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## Emerging Solutions to Challenges in Greenhouse Nematode & Plant Nutrition Tomato Production

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### ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) is a high-value vegetable. However, nematode infestation and nutrient depletion are major constraints in greenhouse tomato production, causing 35% to 40% losses. Chemical control options in Kenya compromises environmental safety. This study investigated the impact of soil amendments with fresh biomass from *Lippia kituensis* Vatke (LK) and *Ocimum gratissimum* L. (OG) on *Meloidogyne* sp., tomato growth, yield and quality. Unbalanced pot experiment in factorial format, embedded in RCBD, with 4 levels of plant biomass replicated 3 times was used. Biomass rates were 0, 2%, 4% and 8% in 10 kg potted soil mixes, singly and in all possible combinations. Neem extract (Azadirachtin 0.3 w/w) was used as positive control. Nematode population reduced by 82.2% at 8% of both *Lippia* and *Ocimum* combined, compared to 91.4% of Azadirachtin. Gall numbers reduced by 98.3% in roots compared to 98.7% recorded in Azadirachtin. Total root volume of 308.7 cm<sup>3</sup> was recorded in 8% LK + 8% OG compared to 69.33 cm<sup>3</sup> and 89 cm<sup>3</sup> for non-amended and Azadirachtin treatments, respectively. Means on leaf numbers were 29.90 compared to 22.67 and 28.13 of non-amended and Azadirachtin, respectively. Shoot and root dry weights of 53.17 g and 100.85 g were recorded compared to 24.17 g and 46.12 g and 51.75 g and 86.23 g for non-amendment and Azadirachtin, respectively. The yields were 2.71 kg per plant at 8% LK + 4% OG compared to 0.53 kg of non-amended treatment. Thus, *L. kituensis* and *O. gratissimum* L. are potential nematicides and can be used in tomato nutrition for sustainable production in the greenhouse.

**Key words:** *Plant biomass, Lippia kituensis, Ocimum gratissimum, Meloidogyne sp.*

## INTRODUCTION

Tomato is the leading greenhouse vegetable crop grown in both soil and soilless media. Soil based media often require appropriate amendments with compost and other additives to be effective. Under soil based greenhouse tomato production, nematode infestations become a serious constraint leading to yield reductions of 35-40%. Root-knot nematodes (*Meloidogyne spp.*) are the most damaging nematodes in tomatoes grown in the tropics (Walker, 2007). Control of nematodes in the greenhouse is largely done with chemicals - nematicides, which are costly and tend to persist in the soil after harvest, causing contamination of ground water. Consequently, several nematicidal compounds have been withdrawn from the market. There is growing interest in use of a wide variety of plant based amendments and cover crops for the management nematodes (Walker, 2007). Olabiyi *et al.* (2007) reported significant reduction in the soil population of *Meloidogyne spp.*, *Helicotylenchus sp.* and *Xiphinema sp* in field grown with cowpea after application of high levels of organic plant based manures. Use of composted dry cork, dry grape marc and a mixture of dry olive marc has been reported to significantly reduce root galling and populations of *Meloidogyne incognita* and *M. javanica* in plots planted with tomato compared to controls (Andres *et al.*, 2004). Similarly, Garcia *et al.* (2006) reported effective control of root knot nematodes using pepper crop residues within 20 days of application.

It has also been suggested that reduction of nematodes and associated damages in fields treated with plant biomass wastes results from improved soil structure and fertility, alteration of plant resistance from release of nematode toxins, or increased population of fungal and bacterial parasites or other nematode antagonistic agents (McSorley, 2011). Apart from nematode control, these biomass based also provide essential nutrients (such as N and P), help rebuild soil organic matter contents, and aid in the re-establishment of beneficial microbial populations (Suresh *et al.*, 2004; EPA, 2007; Dauda *et al.*, 2008). Although these organic materials provide plant nutrients in small quantities compared to inorganic fertilizers, they also complement plants by releasing other growth factors such as hormones thereby improving overall plant productivity (Sanwal *et al.*, 2007). Additionally, higher organic matter content increases soil water holding capacity and supports thriving communities of decomposers and predators in the soil system.

The nematicidal properties of plants may be contributed from extracted biomolecules resident in the plant bodies. The major classes of compounds with proven nematicidal activity include alkaloids, fatty acids, glucosinolates, isothiocyanates, phenols, diterpenes and a variety of essential oils (Chitwood, 2002). Neem cake, known to be rich in azadirachtin is also associated with strong nematicidal activity (Riga and Lazarovits, 2001; Abbasi *et al.*, 2005). Laboratory extracted essential oils have also been reported to affect development of nematode eggs and second juvenile stage under *in vitro* conditions (Onifade, 2007). Other extracts with strong pesticidal properties include rotenone, nicotine and pyrethrins (Berger, 1994).

