculum levels had higher and significant differences (P=0.05) in galling indices than those at lower inoculum levels. The egg mass indices increased with increasing inoculum densities but were significantly fewer in the roots of plants amended with NPK 1.6 NPK/kg, 2.7 NPK/kg and 4.1 NPK/kg compared to those of roots grown without NPK at 2.2 NPK/kg, 3.1 NPK/kg and 4.6 NPK/kg on a rating scale of 1-6, except the control that had no nematodes. The galling indices were highly significantly (P=0.05) different at all levels of nematode densities and nutritional status (Table 3).

The damage by nematodes was highly significantly (P=0.05) and positively correlated to nematode population recovered from soil and the number of juveniles was highly negatively correlated to the fresh weights of roots. The coffee seedlings grown without NPK were highly susceptible to nematode damage under very high nematode inoculum densities as indicated by high galling and egg mass indices (Fig. 3 and 4). Despite the high galling indices in roots of seedlings amended with NPK and inoculated with 5000 eggs, the rooting system was significantly better developed with root weight of 3.2g compared to NPK deficient conditions which had root weight of 1.1 g (Table 4).

Table 4. Influence of varying inoculum densities and nutrient regimes on fresh weights of root and shoot and SRR of coffee seedlings after 90 days of inoculation.

Treatments	1000	2000	5000
	Shoots		
NPK0	5.7e	5.2e	2.1f
NPK1	12.6b	11.5c	7.9d
	Roots		
NPK0	4.2e	3.3f	1.1g
NPK1	7.6b	6.1c	3.2f
	SRR		
NPK0	1.4e	1.6d	1.9c
NPK1	1.7d	1.9c	2.5a

Key: SRR-shoot to root ratio.

Discussion

Influence of NPK on plant growth parameters

Application of NPK fertilizer significantly increased all growth parameters i.e. height, root and shoot weights, leaf size, length of internodes. Plants treated with NPK were taller, with branching commencing earlier than those grown without NPK regardless of *M. incognita* infestation levels. When challenged with higher levels of inoculum, FSWs were greater for plants amended with NPK than in plants grown without NPK. Plants grown without NPK when inoculated became stunted with glaring nutrient deficiency symptoms unlike plants grown in soil amended with NPK, where enhanced root growth compensated for losses through nematode damage.

The height of a plant is reported to be an important character directly linked to plant potential and an optimum plant height is correlated to productivity (Jones, 2003; Omotoso, 2007). Fresh weights of roots infested with high intensities of nematode were significantly lower than those infested with lower inoculum intensities under both nutrient regimes.

The seedlings treated with NPK at different levels of inoculum responded with much increased shoot:root ratio indicating a more efficiently functioning root system under conditions of higher fertility.

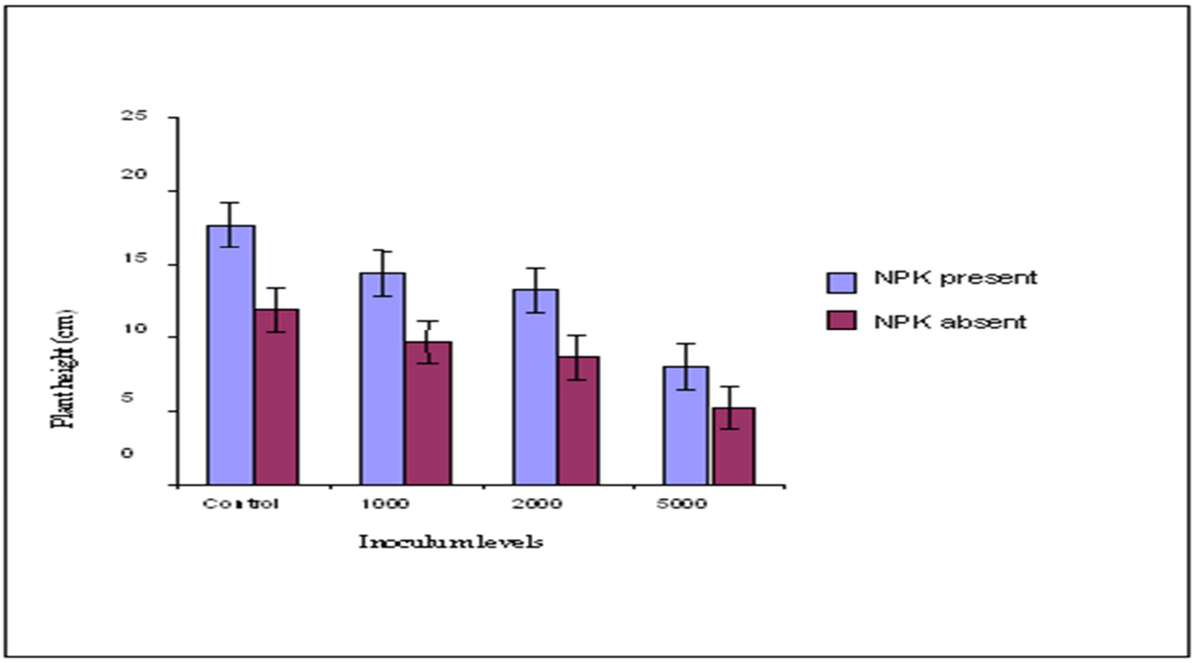

Fig. 1. Height of plants inoculated with varying levels of *M. incognita* inoculum and grown under optimal and deficient nutrient conditions.