Control of nematode infestations in soil based greenhouse tomato production using fresh plant derived biomass from species known to be rich in essential oils has not been reported. In this study we report results of a greenhouse pot experiment to evaluate management of root knot nematodes using fresh plant derived biomass soil amendments with *Lippia kituensis* Vatke. (Verbenaceae) and *Ocimum gratissimum* L. (Lamiaceae).

## **MATERIALS AND METHODS**

### **Site Description**

The study was conducted for two growing seasons at the Horticulture Research and Teaching Field, Egerton University, Kenya in 2012. The field is located on latitude 0 23'S, longitude 35 35'E in the lower highland III (LH3) agro ecological zone at an altitude of 2238 m a.s.l (Jaetzold and Schmidt, 1983). The site receives a mean rainfall of 1012 mm with a mean day temperature of 22°C and night ranges of 5 - 10°C. The pot experiment was conducted in a polytunnel greenhouse measuring 8 m wide × 60 m length and 3 m height, covered with UV stabilized polythene sheet gauge 200µm.

### ***Biofumigant plant materials preparation***

Leafy twigs of *L. kituensis* Vatke. and *O. gratissimum* L were collected in the wild around Egerton University from flowering plants. The materials were chopped into aggregates approx. 0.5 cm to enable proper mixing with soil and to increase the surface area for decomposition activity. The chopped aggregates were incorporated into 10kg of potted solarized forest soil at four levels (0% (control), 2%, 4% and 8%g w/w), singly and in all possible combinations. A positive control treatment of a commercial organic nematicide from a neem extract (Azadirachtin) was also included.

### ***Crop establishment and maintenance***

Tomato seedlings were raised in a protected nursery bed for four weeks and transplanted into plastic sleeves (8 cm x 14 cm x 14 cm), Gauge 300, filled with the various plant biomass and solarized soil mixtures. The pot soil/biomass mixtures were single rate treatments (0% (control), 2%, 4% and 8% w/w) of the two plant species and all possible combinations of the rates. To each pot 10 grams of diammonium phosphate (DAP- 18:46:0) was added as basal fertilizer, an equivalent of 200 kg/ha DAP. On attaining a height of 30 cm, plants were trained on to a binding wire trellis placed 150 cm above the pots. Top dressing with calcium ammonium nitrate (CAN, 26% N) at the rate of 10 g per pot was applied 21 days after transplanting (DAT). Plants were pruned to maintain two stems per plant and watering was done continuously during the growing period with rates being adjusted according to plant growth

phases. In the first 30 DAT, 2 litres of water was applied per plant per day and thereafter, the rate was increased to 3 litres per day as the plants developed. Weeding and pest and disease control were performed as required.

### **Nematode augmentation, extraction and inoculum preparation**

Nematodes were collected from a field previously grown with infested tomatoes and augmented on two weeks old potted tomato seedlings established in a greenhouse following the method of Siddiqui and Akhtar, (2007). Specifically, Galls were extracted from the roots of infested tomatoes, chopped and mixed with the native soil. The mixture was added to pots planted with 2 week old tomato seedlings and the inoculum allowed to infest and multiply for 8 weeks.

After augmentation, nematode egg masses were extracted from the heavily galled tomato roots by chopping the roots to lengths of 0.5 cm and macerating the tissues to release egg masses. These were placed in 15 cm diameter sieves of 1 mm pore size, lined with cross-layered tissue papers and incubated at 27°C to hatching in glass petri-dishes containing distilled water. After hatching, the second instar juveniles (J2) were transferred into 2 litre conical flasks. Quantification of juveniles was done under a stereoscope with gridded petri dishes. Ten 1 ml replicate samples were drawn from the well mixed suspensions to establish the average number of juveniles per ml. The determined quantity was 20 juveniles per ml. Finally, the nematode inoculum suspension samples were adjusted to contain approx. 1000 juveniles in 50 ml of distilled water.

### **Nematode inoculation and determination of infestation parameters**

The 50 ml J2s inoculum suspensions were added to pots containing the various plant biomass amended media planted with 28 day old tomato transplants. The inoculum was allowed to develop under normal tomato culture conditions in a polytunnel greenhouse. Destructive sampling of tomato plants to determine nematode infestation was conducted 100 DAT. Four plants from each replicate were sampled at the peak of flowering.

**Nematodes population:** To determine the nematode population in the biomass amended pot soil treatments, second stage juveniles (J2) were extracted from 100 cm<sup>3</sup> composite sample of soil from each replicate, using the method described by Kimenju et al., (2010). Specifically, at 100 DAT, the soils from each of the four pots were sampled by taking 100 cm<sup>3</sup> of sample. The samples were placed in 9 cm diameter sieves with pore diameters of 1 mm lined with double layered tissue paper. The sieves were half immersed in metallic troughs containing 250 ml of distilled water to allow nematode migration into

the water underneath for 24 hours. Nematode counts were determined in 10 replicate samples of 1 ml for each soil sample as previously described.

**Gall numbers and galling index:** For gall assessments, plants were gently uprooted and their roots thoroughly washed under tap water to remove all the adhering soil. Galling was determined by counts of galls size 1 mm diameter and above. The galling index was scored on a scale of 1-10, where 0= no gall, 1= 1-50 galls, 2= 51-100 galls, 3= 101-150 galls, 4= 151-200 galls, 5= 201-250 galls, 6= 251-300 galls, 7= 301-350 galls, 8= 351-400, 9= 401–450 and 10= 451 and above (Kimenju et al., 2010). The scores were converted into numerical entries and their means worked out for analysis of variance.

### **Experimental design and data analysis**

The experimental design was an unbalanced factorial in a Randomized Complete Block Design (RCBD) with a total of 17 treatments. Each replicate treatment consisted of 6 pots which represented a plot. Treatments were replicated three times including the positive control treated with 0.3% w/w Azadirachtin. The pots were arranged in rows spaced at 60cm X 40cm. Data was analysed by analysis of variance (ANOVA) and means separated by the Tukey's LSD using The SAS statistical program version 12e.

## **RESULTS**

### **Effect of soil organic amendment on nematode population**

Different levels of amendments with *Lippia kituensis* Vatke and *Ocimum gratissimum* L. biomass significantly influenced nematode populations in the amended soils (Table 1). At 100 DAT in season 1 and 2, the various rates of the two plant organic amendments indicated suppression of juvenile populations in the soil compared with the control. In season 1, single treatments of *Lippia* and *Ocimum* at 200g, 400g and 800g reduced nematode population in the soil by 14%, 61.3%, and 67% and 4.7%, 14% and 47.7%, respectively, compared with the control (Table 1).

A combination of *Lippia* and *Ocimum* at rates of 800 each produced the best nematode population reduction of 82.2%. This effect was not significantly different from treatment with Azadirachtin recorded a 91.4% population reduction. Similarly, no statistically significant differences in nematode population reduction were observed when *Lippia* and *Ocimum* were applied in combinations of 800LK + 400OG (81.5%) and 400LK + 800OG (79.8%), respectively. Similar observations were made in season 2. Figure 1 shows a general trend of nematode reduction with the increasing rates of organic amendments, used singly or in combinations.

**Table 1. Effect of plant biomass rates on the nematode populations extracted from 100 cm<sup>3</sup> of potting mixture**

<b>Season 1</b>		<i>Lippia</i> levels (g)			
<i>Ocimum</i> levels (g)	0	200	400	800	<i>Ocimum</i> means
0	53.42a**	39.25c	19.42fg	21.67f	<b>33.44a</b>
200	46.33b	39.50c	17.08gh	12.08i	<b>28.75b</b>
400	35.83d	34.67d	16.50h	9.7ij	<b>24.17c</b>
800	29.50	28.425e	10.58ij	9.5j	<b>19.50d</b>
<b><i>Lippia</i> means</b>	<b>41.25a</b>	<b>35.46b</b>	<b>15.90c</b>	<b>13.23d</b>	
<b><i>Azadirachtin</i></b>		<b>4.25</b>			
<b>Season 2</b>					
0	51.58a	38.50b	19.17f	19.50f	<b>32.19a</b>
200	48.42a.	36.92bc	17.92f	12.42gh	<b>28.92b</b>
400	33.33cd	31.92cde	15.42fg	9.17h	<b>22.46c</b>
800	31.75de	28.25e	10.17h	9.33h	<b>19.87d</b>
<b><i>Lippia</i> means</b>	<b>41.27a</b>	<b>33.90b</b>	<b>15.67c</b>	<b>12.60d</b>	
<b><i>Azadirachtin</i></b>		<b>4.08</b>			

\*\*Means followed by the same letter series within a column and a row per season are not significantly different according to Tukey's LSD at  $P \leq 0.05$ .

### **Effect of organic amendments on gall numbers and galling index**

Gall numbers and galling index were destructively determined 100 days after transplanting (DAT). Gall numbers and galling index were significantly influenced by organic amendments in both seasons I and 2 (Table 2). There was a general decrease of gall numbers in the roots of tomato with increased levels of *Lippia* and *Ocimum*. In season 1, application of *Lippia* alone at rates of 4% and 8% reduced the galls in the roots by 89.8% and 93.8% respectively, in comparison to the non-amended soil. *Ocimum* applied at the same rates had a lower response in root galls, registering reductions of 35.5% and 41.1%, respectively. Similar result trends were observed in season 2, only that gall numbers were higher in

season 2. Combination treatments of *Lippia* and *Ocimum* were most effective in terms of reducing gall numbers but no significant differences were evident among the various combinations. The combination treatments were also comparable with Azadirachtin treatment (Table 2). The root-knot galling of tomatoes generally varied according to the rates of *Lippia* and *Ocimum* applied. As with gall numbers, the galling index also showed a reducing trend with increasing levels of the two soil organic amendments (Figure 2).