The study findings are consistent with other studies on the effects of root knot nematodes on plant roots where normal roots were reduced to a limited number of galled roots with necrotic sections, vascular system destroyed and rootlets virtually absent when grown under nutrient deficient conditions (Duncan and Cohn, 1990; Walter *et al.,* 1992; Campos and Villain, 2005; Alban and Guerrero, 2013; Kimenju *et al.*, 2014). Studies conducted in Sri Lanka showed that the leaves of infested tea seedlings with *Meloidogyne incognita* were small, yellow and dull in appearance as reported by Campos *et al.,* (1990).

Fig. 2. Coffee seedlings treated with complete NPK fertilizer (left), without fertilizer and inoculated with 5000 eggs/plant (right) exhibiting general chlorosis and necrotic symptoms (right).

Influence of NPK on nematode damage

High numbers of *M. incognita* juvenile in soils with low fertility has been reported on cherry rootstocks by other researchers (Zhang and Schmitt, 1995; Melakeberhan *et al.,* 1997). The lower number of nematodes recovered from roots of plants amended with NPK compared with juvenile counts recorded for the roots grown without NPK suggests that nutrients may change soil chemistry thus adversely affecting nematode mortality (Melakeberhan *et al.,* 1997). Nutrient rich environment may improve ability of plants to resist nematode infection or negatively influence their reproduction (Melakeberhan *et al.,* 1997).

Fig. 3. Differential growth of root systems of coffee seedlings grown under optimal (Left) and deficient (Right) nutrient conditions and inoculated with 5000 *M. incognita* eggs.

The population densities under deficient and optimal nutrient conditions support the theory that coffee seedlings planted in nutrient deficient soil suffer more from root knot nematode attack especially *M. exigua* and *M. incognita* infection than those in balanced nutrient conditions and results in low yields or total seedling loss (Walker, 1987). Other studies have shown that application of K_2O has significant positive influence on plant height, leaf size, seed yield, seed weight and seed size (Melakeberhan *et al.,* 1997; Jones, 2003). Provision of balanced fertilizer mixtures may have influenced the physiological status of the plant and in turn the population dynamics of plant parasitic nematodes as per earlier reports (Campos *et al.,* 1990). This is an indication that nutrients play a major role in plant growth and despite the existence of an extensive root system once the initial nutrients are depleted, growth is arrested and the plant becomes more vulnerable to nematodes and the few existing roots can be destroyed completely (Tisdale *et al.*, 1993).

The probable reason for plant tolerance to nematode damage as explained by Omotoso, (2007) is that nitrogen and potassium increases plant growth and yields or negatively influences nematode reproduction (Melakeberhan *et al.,* 1997). Research has shown that galled roots are hampered in their functions of water and nutrient uptake, plant growth is retarded, leaves become chlorotic and in case of seedlings infestation, plants may die or may not survive transplanting (Nestcher and Sikora, 1990). The changes in soil conditions and plant physiology with fertilizer application may also be detrimental to plant parasitic nematodes hence increasing mortality of juveniles, inhibiting egg hatch thus suppressing nematode reproduction (Carvalho *et al.*, 2008).

Fig. 4. Seedlings treated with NPK and inoculated with *M. incognita* at varying inoculum densities exhibiting nutrients deficiency symptoms.
Key: (A- water or control), B-1000, C-2000 and D-5000 eggs/kg).

This is an indication that balanced nutrition leads to vigorous growth of plants that outweighs an effect of pest attack.

Conclusion

Coffee plants growing under optimal nutrients express resistance to nematode attack resulting to reduce damage due to suppression of growth and multiplication of nematodes.

Plants growing in nutrient deficient soil suffer more from nematodes than those planted in optimal soil nutrient conditions. Growers should adhere to proper crop management by ensuring soil fertility in coffee farms either by adding organic amendments like manure or inorganic fertilizers at the recommended rates. Nutrition should be considered as part of a integrated strategy in the management of root knot nematodes.

References

Alban R, Guerrero R, Toro M. 2013. Interactions between a Root Knot Nematode (*Meloidogyne exigua*) and *Arbuscular mycorrhizae* in Coffee Plant Development (*Coffea arabica*). American Journal of Plant Sciences **4,** 19-23.
http://dx.doi.org/10.4236/ajps.2013.47A2003

Berberchek M, Duncan L. 2004. Nematode behaviour. Department of Entomology, Pennsylvania State University, USA. Pg 309-343.

Campos VP, Sivaplan P, Gnanapragasam NC. 1990. Namatode parasites of coffee, cocoa and tea. In Plant parasitic nematodes in sub tropical and tropical agriculture (Eds). Luc, M, Sikora, R.A. and Bridge, J. (Eds.). CAB International. Wallingford, Uk. 387-430.