**Table 2. Effect of fresh organic amendments on gall numbers**

Amendments* (%)	Season 1	Season 2
0	718.33a**	773.00a
200 OG	671.33a	585.00b
400 OG	463.00b	435.67b
800 OG	423.00b	334.00c
200LK	54.33c	449.00b
400 LK	73.00c	280.00cd
800 LK	47.00c	143.00c
200LK +200 OG	97.33c	470.67b
200LK+400 OG	57.33c	271.33b
200LK +800G	20.33c	210.00d
400LK+ 200 OG	52.33c	246.00d
400LK+ 400 OG	14.67c	17.33d
400LK+800 OG	15.33c	72.67cd
800LK +200 OG	27.33c	45.00d
800LK +400 OG	15.00c	99.00cd
800LK +800 OG	12.00c	36.00d
Azad.	9.00c	8.67d

\*OG = *Ocimum gratissimum* L. and LK = *Lippia kituensis* Vatke, 0 = control Azad = Azadirachtin.

\*\*Means with the same letter within a column are not significantly different according to Tukey's LSD at  $P \geq 0.05$ .

Generally the root systems from pots amended with a mixture of 800 g of each of *Lippia* and *Ocimum* biomasses consisted of numerous fibrous roots with few small galls compared to those without amendments (Plate 1A). Plants from non-amended soil had comparably larger diameter roots with more pronounced galling (Plate 3B).

### Marketable and non-marketable fruit yield

The effects of *Lippia* and *Ocimum* biomass significantly ( $P \leq 0.05$ ) influenced marketable tomatoes (Table 3).

**Table 3. Effect of fresh plant biomass on marketable, non-marketable yields and number of fruits per plant.**

	Fruit/plant (Numbers)	Mkt wt/ plant (kg)	Non-mkt/ plant (kg)	Fruit/plant (Numbers)	Mkt wt/ plant (kg)	Non-mkt/ Plant (kg)
Amendments (mt/ha)	Season one			Season two		
0	22.25d	0.53j	0.35a	22.23d	0.50i	0.44a
2% OG	24.33d	0.82i	0.36a	24.36d	0.95h	0.36abcd
4% OG	28.83cd	1.80de	0.31abc	28.92cd	1.79cd	0.30abcd
8% OG	28.25d	2.16bc	0.35a	28.28d	2.15b	0.33abcd
2% LK	27.17d	1.35gh	0.25abcd	27.13d	1.35fg	0.24cdef
4% LK	35.63cd	1.25h	0.37a	35.83cd	1.25g	0.37abc
8% LK	49.83abc	1.65ef	0.32ab	49.87abc	1.61de	0.28bcde
2% LK + 2% OG	39.27bcd	1.49fg	0.31abcd	39.37bcd	1.50efg	0.29bcde
2% LK + 4% OG	35.58cd	1.95cd	0.36a	35.78cd	1.94bc	0.29bcde
2% LK + 8% OG	31.10cd	2.19b	0.28abcd	31.30cd	2.18b	0.0.22def

4% LK + 2% OG	30.43cd	1.87d	0.21abcd	30.48cd	1.87c	0.23def
4% LK + 4% OG	40.58bcd	1.61ef	0.25abcd	40.46bcd	1.54def	0.24cdef
4% LK + 8% OG	57.83ab	2.6a	0.14d	57.63ab	2.60a	0.13f
8% LK + 2% OG	36.25cd	2.16bc	0.17bcd	36.21cd	2.16b	0.17ef
8% LK + 4% OG	41.40bcd	2.71a	0.17bcd	41.51bcd	2.71a	0.13f
8% LK + 8% OG	62.67a	2.62a	0.15cd	62.77a	2.61a	0.14f
Azad.	32.58cd	2.23b	0.38a	32.42cd	2.20b	0.39ab

\*OG = *Ocimum gratissimum* L. and LK = *Lippia kituensis* Vatke, are Soil organic amendments, 0 = no amendment and Azad = Azadirachtin.

\*\*Means followed by the same letter in a letter series within a column per season are not significantly different according to Tukey's honestly significant difference (THSD) at  $P \leq 0.05$ .

In season 1, the highest weight of marketable fruits from single treatments was harvested from treatment of *Ocimum* at 8%OG with 2.16 Kg per plant while non-amended treatment yielded the lowest weight (0.53 kg) per plant. The result was similar in season 2. The single *Lippia* treatment at 8% LK produced 1.65 kg of tomatoes per plant, which was significantly ( $P \leq 0.05$ ) lower than those from *Ocimum* at the same rate of amendment. In the combination of the two amendments, 8% LK+ 4% OG produced the highest weight of tomatoes per plant (2.72 kg), while from Azadirachtin treatment 2.23 kg per plant was harvested.

Non-marketable fruit yield trends were opposite to those of marketable fruit weight. In both seasons, the highest number of non-marketable fruits was obtained from Azadirachtin and non-amended treatments. However there were no significant difference between the single rates and non-amended treatments on weight of non-marketable tomatoes in seasons 1 and 2. The combination with the lowest non-marketable weight was 4%LK + 8%OG (0.14 kg) per plant and this was not significantly different from other various combinations of treatments (Table 3). Generally, for any given treatment there was very little significant variation on non-marketable weights in both seasons (Plate 2). On marketable quality, significant relationship was also observed between nematode population and marketable yield ( $P= 0.0001$ ). Analysis revealed that increase in nematode population resulted to a decrease in marketable tomato fruit weight as explained by the equation {Marketable yield (kg/plant) =  $2.4019876 - 0.0320059 \times \text{Nematode population (no./plant)}$ } (Figure 4A). The relationship between nematode population and fruit was also significant ( $P= 0.0001$ ). Firmness of tomato fruit decreased with increase in nematode population as explained by the equation; Fruit firmness (KgF) =  $4.4193861 - 0.0405667 \times \text{Nematode population (no. / plant)}$  (Figure 4B).

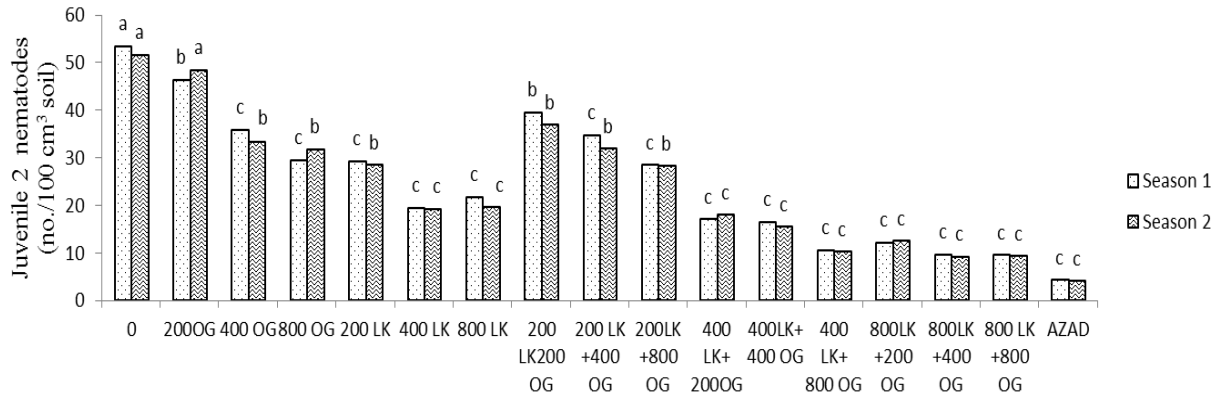


Figure 1. Trends of nematode populations against fresh plant biomass treatments.