Campos VP, Villain L. 2005. Nematodes parasites of coffee and cocoa. In: Plant parasitic nematodes in subtropical and tropical agriculture. Luc, M., Sikora, A. & Bridge, J. (Eds.), CABI Publishing, Wallingford, UK, pp: 529-579.

Coyne DL, Sahrawat KL, Plowright RA. 2004. The influence of mineral fertilizer application and plant nutrition on plant-parasitic nematodes in upland and Lowland rice in Cote D'Voire and its implications in long term Agricultural Research trials. Experimental Agriculture, **40**, 245-256 p.
http://dx.doi.org/10.1017/S00144797031595.

Carvalho MP, Rodrigues FA, Siveira PR, Andrade CC, Baron JCP, Paye HAS, Louriro JE. 2008. Rice resistance to brown spot mediated by Nitrogen and Potassium. Nutrition journal **158(3),** 160-166.
http://dx.doi.org/10.1111/j.1439-0434.2009.01593.x

Duncan L, Cohn E. 1990. Nematode parasites of citrus pp. 321-337 In: Luc M., Sikora R.A & Bridge J. (Eds). Plant parasitic nematodes in subtropical and Tropical Agriculture. 2nd Edition, CAB international, Institute of Parasitology. UK ISBN 0-85198-630-7.

Hooper DJ, Hallman J, Subbolin S. 2005. Methods of extracting and processing of plant and soil nematodes in Plant parasitic nematodes in subtropical and Tropical Agriculture. 2nd Edition edited by Luc M., Sikora R.A & Bridge J. (2005). CAB international, Institute of Parasitology. UK ISBN 0-85198-630-7, 45-68 p.

Jones JB. 2002. Agronomic handbook: management of crops, soils and their fertility. CRC Press. ISBN 0-8493-08977-6.

Khan MR. 2008. Current options for managing nematode pests of crops in India. Department of agricultural entomology, West Bengal, India. www.articlebase.com/science-articles/current-options-for-managing-nematodes

Kimenju JW, Maundu JN, Muiru WM, Wachira MP, Gichuru KE. 2014. Plant Parasitic Nematodes Associated With Coffee in Kenya and Factors Influencing their Occurrence, Abundance and Diversity. Journal of Biology, Agriculture and Healthcare. **4,** No.3, 2014. http://dx.doi.org/10.1.1.942.9516

Melakeberhan H, Bird GW, Gore R. 1997. Impact of plant nutrition on *Pratylenchus penetrans* infection of *Prunus avium* rootstocks. Journal of Nematology **29(3),** 381-388.

Maredia KM, Dakon D. Mota SD. 2003. Integrated Pest Management in the Global Arena. ISBN 0-85199-652-3. CABI Publishing.

Netscher C, Sikora A. 1990. Nematode parasites of vegetables 237-272 p. In: Plant parasitic nematodes in subtropical and Tropical Agriculture. 2nd Edition, edited by Luc M., Sikora R.A & Bridge J. (Eds). CAB international, Institute of Parasitology. UK ISBN 0-85198-630-7).

Nyasani JO, Kimenju JW, Olubayo FM, Shibairo SI, Mutua GK. 2008. Occurrence of Entomopathogenic Nematodes and their Potential in the Management of Diamondback Moth in Kale. Asian Journal of Plant Sciences. **7(3),** 314-318. http://dx.doi.org/ajps.2008.314.318

Omotoso SO. 2007. Effect of NPK fertilizer application rates and method of application on growth and yield of Okra (*Abelmosdus esculentus* L.). International Journal of Agricultural Research **2(7),** 614-619. http://dx.doi.org/10.3923/ijar.2007.614.619

Orisajo SB, Fademi OA. 2013. Plant-Parasitic Nematodes Associated With Coffee in Nigeria. International Journal of Science and Nature **3(4),** 768-772 http://dx.doi.org/10.1080/09670874.2013.766776.

Tisdale SL, Nelson WL, Beaton JD, Havlin JL. 1993. Soil fertility and fertilizers. Fifth edition. ISBN 0-02-42.835-3. U.S.A By Prentice-Hall, inc. upper Sandle River, New Jersey 07458. U.S.A.

Walker PT. 1987. Quantifying the relationship between pest population and damage yield loss. In Crop loss Assessment and Pest management. Teng, P.S. (Ed). The American phytopathological Society, Minesoa, USA. APs Press, 6-125.

Walter SA, Wehner TC, Barker KR. 1992. Effects of Root decay on the Relationship between *Meloidogyne* spp. gall index and egg mass number in cucumber and horned cucumber 1. Supplement to Journal of Nematology, **24(4),** 707-711.

Whitehead AG. 1998. Plant Nematode Control., CAB International, Wallingford, UK and USA . ISBN 0 85199 188 2. Reprinted in 2002.

Wintgens JN. 2009. Coffee: growing, processing, sustainable production. A Guidebook for growers, processors, traders and researchers. Second Revised Edition. Switzerland ISBN 978-3-527-32286-2.

Zhang F, Schmitt DP. 1995. Relationship of *Meloidogyne konaensis* population densities to coffee growth, Plant disease: **79,** 446-449.