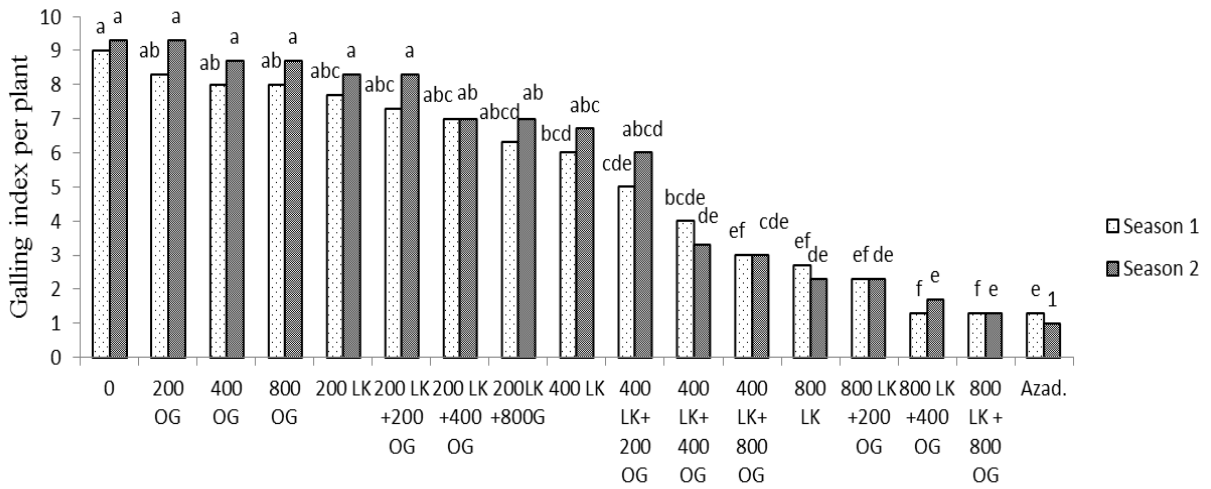
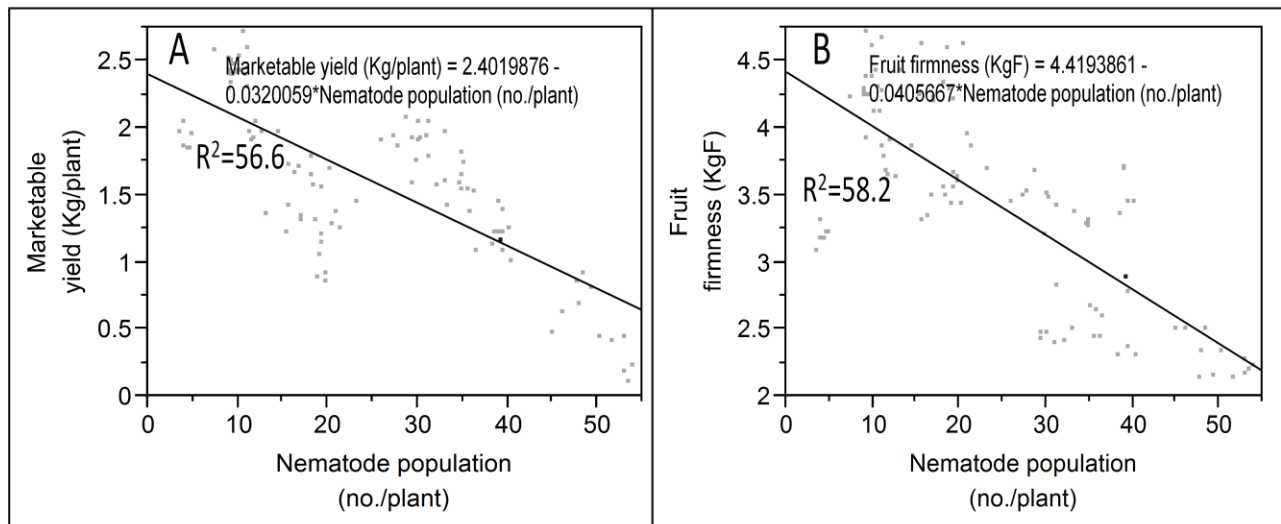


Figure 2. Gall index trends against soil amendment treatments



**Figure 3. Relationship between nematode population, marketable yield (A) and fruit firmness (B)**



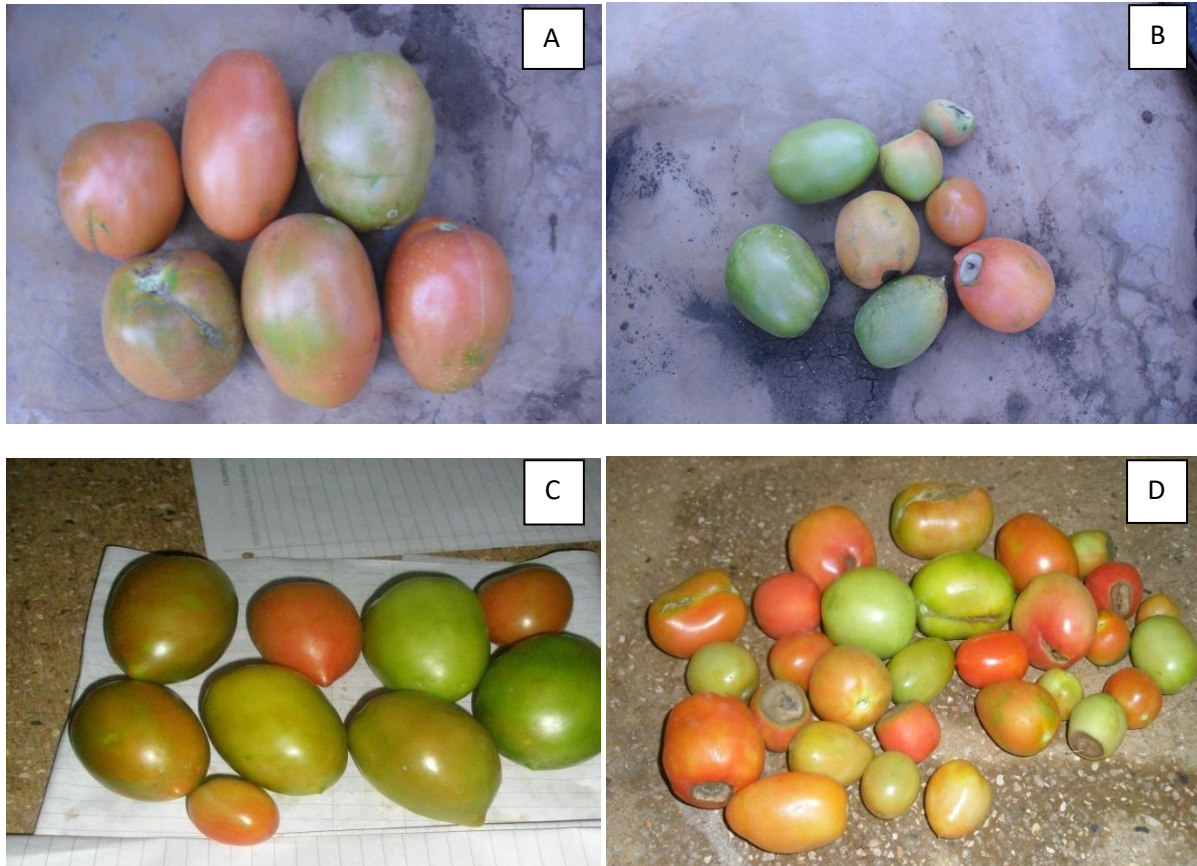
**Figure 1. Root characteristics of tomato plants grown in soil treated with a combination of 8% Lippia + 8% Ocimum showing clean healthy roots (A) and tomato roots from a control treatment showing galls (B).**

## DISCUSSION

Use of various indigenous plants and botanical extracts as sources of organic soil amendments has become an important option in pest management. Apart from direct toxicity, plant derived soil amendment acts in various ways against plant parasitic nematodes. For instance, increased microbial activity in the soil amended with certain plant materials may enhance enzymatic activities, accumulation and decomposition of organic matter and also microbial metabolites which are deleterious to nematodes (Kagai et al 2012). This study has revealed positive effects of *Lippia kituensis* Vatke and

*Ocimum gratissimum* L. fresh biomass for the control of nematodes (*Meloidogyne* spp.) in greenhouse tomatoes. Overall, the results indicated effective nematode control in the tomato crop treated with the plant biomass compared to the control treatments where no amendments were applied. Additionally, it was generally observed that combination biomass treatments of the two species at the higher rates tested (i.e. 400g and 800g) were more effective than the single treatments. Oka et al. (2007) reported that sensitivity of plant-parasitic nematodes to plant derived nematicides, however, indicated that the effect varied with the nematodes species targeted and the rates applied. He further reported that the second instar juveniles (J2) of *Meloidogyne* spp were more susceptible to the treatments. Proliferation of the second instar juveniles, which is the more destructive stage of the nematode, was effectively suppressed when growing soils were treated with the fresh biomass of the two plant species tested.

Several postulations on the mechanisms of action of these fresh biomass materials have been put forward. One of them is that during the decomposition of organic materials, volatile fatty acids, ammonia and hydrogen sulphide gases are released (McSorley, (2011) and these enhance nematode control. In this study *L. kituensis* and *O. gratissimum* biomass additions to soil proved toxic to *Meloidogyne* spp. under greenhouse conditions. These treatments effectively reduced the nematode J2 population in the soil to tolerable levels by these plants derived soil amendments (Table 2). Similar results have been reported by Kagai et al. (2012) with selected plant biofumigants in the management of plant parasitic nematodes in *Asclepias tuberosa* L. Claudia et al. (2004) reported that essential oils from Argentine *Lippia* (*L. juneliana* and *L. turbinata*), has effect on nematodes control in the laboratory condition. The same has been confirmed by Onifade, (2007) using essential oils from basil (*Ocimum basilicum*) to have nematicidal effect on parasitic nematodes, especially RKN *Meloidogyne* spp and root lesion nematode *Pratylenchus penetrance*. In this study, *Lippia* and *Ocimum* decomposition and mineralization probably released essential oils that were effective in RKN control. Other possible mechanisms for nematode suppression by these organic amendments include direct inhibition or reduced infectivity of nematodes on the plant host. In the present context, it may be that the use of *Lippia* and *Ocimum* as fresh soil organic amendment increased antagonism in the soil mixes by increasing the abundance of other competing beneficial organisms as also reported by Akhtar and Alam (1993a).



**Plate 2. Differences in the quality of tomatoes produced with different rates of *Lippia* and *Ocimum* amendments. A=4 LK+4% OG; B= 0 LK+2%OG; C= 8% LK+8% OG and D= Non- amended soil.**

A number of other mechanisms have been proposed to explain the beneficial effects of organic amendments on plants infested with nematodes (McSorley, 2011). These include the release of nematicidal compounds from decomposing materials, stimulating the natural enemies of nematodes and improving plant tolerance to nematodes. Chen et al., (2000) reported that breakdown of plant organic material releases nematicidal substances that contribute to nematode control. Akhtar and Malik (2000) further reported that crops and weeds release biochemicals that counteract the activities of nematodes. *Lippia* and *Ocimum* have been reported to yield essential oils of diverse nature (Atuboyedia et al., 2010). In particular, *Lippia* has been reported to possess piperitenone oxide, limonene, camphor and spathulenol, piperitenone oxide (Claudia et al., 2004). Analysis of *Ocimum* yielded eugenol, citrol linalol, charvicol, thymol, gerianol, triterpenoids, saponins and alkaloids (Matasyoh et al., 2007; Ogendo, 2008). Based on the findings of the present investigation, it is may be plausible to suggest that these biomolecules would have been extracted during decomposition of the plants biomasses to inhibit nematode activity in the amended soil.

The efficacies of soil amendments in this study increased with the dosage of application. This is consistent with the work of Ogwulumba et al. (2010). Similarly, Faruk et al. (2011) reported similar results of the effect of plant organic amendment for controlling RKN *Meloidogyne incognita* on country bean. Further observations by Onifade (2007) indicated that use of essential oils of *O. gratissimum* and *O. basillicum* *in vitro* at rates ranging from 25-100  $\mu\text{g mL}^{-1}$  completely inhibited egg hatching and larval survival of nematodes. Attacks by RKN can significantly limit tomato production worldwide particularly in green house production. Based on the findings from the current studies, the organic soil amendment is a nematode management option, and numerous aspects of this research have practical applications in commercial agriculture for the solution of the pest problems. Management of nematode population and reduction of gall numbers, were contribution from *Lippia kituensis* Vatke and *Ocimum gratissimum* L. applied at different rates. *L. kituensis* Vatke and *O. gratissimum* L. significantly reduced the nematode population in the soil and the gall numbers in the roots (Table 2); hence they have the potential to manage RKN *Meloidogyne* spp. From this study, *L. kituensis* Vatke and *O. gratissimum* L. combined, or as single rates at the range of 400 g to 800 g of either of the plant biomass, was the most suitable rate for nematode management in the greenhouse. Based on the findings of the present investigation, different levels of fresh plant biomass from *Lippia kituensis* Vatke and *Ocimum gratissimum* L. have significant effect on the management of root knot nematodes in greenhouse produced tomatoes.

Besides direct toxicity to RKN, *Lippia* and *Ocimum* influenced tomato yield in terms reducing plant photosynthate's sinks (giant cells), which formed root galls. These root galls are feeding sites in the plant that attracts plant photosynthates down to the roots at expense of other plant parts, thus reducing the growth and yield. Reduction of the RKN population and consequently gall number (Table 2), also meant reduced photosynthate sinks in the root, which increased tomato marketable yield (Table 3). Volvas *et al.* (2005) reported that parasitism by RKN involves the establishment of permanent feeding sites called giant cells in the root cortex, endodermis, pericycle and vascular parenchyma which is sinks for photosynthates, leading to impaired growth of plants low productivity of Chick pea. The effect of biomass of the two plants *Lippia* and *Ocimum* may have probably reduced the sink sites in roots leading to better yield in amended soil treatments.

The biomass used caused increase in plant growth vigor and yield by suppressing nematode population and increasing the growth through the nutrients available in *Lippia* and *Ocimum* amendments. Similar observation was reported by Walker (2007) in the study on the effect of organic amendments, fertilizers and fenamiphos on parasitic and free-living nematodes on tomato, where growth and yield were increased on amended soil. This is further supported by the studies of Claudius-Cole *et al.* (2010) who evaluated plant extract in the management of *Meloidogyne incognita* on cow pea *Vigna unguiculata* (L) Walp. In their work the boost on yield was connected with nutrient derived from *O. basillicum*, used as organic amendment. In earlier study by Hasabo and Noweer (2005), it was reported that the extract of *Ocimum* reduced nematode population on eggplant, which resulted in higher yields. In the current investigation, it was apparent that those plants established in higher rates of *Lippia* and *Ocimum*

produced fruits of better quality and marketable size, compared to those in non-amended treatment (Plate 2). From the regression equation (Figure 4A), it was evident that nematode reduced tomato fruit marketable yield. This implied that 56.6% of the reduction in marketable yield was due to the effects of nematode population. However, in tomato quality, only fruit firmness was significantly affected by nematode population at 58.2% (Figure 4B). These plant organic amendments can be used combined or as single rates at the range of 400 g to 800 g of either of the biomass, for nematode management in the greenhouse tomatoes production.

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